INFLAMMATORY CYTOKINES AND THEIR RECEPTORS IN PSORIATIC SKIN

Emphasis on the epidermal interleukin 1 system

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ONTSTEKINGSCYTOKINEN EN HUN RECEPTOREN IN PSORIATISCHE HUID

Nadruk op het epidermale interleukine 1 systeem

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The figure on the cover is explained in chapter 5.

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"Stand tall, touch the clouds"
Mick Jagger

CONTENTS

Chapter 1

GENERAL INTRODUCTION

General introduction

Chapter 1

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Nearly two centuries have passed since the first accurate description of psoriasis by Wilan in the year 1808 (1). Psoriasis is a common, well-defined skin disease featuring the interplay of genetic, environmental, and immunological factors. Extensive reviews on psoriasis are given elsewhere (2). Despite numerous reports, the exact etiology is still unknown. Moreover, 70 % of psoriatic patients are not satisfied with the currently available anti-psoriatic treatments (Gottlieb AB, oral presentation, Psoriasis Meeting, Dec 4-5 1995, Lake Buena Vista, FL), which stresses the contemporary societal interest of psoriasis research.

Recent studies have identified a dysregulated crosstalk between resident skin cells and infiltrating leukocytes in psoriasis. Several lines of evidence point to the cytokine interleukin 1 (IL-1) as an integral component of the cellular immune basis of psoriasis. The regulation of cytokine activity in general, and that of epidermal IL-1 in particular, is described in chapter 1.1. Skin as a site of immune responses, exemplified by the cutaneous inflammatory response, is described in chapter 1.2. Finally, the cellular immunopathogenesis of psoriasis, with special emphasis on IL-1, is reviewed in chapter 1.3.

1.1 CYTOKINES AS CELLULAR COMMUNICATORS

For over two decades, it is recognized that immune-competent cells produce peptide mediators, now termed cytokines, which act as chemical communicators between cells, but mostly not as effector molecules in their own right. In general, cytokines are not constitutively expressed, but are rapidly produced after stimulation and act locally to restore homeostasis (e.g. insulin, a soluble peptide mediator which is continuously present in the circulation and serves to maintain glucose homeostasis, is not considered a cytokine). Cytokines bind with high affinity to specific receptors on the surface of target cells. A characteristic feature of cytokines is their functional pleiotropy and redundancy, which can be largely explained at the molecular level of receptor systems (see below).

Families of cytokines

Table I presents a scheme in which cytokines are grouped according to their three-dimensional structure and receptor usage. This scheme is still incomplete and inconclusive. At least six different families of cytokines have been identified: the hemopoietins (four α-helical bundles); IL-1 and fibroblast growth factor (FGF) (capped β-barrel of 12 antiparallel β-strands); tumor necrosis factor (TNF) (sandwich of antiparallel β-sheets in jelly roll motif);

transforming growth factor-alpha (TGF-α) and epidermal growth factor (EGF) (β-sheets); TGF-β, platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and nerve growth factor (NGF) (cysteine knots); and chemotactic cytokines (chemokines) (triple-stranded, anti-parallel β-sheets in Greek key motif) (3,4). The large family of chemokines can be subdivided into α-chemokines (the two most amino-proximal cysteine residues are separated by an intervening amino acid: CXC) and β-chemokines (CC), which are functionally distinct, as they predominantly attract neutrophils and monocytes, respectively (6). It is intriguing that different structural domains of cytokines (e.g. IL-1β) may display different or even antagonistic cytokine activities (7).

Table I. Structural families of cytokines and their receptors.[a,b]

Family of cytokine[c]	Members	Superfamily (SF) of cytokine receptor[d]
Hemopoietins[e]		Cytokine receptor SF class I
	IL-6, IL-11, LIF, OM, CNTF, CT-1	gp130
	IL-3, IL-5, GM-CSF	common β-chain
	IL-2, IL-4, IL-7, IL-9, IL-13, IL-15	common γ-chain
	IL-10, IFN-α, IFN-β, IFN-γ	Cytokine receptor SF class II
	M-CSF	Immunoglobulin SF
IL-1	IL-1α, IL-1β, IL-1ra, FGF-α, FGF-β	Immunoglobulin SF
TNF	TNF-α, TNF-β, LT-β	TNF receptor SF
TGF-α	TGF-α, EGF	Protein tyrosine kinase receptor SF
TGF-β	TGF-β1, TGF-β2, TGF-β3	TGF-β receptors[f]
	PDGF, VEGF	Immunoglobulin SF
	NGF	TNF receptor SF
Chemokines		Chemokine receptor SF
α subfamily	IL-8, GRO-α, GRO-β, GRO-γ, ENA-78, IP-10, MIG, GCP-2, PBP (and its cleavage products: CTAP-III, β-thromboglobulin, NAP-2), PF-4, SDF-1α, SDF-1β	
β subfamily	MCP-1, MCP-2, MCP-3, RANTES, I-309, MIP-1α, MIP-1β	

[a] Refs 3-5.
[b] Abbreviations: CNTF, ciliary neurotrophic factor; CT, cardiotrophin; CTAP, connective tissue activating protein; EGF, epidermal growth factor; ENA, epithelial-derived neutrophil attractant; FGF, fibroblast growth factor; GCP, granulocyte chemotactic protein; (G)M-CSF, (granulocyte) macrophage-colony stimulating factor; IFN, interferon; IL, interleukin; IP, IFN-γ-inducible protein; LIF, leukemia inhibitory factor; LT, lymphotoxin; MCP, monocyte chemotactic protein; MIG, monokine induced by IFN-γ; MIP, macrophage inflammatory protein; NAP, neutrophil-activating peptide; NGF, nerve growth factor; OM, oncostatin M; PBP, platelet basic protein; PDGF, platelet-derived growth factor; PF, platelet factor; ra, receptor antagonist; RANTES, regulated on activation, normal T-cell expressed and secreted; SDF, stromal cell-derived factor; SF, superfamily; TGF, transforming growth factor; TNF, tumor necrosis factor; and VEGF, vascular endothelial growth factor.
[c] The families of cytokines are named after a representative member (except for the hemopoietins and chemokines).
[d] Members of cytokine families were subclassified according to receptor usage.
[e] The family of hemopoietins also comprise G-CSF and erythropoietin (Epo), but data on their receptors are insufficient for receptor classification.
[f] To date, the TGF-β receptors are not grouped into a SF of cytokine receptors (see text).

Families of cytokine receptors

Cytokine receptors are classified into several superfamilies based on common homology regions. The main superfamilies recognized today are the cytokine receptor superfamilies class I and class II, the immunoglobulin superfamily, the TNF receptor superfamily, the protein tyrosine kinase receptor superfamily, and the chemokine receptor superfamily (4). It should be noted that the superfamilies are not restricted to cytokine receptors (for example, several hormone receptors and leukocyte membrane polypeptides also belong to cytokine receptor superfamilies) and that several cytokine receptors belong to more than one superfamily (e.g. IL-6 receptor belongs to both the class I receptor and immunoglobulin superfamilies). All cytokine receptors are single transmembrane proteins, except for the chemokine receptors which contain seven transmembrane domains.

The cytokine receptor superfamily class I receptors are characterized by the presence of combinations of cytokine and fibronectin III domains in the extracellular regions. The amino terminal cytokine domain(s) contains four conserved cysteine residues, and (one of) the membrane proximal fibronectin III domains contains the Trp-Ser-X-Trp-Ser (WSXWS) motif required for ligand binding (8). Functional class I receptors consist of a complex of two or three receptor chains. These multisubunit receptors can be grouped according to the identity of common chains which are required for high affinity ligand binding and signal transduction. Three subgroups of class I receptors were identified sharing either gp130 (a glycoprotein with a relative molecular mass (M_r) of 130-kDa, cluster of differentiation (CD) 130), the common β-chain (M_r: 120-kDa, KH97) or the common γ-chain (M_r: 64-kDa) (9) (Table I). The gp130 chain is shared by the receptors (R) for IL-6, IL-11, ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), oncostatin M (OM) and cardiotrophin-1 (CT-1) (10). Ligand binding by this group of receptors induces homodimerization of gp130 (in case of IL-6R and IL-11R) or heterodimerization of gp130 and LIFR (in case of CNTFR). LIF, OM and CT-1 are believed to directly induce heterodimerization of gp130 and LIFR (OM and perhaps CT-1 also induce heterodimerization of gp130 and the ligand-specific receptor). The LIFR, granulocyte-colony stimulating factor (G-CSF) R and IL-12R are highly homologous with gp130 and belong to the so-called gp130 family. The common β-chain is shared by IL-3R, IL-5R and granulocyte and macrophage-CSF (GM-CSF) R, whereas the common γ-chain is shared by IL-2R, IL-4R, IL-7R, IL-9R, IL-13R and IL-15R.

Recent evidence suggests that the cytoplasmic parts of these signal transducing chains harbor two functional domains: a membrane-proximal region which is essential for proliferation signals (*c-myc* induction), and a membrane-distal region which is a essential for differentiation signals (*c-fos* and *c-jun* induction) (11,12). Ligand-induced receptor di- or oligomerization typically results in activation of (receptor-associated) protein tyrosine kinases (such as members of the SRC and Janus kinases (JAK)) and subsequent phosphorylation of several substrates (such as the receptors themselves,

members of the RAS-mitogen activated protein (MAP) kinase cascades, and transcription factors). Phosphorylation of tyrosine-based motifs in the cytoplasmic domains of the receptors is followed by specific docking and activation of signal transducing and activator of transcription factors (STATs) (12,13). Activated STATs form homodimers, translocate into the nucleus, and bind to responsive elements to initiate gene transcription. The JAK-STAT pathway results in rapid and specific transcriptional activation of target genes, and may, at least in part, contribute to cytokine-specific responses through selective activation of individual JAK kinases (JAK1, JAK2, JAK3 and TYK2) and STAT factors (STAT1 through STAT6) (14,15).

The cytokine receptor superfamily class II receptors (interferon (IFN) R and IL-10R) contain one or two extracellular fibronectin III domains, which lack the WSXWS motif characteristic of the class I receptors (8). Ligand binding may induce receptor homodimerization (e.g. IFN-γR) and activate the JAK-STAT pathway. The immunoglobulin superfamily receptors (IL-1R, IL-6R, FGFR, M-CSFR, PDGFR, VEGFR, stem cell factor R (SCFR)) contain one or more domains with the characteristic immunoglobulin fold (16). Ligand binding and signal transducing properties of the IL-1R are described in detail in chapter 1.1.1. Receptors belonging to the protein tyrosine kinase receptor superfamily (EGFR, Insulin-like growth factor-1 (IGF-1) R, FGFR, M-CSFR, PDGFR, VEGFR, SCFR) are in general growth factor receptors with an intracellular tyrosine kinase catalytic domain (17). Ligand binding by these receptors induces receptor homo- or oligomerization, autophosphorylation and subsequent signaling. Members of the TNF receptor superfamily (TNFR and NGFR) are characterized by three or four cysteine-rich repeats in the extracellular part of the molecule (18). These receptors may bind more than one ligand. Little is known about the mode of signal transduction by receptors of this superfamily. Receptors of the chemokine receptor superfamily are coupled to GTP binding proteins which, after ligand binding, activate lipases, kinases, phosphatases and ion channels (19).

Soluble cytokine receptors

It seems that essentially all single transmembrane cytokine receptors exist as soluble forms (20). Soluble receptors can be translated from differentially spliced pre-mRNA molecules lacking the transmembrane domain (e.g. IL-4R, IL-5R, IL-6R, IL-7R, IL-9R, GM-CSFR, G-CSFR, TGF-αR). However, the presence of mRNA for soluble receptors not always seems to represent protein synthesis. Soluble receptors can also be generated by proteolysis of cell surface receptors (e.g. IL-1R, IL-2Rα, IL-6R, gp130, PDGFR, NGFR, TNFR, TGF-βR) and by phospholipase action on the glycosyl-phosphatidylinositol (GPI) anchor of CNTFR. Proteolytic shedding may be controlled by protein kinase C and may involve a metalloprotease, since phorbol myristate acetate (PMA)-induced shedding of IL-6R, TNFR type I and II as well as membrane TNF-α is blocked by a metalloprotease inhibitor, which was first identified as TNF-α protease inhibitor (TAPI) (21,22).

Virokines

The immunological relevance of cytokines and (soluble) cytokine receptors is clearly illustrated by the observation that several pathogenic virus strains, particularly pox viruses, encode proteins which counteract the anti-viral cytokine activity during the host response (23). Examples of theses so-called virokines include a homologue of the host suppressive cytokine IL-10 encoded by Epstein-Barr virus (24) and several poxviral homologues of soluble IL-1R (sIL-1R) (see chapter 1.1.1), sTNFR and sIFNR, which prevent interaction of the ligands with cellular receptors (23,25). In addition, herpesviruses and cytomegalovirus encode chemokine receptors which are expressed on the membrane of infected cells, but probably display an altered function (26). Furthermore, intracellular viral proteins have been described that interfere with cytokine maturation and cytokine-mediated responses (23).

Cytokine biology

The current view on cytokine biology is that of a network of cytokines with additive, synergistic and opposite effects, and inducers and inhibitors of the expression of cytokines and/or their receptors, which combine to give an overall biological (or clinical) response (27). Cytokines are potent molecules, of which the activity is regulated at multiple levels.

First, stimuli are required to induce as well as to suppress the transcription of most cytokine genes (4). For example, infectious or toxic agents, mechanical injury, inflammatory mediators, and cytokines themselves (e.g. IL-1, TNF, IFN-γ) are potent cytokine inducers, whereas glucocorticoids, prostaglandins and cytokines (e.g. IL-10 and TGF-β) are potent inhibitors of cytokine gene expression. Note that genetic polymorphisms (e.g. IL-1α, IL-1 receptor antagonist (IL-1ra) and TNF-β, see chapter 1.1.1 and ref 28) may contribute to inter-individual differences in cytokine expression upon stimulation.

Second, the extent of cytokine protein production is limited, since the consensus sequence UUAUUUAU in the 3' end of most cytokine mRNAs promotes message instability (29), and translation of transcripts may require an additional signal (e.g. IL-1β) (30).

Third, several cytokines are not released through the classic secretory pathway. These cytokines are produced as larger biologically inactive precursor molecules, which are stored within the cytoplasm (e.g. IL-1, IL-8, GM-CSF, TGF-β and PDGF) or expressed on the plasma membrane (e.g. TGF-α, IL-1α and TNF-α), and need to be proteolytically cleaved to release the mature molecule (e.g. TGF-α, IL-1α, IL-1β and TNF-α) (31-33). Extracellular enzymes may also contribute to the processing of precursor molecules (e.g. IL-1β and TGF-β) (34,35).

Fourth, post-translational modifications (e.g. cleavage-site of leader peptide, glycosylation, phosphorylation and/or multimerization) affect the biological activity of mature cytokines (e.g. IL-6 and IL-8) (36-38).

Fifth, once secreted, several cytokines are trapped by cell surface binding

proteins (e.g. TGF-β), extracellular matrix (e.g. LIF, IL-1 and FGF), stromal cells (e.g. IL-3, IL-5 and GM-CSF) and endothelial cells (e.g. IL-8) (4,39). It should be noted that the intracellular, membrane and extracellular pools of cytokines are available for rapid maturation and/or release in response to stimulation.

Sixth, soluble proteins which bind to cytokines can agonize or antagonize cytokine activity. The proteins bind to cytokines either nonspecifically such as α2-macroglobulin (40) or specifically such as autoantibodies to cytokines (e.g. IL-1α, IL-6, IL-8, IL-10, monocyte chemotactic protein-1 (MCP-1) and IFN-α) (41,42) and soluble cytokine receptors (20). On the one hand, these soluble proteins may agonize cytokine activity by acting as chaperons, increasing the cytokine's persistence (5,43). Moreover, in case of sIL-6R and sCNTFR, the soluble proteins form a biologically active binary complex with their ligands (IL-6 - sIL-6R and CNTF - sCNTFR) which may act on cells only expressing gp130 on their cell surface, but not IL-6R or CNTFR (normally anticipated as cells unresponsive to IL-6 or CNTF) (20). Note that in line with this 'trans-signaling pathway' is the fact that gp130 is present essentially on all cell types, whereas IL-6R and CNTFR are not expressed ubiquitously. On the other hand, soluble proteins may antagonize cytokine activity by acting as scavengers of cytokines, neutralizing their activity, enhancing antibody-dependent cell-mediated cytotoxicity, preventing extravascular escape and promoting excretion (5). Whether soluble proteins will act as agonists or antagonists of cytokine activity is, at least in part, determined by the ratio between membrane receptors and soluble proteins: the predominance of membrane receptors favors agonistic activity, whereas the predominance of soluble proteins favors antagonistic activity (43).

Finally, cytokine activity can be regulated by modulating the number and/or function of membrane receptors. Receptor number may be modulated by controlling gene expression, internalization or the generation of soluble receptors. Receptor affinity and/or function may be modulated by affecting receptor phosphorylation and/or glycosylation, by competition for common receptor chains or signal transduction molecules (17,40) or by binding cytokine receptor antagonists which bind to the receptor but do not cause signal transduction (for example, IL-1ra and $(p40)_2$ antagonize IL-1 and IL-12 functions, respectively) (44,45).

1.1.1 The IL-1 system

In 1972, Gery *et al.* reported the finding of a human leukocyte-derived factor which promoted murine thymocyte proliferation (46). This factor has been further characterized and subsequently termed IL-1 (47). In 1985, two distinct, but distantly related, human macrophage cDNAs of IL-1 (referred to as IL-1α and IL-1β) were reported which encoded proteins sharing IL-1 activity (48). Nowadays, we realize that IL-1 activity is in fact the net outcome of interacting IL-1 agonists and IL-1 antagonists, constituting the IL-1 system (49).

Family members

The human IL-1 system comprises ligands and receptors, which are all located on chromosome 2, suggesting that these factors are expressed in a coordinate fashion following cell activation (49). There exist three ligand isoforms: IL-1α, IL-1β and IL-1ra, and two receptor isoforms: IL-1R type I (IL-1RI) and IL-1RII. The naturally occurring anti-IL-1α autoantibody is for clarity reasons not included in the description of the IL-1 system.

Ligands

IL-1α and IL-1β: Genetic analysis of IL-1α identified the existence of at least six different alleles (50). This IL-1α polymorphism is caused by a variable number of 46 base pairs (bp) tandem repeats in intron six, with each repeat containing binding sites for transcription factors, and may therefore affect IL-1α expression. The induction of IL-1 gene expression, in particular IL-1β, is rapid and transient, involving not only rapid activation but also rapid repression (similar to the response exhibited by the immediate early (IE) response genes such as *c-fos*) (51). IL-1β gene transcription is more tightly regulated than that of IL-1α, and depends on a combination of cell type-specific (e.g. SPI-1, which is abundantly present in monocytic cells) and ubiquitous transcription factors (e.g. nuclear factor IL-6 (NFIL-6) and cyclic AMP response element binding protein (CREB)) which bind to promoter-proximal sites and the upstream inducer sequence (UIS) enhancer, respectively (51). These proteins are probably involved in releasing the repression exhibited by the IL-1β promoter. IL-1α and IL-1β translation products both occur as 31-kDa precursor proteins lacking a leader peptide (48) which, therefore, do not enter the rough endoplasmic reticulum and the Golgi complex and remain in a non-glycosylated fashion in the cytosol (52). Approximately 10 to 15 % of the pro-IL-1α is myristoylated (53) and transported to the cell surface, where it is called membrane IL-1 (54).

Pro-IL-1α and pro-IL-1β differ in their post-translational modification, as the IL-1α and IL-1β precursors are differentially processed to the 17-kDa mature IL-1α and IL-1β forms (32,55). Processing of pro-IL-1α is selectively mediated by the calcium-activated neutral protease calpain (CANP) (55), whereas that of pro-IL-1β is mediated by IL-1β converting enzyme (ICE) (32). Both CANP and ICE are intracellular cysteine proteases that are synthesized as inactive proenzymes which are activated by autocatalysis (56,57). Autoprocessing of pro-CANP probably occurs at the inner surface of the plasma membrane in the presence of calcium and phospholipid, resulting in membrane-bound CANP (56). Cleavage of membrane-associated pro-IL-1α by CANP into mature N-terminal Leu-119 IL-1α (58) probably results in release of IL-1α (55). Autocleavage of pro-ICE, present in monocytic cells, but not in keratinocytes (59) may be induced by potassium (60) and results in a homodimer of catalytic domains, each of which contains a 20- and a 10-kDa subunit (61,62). Activation of ICE is critical in processing of pro-IL-1β into mature N-terminal Ala-117 IL-1β and release of IL-1β (32,63). Released pro-

IL-1β (e.g. pro-IL-1β leaked from necrotic cells or via a separate secretion pathway) can still be processed to active products (with additional residues at the N-terminus when compared to ICE-processed IL-1β) by extracellular proteases (e.g. chymotrypsin, collagenase, elastase and cathepsin G) (34,59). ICE has recently been recognized to be a member of a new subfamily of cysteine proteases (64) and may take part in an amplified protease cascade, which is characteristic of apoptosis (63,65). Cells undergoing apoptosis acquire the capacity to release IL-1β more efficiently before cell death (66-68). ICE may activate CANP, as reports with ICE knockout mice have shown that ICE is also involved in the release of mature IL-1α (63,69).

The precise mechanism of release of IL-1 from cells remains enigmatic and has been incompletely explained (52,70). IL-1α and IL-1β proteins are not secreted by the classic secretory pathway. Cell death may result in release of IL-1, with the nature of the injury profoundly affecting the post-translational modification of the released IL-1 (60,66). In addition to cell death, recent studies support an alternative secretion pathway(s) in which a conformational change of IL-1, as a consequence of processing, induces interaction with components of the classic secretory pathway and results in secretion (71,72). Some IL-1 is found in lysosomes (73) or intracellular vesicles (74), and either localization may play a role in the secretion of IL-1. In accordance with the alternative secretion model is the release of a small amount of pro-IL-1β by intact mononuclear phagocytes, which may represent an event separate from secretion of the mature molecule (i.e. processed IL-1) (57). Recently it has been hypothesized that IL-1 secretion may represent a multistep process that is subject to regulation in a cell-type specific manner (for example, monocytes versus keratinocytes) (72).

IL-1ra: The IL-1ra gene is polymorphic in that intron two contains a variable copy number of an 86 bp sequence, which affects the production of both IL-1α and IL-1ra by human monocytes (75,76). There exist two alternative splice products of the same IL-1ra gene, which are transcribed from unique promoters and use distinct first exons (77). The form of IL-1ra first described was a 22-kDa product secreted by monocytes (secreted IL-1ra (sIL-1ra)) (44,78,79). This form contains a leader peptide, is glycosylated, and is released by cells through the classic secretion pathway. More recently, a second, cell-associated variant was described, termed intracellular IL-1ra (icIL-1ra). icIL-1ra (Mr: 22-kDa) contains an additional seven N-terminal amino acids, lacks the leader peptide, is non-glycosylated and predominantly resides in epithelial cells (80). There is evidence supporting the existence of a third splice variant generating another form of icIL-1ra (Mr: 25-kDa) (81).

Recently, yet another novel, IFN-γ-inducing cytokine has been cloned which demonstrated a remarkable structural homology with the IL-1 ligands and was termed IL-1γ (82).

Receptors

IL-1RI and IL-1RII: The IL-1RI and IL-1RII genes express multiple transcriptional start sites spread over multiple exons. As a consequence, three different IL-1RI mRNAs and two IL-1RII mRNAs can be generated which contain different 5' untranslated regions, but similar exons coding for the receptor proteins (83). The IL-1RI gene promoter does not contain a TATA box but rather falls into the category of housekeeping gene promoters (84). Translation of mRNA coding for IL-1RI is limited because there are considerable secondary RNA structures in each of the 5' untranslated regions (84). Consequently, IL-1RI is constitutively expressed at low levels consistent with the low numbers of surface receptors on most cells.

The most striking structural difference between IL-1RI (CDw121a) and IL-1RII (CDw121b) is the size of the cytoplasmic domains, being 213 amino acids for IL-1RI and 29 amino acids for IL-1RII, which accounts for the different molecular masses of the two receptors (M_r: 80-kDa and M_r: 68-kDa, respectively). The extracellular domains of both types of receptors can be shed by proteolysis, generating soluble receptors (85). All IL-1 ligands bind to both membrane-bound and sIL-1Rs, although with varying affinities (85,86). The reported affinities vary considerably between studies and show an apparent heterogeneity among different cell types. However, in general, membrane-bound IL-1RI preferentially binds IL-1α and IL-1ra, whereas membrane-bound IL-1RII preferentially binds IL-1β (86). Of importance is the observation that IL-1RI binds IL-1ra with greater affinity when compared to IL-1RII, and that the binding of IL-1ra by the former receptor is nearly irreversible. In contrast to IL-1RII on leukocytic cells, the keratinocyte IL-1RII, being the dominant receptor form on these cells, binds IL-1α and IL-1β with similar affinities (87). Naturally occurring sIL-1RI seems to preferentially bind IL-1ra (88), whereas sIL-1RII seems to preferentially bind IL-1β (89). Similar results were found with the recombinant forms of the soluble receptors (90). Recently, sIL-1RII, but not membrane-bound IL-1RII, was also reported to bind pro-IL-1β and to block its processing into mature IL-1β (91).

IL-1RI is the sole known signal-transducing receptor for IL-1 (92). It is generally believed that the IL-1RI is monomeric and binds one IL-1 molecule (93). Recently an additional protein, a putative second subunit of the IL-1R complex, has been characterized that affects receptor binding and activity of IL-1 (94). The cytoplasmic domain of IL-1RI is required for receptor function as demonstrated by deletion experiments (95). The IL-1RII, which has a truncated cytoplasmic domain, does not transduce a signal, and appears to act as a scavenger of IL-1β, preventing it from binding to the signaling IL-1RI (96). Mutational analyses of IL-1 ligands have revealed two distinct sites for binding to IL-1RI. One site is present on all ligands and accounts for binding and receptor occupancy, and the other site is only present on IL-1α and IL-1β and accounts for receptor activation (97). The binding of IL-1ra to IL-1RI competitively prevents the binding of other ligands, but does not engage the critical amino acids for receptor triggering (44). IL-1ra is structurally related

to IL-1α and IL-1β, but differs at the open end of the β-barrel (98), a stretch which is possibly involved in receptor activation. Receptor binding and activation are, thus, two physically separate events. Pro-IL-1α and pro-IL-1β differ in the expression of the receptor binding sites, as pro-IL-1α is biologically active (i.e. binds to and activates IL-1RI), whereas pro-IL-1β does not bind to IL-1RI (99). Processing of pro-IL-1β, but not pro-IL-1α, is probably associated with conformational changes necessary to reveal both binding sites of IL-1β to become functionally active. Recent structural data show furthermore that mature IL-1α and IL-1β do not express identical receptor binding sites (100,101), which may explain differences between IL-1α and IL-1β responses in some cells (102), and that IL-1β binds to IL-1RI and IL-1RII through different sites (103).

Other molecules have been found which share significant sequence similarity to all or part of the IL-1Rs (104). Notably, the extracellular as well as the intracellular portion of the T1/ST2 molecule resemble those of IL-1RI. The IL-1RI, IL-1RII and T1/ST2 genes are probably derived from a common ancestor gene. Interestingly, T1/ST2 and its soluble alternative splice product do not bind any of the IL-1 ligands, but the putative signaling domains of T1/ST2 appeared functional (105,106). Moreover, a group of several proteins which are homologous to the cytoplasmic portion of the IL-1RI and T1/ST2 (e.g. the Drosophila gene Toll, and the mammalian genes MyD88 and hRSC786) may represent a family of signaling molecules (104).

Signal transduction

IL-1 signaling is unusually efficient, as only one to ten IL-1 molecules bound per cell are required to trigger an IL-1 response (107). The cytoplasmic domain of IL-1RI bears no resemblance to known protein kinases (108), and the cytoplasmic amino acids do not serve as substrates for tyrosine kinases (109). Still, a variety of signaling pathways have been implicated in the IL-1 response, including: i) activation of a GTP binding protein with no associated increase in adenylate cyclase (110); ii) activation of adenylate cyclase (111); iii) hydrolysis of phospholipids by non-phosphatidylinositol phospholipase Cs (112); iv) release of ceramide from sphingomyelin after activation of sphingomyelinase (113); and v) release of arachidonic acid from phospholipids after activation of cytosolic phospholipase A_2 by phospholipase A_2 activating protein (114). However, in many cases evidence is conflicting.

IL-1 receptor binding causes rapid changes in the phosphorylation state of cellular proteins as a result of activated MAP kinases, novel protein kinases and phosphatases (115). Novel IL-1-activated protein kinases which may initiate specific signaling cascades comprise an IL-1RI-associated kinase (116), a 54-kDa MAP kinase (117) and a β-casein kinase (118). Only recently it was observed that in human epidermoid carcinoma cells yet another novel protein kinase cascade was activated in response to IL-1. This cascade, quite similar to stress-induced signal transduction, resulted in the

sequential phosphorylation of three kinases (a 35-, 40- and a 50-kDa kinase) and the 27-kDa heat shock protein (119). The cascade of events closely resembles, but is molecularly distinct from, the microtubule assembly protein kinase cascade (120). The extent to which different protein kinases, as mentioned above, are activated may be cell-type specific (115). In addition, it is suggested that IL-1 inhibits protein phosphatase 2A which could lead to inactivation of a kinase suppressor (121). Taken together, IL-1 signaling presumably involves a coordinate activation of kinases and inactivation of phosphatases resulting in sequential activation of kinases that likely contributes to the exquisite sensitivity of the IL-1 response.

The IL-1-initiated signaling cascade results in the activation of transcription factors. IL-1 was found to induce phosphorylation and subsequent degradation of the inhibitor of NFκB (122), thereby activating NFκB. Activation of NFκB and activating protein-1 (AP-1) is common to many IL-1 induced genes (123,124).

G-protein coupling, phospholipid hydrolysis, protein phosphorylations and translocation of transcription factors may take place within 10 min (some earlier) after receptor activation. Note that the continued presence of IL-1 on the IL-1RI is part of the amplification mechanism, as displacement of IL-1 from the receptor results in a ceased IL-1 response (125). Ligand binding may result in rapid internalization of IL-1/IL-1RI complexes, of which some are degraded and others are found in the nucleus (126,127). Nuclear translocation motifs have been demonstrated in IL-1RI (128), suggesting that such a translocation is important for IL-1-induced gene expression. IL-1R were also observed on the nuclear membrane (129). They may complex with cytosolic IL-1 and subsequently become internalized into the nucleus, express DNA binding activity and initiate gene expression (130).

Biology of IL-1

IL-1 has a very wide range of biological activities on many different target cell types *in vitro*, and mediates local and systemic responses, from fever, anorexia and slow-wave sleep, to immunoregulation, generation of the acute phase response and local inflammatory reactions *in vivo*. IL-1 is specifically found to have profound effects on skin cells (131,132). Others have already excellently reviewed the biological effects of IL-1 in more detail (133,134). The pleiotropic actions of IL-1 can be attributed to the induction of a wide variety of nonstructural, function-associated genes that are characteristically expressed during inflammation, particularly cytokines (e.g. IL-6, IL-8 and TNF-α) and enzymes (e.g. nitric oxide synthase, phospholipase A_2 and cyclooxygenase) (49). IL-1 suppresses the constitutive expression of several housekeeping genes. These divergent effects of IL-1 on gene expression may be a coordinated effort by IL-1 to alter host responses to an inflammatory, infectious or immunological challenge. Hence, the term 'primary' cytokine based upon the prediction that the release of IL-1, as an isolated event, would be sufficient to induce inflammation. How IL-1 may orchestrate

cutaneous inflammation will be discussed in chapter 1.2.2.

Virus biology underscores the importance of IL-1 in immune responses. The virulence of vaccinia and cowpox viruses is related to the expression of proteins homologous to IL-1RII (25). These proteins (e.g. B15R from the Western Reserve strain of the vaccinia virus) antagonize IL-1β activity, and thus diminish the host's ability to mount an inflammatory response (23). There is also significant homology between the transmembrane regions of IL-1RII and the Epstein-Barr virus protein BHRF1 (86). Pox viruses even evolved a second mechanism to circumvent IL-1β activity, as viral products are reported to inhibit the activity of ICE (e.g. B13R from the Copenhagen strain of the vaccinia virus, and CRMA, a cytokine-response modifier from cowpox virus) (23,135).

The primary role of the IL-1 system in skin immune responses is substantiated by the transgenic approach (136). A series of transgenic mice have been generated in the laboratories of Kupper and coworkers using the keratin 14 (K14) promoter to target overexpression of the transgene to basal epidermis. Interestingly, transgenic mice that overexpress IL-1α (TgIL-1.1 line) do develop inflammation and skin disease (137). Similar results were found with overexpression of TNF-α (138), but not adhesion molecules (e.g. intercellular adhesion molecule-1 (ICAM-1), costimulatory molecules (e.g. B7-1) or chemokines (e.g. MCP-1 or GRO-α) (136,139,140). In addition, IL-1β knockout mice exhibited an impaired inflammatory response to tissue injury (141). Moreover, IL-1 injected into normal human skin gives rise to a wheal and flare reaction and a mixed leukocyte infiltrate in dermis as well as epidermis (142). Taken together, these experiments provide *in vivo* prove that IL-1 is sufficient to induce cutaneous inflammation.

Regulation of IL-1 activity

Human epidermis contains in total approximately 20 to 60 μg IL-1, equivalent to 4 to 12 nM IL-1, which can be considered life-threatening, knowing that IL-1 is biologically active in the pM to fM range (143,144). Recent advances in understanding the regulation of IL-1 activity underscore that this is one of the most highly regulated cytokine systems known.

Epidermal keratinocytes (and other epithelial cells lining the external environment) constitutively express pro-IL-1α and IL-1ra intracellularly (143,145). Pro-IL-1α functions as an autocrine, intracellular messenger with its activity controlled by the co-presence of icIL-1ra which is thought to compete with pro-IL-1α for nuclear IL-1RI (49). Moreover, the balance between icIL-1ra and pro-IL-1α may control terminal keratinocyte differentiation (145). Several cytokines such as IFN-γ, IL-6 and TNF-α, but not IL-4 and IL-10 stimulate the expression of intracellular IL-1α without affecting the expression of icIL-1ra, thereby increasing the intracellular IL-1 activity in keratinocytes (146,147).

IL-1RII, expressed in the basal layer of epithelia (148), may act as a barrier mechanism to neutralize locally released IL-1 agonists and prevent them from

mediating a cellular response or entering the circulation (5). In fact, transfection experiments recently provided evidence that IL-1RII impairs the IL-1 responsiveness of keratinocytes (149). The expression of keratinocyte IL-1RII is also under the control of cytokines (e.g. IFN-γ, see chapter 1.2.2 and ref 87).

Of the two IL-1 agonists (IL-1α and IL-1β), the production and activity of IL-1β is more effectively regulated (which is confirmed by natural poxviral strategies).

First, the IL-1β gene is expressed transiently and in a cell type-specific manner (e.g. dependent upon the monocytic transcription factor SPI-1).

Second, IL-1β transcripts are not readily translated into IL-1 precursor proteins, but generally require an additional signal for translation (30).

Third, pro-IL-1β is biologically inactive and its maturation depends on the activation of ICE, whereas pro-IL-1α is biologically active.

Fourth, secreted pro-IL-1β is bound by sIL-1RII, which thereby blocks the processing and activation of pro-IL-1β.

Fifth, mature IL-1β (present during cutaneous inflammation) preferentially binds to the nonsignaling IL-1RII (i.e. scavenged by IL-1RII), whereas (pro-)IL-1α preferentially binds to the signaling IL-1RI.

Sixth, IL-1β activity is antagonized at both the receptor and ligand level by IL-1ra and sIL-1RII, respectively. IL-1ra almost irreversibly binds to IL-1RI, whereas sIL-1RII preferentially binds (pro)IL-1β, but has lost affinity for IL-1ra. IL-1ra is therefore unlikely to compete with IL-1β for binding to sIL-1RII, resulting in a synergistic reduction of IL-1β activity by IL-1ra and sIL-1RII. During an inflammatory response, IL-1ra and sIL-1RII enter the circulation and are present in substantial molar excess to prevent systemic effects of IL-1β (89,150).

Finally, IL-1β responses may be limited at a point downstream of the IL-1β - IL-1RI interaction since icIL-1ra was observed to reduce the stability of IL-1β-induced cytokine mRNAs in human epithelial cells (151).

Taken together, the regulatory differences to control IL-1α and IL-1β activity are consistent with the idea that pro-IL-1α delivers signals intracellularly and during cell-cell interactions (as membrane IL-1 form), whereas IL-1β is more important in the inflammatory response and functions as a soluble molecule.

The cellular response to IL-1 is ultimately determined by the balance between IL-1 agonists and IL-1 antagonists (see Figure 1). Regulation of the expression of the IL-1 antagonists (e.g. IL-1ra and IL-1RII) is an efficient means to control the IL-1 responsiveness. Indeed, mediators of inflammation (e.g. prostaglandins, corticosteroids and cytokines) often increase secretion of IL-1ra (77), and surface expression of IL-1RII (93). In monocytic cells for example, IL-4 increases gene transcription and production of sIL-1ra (152). In addition, both IL-4 and glucocorticoids increase surface expression and release of sIL-1RII, but not IL-1RI, at the level of gene transcription and mRNA stabilization (96,153). Furthermore, IL-4 and glucocorticoids also affect the

expression of IL-1 agonists by decreasing mRNA stability and genetranscription, respectively (51). The most important negative-feedback loops controlling IL-1 activity are presented in Figure 2 (154).

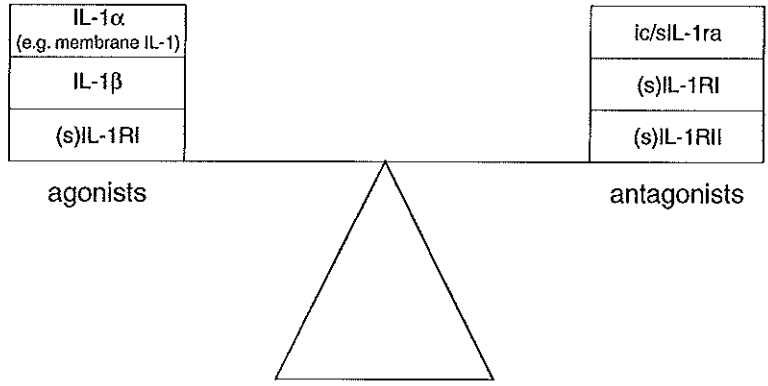

Figure 1. IL-1 axis in skin. Balance between agonists and antagonists determines the epidermal responsiveness to IL-1. See text for details (modified from ref 136).

1.1.2 IL-1 system-related cytokines

The IL-1 system is an integral part of (epidermal) cytokine networks. Cytokines may be produced under the control of IL-1, display overlapping signal transducing pathways and biological properties with IL-1, synergize or antagonize with IL-1, and/or affect the production of IL-1 family members (134,154,155). Examples of such IL-1 system-related cytokines, which fulfil key functions in cutaneous inflammation, are IL-4, IL-6, IL-8 and TNF-α.

IL-4 and IL-4R

The IL-4 promoter demonstrates allelic variation which affects the transcriptional activity of the IL-4 gene (156). Gene expression is at least in part under control of T cell specific transcription factors (157). Alternative splicing results in an IL-4 variant which lacks exon 2 and seems to act as an IL-4 antagonist as it competes with IL-4 for receptor binding and inhibits IL-4-stimulated T cel proliferation (158). IL-4 is translated as a pro-peptide with a leader peptide, processed to the mature molecule and secreted as a 20-kDa cytokine (see for review on IL-4 ref 159). IL-4 is important for the skewing towards the production of T_H2-type cytokines (160). In addition, IL-4 is a potent negative regulator of the monocytic IL-1 system (see chapter 1.1.1), and inhibits the production of IL-1 system-related cytokines (e.g. IL-6 and TNF-α) (161-163). In turn, IL-4 expression by T cells is inhibited by IL-1 (164).

Membrane-bound IL-4R (M_r: 140-kDa, CD124) associated with the common-γ chain (see Table I) binds IL-4 with high affinity. Alternative splicing is responsible for the generation of sIL-4R. Studies with cells of myeloid lineage

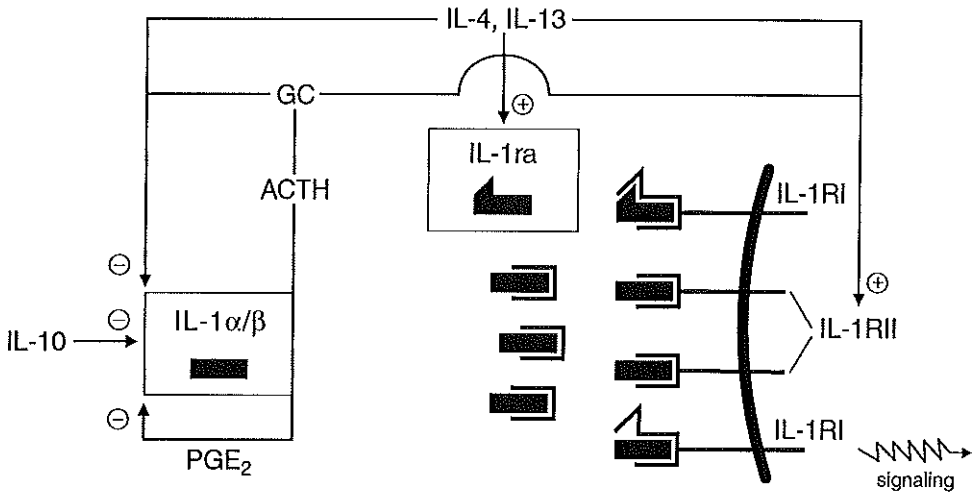

Figure 2. Regulatory pathways of the IL-1 system. The release and action of the IL-1α and IL-1β are tightly regulated by negative-feedback loops. For example, prostaglandins, glucocorticoids, T_H2-type cytokines (e.g. IL-4, IL-10 and IL-13), and members of the IL-1 system (e.g. IL-1ra, IL-1RI and IL-1RII) all affect the IL-1 activity. +/- indicates stimulation/inhibition of expression. Note that no distinction is made between IL-1α and IL-1β, and that IL-1RII is depicted as unable to bind IL-1ra. See text for details (modified from ref 154). Abbreviations: GC, glucocorticoids; ACTH, adrenocorticotropic hormone; and PGE_2, prostaglandin E_2.

have shown that IL-4-induced cell growth uses an intracellular activation pathway of which the initial events are characterized by phosphorylation of the insulin receptor/IL-4R motif (I4R), present in the cytoplasmic domains of the IL-4R and members of the insulin receptor family, and subsequent phosphorylation of the 4PS/insulin receptor substrate-1 (165). Other IL-4-induced responses, such as lymphocytic differentiation, may use the JAK-STAT pathway. Recently, a second IL-4R, which binds IL-4 with low affinity and uses different signaling pathways, has been described (166).

IL-6 and IL-6R

The regulation of IL-6 gene expression, in analogy with that of IL-1, shows some similarities with IE response gene regulation, as evidenced by the location of the *c-fos* serum-responsive element homology (*c-fos* SRE) within the IL-6 promoter (167). IL-6 mRNA is translated into an IL-6 precursor protein containing a leader peptide. The mature secreted IL-6 polypeptide is characterized by cell type-specific differences in N-termini (as a result of heterogeneity in the leader peptide cleavage site), glycosylation, phosphorylation and multimerization (36,168). As a consequence, different IL-6 molecules may vary considerably in molecular mass (M_r: 21-kDa to 28-kDa) and biological activity. IL-6 is a multifunctional cytokine the biology of which is excellently reviewed in ref 169. Keratinocyte IL-6 is induced by IL-1 and

TNF-α, and significantly increased during inflammation. IL-6 in turn may enter the circulation, and is considered an anti-inflammatory cytokine. This cytokine is part of a negative-feedback loop, as it decreases stimulated IL-1 and TNF-α production (170), and increases the production of IL-1ra and sTNFRI (171). Furthermore, the spectrum of acute phase proteins induced by IL-6 includes many anti-proteases with anti-inflammatory properties (171).

Membrane-bound IL-6R (M_r: 80-kDa, CD126) exhibits a low affinity binding site for IL-6, but together with gp130 a high-affinity binding site for IL-6 is formed (172). The sIL-6R form is unique in that it is generated by alternative splicing as well as shedding (20). Interestingly, the IL-6 - sIL-6R complex shows significant sequence similarity to IL-12, composed of a 35- and 40-kDa subunit. In the light of this observation one could view the IL-6 - sIL-6R complex as an agonist heterodimer involved in 'trans-signaling' routes (see chapter 1.1 and refs 20,173). In addition, soluble gp130, generated by shedding, may form antagonistic ternary complexes with IL-6 and sIL-6R (174).

IL-6, when complexed with membrane-bound IL-6R, triggers homodimerization of gp130, resulting in signal transduction through gp130, as described previously (chapter 1.1). At least for acute phase protein gene expression, there are two signal transduction pathways activated by homodimerization of gp130: the JAK-STAT pathway and a pathway involving a not yet identified tyrosine kinase responsible for the critical threonine-phosphorylation of NFIL-6 through the RAS-MAP kinase cascades (10). IL-6-induced growth responses only require conserved motifs in the membrane-proximal cytoplasmic region of gp130 (to which JAK kinases associate), and are independent of a conserved tyrosine-based motif in the membrane-distal region (which is required for specific docking of STAT factors) (11). It should be noted that NFIL-6 is a ubiquitous transcription factor that, at least in monocytes and hepatocytes, is induced by LPS, IL-1, IL-6 and TNF-α, and mediates the expression of inflammatory cytokines (e.g. IL-1, IL-6, IL-8 and TNF-α) as well as IL-6-inducible genes (10).

IL-8 and IL-8R

The regulation of IL-8 gene expression in keratinocytes involves IL-8 mRNA length polymorphism (33). It is speculated that this reflects a cytokine-controlled two-staged IL-8 release mechanism:

1) TNF-α and IFN-γ induce the formation of more and larger IL-8 mRNA species, which may be translated into biologically inactive high-molecular mass IL-8 protein (present in normal human epidermis), being protein-bound (constituting an IL-8 storage) and released slowly;
2) IL-2 induces the cleavage of preformed IL-8, the products of which are released fast.

IL-8 is synthesized as a pro-form containing a leader peptide. Several distinct N-termini are found in the natural protein, resulting in differences in molecular mass (M_r: 6 to 8-kDa) and biological activity (e.g. the 69 and 72

residue IL-8 forms are more potent than the 77 residue form) (37,38). See ref 5 for a review on the biological activities of IL-8. IL-8, which appears to exist as a dimer in solution, is produced by nearly all cells of the organism when stimulated with primary cytokines. Once entered the circulation, the Duffy antigen receptor for chemokines (DARC) on erythrocytes (in addition to autoantibodies) scavenges free IL-8 and other chemokines in the blood and limits their biological activities (175). Chemokines may also exhibit anti-inflammatory properties, as they induce rapid shedding of IL-1RII by polymorphonuclear cells (176) and, when accumulated predominantly intravascularly, inhibit their own activity (175).

Two types of IL-8R have been described: IL-8RA and B (both have an M_r of 60-kDa, CD128). The receptors express varying affinities for several CXC chemokines: IL-8RA binds IL-8 exclusively, whereas IL-8RB binds both IL-8 and GRO-α with high affinity and NAP-2 with lesser affinity (177). Both receptors are seven transmembrane domains spanning, G-protein-linked receptors (see chapter 1.1).

TNF-α and TNFR

The TNF-α gene is expressed rapidly and transiently upon stimulation (178). TNF-α protein is produced as a pro-form containing a signal anchor domain, which enables expression as a biologically active type II membrane protein (i.e. the carboxyl terminus is expressed extracellularly) (1 to 2 % of TNF-α is membrane-bound) (179). Maturation of TNF-α involves cleavage of an atypical propeptide of 76 residues by a matrix metalloprotease, and the formation of a homotrimer of TNF-α monomers (M_r: 52-kDa). Note that monomeric TNF-α is not biologically active. The biological properties of TNF-α share remarkable similarities to those of IL-1, and both are primary cytokines. Moreover, IL-1 and TNF-α often act synergistically (134). TNF-α may also affect IL-1 responses as it enhances the release of IL-1RII by neutrophils (180).

There are two receptors for TNF: TNFRI (M_r: 55-kDa, CD120a) and TNFRII (M_r: 75-kDa, CD120b). Both receptors bind TNF-α and TNF-β, are active in signal transduction and are responsible for nonredundant TNF activities (181). Keratinocytes predominantly express TNFRI (182) and most immunomodulatory effects of TNF-α (e.g. on keratinocytes and fibroblasts) are mediated by this receptor. Both types of receptors are shed either by protein kinase C dependent proteolysis (induced by PMA) or by protein kinase C independent proteolysis (induced by IL-1 and IL-4) (183,184). Receptor shedding and TNF-α processing may be coordinately regulated as both processes involve a similar enzyme (21). Receptor crosslinking by the TNF trimer is important for signal transduction. The signal transduction of TNF-α appears to be strikingly similar to that of IL-1 (134).

1.2 SKIN, AN ACTIVE SITE OF IMMUNE RESPONSES

The human integument, the principal interface between host and environment, is an active immunological microenvironment and often the site of inflammatory or allergic responses to external agents (185).

1.2.1 Cellular constituents

The skin, composed of epidermis, dermis and subcutaneous fat, contains a large number and variety of resident and recirculating cells (186). The cellular constituents of the epidermal compartment comprise predominantly keratinocytes, but also some melanocytes, Merkel cells, Langerhans cells, macrophages and T cells (186). The dermal compartment contains fibroblasts, endothelial cells, mast cells, dendrocytes, macrophages, T cells, (some) B cells, natural killer cells, granulocytes, neuronal and glandular cells, whereas the subcutis contains fat cells, some endothelial cells and glandular cells. All these cells are in principal immunologically competent.
A more detailed description of the most predominant skin cells is given below.

Resident cells

Resident keratinocytes are responsible for production and maintenance of the epidermal barrier. The human keratinocyte stem cell population is contained within the basally located β1 integrin$^+$ (CD29), β4 integrin$^+$ (CD104), K1$^-$ and K10$^-$ population of keratinocytes of which 95 % is in the G_0 phase of the cell cycle (187). Progeny of these stem cells may give rise to a highly proliferative population (β1$^+$, K1/K10$^+$) which have committed to differentiation (K1/K10 expression) but transiently amplify before they become terminally differentiated (β1$^-$, K1/K10$^+$). As keratinocytes differentiate, they produce different patterns of intermediate filament proteins (e.g. keratins and tonofilaments), enzymes (e.g. transglutaminase), and structural proteins (e.g. involucrin and loricrin) that lead to the formation of cornified cells. Terminal differentiation may be considered as a specialized form of the apoptotic program to serve skin-specific functions (e.g. the squamous product provides the skin biochemical and physical integrity) (188). The proto-oncogene B cell lymphoma 2 (BCL-2), a major anti-apoptotic protein, is found in basal keratinocytes and its expression is lost during differentiation (188). Triggers for the induction of apoptosis in differentiating epidermis may include the withdrawal of proliferation-inducing cytokines (e.g. IL-1 or TGF-α) or the presence of proliferation-inhibiting cytokines (TGF-β) (188,189).

Human keratinocytes seem to control T cell activation by the expression of major histocompatibility complex (MHC) molecules, costimulatory molecules and cytokines (190). Keratinocytes differ from the classical bone marrow-derived antigen presenting cells (APCs), such as macrophages and Langerhans cells. These differences are presented in Table II.
First, keratinocytes only express MHC class II antigens when activated (after

exposure to T cell IFN-γ).

Second, keratinocytes lack or express only low levels of the non-polymorphic class II-associated invariant chain (Ii, CD74), which is intimately involved in processing, transport and peptide presentation of MHC class II molecules. This may explain the observation that keratinocytes fail to induce a significant allogeneic T cell proliferative response (191), but are able to induce a vigorous autologous T cell proliferation in the presence of stimuli which provide a T cell receptor (TCR)-mediated signal (such as phytohemagglutinin (PHA), bacterial-derived superantigens, or immobilized anti-CD3 monoclonal antibody) (192).

Third, keratinocytes lack the B7-1 (CD80) and B7-2 (CD86) costimulatory molecules, but do express the B7-3 (BB-1) molecule (193,194). The B7-1 and B7-2 molecules, expressed by classical APC, bind to the T cell CD28 molecule which results in a unique signal-transduction pathway that synergistically interacts with TCR/CD3 receptor stimulation to produce maximum cell proliferation and cytokine secretion (195). Moreover, the presence or absence of B7-1 on APC was observed to dictate whether T helper cell type 1 (T_H1)-type cytokines (e.g. IL-2, IFN-γ) or T_H2-type cytokines (e.g. IL-4, IL-5, IL-10) are produced, respectively (196). In contrast, B7-3 binding to CD28 may represent a nonactivating signal resulting in a functional state of anergy, which could explain the tolerogenic capacity of keratinocytes with nominal antigen (197). Nickoloff *et al.* even speculated that keratinocyte B7-3 may actually trigger antigen-specific apoptosis (190).

Finally, keratinocyte-supported T cell cultures produce relatively low amounts of IFN-γ (194). The IL-12 dependence of IFN-γ production (198), together with the observation that keratinocytes produce no or only minute quantities of IL-12 (the inducible 40-kDa chain was barely detectable in activated keratinocytes) (199), explain the lack of keratinocytes to induce IFN-γ production. Moreover, IL-1β derived from the classical APC is required for IL-12 to induce IFN-γ production (200,201), whereas IL-1α derived from keratinocytes favors the generation of T_H2-type cytokines (202). Presentation of superantigens by keratinocytes to autologous T cells results in the generation of the T_H2-type cytokines IL-4, IL-5 and IL-10, but not the T_H1-type cytokine IFN-γ (199). This situation is reversed with the use of classical APC, which are potent producers of IL-12. It should be noted that both types of APC induce the production of the T_H1-type cytokine IL-2 (199,203).

Thus, the expression and conformation of the MHC class II molecules, the nature of the costimulatory pathway and the profile of cytokines produced make keratinocytes unique APC when compared to Langerhans cells and macrophages with respect to the quantity and quality of the T cell response (Table II).

Fibroblasts are responsible for the production and maintenance of the connective tissue matrix. Fibroblasts or even connective tissue-derived small molecular weight material support the growth of human keratinocytes *in vitro* (204,205). For instance, human dermal fibroblasts are able to produce

IL-1, IL-6 and IL-8 (206-208) which are not only actively involved in inflammation but are also mitogenic for epidermal cells (209-211). Fibroblasts may also affect shape, movement and state of activation of cells via the production of extracellular matrix proteins (212,213). These proteins play prominent roles in the homing of inflammatory cells and the sequestration of cytokines (e.g. IL-8). Fibroblasts and endothelial cells may, by virtue of their capacity to function as accessory cells and/or APC, selectively affect the transmigration and function of T cell subsets (214,215).

Table II. Antigen presenting capacity of human keratinocytes.[a]

	MHC class II Ii chain[b]	B7 molecules			Production of cytokines				Allogeneic response	Autologous response	
		1	2	3	IL-1α	IL-1β	IL-10	IL-12		Tolerogenic effect	Generation of T_H1-type cytokines
KC[c]	-	-	-	+	++	-	+	-	-	+	-
LC[c]	+	+	+	?	+	++	-	+	+	-	+
Mϕ[c]	+	+	+	?	+	++	+	+	+	-	+

[a] See text for details and references.
[b] MHC class II-associated invariant chain (Ii, CD74).
[c] Abbreviations: KC, kerotinocyte; LC, Langerhans cell; Mϕ, macrophage.

Recirculating cells

Extravasation and skin-infiltration of leukocytes involve a multistep cascade:

1) initial 'rolling' of leukocytes on the endothelium via selectin-mediated interactions;
2) activation of leukocyte integrins (leukocyte function-associated antigen-1 (LFA-1), MAC-1 and very late activation antigen 4 (VLA-4)) by chemokines or divalent cations, resulting in high-affinity ligand binding and strong adhesion; and
3) emigration of the leukocyte in response to chemokine gradients (216).

Properties and functions of epidermal APC are given in Table II. In normal skin, only one type of epidermal APC predominates: the human leukocyte antigen (HLA)-DR^+, $CD1a^+$, $Fc\gamma R^+$ and $C3R^+$ Langerhans cell (217,218). Langerhans cells are in fact sentinel dendrocytes that are designed to perceive local damage (219). Activated Langerhans cells mature into potent immunostimulatory dendrocytes and migrate into draining lymph nodes where they contact naive T cells and initiate a primary immune response (220). Cytokines control maturation and migration as they, at least in part, result in extensive remodeling of the cell surface. GM-CSF, alone or in synergy with IL-1, IL-4 or TNF-α, mediates maturation (accompanied by upregulation of MHC molecules, B7-1, ICAM-1, LFA-3, and CD40 as well as

downregulation of CD32 and F4/80) (219,220), whereas TNF-α mediates migration (downregulation of E-cadherin) (221). An important dermal APC is the dermal dendrocyte, which can be distinguished from other dendritic cells of the skin by their expression of clotting factor XIIIa (222). These cells may locally contact memory T cells, which circulate through the skin (220).

A unique set of skin-seeking T cells is selectively recruited into the skin via the interaction between cutaneous lymphocyte-associated antigen (CLA, a sialylated carbohydrate) and E-selectin on endothelial cells (223). Moreover, VLA-3$^+$ and VLA-4$^+$ T cells preferentially home to skin as they are able to adhere to epiligrin, a basement membrane component, and dermal fibronectin, respectively (224,225). The expression of CLA and other specific adhesion molecules by skin-homing T cells suggested that selective recruitment of resting T cells precedes their activation and proliferation within the skin (190). Variable gene region (V)β2 and Vβ6 positive TCRs are characteristic of T cells that convey immunosurveillance in skin tissue (226).

1.2.2 Cutaneous inflammation

A general consequence of most, if not all agents that trigger cutaneous inflammation (e.g. microbial agents, allergens which may exist in association with toxic molecules, wounding, ultraviolet irradiation etc.) is damage of skin tissue (227,228). IL-1, released by dying keratinocytes, is sufficient to *induce inflammation*, as mentioned in chapter 1.1.1 (relevant *in vivo* proof is provided by IL-1 gene-manipulated mice and intradermal injections of IL-1 in humans). IL-1 mediates inflammation via induction/upregulation of: i) adhesion molecule expression on keratinocytes (ICAM-1), fibroblasts (ICAM-1) and endothelial cells (E-selectin, ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1)) (229,230); ii) cytokine production (IL-1 through IL-8, TNF-α and CSFs) by skin cells; and iii) proliferation, migration and activation of skin cells (134).

Adhesion molecules and chemokines selectively recruit leukocytes into the skin. Chemokines, all of which are induced by IL-1 *in vitro*, are trapped by glycosaminoglycans (e.g. heparan sulphate) which enables the guidance of leukocytes towards the original site of injury (i.e. the original site of IL-1 release). IL-1-induced synthesis of leukotrienes (e.g. by mast cells and keratinocytes) are also reported to stimulate keratinocyte proliferation, activate endothelial cells and act as chemoattractants for leukocytes (134, 231).

The *inflammatory response amplifies and is maintained*, as a result of the reinforcing nature of inflammatory cytokines, the accumulation of macrophages, T cells and neutrophils, the release of enzymes, eicosanoids, oxygen radicals and nitric oxide from the attracted cells, and the activation of complement. IL-1 is at least in part involved in these processes as this cytokine induces the generation of inflammatory mediators such as eicosanoids, reactive oxygen intermediates and nitric oxide (134). During the amplification phase skin-infiltrating CLA$^+$ T cells interact with resident cells

(e.g. endothelial cells, fibroblasts and keratinocytes, (epi)dermal macrophages and dendrocytes) which results in bidirectional cellular activation affecting phenotype and function (e.g. cytokine production) of the interacting cells. In addition, the interaction of T cells with extracellular matrix components may lead to persistent cytokine production (232). It is important to note that an antigen-dependent component cannot be excluded during the amplification phase of the cutaneous inflammatory response, whereas the initiation of inflammation may in principle be antigen-independent (see above). Exogenously acquired antigen(s) or a modified endogenously-derived antigen(s) drives the selective expansion of T cells. Antigen presentation by classical APC will result in the generation of T_H1-type cytokines (in part depending on IL-1β production, see chapter 1.2.1). In addition, damaged keratinocytes activate Langerhans cells (219). Langerhans cell activation may occur through an altered pattern of surface glycoproteins expressed by sublethally injured keratinocytes; through IL-1 (e.g. released by necrotic keratinocytes) and other (secondary) cytokines such as IL-6, IL-12, TNF-α and GM-CSF; or through apoptotic keratinocytes as a potential source of (auto)antigen (233,234) in the presence of free oxygen radicals (235). Langerhans cells mature and migrate into draining lymph nodes (controlled by cytokines such as IL-1, TNF-α and GM-CSF), where they activate naive, antigen-specific T cells which may furthermore contribute to the local inflammatory response (220).

During inflammation, the activation of effector leukocytes (e.g. macrophages, neutrophils, T cells, B cells and NK cells) ultimately results in the removal of damaged tissue, and the release of several cytokines (e.g. IL-1, IL-6, IL-8, TGF-α and IFN-γ) which induce keratinocyte proliferation that finally leads to the formation of new skin tissue (209-211,236,237). Control of local inflammation is of vital importance to prevent pathological tissue destruction and spreading of the inflammatory response to other sites of the body. Normally, the (auto)immune response is self-limiting and cutaneous inflammation resolves as a consequence of activated *downregulatory pathways of inflammation*.

First of all, initiators of the inflammatory response (e.g. primary cytokines, most notably IL-1) control inflammation, at least in part, through negative-feedback loops, which are inherent to the tight regulation of cytokine activity. For example, IL-1 induces glucocorticoids, prostaglandins, TGF-β and T_H2-type cytokines, which are all potent inhibitors of cytokine production (134,202). Glucocorticoids, prostaglandins and T_H2-type cytokines display an inhibiting effect on the IL-1 system and on the expression of secondary cytokines (see chapters 1.1.1 and 1.1.2) (163,238). Moreover, secondary cytokines themselves (e.g. IL-6 and chemokines) can display anti-inflammatory properties (see chapter 1.1.2). In addition, IL-1 downregulates the IL-1 and TNF receptors, and induces oxygen scavengers (134). Cytokine binding proteins (soluble cytokine receptors and anti-cytokine autoantibodies), which may antagonize cytokine activity, are also upregulated during

the inflammatory response. Furthermore, IFN-γ, another primary cytokine, negatively affects the IL-1 activity by downregulating the production of IL-1-induced IL-1 by mononuclear cells (239) and upregulating the expression and shedding of keratinocyte IL-1RII (87). It is important to note that in general the anti-inflammatory activities of cytokines are expressed at lower concentrations and with delayed kinetics (requiring the induction of intermediate genes) when compared to the inflammatory activities of cytokines, resulting in an efficient control of inflammation (134).

Second, apoptosis eliminates resident (target) cells and effector leukocytes. For instance, IFN-γ upregulates the expression of FAS by keratinocytes which may undergo apoptosis after interacting with FAS ligand$^+$ T cells (240). The FAS system is also thought to be involved in the clonal deletion of locally activated autoreactive T cells (241). Apoptosis of macrophages is enhanced by IL-4 (another downregulatory effect of a T_H2-type cytokine) (242) and suppressed by IL-1β, TNF-α, IFN-γ and CSFs (243,244), whereas apoptosis of neutrophils is enhanced by TNF-α and suppressed by CSFs and C5a (244). Apoptosis results in clearance of cells by phagocytosis without release of the cellular contents (i.e. not eliciting an inflammatory response), which is of paramount importance for the cessation of inflammation. Cytokines such as IL-1β, TNF-α and IFN-γ were observed to potentiate the removal of apoptotic neutrophils (245).

Third, keratinocytes are thought to play a role in peripheral tolerance to self-antigens, possibly via keratinocyte B7-3 and the induction of T_H2-type cytokines (which may in part depend on keratinocyte IL-1α) (see chapter 1.2.1).

Table III. IL-1 in cutaneous inflammation.

Initiation
- Induction/upregulation of adhesion molecule expression (ICAM-1 on keratinocytes, fibroblasts and endothelial cells, and E-selectin and VCAM-1 on endothelial cells).
- Induction/upregulation of cytokine production (e.g. IL-1 through IL-8, TNF-α and CSFs).
- Induction of proliferation of keratinocytes and fibroblasts.

Amplification
- IL-12-induced generation of T_H1-type cytokines (dependent upon IL-1β).
- Induction of synthesis of eicosanoids, reactive oxygen intermediates and nitric oxide.
- Suppression of apoptosis of macrophages.
- Activation/maturation of Langerhans cells.

Downregulation
- Induction of T_H2-type cytokines (via IL-1α).
- Induction of anti-inflammatory cytokines (e.g. TGF-β).
- Downregulation of IL-1R and TNFR.
- Induction of glucocorticoids and PGE_2.
- Induction of oxygen scavengers.
- Repair of skin tissue.

Finally, repair of skin tissue (i.e. decrease of activation signals) results in a decrease of IL-1 release and IL-1-dependent cascades of events. In addition, the concomitant decrease of dendritic cell activation and maturation together with the influx of new dendritic cell precursors into the repaired skin force the system back into tolerance mode (219). Furthermore, activated natural killer cells (via tissue injury) may downregulate antigen presenting dendritic cells.

Taken together, keratinocyte-derived IL-1 is integrally involved in the initiation, and amplification as well as cessation of local cutaneous inflammation, as illustrated in Table III.

1.3 DYSREGULATED CELLULAR CROSS-TALK IN PSORIASIS

Cellular immunopathologic mechanisms may initiate and/or mediate psoriasis, as is suggested by the following.

1) acquisition or disappearance of psoriasis after bone marrow transplantation (246,247);
2) appearance of skin-infiltrating monocytes and T cells is one of the earliest events in the pathologic changes occurring in psoriasis (248,249);
3) HLA associations with (subtypes of) psoriasis (e.g. B13, B17, Cw6 and DR7) (250);
4) triggering of psoriasis by microbial agents (e.g. streptococci and human immunodeficiency virus) (251,252);
5) appearance of psoriasis upon administration of IL-2 and IFN-γ (253,254);
6) sensitivity of psoriasis to T cell-selective immunosuppressive agents such as anti-CD3 or CD4 monoclonal antibodies (255,256) or a lymphocyte-selective toxin composed of IL-2 and fragments of diphtheria toxin (257);
7) overstimulated crosstalk between T cells and epidermal cells in lesional psoriatic skin (258-261);
8) an altered expression of IL-1 and IL-1 system-related cytokines and their receptors in psoriatic plaques (see below and chapter 1.3.2); and
9) the activation state of skin cells (e.g. production of cytokines) are the target of several effective anti-psoriatic therapies (262).

To date, there is no validated animal model for psoriasis. However, the flaky skin mouse, a spontaneous autosomal recessive mutation, shows a high degree of correlation between skin pathology (i.e. epidermal abnormalities and a prominent inflammatory cell infiltrate) and human psoriasis, and may represent a naturally occurring analog to at least one variety of psoriasis (263,264). Moreover, only recently the intriguing observation was made that mice overexpressing epidermal β1 integrin also expressed a phenotype accurately resembling human psoriasis (265). In addition, gene-manipulated mice underscore the pathogenic relevance of cytokines and their receptors in psoriasis. Overexpression of transgenes targeted to the epidermis (e.g. TGF-α, keratinocyte growth factor (KGF), IL-6 and dominant-negative mutants of KGFR and FGFR) as well as deficiency of genes (e.g. IGF-1, IGF-2, IGF-1R)

demonstrated moderate to severe abnormalities in epidermal proliferation and/or differentiation (266-270, and Gottlieb AB, oral presentation, Psoriasis Meeting, Dec 4-5 1995, Lake Buena Vista, FL). These studies stress that the aberrant expression of a single cytokine(receptor), being inherent to epidermal cell proliferation under physiological conditions, may lead to epidermal alterations but does not exert direct proinflammatory actions. Epidermal overexpression of IL-1 agonists (e.g. IL-1α and IL-1RI), however, induced scaly inflammatory skin lesions with marked erythema, crusting and psoriasis-like histological features such as hyperkeratosis and a dense dermal inflammatory infiltrate (see chapter 1.1.1 on IL-1 biology and refs 137,271).

Lesional psoriatic skin

The human cellular pathology in psoriasis, with special focus on cytokine-based intercellular communication is reviewed. Alterations in intracellular signal transduction and in the synthesis of arachidonic acid-derived mediators in psoriatic epidermis, often a measure of the cellular activation state, are thoroughly reviewed elsewhere (231,272). In addition, epidermal changes regarding mast cells and the expression of neuropeptides, which may contribute to the neuronal element implicated in the pathogenesis of psoriasis are described by others (273-275).

Keratinocytes

Lesional psoriatic keratinocytes are hyperproliferative (276). The hyperproliferative defect is localized to the β1 and $\beta4^+$, $K1/K10^-$ stem cell population (187). Terminal differentiation is accelerated. The proliferation- and differentiation-related alterations constitute a phenotype of regenerative epidermal maturation (similar to that in wound healing) (277). Most notably, the expression of Ki67, K16, B7-3 and the α3, α5 and α6 integrins are increased and there is no expression of filaggrin (278,279, and Gottlieb AB, oral presentation, Psoriasis Meeting, Dec 4-5 1995, Lake Buena Vista, FL). Lesional psoriatic keratinocytes also display an altered expression of inflammation-associated enzymes such as skin-derived anti-leukoproteinase (SKALP) and manganese superoxide dismutase (280,281). The expression of MHC class II molecules, ICAM-1 and IFN-γ-inducible protein-10 (γIP-10) on psoriatic keratinocytes, in contrast to normal keratinocytes, implies *in vivo* exposure to T cell IFN-γ (282-284). Adhesion of T cells and neutrophils to lesional psoriatic epidermis is increased when compared to normal skin, and most pronounced in areas of maximum ICAM-1 expression (285). Furthermore, apoptosis may also be altered in psoriatic epidermis, as suggested by aberrant expressions of BCL proteins in basal keratinocytes (286,287).

Cytokine abnormalities of psoriatic keratinocytes *in vivo* comprise a dysregulated expression of IL-1 (see chapter 1.3.2) and an increased expression of IL-6, IL-8, GRO-α, MCP-1, TGF-α and VEGF (210,288-292). Psoriatic keratinocytes overexpress IL-6R, EGF/TGF-αR and IGF-1R, and show an decreased expression of IFN-γR (293-296). Lesional psoriatic skin cells (with

no distinction made between different cell types) also express increased levels of IL-8R (297). Furthermore, lesional psoriatic keratinocytes *in vitro* demonstrated both an altered response to cytokines (e.g. less inhibition of cell growth on stimulation with IFN-γ and a decreased proliferative response to IL-6) (298-300) and an enhanced production of cytokines (e.g. IL-6, IL-8 and TGF-α) (210,299,301). Moreover, cytokines (e.g. IL-1) released by primary *ex vivo* cultures of lesional psoriatic, but not normal, epidermal cells potentiate T cell activation (261).

Fibroblasts

Psoriatic fibroblasts display a hyperactive state *in vitro*. These cells produce elevated amounts of extracellular matrix proteins (e.g. fibronectin and tenascin), superoxide anion, PDGF-BB and IL-1β, express elevated levels of surface proteases (e.g. exoaminopeptidases and dipeptidylpeptidase IV) and PDGFRβ, and show an altered proliferative response to serum factors, growth factors and several therapeutics (302-309). In addition, fibroblasts are a potential source for the locally produced IL-6 in psoriasis (310,311). Lesional fibroblasts also express elevated levels of PDGFRβ *in vivo*, and show an increased biological response to PDGF *in vitro* (312,313).

Transplantation experiments using athymic nude mice showed that psoriatic keratinocytes need to be in contiguity with the underlying dermis to maintain epidermal hyperplasia *in vivo* (314). In line with this observation are the recent findings that full-thickness human psoriatic plaque skin grafts to severe combined immunodeficiency mice as well as skin grafts from flaky skin mice to nude mice maintained their psoriatic phenotype (315,316). Moreover, Saiag and colleagues found that upper dermal fibroblasts from psoriatic patients cultured in a collagen lattice induced hyperproliferation in normal epidermal biopsies (317). These studies suggest the involvement of fibroblast-derived cytokines (e.g. IL-1β, IL-6 and PDGF) in the pathogenesis of psoriasis.

Endothelial cells

Papillary dermal microvascular endothelium in psoriasis is activated, as illustrated by increased tortuosity, dilatation, permeability and angiogenesis (318-320). In addition, dermal endothelia in psoriatic plaques, but not in uninvolved psoriatic or normal control skin allow specific binding of $CD4^+$ T cells as a consequence of increased expression of ICAM-1 and E-selectin (321-323). In psoriasis, VCAM-1, endoglin, TNF-α, TNFR, PDGFRβ and VEGFR are also upregulated on dermal endothelium (182,292,312,324,325). In addition, adhesion molecules on psoriatic endothelial cells have been shown to be less sensitive to the downregulatory effects of TGF-β1 (326).

Classical APC

The number and function of epidermal (non-Langerhans cell) APC are increased in psoriatic lesions (327). These bone marrow-derived $CD1a^-$ and

HLA-DR$^+$ cells express macrophage markers (e.g. CD36), adhesion molecules (e.g. LFA-1, LFA-2, LFA-3 and ICAM-1) and membrane-associated IL-1, and possess an elevated ability to present alloantigen and to stimulate autologous T cells in the absence of exogenous antigen (327-329). Psoriatic dermis harbors increased numbers of Langerhans cells showing a phenotype which is consistent with migration (282,330). The number of another dendritic cell type (being positive for HLA-DR, LFA-1, B7 and factor XIIIa molecules) is also increased in psoriatic dermis and is often observed in direct apposition to T cells (203,331,332). Moreover, dermal dendrocytes isolated from lesional psoriatic skin are much more potent stimulators of T cell proliferation and generation of T_H1-type cytokines relative to normal skin or peripheral blood-derived dendritic cells (203). Macrophages, often located directly beneath and lining the border between epidermis and dermis in lesional psoriatic skin (333), also show an activated state as evidenced by an increased expression of IL-6 and TNF-α (334,335). In addition, peripheral monocytes from psoriatic patients release increased levels of IL-6, TNF-α and GM-CSF, and activate neutrophils more efficiently when compared to normal monocytes (335,336). Patients with severe psoriasis even show elevated serum levels of IL-6 (335).

T cells

The number of T cells present in lesional psoriatic skin is increased. These cells primarily express the activated memory cell phenotype (e.g. HLA-DR$^+$, IL-2R$^+$, CD45RO$^+$, CLA$^+$, LFA-1$^+$, ICAM-1$^+$, VLA-4$^+$ and CDw60$^+$) (248,258,328,337-339). In the dermis of early and developed lesions, CD4$^+$ T cells are predominant, whereas in spontaneously resolving lesions dermal CD8$^+$ T cells become more prominent (340,341). Analysis of TCR usage by T cells in psoriatic plaques *in vivo* revealed that Vβ3 and Vβ13.1 were predominantly used by intra-epidermal CD8$^+$ T cells, whereas Vβ2 and Vβ6 dominate dermal T cell infiltrates (of which the CD4/CD8 phenotype was not determined) (226,342,343). Furthermore, sequence analysis of the junctional complementarity-determining region 3 motifs revealed a marked TCR oligoclonality in psoriatic lesions (226,343).

The cytokine network of psoriatic plaques *in vivo* is primarily dominated by T_H1-type cytokines (IL-2, IL-5 and IFN-γ, but no IL-4 or IL-10) (322,344,345). Cytokines released by lesional skin-derived T cell clones induce proliferation, activation and a 'psoriatic phenotype' in cultured keratinocytes (258-260). These T cell clones (predominantly of the CD4 phenotype) primarily secrete T_H1-type cytokines, but also secrete IL-4, in a minority of clones, and express IL-5 mRNA (344-346). To date there is no consensus on the predominant phenotype of T cells (CD4$^+$ versus CD8$^+$ cells) present in active lesional epidermis (257,347), and of T cell clones producing keratinocyte clonal growth stimulatory cytokines (260,346).

When analyzing the peripheral compartment, psoriatic T cells display augmented binding to normal endothelium as compared with normal controls

(348). CD3-triggered peripheral T cell proliferation is decreased in psoriatic individuals when compared to normal subjects, but is enhanced by extracellular matrix components (349). In peripheral blood of psoriatic patients signs of T cell activation have been observed, such as an increased number of activated T cells, and elevated levels of IFN-γ, sIL-2R, sCD8 and sICAM-1 (328,350-353).

Neutrophils

Active psoriatic tissue reactions are characterized by the presence of intra-epidermal neutrophils (354). Peripheral blood neutrophils of psoriatic patients demonstrate an enhanced cellular activation, as these cells express increased levels of IL-8R (355) and show enhanced antibody-dependent cell-mediated cytotoxicity, respiratory burst and chemotaxis (356-358).

Non-lesional psoriatic skin

It is important to realize that non-lesional psoriatic skin is not normal. Non-lesional skin shows enhanced epidermal proliferation, biochemical and enzymatic alterations of the epidermis, dilated capillaries and a variable degree of leukocyte infiltration relative to normal control skin (337). Non-lesional psoriatic skin cells appear to be primed for leukocyte adherence, as suggested by increased expression of adhesion molecules (β1 integrin on keratinocytes, and E-selectin, ICAM-1 and VCAM-1 on endothelial cells) (322,359). When transplanted onto severe combined immunodeficiency mice, non-lesional psoriatic skin expressed enhanced IL-8 levels and became thicker relative to normal skin (315). *In vitro* cultures of non-lesional psoriatic keratinocytes have also been shown to produce elevated amounts of IL-8 (301). Using primary *ex vivo* cultures, T cell cytokines (e.g. IFN-γ) induced accelerated recruitment of non-lesional psoriatic stem keratinocytes from their quiescent state (G_0) into cell cycle relative to normal stem keratinocytes (346). Furthermore, of special interest to this thesis are the findings that non-lesional psoriatic skin shows an increased expression of IL-1α, IL-1β, IL-6 and IL-6R *in vivo* (293,322,360) as well as an increased production of biologically active IL-1 and IL-6 in organ culture (361).

We believe that it may prove valuable to subdivide non-lesional psoriatic skin into skin that has never been lesional before and post-lesional skin. Although such a distinction is difficult to make, it may enable the discrimination between in-born alterations of non-lesional psoriatic skin and those that are the consequence of a former psoriatic response.

1.3.1 Current view on the immunopathogenesis of psoriasis

The last few years, some consensus is reached on the view that clonally expanded T cells stimulate keratinocytes into a 'regenerative' program which is normally activated in response to injury or wounding and gives rise to the 'psoriatic phenotype' (Krueger JG, oral presentation, Psoriasis Meeting, Dec 4-5 1995, Lake Buena Vista, FL). Although circumstantial evidence supports

an autoimmune basis for psoriasis, at the moment of writing there is no identifiable self-antigen. Putative psoriasis-related antigens have been described elsewhere (262). Microbial antigens, to which the skin is constantly exposed to, such as bacterial toxins derived from *Staphylococcus aureus* and streptococci, acting as superantigens, mediate T cell activation in at least a subset of patients (362,363). Hypothetically, superantigens from epicutaneous sources (via physical injury, Koebner phenomenon) or from the circulation (via a throat infection with exotoxin C-secreting *Streptococcus pyogenes*) may bind with sufficient affinity to MHC class II molecules on skin APC (Langerhans cells, dendrocytes and macrophages) and drive the selective expansion of T cell populations (the superantigen-MHC unit is recognized primarily by polyclonal TCR Vβ chains) (362,364). This may hold true for any (auto)antigen. MHC class II binding of superantigens, however, induces the release of IL-1β, TNF-α (365) and IL-12 (366) and increases the expression of CLA on activated T cells either in the skin, skin-associated lymph nodes (via migrated Langerhans cells) or spleen which results in the generation of additional skin-recirculating and homing T cells (366). Interaction of T cells with dermal dendrocytes may result in the generation of T_H1-type cytokines, which are characteristic of the *in vivo* cytokine profile of psoriasis (203,322). An alternative hypothesis for inflammation to ensue in the skin of psoriatic patients might be an antigen-independent one. In line with such a hypothesis is the observation that T cell binding to dermal dendrocytes does not necessarily need cognate antigen (220).

Patients with psoriasis can demonstrate divergent T cell responses (with respect to the Vβ repertoire), but lesional T cells were found to be oligoclonal with respect to the junctional motifs (226,343). This oligoclonal T cell subset probably expanded *in situ* from a few progenitor T cells in response to common epitopes on the putative psoriasis antigen. Thus, the contribution of (auto)antigen(s) to, at least, the persistence of psoriatic lesions is most likely. Sensitization to multiple autoantigens or cryptic epitopes may occur as a consequence of chronic inflammation, epidermal hyperplasia and accelerated terminal keratinocyte differentiation (367). Expression of autoantigens by the psoriatic keratinocyte may for example result from alterations in (the glycosylation of) plasma membrane proteins (368) or from apoptosis (233,234). Molecular mimicry between microbial antigens and keratins may also contribute to the autoimmune response (369). Persistence of psoriasis is likely the consequence of a self-sustaining cycle of T cell-derived cytokines that preferentially stimulate psoriatic epidermal stem cells ($\beta1^+$ and $K1/K10^-$ keratinocytes) to proliferate, and further epidermal potentiation of the T cell mediated lesions (370). Transplantation experiments with mice have indicated that the persistence of the psoriatic phenotype is independent of newly infiltrating T cells and that other cells such as fibroblasts may be actively involved in the maintenance of psoriasis (see chapter 1.3 on fibroblasts).

Psoriatic lesions can spontaneously resolve, in which dermal $CD8^+$ T cells may play a prominent role (340).

1.3.2 IL-1 in psoriasis

An inventory of the dysregulated epidermal expression of IL-1 in psoriasis is given in Table IV. In short, lesional psoriatic skin, and to a lesser extent non-lesional psoriatic skin, showed an increased expression of mRNA for IL-1β, icIL-1ra and sIL-1ra (322,360,371,372) when compared to normal control skin. Cytosolic extracts of lesional psoriatic keratome specimens contained decreased amounts of IL-1α protein

Table IV. Overview of IL-1 network in psoriatic lesions.[a,b]

Type of skin sample	IL-1α			IL-1β			IL-1ra		
	mRNA	protein		mRNA	protein		mRNA	protein	
		IR	BA		IR	BA		IR	BA
Cytosolic extract	↔[c]	↓↓	↓↓	↑	↑	-		↓[d]	
Scale extract		↓	↓		↓	-			-
Suction blister fluid		↔			-				
Biopsy	↔	↔[e]	↑	-	↑[f]			↓	
Culture	↑	↔	↔	↑	↑	↔			

Type of skin sample	IL-1RI		IL-1RII	
	mRNA	protein	mRNA	protein
Biopsy[g]				↑

[a] See text for details and references.
[b] Abbreviations: IR, immunoreactivity; BA, biological activity.
[c] ↑/↓ indicates increase/decrease of the expression in lesional samples relative to normal control samples; ↔ indicates a similar expression in both types of samples; and - indicates no expression in both types of samples.
[d] Ratio between IL-1ra and IL-1α is increased in psoriasis.
[e] Intracellular IL-1α staining. A strong membrane staining for IL-1β was observed in lesional psoriatic epidermal cell suspensions.
[f] RNA for icIL-1ra and sIL-1ra is increased in psoriasis.
[g] To date, the only type of psoriatic skin sample analyzed for the expression of IL-1R is biopsy material.

but increased amounts of IL-1β protein (360,373). Moreover, in lesional psoriatic cytosolic extracts, the ratio between IL-1ra and IL-1α has been found to be increased (371). When aqueous extracts of the stratum corneum (scale extracts) were used, it was observed that IL-1α was decreased in psoriatic lesions when compared to normal control skin and that IL-1β immunoreactivity and IL-1 inhibitory activity were barely detectable in both lesional psoriatic and normal control skin (374-376). In addition, suction blister fluids from lesional psoriatic as well as from normal control skin

contained similarly low levels of IL-1α and no IL-1β (311). Furthermore, IL-1 immunohistochemistry revealed either no differences in the expression of IL-1α and IL-1β between lesional psoriatic and normal control epidermis (360), a predominantly intracellular IL-1α staining in lesional psoriatic epidermis relative to the predominantly intercellular IL-1α staining in normal control epidermis (377), or a decreased expression of IL-1ra in lesional psoriatic epidermis (378). Immunofluorescent staining of epidermal cell suspensions showed that IL-1β was overexpressed on the plasma membrane and in the intracellular compartment of lesional psoriatic epidermal cells (311). Despite the before mentioned elevation of IL-1β in lesional psoriatic skin, all bioactivity was confined to IL-1α. The non-functionality of IL-1β was not due to lack of processing or the co-presence of IL-1ra (360,374,379). With respect to the IL-1 receptors, it was recently observed that the immunohistochemical expression of IL-1RII was enhanced in lesional psoriatic epidermis (148).

Chapter 5 discusses our own findings in relation to the above mentioned data on the aberrant expression of IL-1 in psoriatic skin.

Taken together, the pathological relevance of IL-1 to psoriasis is supported by the following.

1) the controlling effects of IL-1 on keratinocyte proliferation and differentiation (145,209);
2) the prominent regulatory role of IL-1 in cutaneous immunological responses, especially the inflammatory response (see chapter 1.2.1);
3) the transgenic approach which shows that overexpression of IL-1 agonists induce a psoriasis-like phenotype (see chapter 1.3);
4) the altered expression of the components of the IL-1 system (Table IV); and
5) several effective anti-psoriatic therapeutic modalities (such as retinoids, glucocorticoids, ultraviolet irradiation) affect the expression of epidermal IL-1 *in vivo* and/or *in vitro* (373,380-384).

REFERENCES

1. Wilan R. On cutaneous diseases (1808) Kimber and Conrad, London, pp. 115.
2. Dubertret L (ed) (1994) Psoriasis. ISED Publ. Co. Brescia.
3. Boulay JL, Paul WE (1993) The interleukin-4 family of lymphokines. Curr Opin Immunol 3:573-581.
4. Callard R, Gearing A (1994) The cytokine factsbook. Academic press, London, pp. 3, 12-27.
5. Debets R, Savelkoul HFJ (1993) Cytokine antagonists and their potential therapeutic use. Immunol Today 15:455-458.
6. Baggiolini M, Dewald B, Moser B (1993) IL-8 and related chemotactic cytokines-CXC and CC chemokines. Adv Immunol 55:97-179.
7. Boraschi D, Nencioni L, Villa L, Censini S, Bossù P, Ghiara P, Presentini R, Perin F, Frasca D, Doria G, Forni G, Musso T, Giovarelli M, Ghezzi P, Bertini R, Besedovsky HO, Del Rey A, Sipe JD, Antoni G, Silvestri S, Tagliabue A (1988) In vivo stimulation and restoration of the immune response by the non-inflammatory fragment 163-171 of human interleukin 1β.

J Exp Med 168:675-686.
8. Bazan JF (1990) Structural design and molecular evolution of a cytokine receptor superfamily. Proc Natl Acad Sci USA 87:6934-6938.
9. Kishimoto T, Taga T, Akira S (1994) Cytokine signal transduction. Cell 76:253-262.
10. Kishimoto T, Akira S, Narazaki M, Taga T (1995) Interleukin-6 family of cytokines and gp130. Blood 86:1243-1254.
11. Murakami M, Narazaki M, Hibi M, Yawata H, Yasukawa K, Hamaguchi M, Taga T, Kishimoto T (1991) Critical cytoplasmic region of the IL-6 signal transducer, gp130, is conserved in the cytokine receptor family. Proc Natl Acad Sci USA 88:11349-11353.
12. Dower SK (1993) Cytokine receptor families. Adv Second Messenger Phosphoprotein Res 28:19-25.
13. Stahl N, Farruggella J, Boulton TG, Zhong Z, Darnell JE, Yancopoulos GD (1995) Choice of STATs and other substrates specified by modular tyrosine-based motifs in cytokine receptors. Science 267:1349-1353.
14. Ivashkiv LB (1995) Cytokines and STATs: How can signals achieve specificity? Immunity 3:1-4.
15. Ihle JN (1995) Cytokine receptor signalling. Nature 377:591-594.
16. Amzel LM, Poljak RJ (1979) Three-dimensional structure of immunoglobulins. Annu Rev Biochem 48:961-967.
17. Ullrich A, Schlessinger J (1990) Signal transduction by receptors with tyrosine kinase activity. Cell 61:203-212.
18. Mallett S, Barclay AN (1991) A new superfamily of cell surface proteins related to the nerve growth factor receptor. Immunol Today 12:220-223.
19. Gerard C, Gerard NP (1994) The pro-inflammatory seven-transmembrane segment receptors of the leucocyte. Curr Opin Immunol 6:140-145.
20. Rose-John S, Heinrich PC (1994) Soluble receptors for cytokines and growth factors: generation and biological function. Biochem J 300:281-290.
21. Crowe PD, Walter BN, Mohler KM, Otten-Evans C, Black RA, Ware CF (1995) A metalloprotease inhibitor blocks shedding of the 80-kD TNF receptor and TNF processing in T lymphocytes. J Exp Med 181:1205-1210.
22. Müllberg J, Durie FH, Otten-Evans C, Alderson MR, Rose-John S, Cosman D, Black RA, Mohler KM (1995) A metalloprotease inhibitor blocks shedding of the IL-6 receptor and the p60 TNF receptor. J Immunol 155:5198-5205.
23. Alcamí A, Smith GL (1995) Cytokine receptors encoded by pox viruses: a lesson in cytokine biology. Immunol Today 16:474-478.
24. Hsu D-H, De Waal Malefyt R, Fiorentino DF, Dang MN, Vieira P, De Vries J, Spits H, Mosmann TR, Moore KW (1990) Expression of interleukin-10 activity by Epstein-Barr virus protein BCRF1. Science 250:830-832.
25. Spriggs MK, Hruby DE, Maliszewski CR, Pickup DJ, Sims JE, Buller RM, Van Slyke J (1992) Vaccinia and cowpox viruses encode a novel secreted interleukin-1 binding protein. Cell 71:153-167.
26. Ahuja SK, Murphy PM (1993) Molecular piracy of mammalian interleukin-8 receptor type B by herpesvirus saimiri. J Biol Chem 268:20691-20694.
27. Chatenoud L (1992) Allograft rejection: the role of the cytokine network. Eur Cyt Network 3:509-513.
28. Ruddle NH (1992) Tumor necrosis factor (TNF-α) and lymphotoxin (TNF-β). Curr Opin Immunol 4:327-332.
29. Cosman D (1987) Control of messenger RNA stability. Immunol Today 8:16-17.
30. Schindler R, Clark BD, Dinarello CA (1990) Dissociation between interleukin-1β mRNA and protein synthesis in human peripheral blood mononuclear cells. J Biol Chem 265:10232-10237.
31. Massague J, Pandiella A (1993) Membrane-anchored growth factors. Annu Rev Biochem 62:515-541.
32. Howard AD, Kostura MJ, Thornberry N, Ding G-JF, Limjuco G, Weidner J, Salley JP, Hogquist KA, Chaplin DD, Mumford RA, Schmidt JA, Tocci MJ (1991) IL-1-converting

enzyme requires aspartic acid residues for processing of the IL-1β precursor at two distinct sites and does not cleave 31-kDa IL-1α. J Immunol 147:2964-2969.
33. Schröder JM (1995) Cytokine networks in the skin. J Invest Dermatol 105:20S-24S.
34. Hazuda DJ, Strickler J, Kueppers F, Simon PL, Young PR (1990) Processing of precursor interleukin 1 beta and inflammatory disease. J Biol Chem 265:6318-6322.
35. Harpel JG, Metz CN, Kojima S, Rifkin DB (1993) Control of transforming growth factor-beta activity: latency vs. activation. Prog Growth Factor Res 4:321-335.
36. Sehgal PB (1990) Interleukin 6: molecular pathophysiology. J Invest Dermatol 94:2S-6S.
37. Van Damme J, Van Beeumen J, Conings R, Decock B, Billiau A (1989) Purification of granulocyte chemotactic peptide/interleukin-8 reveals N-terminal sequence heterogeneity similar to that of beta-thromboglobulin. Eur J Biochem 181:337-344.
38. Opdenakker G, Rudd PM, Wormald M, Dwek RA, Van Damme J (1995) Cells regulate the activities of cytokines by glycosylation. FASEB J 9:453-457.
39. Tanaka Y, Adams DH, Shaw S (1993) Proteoglycans on endothelial cells present adhesion-inducing cytokines to leucocytes. Immunol Today 14:111-115.
40. Cosman D (1993) The hematopoietin receptor superfamily. Cytokine 5:95-106.
41. Bendtzen K, Svenson M, Jǿnsson V, Hippe F (1990) Autoantibodies to cytokines--friends or foes? Immunol Today 11:167-169.
42. Sylvester I, Suffredini AF, Boujoukos AJ, Martich GD, Danner RL, Yoshimura T, Leonard EJ (1993) Neutrophil attractant protein-1 and monocyte chemoattractant protein-1 in human serum. Effects of intravenous lipopolysaccharide on free attractants, specific IgG autoantibodies and immune complexes. J Immunol 151:3292-3298.
43. Klein B, Brailly H (1995) Cytokine-binding proteins: stimulating antagonists. Immunol Today 16:216-220.
44. Eisenberg SP, Evan RJ, Arend WP, Verderber E, Brewer MT, Hannum CH, Thompson RC (1990) Primary structure and functional expression from complementary DNA of a human interleukin-1 receptor antagonist. Nature 343:341-346.
45. Ling P, Gately MK, Gubler U, Stern AS, Lin P, Hollfelder K, Su C, Pan YC, Hakimi J (1995) Human IL-12 p40 homodimer binds to the IL-12 receptor but does not mediate biologic activity. J Immunol 154:116-127.
46. Gery I, Gershon RK, Waksman BH (1972) Potentiation of the T-lymphocyte response to mitogens. I. The responding cell. J Exp Med 136:128-142.
47. Mizel SB, Farrar JJ (1979) Revised nomenclature for antigen-nonspecific T-cell proliferation and helper factors. Cell Immunol 48:433-436.
48. March C, Mosley B, Larsen A, Cerretti D, Braedt G, Price V, Gillis S, Henney C, Kronheims S (1985) Cloning sequence and expression of two distinct human interleukin-1 complementary DNA's. Nature 315:641-647.
49. Dinarello CA (1994) The interleukin-1 family: 10 years of discovery. FASEB J 8:1314-1325.
50. Bailly S, di Giovine FS, Blakemore AI, Duff GW (1993) Genetic polymorphism of human interleukin-1 alpha. Eur J Immunol 23:1240-1245.
51. Auron PE, Webb AC (1994) Interleukin-1: a gene expression system regulated at multiple levels. Eur Cytokine Netw 5:573-592.
52. Stevenson FT, Torrano F, Locksley RM, Lovett DH (1992) IL-1: the patterns of translation and intracellular distribution support alternative secretory mechanisms. J Cell Physiol 152:223-231.
53. Stevenson FT, Bursten SL, Fanton C, Locksley RM, Lovett DH (1993) The 31-kDa precursor of interleukin-1α is myristoylated on specific lysines within the 16-kDa N-terminal propiece. Proc Natl Acad Sci USA 90:7245-7249.
54. Kurt-Jones EA, Beller DI, Mizel SB, Unanue ER (1985) Identification of a membrane-associated interleukin-1 in macrophages. Proc Natl Acad Sci USA 82:1204-1208.
55. Kobayashi Y, Yamamoto K, Saido T, Kawasaki H, Oppenheim JJ, Matsushima K (1990) Identification of a calcium-activated neutral protease as a processing enzyme of human interleukin 1α. Proc Natl Acad Sci USA 87:5548-5552.
56. Pontremoli S, Salamino F, Sparatore B, Michetti M, Sacco O, Melloni E (1985) Following

association to the membrane, human erythrocyte procalpain is converted and released as fully active calpain. Biochim Biophys Acta 831:335-339.

57. Thornberry NA, Bull HG, Calaycay JR, Chapman KT, Howard AD, Kostura MJ, Miller DK, Molineaux SM, Weidner JR, Aunins J, Elliston KO, Ayala JM, Casano FJ, Chin J, Ding GJ-F, Egger LA, Gaffney EP, Limjuco G, Palyha OC, Raju SM, Rolando AM, Salley JP, Yamin T-T, Lee TD, Shively JE, MacCross M, Mumford RA, Schmidt JA, Tocci MJ (1992) A novel heterodimeric cysteine protease is required for interleukin-1β processing in monocytes. Nature 356:768-774.
58. Cameron PM, Limjuco GA, Chin J, Silberstein L, Schmidt JA (1986) Purification to homogeneity and amino acid sequence analysis of two anionic species of human interleukin-1. J Exp Med 164:237-250.
59. Mizutani H, Black R, Kupper TS (1991) Human keratinocytes produce but do not process pro-interleukin-1 (IL-1) beta. Different strategies of IL-1 production and processing in monocytes and keratinocytes. J Clin Invest 87:1066-1071.
60. Perregaux D, Barberia J, Lanzetti AJ, Geoghegan KF, Carty TJ, Gabel CA (1992) IL-1β maturation: evidence that mature cytokine formation can be induced specifically by nigericin. J Immunol 149:1294-1303.
61. Wilson KP, Black J-AF, Thomson JA, Kim EE, Griffith JP, Navia MA, Murcko MA, Chambers SP, Aldape RA, Raybuck SA, Livingston DJ (1994) Structure and mechanism of interleukin-1β converting enzyme. Nature 370:270-275.
62. Walker NPC, Talanian RV, Brady KD, Dang LC, Bump NJ, Ferenz CR, Franklin S, Ghayur T, Hackett MC, Hammill LD, Herzog L, Hugunin M, Houy W, Mankovich JA, McGuiness L, Orlewicz E, Paskind M, Pratt CA, Reis P, Summani A, Terranova M, Welch JP, Xiong L, Möller A, Tracey DE, Kamen R, Wong WW (1994) Crystal structure of the cysteine protease interleukin-1β-converting enzyme: A $(p20/p10)_2$ homodimer. Cell 78:343-352.
63. Kuida K, Lippke JA, Ku G, Harding MW, Livingston DJ, Su MS-S, Flavell RA (1995) Altered cytokine export and apoptosis in mice deficient in interleukin-1β converting enzyme. Science 267:2000-2003.
64. Yuan J, Shaham S, Ledoux S, Ellis HM, Horvitz HR (1993) The *C. elegans* cell death gene ced-3 encodes a protein similar to mammalian interleukin-1β-converting enzyme. Cell 75:641-652.
65. Martin SJ, Green DR (1995) Protease activation during apoptosis. Death by a thousand cuts? Cell 82:349-352.
66. Hogquist KA, Nett MA, Unanue ER, Chaplin DD (1991) Interleukin 1 is processed and released during apoptosis. Proc Natl Acad Sci USA 88:8485-8489.
67. Zychlinsky A, Fitting C, Cavaillon J-M, Sansonetti PJ (1994) Interleukin 1 is released by murine macrophages during apoptosis induced by *Shigella flexneri*. J Clin Invest 94:1328-1332.
68. Sarih M, Souvannavong V, Brown SC, Adam A (1993) Silica induces apoptosis in macrophages and the release of interleukin-1 alpha and interleukin-1 beta. J Leukoc Biol 54:407-413.
69. Li P, Allen H, Banerjee S, Franklin S, Herzog L, Johnston C, McDowell J, Paskind M, Rodman L, Salfeld J, Towne E, Tracey D, Wardwell S, Wei F-Y, Wong W, Kamen R, Seshadri T (1995) Mice deficient in IL-1β-converting enzyme are defective in production of mature IL-1β and resistant to endotoxic shock. Cell 80:401-411.
70. Rubartelli A, Bajetto A, Allavena G, Cozzolino F, Sitia R (1993) Post-translational regulation of IL-1 beta secretion. Cytokine 5:117-124.
71. Siders WM, Klimovitz JC, Mizel SB (1993) Characterization of the structural requirements and cell type specificity of IL-1α and IL-1β secretion. J Biol Chem 268:22170-22174.
72. Siders WM, Mizel SB (1995) Interleukin-1β secretion. A possible multistep process that is regulated in a cell-type specific manner. J Biol Chem 270:16258-16264.
73. Bakouche O, Brown DC, Lachman LB (1987) Subcellular localization of human monocyte interleukin 1: evidence for an inactive precursor molecule and a possible mechanism for IL-1 release. J Immunol 138:4249-4255.
74. Rubartelli A, Cozzolino F, Talio M, Sitia R (1990) A novel secretory pathway for interleu-

kin-1 beta, a protein lacking a signal sequence. EMBO J 9:1503-1510.
75. Tarlow JK, Blakemore AI, Lennard A, Solari R, Hughes HN, Steinkasserer A, Duff GW (1993) Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. Hum Genet 91:403-404.
76. Danis VA, Millington M, Hyland VJ, Grennan D (1995) Cytokine production by normal human monocytes: inter-subject variation and relationship to an IL-1 receptor antagonist (IL-1ra) gene polymorphism. Clin Exp Immunol 99:303-310.
77. Butcher C, Steinkasserer A, Tejura S, Leonard AC (1994) Comparison of two promoters controlling expression of secreted or intracellular IL-1 receptor antagonist. J Immunol 153:701-711.
78. Hannum CH, Wilcox CS, Arend WP, Joslin FG, Dripps DJ, Heimdal PL, Armes LG, Somner A, Eisenberg SP, Thompson RC (1990) Interleukin-1 receptor antagonist activity of a human interleukin 1 inhibitor. Nature 343:336-340.
79. Carter DB, Deibel MR Jr., Dunn CJ, Tomich C-SC, Slightom AL, Berger AE, Bienkowski MJ, Sun FF, McEwan RN, Harris PKW, Yem AW, Waszak GA, Chosay JG, Sieu LC, Hardee MM, Zurcher-Neely HA, Reardon IM, Heinrikson RL, Truesdell SE, Shelly JA, Eessalu TE, Taylor BM, Tracey DE (1990) Purification, cloning, expression and biological characterization of an interleukin-1 receptor antagonist. Nature 344:633-638.
80. Haskill S, Martin G, Van Le L, Morris J, Peace A, Bigler CF, Jaffe GJ, Hammerberg C, Sporn SA, Fong S, Arend WP, Ralph P (1991) cDNA cloning of an intracellular form of the human interleukin 1 receptor antagonist associated with epithelium. Proc Natl Acad Sci USA 88:3681-3685.
81. Muzio M, Polentarutti N, Sironi M, Poli G, De Gioia L, Introna M, Mantovani A, Colotta F (1995) Cloning and characterization of a new isoform of the interleukin 1 receptor antagonist. J Exp Med 182:623-628.
82. Bazan JF, Timans JC, Kastelein RA (1996) A newly defined interleukin-1? Nature 379:591.
83. Sims JE, Painter SL, Gow IR (1995) Genomic organization of the type I and type II IL-1 receptors. Cytokine 7:483-490.
84. Ye K, Dinarello CA, Clark BD (1993) Identification of the promoter region of the human interleukin 1 type I receptor gene: multiple initiation sites, high G+C content, and constitutive expression. Proc Natl Acad Sci USA 90:2295-2299.
85. Slack J, McMahan CJ, Waugh S, Schooley K, Spriggs MK, Sims JE, Dower SK (1993) Independent binding of interleukin-1α and interleukin-1β to type I and type II interleukin-1 receptors. J Biol Chem 268:2513-2524.
86. McMahan C, Slack J, Mosley B, Cosman D, Lupton SD, Brunton LL, Grubin CE, Wignall JM, Jenkins NA, Brannan CI, Copeland NG, Huebner K, Croce CM, Cannizzarro LA, Benjamin D, Dower S, Spriggs M, Sims J (1991) A novel IL-1 receptor, cloned from B cells by mammalian expression, is expressed in many cell types. EMBO J 10:2821-2832.
87. Groves RW, Giri J, Sims J, Dower S, Kupper TS (1995) Inducible expression of type 2 IL-1 receptors by cultured human keratinocytes. Implications for IL-1-mediated processes in epidermis. J Immunol 154:4065-4072.
88. Svenson M, Hansen MB, Heegaard P, Abell K, Bendtzen K (1993) Specific binding of interleukin-1 (IL-1)β and IL-1 receptor antagonist (IL-1ra) to human serum: high-affinity binding of IL-1ra to soluble IL-1 receptor type I. Cytokine 5:427-435.
89. Symons JA, Eastgate JA, Duff GW (1991) Purification and characterization of a novel soluble receptor for interleukin 1. J Exp Med 174:1251-1254.
90. Arend WP, Malyak M, Smith MF Jr., Whisenand TD, Slack JL, Sims JE, Giri JG, Dower SK (1994) Binding of IL-1α, IL-1β, and IL-1 receptor antagonist by soluble IL-1 receptors and levels of soluble IL-1 receptors in synovial fluids. J Immunol 153: 4766-4774.
91. Symons JA, Young PR, Duff GW (1995) Soluble type II interleukin 1 (IL-1) receptor binds and blocks processing of IL-1 beta precursor and loses affinity for IL-1 receptor antagonist. Proc Natl Acad Sci USA 92:1714-1718.
92. Sims J, Gayle M, Slack J, Alderson M, Bird T, Giri J, Colotta FR, Mantovani A, Shanebeck K, Grabstein K, Dower S (1993) Interleukin-1 signaling occurs exclusively via the type 1

receptor. Proc Natl Acad Sci USA 90:6155-6159.
93. Dower SK, Fanslow W, Jacobs C, Waughs S, Sims JE, Widmer MB (1994) Interleukin-1 antagonists. Therapeutic Immunol 1:113-122.
94. Greenfeder SA, Nunes P, Kwee L, Labow M, Chizzonite RA, Ju G (1995) Molecular cloning and characterization of a second subunit of the interleukin 1 receptor complex. J Biol Chem 270:13757-13765.
95. Curtis BM, Gallis B, Overell RW, McMahan CJ, De Roos P, Ireland R, Eisenman J, DowerSK, Sims JE (1989) T cell interleukin 1 receptor cDNA expressed in Chinese hamster ovary cells regulates functional responses to IL-1. Proc Natl Acad Sci USA 86:3045-3049.
96. Colotta F, Re F, Muzio M, Bertini R, Polentarutti N, Sironi M, Giri JG, Dower SK, Sims JE, Mantovani A (1993) Interleukin-1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. Science 261:472-475.
97. Evans RJ, Bray J, Childs JD, Vigers GPA, Brandhuber BJ, Skalicky JJ, Thompson RC, Eisenberg SP (1995) Mapping receptor binding sites in the IL-1 receptor antagonist and IL-1 beta by site-directed mutagenesis: identification of a single site in IL-1ra and two sites in IL-1 beta. J Biol Chem 270:11477-11483.
98. Vigers GP, Caffes P, Evans RJ, Thompson RC, Eisenberg SP, Brandhuber BJ (1994) X-ray structure of interleukin-1 receptor antagonist at 2.0-A resolution. J Biol Chem 269:12874-12879.
99. Mosley B, Urdal DL, Prickett K, Larsen A, Cosman D, Conlan PJ, Gillis S, Dower SK (1987) The interleukin-1 receptor binds the human interleukin-1α precursor but not the interleukin-1β precursor. J Biol Chem 262:2941-2944.
100. Lambriola-Tomkins E, Chandran C, Varnell TA, Madison VS, Ju G (1993) Structure-function analysis of human IL-1α: identification of residues required for the binding to the human type I IL-1 receptor. Protein Eng 6:535-539.
101. Grutter MG, Van Oostrum J, Priestle JP, Edelmann E, Joss U, Feige U, Vosbeck K, Schmitz A (1994) A mutational analysis of receptor binding sites of interleukin-1β: differences in binding of human interleukin-1β mutants to human and mouse receptors. Protein Eng 7:663-671.
102. Boraschi D, Villa L, Volpini G, Bossù P, Censini S, Ghlara P, Scapigliat G, Nencioni L, Bartalini M, Matteucci G (1990) Differential activity of interleukin 1 alpha and interleukin 1 beta in the stimulation of the immune response in vivo. Eur J Immunol 20:317-320.
103. Labriola-Tompkins E, Chandran C, Kaffka KL, Biondi D, Graves BJ, Hatada M, Madison VS, Karas J, Kilian PL, Ju G (1991) Identification of the discontinuous binding site in human interleukin 1 beta for the type-I interleukin-1 receptor. Proc Natl Acad Sci USA 88:11182-11186.
104. Sims J, Dower S (1994) Interleukin-1 receptors. Eur Cytokine Netw 5:539-546.
105. Gayle MA, Slack JL, Bonnert TP, Renshaw BR, Sonoda G, Taguchi T, Testa JR, Dower SK, Sims JE (1996) Cloning of a putative ligand for the T1/ST2 receptor. J Biol Chem 271:5784-5789.
106. Mitcham JL, Parnet P, Bonnert TP, Garka KE, Gerhart MJ, Slack JL, Gayle MA, Dower SK, Sims JE (1996) T1/ST2 signaling establishes it as a member of an expanding interleukin-1 receptor family. J Biol Chem 271:5777-5783.
107. McKean DJ, Podzorski RP, Bell MP, Nilson AE, Huntoon CJ, Slack J, Dower SK, Sims J (1993) Murine T helper cell-2 lymphocytes express type I and type II IL-1 receptors, but only the type I receptor mediates costimulatory activity. J Immunol 151:3500-3510.
108. Hanks SK, Quinn AM, Hunter T (1988) The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science 241:42-52.
109. Sims J, March CJ, Cosman D, Widmer MB, MacDonald HR, McMahan C, Grubin CE, Wignall JM, Jackson J, Call SM, Friend D, Alpert A, Gillis S, Urdal DL, Dower S (1988) cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin superfamily. Science 241:585-589.
110. O'Neill LAJ, Bird TA, Saklatvala J (1990) Interleukin-1 signal transduction. Immunol Today 11:392-394.
111. Mizel SB (1990) Cyclic AMP and interleukin-1 signal transduction. Immunol Today

11:390-391.
112. Rosoff PM, Savage N, Dinarello CA (1988) Interleukin-1 stimulates diacylglycerol production in T lymphocytes by a novel mechanism. Cell 54:73-81.
113. Mathias S, Younes A, Kan C-C, Orlow I, Joseph C, Kolesnick RN (1993) Activation of the sphingomyelin signaling pathway in intact EL4 cells and in a cell-free system by IL-1β. Science 259:519-522.
114. Gronich J, Konieczkowski M, Gelb MH, Nemenhoff RA, Sedor JR (1994) Interleukin-1α causes a rapid activation of cytosolic phospholipase A_2 by phosphorylation in rat mesangial cells. J Clin Invest 93:1224-1233.
115. O'Neill LAJ (1995) Towards an understanding of the signal transduction pathways for interleukin 1. Biochim Biophys Acta 1266:31-44.
116. Cao Z, Henzel WJ, Gao X (1996) IRAK: a kinase associated with the interleukin-1 receptor. Science 271:1128-1131.
117. Bird TA, Sleath PR, De Roos PC, Dower SK, Virca GD (1991) Interleukin-1 represents a new modality for the activation of extracellular signal-regulated kinases/microtubule-associated protein-2 kinases. J Biol Chem 266:22661-22670.
118. Guesdon F, Waller RJ, Saklatvala J (1994) Specific activation of beta-casein kinase by the inflammatory cytokines interleukin 1 and tumour necrosis factor. Biochem J 304:761-768.
119. Freshney NW, Rawlinson L, Guesdon F, Jones E, Cowley S, Hsuan J, Saklatvala J (1994) Interleukin-1 activates a novel protein kinase cascade that results in the phosphorylation of Hsp27. Cell 78:1039-1049.
120. Cohen P (1993) Dissection of the protein phosphorylation cascades involved in insulin and growth factor action. Biochem Soc Trans 21:555-567.
121. Guy GR, Cairns J, Ng SB, Tan YH (1993) Inactivation of a redox-sensitive protein phosphatase during the early events of tumor necrosis factor/interleukin-1 signal transduction. J Biol Chem 268:2141-2148.
122. Guesdon F, Ikebe T, Stylianou E, Warwick-Davies J, Haskill S, Saklatvala J (1995) Interleukin 1-induced phosphorylation of MAD3, the major inhibitor of nuclear factor kappa B of HeLa Cells. Interference in signalling by the proteinase inhibitors 3,4-dichloroisocoumarin and tosylphenylalanyl chloromethylketone. Biochem J 307:287-295.
123. Stylianou E, O'Neill LAJ, Rawlinson L, Edbrooke MR, Woo P, Saklatvala J (1992) Interleukin-1 induces NFκB through its type I but not type II receptor in lymphocytes. J Biol Chem 267:15836-15841.
124. Muegge K, Williams TM, Kant J, Karin M, Chiu R, Schmidt A, Siebenlist U, Young HA, Durum SK (1989) Interleukin-1 costimulatory activity on the interleukin-2 promoter via AP-1. Science 246:249-251.
125. Granowitz EV, Clark BD, Vannier E, Callahan MV, Dinarello CA (1992) Effect of interleukin-1 (IL-1) blockade on cytokine synthesis. I. IL-1 receptor antagonist inhibits IL-1-induced cytokine synthesis and blocks the binding of IL-1 to its type II receptor on human monocytes. Blood 79:2356-2363.
126. Mizel SB, Kilian PL, Lewis JC, Paganelli KA, Chizzonite RA (1987) The interleukin-1 receptor. Dynamics of interleukin 1 binding and internalization in T cells and fibroblasts. J Immunol 138:2906-2912.
127. Qwarnstrom EE, Page RC, Gillis S, Dower SK (1988) Binding, internalization, and intracellular localization of interleukin-1 beta in human diploid fibroblasts. J Biol Chem 263:8261-8269.
128. Sims JE, Acres RB, Grubin CE, McMahan CJ, Wignall JM, March CJ, Dower SK (1989) Cloning the interleukin 1 receptor from human T cells. Proc Natl Acad Sci USA 86:8946-8950.
129. Curtis BM, Widmer MB, De Roos P, Qwarnstrom EE (1990) IL-1 and its receptor are translocated to the nucleus. J Immunol 144:1295-1303.
130. Weizmann MN, Savage N (1992) Nuclear internalization and DNA binding activities of interleukin-1, interleukin-1 receptor complexes. Biochem Biophys Res Commun 187:1166-1171.
131. Kupper TS, Lee F, Birchall N, Clark S, Dower SK (1988) Interleukin 1 binds to specific

receptors on human keratinocytes and induces granulocyte macrophage colony-stimulating factor mRNA and protein. J Clin Invest 82:1787-1792.
132. Camp RDR, Fincham NJ, Ross J, Bird CR, Gearing AJH (1990) Potent inflammatory properties in human skin of interleukin-1 alpha-like material isolated from normal skin. J Invest Dermatol 94:735-741.
133. Oppenheim JJ, Kovacs EJ, Matsushima K, Durum SK (1986) There is more than one interleukin 1. Immunol Today 7:45-56.
134. Dinarello CA (1994) The biological properties of interleukin-1. Eur Cytokine Netw 5:517-531.
135. Ray CA, Black RA, Kronheim SR, Greenstreet TA, Sleath PR, Salvesen GS, Pickup DJ (1992) Viral inhibition of inflammation: cowpox virus encodes an inhibitor of the interleukin-1 beta converting enzyme. Cell 69:597-604.
136. Kupper TS, Groves RW (1995) The interleukin-1 axis and cutaneous inflammation. J Invest Dermatol 105:62S-66S.
137. Groves RW, Mizutani H, Kieffer JD, Kupper TS (1995) Inflammatory skin disease in transgenic mice that express high levels of interleukin 1α in basal epidermis. Proc Natl Acad Sci USA 92:11874-11878.
138. Cheng J, Turksen K, Yu Q-C, Schreiber H, Teng M, Fuchs E (1992) Cachexia and graft-vs. host-disease-type skin changes in keratin promoter-driven TNF-α transgenic mice. Genes Dev 6:1444-1456.
139. Williams IR, Kupper TS (1994) Epidermal expression of ICAM-1 is not a primary inducer of cutaneous inflammation in transgenic mice. Proc Natl Acad Sci USA 91:9710-9714.
140. Williams IR, Ort RJ, Kupper TS (1994) Keratinocyte expression of B7-1 in transgenic mice amplifies the primary immune response to cutaneous antigens. Proc Natl Acad Sci USA 91:12780-12784.
141. Zheng H, Fletcher D, Kozak W, Jiang M, Hofmann KJ, Conn CA, Soszynski D, Grabiec C, Trumbauer ME, Shaw A, Kostura MJ, Stevens K, Rosen H, North RJ, Chen HY, Tocci MJ, Kluger MJ, Van Der Ploeg LHT (1995) Resistance to fever induction and impaired acute-phase response in interleukin-1β-deficient mice. Immunity 3:9-19.
142. Dowd PM, Camp RDR, Greaves RW (1989) Human recombinant IL-1a is proinflammatory in normal human skin. Skin Pharmacol 1:30-37.
143. Hauser C, Saurat J-H, Schmitt A, Jaunin F, Dayer J-M (1986) Interleukin 1 is present in normal human epidermis. J Immunol 136:3317-3321.
144. Kupper TS (1989) Mechanisms of cutaneous inflammation (interactions between epidermal cytokines, adhesion molecules, and leucocytes). Arch Dermatol 125:1406-1412.
145. Arend WP (1993) Interleukin-1 receptor antagonist. Adv Immunol 54:167-227.
146. Phillips WG, Feldmann M, Breathnach SM, Brennan FM (1995) Modulation of the IL-1 cytokine network in keratinocytes by intracellular IL-1α and IL-1 receptor antagonist. Clin Exp Immunol 101:177-182.
147. Kutsch CL, Norris DA, Arend WP (1993) Tumor necrosis factor-alpha induces interleukin-1 alpha and interleukin-1 receptor antagonist production by cultured human keratinocytes. J Invest Dermatol 101:79-85.
148. Groves RW, Sherman L, Mizutani H, Dower SK, Kupper TS (1994) Detection of interleukin-1 receptors in human epidermis. Induction of the type II receptor after organ culture and in psoriasis. Am J Pathol 145:1048-1056.
149. Bossù P, Visconti U, Ruggiero P, Macchia G, Muda M, Bertini R, Bizzarri C, Colagrande A, Sabbatini V, Maurizi G, Delgrosso E, Tagliabue A, Boraschi D (1995) Transfected type II interleukin-1 receptor impairs responsiveness of human keratinocytes to interleukin-1. Am J Pathol 147:1852-1861.
150. Granowitz EV, Santos A, Poutsiaka DD, Cannon JG, Wilmore DA, Wolff SM, Dinarello CA (1991) Circulating interleukin-1 receptor antagonist levels during experimental endotoxemia in humans. Lancet 338:1423-1424.
151. Watson JM, Lofquist AK, Rinehart CA, Olsen JC, Makarov SS, Kaufman DG, Haskill JS (1995) The intracellular IL-1 receptor antagonist alters IL-1-inducible gene expression without blocking exogenous signaling by IL-1β. J Immunol 155:4467-4475.

152. Orino E, Sone S, Nii A, Ogura T (1992) IL-4 up-regulates IL-1 receptor antagonist gene expression and its production in human blood monocytes. J Immunol 149:925-931.
153. Re F, Muzio M, De Rossi M, Polentarutti N, Giri JG, Mantovani A, Colotta F (1994) The type II 'receptor' as a decoy target for interleukin 1 in polymorphonuclear leucocytes: characterization of induction by dexamethasone and ligand binding properties of the released decoy receptor. J Exp Med 179:739-743.
154. Colotta F, Dower SK, Sims JE, Mantovani A (1994) The type II 'decoy' receptor: a novel regulatory pathway for interleukin 1. Immunol Today 15:562-566.
155. Dinarello CA (1989) Interleukin 1 and its biologically related cytokines. Adv Immunol 44:153-205.
156. Song Z, Casolaro V, Chen R, Georas SN, Monos D, Ono SJ (1996) Polymorphic nucleotides within the human IL-4 promoter that mediate overexpression of the gene. J Immunol 156:424-429.
157. Lee HJ, Matsuda I, Naito Y, Yokota T, Arai N, Arai K (1994) Signals and nuclear factors that regulate the expression of interleukin-4 and interleukin-5 genes in helper T cells. J Allergy Clin Immunol 94:595-604.
158. Atamas SP, Choi J, Yurovsky VV, White B (1996) An alternative splice variant of human IL-4, IL-4δ2, inhibits IL-4-stimulated T cell proliferation. J Immunol 156:435-441.
159. Paul WE, Ohara J (1987) B-cell stimulatory factor-1/interleukin-4. Annu Rev Immunol 5:429-459.
160. Swain SL, Weinberg AD, English M, Huston G (1990) IL-4 directs he development of Th2-like helper effectors. J Immunol 145:3796-3806.
161. Hart PH, Vitti GF, Burgess DR, Whitty GA, Piccoli DS, Hamilton JA (1989) Potential antiinflammatory effects of interleukin 4. Suppression of human monocyte tumor necrosis factor α, interleukin 1, and prostaglandin E_2. Proc Natl Acad Sci USA 86:3803-3807.
162. Lee JD, Swisher SG, Minehart EH, McBride WH, Economou JS (1990) Interleukin-4 downregulates interleukin 6 production in human peripheral blood mononuclear cells. J Leuk Biol 47:475-479.
163. Te Velde AA, Huijbens RJF, Heije K, De Vries JE, Figdor CG (1990) Interleukin-4 (IL-4) inhibits secretion of IL-1β, tumor necrosis factor α, and IL-6 by human monocytes. Blood 76:1392-1397.
164. Sandborg CI, Imfeld KL, Zaldivar F Jr., Wang Z, Buckingham BA, Berman MA (1995) IL-4 expression in human T cells is selectively inhibited by IL-1α and IL-1β. J Immunol 155:5206-5212.
165. Keegan AD, Nelms K, Wang L-M, Pierce JH, Paul WE (1994) Interleukin 4 receptor: signaling mechanisms. Immunol Today 15:423-432.
166. Fanslow WC, Spriggs MK, Rauch CT, Clifford KN, Macduff BM, Ziegler SF, Schooley KA, Mohler KM, March CJ, Armitage RJ (1993) Identification of a distinct low-affinity receptor for human interleukin-4 on pre-B cells. Blood 81:2998-3005.
167. Ray A, Sassone-Corsi P, Sehgal PB (1989) A multiple cytokine- and second messenger-responsive element in the enhancer of the human interleukin-6 gene: similarities with c-fos gene regulation. Mol Cell Biol 9:5537-5547.
168. May LT, Shaw JE, Khanna AK, Zabriskie JB, Sehgal PB (1991) Marked cell-type-specific differences in glycosylation of human interleukin-6. Cytokine 3:204-214.
169. Kishimoto T (1989) The biology of interleukin-6. Blood 74:1-10.
170. Schindler R, Mancilla J, Endres S, Ghorbani R, Clark SC, Dinarello CA (1990) Correlations and interactions in the production of interleukin-6 (IL-6), IL-1, and tumor necrosis factor (TNF) in human blood mononuclear cells: IL-6 suppresses IL-1 and TNF. Blood 75:40-47.
171. Tilg H, Trehu E, Atkins MB, Dinarello CA, Mier JW (1994) Interleukin-6 (IL-6) as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55. Blood 83:113-118.
172. Hibi M, Murakami M, Saito M, Hirano T, Taga T, Kishimoto T (1990) Molecular cloning and expression of an IL-6 signal transducer, gp130. Cell 63:1149-1157.
173. Gearing DP, Cosman D (1991) Homology of the p40 subunit of natural killer cell stimulatory factor (NKSF) with the extracellular domain of the interleukin-6 receptor. Cell 66:9-10.

174. Narazaki M, Yasukawa K, Saito T, Ohsugi Y, Fukui H, Koishihara Y, Yancopoulos GD, Taga T, Kishimoto T (1993) Blood 82:1120-1126.
175. Furie MB, Randolph GJ (1995) Chemokines and tissue injury. Am J Pathol 146:1287-1301.
176. Colotta F, Orlando S, Fadlon EJ, Sozzani S, Matteucci C, Mantovani A (1995) Chemoattractants induce rapid release of the interleukin 1 type II decoy receptor in human polymorphonuclear cells. J Exp Med 181:2181-2188.
177. La Rosa GJ, Thomas KM, Kaufmann ME, Mark R, White M, Taylor L, Gray G, Witt D, Navarro J (1992) Amino terminus of the interleukin-8 receptor is a major determinant of receptor subtype specificity. J Biol Chem 267:25402-25406.
178. English K, Weaver WM, Wilson CB (1991) Differential regulation of lymphotoxin and tumor necrosis factor genes in human T lymphocytes. J Biol Chem 266:7108-7113.
179. Gearing AJH, Beckett P, Christodoulou M, Churchill M, Clements J, Davidson AH, Drummond AH, Galloway WA, Gilbert R, Gordon JL, Leber TM, Mangan M, Miller K, Nayee P, Owen K, Patel S, Thomas W, Wells G, Wood LM, Woolley K (1994) Processing of tumour necrosis factor-alpha precursor by metalloproteinases. Nature 370:555-557.
180. Giri JG, Wells J, Dower SK, McCall CE, Guzman RN, Slack J, Bird TA, Shanebeck K, Grabstein KH, Sims JE, Alderson MR (1994) Elevated levels of shed type II IL-1 receptor in sepsis. Potential role for type II receptor in regulation of IL-1 responses. J Immunol 153:5802-5809.
181. Tartaglia LA, Goeddel DV (1992) Two TNF receptors. Immunol Today 13:151-153.
182. Kristensen M, Chu CO, Eedy DJ, Feldmann M, Brennan FM, Breathnach SM (1993) Localization of tumor necrosis factor-alpha (TNF-alpha) and its receptors in normal and psoriatic skin: Epidermal cells express the 55-kD but not the 75-kD TNF receptor. Clin Exp Immunol 94:354-362.
183. Holtmann H, Wallach D (1987) Down regulation of the receptors for tumor necrosis factor by interleukin 1 and 4 beta-phorbol-12-myristate-13-acetate. J Immunol 139:1161-1167.
184. Taylor DJ (1994) Interleukin-4 (IL-4) induces down-modulation and shedding of the p55 tumour necrosis factor receptor and inhibits TNF alpha's effect on rheumatoid synovial fibroblasts. Rheumatol Int 14:21-25.
185. Bos JD, Kapsenberg ML (1993) The skin immune system: progress in cutaneous biology. Immunol Today 14:75-78.
186. Uitto J, Oikarinen A, Thody AJ (1986) Mechanical and physical functions of skin. In Thody AJ, Friedmann PS (eds). Scientific basis of dermatology (a physiological approach). Churchill Livingstone Publ, London, pp. 6-25.
187. Bata-Csorgo Z, Hammerberg C, Voorhees JJ, Cooper KD (1993) Flow cytometric identification of proliferative subpopulations within normal human epidermis and the localization of the primary hyperproliferative population in psoriasis. J Exp Med 178:1271-1281.
188. Haake AR, Polakowska RR (1993) Cell death by apoptosis in epidermal biology. J Invest Dermatol 101:107-112.
189. Norris DA (1995) Differential control of cell death in the skin. Arch Dermatol 131:945-948.
190. Nickoloff BJ, Turka LA, Mitra RS, Nestle FO (1995) Direct and indirect control of T-cell activation by keratinocytes. J Invest Dermatol 105:25S-29S.
191. Nickoloff BJ, Basham TY, Torseth J, Merigan TC, Morhenn VB (1986) Human keratinocyte-lymphocyte-reactions *in vitro*. J Invest Dermatol 87:11-18.
192. Nickoloff BJ, Mitra RS, Green J, Zheng XG, Shimuzu Y, Thompson C, Turka LA (1993) Accessory cell function of keratinocytes for superantigens. J Immunol 150:2148-2159.
193. Fleming TE, Mirando WS, Trefzer U, Tubesing KA, Elmets CA (1993) In situ expression of a B7-like adhesion molecule on keratinocytes from human epidermis. J Invest Dermatol 101:754-758.
194. Nickoloff BJ, Turka LA (1994) Immunological functions of non-professional antigen-presenting cells: new insights from studies of T-cell interactions with keratinocytes. Immunol Today 15:464-469.

195. June CH, Ledbetter JA, Linsley PA, Thompson CB (1990) Role of the CD28 receptor in T-cell activation. Immunol Today 11:211-216.
196. Tan P, Anasetti C, Hansen JA, Melrose J, Brunvand M, Bradshaw J, Ledbetter JA, Linsley PA (1993) Induction of alloantigen-specific hyporesponsiveness in human T lymphocytes by blocking interaction of CD28 with its natural ligand B7/BB-1. J Exp Med 177:165-170.
197. Bal V, McIndoe A, Denton G, Hudson D, Lombardi G, Lamb J, Lechler R (1990) Antigen presentation of keratinocytes induces tolerance in human T cells. Eur J Immunol 20:1893-1897.
198. Seder RA, Gazzinelli R, Sher A, Paul WE (1993) Interleukin 12 acts directly on CD4+ T cells to enhance interferon gamma production and diminishes interleukin 4 inhibition of such priming. Proc Natl Acad Sci USA 90:10188-10192.
199. Goodman RE, Nestle F, Naidu Y, Green J, Thompson C, Nickoloff BJ, Turka LA (1994) Keratinocyte-derived T cell costimulation induces preferential production of IL-1 and IL-4 but not IFN-γ. J Immunol 152:5189-5198.
200. Hunter CA, Chizzonite R, Remington JS (1995) IL-1β is required for IL-12 to induce production of IFN-γ by NK cells. A role for IL-1β in the T cell-independent mechanism of resistance against intracellular pathogens. J Immunol 155:4347-4354.
201. O'Garra A, Murphy K (1994) Role of cytokines in determining T-lymphocyte function. Curr Opin Immunol 6:458-466.
202. Manetti R, Barak V, Piccinni M-P, Sampognaro S, Parronchi P, Maggi E, Dinarello CA, Romagnani S (1994) Interleukin-1 favours the *in vitro* development of type 2 T helper (Th2) human T-cell clones. Res Immunol 145:93-100.
203. Nestle FO, Turka LA, Nickoloff BJ (1994) Characterization of dermal dendritic cells in psoriasis: autostimulation of T lymphocytes and induction of TH-1 type cytokines. J Clin Invest 94:202-209.
204. Baden HP, Kubilus J, Macdonald MJ (1981) Normal and psoriatic keratinocytes and fibroblasts compared in culture. J Invest Dermatol 76:53-55.
205. Karasek MA (1972) Dermal factors affecting epidermal cells in vitro. J Invest Dermatol 59:99-101.
206. Mauviel A, Temime M, Charron D, Loyau G, Pujol J-P (1991) Induction of interleukin-1β production in human dermal fibroblasts by interleukin-1α and tumor necrosis factor-α. Involvement of protein kinase-dependent and adenylate cyclase-dependent regulatory pathways. J Cell Biochem 47:174-183.
207. Van Damme J, Cayphas S, Opdenakker G, Billiau A, Van Snick J (1987) Interleukin 1 and poly(rI)·poly(rC) induce production of a hybridoma growth factor by human fibroblasts. Eur J Immunol 17:1-7.
208. Larsen CG, Anderson AO, Oppenheim JJ, Matsushima K (1989) Production of interleukin-8 by human dermal fibroblasts and keratinocytes in response to interleukin-1 or tumour necrosis factor. Immunology 68:31-36.
209. Ristow HJ (1987) A major factor contributing to epidermal proliferation in inflammatory skin diseases appears to be interleukin 1 or a related protein. Proc Natl Acad Sci USA 84:1940-1944.
210. Grossman RM, Krueger J, Yourish D, Granelli-Piperno A, Murphy DP, May LT, Kupper TS, Sehgal PB, Gottlieb AB (1989) Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proc Natl Acad Sci USA 86:6367-6371.
211. Tuschil A, Lam C, Halsberger A, Lindley I (1992) Interleukin-8 stimulates calcium transients and promotes epidermal cell proliferation. J Invest Dermatol 99:294-298.
212. Jordana M, Sarnstrand B, Sime PJ, Ramis I (1994) Immune-inflammatory functions of fibroblasts. Eur Resp J 7:2212--2222.
213. Pakianathan DR (1995) Extracellular matrix proteins and leucocyte function. J Leuk Biol 57:699-702.
214. St.-Louis JD, Lederer JA, Lichtman AH (1993) Costimulator deficient antigen presentation by an endothelial cell line induces a non-proliferative T-cell activation response without anergy. J Exp Med 178:1597-1605.

215. Pawlowski T, Elliott JD, Loh DY, Staerz UD (1993) Positive selection of T lymphocytes on fibroblasts. Nature 364:642-645.
216. Singer TA (1994) Traffic signals for lymphocyte recirculation and leucocyte emigration: the multistep paradigm. Cell 76:301-314.
217. Stingl G, Wolff-Schreiner EC, Pichler EJ, Gschnait F, Knapp W, Wolff K (1977) Epidermal Langerhans cells bear Fc and C3 receptors. Nature 268:245-246.
218. Sontheimer RD (1985) The mixed epidermal cell-lymphocyte reaction. II. Epidermal Langerhans cells are responsible for the enhanced allogeneic lymphocyte-stimulating capacity of normal human epidermal cell suspensions. J Invest Dermatol 85:21S-25S.
219. Ibrahim MAA, Chain BM, Katz DR (1995) The injured cell: the role of the dendritic cell system as a sentinel receptor pathway. Immunol Today 16:181-186.
220. Steinman R, Hoffman L, Pope M (1995) Maturation and migration of cutaneous dendritic cells. J Invest Dermatol 105:2S-7S.
221. Cumberbatch M, Fielding I, Kimber I (1994) Modulation of epidermal Langerhans' cell frequency by tumour necrosis factor-alpha. Immunology 81:395-401.
222. Nickoloff BJ, Griffiths CEM (1990) Abnormal cutaneous topobiology: The molecular basis for dermatopathologic mononuclear cell patterns in inflammatory skin diseases. J Invest Dermatol 95:128S-131S.
223. Picker LJ, Treer JR, Ferguson-Darnell B, Collins PA, Bergstresser PR, Terstappen LW (1993)' Control of lymphocyte recirculation in man. II. Differential regulation of the cutaneous lymphocyte-associated antigen, a tissue-selective homing receptor for skin-homing T cells. J Immunol 150:1122-1136.
224. Wayner EA, Gil SG, Murphy GF, Wilke MS, Carter WG (1993) Epiligrin, a component of epithelial basement membranes, is an adhesive ligand for alpha 3 beta 1 positive T lymphocytes. J Cell Biol 121:1141-1152.
225. MacKay CR, Marston WL, Dudler L, Spertini O, Tedder TF, Hein WR (1992) Tissue-specific migration pathways by phenotypically distinct subpopulations of memory T cells. Eur J Immunol 22:887-895.
226. Menssen A, Trommler P, Vollmer S, Schendel D, Albert E, Gürtler L, Riethmüller G, Prinz JC (1995) Evidence for an antigen-specific cellular immune response in skin lesions of patients with psoriasis vulgaris. J Immunol 155: 4078-4083.
227. Kupper TS (1990) The activated keratinocyte. A model for inducible cytokine production by non-bone marrow derived cells in cutaneous inflammatory and immune responses. J Invest Dermatol 94:146S-150S.
228. Molfino NA, Wright SC, Katz I, Tarlo S, Silverman F, McClean PA, Szalai JP, Raizenne M, Slutsky AS, Zamel N (1991) Effects of low concentrations of ozone on inhaled allergen responses in asthmatic subjects. Lancet 338:199-203.
229. Dustin ML, Rothlein R, Bhan AK, Dinarello CA, Springer TA (1986) Induction by IL-1 and interferon-gamma: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). J Immunol 137:245-254.
230. Groves RW, Ross E, Barker JNWN, Ross J, Camp RDR, MacDonald DM (1992) Effect of in vivo interleukin 1 on adhesion molecule expression in normal human skin. J Invest Dermatol 98:384-387.
231. Schröder JM (1995) Inflammatory mediators and chemoattractants. Clin Dermatol 13:137-150.
232. Yabkowitz R, Dixit VM, Guo N, Roberts DD, Shimizu Y (1993) Activated T-cell adhesion to thrombospondin is mediated by the alpha 4 beta 1 (VLA-4) and alpha 5 beta 1 (VLA-5) integrins. J Immunol 151:149-158.
233. Grubauer G, Romani N, Kofler H, Stanzl U, Fritsch P, Hintner H (1986) Apoptotic keratin bodies as autoantigen causing the production of IgM-anti-keratin intermediate filament autoantibodies. J Invest Dermatol 87:466-471.
234. Rosen A, Casciola-Rosen L, Ahearn J (1995) Novel packages of viral and self-antigens are generated during apoptosis. J Exp Med 181:1557-1561.
235. Casciola-Rosen LA, Anhalt G, Rosen A (1994) Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic

keratinocytes. J Exp Med 179:1317-1330.
236. Krueger JG, Krane JF, Carter DM, Gottlieb AB (1990) Role of growth factors, cytokines and their receptors in the pathogenesis of psoriasis. J Invest Dermatol 94:135S-140S.
237. Barker JN, Goodlad JR, Ross EL, Yu CC, Groves RW, MacDonald DM (1993) Increased epidermal cell proliferation in normal human skin in vivo following local administration of interferon-gamma. Am J Pathol 142:1091-1097.
238. Standiford TJ, Strieter RM, Chensue SW, Westwick J, Kasahara K, Kunkel SL (1990) IL-4 inhibits the expression of IL-8 by stimulated human monocytes. J Immunol 145:1435-1439.
239. Ghezzi P, Dinarello CA (1988) IL-1 induces IL-1. III. Specific inhibition of IL-1 production by IFN-gamma. J Immunol 140:4238-4244.
240. Sayama K, Yonehara S, Watanabe Y, Miki Y (1994) Expression of Fas antigen on keratinocytes *in vivo* and induction of apoptosis in cultured keratinocytes. J Invest Dermatol 103:330-334.
241. Nagata S, Suda T (1995) Fas and Fas ligand: lpr and gld mutations. Immunol Today 16:39-43.
242. Mangan DF, Robertson B, Wahl SM (1992) IL-4 enhances programmed cell death (apoptosis) in stimulated human monocytes. J Immunol 148:1812-1816.
243. Mangan DF, Welch GR, Wahl SM (1991) Lipopolysaccharide, tumor necrosis factor-α, and IL-1β prevent programmed cell death (apoptosis) in human peripheral blood monocytes. J Immunol 146:1541-1546.
244. Haanen C, Vermes I (1995) Apoptosis and inflammation. Med Inflamm 4:5-15.
245. Ren Y, Savill J (1995) Proinflammatory cytokines potentiate thrombospondin-mediated phagocytosis of neutrophils undergoing apoptosis. J Immunol 154: 2366-2374.
246. Eedy DJ, Burrows D, Bridges JM, Jones FCC (1990) Clearance of severe psoriasis after allogeneic bone marrow transplantation. Br Med J 300:908-909.
247. Gardembas-Pain M, Ifrah N, Foussard C, Boasson M, Saint Andre JP, Verret JL (1990) Psoriasis after allogeneic bone marrow transplantation. Arch Dermatol 126:1523-1525.
248. Christophers E, Parzefall R, Braun-Falco O (1973) Initial events in psoriasis: quantitative assessment. Br J Dermatol 89:327-334.
249. Baadsgaard O, Fisher G, Voorhees JJ, Cooper KD (1990) The role of the immune system in the pathogenesis of psoriasis. J Invest Dermatol 95:32S-34S.
250. Elder JT, Henseler T, Christophers E, Voorhees JJ, Nair RP (1994) Of genes and antigenes: the inheritance of psoriasis. J Invest Dermatol 103:150S-153S.
251. Rosenberg EW, Noel PW (1988) The Koebner phenomenon and the microbial basis of psoriasis. J Am Acad Dermatol 18:151-158.
252. Mahoney SE, Duvic M, Nickoloff BJ, Minshall M, Smith LC, Griffiths CE, Paddock SW, Lewis DE (1991) Human immunodeficiency virus (HIV) transcripts identified in HIV-related psoriasis and Kaposi's sarcoma lesions. J Clin Invest 88:174-185.
253. Lee RE, Gaspari AA, Lotze MT, Chang AE, Rosenberg SA (1988) Interleukin 2 and psoriasis. Arch Dermatol 124:1811-1815.
254. Fierlbeck G, Rassner G, Muller C (1990) Psoriasis induced at the injection site of recombinant interferon gamma. Results of immunohistologic investigations. Arch Dermatol 126:351-355.
255. Wernschenker BG, Bass BH, Ebers G (1989) Remission of psoriatic lesions with muromab-CD3 (Orthoclone OKT3) treatment. J Am Acad Dermatol 20:1132-1133.
256. Prinz JC, Braun-Falco O, Meurer M, Daddona P, Reiter C, Rieber EP, Riethmüller G (1991) Chimeric CD4 monoclonal antibody in the treatment of generalized pustular psoriasis. Lancet 338:320-321.
257. Gottlieb SL, Gilleaudeau P, Johnson R, Estes L, Woodworth TG, Gottlieb AB, Krueger JG (1995) Response of psoriasis to a lymphocyte-selective toxin (DAB_{389}IL-2) suggests a primary immune, but not keratinocyte, pathogenic basis. Nature Med 1:442-447.
258. Baadsgaard O, Tong P, Elder JT, Hansen ER, Ho V, Hammerberg C, Lange-Vejlsgaard G, Fox DA, Fisher G, Chan LS, Voorhees JJ, Cooper KD (1990) UM4D4+ (CDw60) T cells are compartmentalized into psoriatic skin and release lymphokines that induce a keratino-

cyte phenotype expressed in psoriatic lesions. J Invest Dermatol 95:275-282.
259. Strange P, Cooper KD, Hansen ER, Fisher G, Larsen JK, Fox DA, Krag C, Voorhees JJ, Baadsgaard O (1993) T-lymphocyte clones initiated from lesional psoriatic skin release growth factors that induce keratinocyte proliferation. J Invest Dermatol 101:695-700.
260. Prinz JC, Groß B, Vollmer S, Trommler P, Strobel I, Meurer M, Plewig G (1994) T cell clones from psoriatic skin lesions can promote keratinocyte proliferation *in vitro* via secreted products. Eur J Immunol 24:593-598.
261. Chang EY, Hammerberg C, Fisher G, Baadsgaard O, Ellis CN, Voorhees JJ, Cooper KD (1992) T-cell activation is potentiated by cytokines released by lesional psoriatic, but not normal, epidermis. Arch Dermatol 128:1479-1485.
262. Prens EP, Debets R, Hegmans J (1995) T lymphocytes in psoriasis. Clin Dermatol 13:115-129.
263. Sundberg JP, Boggess D, Sundberg BA, Beamer WG, Schultz LD (1993) Epidermal dendritic cell populations in the flaky skin mutant mouse. Immunol Invest 22:389-401.
264. Morita K, Hogan ME, Nanney LB, King LE Jr., Manabe M, Sun TT, Sundberg JP (1995) Cutaneous ultrastructural features of the flaky skin (fsn) mouse mutation. J Dermatol 22:385-395.
265. Carroll JM, Romero MR, Watt FW (1995) Suprabasal integrin expression in the epidermis of transgenic mice results in developmental defects and a phenotype resembling psoriasis. Cell 83:957-968.
266. Dominey AM, Wang XJ, King LE Jr., Nanney LB, Gagne TA, Sellheyer K, Bundman DS, Longley MA, Rothnagel JA, Greenhalgh DA, Roop DR (1993) Targeted overexpression of transforming growth factor alpha in the epidermis of transgenic mice elicits hyperplasia, hyperkeratosis, and spontaneous, squamous papillomas. Cell Growth Diff 4:1071-1082.
267. Guo L, Yu QC, Fuchs E (1993) Targeting expression of keratinocyte growth factor to keratinocytes elicits striking changes in epithelial differentiation in transgenic mice. EMBO J 12:973-986.
268. Turksen K, Kupper T, Degenstein L, Williams I, Fuchs E (1992) Interleukin-6: insights to its function in skin by overexpression in transgenic mice. Proc Natl Acad Sci USA 89:5068-5072.
269. Werner S, Smola H, Liao X, Longaker MT, Krieg T, Hofschneider PH, Williams LT (1994) The function of KGF in morphogenesis of epithelium and reepithelialization of wounds. Science 266:819-822.
270. Werner S, Weinberg W, Liau X, Peters KG, Blessing M, Yuspa SH, Weiner RL, Williams LT (1993) Targeted expression of a dominant-negative FGF receptor mutant in the epidermis of transgenic mice reveals a role of FGF in keratinocyte organization and differentiation. EMBO J 12:2635-2643.
271. Groves RW, Williams IR, Kupper TS (1995) Overexpression of keratinocyte type 1 IL-1 receptor in transgenic mice results in exaggerated IL-1 dependent cutaneous inflammation in vivo. J Invest Dermatol 105:453. (Abstr.)
272. Van Ruissen F, Van De Kerkhof PCM, Schalkwijk J (1995) Signal transduction pathways in epidermal proliferation and cutaneous inflammation. Clin Dermatol 13:161-190.
273. Schubert C, Christophers E (1985) Mast cells and macrophages in early relapsing psoriasis. Arch Dermatol Res 277:352-358.
274. Pincelli C, Fantini F, Romualdi P, Sevignani C, Lesa G, Benassi L, Giannetti A (1992) Substance P is diminished and vasoactive intestinal polypeptide is augmented in psoriatic lesions and these peptides exert disparate effects on the proliferation of cultured human keratinocytes. J Invest Dermatol 98:421-427.
275. Farber EM, Nickoloff BJ, Recht B, Fräki JE (1986) Stress, symmetry, and psoriasis: possible role of neuropeptides. J Am Acad Dermatol 14:305-311.
276. McKay IA, Leigh IM (1995) Altered keratinocyte growth and differentiation in psoriasis. Clin Dermatol 13:105-114.
277. Mansbridge JN, Knapp AM (1987) Changes in keratinocyte maturation during wound healing. J Invest Dermatol 89:253-263.
278. Kellner I, Konter U, Sterry W (1991) Overexpression of extracellular matrix receptors

(VLA-3, 5 and 6) on psoriatic keratinocytes. Br J Dermatol 125:211-216.
279. Nickoloff BJ, Mitra RS, Lee K, Turka LA, Green J, Thompson C, Shimizu Y (1993) Discordant expression of CD28 ligands, B8-1, and B7 on keratinocytes in vitro and psoriatic cells in vivo. Am J Pathol 142:1029-1040.
280. Schalkwijk J, Van Vlijmen IM, Alkemade JA, De Jongh GJ (1993) Immunohistochemical localization of SKALP/elafin in psoriatic epidermis. J Invest Dermatol 100:390-393.
281. Lontz W, Sirsjo A, Liu W, Lindberg M, Rollman O, Torma H (1995) Increased mRNA expression of manganese superoxide dismutase in psoriasis skin lesions and in cultured human keratinocytes exposed to IL-1 beta and TNF-alpha. Free Radic Biol Med 18:349-355.
282. Gottlieb AB, Lifshitz B, Fu SM, Siaiano-Coico L, Wang CY, Carter DM (1986) Expression of HLA-DR molecules by keratinocytes and the presence of Langerhans cells in the dermal infiltrate of active psoriatic plaques. J Exp Med 164:1013-1028.
283. Griffiths CEM, Voorhees JJ, Nickoloff BJ (1989) Characterization of intercellular adhesion molecule-1 and HLA-DR expression in normal and inflamed skin: modulation by recombinant gamma interferon and tumor necrosis factor. J Am Acad Dermatol 20:617-629.
284. Gottlieb AB, Luster AD, Posnett DN, Carter DM (1988) Detection of a (gamma) interferon induced protein γIP-10 in psoriatic plaques. J Exp Med 168:941-948.
285. Barker JNWN, Groves RW, Allen MH, MacDonald DM (1992) Preferential adherence of T-lymphocytes and neutrophils to psoriatic epidermis. Br J Dermatol 127:205-211.
286. Bianchi L, Farrace MG, Nini G, Piacentini M (1994) Abnormal Bcl-2 and 'tissue' transglutaminase expression in psoriatic skin. J Invest Dermatol 103:829-833.
287. Wrone-Smith T, Johnson T, Nelson B, Boise LH, Thompson CB, Núñez G, Nickoloff BJ (1995) Discordant expression of Bcl-x and Bcl-2 by keratinocytes *in vitro* and psoriatic keratinocytes *in vivo*. Am J Pathol 146:1079-1088.
288. Gillitzer R, Berger R, Mielke V, Müller C, Wolff K, Stingl G (1991) Upper keratinocytes of psoriatic lesions express high levels of NAP-1/IL-8 mRNA in situ. J Invest Dermatol 97:73-79.
289. Gillitzer R, Wolff K, Tong D, Müller C, Yoshimura T, Hartmann AA, Stingl G, Berger R (1993) MCP-1 mRNA expression in basal keratinocytes of psoriatic lesions. J Invest Dermatol 101:127-131.
290. Kojima T, Cromie MA, Fisher GJ, Voorhees JJ, Elder JT (1993) Gro-alpha mRNA is selectively overexpressed in psoriatic epidermis and is reduced by cyclosporin A in vivo, but not in cultured keratinocytes. J Invest Dermatol 101:767-772.
291. Elder JT, Fisher GJ, Lindquist PB, Bennett GL, Pittelkow MR, Coffey RJ Jr., Ellingsworth L, Derynck R, Voorhees JJ (1989) Overexpression of transforming growth factor-alpha in psoriatic epidermis. Science 243:811-814.
292. Detmar M, Brown LF, Claffey KP, Yeo K-T, Kocher O, Jackman RW, Berse B, Dvorak HF (1994) Overexpression of vascular permeability factor/Vascular endothelial growth factor and its receptors in psoriasis. J Exp Med 180:1141-1146.
293. Ohta Y, Katayama I, Funato T, Yokozeki H, Nishiyama S, Hirano T, Kishimoto T, Nishioka K (1991) In situ expression of messenger RNA of interleukin-1 and interleukin-6 in psoriasis: interleukin-6 involved in formation of psoriatic lesions. Arch Dermatol Res 283:351-356.
294. Nanney LB, Stoscheck CM, Magid M, King LE Jr. (1986) Altered epidermal growth factor binding and receptor distribution in psoriasis. J Invest Dermatol 86:260-265.
295. Krane JF, Gottlieb AB, Carter DM, Krueger JG (1992) The insulin-like growth factor I receptor is overexpressed in psoriatic epidermis, but is differentially regulated from the epidermal growth factor receptor. J Exp Med 175:1081-1090.
296. Scheynius A, Fransson J, Johansson C, Hammar H, Baker B, Fry L, Valdimarsson H (1992) Expression of interferon-gamma receptors in normal and psoriatic skin. J Invest Dermatol 98:255-258.
297. Schulz BS, Michel G, Wagner S, Süss R, Beetz A, Peter RU, Kemény L, Ruzicka T (1993) Increased expression of epidermal IL-8 receptor in psoriasis. Down-regulation by FK-506 in vitro. J Immunol 151:4399-4406.

298. Baker BS, Powles AV, Valdimarsson H, Fry L (1988) An altered response by psoriatic keratinocytes to gamma interferon. Scand J Immunol 28:735-740.
299. Nickoloff BJ, Mitra RS, Elder JT, Fisher GJ, Voorhees JJ (1989) Decreased growth inhibition by recombinant gamma interferon is associated with increased transforming growth factor-alpha production in keratinocytes cultured from psoriatic lesions. Br J Dermatol 121:161-174.
300. Olaniran AK, Baker BS, Garioch JJ, Powles AV, Fry L (1995) A comparison of the stimulatory effects of cytokines on normal and psoriatic keratinocytes in vitro. Arch Dermatol Res 287:231-236.
301. Nickoloff BJ, Mitra RS, Varani J, Dixit VM, Polverini PJ (1994) Aberrant production of interleukin-8 and thrombospondin-1 by psoriatic keratinocytes mediates angiogenesis. Am J Pathol 144:820-828.
302. Fyrand O (1979) Studies on fibronectin in the skin. II. Indirect immunofluorescence studies in psoriasis vulgaris. Arch Dermatol Res 266:33-41.
303. Schalkwijk J, Van Vlijmen I, Oosterling B, Perret C, Koopman R, Van Den Born J, Mackie EJ (1991) Tenascin expression in hyperproliferative skin diseases. Br J Dermatol 124:13-20.
304. Er-raki A, Charveron M, Bonafe JL (1993) Increased superoxide anion production in dermal fibroblasts of psoriatic patients. Skin Pharmacol 6:253-258.
305. Espinoza LR, Espinoza CG, Cuéllar ML, Scopelitis E, Silveira LH, Grotendorst GR (1994) Fibroblast function in psoriatic arthritis. II. Increased expression of β platelet derived growth factor receptors and increased production of growth factor and cytokines. J Rheumatol 21:1507-1514.
306. Raynaud F, Bauvois B, Gerbaud P, Evain-Brion D (1992) Characterization of specific proteases associated with the surface of human skin fibroblasts, and their modulation in pathology. J Cell Physiol 151:378-385.
307. Espinoza LR, Aguilar JL, Espinoza CG, Cuéllar ML, Scopelitis E, Silveira LH (1994) Fibroblast function in psoriatic arthritis. I. Alteration of cell kinetics and growth factor responses. J Rheumatol 21:1502-1506.
308. Priestley GC, Adams LW (1985) Mitogenic effects of sera from normal and psoriatic subjects on human skin fibroblasts. Arch Dermatol Res 277:13-15.
309. MacLaughlin JA, Gange W, Taylor D, Smith E, Holick MF (1985) Cultured psoriatic fibroblasts from involved and uninvolved sites have a partial but not absolute resistance to the proliferation-inhibition activity of 1,25-dihydroxyvitamin D3. Proc Natl Acad Sci USA 82:5409-5412.
310. Elder JT, Sartor CI, Boman DK, Benrazavi S, Fisher GJ, Pittelkow MR (1992) Interleukin-6 in psoriasis: expression and mitogenicity studies. Arch Dermatol Res 284:324-332.
311. Prens EP, Benne K, Van Damme J, Bakkus M, Brakel K, Benner R, Van Joost T (1990) Interleukin-1 and interleukin-6 in psoriasis. J Invest Dermatol 95:121S-124S.
312. Krane JF, Murphy DP, Gottlieb AB, Carter DM, Hart CE, Krueger JG (1991) Increased dermal expression of platelet-derived growth factor receptors in growth activated skin wounds and psoriasis. J Invest Dermatol 96:983-986.
313. Gu XF, Raynaud F, Evain-Brion D (1988) Increased chemotactic and mitogenic response of psoriatic fibroblasts to platelet-derived growth factor. J Invest Dermatol 91:599-602.
314. Fräki JE, Briggaman RA, Lazarus GS (1983) Transplantation of psoriatic skin onto nude mouse. J Invest Dermatol 80:31S-35S.
315. Nickoloff BJ, Kunkel SL, Burdick M, Strieter RM (1995) Severe combined immunodeficiency mouse and human psoriatic skin chimeras. Validation of a new animal model. Am J Pathol 146:580-588.
316. Sundberg JP, Dunstan RW, Roop DR, Beamer WG (1994) Full-thickness skin grafts from flaky skin mice to nude mice: maintenance of the psoriasiform phenotype. J Invest Dermatol 102:781-788.
317. Saiag P, Coulomb B, Lebreton C, Bell E, Dubertret L (1985) Psoriatic fibroblasts induce hyperproliferation of normal keratinocytes in a skin equivalent model in vitro. Science 230:669-672.

318. Lowe PM, Lee ML, Jackson CJ, To SS, Cooper AJ, Schrieber L (1995) The endothelium in psoriasis. Br J Dermatol 132:497-505.
319. Braverman IM, Keh-Yen A (1986) Three-dimensional reconstruction of endothelial cell gaps in psoriatic vessels and their morphologic identity with gaps produced by the intradermal injection with histamine. J Invest Dermatol 86:577-581.
320. Malhotra R, Stenn KS, Fernandez LA, Braverman IM (1989) The angiogenic properties of normal and psoriatic skin associate with the epidermis - not the dermis. Lab invest 61:162-165.
321. Chin YH, Falanga V, Taylor J, Cai JP, Bax J (1990) Adhesion of human helper/memory T cell subsets to psoriatic dermal endothelium: Mechanisms and modulation. J Invest Dermatol 94:413-417.
322. Uyemura K, Yamamura M, Fivenson DP, Modlin RL, Nickoloff BJ (1993) The cytokine network in lesional and lesion-free psoriatic skin is characterized by a T-helper type 1 cell-mediated response. J Invest Dermatol 101:701-705.
323. Groves RW, Allen MH, Barker JNWN, Haskard DO, MacDonald DM (1991) Endothelial leukocyte adhesion molecule-1 (ELAM-1) expression in cutaneous inflammation. Br J Dermatol 124:117-123.
324. Groves RW, Ross EL, Barker JN, MacDonald DM (1993) Vascular cell adhesion molecule-1: Expression in normal and diseased skin and regulation in vivo by interferon gamma. J Am Acad Dermatol 29:62-72.
325. Westphal JR, Willems HW, Schalkwijk CJ, Ruiter DJ, De Waal RM (1993) A new 180-kDa dermal endothelial cell activation antigen: In vitro and in situ characteristics. J Invest Dermatol 100:27-34.
326. Cai JP, Falaga V, Taylor JR, Chin YH (1992) Transforming growth factor-beta differentially regulates the adhesiveness of normal and psoriatic dermal microvascular endothelial cells for peripheral blood mononuclear cells. J Invest Dermatol 98:405-409.
327. Baadsgaard O, Gupta AK, Taylor RS, Ellis CN, Voorhees JJ, Cooper KD (1989) Psoriatic epidermal cells demonstrate increased numbers and function of non-Langerhans antigen presenting cells. J Invest Dermatol 92:190-195.
328. Prens EP, Benne SK, Van Joost T, Benner R (1991) The autologous mixed epidermal cell-T lymphocyte reaction is elevated in psoriasis: a crucial role for epidermal HLA-DR+/CD1a- antigen-presenting cells. J Invest Dermatol 96:880-887.
329. Prens E, 't Hooft-Benne K, Tank B, Van Damme J, Van Joost T, Benner R (1996) Adhesion molecules and IL-1 costimulate T lymphocytes in the autologous MECLR in psoriasis. Arch Dermatol Res, in press.
330. Koszik F, Strunk D, Simonitsch I, Picker LJ, Stingl G, Payer E (1994) Expression of monoclonal antibody HECA-452 defined E-selectin ligands on Langerhans cells in normal and diseased skin. J Invest Dermatol 102:773-780.
331. Cerio R, Griffiths CEM, Cooper KD, Nickoloff BJ, Headington JT (1989) Characterization of factor XIIIa positive dermal dendritic cells obtained from normal and inflamed skin. Br J Dermatol 121:421-431.
332. Morganroth GS, Chan LS, Weinstein GD, Voorhees JJ, Cooper KD (1991) Proliferating cells in psoriatic dermis are comprised primarily of T cells, endothelial cells, and factor XIIIa+ perivascular dendritic cells. J Invest Dermatol 96:333-340.
333. Van Den Oord JJ, De Wolf-Peeters C (1994) Epithelium-lining macrophages in psoriasis. Br J Dermatol 130:589-594.
334. Nickoloff BJ, Karabin GD, Barker JNWN, Griffiths CEM, Sarma V, Mitra RS, Elder JT, Kunkel SL, Dixit VM (1991) Cellular localization of interleukin-8 and its inducer, tumor necrosis factor-alpha in psoriasis. Am J Pathol 138:129-140.
335. Neuner P, Urbanski A, Trautinger F, Möller A, Kirnbauer R, Kapp A, Schöpf E, Schwarz T, Luger TA (1991) Increased IL-6 production by monocytes and keratinocytes in patients with psoriasis. J Invest Dermatol 97:27-33.
336. Pigatto PD, Pigatto LB, Bigardi A, Altomare G, Finzi AF (1990) Factors secreted by untreated psoriatic monocytes enhance neutrophil functions. J Invest Dermatol 94:372-376.

337. Baker BS, Swain AF, Fry L, Valdimarsson H (1984) Epidermal T lymphocytes and HLA-DR expression in psoriasis. Br J Dermatol 110:555-564.
338. Bos JD, Hulsebosch HJ, Krieg SR, Bakker PM, Cormane RH (1983) Immunocompetent cells in psoriasis: in situ immunophenotyping by monoclonal antibodies. Arch Dermatol Res 275:181-189.
339. Picker LJ, Michie SA, Rott LS, Butcher E (1990) A unique phenotype of skin-associated lymphocytes in humans. Preferential expression of the HECA-452 epitope by benign and malignant T cells at cutaneous sites. Am J Pathol 136:1053-1068.
340. Valdimarsson H, Baker BS, Jónsdóttir I, Fry L (1986) Psoriasis: a disease of abnormal keratinocyte proliferation induced by T lymphocytes. Immunol Today 7:256-259.
341. Bos JD, Hagenaars C, Das PK, Krieg SR, Voorn WJ, Kapsenberg LM (1989) Predominance of 'memory' T cells (CD4+, CDw29+) over 'naive' T cells (CD4+, CD45R+) in both normal and diseased human skin. Arch Dermatol Res 281:24-30.
342. Lewis HM, Baker BS, Bokth S, Powles AV, Garioch JJ, Valdimarsson H, Fry L (1993) Restricted T-cell receptor V beta gene usage in the skin of patients with guttate and chronic plaque psoriasis. Br J Dermatol 129:514-520.
343. Chang JCC, Smith LR, Froning KJ, Schwabe BJ, Laxer JA, Caralli LL, Kurland HH, Karasek MA, Wilkinson DI, Carlo DJ, Brostoff SW (1994) $CD8^+$ T cells in psoriatic lesions preferentially use T-cell receptor $V_\beta 3$ and/or $V_\beta 13.1$ genes. Proc Natl Acad Sci USA 91:9282-9286.
344. Schlaak JF, Buslau M, Jochum W, Hermann E, Girndt M, Gallati H, Buschenfelde K-H, Fleischer B (1994) T cells involved in psoriasis vulgaris belong to the Th1 subset. J Invest Dermatol 102:145-149.
345. Vollmer S, Menssen A, Trommler P, Schendel D, Prinz JC (1994) T lymphocytes derived from skin lesions of patients with psoriasis vulgaris express a novel cytokine pattern that is distinct from that of T helper type 1 and T helper type 2 cells. Eur J Immunol 24:2377-2382.
346. Bata-Csorgo ZS, Hammerberg C, Voorhees JJ, Cooper KD (1995) Kinetics and regulation of human keratinocyte stem cell growth in short-term primary *ex vivo* culture. Cooperative growth factors from psoriatic lesional T lymphocytes stimulate proliferation among psoriatic uninvolved, but not normal, stem keratinocytes. J Clin Invest 95:317-327.
347. Baker BS, Powles AV, Lambert S, Valdimarsson H, Fry L (1988) A prospective study of the Koebner reaction and T lymphocytes in uninvolved psoriatic skin. Acta Derm Venereol (Stockh) 68:430-434.
348. Le Roy F, Brown KA, Greaves MW, Vora AJ, Slavin B, Robinson M, Ellis BA, Dowd PM, Dumonde DC (1991) Blood mononuclear cells from patients with psoriasis exhibit an enhanced adherence to cultured vascular endothelium. J Invest Dermatol 97:511-516.
349. Glinski W, Stepien-Sopniewska B, Majewski S, Glinska-Ferenz M, Gorski A (1993) Alterations of T cell: extracellular matrix proteins interactions in psoriasis. Immunol Lett 35:153-157.
350. Gomi T, Shiohara T, Munakata T, Imanishi K, Nagashima M (1991) Interleukin 1 alpha, tumor necrosis factor alpha, and interferon-gamma in psoriasis. Arch Dermatol 127:827-830.
351. Kapp A, Piskorski A, Schopf E (1988) Elevated levels of interleukin 2 receptor in sera of patients with atopic dermatitis and psoriasis. Br J Dermatol 119:707-710.
352. Kapp A (1990) Elevated levels of soluble CD8 antigen in sera of patients with psoriasis. A possible sign of suppressor/cytotoxic T cell activation. Arch Dermatol Res 282:6-7.
353. Schopf RE, Naumann S, Rehder M, Morsches B (1993) Soluble intercellular adhesion molecule-1 levels in patients with psoriasis. Br J Dermatol 128:34-37.
354. Christophers E, Mrowietz U (1995) The inflammatory infiltrate in psoriasis. Clin Dermatol 13:131-136.
355. Arenberger P, Kemény L, Suss R, Michel G, Peter RU, Ruzicka T (1992) Interleukin-8 receptors in normal and psoriatic polymorphonuclear leukocytes. Acta Derm Venereol 72:334-336.
356. Herlin T, Borregaard N, Kragballe K (1982) On the mechanism of enhanced monocyte and

neutrophil cytotoxicity in severe psoriasis. J Invest Dermatol 79:104-108.
357. Schopf RE, Altmeyer P, Lemmel EM (1982) Increased respiratory burst activity of monocytes and polymorphonuclear leukocytes in psoriasis. Br J Dermatol 107:505-510.
358. Ternowitz T (1986) Monocyte and neutrophil chemotaxis in psoriasis. J Am Acad Dermatol 15:1191-1199.
359. Pellegrini G, De Luca M, Orecchia G, Balzac F, Cremona O, Savoia P, Cancedda R, Marchisio PC (1992) Expression, topography and function of integrin receptors are severely altered in keratinocytes from involved and uninvolved psoriatic skin. J Clin Invest 89:1783-1795.
360. Cooper KD, Hammerberg C, Baadsgaard O, Elder JT, Chan LS, Sauder DN, Voorhees JJ, Fisher G (1990) IL-1 activity is reduced in psoriatic skin. Decreased IL-1α and increased nonfunctional IL-1β. J Immunol 144:4593-4603.
361. Yoshinaga Y, Higaki M, Terajima S, Ohkubo E, Nogita T, Miyasaka N, Kawashima M (1995) Detection of inflammatory cytokines in psoriatic skin. Arch Dermatol Res 287:158-164.
362. Leung DYM, Travers JB, Norris DA (1995) The role of superantigens in skin diseases. J Invest Dermatol 105:37S-42S.
363. Valdimarsson H, Baker BS, Jónsdóttir I, Powles A, Fry L (1995) Psoriasis: a T-cell-mediated autoimmune disease induced by streptococcal superantigens? Immunol Today 16:145-149.
364. Leung DYM, Travers JB, Giorno R, Norris DA, Skinner R, Aelion J, Kazemi LV, Kim MH, Trumble AE, Kotb M, Schlievert PM (1995) Evidence for a streptococcal superantigen-driven process in acute guttate psoriasis. J Clin Invest 96:2106-2112.
365. Fast DJ, Schlievert PM, Nelson RD (1989) Toxic shock syndrome-associated staphylococcal and streptococcal pyrogenic toxins are potent inducers of tumor necrosis factor production. Infect Immunol 57:291-296.
366. Leung DYM, Gately M, Trumble A, Ferguson-Darnell B, Schlievert PM, Picker LJ (1995) Bacterial superantigens induce T cell expression of the skin-selective homing receptor, the cutaneous lymphocyte-associated antigen, via stimulation of interleukin 12 production. J Exp Med 181:747-753.
367. Bach F (1995) Organ-specific autoimmunity. Immunol Today 16:353-355.
368. Di Cicco LM, Fräki JE, Mansbridge JN (1987) The plasma membrane in psoriasis. Int J Dermatol 26:631-638.
369. McFadden J, Valdimarsson H, Fry L (1991) Cross-reactivity between streptococcal M surface antigen and human skin. Br J Dermatol 125:443-447.
370. Bata-Csorgo Z, Hammerberg C, Voorhees JJ, Cooper KD (1995) Intralesional T-lymphocyte activation as a mediator of psoriatic epidermal hyperplasia. J Invest Dermatol 105:89S-94S.
371. Hammerberg C, Arend WP, Fisher GJ, Chan LS, Berger AE, Haskill SJ, Voorhees JJ, Cooper KD (1992) Interleukin-1 receptor antagonist in normal and psoriatic epidermis. J Clin Invest 90:571-583.
372. Schmid P, Cox D, McMaster GK, Itin P (1993) In situ hybridization analysis of cytokine, proto-oncogene and tumour suppressor gene expression in psoriasis. Arch Dermatol Res 285:334-340.
373. Gruaz D, Didierjean L, Grassi J, Frobert Y, Dayer J-M, Saurat J-H (1989) Interleukin 1 alpha and beta in psoriatic skin: enzymoimmunoassay, immunoblot studies and effect of systemic retinoids. Dermatologica 179:202-206.
374. Gearing AJH, Fincham NJ, Bird CR, Wadhwa M, Meager A, Cartwright JE, Camp RDR (1990) Cytokines in skin lesions of psoriasis. Cytokine 2:68-75.
375. Takematsu H, Ohmoto Y, Tagami H (1990) Decreased levels of IL-1 alpha and beta in psoriatic lesional skin. Tohoku J Exp Med 161:159-169.
376. Terui T, Suzuki R, Kumagai K, Takematsu H, Kato T, Tagami H (1988) Absence of IL-1 inhibitor in psoriatic scale extracts. Tohoku J Exp Med 156:149-156.
377. Romero LI, Ikejima T, Pincus SH (1989) In situ localization of interleukin-1 in normal and psoriatic skin. J Invest Dermatol 93:518-522.

378. Kristensen M, Deleuran B, Eedy DJ, Feldmann M, Breathnach SM, Brennan FM (1992) Distribution of interleukin 1 receptor antagonist protein (IRAP), interleukin 1 receptor, and interleukin 1α in normal and psoriatic skin. Decreased expression of IRAP in psoriatic lesional epidermis. Br J Dermatol 127:305-311.
379. Takematsu H, Suzuki R, Tagami H, Kumagai K (1986) Interleukin-1-like activity in horny layer extracts: decreased activity in scale extracts of psoriasis and sterile pustular dermatoses. Dermatologica 172:236-240.
380. Blanton RA, Kupper TS, McDougall JK, Dower S (1989) Regulation of interleukin 1 and its receptor in human keratinocytes. Proc Natl Acad Sci USA 86:1273-1277.
381. Lee SW, Morhenn VB, Ilnicka M, Eugui EM, Allison AC (1991) Autocrine stimulation of interleukin-1α and transforming growth factorα production in human keratinocytes and its antagonism by glucocorticoids. J Invest Dermatol 97:106-110.
382. Debets R, Van Joost T, Benner R, Prens EP (1993) Psoriatic epidermal cells release elevated levels of immunoreactive and biologically active interleukins 1 and 6: modulation of corticosteroid treatment. In Bernard BA, Schroot B (eds). Pharmacol skin: From molecular biology to therapeutics. Kärger, Basel, 5:158-166.
383. Schwarz T, Urbanska A, Gschnait F, Luger TA (1987) UV-irradiated epidermal cells produce a specific inhibitor of interleukin 1 activity. J Immunol 138:1457-1463.
384. Maruyama K, Zhang JZ, Nihei Y, Ono I, Kaneko F (1995) Regulatory effects of antipsoriatic agents on interleukin-1 alpha production by human keratinocytes stimulated with gamma interferon in vitro. Skin Pharmacol 8:41-48.

Chapter 2

THESIS

Thesis

Chapter 2

THESIS

Recent research provided several arguments that favor the pathological relevance of cytokines and cytokine receptors in psoriatic skin (see chapter 1). This chapter briefly describes the hypothesis, the study design and the outline of the thesis.

2.1 HYPOTHESIS

The hypothesis, which directed the research presented in the subsequent chapters, is formulated as follows.

The (epi)dermal expression of members of the interleukin-1 (IL-1) system and IL-1 system-related cytokines and cytokine receptors is coordinately altered and is involved in the initiation and/or maintenance of psoriasis.

Testing this hypothesis is clinically relevant as the identification of a cytokine or a cytokine receptor (or a combination of cytokines and/or cytokine receptors) with a prominent role in the pathogenesis of psoriasis provides a basis for developing novel, more selective and effective anti-psoriatic therapies.

2.2 STUDY DESIGN

The hypothesis is stated in general terms and therefore needs to be made operational (i.e. testable). Testing the hypothesis can be subdivided into two phases: i) the analysis of the (epi)dermal expression of cytokines and cytokine receptors in psoriasis; and ii) the analysis of the relation between the expression of cytokines and/or cytokine receptors and the psoriatic phenotype (see for a description of this phenotype chapter 1.3). This thesis deals with the first phase of hypothesis testing.

It is important to note that although data from related research was available at the start of the study, it provided no clarity as it was inconsistent (see chapter 1). Data interpretation was (and still is) difficult as a consequence of: i) heterogeneity with regard to types of skin samples (see chapter 1, Table IV) as well as detection techniques; ii) analysis either *in vivo* or *in vitro*; iii) analysis of only one or a limited number of cytokines; iv) limited analysis of cytokine receptors; and v) the use of only one detection technique (i.e. analysis of only a single level of expression: mRNA, immunoreactive protein or biologically active protein).

The present study design therefore aimed at an approach that integrated several study models and multiple assays to thoroughly analyze the expressi-

on of multiple cytokines and their receptors simultaneously and at different levels of expression in parallel. The study models comprised an *in vivo* model (i.e. skin biopsies), an *ex vivo* model (i.e. freshly isolated epidermal cells and non-stimulated, short-term primary cultures of these cells), and an *in vitro* model (i.e. early passages of dermal fibroblast cultures). The latter two models enabled monitoring of the synthesis of cytokines and their receptors under defined culture conditions. Assays which were set up, optimized and used in the different models to analyze the expression of cytokines and their receptors are given in Table I. The study design used to analyze the epidermal compartment is somewhat different from the one used to analyze the dermal compartment. Both designs are schematically presented in Figures 1 and 2.

Table I. Multi-assay approach to analyze the (epi)dermal expression of cytokines and cytokine receptors.[a]

Model	Cytokine and/or cytokine receptor		
	mRNA	protein	
		IR	BA
in vivo	ISH	Immunohistochemistry	-
ex vivo	RT-PCR	Immunocytochemistry Flow cytometry ELISA	Bioassays
in vitro	Northern hybridization	Immunocytochemistry Flow cytometry ELISA	Bioassays

[a] Abbreviations: BA, biologically active; ELISA, enzyme-linked immunosorbent-assay; IR, immunoreactive; ISH, in situ hybridization; RT-PCR, reverse transcriptase - polymerase chain reaction.

2.3 OUTLINE OF THE THESIS

The primary goal was to investigate the expression of cytokines and their receptors in psoriatic skin, with special focus on the epidermal IL-1 system.

The epidermal compartment is dealt with in **chapter 3.** An in-depth analysis of the complete epidermal IL-1 system *in vivo*, using samples derived from lesional psoriatic, non-lesional psoriatic as well as normal control skin is given in **chapter 3.1.** The individual members of the IL-1 system were localized *in situ* by immunohistochemistry and in situ hybridization (ISH). These techniques together with reverse transcriptase - polyme-

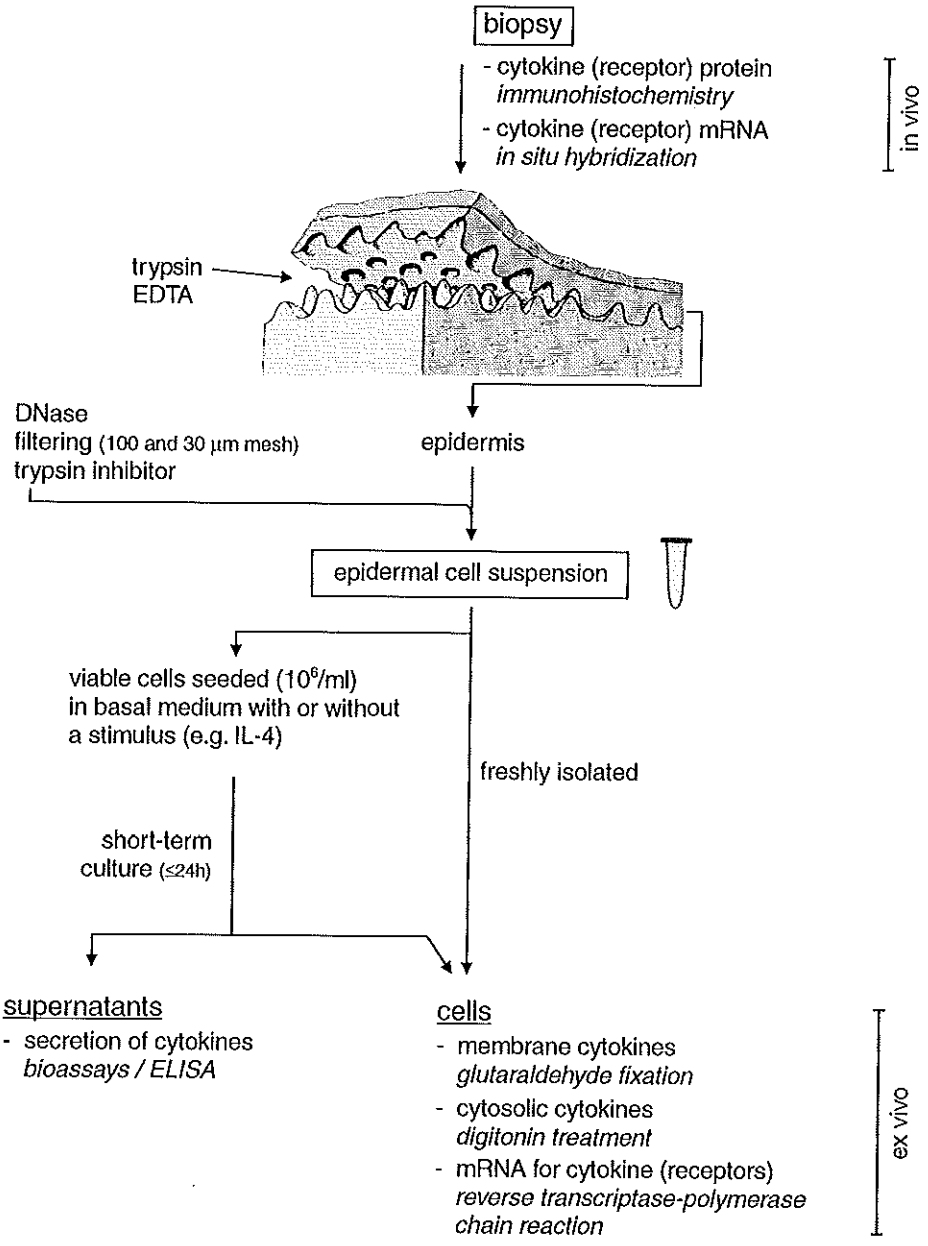

Figure 1. Study design to analyze the expression of cytokines and cytokine receptors in the epidermal compartment of human skin. This simplified scheme illustrates the study plan and techniques used to analyze the expression of cytokines and their receptors in human epidermis *in vivo* and in single epidermal cell suspensions *ex vivo*.

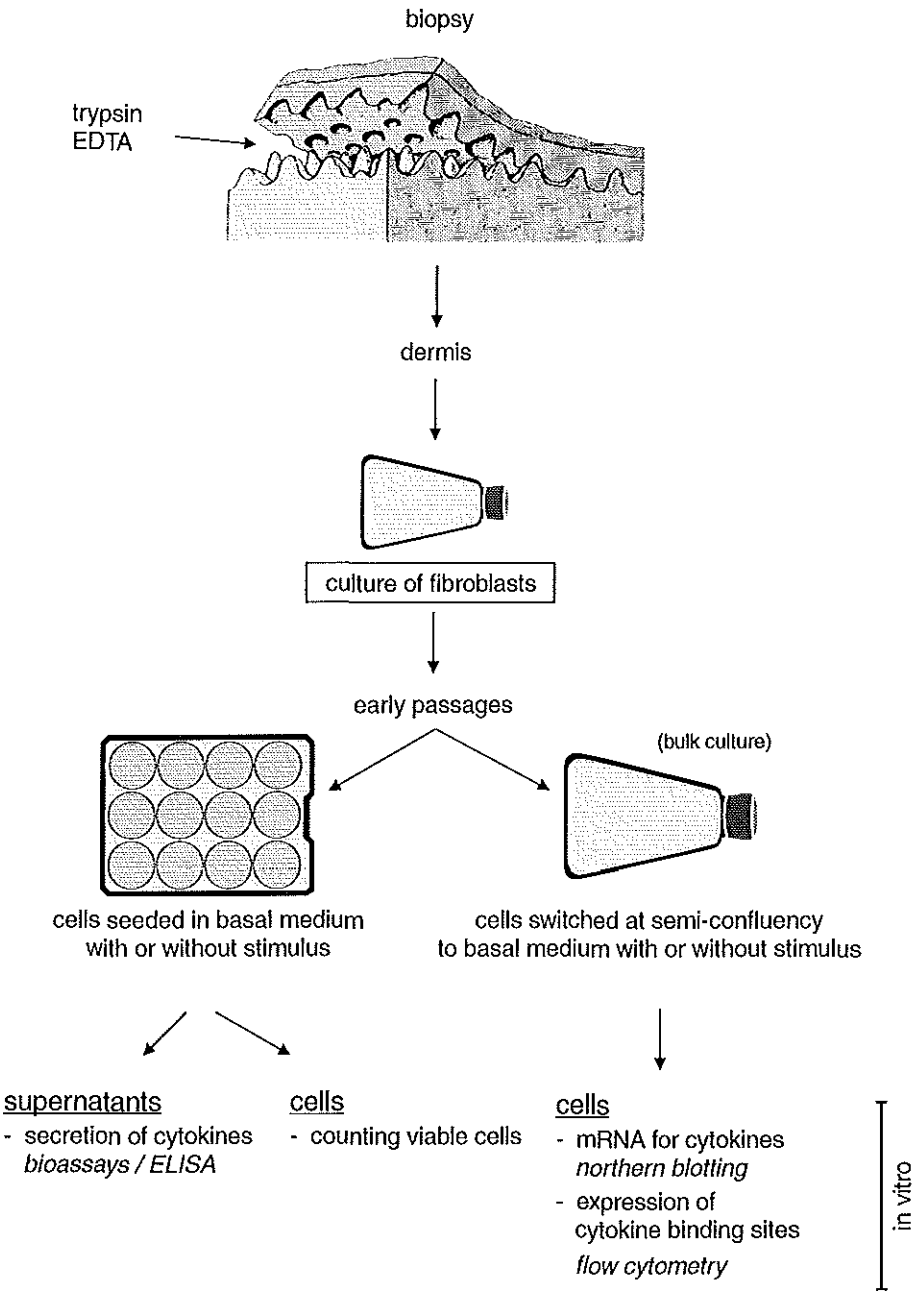

Figure 2. Study design to analyze the expression of cytokines and cytokine receptors in the dermal compartment of human skin. This simplified scheme illustrates the study plan and techniques used to analyze the expression of cytokines and their receptor in human dermal fibroblasts *in vitro*.

rase chain reaction (RT-PCR) performed on RNA from freshly isolated epidermal cells were used in a semi-quantitative manner. This approach enabled analysis of the expressions of single IL-1 and IL-1 receptor (IL-1R) isoforms at the mRNA and protein level as well as analysis of the interrelationships among the expressions of different IL-1 and IL-1R isoforms. In **chapter 3.2** an *ex vivo* model is introduced. Bioassays in combination with neutralizing antibodies and enzyme-linked immunosorbent assay (ELISA) were used to characterize the secretion of IL-1α, IL-1β, IL-6 and tumor necrosis factor-α (TNF-α) by lesional psoriatic epidermal cells relative to normal control epidermal cells. Analysis of different cellular compartments before and after short-term culture enabled the monitoring of the IL-1 synthesis. Further studies to reveal whether apoptosis is inherent to the IL-1 synthesis by lesional psoriatic epidermal cells are described in **chapter 3.3**. DNA fragmentation (i.e. a feature of apoptosis), measured by ELISA and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL), was assessed in epidermal cells both *in vivo* and *ex vivo*. Kinetic studies with epidermal cells *ex vivo* as well as immunohistochemical stainings were performed to relate apoptosis to IL-1β maturation. Finally, in **chapter 3.4** the epidermal expression of the IL-4R in psoriasis was analyzed, as its ligand may affect the IL-1 system. The functionality of this receptor on lesional psoriatic epidermal cells was tested by analyzing the IL-4-mediated effect on IL-1 and IL-6 mRNA levels and protein secretion *ex vivo*.

The dermal compartment is dealt with in **chapter 4. Chapter 4.1** describes an *in vitro* study in which cultures of lesional psoriatic and normal control fibroblasts were used to analyze the secretion of IL-1, IL-6, IL-8 and TNF-α, and the expression of their corresponding mRNAs, by bioassays, ELISA and Northern hybridization, respectively. Calcium, growth factors, several types of sera and cytokines were evaluated for their effect on cytokine production by both types of fibroblasts. As cytokine receptors may be involved in auto/paracrine induction of cytokines, subsequent studies, described in **chapter 4.2**, were performed to investigate the expression of receptors for IL-1, IL-4, IL-6 and TNF-α by lesional psoriatic fibroblasts. The before mentioned stimuli were analyzed for their effect on the expression and shedding of receptors by both types of fibroblasts using flow cytometry, immunocytochemistry and ELISA, respectively.

Chapter 5 discusses the findings of chapters 3 and 4 in the light of the hypothesis. Results of the different models presented in this thesis and recent data reported in the literature are integrated. Finally, a scheme is presented that illustrates our current view on the pathological relevance of the IL-1 system and IL-1 system-related cytokines in psoriasis.

Chapter 3

THE EPIDERMAL IL-1 SYSTEM IN PSORIASIS

The epidermal IL-1 system in psoriasis

Chapter 3.1

THE IL-1 SYSTEM IN PSORIATIC SKIN. IL-1 antagonist sphere of influence in lesional psoriatic epidermis.*

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ABSTRACT

The epidermal expression of IL-1 is altered in psoriasis, a disease characterized by chronic cutaneous inflammation and keratinocyte hyperproliferation. To understand the regulation of IL-1 expression in psoriasis, we semiquantitatively analyzed the epidermal expression of all currently characterized IL-1 isoforms and their receptors in this skin disease. Immunostaining of skin sections showed that IL-1α was located in the basal keratinocytes of normal control (NN) and non-lesional psoriatic (PN) epidermis, and that its expression was significantly decreased to negligible levels in lesional psoriatic (PP) epidermis. IL-1 receptor antagonist (IL-1ra) and IL-1 receptor type II (IL-1RII) were both significantly overexpressed in mutually exclusive compartments of PP epidermis, the suprabasal and basal cell layer(s), respectively. A significant inverse correlation was found between the expressions of IL-1α and these two IL-1 antagonists, which may be inherent to the accelerated terminal differentiation of the psoriatic keratinocyte. In situ hybridization of IL-1 and IL-1R mRNAs in corresponding skin sections confirmed the staining results. Levels of IL-1ra mRNA, however, were not increased in PP epidermis, suggesting that the overexpression of IL-1ra protein is due to enhanced post-transcriptional synthesis. The more sensitive PCR demonstrated a clearly increased expression of IL-1β mRNA in PP epidermal cells (EC), which may contribute to the cutaneous inflammatory response in psoriasis. IL-1RI mRNA was clearly present in both PP and NN EC. The mRNA levels of the secreted IL-1ra isoform and IL-1RII were elevated in PP EC and paralleled those of IL-1β. The upregulated expression of IL-1 antagonists may represent a negative-feedback response to IL-1β. Taken together, this study shows for the first time that the expressions of IL-1 and IL-1R isoforms are coordinatedly altered in PP epidermis, resulting in a predominance of IL-1 antagonists, which favors a decreased IL-1 responsiveness.

* Submitted for publication

INTRODUCTION

Interleukin 1 (IL-1) is abundantly present in human epidermis (1) and has pleiotropic effects on epithelial cells *in vitro* (2) and *in vivo* (3). This cytokine influences cutaneous inflammation (4) and the proliferative state of keratinocytes profoundly (5). The epidermal IL-1 activity is therefore highly regulated. The human epidermal IL-1 system comprises agonists, antagonists and receptors. The IL-1 agonists pro-IL-1α and IL-1α are able to bind to and activate the IL-1 receptors (IL-1R), and are predominantly retained within the keratinocyte (6). Pro-IL-1α and pro-IL-1β differ in the way they are processed in that pro-IL-1α is biologically active, whereas pro-IL-1β has minimal biological activity (7). Normal keratinocytes do not possess the classical IL-1β converting enzyme (ICE), and are therefore unable to cleave pro-IL-1β to the active mature IL-1β (8). Two splice variants of the IL-1 receptor antagonist (IL-1ra) gene product occur in human keratinocytes, the intracellular (icIL-1ra) and the secreted (sIL-1ra) form. The former, which is the predominant form, lacks a signal peptide and resides in the cytoplasm of the keratinocyte (9). The icIL-1ra may antagonize intracellular IL-1α by binding to IL-1R present on the nuclear membrane (10). Two species of IL-1R have been characterized (11,12). The type I IL-1R (IL-1RI) is responsible for IL-1-mediated signaling, whereas the type II IL-1R (IL-1RII) has a short cytoplasmic domain and appears to function as a scavenger for IL-1β (13). Both types of IL-1R bind all IL-1 ligands, but, at least in leukocytes, IL-1RI has the highest affinity for IL-1α and IL-1ra binding while IL-1RII has the highest affinity for IL-1β binding (14). However, the keratinocyte IL-1RII displays similar affinities for both IL-1α and IL-1β (15). Finally, the extracellular portions of both types of IL-1R may be shed and then act as IL-1 inhibitors (16).

The pathophysiological relevance of IL-1 in psoriasis is evidenced by transgenic mice in which epidermal cell (EC)-derived IL-1 induced scaly inflammatory skin lesions with marked erythema, crusting and psoriasis-like histological features such as hyperkeratosis and a dense inflammatory infiltrate (17). Cooper *et al.* were one of the first to report an altered expression of epidermal IL-1α and IL-1β in psoriasis (18). It was observed that IL-1α protein was considerably decreased in cytosolic extracts of lesional psoriatic (PP) skin, whereas IL-1β protein as well as its mRNA were increased when compared to normal control (NN) skin (18,19). Despite the elevation of IL-1β in PP skin, all bioactivity was confined to intracellular IL-1α. We recently extended these findings using non-stimulated, short-term primary cultures of PP EC (EC *ex vivo*) which were capable of producing both functionally active IL-1α and IL-1β (chapter 3.2). However, levels of IL-1α, IL-1β and IL-1ra were negligible when using extracts of the stratum corneum or blister fluids (20-23) and immunostaining data on the epidermal expression of these IL-1 isoforms in both PP and NN skin showed considerable variation (18,22,24-28). The expression of IL-1 in PP epidermis is thus clearly dysre-

gulated. For a full understanding of the regulation of the epidermal IL-1 expression in psoriasis, it is essential to determine the expression of all the different IL-1 and IL-1 receptor isoforms in parallel, preferably using different types of samples and techniques.

Therefore, in the present study, we semiquantitatively assessed the expression of epidermal IL-1α, IL-1β, IL-1ra, IL-1RI and IL-1RII in both skin biopsies and freshly prepared EC suspensions, which closely represent the *in vivo* situation (chapter 3.2, and ref 29). The expressions of IL-1 and IL-1R isoforms were analyzed at the protein and mRNA level using immunohistochemistry, in situ hybridization (ISH) and reverse transcriptase - polymerase chain reaction (RT-PCR). In PP epidermis, the expressions of the IL-1 and IL-1R isoforms are altered in a coordinated manner, resulting in a predominance of IL-1 antagonists. This suggests an effective inhibition of IL-1 responsiveness in PP epidermis.

MATERIALS AND METHODS

Patients and controls

The intake of nineteen patients with untreated plaque-type psoriasis (Outpatient Department of Dermatology, Hospital Walcheren, Vlissingen, The Netherlands, and University Hospital Rotterdam-Dijkzigt, Rotterdam, The Netherlands) was done as described (chapter 3.2). The disease activity, severity and extent were expressed as psoriasis area and severity index (PASI) scores (30). PP skin was obtained from all patients and non-lesional psoriatic (PN) skin from nine patients. Twelve healthy volunteers undergoing abdominal or breast plastic surgery (Sint Franciscus Hospital, Rotterdam) served as controls.

Immunohistochemistry

Punch biopsies (3 mm) were obtained from PP, PN and NN skin. Serial 6 µm cryostat sections were prepared of Tissue-Tek® (Miles Inc., Elkhart, IN) embedded biopsies. Sections were air-dried, acetone-fixed, incubated with the primary monoclonal antibody (Table I) and stained with the supersensitive alkaline phosphatase kit (Biogenex, San Ramon, CA) using new fuchsin (Chroma-Gesellschaft, Köngen, Germany) as the chromogen. Slides were counterstained with Mayer's hematoxylin and mounted in glycerol gelatin (Merck, Darmstadt, Germany). The negative controls comprised concentration-matched mouse IgG_1 (Becton Dickinson, Plymouth, UK) and omitting the first and second step. Human lymphoid tissues, such as lymph node and thymus, and spins of lipopolysaccharide (LPS)-treated peripheral blood mononuclear cells (PBMC) were used as positive controls. The extent and intensity of the stainings were semiquantitatively assessed by two of the authors (RD and JPJJH). The epidermal reaction was scored on a scale of 0-4: 0, no reactivity; 1, weak reactivity; 2, moderate reactivity; 3, strong

Table I. Antibodies for immunostaining of IL-1 and IL-1R isoforms in skin.

IL-1 (R)	Antibody[a]	[protein][b]	Source
IL-1α	1C12.1	5	Oncogene Science, Inc., Uniondale, NY
IL-1β	1886-01	5	Genzyme Corp., Cambridge, MA
IL-1ra	M5	80	Immunex Corp., Seattle, WA
IL-1RI	M1	20	Immunex Corp.
IL-1RII	M2	15	Immunex Corp.

[a] All antibodies are of the mouse IgG_1 isotype.
[b] [protein] in μg/ml.

reactivity; and 4, very strong reactivity (modified from ref 31).

In situ hybridization

Synthesis of RNA probes

cDNAs for IL-1 and IL-1R isoforms were cloned into pBluescriptII KS+ (Stratagene, La Jolla, CA). The cDNAs are given in Table II. Antisense and sense cRNA were synthesized using a digoxigenin RNA labeling kit (Boehringer Mannheim, Germany). The sizes of the transcripts were analyzed by electrophoresis and subsequently reduced to an average length of 100 base pairs (bp) by limited alkaline hydrolysis (32). The labeling efficiency was checked by Southern hybridization of the cDNA clones, and the specificity by Northern hybridization of total cellular RNA extracts of lymphoid cells or cell lines.

In situ hybridization

ISH and immunostaining were performed on serial sections. Skin sections were adhered to slides coated with aminopropyltriethoxysilane (Sigma, St. Louis, MO). Sections were immediately fixed in freshly prepared 4 % paraformaldehyde in PBS for 10 min at 4 °C, permeabilized with 0.5 μg/ml proteinase K (Boehringer Mannheim) for 10 min at 37 °C, and refixed in 4 % paraformaldehyde. Subsequently, sections were rinsed with PBS, acetylated with acetic anhydride in 0.1 M triethanolamine (pH 8.0) for 10 min at room temperature (RT), dehydrated and dried in a vacuum desiccator. Next, sections were overlaid with hybridization solution consisting of 300 mM NaCl, 20 mM NaH_2PO_4, 2 mM EDTA (pH 7.4), 10 % dextran sulphate, 10 x Denhardt's solution, 40 % formamide and 60 U/ml RNA guard (Pharmacia LKB, Piscataway, NJ), which was supplemented with a heat-denatured mixture of probe (final concentration of 0.3 ng/μl/kb), 0.1 mg/ml salmon sperm DNA and 0.1 mg/ml *Escherichia coli* transfer RNA. Sections were

Table II. Digoxigenin-labeled cRNA probes for ISH of IL-1 and IL-1R isoform mRNAs in skin.

IL-1 (R)	cDNA fragments	Source	Synthesis of RNA probes[a]	
			antisense	sense
IL-1α	0.60 kb *Eco*RI-*Hin*dIII	Biogen, Basel	EcoRI/T3	*Hin*dIII/T7
IL-1β	1.30 kb *Pst*I	Genetics Inst., Cambridge	*Hin*dIII/T7	*Bam*HI/T3
IL-1ra	0.47 kb *Cla*I-*Eco*RV	Immunex Corp., Seattle	*Bam*HI/T3	*Cla*I/T7
IL-1RI	0.48 kb *Hin*dIII-*Eco*RI	Immunex Corp., Seattle	*Hin*dIII/T7	*Eco*RI/T3
IL-1RII	0.75 kb *Eco*RI-*Sal*I	Immunex Corp., Seattle	*Eco*RI/T3	*Sal*I/T7
GAPDH	0.78 kb *Eco*RI/*Pst*I	Dr. F.J. Benham, London	*Pst*I/T3	*Eco*RI/T7

[a] The restriction enzyme and the RNA polymerase used to generate the RNA probes are indicated.

covered with silanized slips, sealed with rubber cement and incubated for 16-20 h at 37 °C in a humid chamber. Subsequently, non-hybridized probes were removed by four high-stringency washings with 0.01 x standard sodium citrate (SSC) at 37 °C. To minimize non-specific background, unbound probes were digested with 10 μg/ml RNAse A (Boehringer Mannheim) for 30 min at 37 °C. Hybridization was on average 30 °C below the calculated melting temperature, and washing conditions were just slightly below this temperature, i.e. optimal conditions for the detection of RNA-RNA duplexes (33).

Immunological detection

Hybridization signals were detected using sheep anti-digoxigenin Fab fragments conjugated with alkaline phosphatase (Boehringer Mannheim, diluted 1:800) applied to 0.1 % triton-X-100-treated sections (15 min at RT). Nitro Blue Tetrazolium/X-phosphate (Sigma) was used as the substrate. The addition of polyvinyl alcohols (Mr: 31-50-kDa, 10 % (m/v), Aldrich Chemical Company Inc., Milwaukee) enhanced and localized the enzyme reaction (34). Slides were left to develop in the dark for 2-16 h at 37 °C. The reaction was stopped in water and slides were mounted. The negative controls comprised omission of probe or antibody, hybridization with the sense probe, and RNAse (10 mg/ml) treatment for 1 h at 37 °C before hybridization. Hybridizations on lymphoid tissues were performed as positive controls. *In situ* expression of IL-1 and IL-1R mRNAs was only evaluated in slides showing no hybridization signal with the corresponding sense probe and a positive hybridization signal with the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antisense probe which confirmed the preservation of cellular RNA. The epidermal ISH findings were ranked to correlate the mRNA expression with the protein expression. Ranking was performed as described for immunostaining.

Reverse transcriptase - polymerase chain reaction

RNA isolation and cDNA synthesis

Split-skin specimens of 10-15 cm^2 were obtained from PP and NN skin and used to prepare single EC suspensions as reported in chapter 3.2. Total cellular RNA was isolated using the guanidine thiocyanate extraction procedure (35). Prior to amplification, the samples were treated with RNAse-free DNAse (DNase I, Amplification Grade, GIBCO BRL, Gaithersburg, MD) at a final concentration of 1 U/µg RNA for 15 min at RT. Samples were reverse-transcribed into cDNA by incubating 1 µg RNA in 20 µl of 50 mM Tris-HCl buffer (pH 8.3) supplemented with 10 mM $MgCl_2$, 50 mM KCl, 1 mM dithiothreitol (Sigma), 1 mM EDTA, 10 µg/ml BSA (Fraction V, Boehringer Mannheim), 10 µg/ml oligo-$d(T)_{12-18}$ (Pharmacia LKB), 85 µg/ml random hexamer primers ($(dN)_6$, Pharmacia LKB), 1 mM deoxynucleotide triphosphates (dNTPs, Pharmacia LKB), 1 mM spermidine-HCl, 1125 U/ml RNA guard and 250 U/ml avian myeloblastosis virus reverse transcriptase (Promega Corp., Madison, WI) for 1 h at 42 °C (36).

Table III. Oligonucleotides for PCR amplification and detection of EC-derived IL-1 and IL-1R isoform cDNAs.

IL-1 (R)	Oligonucleotide	Sequence (5′ to 3′)	Size of amplimer[a]	Reference
IL-1α	sense primer	CATCGCCAATGACTCAGAGGAAG	469	37
	antisense primer	TGCCAAGCACACCCAGTAGTCTTGCTT		37
	probe	CATGGGTGCTTATAAGTCATC		37
IL-1β	sense primer	CCAGCTACGAATCTCGGACCACC	667	37
	antisense primer	TTAGGAAGACACAAATTGCATGGTGAA GTCAGT		37
	probe	CGATCACTGAACTGCACGCTCCGGG		37
icIL-1ra	sense primer	CAGAAGACCTCCTGTCCTATGAGG	511	9
	antisense primer	CTACTCGTCCTCCTGGAAGTAG		9
	probe	CCCTCCCCATGGCTTTAG		
sIL-1ra	sense primer	GAATGGAAATCTGCAGAGGCCTCCGC	146	9
	antisense primer	GAAGGTCTTCTGGTTAACATCCCAG		9
	probe	TCACTCTCCTCCTCTTCCTG		
IL-1RI	sense primer	TGCCTGAGGTCTTGGAAAAAC	340	38
	antisense primer	TGTGGTCCCTGTGTAAAGTCC		38
	probe	GCTCTTGTTCAGGATGGAAT		
IL-1RII	sense primer	TCCTGCCGTTCATCTCATACC	574	38
	antisense primer	TCCATGTGCAAATCCTCTCTT		38
	probe	GAGACCATTCCTGTGATCAT		
GAPDH	sense primer	CCGAGCCACATCGCTCAGACAC	594	39
	antisense primer	GGCCATCCACAGTCTTCTGGGT		39
	probe	CTTCCAGGAGCGAGATCCCTC		39

[a] size of amplimer in bp

Polymerase chain reaction

The reaction mixture contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.125 mM dNTPs, 335 µg/ml 5' and 3' oligonucleotide primers (Table III), 20 U/ml Taq polymerase (Amplitaq, Perkin-Elmer Cetus, Norwalk, CT) and 0.1 % gelatine in a total volume of 50 µl. The oligonucleotides were generated with a DNA synthesizer (Applied Biosystems, Forster City, CA). Samples were added to the reaction mixture and amplified in a DNA Thermocycler (Perkin-Elmer Cetus) for 35 cycles. The PCR protocol started with an adapted hot start, for 3 min at 94 °C, to prevent non-specific primer annealing (40). Each cycle consisted of denaturation for 30 sec at 94 °C, annealing for 30 sec at 55 °C and extension for 1 min at 72 °C . Aliquots of PCR products were electrophoresed on 2 % agarose gels and visualized by ethidium bromide staining (41). GAPDH was used as an internal control to standardize for total cellular mRNA. Serial twofold dilutions of individual cDNAs, starting from approximately 5 ng, were amplified using GAPDH primers and demonstrated concomitant decreases of amplified products. cDNA concentrations were selected which yielded equivalent quantities of GAPDH products and fell within the linear range of amplification (42). Each experiment included a positive control (cDNA from LPS-treated PBMC) and a negative control (RNA that had not been reverse-transcribed or water).

Radioactive hybridization of PCR product

Cytokine cDNAs were verified by Southern hybridization. Prior to transfer (see ref 41) to Nytran N nylon membranes (Schleicher and Schuell, Dassel, Germany), large DNA fragments were nicked by partial hydrolysis. Oligonucleotides to probe PCR products (Table III), being complementary to internal sequences of the PCR products, were end-labeled with ^{32}P-γATP (ICN Pharmaceuticals Inc., Irvine, CA) by T4 polynucleotidyl kinase (Pharmacia LKB) (41). Blots were hybridized in 6 x SSC, 10 x Denhardt's solution, 1 % sodium dodecyl sulphate (SDS) and 50 µg/ml salmon sperm DNA for 18 h at 55 °C (41). Subsequently, blots were rinsed twice for 20 min at RT with 2 x SSC and 0.1 % SDS, and exposed to Fuij film.

Statistical analysis

Results were analyzed with the Wilcoxon Matched-Pairs Signed-Rank Test (PP versus PN samples), the Wilcoxon Rank Sum Test (PP or PN versus NN samples) and the Spearman's Rank Correlation Coefficient (r_s) using STATA™ (Computing Resource Center, Los Angeles, CA). Significant differences or correlations are indicated by P values smaller than 0.05.

RESULTS

PP epidermis expresses decreased levels of IL-1α protein

Immunostaining showed that IL-1α protein was located primarily in the intracellular compartment of keratinocytes in the stratum basale of PN and NN epidermis (Figs 1A and C, annex 'Color Plates'). In PP epidermis, its expression was significantly decreased to almost negligible levels (PP versus PN: $P < 0.03$, and PP versus NN: $P < 0.02$) (Fig 1, annex 'Color Plates', and Table IV). We were unable to detect epidermal IL-1β immunoreactivity in any PP, PN or NN skin sample. Some dermal IL-1β staining, however, was observed.

Table IV. Interrelated epidermal expression of IL-1 and IL-1R isoforms.[a]

	PP[b] Patient number									PN Patient number									NN Control number						
	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7
protein																									
IL-1α	0	0	ND[c]	0	0	1	ND	1	0	3	0	0	1	1	1	2	2	1	ND	1	1	2	ND	2	1
IL-1ra	3	2	4	2	4	2	2	2	1	ND	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0
IL-1RII	2	2	2	ND	2	1	0	1	2	1	1	1	0	1	0	0	1	0	1	0	ND	2	ND	0	0
mRNA																									
IL-1ra	1	0	0	1	ND	2	2	0	0	0	0	2	2	2	1	1	ND	ND	0	0	1	ND	1	1	1
IL-1RII	1	3	3	1	ND	0	2	1	2	2	1	2	0	1	0	1	ND	ND	2	1	2	ND	1	1	1
PASI	ND	16	24	25	7	12	7	4	6																

[a] Serial skin sections were analyzed using immunohistochemistry and ISH for the expression of IL-1 and IL-1R isoforms at the protein and mRNA level, respectively. Findings were scored on a 0-4 scale based on the extent and intensity of the signal. The epidermal immunostaining for IL-1β and IL-1RI as well as ISH for IL-1α, IL-1β and IL-1RI were negligible. See text for details.
[b] Type of skin (PP, lesional psoriasis; PN, non-lesional psoriasis; and NN, normal control).
[c] ND, not done.

Levels of IL-1ra and IL-1RII protein are increased in PP epidermis

Weak staining for IL-1ra was observed in the suprabasal keratinocytes of PN and NN epidermis (Figs 2B and C, annex 'Color Plates'). The expression of IL-1ra was significantly increased suprabasally in PP epidermis (PP versus PN: $P < 0.01$, and PP versus NN: $P < 0.001$) (Fig 2C, annex 'Color Plates', and Table IV). In PP skin, some cells in the papillary dermis were also slightly positive for IL-1ra. We were unable to detect positive epidermal staining for IL-1RI in any type of skin sample. Only in PP skin, sporadically a slight dermal expression of IL-1RI was seen. Immunoreactive IL-1RII, however, was detectable in all types of skin samples and its expression was restricted to the basal epidermal layer(s) and the dermis. The expression of IL-1RII in PP skin samples was increased when compared to the corresponding PN skin samples ($P < 0.02$), and showed a trend of increase when compared to NN skin samples ($P < 0.1$) (Figs 2D-F, annex 'Color PLates', and Table IV).

Semiquantitative scores of epidermal IL-1ra immunoreactivity correlated nicely with those of IL-1RII (r_s = 0.71, n = 21, P < 0.0025), whereas IL-1α, which was down-regulated in PP skin, correlated inversely with both IL-1ra (r_s = - 0.39, n = 20, P < 0.05) and IL-1RII (r_s = - 0.46, n = 19, P < 0.05). It is of note that the inverse correlations between IL-1α and both IL-1ra and IL-1RII showed the most negative coefficients when analyzing only PP samples.

PP epidermis expresses increased levels of IL-1RII mRNA, but not IL-1ra mRNA

ISH of mRNA for the IL-1 and IL-1R isoforms with digoxigenin-labeled cRNA probes appeared not sensitive enough for detecting IL-1α, IL-1β and IL-1RI mRNAs in skin sections. Epidermal mRNAs for IL-1ra and IL-1RII, however, were readily detectable. IL-1ra mRNA was present in all epidermal cell layers and to some extent in the dermis, with the level of expression being similar among the different types of skin samples (Fig 3A, annex 'Color PLates'). This is in contrast with the IL-1ra immunostainings (Table IV). IL-1RII mRNA was easily detectable in the basal and suprabasal cell layers and the dermal compartment of nearly all PN and NN skin samples, but levels were increased in PP skin samples (Figs 3B-D, annex 'Color PLates'). ISH of IL-1RII mRNA was in line with the corresponding immunostaining, as the protein and mRNA scores of IL-1RII correlated well (r_s = 0.54, n = 18, P < 0.025). An overview of the immunostaining and ISH scores of individual patients is shown in Table IV.

PP EC display an increased expression of IL-1β, secreted IL-1ra and IL-1RII mRNA levels

The presence of mRNAs for IL-1 and IL-1R isoforms was more sensitively analyzed by RT-PCR performed on total cellular RNA from freshly isolated EC. GAPDH-standardized amounts of cDNA from PP EC were on average 4 times less than those from NN EC, which reflected the enhanced metabolic state of PP cells. Unexpectedly, IL-1α mRNA was hardly detectable in any EC sample. A second set of primers (kindly provided by Dr. K. Uyemura, Los Angeles, CA) and extension of PCR to 45 cycles did not change the results. IL-1β mRNA was absent in all 5 NN samples, but clearly detectable in 8 out of 10 PP samples (Fig 4). IL-1ra mRNA was present in all samples investigated. Using different sets of primers to amplify IL-1ra cDNA, we were able to distinguish the icIL-1ra isoform from the sIL-1ra form (Table III). icIL-1ra appeared to be the dominant isoform present in EC. All PP and NN samples displayed a similar expression of icIL-1ra mRNA, whereas sIL-1ra mRNA was present in increased amounts in part of the PP samples (Fig 4). The expression of IL-1RI mRNA was clear and quite homogeneous among the individual NN and PP samples. The amounts of IL-1RII mRNA varied, especially among the PP samples, and were increased in part of the PP samples when compared to NN samples (Fig 4). It is of note that the absence of IL-1β mRNA in PP

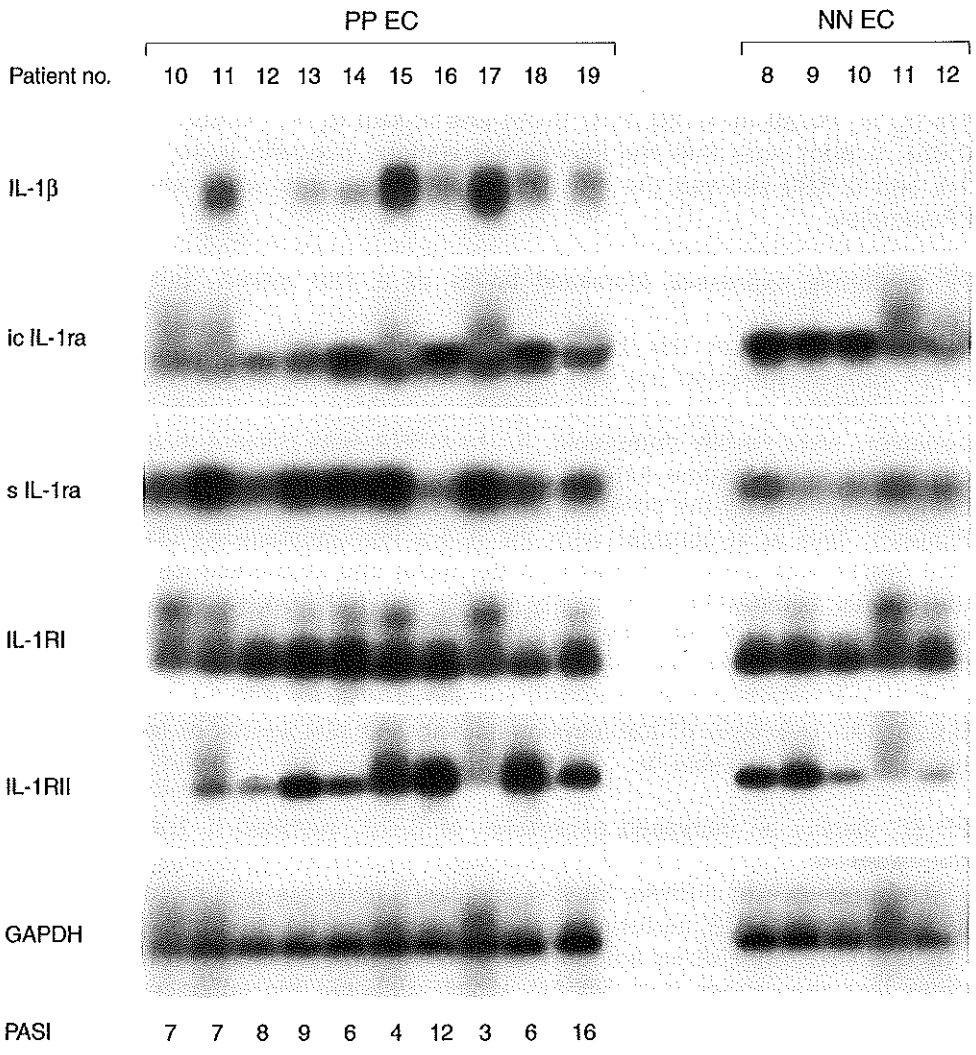

Figure 4. PCR analysis of mRNAs for IL-1 and IL-1R isoforms in PP EC. cDNAs were synthesized from mRNAs isolated from freshly prepared EC suspensions from PP and NN skin specimens. Initially, individual cDNA concentrations for PCR were standardized to yield equivalent quantities of GAPDH mRNA. Water or non-reverse transcribed RNA gave no signals, whereas cDNA from LPS-treated PBMC was clearly positive in all PCRs performed.

EC samples was always accompanied by normal expression, i.e. identical when compared to NN EC, or even absence of sIL-1ra and IL-1RII mRNA (Fig 4, i.e. patient numbers 10 and 12). Presence of IL-1β mRNA was, however, always accompanied by increased expression of either sIL-1ra or IL-1RII mRNA (Fig 4, i.e. patient numbers 16 and 17) or both types of mRNAs (Fig

4, i.e. patient numbers 15 and 18) when compared to NN EC.

The differences in electrophoretic mobility between the individual icIL-1ra and IL-1RI PCR products (Fig 4) were probably due to the presence of splice variants. The occurrence of these variants, however, does not seem to be psoriasis-specific as they are present in both types of EC. To check for the presence of intra-epidermal T cells, EC samples were further analyzed for IL-2 mRNA (see ref 42 for primers and probes). The signals for this marker of T cell activation, although increased in some PP samples, were weak and did not correlate with the IL-1 findings (not shown).

Table V. Overview of the IL-1 system in lesional psoriatic epidermis.[a]

IL-1 (R)	Immunostaining	ISH/RT-PCR	Epidermal compartment
IL-1α	↓	-	Basal cell layer
IL-1β	-	↑	-
IL-1ra	↑	↔/↑[b]	Suprabasal cell layers
IL-1RI	-	↔	-
IL-1RII	↑	↑	Basal cell layer

[a] The IL-1 phenotype of PP epidermis based on immunostaining, ISH and RT-PCR. Symbols; ↓ and ↑: significantly decreased and increased expression, respectively, when compared to PN and NN epidermis; ↔: similar expression when compared to PN and NN epidermis; and -: not detectable. The epidermal compartment indicates the *in situ* localization as observed with immunostaining. Note that the expressions of IL-1 and IL-1R in PP epidermis are interrelated (see text for details), and that the expressions of IL-1 and IL-1R in PN and NN epidermis are similar.

[b] The expression of icIL-1ra mRNA is unchanged in PP epidermis when compared to PN and NN epidermis (results of ISH and RT-PCR), whereas the expression of sIL-1ra is increased in PP epidermis when compared to PN and NN epidermis (RT-PCR).

DISCUSSION

A thorough analysis was made of the complete epidermal IL-1 system in psoriasis resulting in a defined phenotype, as outlined in Table V. In short, we provide the *in situ* evidence that IL-1α is decreased in PP epidermis, but not in PN epidermis, when compared to NN epidermis (Fig 1, annex 'Color Plates', and Table IV). This is in line with findings by others using cytosolic extracts of PP and NN skin (18,19,24). Immunostaining showed that epidermal IL-1α resides in the cytoplasm of basal keratinocytes, which confirmed the preferential reaction of polyclonal anti-IL-1α antibodies with the basal cell layer, as reported by Anttila *et al.* (43). The observed decrease of cytosolic IL-1α protein in PP epidermis may reflect increased secretion of this cytokine *in vivo*, as we demonstrated that PP EC show an increased release of biologically active IL-1α *ex vivo* when compared to NN EC (chapter 3.2). Unexpectedly, we were unable to detect epidermal IL-1β immunoreactivity in either PP, PN or NN skin. This contrasted with the report that basal keratinocytes in cytofuge preparations of NN EC express IL-1β immunoreactivity (43),

probably representing pro-IL-1β (8). Moreover, we and others observed increased levels of IL-1β in cytosolic extracts of PP EC when compared to NN EC (chapter 3.2, and ref 18), and activation of this cytosolic IL-1β concomitant with its release *ex vivo* (chapter 3.2). Nevertheless, the lack of consistent epidermal IL-1β immunoreactivity *in situ* was validated by the use of a panel of anti-IL-1β monoclonal and polyclonal antibodies, and different fixation and detection protocols (Hegmans JPJJ, Schrama M, Troost RJJ, Prens EP, Debets R. IL-1 in the skin. Immunohistochemistry revisited. Manuscript in preparation). Furthermore, an anti-IL-1β polyclonal antibody, which binds with similar avidity to pro-IL-1β and mature IL-1β (generously provided by Dr. B. Ferrua, Nice, France), did not enable us to detect epidermal IL-1β. Immunohistochemical techniques may thus not be sufficiently sensitive to detect epidermal IL-1β, or unable to detect masked epitopes or epitopes exposed during IL-1β processing, which may be incomplete in PP epidermis (18).

IL-1ra and IL-1RII proteins are compartmentalized to reciprocal regions of NN epidermis, the suprabasal layers and the basal layer, respectively (Fig 2, annex 'Color Plates'). Thus, basal keratinocytes, which contain cytosolic IL-1α, but lack IL-1ra, are probably responsive to the mitogenic effects of IL-1α in an autocrine fashion. This is in line with normal keratinocyte proliferation in that cycling cells reside in the stratum basale. The expressions of both IL-1ra and IL-1RII were significantly increased in PP epidermis when compared to PN and NN epidermis, which confirms previous observations by others (24,39). In addition, our data show for the first time that the expressions of IL-1α, IL-1ra and IL-1RII are significantly interrelated (Table IV).

First, IL-1ra and IL-1RII are both negatively correlated with IL-1α. This relation may simply reflect accelerated terminal differentiation in clinically stable psoriatic lesions, as the expression of IL-1α decreases during the differentiation process of keratinocytes, whereas the expressions of both IL-1ra and IL-1RII increase during this process *in vitro* (44,45). However, the observations that IL-1RII protein is located in the basal cell layer and that the intensity of its expression is positively related to the length of epidermal elongations in PP skin (not shown) suggest that the expression of this receptor *in vivo* may be limited to proliferating cells. Vice versa, one cannot exclude the possibility that the shift in the ratio between IL-1 agonists (IL-1α) and IL-1 antagonists (IL-1ra and IL-1RII) in PP EC may in fact induce keratinocyte differentiation (24,46).

Second, there is a positive correlation between IL-1ra and IL-1RII, suggesting an effective inhibition of IL-1 responsiveness in PP epidermis. These inhibitors of IL-1 activity work synergistically. IL-1ra antagonizes IL-1 activity by binding to IL-1RI, whereas IL-1RII inhibits IL-1 responses by acting as a scavenger to prevent an IL-1RI-mediated response. Moreover, soluble IL-1RII binds pro-IL-1β and blocks processing of this isoform (47). The expression and shedding of keratinocyte IL-1RII is induced by IFNγ (15), which is present in PP skin (42). This may explain the overexpression of this receptor

on PP keratinocytes.

PP, PN and NN keratinocytes all lack IL-1RI immunoreactivity, which is in agreement with the report that in keratinocytes IL-1RII molecules significantly outnumber the IL-1RI molecules (15). This does not necessarily imply unresponsiveness to IL-1, as only a few IL-1R type I molecules per cell are sufficient for an IL-1-mediated response (48). The noted increase in the dermal expressions of most IL-1 and IL-1R forms probably reflects activated fibroblasts and infiltrating cells.

IL-1α and IL-1β mRNAs could hardly be detected with ISH. IL-1α mRNA levels were also negligible in all EC samples tested with the more sensitive RT-PCR. Others, however, have observed a clear expression of IL-1α mRNA in full thickness skin biopsies (42). This can be explained by the presence of resident and infiltrating dermal cells in such biopsies, as our EC suspensions were made of pure epidermal sheets (chapter 3.2) resulting in an enhanced EC purity of these samples when compared to complete skin biopsies. Although the decrease of IL-1α protein in PP epidermis might be due to translational arrest of IL-1 synthesis, findings *ex vivo* favor increased IL-1 secretion by PP EC (chapter 3.2). PCR experiments showed that 8 out of 10 EC suspensions derived from PP skin expressed IL-1β mRNA, whereas NN EC did not (Fig 4). Up to now, this was only reported with complete skin biopsies (18,42,49). The increased expression of cytosolic IL-1β protein by PP EC, as we found with enzyme-linked immunosorbent assay (ELISA) (chapter 3.2), is thus probably due to enhanced transcription of the IL-1β gene. The observation that IL-1β mRNA was also increased in PN skin (42), favors a primary role of IL-1β in the inflammatory response in psoriasis.

When performing ISH, the IL-1ra probe revealed no clear-cut differences between the different types of skin samples. EC-derived icIL-1ra as well as sIL-1ra mRNAs were detectable with PCR, confirming the presence of both splice variants in keratinocytes (9). Levels of icIL-1ra mRNA, although variable, did not differ between PP and NN samples, whereas levels of sIL-1ra mRNA were increased in PP samples when compared to NN samples. Based on these observations, an increased synthesis of icIL-1ra protein by PP EC at the level of translation or post-translational modification, cannot be excluded. Increased steady-state levels of IL-1RII mRNA were found in part of the PP samples with both ISH (Fig 3, annex 'Color Plates') and PCR (Fig 4). Results of immunostaining and ISH correlated nicely (Table IV), which suggested an enhanced transcription of the IL-1RII gene in PP EC. Although IL-1RII mRNA is present in all epidermal cell layers, protein synthesis only takes place in proliferating basal cells. Epidermal IL-1RI mRNA was not detectable in any type of skin sample using ISH, which is in accordance with our immunostaining results. In line with findings by Groves *et al.* (38), the presence of IL-1RI mRNA was readily detectable with the sensitive PCR in both PP and NN EC (Fig 4). Although this implies in principle similar IL-1 responsiveness between both types of EC, the overexpression of the IL-1 inhibitors, IL-1ra and IL-1RII, by PP EC may downmodulate the response to IL-1.

In analogy with immunostainings, the PCR findings showed that the expressions of IL-1 agonists were interrelated with those of IL-1 antagonists. The increased levels of the sIL-1ra and IL-1RII mRNA, as observed in part of the PP samples, were interrelated with IL-1β mRNA levels (Fig 4). The cellular environment of PP EC, due to an altered expression of transcription factors, may favor initiation of transcription at the otherwise repressed promoter of the IL-1β gene (50). In PP samples with an elevated IL-1β expression, a negative-feedback signal may have occurred resulting in an up-regulated expression of IL-1ra and/or IL-1RII in these samples. At the moment, it is not clear whether psoriatic patients, not expressing epidermal IL-1β levels, constitute a clinically different subgroup.

Taken together, this is the first report of a complete analysis of the IL-1 system in lesional as well as non-lesional psoriatic epidermis using immunostaining, ISH and PCR. Our study is unique in that the expressions of IL-1 and IL-1R isoforms were investigated in parallel, which revealed an interrelated expression of the IL-1 and IL-1R forms in PP epidermis. The inverse relation between IL-1α and the IL-1 antagonists and the positive relation between IL-1β and the IL-1 antagonists may be linked to keratinocyte differentiation and cutaneous inflammation, respectively, which are the two hallmarks of psoriasis. Patient's PASI scores, however, did not correlate with the expression of any IL-1 marker. Moreover, genetic analysis excluded, for example, IL-1ra as a single primary disease determinant for psoriasis (51). We therefore believe that in psoriasis the study of multiple IL-1 and IL-1R forms rather than any single isoform is more valuable. In our view, the expressions of IL-1 and IL-1R isoforms are coordinatedly altered in PP epidermis, favoring a local IL-1 antagonist sphere of influence.

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REFERENCES

1. Hauser C, Saurat J-H, Schmitt A, Jaunin F, Dayer J-M (1986) Interleukin 1 is present in normal human epidermis. J Immunol 136:3317-3321.
2. Kupper TS, Lee F, Birchall N, Clark S, Dower SK (1988) Interleukin 1 binds to specific receptors on human keratinocytes and induces granulocyte macrophage colony-stimulating factor mRNA and protein. J Clin Invest 82:1787-1792.
3. Camp RDR, Fincham NJ, Ross J, Bird CR, Gearing AJH (1990) Potent inflammatory properties in human skin of interleukin-1 alpha-like material isolated from normal skin. J Invest Dermatol 94:735-741.
4. Kupper TS (1990) Immune and inflammatory processes in cutaneous tissues. Mechanisms and speculations. J Clin Invest 86:1783-1789.
5. Ristow HJ (1987) A major factor contributing to epidermal proliferation in inflammatory skin diseases appears to be interleukin 1 or a related protein. Proc Natl Acad Sci USA

84:1940-1944.
6. McGuire J, Didierjean L, Langdon RC, Dayer J-M, Saurat J-H (1988) The subcellular distribution of different molecular weight forms of interleukin-1 alpha and beta in the human keratinocyte. J Invest Dermatol 90:587. (Abstr.)
7. Mosley B, Urdal DL, Prickett K, Larsen A, Cosman D, Conlan PJ, Gillis S, Dower SK (1987) The interleukin-1 receptor binds the human interleukin-1α precursor but not the interleukin-1β precursor. J Biol Chem 262:2941-2944.
8. Mizutani H, Black R, Kupper TS (1989) Human keratinocytes produce but do not process pro-interleukin-1 (IL-1) beta. Different strategies of IL-1 production and processing in monocytes and keratinocytes. J Clin Invest 87:1066-1071.
9. Haskill S, Martin G, Van Le L, Morris J, Peace A, Bigler CF, Jaffe GJ, Hammerberg C, Sporn SA, Fong S, Arend WP, Ralph P (1991) cDNA cloning of an intracellular form of the human interleukin 1 receptor antagonist associated with epithelium. Proc Natl Acad Sci USA 88:3681-3685.
10. Curtis BM, Widmer MB, De Roos P, Qwarnstrom EE (1990) IL-1 and its receptor are translocated to the nucleus. J Immunol 144:1295-1303.
11. Sims J, March CJ, Cosman D, Widmer MB, MacDonald HR, McMahan C, Grubin CE, Wignall JM, Jackson J, Call SM, Friend D, Alpert A, Gillis S, Urdal DL, Dower S (1988) cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin superfamily. Science 241:585-589.
12. McMahan C, Slack J, Mosley B, Cosman D, Lupton SD, Brunton LL, Grubin CE, Wignall JM, Jenkins NA, Brannan CI, Copeland NG, Huebner K, Croce CM, Cannizzarro LA, Benjamin D, Dower S, Spriggs M, Sims J (1991) A novel IL-1 receptor, cloned from B cells by mammalian expression, is expressed in many cell types. EMBO J 10:2821-2832.
13. Colotta F, Re F, Muzio M, Bertini R, Polentarutti N, Sironi M, Giri JG, Dower SK, Sims JE, Mantovani A (1993) Interleukin-1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. Science 261:472-475.
14. Sims J, Dower S (1994) Interleukin-1 receptors. Eur Cytokine Netw 5:539-546.
15. Groves RW, Giri J, Sims J, Dower S, Kupper TS (1995) Inducible expression of type 2 IL-1 receptors by cultured human keratinocytes. Implications for IL-1-mediated processes in epidermis. J Immunol 154:4065-4072.
16. Symons JA, Eastgate JA, Duff GW (1991) Purification and characterization of a novel soluble receptor for interleukin 1. J Exp Med 174:1251-1254.
17. Groves RW, Kupper TS (1994) Spontaneous inflammatory skin disease in mice which overexpress IL-1α in basal keratinocytes. J Invest Dermatol 103:442. (Abstr.)
18. Cooper KD, Hammerberg C, Baadsgaard O, Elder JT, Chan LS, Sauder DN, Voorhees JJ, Fisher G (1990) IL-1 activity is reduced in psoriatic skin. Decreased IL-1α and increased nonfunctional IL-1β. J Immunol 144:4593-4603.
19. Gruaz D, Didierjean L, Grassi J, Frobert Y, Dayer J-M, Saurat J-H (1989) Interleukin 1 alpha and beta in psoriatic skin: enzymoimmunoassay, immunoblot studies and effect of systemic retinoids. Dermatologica 179:202-206.
20. Gearing AJH, Fincham NJ, Bird CR, Wadhwa M, Meager A, Cartwright JE, Camp RDR (1990) Cytokines in skin lesions of psoriasis. Cytokine 2:68-75.
21. Takematsu H, Ohmoto Y, Tagami H (1990) Decreased levels of IL-1 alpha and beta in psoriatic lesional skin. Tohoku J Exp Med 161:159-169.
22. Prens EP, Benne K, Van Damme J, Bakkus M, Brakel K, Benner R, Van Joost T (1990) Interleukin-1 and interleukin-6 in psoriasis. J Invest Dermatol 95:121S-124S.
23. Terui T, Suzuki R, Kumagai K, Takematsu H, Kato T, Tagami H (1988) Absence of IL-1 inhibitor in psoriatic scale extracts. Tohoku J Exp Med 156:149-156.
24. Hammerberg C, Arend WP, Fisher GJ, Chan LS, Berger AE, Haskill SJ, Voorhees JJ, Cooper KD (1992) Interleukin-1 receptor antagonist in normal and psoriatic epidermis. J Clin Invest 90:571-583.
25. Didierjean L, Salomon D, Mérot Y, Siegenthaler G, Shaw A, Dayer J-M, Saurat J-H (1989) Localization and characterization of the interleukin 1 immunoreactive pool (IL-1 alpha and beta forms) in normal human epidermis. J Invest Dermatol 92:809-816.

26. Tron VA, Rosenthal D, Sauder DN (1988) Epidermal interleukin-1 is increased in cutaneous T-cell lymphoma. J Invest Dermatol 90:378-381.
27. Romero LI, Ikejima T, Pincus SH (1989) In situ localization of interleukin-1 in normal and psoriatic skin. J Invest Dermatol 93:518-522.
28. Kristensen M, Deleuran B, Eedy DJ, Feldmann M, Breathnach SM, Brennan FM (1992) Distribution of interleukin 1 receptor antagonist protein (IRAP), interleukin 1 receptor, and interleukin 1α in normal and psoriatic skin. Decreased expression of IRAP in psoriatic lesional epidermis. Br J Dermatol 127:305-311.
29. Longley J, Gang Ding T, Cuono C, Durden F, Crooks C, Hufeisen S, Eckert R, Wood GS (1991) Isolation, detection, and amplification of intact mRNA from dermatome strips, epidermal sheets, and sorted epidermal cells. J Invest Dermatol 97:974-979.
30. Fredriksson T, Pettersson U (1978) Severe psoriasis-oral therapy with a new retinoid. Dermatologica 157:238-242.
31. Griffiths CEM, Barker JNWN, Kunkel S, Nickoloff BJ (1991) Modulation of leucocyte adhesion molecules, a T-cell chemotaxin (IL-8) and a regulatory cytokine (TNF-α) in allergic contact dermatitis (rhus dermatitis). Br J Dermatol 124:519-526.
32. Cox KH, De Leon DV, Angerer LM, Angerer RC (1984) Detection of mRNAs in sea urchin embryos by in situ hybridization using asymmetric RNA probes. Dev Biol 101:485-502.
33. Angerer LM, Angerer RC (1991) Localization of mRNAs by in situ hybridization. In Hamkalo BA, Elgin VCR (eds). Methods in Cell Biology, Functional Organization of the Nucleus. A Laboratory Guide. Academic Press, New York, pp. 37-71.
34. De Block M, Debrouwer D (1993) RNA-RNA *in situ* hybridization using digoxigenin-labeled probes. The use of high-molecular-weight polyvinyl alcohol in the alkaline phosphatase indoxyl-nitroblue tetrazolium reaction. Anal Biochem 215:86-89.
35. Chomczynski P, Sacchi N (1987) Single-step method for RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156-159.
36. Krug MS, Berger SL (1987) First strand cDNA synthesis primed with oligo-d(T). Methods Enzymol 152:316-325.
37. De Waal Malefyt R, Abrams J, Bennett B, Figdor CG, De Vries JE (1991) Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes. An autoregulatory role of IL-10 produced by monocytes. J Exp Med 174:1209-1220.
38. Groves RW, Sherman L, Mizutani H, Dower SK, Kupper TS (1994) Detection of interleukin-1 receptors in human epidermis. Induction of the type II receptor after organ culture and in psoriasis. Am J Pathol 145:1048-1056.
39. Koning H, Baert MRM, De Groot R, Neijens HJ, Savelkoul HFJ (1995) Analysis of cytokine gene expression in stimulated T cells in small children by semi-quantitative PCR. Med Inflamm 4:196-204.
40. Mullis KB (1991) The polymerase chain reaction in an anemic mode: how to avoid cold oligodeoxyribonuclear fusion. PCR Methods Appl 1:1-4.
41. Sambrook J, Fritsch EF, Maniatis T (eds) (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York (second edition). Units 6.9-6.15, 9.45-46, 9.52-9.55, and 11.31-33.
42. Uyemura K, Yamamura M, Fivenson DF, Modlin RL, Nickoloff BJ (1993) The cytokine network in lesional and lesion-free psoriatic skin is characterized by a T-helper type 1 cell-mediated response. J Invest Dermatol 101:701-705.
43. Anttila HS, Reitamo S, Erkko P, Miettinen A, Didierjean L, Saurat J-H (1990) Membrane and cytosolic interleukin-1 alpha and beta in normal human epidermal cells. Variability of epitope exposure in immunohistochemistry. J Invest Dermatol 95:31-38.
44. Gruaz-Chatellard D, Baumberger C, Saurat J-H, Dayer J-M (1991) Interleukin 1 receptor antagonist in human epidermis and cultured keratinocytes. FEBS Lett 294:137-140.
45. Blanton RA, Kupper TS, McDougall JK, Dower SK (1989) Regulation of interleukin 1 and its receptor in human keratinocytes. Proc Natl Acad Sci USA 86:1273-1277.
46. Arend WP (1993) Interleukin-1 receptor antagonist. Adv Immunol 54:167-227.
47. Symons JA, Young PR, Duff GW (1995) Soluble type II interleukin 1 (IL-1) receptor binds and blocks processing of IL-1 beta precursor and loses affinity for IL-1 receptor antagonist.

Proc Natl Acad Sci USA 92:1714-1718.
48. Sims J, Gayle M, Slack J, Alderson M, Bird T, Giri J, Colotta FR, Mantovani A, Shanebeck K, Grabstein K, Dower S (1993) Interleukin-1 signaling occurs exclusively via the type 1 receptor. Proc Natl Acad Sci USA 90:6155-6159.
49. Schmid P, Cox D, McMaster GK, Itin P (1993) In situ hybridization analysis of cytokine, proto-oncogene and tumour suppressor gene expression in psoriasis. Arch Dermatol Res 285:334-340.
50. Auron PE, Webb AC (1994) Interleukin-1. A gene expression system regulated at multiple levels. Eur Cytokine Netw 5:573-592.
51. Rosbotham JL, Barker JNWN, Trembath RC (1995) Exclusion of interleukin-1 receptor antagonist as a primary disease determinant for psoriasis. J Invest Dermatol 104:306.

Chapter 3.2

ENHANCED PRODUCTION OF BIOLOGICALLY ACTIVE IL-1α AND IL-1β BY PSORIATIC EPIDERMAL CELLS *EX VIVO*. Evidence of increased cytosolic IL-1β levels and facilitated IL-1 release.*

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ABSTRACT

The expression of IL-1α and IL-1β is altered in psoriatic lesions. However, little is known about the actual production of IL-1 by psoriatic epidermal cells (EC). We monitored IL-1 in the extracellular, the membrane and the intracellular compartment of freshly isolated EC from untreated lesional psoriatic (PP) and normal control (NN) skin during non-stimulated, short-term primary cultures, representing an *ex vivo* psoriasis model. Cytokines were measured using bioassays combined with neutralizing Ab and ELISA in parallel. PP EC released significantly increased amounts of biologically active IL-1α and IL-1β in a ratio of 3:1, whereas NN EC only released IL-1α. Also the release of IL-6, but not of TNF-α, by PP EC was significantly increased. Membrane-associated IL-1 activity, analyzed using glutaraldehyde fixed EC, was low and not unique for PP EC. The cytosol of PP EC contained significantly increased levels of immunoreactive IL-1β. Furthermore, PP EC displayed loss of membrane integrity, as determined by trypan blue exclusion and release of cytosolic lactate dehydrogenase. This facilitated release of intracellular IL-1. Depletion of $CD45^+$ cells showed that intra-epidermal leukocytes did not contribute to the production of IL-1. Our observations show that resident PP EC express enhanced IL-1 production *ex vivo*, which is due to an increased cytosolic IL-1β content and facilitated IL-1 release. This study provides the first evidence that PP EC can produce bioactive IL-1β.

INTRODUCTION

Human epidermal interleukin 1 (IL-1) is safely stored in the cytosol of the keratinocyte and stimuli (e.g. cell injury, T cell interaction) are required for its release (1). Intracellularly stored IL-1 consists of pro-IL-1α and pro-IL-1β, which both lack a signal peptide but differ in the way they are processed and modified after translation (2). Pro-IL-1α is fully biologically active, whereas pro-IL-1β has

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minimal biological activity (3). Cultured normal human keratinocytes are unable to enzymatically cleave pro-IL-β efficiently to the active mature form (4). This implies that in normal human epidermis all IL-1 activity is due to IL-1α (5).

The epidermal expression of IL-1α and IL-1β is altered in psoriasis. IL-1α protein is reported to be decreased in cytosolic extracts of psoriatic skin (PP) specimens and aqueous extracts of the stratum corneum, resulting in a considerable decrease of the total epidermal IL-1 activity (6-8). IL-1α levels were low but not decreased in suction blister fluids from lesional psoriatic skin (9). In contrast to IL-1α, cytosolic extracts of PP skin contained increased levels of a processed but non-functional form of IL-1β (6,7). IL-1β levels were negligible in extracts of the stratum corneum and blister fluids (8,9) and its expression seemed confined to the plasma membrane and the intracellular compartment of epidermal cells (EC) (10,11). The downregulation of IL-1α and upregulation of IL-1β found with cytosolic skin extracts was not observed using immunohistochemistry (10). Conflicting reports also exist on the expression of the IL-1-related cytokines IL-6 and tumor necrosis factor-α (TNF-α) in psoriatic epidermis (8,12,13). Taken together, available data are complex and inconsistent due to detection of cytokines in different cellular compartments and at different levels of expression. To clarify the altered expression of epidermal cytokines in psoriasis, the focus of the present study was to characterize the actual production of IL-1, IL-6 and TNF-α by PP EC.

We investigated the spontaneous release of these cytokines during short-term cultures of freshly isolated EC from untreated PP and normal control (NN) skin, using a defined low calcium basal medium. We considered these cultures an *ex vivo* model which is less prone to *in vitro* artefacts than cell lines. To monitor the epidermal IL-1 production in psoriasis, the extracellular, the membrane and the cytosolic compartment of the same EC samples were analyzed for IL-1. Moreover, EC samples were assayed for several cytokines simultaneously and for different levels of protein expression in parallel. We tested for biologically active and immunoreactive IL-1α, IL-1β, IL-6 and TNF-α.

MATERIALS AND METHODS

Patients and controls

Thirty-five otherwise healthy patients (Outpatient Department of Dermatology, University Hospital Rotterdam-Dijkzigt) with plaque-type psoriasis were studied after informed consent. Patients remained untreated for at least 3 weeks before entering the study. Thirty-five healthy volunteers, individuals without history or signs of skin disease, undergoing abdominal or breast plastic surgery (Sint Franciscus Hospital, Rotterdam) served as controls. Skin samples were collected after approval of the institutional medical ethical committee (MEC 104.050/SPO/1990/30).

EC suspensions

Split-skin specimens from PP and NN skin of 10-15 cm^2 were obtained using a portable dermatome (Davol Inc., Cranston, RI). Preparation of single EC suspensions was based on standard methods (14). Cells were resuspended in a chemically defined keratinocyte basal medium (KBM/unsupplemented MCDB 153, Clonetics, San Diego, CA). Cells were counted microscopically using phase-contrast illumination. Viability was determined by trypan blue exclusion.

EC-conditioned media

EC were seeded at 10^6 viable cells/ml in 2 ml KBM in 25 cm^2 vented culture flasks (Costar, Cambridge, MA). After culture for 24 h, the viability of the cells was checked and supernatants were collected by centrifugation, filter-sterilized, supplemented with 0.1 % BSA and stored at -80 °C.

Fixation of EC

Freshly isolated EC or EC cultured for 24 h, were fixed with freshly prepared 0.08 % glutaraldehyde (Merck, Schuchardt, FRG) in 0.1 M sodium cacodylate buffer (pH 6.9) for 10 sec at 4 °C. Subsequently, cells were washed twice with Hepes-buffered RPMI 1640 medium containing 0.5 % BSA. Next, cells were resuspended at 10^6/ml in RPMI medium and incubated for 24 h at 37 °C in a humidified atmosphere to release unfixed IL-1. Release supernatants and cells were collected separately and stored at 4 °C.

Isolation of EC cytosol

EC were treated with digitonin as described for human monocytes (15). Digitonin treatment perforates the plasma membrane without affecting intracellular organelles resulting in leakage of pure cytoplasm. Digitonin and ethanol were removed from the cytosols by ultrafiltration of PBS-diluted samples using a YM membrane with a 10-kDa cut-off (Centricon-10, Amicon, Danvers, MA). After filter-sterilization, samples were stored at -80 °C. Lactate dehydrogenase (LDH) served as a marker for cytosol. Enzyme activity was determined by the rate of nicotinamide adenine dinucleotide formation measured spectrophotometrically from the decrease in extinction at 340 nm.

Bioassays

IL-1 activity was measured using a subline of the murine T cell line D10.G4.1, designated D10(N4)M (D10) (kindly provided by Dr. S.J. Hopkins, Manchester, UK) (16). Further optimization of the assay at our laboratory resulted in enhanced reproducibility of the test (17). IL-6 activity was measured using the murine hybridoma cell line B9 (kindly donated by Prof. Dr. L.A. Aarden, Amsterdam, The Netherlands) (18). Proliferation of the cytokine dependent cell lines D10 and B9 was measured via [^{3}H]deoxythymidine incorporation. TNF-α activity was measured using the murine fibroblast cell line WEHI 164.13 (kindly provided by Dr. W.A. Buurman, Maastricht, The Netherlands) (19). The MTT cytotoxicity assay (20) served as an indirect

measurement of necrosis of WEHI cells. Recombinant human IL-1β (UBI, Lake Placid, NY), IL-6 (Prof. Dr. L.A. Aarden) and TNF-α (UBI) served as positive controls in the D10, B9 and WEHI assay, respectively. Cytokine activities were corrected for background activity of the culture medium and expressed in U/10^6 viable cells, with 1 U/ml corresponding with the half-maximal response.

Neutralization experiments using the D10 assay were performed with sheep-anti-IL-1α and/or IL-1β antibodies (Glaxo, Geneva, Switzerland). After addition of the antibody to the optimally diluted samples and incubation for 45 min at 37 °C and 5 % CO_2, the D10 cells were added. The anti-IL-1α and IL-1β antibodies, used at a titer of 15,000, neutralized about 10 U/ml natural NN EC-derived IL-1α and peripheral blood mononuclear cell (PBMC)-derived IL-1β activity, respectively. Neutralization results are given as the percentage residual IL-1 activity and were calculated using the formula:

$$\text{Residual IL-1 activity (\%)} = \frac{\text{cpm neutralized sample - cpm background}}{\text{cpm non-neutralized sample - cpm background}} \times 100\ \%$$

ELISA

IL-1α, IL-1β and IL-6 were measured with commercially available specific enzyme-linked immunosorbent assays (ELISA) (Mr. D. Bergman, Eurogenetics, Tessenderlo, Belgium) using the protocols provided by the manufacturers. TNF-α was determined as described elsewhere (21), with minor adaptations to enhance the sensitivity. Recombinant human IL-1α, IL-1β, IL-6 and TNF-α served as positive controls. The amounts of cytokine were corrected for non-specific binding of the culture medium, and expressed in pg/10^6 viable cells.

Depletion of $CD45^+$ cells from EC suspensions

Leukocytes were removed from freshly isolated PP EC by an immunomagnetic rosetting technique using the anti-CD45 monoclonal antibody 2D1 (Becton Dickinson, Mountain View, CA) and goat-anti-mouse conjugated paramagnetic beads (Dynal, Oslo, Norway), as previously described (22). Before and after depletion the number of $CD45^+$ cells were determined using a two-step immunoperoxidase technique on cytocentrifuge preparations. The percentage of $CD45^+$ cells was calculated by counting the number of cells showing positive staining from a total of 1000 cells. The depletion efficiency ranged from 95 % to 99 %.

Statistical analysis

Results were analyzed with the Wilcoxon Rank Sum Test using STATA™ (Computing Resource Center, Los Angeles, CA). Significant differences are indicated by P values smaller than 0.05.

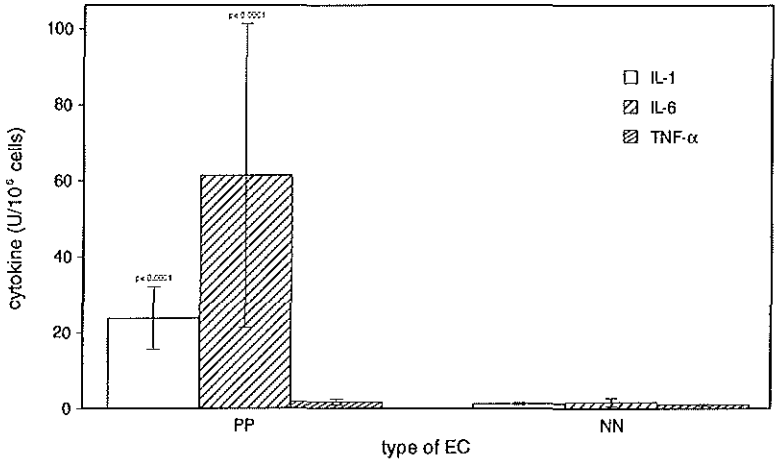

Figure 1. Release of biologically active cytokines by PP and NN EC in basal medium. Levels of IL-1, IL-6 and TNF-α in 24 h supernatants of KBM cultures were measured using bioassays. Results are given in mean ± SEM U/10^6 viable cells. PP: n = 29, 20 and 8; and NN: n = 32, 19 and 12 for IL-1, IL-6 and TNF-α, respectively. The P values indicate significant differences versus NN samples. Human PBMC isolated by Ficoll-Hypaque density gradient centrifugation and cultured for 24 h in RPMI medium supplemented with 5 % human serum were used as a control. PBMC released 52 U IL-1; 24,816 U IL-6; and 14 U TNF per 10^6 cells.

RESULTS

PP EC release increased amounts of biologically active IL-1 and IL-6 but not TNF-α

The amounts of cytokine released by EC from PP and NN skin are given in Figure 1. From this figure it is evident that although NN EC release some units of IL-1, IL-6 and TNF-α under conditions *ex vivo*, PP EC release at least fifteenfold more IL-1 (24 ± 8 U/10^6 EC, $P < 0.0001$) and thirtyfold more IL-6 (61 ± 40 U/10^6 EC, $P < 0.0001$). However, PP EC did not release increased amounts of bioactive TNF-α.

PP EC release biologically active IL-1β

The same samples were tested in the D10 assay using IL-1 neutralizing antibodies as well as in IL-1α and IL-1β ELISA. The effect of neutralizing antibodies against IL-1α and IL-1β on the IL-1 activity of EC supernatants is given in Figure 2. When NN samples were used, anti-IL-1α antibody reduced the [^{3}H]deoxthymidine incorporation to background levels, in contrast to anti-IL-1β antibody that did not affect the D10 proliferation (Fig 2A). Thus, the IL-1 activity of NN samples is completely due to IL-1α. The IL-1 activity of supernatants of PP EC, however, could only be completely blocked when both antibodies were used simultaneously. The residual IL-1 activity of supernatants of PP EC neutralized with either antibody differed significantly from those of NN EC ($P < 0.03$ and $P < 0.05$ for neutralization of IL-1α and IL-1β activity,

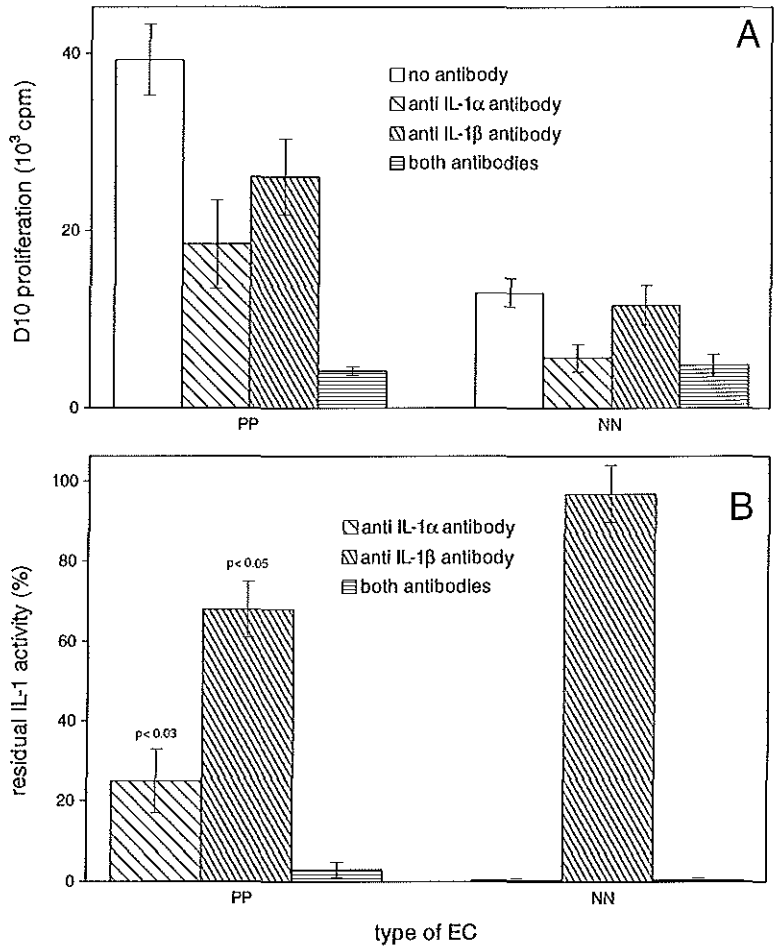

Figure 2. PP EC release biologically active IL-1β. Neutralization of IL-1 activity of 24 h KBM supernatants of EC from PP and NN skin was determined with sheep-anti-IL-1α and/or IL-1β antibodies using the D10 assay. Samples were diluted 5 times. Results are given in mean ± SEM cpm (Fig A). PP and NN groups consisted of 14 and 6 patients, respectively. Controls: recombinant human IL-1β (12.5 U/ml): 42,648 cpm; KBM: 6,967 cpm; anti-IL-1α antibody: 6,874 cpm; anti-IL-1β antibody: 5,671 cpm; and both antibodies: 4,316 cpm. Results are also expressed as the residual IL-1 activity after neutralization (B), calculated as described in materials and methods. Shown are mean ± SEM. Conditioned medium of PBMC was used as a control. See legend to Figure 1 for more details. Neutralization of PBMC supernatants with anti-IL-1α antibody and anti-IL-1β antibody resulted in 97 % and 3 % residual IL-1 activity, respectively.

respectively) (Fig 2B). Approximately 25 % of the IL-1 activity of supernatants of PP EC was due to IL-1β. Supernatants of NN EC contained negligible IL-1β activity. The use of anti-IL-6 antibody as a control had no effect in this system.

The release of IL-1β by PP EC was confirmed using IL-1α and IL-1β ELISA.

Figure 3 shows that both EC types released predominantly IL-1α under non-stimulated short-term culture conditions, with the amounts highest for PP EC (134 ± 45 versus 37 ± 12 pg/10^6 EC, $P < 0.009$). The amounts of immunoreactive IL-1β released by PP EC were about threefold lower than the corresponding amounts of IL-1α (40 ± 14 pg/10^6 EC), but nevertheless significantly increased when compared to the amounts of IL-β released by NN EC ($P < 0.0001$). These ELISA findings are in accordance with the IL-1 neutralization results, indicating that the increased basal release of bioactive IL-1 by PP EC is due to both IL-1α and IL-1β, which are released in a ratio of 3:1. ELISA results for IL-6 and TNF-α are also given in Figure 3, and are in accordance with the data on IL-6 and TNF-α activity presented in Figure 1. Only levels of immunoreactive IL-6 in supernatants of PP EC were significantly increased ($P < 0.0007$).

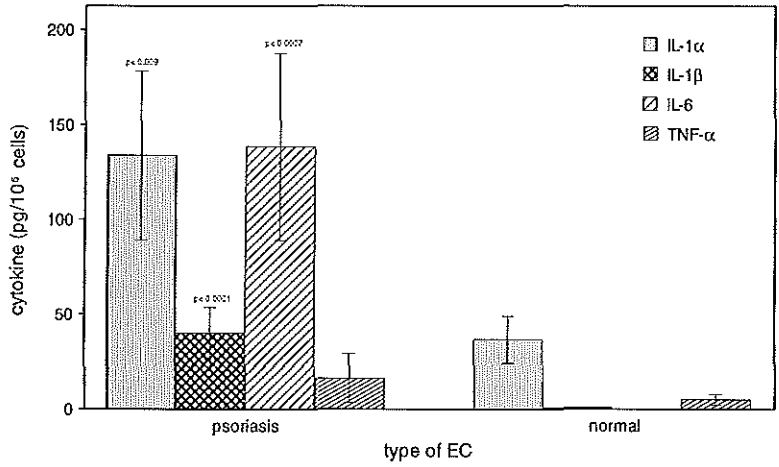

Figure 3. Release of immunoreactive cytokines by PP and NN EC in basal medium. Levels of IL-1α, IL-1β, IL-6 and TNF-α were measured using ELISA. Results are presented as mean ± SEM pg/10^6 viable cells. PP: n = 19, 24, 12 and 8; and NN: n = 20, 19, 11 and 8 for IL-1α, IL-1β, IL-6 and TNF-α, respectively. Conditioned medium of PBMC contained 151 pg IL-1α; 137 pg IL-1β; 70,950 pg IL-6; and 677 pg TNF-α per 10^6 cells.

PP EC do not express membrane IL-1 activity

To study the interrelationship between the increased release of IL-1 and the previously observed increased membrane expression of IL-1β on PP EC (11), the same EC samples were tested for membrane IL-1 activity. EC were fixed with glutaraldehyde and tested in the D10 assay. The possibility that the measured IL-1 had leaked out of the fixed cells instead of representing genuine membrane IL-1 activity, was checked by testing the release supernatants of the fixed cells. These supernatants contained less than 1 U/10^6 cells. Results on IL-1 activity of fixed PP and NN EC samples are shown in Table I. Fixation of freshly isolated PP and NN EC resulted in negligible IL-1 activities. Both types of EC slightly

Table I. IL-1 activity of fixed PP and NN EC.[a]

	Psoriasis	Normal control	P value
Directly fixed	0.9 ± 0.9	1.3 ± 1.3	NS
Fixed after short-term culture	7.2 ± 4.1	9.0 ± 6.9	NS
Unfixed control	41.3 ± 19.5	4.5 ± 1.9	< 0,05

[a] Results are presented as mean ± SEM U IL-1 per 10^6 treated EC. Each experimental condition was performed with 5 to 10 samples of both PP and NN EC. PBMC used as control cells expressed 0, 121 and 267 U IL-1 per 10^6 cells when directly fixed, fixed after short-term culture and unfixed, respectively.

upregulated the expression of IL-1 activity during a 24 h culture in KBM. The levels of IL-1 activity found with unfixed PP EC were significantly increased dueto release of IL-1.

PP EC express increased levels of cytosolic IL-1β

To investigate the intracellular compartment, cell samples were treated with digitonin. The cytosolic fractions were tested in the D10 assay and in the IL-1α and IL-1β ELISA in parallel. The efficiency of the ultrafiltration procedure was more than 95 % as evaluated by the recovery of IL-1 from PBS spiked with 10 U IL-1. Figure 4A shows that the cytosolic IL-1 activity of freshly isolated PP and NN EC was negligible. Short-term culture resulted in an increase of intracellular IL-1 activity in both EC types. However, this increase of intracellular IL-1 activity was only statistically significant for NN EC ($P < 0.05$). In addition, levels of immunoreactive cytosolic IL-1α were significantly upregulated in NN EC during a 24 h culture period ($P < 0.02$) (Fig 4B). The amounts of cytosolic IL-1β were negligible in NN EC. In freshly isolated PP EC, the cytosolic IL-1α levels were somewhat higher than those of NN EC. The cytosolic IL-1β levels were significantly increased in both freshly isolated ($P < 0.002$) and cultured PP EC ($P < 0.03$) when compared to NN EC. However, intracellular IL-1α and IL-1β levels did not significantly increase in PP EC during short-term culture (Fig 4B).

PP EC show facilitated release of IL-1 due to enhanced cytosolic leakage

Viabilities of freshly isolated EC from PP and NN skin, as determined by trypan blue dye exclusion, were similar. However, loss of viability during a 24 h culture in KBM was more than twofold higher for PP EC than for NN EC ($P < 0.0004$), as shown in Figure 5A. This loss of membrane integrity of PP EC correlated well with cytosolic leakage, monitored via release of LDH. The release of LDH from PP EC during a 24 h culture period was about twofold higher when compared to NN EC (Fig 5B, $P < 0.007$). This also applied to the concomitant decrease in cytosolic LDH. Release of cytosolic LDH correlated with that of IL-1.

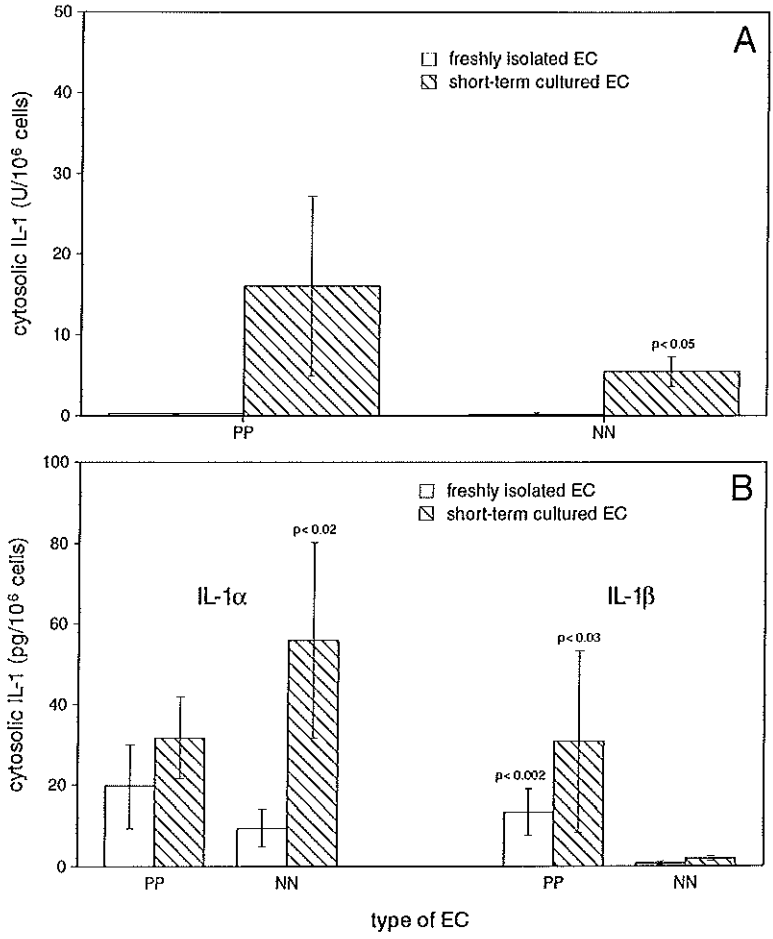

Figure 4. PP EC express an increased cytosolic IL-1β content. Levels of biologically active IL-1 (Fig A) and immunoreactive IL-1α and IL-1β (B) in cytosolic fractions of freshly isolated EC and short-term cultured EC from PP and NN skin are given in mean ± SEM U or pg/10^6 total cells, respectively. PP: n = 12 and 8; and NN: n = 6 and 6 for freshly isolated EC and short-term cultured EC, respectively. Cytosolic fractions were obtained using digitonin. Samples were tested with the D10 assay, IL-1α and IL-1β ELISA. The P values placed above bars of PP samples indicate significant differences between PP and the corresponding NN samples, whereas the P values placed above bars of short-term cultured NN samples indicate significant differences between short-term cultured and freshly isolated NN samples. Short-term cultured PP samples did not differ significantly from freshly isolated PP samples. Freshly isolated and short-term cultured PBMC displayed 0 U IL-1; 11 pg IL-1α; and 2 pg IL-1β, and 109 U IL-1; 688 pg IL-1α; and 1655 pg IL-1β intracellularly per 10^6 total cells, respectively.

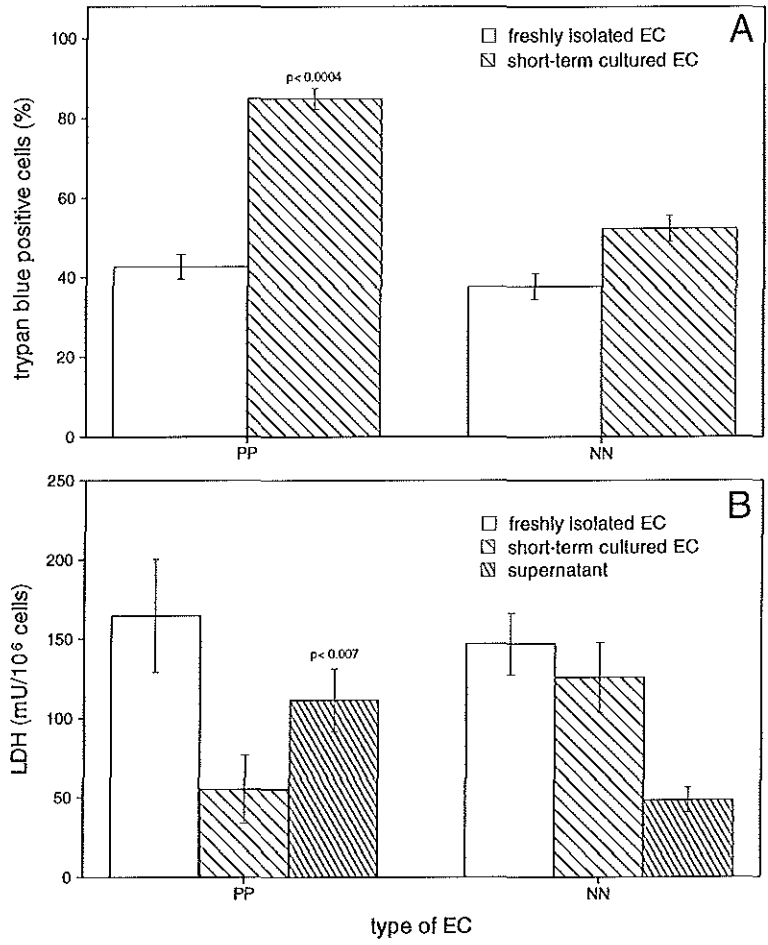

Figure 5. PP EC display enhanced cytosolic leakage. Loss of cellular viability during a 24 h culture of PP and NN EC in KBM was measured by trypan blue exclusion (Fig A). Given are mean ± SEM % trypan blue positive cells of freshly isolated EC and short-term cultured EC. PP: n = 11 and 8; and NN: n = 10 and 10 for freshly isolated EC and short-term cultured EC, respectively. Part of these samples, together with the corresponding conditioned media (termed supernatant) were used to measure cytosolic and released LDH activity (B). Cytosolic fractions were obtained by digitonin treatment of the cells. The LDH activity is given in mean ± SEM mU/10^6 total cells. PP: n = 8, 5 and 8; and NN: n = 5, 5 and 10 for freshly isolated EC, short-term cultured EC and supernatants, respectively. PBMC did not show alterations in their viability using culture conditions as described in the legend to Figure 1. LDH levels corresponding with freshly isolated PBMC, short-term cultured PBMC and supernatants were 46, 100 and 7 mU/10^6 cells, respectively.

Production of IL-1 by PP EC is not due to contaminating intra-epidermal CD45$^+$ cells

Immunophenotyping of freshly isolated PP EC revealed that these cells

Table II. CD45$^-$ EC are the cellular source for IL-1 in psoriasis.[a]

	Experiment I	Experiment II
Total EC	57,550	36,444
CD45$^-$ EC	54,325	32,110
Medium	6,530	5,420

[a] D10 proliferation rates of two separate CD45 depletion experiments are given in cpm (mean of triplicate values with SEM less than 5 % of mean), corresponding with 5 times-diluted samples. The depletion efficiency in both experiments was more than 95 %.

contain about 5 % CD45$^+$ cells as well as HLA-DR$^+$ cells (14). To determine whether intra-epidermal leukocytes were responsible for the PP EC-derived IL-1, CD45$^+$ cells were immunomagnetically depleted before measuring the IL-1 production. From Table II it is evident that CD45$^+$ cells were not responsible for the enhanced production of biologically active IL-1. The presence of IL-1β in supernatants of CD45-depleted EC was confirmed by neutralization studies as well as ELISA.

DISCUSSION

In the present study we show for the first time, using a large number of patients and controls, that PP EC release significantly increased amounts of bioactive IL-1. These results extend preliminary data previously reported by our group (17). Other investigators, however, observed decreased epidermal IL-1 bioactivity in psoriasis (7,8). The reported downregulation of IL-1α and upregulation of a non-functional IL-1β *in vivo* (7) made us investigate the separate contributions of IL-1α and IL-1β to the observed extracellular IL-1 activity. About 25 % of the IL-1 activity of PP samples was due to IL-1β, while the IL-1β activity of NN samples was negligible. This corresponded nicely with the ELISA results. The capacity of PP EC to produce functionally active IL-1β is an unexpected and novel finding. Our observation that PP EC release equivalent amounts of bioactive and immunoreactive IL-1 does not point to the involvement of IL-1 receptor antagonist (IL-1ra) in psoriasis.

Since overexpression of IL-1β in the membrane and the intracellular compartment of PP EC has previously been reported (10,11), we questioned whether PP EC express membrane-associated IL-1 activity. Membrane-associated IL-1 activity has been demonstrated on a variety of cell types, including keratinocytes (23). Recently, it has been suggested that the assumed membrane IL-1 activity may be caused by the leakage of IL-1 from cells inadequately fixed with paraformaldehyde (24). In our studies on membrane IL-1 activity, glutaraldehyde fixed EC were tested in the D10 assay. Glutaraldehyde proved to be a more stringent fixative than paraformaldehyde and abrogated leakage of intracellular IL-1. Our results do not provide evidence of a unique expression of membrane-bound IL-1 by PP EC.

Analysis of the cytosolic compartment indicated that PP EC have a significantly increased cytosolic IL-1β content and that newly formed IL-1α and IL-1β are not retained intracellularly under conditions *ex vivo*. The opposite is true for NN EC which express negligible levels of cytosolic IL-1β, accumulate IL-1α synthesized *de novo* but do not release it *ex vivo* (Figs 3 and 4B). Antibodies directed against the mature IL-1 isoforms often underestimate the actual intracellular pro-IL-1 levels due to incomplete crossreactivity. This underlines the significance of the differences found at the cytosolic level with the IL-1 ELISA. Using freshly isolated EC, we could not confirm the decreased expression of cytosolic IL-1α protein in extracts from PP skin as reported by others (6,7). Our findings of increased expression of intracellular IL-1β protein are in accordance with other reports (6,7,11). In line with our results is also the recent report of increased expression of IL-1β mRNA in lesional as well as non-lesional psoriatic skin (25).

Loss of membrane integrity may be associated with processing and secretion of IL-1. In this respect, ionic perturbation could be necessary for activation of the IL-1β converting enzyme (ICE) (26). The issue whether membrane processes are involved in the production of biologically active IL-1β by PP EC was addressed. We demonstrated profound loss of membrane integrity and enhanced cytosolic leakage of PP EC under conditions *ex vivo* using trypan blue exclusion, flow cytometric analysis of propidium iodide-labeled cell samples (not shown), and measurement of LDH release. Thus, release of intracellularly stored proteins such as IL-1 is facilitated by cytosolic leakage in PP EC. For human monocytes, it has already been shown that the release of IL-1 paralleled that of LDH (27). IL-1β processing may precede the secretion process, as previously suggested by Perregaux and others for monocytic cells (26). Moreover, rapid apoptosis, as in macrophages that are targets for allospecific cytotoxic T cells, is also characterized by intracellular IL-1β processing (28). The partial IL-1-neutralizing effect of anti-IL-1β antibody using cytosolic PP samples suggests that pro-IL-1β is processed intracellularly by PP EC. This is in accordance with the finding that intracellular levels of bioactive IL-1 correlate positively with the intracellular levels of immunoreactive IL-1β (not shown). In our laboratory, we have recently investigated to what extent necrosis and apoptosis contribute to the observed processing of cytosolic IL-1β (chapter 3.3).

The enhanced IL-1 production by PP EC could be attributed to contaminating intra-epidermal leukocytes. To identify the potential contribution of bone-marrow-derived cells, we tested PBMC and a variety of cell lines such as a B cell line (JY), a T cell line (HSB) and a myelomonocytic cell line (HL-60) using serum containing media with and without lipopolysaccharide (LPS) as a stimulus (not shown). These experiments showed that PBMC, as expected, are a prominent source of IL-1 in all cellular compartments. Nevertheless, results of the depletion experiments indicate that $CD45^-$ EC were responsible for the observed IL-1 production *ex vivo*. These results were confirmed by testing normal human PBMC for their IL-1 levels under similar conditions *ex vivo*. Extracellular as well as intracellular IL-1 levels were not detectable when PBMC

were cultured in the basal medium KBM at 10^4/ml and 10^5/ml, reflecting 1 % and 10 % potential leukocyte contamination of EC samples, respectively. Our results suggest the existence of an IL-1 convertase associated with resident PP EC, which up to now is thought to be confined to myelomonocytic cells. This could be in line with reports on enzyme alterations in PP EC (29).

Bioassay and ELISA findings showed that PP EC also released increased amounts of IL-6 but not TNF-α. Our IL-6 results are in agreement with findings based on skin biopsies (12) and blister fluids (11), but in disagreement with findings based on extracts of the stratum corneum (8). We found low, but consistent levels of biologically active and immunoreactive TNF-α in supernatants of both types of EC, while others could not detect TNF-α activity using extracts of the stratum corneum or blister fluids from either PP or NN skin (8,13). Our findings may be attributable to the use of viable EC to investigate cytokine production levels.

Cytokine production measured *ex vivo* is not merely based on the passive release of preformed cytosolic proteins (e.g. IL-1), and is, in our view, closely related to the cytokine production *in vivo* for the following reasons. First, upregulation of cytosolic IL-1 proteins (Fig 4, NN EC) and production of functionally active IL-1β during short-term culture (Fig 2, PP EC) indicate active synthesis and processing of IL-1. Second, the release of increased amounts of IL-6, but not TNF-α, by PP EC point to selective cytokine synthesis *de novo*. Third, the reported upregulation of cytosolic IL-1β by normal human keratinocytes *in vitro* (30) was not observed in our model, and the increased expression of cytosolic IL-1β in PP EC is in agreement with reported findings *in vivo* (7,25). Finally, treatment of psoriatic lesions *in vivo* with a class III topical corticosteroid resulted in normalization of the amounts of IL-1 and IL-6 released by PP EC under conditions *ex vivo* (17).

Taken together, the results provide clear evidence that PP EC express enhanced production of functionally active IL-1α and IL-1β *ex vivo* due to enhanced release of IL-1 and activation of cytosolic IL-1β concomitant with its release. PP EC also release increased levels of IL-6, but not TNF-α. Others have shown that normal human keratinocytes need a stimulus to produce substantial amounts of inflammatory cytokines *in vitro* (31). Furthermore, 48 h serum-free culture supernatants of PP EC, but not NN EC, potentiate T cell activation which was shown to be partly due to IL-1 (32). The pathophysiological relevance of IL-1 *in vivo* is evidenced by transgenic mice in which EC-derived IL-1 induced scaly inflammatory skin lesions with marked erythema, crusting and psoriasis-like histological features such as hyperkeratosis and a dense inflammatory infiltrate (33). Mice which express an IL-6 transgene in basal keratinocytes demonstrated a thickened stratum corneum, but IL-6 did not exert direct proinflammatory actions and probably acts synergistically with EC-derived IL-1 (34). We speculate that PP EC display an activated state *in vivo* and are easily triggered to express an enhanced production of IL-1 and IL-6.

ACKNOWLEDGEMENT. We thank Prof. Dr. T. van Joost for his advice, Dr. J. Lindemans for the LDH measurements and Dr. H.F.J. Savelkoul and Dr. W.A. Buurman for critically reading the manuscript. Dr. W.A. Buurman is also thanked for providing the antibodies used in the TNF-α ELISA. Miss A.C. De Vries and Miss P. Assems are thanked for typing the manuscript and Mr. T.M. van Os for preparing the figures.

REFERENCES

1. Kupper TS (1990) The activated keratinocyte: a model for inducible cytokine production by non-bone marrow derived cells in cutaneous inflammatory and immune responses. J Invest Dermatol 94:146S-150S.
2. Oppenheim JJ, Kovacs EJ, Matsushima K, Durum SK (1986) There is more than one interleukin 1. Immunol Today 7:45-56.
3. Mosley B, Urdal DL, Prickett K, Larsen A, Cosman D, Conlan PJ, Gillis A, Dower SK (1987) The IL-1 receptor binds the human interleukin 1α precursor but not the interleukin 1β precursor. J Biol Chem 262:2941-2944.
4. Mizutani H, Black R, Kupper TS (1991) Human keratinocytes produce but do not process pro-interleukin-1 (IL-1) beta. Different strategies of IL-1 production and processing in monocytes and keratinocytes. J Clin Invest 87:1066-1071.
5. Elder JT, Sartor CI, Boman DK, Benrazavi S, Fisher GJ, Pittelkow MR (1992) Interleukin-6 in psoriasis: expression and mitogenicity studies. Arch Dermatol Res 284:324-332.
6. Gruaz D, Didierjean L, Grassi J, Frobert Y, Dayer J-M, Saurat J-H (1989) Interleukin 1 alpha and beta in psoriatic skin: enzymoimmunoassay, immunoblot studies and effect of systemic retinoids. Dermatologica 179:202-206.
7. Cooper KD, Hammerberg C, Baadsgaard O, Elder JT, Chan LS, Sauder DN, Voorhees JJ, Fisher G (1990) IL-1 activity is reduced in psoriatic skin. Decreased IL-1 alpha and increased nonfunctional IL-1 beta. J Immunol 144:4593-4603.
8. Gearing AJ, Fincham NJ, Bird CR, Wadhwa M, Meager A, Cartwright JE, Camp RD (1990) Cytokines in skin lesions of psoriasis. Cytokine 2:68-75.
9. Takematsu H, Ohmoto Y, Tagami H (1990) Decreased levels of IL-1 alpha and beta in psoriatic lesional skin. Tohoku J Exp Med 161:159-169.
10. Romero LI, Ikejima T, Pincus SH (1989) In situ localization of interleukin-1 in normal and psoriatic skin. J Invest Dermatol 93:518-522.
11. Prens EP, Benne K, Van Damme J, Bakkus M, Brakel K, Benner R, Van Joost T (1990) Interleukin-1 and interleukin-6 in psoriasis. J Invest Dermatol 95:121S-124S.
12. Grossman RM, Krueger J, Yourish D, Granelli-Piperno A, Murphy DP, May LT, Kupper TS, Sehgal PB, Gottlieb AB (1989) Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proc Natl Acad Sci USA 86:6367-6371.
13. Takematsu H, Otta H, Tagami H (1989) Absence of tumor necrosis factor α in suction blister fluids and stratum corneum from patients with psoriasis. Arch Dermatol Res 281:398-400.
14. Prens EP, Benne K, Van Joost T, Benner R (1991) The autologous mixed epidermal cell - T lymphocyte reaction is elevated in psoriasis. A crucial role for epidermal HLA-DR^+/$CD1a^+$ antigen presenting cells. J Invest Dermatol 96:880-887.
15. Bakouche O, Brown DC, Lachman LB (1987) Subcellular localization of human monocyte interleukin 1: evidence for an inactive precursor molecule and a possible mechanism for IL-1 release. J Immunol 138:4249-4255.
16. Hopkins SJ, Humphreys M (1989) Simple, sensitive and specific bioassay of interleukin 1. J Immunol Meth 120:271-276.
17. Debets R, Van Joost T, Benner R, Prens EP (1993) Psoriatic epidermal cells release elevated levels of immunoreactive and biologically active interleukins 1 and 6: modulation of

corticosteroid treatment. In Bernard BA, Schroot B (eds). Pharmacol Skin: From Molecular Biology to Therapeutics. Kärger, Basel, vol 5, pp. 158-166.
18. Aarden LA, De Groot ER, Schaap OL, Lansdorp PM (1987) Production of hybridoma growth factor by human monocytes. Eur J Immunol 17:1411-1416.
19. Espevik T, Nissen-Meyer J (1986) A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumor necrosis factor from human monocytes. J Immunol Meth 95:99-105.
20. Mosmann TJ (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Meth 65:55-63.
21. Debets JMH, Van der Linden CJ, Spronken IEM, Buurman WA (1988) T cell mediated production of tumor necrosis factor by monocytes. Scand J Immunol 27:601-608.
22. Nilsson H, Johansson C, Scheynius A (1987) Removal of Langerhans cells from human epidermal cell suspensions by immunomagnetic particles. J Immunol Meth 105:165-169.
23. Goldminz D, Kupper TS, McGuire J (1987) Keratinocyte membrane-associated epidermal cell-derived thymocyte-activating factor (ETAF). J Invest Dermatol 88:97-100.
24. Suttles J, Carruth LM, Mizel SB (1990) Detection of IL-1α and IL-1β in the supernatants of paraformaldehyde-treated human monocytes: evidence against a membrane form of IL-1. J Immunol 144:170-174.
25. Uyemura K, Yamamura M, Fivenson DF, Modlin RL, Nickoloff BJ (1993) The cytokine network in lesional and lesion-free psoriatic skin is characterized by a T-helper type 1 cell-mediated response. J Invest Dermatol 101:701-705.
26. Perregaux D, Barberia J, Lanzetti AJ, Geoghegan KF, Carty TJ, Gabel CA (1992) IL-1 beta maturation: evidence that mature cytokine formation can be induced specifically by nigericin. J Immunol 149:1294-1303.
27. Hogquist KA, Unanue ER, Chaplin DD (1991) Release of IL-1 from mononuclear phagocytes. J Immunol 147:2181-2186.
28. Hogquist KA, Nett MA, Unanue ER, Chaplin DD (1991) Interleukin 1 is processed and released during apoptosis. Proc Natl Acad Sci USA 88:8485-8489.
29. Schalkwijk J, Van Vlijmen IM, Alkemade JA, De Jongh GJ (1993) Immunohistochemical localization of SKALP/elafin in psoriatic epidermis. J Invest Dermatol 100:390-393.
30. Gatto H, Viac J, Charveron M (1991) Levels of immunoreactive interleukin 1a and 1b in basal, supra-basal and cornified epidermal cell populations of normal human epidermis. Comparison with keratinocytes cultured in defined medium. Eur J Dermatol 1:55-59.
31. Kupper TS (1990) Role of epidermal cytokines. In Shevach E, Oppenheim JJ (eds). Immunophysiology: The role of cells and cytokines in immunity and inflammation, Oxford University Press, New York, pp. 285-305.
32. Chang EY, Hammerberg C, Fisher G, Baadsgaard O, Ellis CN, Voorhees JJ, Cooper KD (1992) T-cell activation is potentiated by cytokines released by lesional psoriatic, but not normal, epidermis. Arch Dermatol 128:1479-1485.
33. Groves RW, Kupper TS (1994) Spontaneous inflammatory skin disease in mice which overexpress IL-1α in basal keratinocytes. J Invest Dermatol 103:442. (Abstr.)
34. Turksen K, Kupper TS, Degenstein L, Williams I, Fuchs E (1992) Interleukin-6: insights to its function in skin by overexpression in transgenic mice. Proc Natl Acad Sci USA 89:5068-5072.

Chapter 3.3

ENHANCED APOPTOSIS IN PSORIATIC EPIDERMAL CELLS. IL-1β maturation is related to apoptosis *ex vivo*.*

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ABSTRACT

We previously demonstrated that non-stimulated, short-term primary cultures of epidermal cells (EC) (*ex vivo* model) from lesional psoriatic (PP) skin secrete functionally active IL-1β in contrast to EC from normal control (NN) skin. The generation of functionally active, mature IL-1β and its secretion depend on IL-1β processing. As IL-1β processing may be a feature of apoptosis, at least in monocytic cells, the present study was conducted to investigate whether IL-1β maturation in PP EC is related to apoptosis. Analysis of intra- and extracellular amounts of IL-1 revealed that the increased secretion of IL-1β by PP EC was due to increased *de novo* synthesis of IL-1β, which was inversely correlated with loss of trypan blue exclusion by EC *ex vivo* (i.e. necrosis). In fact, apoptosis was increased in freshly isolated PP EC relative to NN EC, as measured by TUNEL and ELISA specific for cytosolic internucleosome-sized DNA fragments. Double-staining of cytofuge preparations of PP EC for the CD45 marker showed that intra-epidermal leukocytes were not responsible for the observed DNA end-labeling. In our experiments, DNA fragmentation represented genuine apoptosis as kinetic studies with PP EC showed that DNA fragments were most abundantly present intracellularly, showing peak levels at 8 h, which clearly preceded those of extracellular DNA fragments at 24 h after seeding EC. The amounts of cytosolic and extracellular IL-1β, as measured by ELISA, paralleled those of DNA fragments. Moreover, the use of the D10 bioassay combined with neutralizing antibodies revealed a nice correlation between released biologically active IL-1β and DNA fragmentation in PP EC. In accordance to the findings *ex vivo*, the *in situ* DNA end-labeling was enhanced in PP skin when compared to NN skin, and was localized to the upper stratum granulosum. Although epidermal IL-1β immunoreactivity was generally not detectable, in a few patients IL-1β staining clearly co-localized with DNA end-labeling. Taken together, our studies provide evidence that resident PP EC express enhanced apoptosis and suggest that these cells are a source of mature IL-1β synthesized *de novo*.

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* Submitted for publication

INTRODUCTION

Psoriasis is histologically characterized by skin infiltrating leukocytes and keratinocyte hyperproliferation (1). Human epidermis contains substantial amounts of interleukin 1 (IL-1) (2), and this cytokine is able to profoundly influences cutaneous inflammation (3) and the proliferative state of keratinocytes (4). The epidermal expression of IL-1 is dysregulated in psoriasis (5). We have recently reported that non-stimulated, short-term primary cultures of epidermal cells (EC) (termed *ex vivo* model) derived from lesional psoriatic (PP) skin, but not normal control (NN) skin, produce functionally active IL-1β due to activation of cytosolic IL-1β concomitant with its release (chapter 3.2). IL-1β is synthesized as a 31-kDa pro-form devoid of a conventional signal sequence (6). IL-1β converting enzyme (ICE), a member of a new subfamily of cysteine proteases (7), processes the IL-1β precursor to its biologically active 17-kDa mature form and is also involved in secretion of mature IL-1β (8,9). ICE is activated by autocleavage of pro-ICE, mainly in monocytes, but not normal human keratinocytes (10), and takes part in an amplified protease cascade, which characterizes apoptosis (9,11). In macrophages, apoptosis may be accompanied by the processing and liberation of IL-1β (12-14).

Apoptosis is a gene-directed physiological cell death, which is clearly different from pathological cell death (necrosis) (15). This form of cell death is controlled by the coordinated expression of a number of intracellular B cell lymphoma (BCL)-homologous proteins (e.g. BAX, BCL-2, BCL-X and BAD) (16). The formation of BAX homodimeric complexes activates proteases homologous to ICE resulting in the subsequent activation of ICE and other proteases, which are responsible for the morphological alterations associated with apoptosis (i.e. apoptotic phenotype), whereas the formation of heterodimers with BCL proteins inhibit apoptosis (17). The final common apoptotic pathway causes contraction of the cytoplasmic volume, nuclear DNA cleavage at the internucleosomal linker regions, condensation of chromatin and the formation of membrane-enclosed apoptotic bodies, which are phagocytosed and digested by nearby resident cells or leukocytes and do not elicit any inflammatory reaction (18). In contrast, necrosis is featured by cytoplasmic swelling and leakage of the cellular content as a result of loss of plasma membrane integrity, which leads to inflammation (18). Suprabasal keratinizing keratinocytes of normal human epidermis show condensed cytoplasm and chromatin (19), as well as DNA fragmentation (20), confirming the establishment of the mature apoptotic phenotype. Terminal differentiation of the keratinocyte, accelerated in psoriasis, may be considered a specialized form of the apoptotic program that has evolved to provide a barrier function of the skin (19,21). Reports on the expressions of anti-apoptotic BCL proteins in PP epidermis are inconclusive (both a decrease of BCL-2 in basal keratinocytes and an increase of BCL-X throughout all cell layers were reported (22,23)), suggesting that apoptosis of PP keratinocytes

may be altered. Transglutaminases, Ca^{2+}-dependent effector enzymes of the apoptotic program which catalyze the formation of the keratinocyte cell envelope (24), were increased in PP epidermis and localized to suprabasal cells (22).

In the present study, we addressed the question whether IL-1β maturation in PP EC *ex vivo* is related to apoptosis. DNA fragmentation, representing late apoptosis, was measured by both terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL) and enzyme-linked immunosorbent assay (ELISA) against oligonucleosome-sized DNA fragments, and was found to be significantly increased in freshly isolated PP EC when compared to NN EC. IL-1β maturation, measured by ELISA and bioassay, was monitored *ex vivo* and correlated with apoptosis. The *in situ* TUNEL staining was enhanced in PP epidermis and localized to the suprabasal cell layers. In a few patients epidermal IL-1β immunoreactivity co-localized with TUNEL staining, confirming the notion that apoptotic PP EC produce mature IL-1β.

MATERIALS AND METHODS

Patients and controls

Twenty otherwise healthy patients with plaque-type psoriasis (Outpatient Department of Dermatology, Hospital Walcheren, Vlissingen, The Netherlands, and University Hospital Rotterdam-Dijkzigt, Rotterdam, The Netherlands) were studied after informed consent. Patients remained untreated for at least 3 weeks before entering the study. Ten healthy volunteers, individuals without history or signs of skin disease, undergoing abdominal or breast plastic surgery (Sint Franciscus Hospital, Rotterdam) served as controls. All samples were collected after approval of the institutional medical ethical committee (MEC 104.050/SPO/1990/30).

Skin samples

Split-skin specimens of 10-15 cm^2 and punch biopsies (3 mm) were obtained from PP and NN skin. Preparation of single EC suspensions was performed as previously described (chapter 3.2). Cells were counted microscopically using phase-contrast illumination and viability was determined by trypan blue exclusion. Freshly isolated EC were used to collect cytosol and to make cytofuge preparations.

Isolation of EC cytosol

EC were treated with digitonin as described for human monocytes (25). Digitonin and its solvent iso-propanol were removed from the cytosols by ultrafiltration of PBS-diluted samples using a YM membrane with a 10-kDa cut-off (Centricon-10, Amicon, Danvers, MA). After filter-sterilization, samples were stored at -80 °C.

EC-conditioned media

Freshly isolated EC were seeded at 10^6 viable cells/ml in 2 ml of a chemically defined keratinocyte basal medium (KBM/unsupplemented MCDB 153, Clonetics, San Diego, CA) and cultured for 2, 4, 8, 16 and 24 h at 37 °C and 5 % CO_2 (i.e. EC *ex vivo*). Supernatants were collected by centrifugation, filter-sterilized, supplemented with 0.1 % BSA and stored at -80 °C. Viability of EC *ex vivo* was checked, cytosol was collected and cytofuge preparations were made.

ELISA

IL-1α and IL-1β were measured with commercially available specific ELISA (Mr. M. Kempen, Eurogenetics, Tessenderlo, Belgium) using the protocols provided by the manufacturers. Recombinant human IL-1α and IL-1β served as positive controls. The amounts of cytokine were corrected for non-specific binding of the culture medium, and expressed in pg/10^6 total cells. The cell death detection ELISA was performed to semiquantitatively assess the concentration of mono- and oligonucleosomes in cell lysates according to the manufacturer's protocol (Boehringer Mannheim, Germany). NN EC incubated for 4 h with a hypertonic buffer (10 mM Tris pH 7.4, 400 mM NaCl, 5 mM $CaCl_2$ and 10 mM $MgCl_2$), which induced apoptosis, were used as a positive control. The amounts of DNA fragments were expressed in OD, corrected for non-specific binding of lysis buffer, per 10^5 cell-equivalents.

IL-1 Bioassay

IL-1 activity was measured using a subline of the murine T cell line D10.G4.1, designated D10(N4)M (D10) (kindly provided by Dr. S.J. Hopkins, Manchester, UK) (26). Proliferation of the D10 cells was measured via [^{3}H] deoxythymidine incorporation. Recombinant human IL-1β (UBI, Lake Placid, NY) served as a positive control. Cytokine activity was corrected for background activity of the culture medium and expressed in U/10^6 total cells, with 1 U/ml corresponding with the half-maximal response. Neutralization experiments were performed with sheep-anti-IL-1α and/or IL-1β antibodies (Glaxo IMB, Geneva, Switzerland). After addition of the antibodies to optimally diluted samples, and incubation at 37 °C and 5 % CO_2 for 45 min, the D10 cells were added. The anti-IL-1α and IL-1β antibodies, used at a titer of 5,000, neutralized about 10 U/ml recombinant human IL-1α (Glaxo) and IL-1β, respectively. IL-1α and IL-1β activity are given as the percentage of total IL-1 activity which was neutralized by anti-IL-1α and IL-1β antibody, respectively, and was calculated using the formula:

$$\text{IL-1}\alpha \text{ or IL-1}\beta \text{ activity (\%)} = \left(1 - \frac{\text{cpm neutralized sample - cpm background}}{\text{cpm non-neutralized sample - cpm background}}\right) \times 100\ \%$$

TUNEL assay

The TUNEL assay was performed on cytofuged EC preparations and skin sections, modified from Gavrieli *et al.* (20). EC suspensions, supplemented with a few percent of human serum to reduce background staining, were cytocentrifuged on slides coated with poly-L-lysine (Sigma, St. Louis, MO) using 2.5 x 10^4 cells per slide, which were stored at -70 °C until use. Cytospin preparations were fixed in 1.6 % paraformaldehyde for 10 min at room temperature (RT). Six µm thick cryostat sections of Tissue-Tek ® (Miles Inc., Elkhart, IN) embedded skin biopsies were prepared just before use, air dried, fixed in 4 % paraformaldehyde for 10 min at 4 °C, rinsed in PBS and refixed in ethanol:acetic acid glacial (2:1). Apart from fixation both types of samples were treated similarly. Samples were rinsed in PBS, dried and rinsed with TdT buffer, consisting of 0.1 M $NaC_2H_6AsO_2$, 1 mM $CoCl_2$, 0.1 mM dithiothreitol and 0.005 % BSA (Fraction V, Sigma) (pH 8.6) for 5 min at RT. Samples were dried again, overlaid with 50 µl TdT buffer containing 20 U TdT (Pharmacia LKB, Piscataway, NJ), 20 µM deoxynucleotide triphosphates (dNTPs) and digoxigenin-labeled 11-dUTP (Boehringer Mannheim), covered with slips and incubated in a humid chamber for 1 h at 37 °C. The reaction was stopped in 2 x standard sodium citrate (SSC) for 30 min at 37 °C. After rinsing in PBS, immunological detection was performed using anti-digoxigenin sheep Fab fragments conjugated with alkaline phosphatase (Boehringer Mannheim) and Fast Blue BB Base (Sigma) as the substrate according to the manufacturer's protocols.

Omitting the TdT enzyme from the nucleotide mixture served as a negative control. Positive controls comprised normal human thymus and DNAse I treatment of skin samples to introduce DNA breaks in all nuclei. For this purpose, cytofuge preparations and adjacent skin sections were rinsed in DNAse I buffer, consisting of 10 mM Tris-HCl, 10 mM NaCl, 5 mM $MgCl_2$, 0.1 mM $CaCl_2$ and 25 mM KCl (pH 7.4), incubated in DNAse I buffer containing 10 ng DNAse I (Boehringer Mannheim) in a humid chamber for 15 min at 37 °C and rinsed extensively before end-labeling. The skin samples were analyzed using standard Zeiss 16 microscopes (Carl Zeiss, Oberkochen, Germany). It is of note that staining intensity varied from light, confined to the margin of the nuclear membrane, to strong, involving the complete nucleus. The percentage of TUNEL-positive cells of cytofuge preparations was calculated by counting the number of cells showing a complete nuclear staining from a total of 500 - 1000 cells.

Immunostaining

Cytospins and skin sections were stained for CD45 (2D1 at 50 µg/ml, Becton Dickinson, New Jersey, NJ) and IL-1β (Vhp20 at 10 µg/ml, kindly donated by Prof. Dr. E. Claassen, Erasmus University Rotterdam, The Netherlands). Slides were air dried, acetone-fixed, incubated with the primary monoclonal antibody and stained with the supersensitive horse radish peroxidase kit (Biogenex, San Ramon, CA) using 3-amino-9-ethylcarbazole

(AEC, Sigma) as the substrate. To perform double-stainings for DNA fragments with either CD45 or IL-1β, TUNEL (omitting refixation in case of skin sections) was executed before the AEC substrate step. Finally, slides were mounted in glycerol gelatin (Merck, Darmstadt, Germany). The controls comprised the use of concentration-matched mouse IgG_1 (Becton Dickinson) and omitting the first and second step.

Computational and statistical analysis

The following formulas were used to calculate loss of viability, the release of IL-1 and *de novo* synthesis of IL-1:

$$\text{Loss of viability (\%)} = \frac{(\text{viability}_{t=0\,h} - \text{viability}_{t=24\,h})}{\text{viability}_{t=0\,h}} \times 100\ \%$$

$$\text{Release of IL-1 (\%)} = \frac{\text{supernatant}}{(\text{cytosol}_{t=24\,h} + \text{supernatant})} \times 100\ \%$$

$$\textit{De novo}\text{ synthesis of IL-1 (pg or U/}10^6\text{ cells/24 h)} = \text{cytosol}_{t=24\,h} + \text{supernatant} - \text{cytosol}_{t=0\,h}$$

where viability equals the percentage of trypan blue-negative cells of freshly isolated EC (t = 0 h) and short-term cultured EC (t = 24 h); cytosol represents the intracellular IL-1 levels (pg IL-1α or IL-1β or U IL-1 per 10^6 cells) of freshly isolated EC and short-term cultured EC; and supernatant represents the released IL-1 levels by short-term cultured EC. Only positive values for *de novo* synthesis of IL-1 were analyzed.

Results were analyzed with the Wilcoxon Rank Sum Test (PP versus NN samples) and the Spearman's Rank Correlation Coefficient (r_s) using STATA™ (Computing Resource Center, Los Angeles, CA). Significant differences or correlations are indicated by P values smaller than 0.05.

RESULTS

PP EC express an enhanced *de novo* synthesis of IL-1β

Non-stimulated, short-term primary cultures of PP EC released significantly increased amounts of immunoreactive IL-1α and IL-1β, and biologically active IL-1 into the medium relative to NN EC (Table I). Viabilities of freshly isolated PP and NN EC were similar (i.e. 60 %), whereas viabilities of short-term cultured PP EC were significantly decreased when compared to NN EC (loss of viability: 67 ± 5 % and 24 ± 4 % for PP and NN EC (both n = 11), respectively, $P < 0.0001$). This "cell death" correlated significantly with the calculated release of IL-1 (loss of viability with release of IL-1α: $r_s = 0.67$, $n = 14$, $P < 0.01$; release of IL-1β: $r_s = 0.55$, $n = 16$, $P < 0.025$; and release of IL-1 activity: $r_s = 0.75$, $n = 14$, $P < 0.005$). Intracellular levels

Table I. IL-1 production by PP EC *ex vivo*.[a]

	PP EC	NN EC	P value
IL-1α			
$Cytosol_{t=0\ h}$	19.7 ± 10.4	8.1 ± 4.2	NS
$Cytosol_{t=24\ h}$	31.8 ± 10.2	56.0 ± 24.3	NS
Supernatant	39.1 ± 13.6	1.9 ± 0.6	0.0006
IL-1β			
$Cytosol_{t=0\ h}$	10.2 ± 3.7	0.5 ± 0.2	0.0001
$Cytosol_{t=24\ h}$	27.1 ± 18.0	1.8 ± 0.6	NS
Supernatant	25.4 ± 13.5	0.5 ± 0.2	0.001
IL-1 activity			
$Cytosol_{t=0\ h}$	2.2 ± 1.4	0.2 ± 0.2	NS
$Cytosol_{t=24\ h}$	16.2 ± 8.9	5.5 ± 1.8	NS
Supernatant	18.2 ± 9.4	0.1 ± 0.1	0.0004

[a] The amounts of immunoreactive IL-1α and IL-1β and bioactive IL-1 present in the cytosol of freshly isolated EC (t = 0 h) as well as short-term cultured EC (t = 24 h) from PP and NN skin, and their corresponding supernatants were measured with IL-1α and IL-1β ELISA and D10 assay, respectively. Cytosolic samples were ultrafiltered to remove salts before testing in the D10 assay. Data represent mean ± SEM pg or U IL-1 per 10^5 cells, with the number of observations varying between 10 to 15 for psoriatic patients and between 5 to 10 for normal controls. P values indicate significant differences between PP and NN samples. NS: not significant.

of IL-1β, but not IL-1α and IL-1 activity, were significantly increased in freshly isolated PP EC and showed a trend of increase in short-term cultured PP EC ($P < 0.07$). As a consequence, the percentage of PP EC-derived IL-1 that is released into the medium was significantly increased for IL-1α and IL-1 activity, but not IL-1β, when compared to NN EC (Fig 1A). However, when calculating *de novo* synthesis of IL-1, the *de novo* synthesis of IL-1β by PP EC, but not IL-1α or IL-1 activity, was significantly increased (Fig 1B). In addition, there was a significant inverse correlation between *de novo* synthesis of IL-1β and loss of viability by PP EC ($r_s = -0.73$, $n = 10$, $P < 0.02$).

PP EC, but not intra-epidermal leukocytes, express enhanced DNA fragmentation

To investigate whether PP EC underwent apoptosis, we first analyzed the extent of DNA fragmentation in these cells. Both TUNEL assay performed on cytofuge preparations as well as ELISA specific for oligonucleosome-sized DNA fragments present in EC-derived cytosol showed that freshly isolated PP EC expressed significantly increased amounts of DNA fragments when compared to NN EC (Figs 2 and 3). TUNEL following DNAse treatment showed that the accessibility to TdT is similar in all nuclei of EC (Fig 2B), with no difference observed between PP and NN EC. Double-stainings showed that TUNEL-positive cells expressed a pan-keratin marker (not shown), whereas intra-epidermal leukocytes, constituting about 5 % of PP EC (27), were predominantly TUNEL-negative, as less than 5 % of apoptotic cells expressed the CD45 marker (Fig 4, annex 'Color Plates').

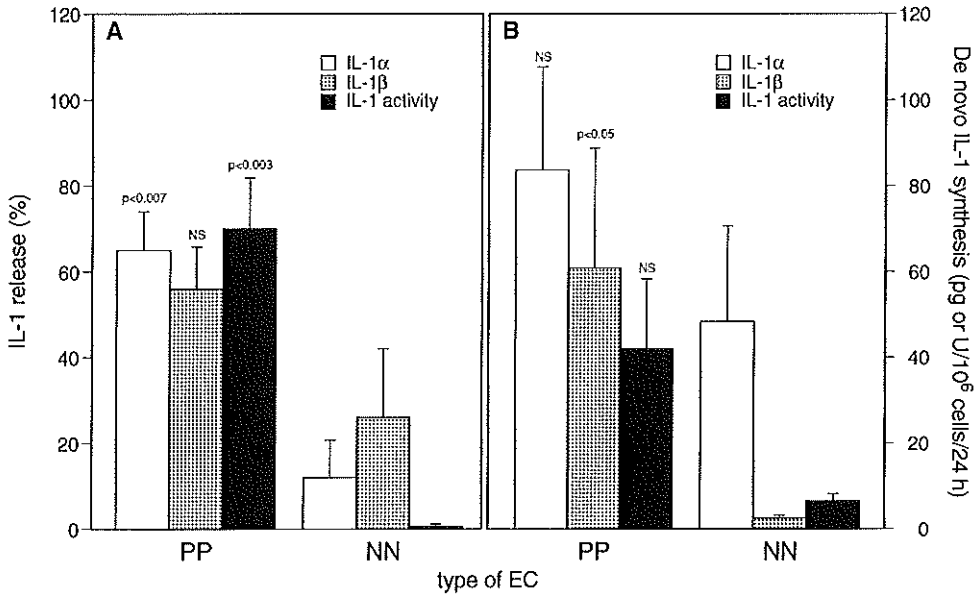

Figure 1. Release and *de novo* synthesis of IL-1 by PP EC *ex vivo*. Computational analyses of the amounts of immunoreactive IL-1α and IL-1β and bioactive IL-1 present in the cytosol of freshly isolated EC as well as short-term cultured EC from PP and NN skin, and in their corresponding supernatants, revealed the individual releases of IL-1 (Fig A) and *de novo* syntheses of IL-1 (B), as described in the materials and methods section. See Table I for details. Bars represent mean ± SEM % and pg or U per 10^6 cells per 24 h for release and *de novo* synthesis of IL-1, respectively. P values indicate significant differences between PP and NN samples.

Processing and release of IL-1β by PP EC is related to apoptosis

To further analyze apoptosis in PP EC and to discern whether it is related to *de novo* synthesis of IL-1β, cells were monitored *ex vivo* for intracellular as well as extracellular levels of DNA fragments, immunoreactive and biologically active IL-1β in parallel. The peak percentage of TUNEL-positive cells was observed at 16 h after seeding PP EC (Fig 5A). ELISA analysis of corresponding EC-derived samples showed that oligonucleosome-sized DNA fragments were predominantly present intracellularly, where they peaked at 8 h, whereas extracellular levels were only maximal at 24 h after seeding EC (Fig 5B). Thus, DNA fragmentation clearly preceded the loss of plasma membrane integrity, which provides evidence for true apoptosis. In addition, the release of DNA fragments into the cytoplasm preceded complete nuclear TUNEL staining, the latter probably representing late apoptosis. Moreover, there was a nice intra-patient correlation between the release of DNA fragments, calculated as described for the release of IL-1 in the materials and methodssection, and the TUNEL staining (r_s = 0.89, n = 6, P < 0.025).
Levels of IL-1β, as measured by ELISA, paralleled those of DNA fragments, with peak levels of intracellular DNA fragments lagging behind those of intracellular IL-1β (Fig 5C). Finally, the use of the D10 assay with neutralizing

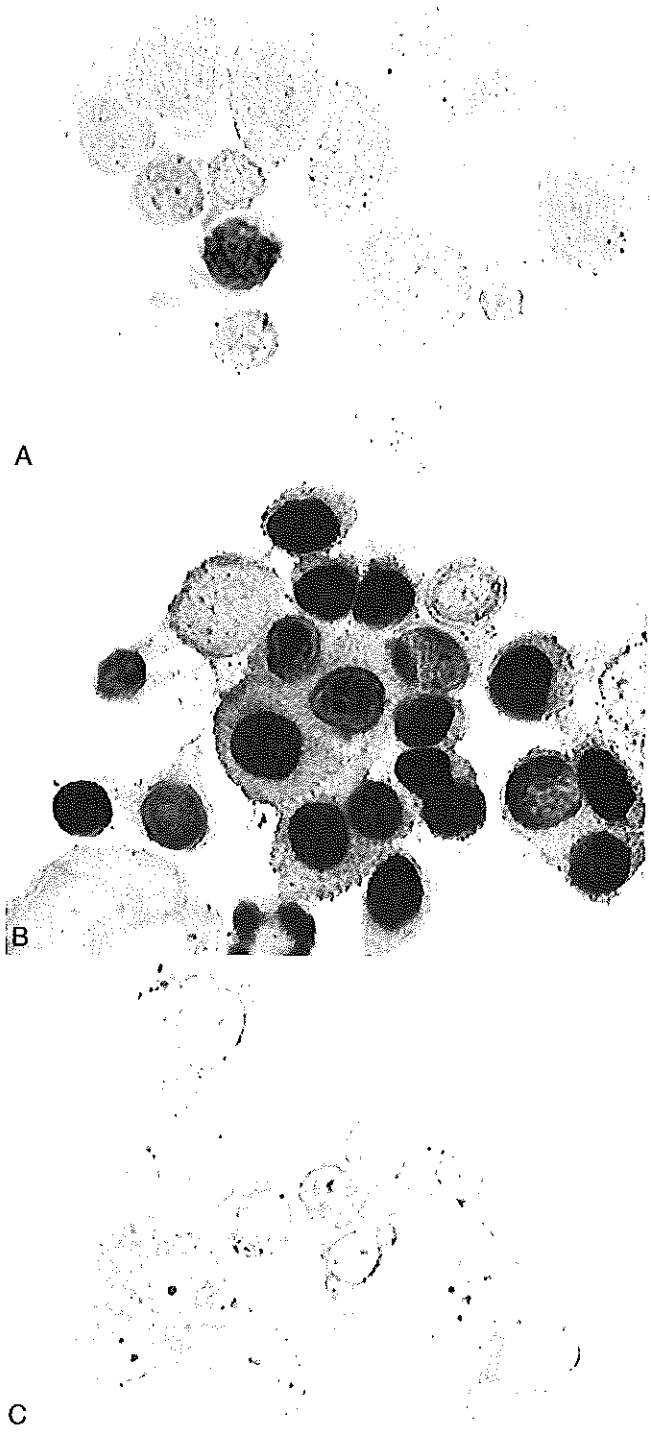

Figure 2.TUNEL staining of PP EC. The TUNEL assay was performed on cytofuge preparations of freshly isolated PP. Controls comprised DNAse I treatment (B) and omitting the enzyme (C). Results of a representative experiment is shown (n = 10). TUNEL staining of NN EC only sporadically revealed a positive cell (n = 5). See also Figure 3. Magn 630 X.

anti-IL-1α and IL-1β antibodies showed that intracellular IL-1β processing occurred at 8 h after EC seeding, which coincided with maximum cellular DNA fragmentation (Fig 5B) as well as total intracellular IL-1 activity (Fig 5D). The releases of DNA fragments, immunoreactive IL-1β and biologically active IL-1 were correlated (both DNA fragments and IL-1 activity with IL-1β: $r_s = 0.83$, $n = 6$, $P < 0.05$, and DNA fragments with IL-1 activity: $r_s = 1.0$, $n = 6$, $P < 0.01$).

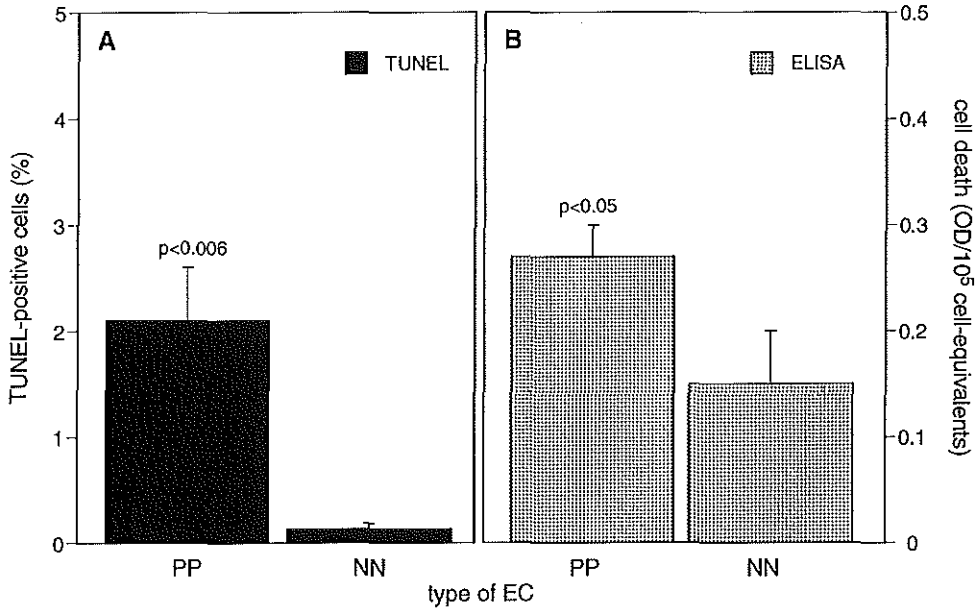

Figure 3. PP EC express enhanced DNA fragmentation. Freshly isolated EC were analyzed for DNA fragmentation using both the TUNEL assay (Fig A) and the cell-death ELISA (B). Results are given in mean ± SEM % positive cells and OD per 10^5 cell-equivalents for TUNEL and ELISA, respectively. Only cells showing a complete nuclear staining were analyzed as TUNEL-positive cells. The number of observations were 10 for PP EC and 5 for NN EC. P values indicate significant differences between PP and NN samples.

PP epidermis expresses enhanced DNA fragmentation which co-localizes with IL-1β to the granular cell layers

We extended our findings to the *in vivo* situation. TUNEL staining of skin sections showed that PP epidermis expressed increased DNA fragmentation when compared to NN epidermis, which was localized to the upper stratum granulosum (Fig 6). Epidermal IL-1β immunoreactivity was only detected in part of the skin samples. However, two out of fifteen psoriatic patients displayed a clear IL-1β staining which co-localized with DNA end-labeling (Fig 7, annex 'Color Plates').

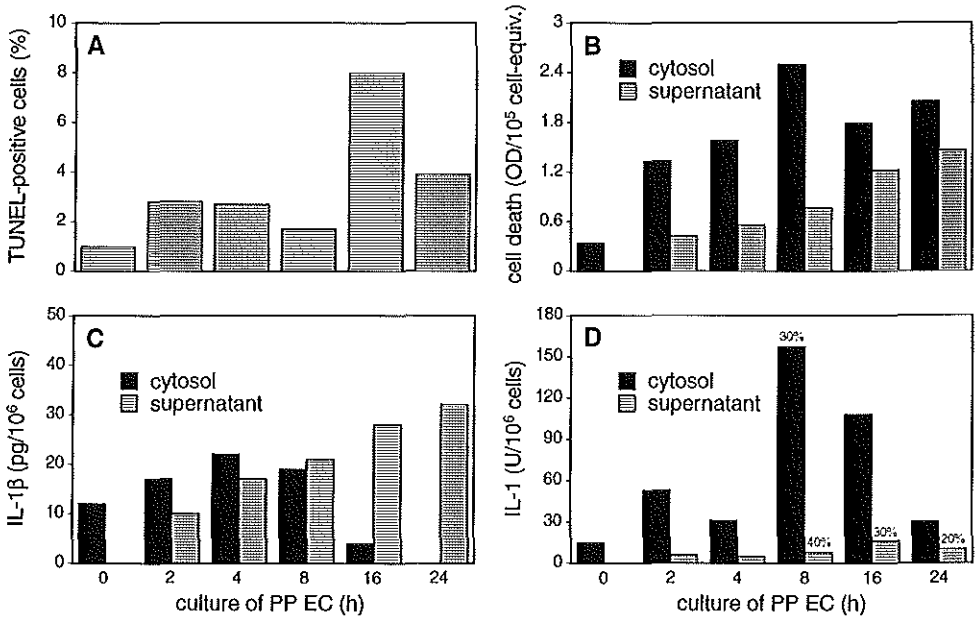

Figure 5. Apoptosis and IL-1β maturation in PP EC. PP EC-derived cytosols and supernatants were collected at 0, 2, 4, 8, 16 and 24 h after seeding in KBM and tested for DNA fragments (Fig B), IL-1β immunoreactivity (C) and IL-1 activity (D) in parallel with cell-death ELISA, IL-1β ELISA and D10 assay, respectively. Corresponding EC cytospins were used to perform the TUNEL assay (A). Cytosolic samples were ultrafiltered to remove salts before testing in the D10 assay. Percentages placed above the bars of total IL-1 activity (D) indicate the percentage of activity due to IL-1β, as analyzed with neutralizing antibodies. When the IL-1 activity is completely due to IL-1α, no percentages are indicated. Results of a representative experiment is shown (n = 2).

DISCUSSION

The present study provides evidence, based on different types of skin samples as well as different detection methods, that resident PP EC express enhanced apoptosis, and that this form of cell death is linked to *de novo* synthesis of IL-1β. We noted that non-stimulated, short-term primary cultures of PP EC showed a significant loss of viability, as measured by trypan blue exclusion, when compared to NN EC, and that this loss of viability correlated significantly with the percentage of EC-derived IL-1 released into the medium. This confirms and extends previous data reported by our group (chapter 3.2). The absolute amounts of IL-1α, IL-1β as well as biologically active IL-1 released by PP EC *ex vivo* were all significantly increased relative to NN EC (Table I). However, only the relative releases of IL-1α and biologically active IL-1 by PP EC, but not IL-1β, were significantly increased (Fig 1A). This suggests necrosis to be responsible for the release of biologically active intracellular (pro-)IL-1α by PP EC. This is in accordance

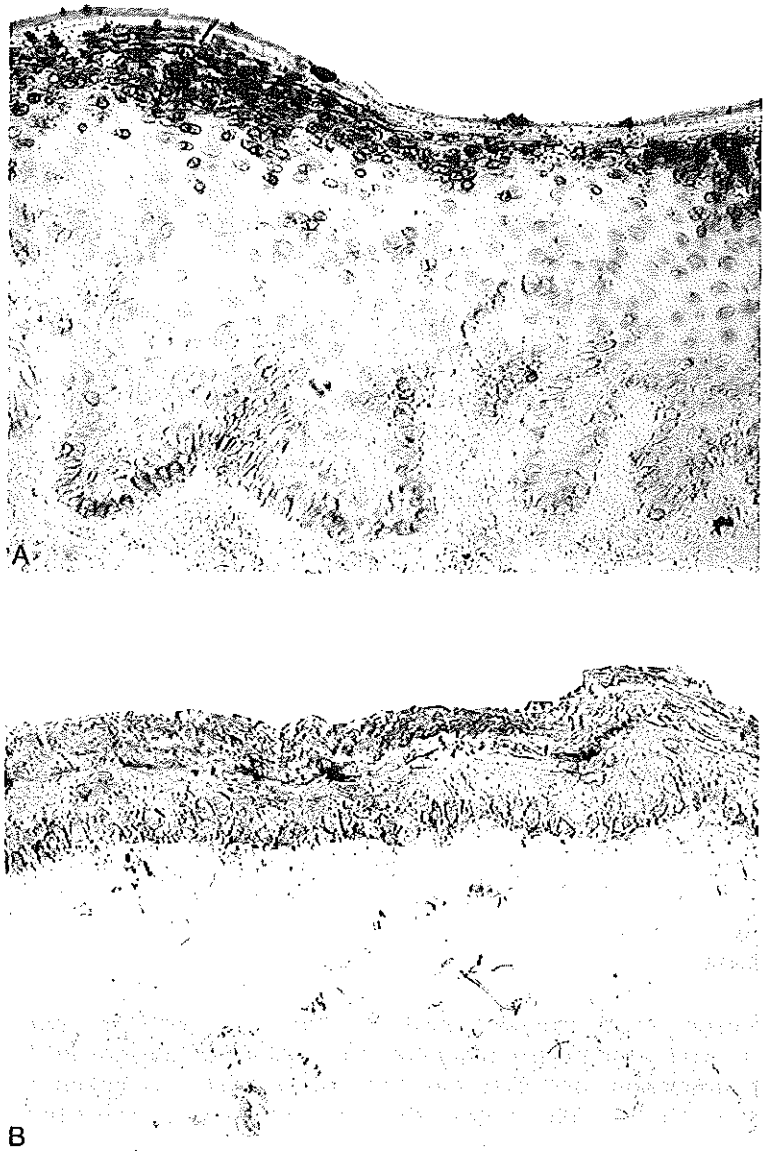

Figure 6. PP epidermis expresses enhanced TUNEL staining, which localized to suprabasal EC. TUNEL assay was performed on sections of PP (Fig A) and NN skin (B). Results of a representative patient are shown (PP skin: n = 15, and NN skin: n = 10). Magn 250 X.

with the previously observed correlation between loss of viability and release of lactate dehydrogenase activity as a marker for cytosol by EC *ex vivo* (chapter 3.2). In contrast, the increased amounts of IL-1β in PP EC supernatants were primarily due to increased *de novo* synthesis of IL-1β (Table I and

Fig 1B). *De novo* synthesis of IL-1β by PP EC was inversely correlated with loss of viability which indicated that trypan-blue negative (i.e. non-necrotic cells) produced IL-1β. Releases as well as *de novo* syntheses of IL-1α, IL-1β and IL-1 activity were nicely interrelated (not shown) being in line with our previous observation that PP EC produce predominantly biologically active (pro-)IL-1α, released by cytosolic leakage, but also process pro-IL-1β into the secreted mature and biologically active IL-1β (chapter 3.2). Keratinocytes may represent the responsible cell-type for the observed IL-1 production by PP EC *ex vivo*, as suggested by the IL-1 production by PP EC depleted for intra-epidermal leukocytes (chapter 3.2) and the ability of human keratinocytes to actively produce IL-1β *in vitro* (28).

The extent of DNA fragmentation, as a measure of apoptosis, was analyzed in freshly isolated PP EC. We showed for the first time that PP EC expressed significantly increased numbers of cells with 3'OH DNA termini, as observed with TUNEL staining, and increased amounts of cytosolic oligonucleosome-sized DNA fragments, as assessed with ELISA, when compared to NN EC (Figs 2 and 3). TUNEL staining of intra-epidermal leukocytes ($CD45^+$ cells) was negligible (Fig 4, annex 'Color Plates'). Thus, keratinocytes predominantly contributed to the increased DNA fragmentation in PP EC. Further analysis of short-term cultures of PP EC revealed that DNA fragmentation represented true apoptosis and correlated with IL-1β maturation (Fig 5). Necrosis cannot be ruled out as a mean of IL-1 processing and secretion, as ionic perturbation may be necessary for the activation of ICE (29). In our *ex vivo* model, DNA fragmentation clearly preceded the loss of plasma membrane integrity (Fig 5B), which provides evidence for apoptosis, but not necrosis, as the primary pathway of cell death. In addition, the release of oligonucleosome-sized DNA fragments into the medium significantly correlated with TUNEL staining, which is reported to be specific for apoptosis (30). This is in line with previous observations by others that both DNA cleavage at internucleosomal sites and complete nuclear TUNEL staining occur during late apoptosis of epithelial cells (20,31). The significant relation between the release of DNA fragments and that of biologically active IL-1β by PP EC is a novel finding. Kinetics showed that production, processing and secretion of mature IL-1β paralleled the generation and release of DNA fragments (Figs 5B, C and D). Synthesis and release of IL-1α by PP EC displayed similar kinetics (not shown). Processing of IL-1β indicates that an ICE-like protease was activated, which may be essential for the release of IL-1α and IL-1β (9) and to the process of apoptosis (11). For instance ICE has been reported to activate CPP32/YAMA, which cleaves poly(ADP-ribose) polymerase (PARP) and inhibits most of its DNA repair activity, thereby accelerating the demise of the cell (32). ICE-like proteases may also activate endonucleases, which could explain the appearance of cytosolic oligonucleosomal DNA fragments (Fig 5B). Finally, complete nuclear DNA fragmentation could be visualized with the TUNEL assay (Figs 2 and 5A). Our results *ex vivo* are in accordance with the recent report that apoptotic keratinocytes *in vitro* show enhanced production of IL-1 (33).

The findings *ex vivo* were extended to the situation *in vivo*. We showed that TUNEL staining of PP epidermis was increased *in situ* when compared to

NN epidermis and localized to the upper granular cell layers (Fig 6). TUNEL staining of PP epidermis was also increased when compared to corresponding non-lesional psoriatic (PN) skin (not shown). Our *in situ* TUNEL stainings of NN epidermis confirmed the findings by Gavrieli *et al.* (20). TUNEL staining of PP epidermis, however, was reported to include all cell layers, but cells were not apoptotic to morphological standards (22,23). This latter finding may in fact represent a false-positive staining as a consequence of technical differences relative to our TUNEL protocol (i.e. proteinase K pretreatment was omitted from our, but not from other protocols). The observation that PP epidermis expressed increased DNA end-labeling *in situ* confirmed the results with freshly isolated single EC (Fig 2). In psoriasis, increased apoptosis may merely represent a homeostatic mechanism to counteract the overproduction of keratinocytes (22). In addition, as apoptotic keratinocytes can express autoantigens (34,35), our finding may be of pathological relevance to psoriasis.

To provide *in vivo* proof that apoptotic epidermal cells produce IL-1β, double-stainings were performed. Although epidermal IL-1β immunoreactivity was hard to detect, a few psoriatic patients did reveal strongly IL-1β-positive epidermal areas which were lined by cells positive for both DNA breaks and IL-1β (Fig 7, annex 'Color Plates'). Further immunohistochemical analysis showed that leukocytes (i.e. neutrophils) were at least in part responsible for the strong epidermal IL-1β immunoreactivity (not shown). Immunohistochemistry may in fact not be sufficiently sensitive to detect keratinocyte-derived IL-1β, perhaps as a consequence of masked epitopes. In addition, PP epidermis may display an altered IL-1β processing *in vivo* (5) resulting in the expression of non-recognizable epitopes. Of a panel of anti-IL-1β monoclonal and polyclonal antibodies only the Vhp20 antibody, used in this study, detected epidermal IL-1β immunoreactivity in part of the skin samples investigated. The Vhp20 reactivity localized to the suprabasal cell layers. This staining pattern confirmed the electron microscopy studies of Didierjean *et al.* showing exocytosis of IL-1β in the stratum granulosum of normal human epidermis (36). Epidermal IL-1β is reported to show important pro-inflammatory properties, as IL-1β may be involved in both the chemotaxis of leukocytes (e.g. via induction of keratinocyte IL-8 (37)) and their protection from apoptosis (38,39). Therefore, epidermal IL-1β, which is increased in at least a subpopulation of psoriatic patients (Fig 7, annex 'Color Plates'), may contribute to the pathogenesis of psoriasis.

Taken together, PP EC show an increased *de novo* synthesis of IL-1β that is linked to apoptosis *ex vivo*. Also, suprabasal PP keratinocytes *in vivo* express increased apoptosis which co-localizes with IL-1β in a small subgroup of psoriatic patients. It is speculated that keratinocyte apoptosis may contribute to the generation of epidermal IL-1β, and thus to the inflammatory response in psoriasis.

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REFERENCES

1. Christophers E, Schubert C (1986) Psoriasis. In Thody AJ, Friedmann PS (Eds). Scientific Basis of Dermatology (a Physiological Approach). London, Churchill Livingstone, pp. 151-174.
2. Hauser C, Saurat J-H, Schmitt A, Jaunin F, Dayer J-M (1986) Interleukin 1 is present in normal human epidermis. J Immunol 136:3317-3321.
3. Kupper TS (1990) Immune and inflammatory processes in cutaneous tissues. Mechanisms and speculations. J Clin Invest 86:1783-1789.
4. Ristow HJ (1987) A major factor contributing to epidermal proliferation in inflammatory skin diseases appears to be interleukin 1 or a related protein. Proc Natl Acad Sci USA 84:1940-1944.
5. Cooper KD, Hammerberg C, Baadsgaard O, Elder JT, Chan LS, Sauder DN, Voorhees JJ, Fisher G (1990) IL-1 activity is reduced in psoriatic skin. Decreased IL-1α and increased nonfunctional IL-1β. J Immunol 144:4593-4603.
6. Auron PE, Webb AC, Rosenwasser LJ, Mucci SF, Rich A, Wolff SM, Dinarello CA (1984) Nucleotide sequence of human monocytic interleukin 1 precursor cDNA. Proc Natl Acad Sci USA 81:7907-7911.
7. Yuan J, Shaham S, Ledoux S, Ellis HM, Horvitz HR (1993) The *C. elegans* cell death gene ced-3 encodes a protein similar to mammalian interleukin-1β-converting enzyme. Cell 75:641-652.
8. Howard AD, Kostura MJ, Thornberry N, Ding GJF, Limjuco G, Weidner J, Salley JP, Hogquist KA, Chaplin DD, Mumford RA, Schmidt JA, Tocci MJ (1991) IL-1-converting enzyme requires aspartic acid residues for processing of the IL-1β precursor at two distinct sites and does not cleave 31-kDa IL-1α. J Immunol 147:2964-2969.
9. Kuida K, Lippke JA, Ku G, Harding MW, Livingston DJ, Su MS-S, Flavell RA (1995) Altered cytokine export and apoptosis in mice deficient in interleukin-1β converting enzyme. Science 267:2000-2003.
10. Mizutani H, Black R, Kupper TS (1991) Human keratinocytes produce but do not process pro-interleukin-1 (IL-1) beta. Different strategies of IL-1 production and processing in monocytes and keratinocytes. J Clin Invest 87:1066-1071.
11. Martin SJ, Green DR (1995) Protease activation during apoptosis. Death by a thousand cuts? Cell 82:349-352.
12. Hogquist KA, Nett MA, Unanue ER, Chaplin DD (1991) Interleukin 1 is processed and released during apoptosis. Proc Natl Acad Sci USA 88:8485-8489.
13. Zychlinsky A, Fitting C, Cavaillon J-M, Sansonetti PJ (1994) Interleukin 1 is released by murine macrophages during apoptosis induced by *Shigella flexneri*. J Clin Invest 94:1328-1332.
14. Sarih M, Souvannavong V, Brown SC, Adam A (1993) Silica induces apoptosis in macrophages and the release of interleukin-1 alpha and interleukin-1 beta. J Leukoc Biol 54:407-413.
15. Kerr JFR, Wyllie AH, Currie AR (1972) Apoptosis. A basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26:239-257.
16. Norris DA (1995) Differential control of cell death in the skin. Arch Dermatol 131:945-948.
17. Miura M, Zhu H, Rotello R, Hartwieg EA, Yuan J (1993) Induction of apoptosis in fibroblasts by IL-1β-converting enzyme, a mammalian homolog of the *C. elegans* cell death gene ced-3. Cell 75:653-660.
18. Wyllie AH, Kerr JFR, Currie AR (1980) Cell death. The significance of apoptosis. Int Rev Cytol 68:251-300.
19. Haake AR, Polakowska RR (1993) Cell death by apoptosis in epidermal biology. J Invest Dermatol 101:107-112.
20. Gavrieli Y, Sherman Y, Ben-Sasson SA (1992) Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 119:493-501.
21. McCall CA, Cohen JJ (1991) Programmed cell death in terminally differentiating keratinocy-

tes: role of endogenous endonuclease. J Invest Dermatol 97:111-114.
22. Bianchi L, Farrace MG, Nini G, Piacentini M (1994) Abnormal Bcl-2 and 'tissue' transglutaminase expression in psoriatic skin. J Invest Dermatol 103:829-833.
23. Wrone-Smith T, Johnson T, Nelson B, Boise LH, Thompson CB, Núñez G, Nickoloff BJ (1995) Discordant expression of Bcl-x and Bcl-2 by keratinocytes *in vitro* and psoriatic keratinocytes *in vivo*. Am J Pathol 146:1079-1088.
24. Fesus L, Thomazy V, Autuori F, Cerú MP, Tarcsa E, Piacentini M (1989) Apoptotic hepatocytes become insoluble in detergents and chaotropic agents as a result of transglutaminase action. FEBS Lett 245:150-154.
25. Bakouche O, Brown DC, Lachman LB (1987) Subcellular localization of human monocyte interleukin 1: evidence for an inactive precursor molecule and a possible mechanism for IL-1 release. J Immunol 138:4249-4255.
26. Hopkins SJ, Humphreys M (1989) Single, sensitive and specific bioassay of interleukin-1. J Immunol Methods 120:271-276.
27. Prens EP, Benne K, Van Joost T, Benner R (1991) The autologous mixed epidermal cell - T lymphocyte reaction is elevated in psoriasis. A crucial role for epidermal HLA-DR$^+$/CD1a$^-$ antigen presenting cells. J Invest Dermatol 96:880-887.
28. McKenzie RC, Arsenault TV, Sauder DN, Harley CB (1990) Expression of interleukin-1β in a human keratinocyte cell line. Lymphokine Res 9:391-403.
29. Perregaux D, Barberia J, Lanzetti AJ, Geoghegan KF, Carty TJ, Gabel CA (1992) IL-1β maturation: evidence that mature cytokine formation can be induced specifically by nigericin. J Immunol 149:1294-1303.
30. Gorczyca W, Bigman K, Mittelman A, Ahmed T, Gong J, Melamed MR, Darzyn-kiewicz Z (1993) Induction of DNA strand breaks associated with apoptosis during treatment of leukemias. Leukemia 7:659-570.
31. Oberhammer F, Wilson JW, Dive C, Morris ID, Hickman JA, Wakeling AE, Walker PR, Sikorska M (1993) Apoptotic death in epithelial cells: cleavage of DNA to 300 and/or 50 kb fragments prior to or in the absence of internucleosomal fragmentation. EMBO J 12:3679-3684.
32. Tewari M, Quan LT, O'Rourke K, Desnoyers S, Zeng Z, Beidler DR, Poirier GG, Salvesen GS, Dixit VM (1995) Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly (ADP-ribose) polymerase. Cell 81:801-809.
33. Marthinuss J, Lawrence L, Seiberg M (1995) Apoptosis in Pam212, an epidermal keratinocyte cell line: a possible role for bcl-2 in epidermal differentiation. Cell Growth Differ 6:239-250.
34. Grubauer G, Romani N, Kofler H, Stanzl U, Fritsch P, Hintner H (1986) Apoptotic keratin bodies as autoantigen causing the production of IgM-anti-keratin intermediate filament autoantibodies. J Invest Dermatol 87:466-471.
35. Rosen A, Casciola-Rosen L, Ahearn J (1995) Novel packages of viral and self-antigens are generated during apoptosis. J Exp Med 181:1557-1561.
36. Didierjean L, Salomon D, Mérot Y, Siegenthaler G, Shaw A, Dayer J-M, Saurat JH (1989) Localization and characterization of the interleukin 1 immunoreactive pool (IL-1 alpha and beta forms) in normal human epidermis. J Invest Dermatol 92:809-816.
37. Larsen CG, Anderson AO, Oppenheim JJ, Matsushima K (1989) Production of interleukin-8 by human dermal fibroblasts and keratinocytes in response to interleukin-1 or tumor necrosis factor. Immunology 68:31-36.
38. Mangan DF, Welch GR, Wahl SM (1991) Lipopolysaccharide, tumor necrosis factor-α, and IL-1β prevent programmed cell death (apoptosis) in human peripheral blood monocytes. J Immunol 146:1541-1546.
39. Colotta F, Re F, Polentarutti N, Sozzani S, Mantovani A (1992) Modulation of granulocyte survival and programmed cell death by cytokines and bacterial products. Blood 80:2012-2020.

Chapter 3.4

PSORIATIC EPIDERMAL CELLS EXPRESS ELEVATED LEVELS OF FUNCTIONAL IL-4 RECEPTORS. IL-4 downregulates the IL-1 and IL-6 production *ex vivo*.*

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ABSTRACT

Our group has recently observed that epidermal cells (EC) derived from involved psoriatic (PP) skin express an increased production of IL-1 and IL-6 relative to normal control (NN) skin. We also found that PP EC express increased levels of IL-4 receptor (IL-4R). The IL-4R mediates the anti-inflammatory properties of its ligand IL-4, which is reported to downregulate the expression of IL-1 and IL-6 in monocytic cells. In the present study, the IL-4R expression by PP, non-lesional psoriatic (PN) and NN EC was further characterized by phenotypic as well as functional analyses. Immunostaining of skin sections localized IL-4R to basal and some suprabasal keratinocytes and cells in the upper dermis. Semiquantitative analyses of immunostaining and in situ hybridization showed that the epidermal expression of IL-4R was highest in PP skin, intermediate in PN skin and lowest in NN skin. The IL-4R protein levels correlated nicely to those of IL-4R mRNA, suggesting that the increased expression of IL-4R in psoriatic epidermis is due to an enhanced transcription of the IL-4R gene. Reverse transcriptase - polymerase chain reaction (RT-PCR) on RNA derived from freshly isolated single EC suspensions confirmed these findings. Short-term primary cultures of PP EC (termed *ex vivo* model), treated with recombinant human IL-4, showed a clear reduction of their basal IL-6 protein production, and to a lesser extent of IL-1β but not IL-1α, as measured by enzyme-linked immunosorbent assay (ELISA). The inhibition of IL-6 production was most profound when PP EC were cultured for 24 h with 100 U/ml of IL-4, resulting in 65 % reduction of the IL-6 production. RT-PCR analysis of the corresponding RNA samples, however, indicated that IL-4 did not affect the steady-state mRNA levels of IL-6, whereas those of IL-1β were reduced in PP EC. IL-4 treatment of IL-1β-stimulated NN EC and peripheral blood mononuclear cells reduced the mRNA levels of both cytokines. This is the first study providing evidence that PP EC express functional IL-4R and that IL-4 exerts anti-inflammatory effects on EC

* Submitted for publication

via downregulating the production of IL-1 and more notably of IL-6 protein, probably at the post-transcriptional level.

INTRODUCTION

Keratinocytes of lesional psoriatic (PP) skin display a typical phenotype which comprises overexpression of markers of proliferation, differentiation and immune activation (1,2). The activated state of PP keratinocytes probably results from a dysregulated cross-talk between skin infiltrating T cells and epidermal keratinocytes (3-5). Cellular communication is directed by cytokines and their receptors. Work from our group and others showed an enhanced expression of interleukin 1β (IL-1β), IL-1ra, IL-1 receptor type II (IL-1RII) and IL-6 by PP epidermis when compared to non-lesional (PN) and normal control (NN) epidermis *in vivo* (chapter 3.1, and refs 6-12). Furthermore, we observed that freshly isolated PP epidermal cells (EC) express increased levels of IL-1β mRNA and cytosolic IL-1β protein (chapters 3.1 and 3.2). In addition, non-stimulated, short-term primary cultures of these cells (termed *ex vivo* model) were found to release highly increased levels of biologically active IL-1α, IL-1β and IL-6, being in line with reports on the situation *in vivo* (chapter 3.2 and ref 9). Moreover, the pro-inflammatory cytokines IL-1 and IL-6 are mitogenic for normal human EC (10,13), suggesting an involvement of these cytokines in the pathogenesis of psoriasis. More recently we have found that PP EC express increased levels of immunoreactive IL-4R and IL-4R mRNA, and an increased number of binding sites for recombinant human IL-4 (14). It is important to note that in psoriatic lesions the IL-4 ligand is, however, only present in negligible amounts as the infiltrating T cells predominantly produce T helper type 1 (T_H1)-type cytokines (12,15).

IL-4, a T_H2-type 2 cytokine, is predominantly produced by T cells and mast cells (see for review ref 16). This cytokine has pleiotropic effects on lymphoid cells as well as non-lymphoid cells, ranging from the promotion of cellular survival, growth and differentiation (17-21), the inhibition of production of pro-inflammatory cytokines (22-24) to the inhibition of human tumor cell growth (25). The modulating effects of IL-4 depend on cell-type (26), stimulating agent (e.g. cytokine) and response-parameter (27), and are probably regulated, at least in mononuclear cells, at the level of activation of transcription factors (26,28,29). IL-4 downregulates the production of IL-1, IL-6 and tumor necrosis factor-α (TNF-α) by human monocytes (22-24), and the production of IL-6 by leukemic cells (30,31). However, IL-4 upregulates IL-6 production by resting human B cells (32), human endothelial cells (33), and human keratinocytes (34). With respect to human dermal fibroblasts there exists some controversy, as one study reports a stimulatory effect (21), whereas another study reports no effect of IL-4 on the IL-6 production (26). At present, the effect of IL-4 on the production of IL-1 and IL-6 by PP

EC is not known. The IL-4R has been observed on a variety of human cell types, such as B and T lymphocytes, hematopoietic precursors, fibroblasts, epithelial cells, endothelial cells (35) and solid tumors of epithelial origin (36,37). The expression of epidermal IL-4R in psoriasis and its functionality need further characterization.

In the current study, we examined the IL-4R expression by PP, PN and NN EC with immunostaining, in situ hybridization (ISH) and reverse transcriptase - polymerase chain reaction (RT-PCR) using skin biopsies and single EC suspensions. The effect of IL-4 on the production of IL-1 and IL-6 by these cells *ex vivo* was analyzed as a measure of the functionality of this receptor. Freshly isolated single EC actively produce IL-1 and IL-6 (chapter 3.2) and provide RNA close to its state *in vivo* (38). The epidermal expression of IL-4R protein and mRNA was highest in PP EC, intermediate in PN EC and lowest in NN EC. Receptors were functional as recombinant human IL-4 reduced the production of IL-1 and more clearly of IL-6 protein by PP EC, probably at the post-transcriptional level.

MATERIALS AND METHODS

Patients and controls

Nineteen otherwise healthy patients (Outpatient Department of Dermatology, Hospital Walcheren, Vlissingen, The Netherlands, and University Hospital Rotterdam-Dijkzigt, Rotterdam, The Netherlands) with untreated plaque-type psoriasis were studied after informed consent. The disease activity, severity and extent were determined for each patient and expressed as psoriasis area and severity index (PASI) scores (39). PP skin was obtained from all patients and PN skin from nine patients. Fourteen healthy volunteers, individuals without history or signs of skin disease, undergoing abdominal or breast plastic surgery (Sint Franciscus Hospital, Rotterdam) served as controls. All samples were collected after approval of the institutional medical ethical committee (MEC 104.050/SPO/1990/30).

Immunohistochemistry

Immunohistochemistry was performed on 6 µm thick cryostat sections of Tissue-Tek® (Miles Inc., Elkhart, IN) embedded punch biopsies (Ø = 3 mm) obtained from PP, PN and NN skin. The immunostaining was performed with the supersensitive alkaline phosphatase kit (Biogenex, San Ramon, CA) using new fuchsin (Chroma-Gesellschaft, Köngen, Germany) as the chromogen. The anti-IL-4R monoclonal antibody MR6 (kindly donated by Dr. M. Larché, London, UK) was used in a concentration of 20 µg/ml. Slides were counterstained with Mayer's hematoxylin, mounted in glycerol gelatin (Merck, Darmstadt, Germany) and evaluated microscopically. The controls comprised concentration-matched control mouse IgG_1 (Becton Dickinson, Plymouth, UK) and omitting the first and second step. The extent and intensity of the

stainings of biopsy specimens were semiquantitatively assessed by two of the authors (RD and JPJJH). The epidermal reaction was scored on a 0-4 scale with the following values: 0, no reactivity; 1, weak reactivity; 2, moderate reactivity; 3, strong reactivity; and 4, very strong reactivity (modified from ref 40).

In situ hybridization

The 0.83 kb human IL-4R cDNA clone (nucleotides 2309-3143, see ref 41) was inserted into pBluescript SK- to construct the hulL-4RPR plasmid, kindly provided by Dr. T. Trout (Immunex Corp., Seattle, WA). The plasmid was linearized with *Cla*I to synthesize the antisense cRNA probe or with *Not*I to synthesize the sense cRNA probe by T3 and T7 RNA polymerase, respectively, using a digoxigenin RNA labeling kit (Boehringer Mannheim, Germany). The 0.78 kb *Eco*RI-*Pst*I fragment of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was cloned and used to synthesize RNA probes as described previously (chapter 3.1). The size of the transcripts was analyzed by electrophoresis and subsequently reduced to an average length of 100 base pairs (bp) by limited alkaline hydrolysis (42). The labeling efficiency was checked by Southern hybridization of the IL-4R cDNA clone, and the specificity by Northern hybridization of total cellular RNA extracts (not shown). The in situ hybridization was performed as described elsewhere (chapter 3.1). Hybridization was detected using sheep anti-digoxigenin Fab fragments conjugated with alkaline phosphatase according to the manufacturer's protocol (Boehringer Mannheim). The negative controls comprised omitting probe or antibody, hybridization with the sense probe, and RNAse (10 mg/ml) treatment for 1 h at 37 °C before hybridization. *In situ* expression of IL-4R mRNA was only evaluated in those slides showing no hybridization signal with the corresponding sense probe and a positive hybridization signal with the GAPDH antisense probe which confirmed the preservation of cellular RNA. ISH and immunostaining were performed on serial sections, and the ISH findings were ranked to relate the mRNA expression to the protein expression. Ranking was performed as described for immunostaining.

EC-conditioned media

EC suspensions were prepared as previously described (chapter 3.2). Cells were counted microscopically using phase-contrast illumination and viability was determined by trypan blue exclusion. Freshly isolated PP EC were seeded at 10^6 viable cells/ml in RPMI 1640 medium (Gibco Ltd., Paisley, Scotland) supplemented with 25 mM HEPES, 2 mM glutamax-I, 100 U/ml penicillin, 100 µg/ml streptomycin and 0.1 % human serum (Red Cross Blood Center, Rotterdam, The Netherlands) using 12 x 75 mm polypropylene tubes (Becton Dickinson) and incubated for 1 to 24 h at 37 °C and 5 % CO_2. This *ex vivo* model was used to monitor PP EC. Supernatants were collected by centrifugation, filter-sterilized and stored at -80 °C until used for enzyme-linked immunosorbent assay (ELISA) measurements. Cells, collected by

centrifugation, were used to check viability and to isolate RNA. Kinetic and dose-response studies were performed with recombinant human IL-4 (43), kindly donated by Ms. A. Van Kimmenade (DNAX Research Inst., Palo Alto, CA). The specific activity of recombinant human IL-4, being 10^7 U/mg protein, was determined with the use of ELISA (Bender MedSystems, Vienna, Austria) and a biological assay (slightly modified from ref 44). One Unit of IL-4 was defined as the amount of IL-4 required to cause half-maximum uptake of [^{3}H]deoxythymidine by 10^5/200 µl peripheral blood mononuclear cells (PBMC) which were preactivated at 10^6 cells/ml with 5 µg/ml phytohemagglutinin (PHA) for 3 days and then extensively washed.

ELISA

The amounts of IL-1α, IL-1β and IL-6 present in supernatants were measured with commercially available specific ELISA kits (Mr. M. Kempen, Eurogenetics, Tessenderlo, Belgium) using the protocols provided by the manufacturers.

Reverse transcriptase - polymerase chain reaction

Total cellular RNA was isolated by the guanidine isothiocyanate extraction procedure (45). Prior to RT-PCR, the samples were treated with RNAse-free DNAse (DNase I, Amplification Grade, GIBCO BRL, Gaithersburg, MD) at a final concentration of 1 U/µg RNA for 15 min at room temperature (RT) to digest contaminating genomic DNA. The DNase I was inactivated by 2 mM EDTA. Samples were reverse transcribed into cDNA and subsequently amplified, as described elsewhere (chapter 3.1). Sets of primers and probes are mentioned in Table I. The IL-4R oligonucleotides, which we developed, displayed no homology to known complementary cDNA sequences, except human IL-4R cDNA, as verified by searching EMBL database (February 1996) using the program BLASTN 1.4.7 (49). The necessity of DNase treatment of the RNA samples indicated that our primers span a region within the same exon of the IL-4R mRNA molecule. PCR products were determined at 30 cycles, except for IL-6 PCR products which were determined at 45 cycles. Transcripts of the GAPDH housekeeping gene were used as an internal control to standardize for total cellular mRNA. Serial dilutions of individual cDNAs, starting from approximately 5 ng cDNA, were amplified using GAPDH primers and showed concomitant decreases of amplified products. cDNA concentrations were selected which yielded equivalent quantities of GAPDH products and fell within the linear range of amplification (12). Each experiment included a positive control (cDNA from lipopolysaccharide (LPS)-treated PBMC) and a negative control (DNase-treated RNA that had not been reverse transcribed or water). The specificity of the PCR products was verified by Southern hybridization.

Table I. Oligonucleotides for PCR amplification and detection of EC-derived cytokine and cytokine receptor cDNAs.

Cytokine(R)	Oligonucleotide	Sequence (5' to 3')	Size of amplimer[a]	Reference
IL-1β	sense primer	CCAGCTACGAATCTCGGACCACC	667	46
	antisense primer	TTAGGAAGACACAAATTGCATGGTG AAGTCAGT		46
	probe	CGATCACTGAACTGCACGCTCCGGG		46
IL-6	sense primer	ATGAACTCCTTCTCCACAAGC	613	47
	antisense primer	TGGACTGCAGGAACTCCTT		47
	probe	GAGGTATACCTAGAGTACCTC		47
IL-4R	sense primer	CTGGAGCACAACATGAAAAGG	510	
	antisense primer	AGTCAGGTTGTCTGGACTCTG		
	probe	TGGCCAGAGAGCATCAGCGT		
GAPDH	sense primer	CCGAGCCACATCGCTCAGACAC	594	48
	antisense primer	GGCCATCCACAGTCTTCTGGGT		48
	probe	CTTCCAGGAGCGAGATCCCTC		48

[a] size of amplimer in bp

Controls

Cellular controls to test IL-4 responsiveness were NN EC and PBMC. NN EC were stimulated with 100 U/ml recombinant human IL-1β (Glaxo, Geneva, Switserland), using the culture conditions as described for PP EC, in the presence or absence of 100 U/ml IL-4 for 4 h. PBMC were cultured at 5 x 10^6 cells/ml in RPMI 1640 medium supplemented with HEPES, glutamax-I, antibiotics, 2 % human serum and 10 μg/ml LPS (*Escherichia coli* 026:B6, Difco Laboratories, Detroit, MI) in the presence or absence of 100 U/ml IL-4 for 16 h. The effects of IL-4 on the expression of IL-1 and IL-6 were compared to those of the anti-inflammatory agent dexamethasone, used at 10^{-7} M for 4 h.

Statistical analysis

Results were analyzed with the Wilcoxon Matched-Pairs Signed-Rank Test (PP versus PN samples), the Wilcoxon Rank Sum Test (PP or PN versus NN samples) and the Spearman's Rank Correlation Coefficient (r_s) using STATA™ (Computing Resource Center, Los Angeles, CA). Significant differences or correlations are indicated by P values smaller than 0.05.

RESULTS

PP and PN epidermis express increased levels of IL-4R protein and mRNA when compared to NN epidermis

Immunohistochemical staining of skin sections clearly showed that IL-4R was localized to basal and some suprabasal keratinocytes and upper dermal cells (Fig 1, annex 'Color Plates', and Table II). The IL-4R expression was highest in PP skin (PP versus PN and PP versus NN: $P < 0.02$ and $P < 0.002$, respectively), intermediate in PN skin (PN versus NN: $P < 0.04$) and lowest in NN skin. Similar findings were observed with ISH of IL-4R mRNA performed on serial sections (Fig 2, annex 'Color Plates', and Table II). In fact, the individual scores of immunostaining correlated well with those of ISH (Table II, $r_s = 0.7$, $n = 20$, $P < 0.0025$). The results with whole skin sections were confirmed by PCR analysis of steady-state IL-4R mRNA levels in freshly isolated PP EC ($n = 10$) and NN EC ($n = 5$) (Fig 3). cDNA samples from DNAse-treated RNA were titrated to standardize for the expression of the GAPDH gene. It is of note that the amounts of cDNA from PP EC were less, on average 4 times, than those from NN EC, which reflected the enhanced metabolic state of PP cells.

Table II. Expression of IL-4R at the mRNA and protein level in skin biopsies.[a]

	PP[b] Patient number									PN Patient number									NN Control number								
	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
protein	3	3	3	1	3	4	4	3	3	1	2	2	ND	3	1	1	2	2	ND	1	1	2	1	1	0	1	0
mRNA	1	1	2	2	ND	2	2	2	ND	1	1	1	1	1	0	1	ND	ND	0	1	1	ND	0	1	0	0	0
PASI	ND	16	24	25	7	12	7	4	6																		

[a] Serial skin sections were analyzed using immunohistochemistry and ISH for the expression of IL-4R at the protein and mRNA level, respectively. Findings were scored on a 0-4 scale based on the extent and intensity of the signal. See text for details.
[b] Type of skin (PP, lesional psoriasis; PN, non-lesional psoriasis; and NN, normal control).
[c] ND, not done.

IL-4 downregulates the production of IL-6 protein, and to a lesser extent of IL-1β but not IL-1α, by PP EC

Exogenous recombinant human IL-4 clearly reduced, although to varying degrees in different patients, the basal production of IL-6 by PP EC *ex vivo* (Fig 4A, $n = 5$). IL-4 reduced the secretion of IL-1β to a lesser extent, but did not affect the secretion of IL-1α. Time-response studies showed that 100 U/ml IL-4 induced an inhibition of the IL-6 production which was already prominent at 2 h (i.e. a two-third reduction of the IL-6 production), and persisted throughout the 24 h test-period (Fig 4A). In absolute terms, the most profound inhibition was observed at 24 h culture, as the basal IL-6

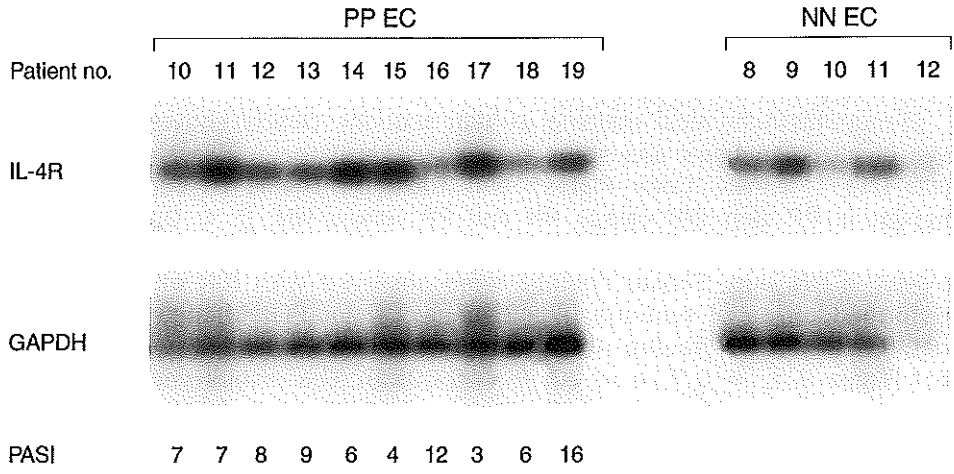

Figure 3. PCR analysis of IL-4R mRNA in PP EC. cDNAs were synthesized from RNA of freshly prepared EC suspensions from PP (n = 10) and NN skin specimens (n = 5). Individual cDNAs were standardized for the level of GAPDH mRNA. Water or non-reverse transcribed RNA gave no signals, whereas cDNA from LPS-treated PBMC was clearly positive in all PCRs performed (not shown).

production by PP EC was maximal at this time point. The inhibitory effect of IL-4 was similar at doses ranging from 1 to 1000 U/ml IL-4 (not shown). IL-4 did not affect the negligible amounts of IL-1 and IL-6 produced by non-stimulated or IL-1β-stimulated NN EC (not shown). As expected, IL-4 treatment of PBMC,as a positive control, resulted in a drastic decrease of the IL-1α, IL-1β as well as IL-6 production (Fig 4B). Dexamethasone, a potent anti-psoriatic therapeutic agent, was less effective than IL-4 in the reduction of cytokine production by PBMC.

IL-4 does not affect the steady-state mRNA levels of IL-6, but reduces those of IL-1β in PP EC

Semiquantitative RT-PCR analysis was performed on corresponding RNA samples. Short-term culture of cells (either EC or PBMC) resulted in upregulation of IL-1 and IL-6 mRNA levels (not shown). Treatment of PP EC with 100 U/ml IL-4 for 4 h did not affect the steady-state mRNA levels of IL-6 *ex vivo*, whereas those of IL-1β were reduced (i.e. IL-4 abrogated the increase of IL-1β mRNA expression *ex vivo*) (Fig 5). IL-4 treatment of NN EC reduced the mRNA levels of both cytokines, most notably those of IL-1β. Time and dose-response studies confirmed these findings. Prolonged treatment of EC with IL-4 (i.e. 24 h) did not result in persistence of the inhibiting effect on cytokine mRNA expression (not shown). PBMC cultured in the presence of IL-4 showed a clear reduction in the expressions of IL-1β and IL-6 mRNA levels, which was not found when cells were cultured in the co-presence of LPS

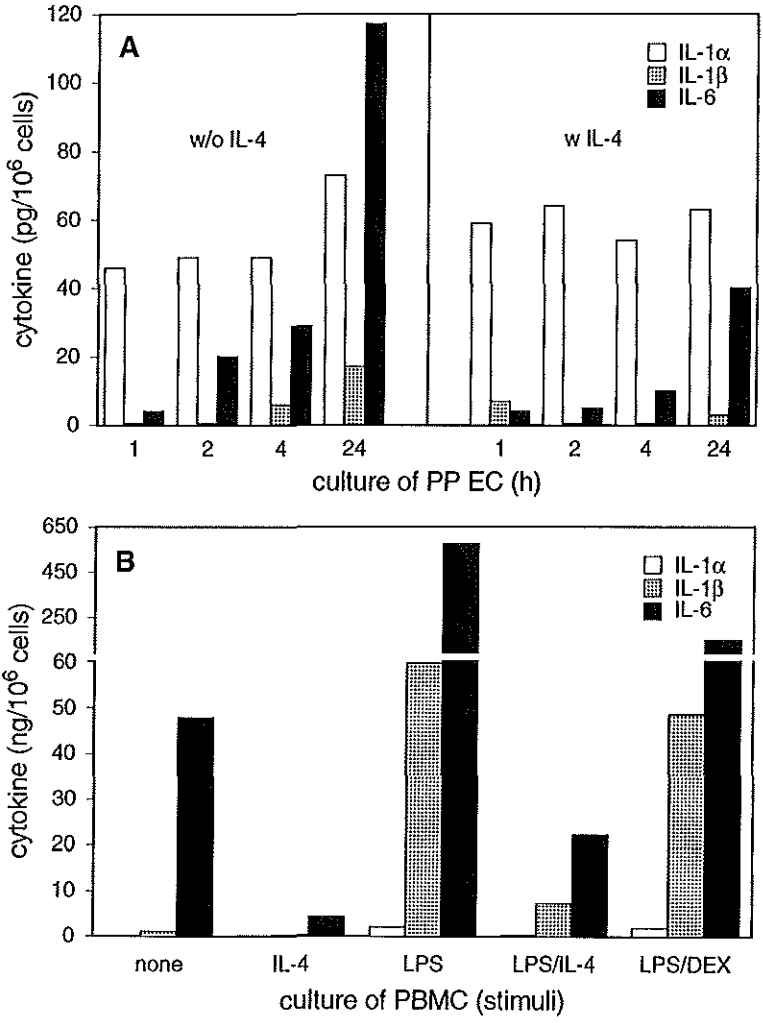

Figure 4. Effect of IL-4 on secretion of IL-1 and IL-6 by PP EC. PP EC were incubated in the absence (w/o: without) or presence of 100 U/ml recombinant human IL-4 (w: with) for 1, 2, 4 or 24 h as described in the materials and methods section. Supernatants were tested for immunoreactive IL-1α, IL-1β and IL-6 by ELISA. Results of a representative PP EC (n = 6) are given (Fig A). The controls included 16 h supernatants of LPS-stimulated PBMC treated with IL-4 or dexamethasone (10^{-7} M, 4 h). A representative experiment is shown (B). Results are given in means of triplicate measurements which deviate less than 5 % from the mean.

(Fig 5). The expression of cytokine mRNAs by EC and PBMC was also reduced by dexamethasone (Fig 5). Note that EC-derived IL-1α mRNA was not detectable with RT-PCR, despite the use of different primer sets or extension of PCR to 45 cycles (chapter 3.1).

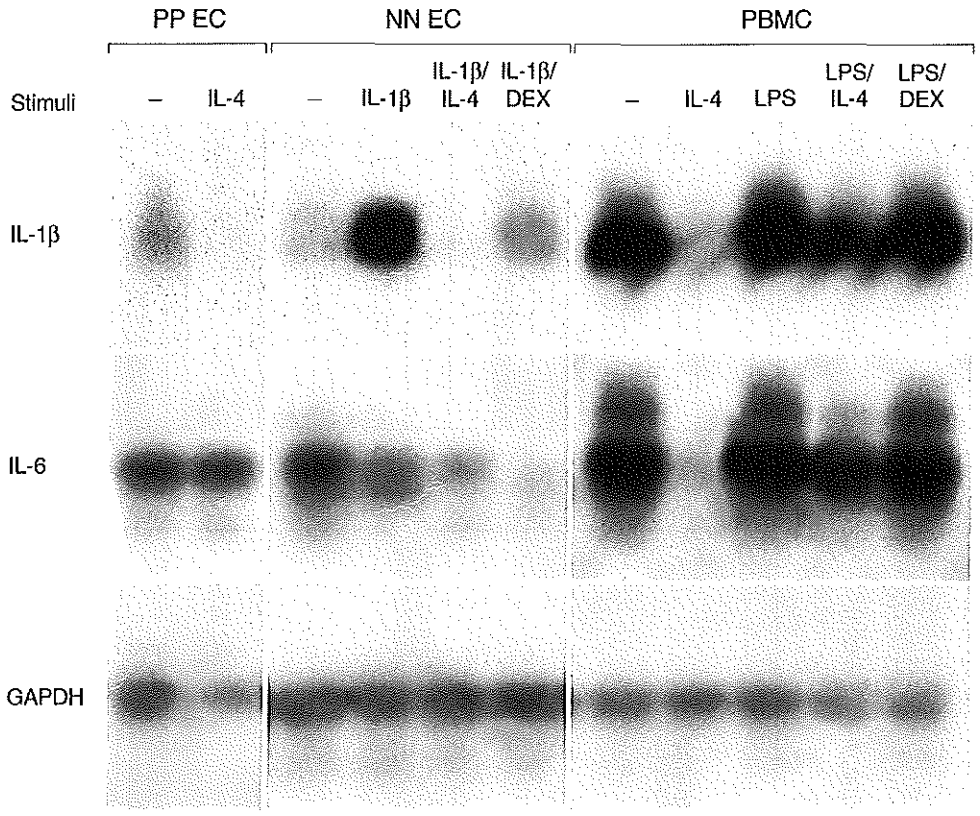

Figure 5. Effect of IL-4 on IL-1 and IL-6 mRNA levels in PP EC. PCR analysis of steady-state IL 1β and IL-6 mRNA levels in PP EC (n = 3) after culture with or without 100 U/ml IL-4 for 4 h. IL-1β-stimulated NN EC (n = 3) and PBMC (n = 2) were used as controls. See legend to Figure 3 for details.

DISCUSSION

Results of the present study indicate that basal keratinocytes of psoriatic epidermis express increased levels of functional IL-4R. Semiquantitative analyses of immunostaining and ISH showed that PP epidermis expressed significantly increased levels of IL-4R protein and mRNA, respectively, when compared to corresponding PN and NN epidermis (Figs 1 and 2, annex 'Color Plates', and Table II), which confirms and extends our previous study (14). Our finding that even PN epidermis expressed significantly increased levels of IL-4R when compared to NN epidermis underlined the relevance of this receptor in the pathophysiology of psoriasis. However, no correlation was found between the patient's whole body clinical PASI scores and the epidermal IL-4R expression. This does not necessarily undermine the pathological

relevance of our findings, as such a score may not be representative for a specific lesion used for biopsy. The observation that the IL-4R expression at the protein and mRNA level correlated well (Table II), suggests that the increased expression of IL-4R by psoriatic epidermis is due to enhanced transcription of the IL-4R gene. It should be noted that the IL-4R expression is also increased in the dermal compartment of psoriatic skin, which probably reflects the activated state of fibroblasts and infiltrating cells. RT-PCR analysis of RNA derived from EC, which is a purer keratinocyte population than complete skin biopsies, supported this notion. The steady-state IL-4R mRNA levels were increased in part of the PP EC samples when compared to NN EC samples (Fig 3). As far as we know, this is the first report which describes and verifies the amplification of human IL-4R transcripts.

The increased IL-4R expression by PP EC may be a consequence of the local increase of IL-6 levels, as IL-6, at least in myeloid cells, induces expression of the IL-4R gene (50). Moreover, the expression of IL-6R is also reported to be increased in PP as well as PN epidermis (51), suggesting an increased IL-6 responsiveness of psoriatic EC. IFN-γ suppresses the IL-6-induced transcription of the IL-4R gene (50). This mechanism may, however, be disturbed in PP EC as these cells display a decreased IFN-γR expression (52) and responsiveness (53), despite the presence of IFN-γ^+ infiltrating T cells (12,15).

The local IL-1 and IL-6 expression is enhanced in PP skin *in vivo* (6,9-12). At least for IL-1 and IL-6 the same is true for PN skin (12,51). Moreover, we have recently observed that PP EC express an increased production of IL-1 and IL-6 *ex vivo* (chapter 3.2). This *ex vivo* model was used to test the functionality of the IL-4R by analyzing the IL-4-mediated effect on IL-1 and IL-6 production by PP EC. IL-4 inhibited the secretion of individual pro-inflammatory cytokines produced by PP EC. The secretion of IL-6 protein, and to a lesser extent IL-1β, but not IL-1α, were downregulated by IL-4 (Fig 4A). There was some donor variation in IL-4 responsiveness, which did, however, not correlate with the level of IL-4R expression as measured by binding of biotinylated recombinant human IL-4 by PP EC samples (not shown). Immunohistochemistry and ISH (Figs 1 and 2, annex 'Color Plates') as well as experiments with EC depleted for CD45$^+$ cells, which showed that CD45$^+$ cells did not substantially contribute to the production of IL-1 (chapter 3.2), favor keratinocytes as the predominant IL-4 responsive cells in our model. Kupper and colleagues observed that IL-4 enhanced the IL-6 production of cultured normal human keratinocytes (34). We could not confirm this finding using primary cultures of NN EC, which may be caused by a different IL-4 responsiveness of cells *ex vivo* relative to cells *in vitro*.

IL-4 responsiveness of PP EC was also measured at the RNA level. The effect of IL-4 on the expression of cytokine mRNAs was both cell-type and cytokine specific. IL-4 reduced both IL-1β and IL-6 mRNA levels in PBMC, which is in agreement with earlier reports (22-24), and NN EC, whereas in PP EC IL-4 only reduced IL-1β mRNA levels (Fig 5). Note that PP EC upregu-

lated the levels of both IL-6 mRNA (not shown) and protein during short-term culture (Fig 4A, w/o IL-4), which is in line with previous reports (54), and point to *de novo* synthesis of IL-6 and not merely passive release of this cytokine *ex vivo*. The discrepancy between the effects of IL-4 on the expression of IL-6 mRNA and IL-6 secretion by PP EC may point to an inhibiting effect of IL-4 on post-transcriptional processes of IL-6 synthesis. IL-4 also inhibited the expression of IL-1β and IL-6 by LPS-stimulated PBMC most notably at the protein level (Fig 4B and 5). Others observed that IL-4 reduced cytokine mRNA expression by LPS-stimulated monocytes rapidly after the start of cell culturing (< 5 h of cell culture), but affected post-transcriptional synthesis (e.g. mRNA stability) rather than transcription (55). Dexamethasone, in contrast to IL-4, was not cell-type and cytokine specific in its suppressive action on cytokine production (Figs 4B and 5). Dexamethasone binds with and activates the intracellular glucocorticoid receptor, which subsequently associates with the transcription factor nuclear factor (NF)IL-6 via a direct protein-protein interaction (56). This suggests a model for glucocorticoid receptor-mediated inhibition of transcription of NFIL-6 dependent genes (e.g. IL-1 and IL-6). Dexamethasone was observed to reduce the cytokine mRNA levels in IL-1β-stimulated NN EC (Fig 5), which is in accordance with reported effects of dexamethasone on the human epidermoid carcinoma cell line KB (57).

In analogy with psoriasis, the expressions of both IL-6 and IL-4R are also increased in many solid tumors of epithelial origin (36,58). IL-6 may act as an autocrine or paracrine growth factor of PP EC or tumor cells (59). It has recently been reported that IL-4 exerts suppressive effects on the spontaneous growth of myeloma cells through the inhibition of IL-6 production (60). We did not observe any effect of IL-4 on the cell number and viability of EC *ex vivo*, as measured by cell counting and trypan blue exclusion, respectively. In fact, the effects of IL-4 on cell growth and IL-6 production may be the result of distinct cellular regulatory pathways (61). More recently, IL-4 was also reported to modulate epithelial cell function, as IL-4 dampened the cellular inflammation in human intestine (62). As PP skin, and even to a lesser extent PN skin, show both an enhanced epidermal expression of IL-4R as well as an enhanced inflammatory response (i.e. increased expression of cytokines and leukocyte infiltration) relative to NN skin, the IL-4-mediated modulatory effects may prove relevant to the therapy of psoriasis.

Taken together, we conclude that IL-4 interferes with the production of IL-6 protein, and to a lesser extent of IL-1β protein, by PP EC. We speculate that the upregulated expression of the IL-4R in psoriatic epidermis may represent a regulatory feedback mechanism to downregulate the local epidermal IL-1 and IL-6 overexpression. In line with this thought, we observed that IL-4 upregulated the expression of IL-1ra and IL-1RII mRNAs in PP EC (not shown). In addition, the epidermal expression of IL-4R protein (Table II) correlated significantly with those of IL-1ra and IL-1RII (IL-4R with IL-1ra: $r_s = 0.69$, $n = 22$, $P < 0.0025$; and IL-4R with IL-1RII: $r_s = 0.58$, $n = 20$,

$P < 0.01$) (see for individual scores of IL-1ra and IL-1RII, chapter 3.1). This suggests that the increased expression of IL-4R by psoriatic epidermis represents a mean to downregulate the IL-1 agonists (i.e. IL-1β) and IL-1-inducible cytokines (i.e. IL-6) and to upregulate the IL-1 antagonists (i.e. IL-1ra and IL-1RII), thereby repressing the epidermal IL-1 system effectively.

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REFERENCES

1. McKay IA, Leigh IM (1995) Altered keratinocyte growth and differentiation in psoriasis. In Van De Kerkhof PCM, Bos JD (eds). Pathogenic aspects of psoriasis. Clin Dermatol 13:105-114.
2. Prens E, Debets R, Hegmans J (1995) T lymphocytes in psoriasis. In Van De Kerkhof PCM, Bos JD (eds). Pathogenic aspects of psoriasis. Clin Dermatol 13:115-130.
3. Baadsgaard O, Tong P, Elder JT, Hansen ER, Ho V, Hammerberg C, Lange-Vejlsgaard G, Fox DA, Fisher G, Chan LS, Voorhees JJ, Cooper KD (1990) UM4D4$^+$ (CDw60) T cells are compartmentalized into psoriatic skin and release lymphokines that induce a keratinocyte phenotype expressed in psoriatic lesions. J Invest Dermatol 95:275-282.
4. Strange P, Cooper KD, Hansen ER, Fisher G, Larsen JK, Fox D, Krag C, Voorhees JJ, Baadsgaard O (1993) T-lymphocyte clones initiated from lesional psoriatic skin release growth factors that induce keratinocyte proliferation. J Invest Dermatol 101:695-700.
5. Chang EY, Hammerberg C, Fisher G, Baadsgaard O, Ellis CN, Voorhees JJ, Cooper KD (1992) T-cell activation is potentiated by cytokines released by lesional psoriatic, but not normal, epidermis. Arch Dermatol 128:1479-1485.
6. Cooper KD, Hammerberg C, Baadsgaard O, Elder JT, Chan LS, Sauder DN, Voorhees JJ, Fisher G (1990) IL-1 activity is reduced in psoriatic skin. Decreased IL-1α and increased nonfunctional IL-1β. J Immunol 144:4593-4603.
7. Hammerberg C, Arend WP, Fisher GJ, Chan LS, Berger AE, Haskill SJ, Voorhees JJ, Cooper KD (1992) Interleukin-1 receptor antagonist in normal and psoriatic epidermis. J Clin Invest 90:571-583.
8. Groves RW, Sherman L, Mizutani H, Dower SK, Kupper TS (1994) Detection of interleukin-1 receptors in human epidermis. Induction of the type II receptor after organ culture and in psoriasis. Am J Pathol 145:1048-1056.
9. Prens EP, Benne K, Van Damme J, Bakkus M, Brakel K, Benner R, Van Joost T (1990) Interleukin-1 and interleukin-6 in psoriasis. J Invest Dermatol 95:121S-124S.
10. Grossman RM, Krueger J, Yourish D, Granelli-Piperno A, Murphy DP, May LT, Kupper TS, Sehgal PB, Gottlieb AB (1989) Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proc Natl Acad Sci USA 86:6367-6371.
11. Neuner P, Urbanski A, Trautinger F, Möller A, Kirnbauer R, Kapp A, Schöpf E, Schwarz T, Luger TA (1991) Increased IL-6 production by monocytes and keratinocytes in patients with psoriasis. J Invest Dermatol 97:27-33.
12. Uyemura K, Yamamura M, Fivenson DF, Modlin RL, Nickoloff BJ (1993) The cytokine network in lesional and lesion-free psoriatic skin is characterized by a T-helper type 1 cell-mediated response. J Invest Dermatol 101:701-705.
13. Ristow HJ (1987) A major factor contributing to epidermal proliferation in inflammatory

skin diseases appears to be interleukin 1 or a related protein. Proc Natl Acad Sci USA 84:1940-1944.
14. Prens E, Hegmans J, Chin A Lien R, Debets R, Troost R, Van Joost T, Benner R (1996) Increased expression of interleukin-4 receptor on psoriatic epidermal cells. Am J Pathol, in press.
15. Schlaak JF, Buslau M, Jochum W, Hermann E, Girndt M, Gallati H, Buschenfelde K-H, Fleischer B (1994) T cells involved in psoriasis vulgaris belong to the Th1 subset. J Invest Dermatol 102:145-149.
16. Paul WE, Ohara J (1987) B-cell stimulatory factor-1/interleukin-4 (review). Annu Rev Immunol 5:429-459.
17. De France T, Vanbervliet T, Aubrey JP, Takebe Y, Arai N, Miyajima A, Yokota T, Lee F, Arai KI, De Vries JE, Banchereau J (1987) B cell growth promoting activity of recombinant human interleukin 4. J Immunol 139:1135-1144.
18. Brown M, Hu-Li J, Paul WE (1988) IL-4/B cell stimulatory factor-1 stimulates T cell growth by an IL-2 independent mechanism. J Immunol 131:504-511.
19. Coffman RL, Ohara J, Bonds MW, Carty J, Zlotnik A, Paul WE (1986) B cell stimulatory factor-1 enhances IgE response of lipopolysaccharide-activated B cells. J Immunol 136:4538-4541.
20. Toi M, Harris AL, Bicknell R (1991) Interleukin 4 is a potent mitogen for capillary endothelium. Biochem Biophys Res Commun 174:1287-1293.
21. Feghali CA, Bost KL, Boulware DW, Levy LS (1992) Human recombinant interleukin-4 induces proliferation and interleukin-6 production by cultured human skin fibroblasts. Clin Immunol Immunopathol 63:182-187.
22. Hart PH, Vitti GF, Burgess DR, Whitty GA, Piccoli DS, Hamilton JA (1989) Potential antiinflammatory effects of interleukin 4. Suppression of human monocyte tumor necrosis factor α, interleukin 1, and prostaglandin E_2. Proc Natl Acad Sci USA 86:3803-3807.
23. Lee JD, Swisher SG, Minehart EH, McBride WH, Economou JS (1990) Interleukin-4 downregulates interleukin 6 production in human peripheral blood mononuclear cells. J Leukocyte Biol 47:475-479.
24. Te Velde AA, Huijbens RJF, Heije K, De Vries JE, Figdor CG (1990) Interleukin-4 (IL-4) inhibits secretion of IL-1β, tumor necrosis factor α, and IL-6 by human monocytes. Blood 76:1392-1397.
25. Hoon DSB, Banez M, Okun E, Morton DL, Irie RF (1991) Modulation of human melanoma cells by interleukin-4 and in combination with γ-interferon or α-tumor necrosis factor. Cancer Res 51:2002-2008.
26. Donnelly RP, Crofford LJ, Freeman SL, Buras J, Remmers E, Wilder RL, Fenton MJ (1993) Tissue-specific regulation of IL-6 production by IL-4. Differential effects of IL-4 on nuclear factor-κB activity in monocytes and fibroblasts. J Immunol 151:5603-5612.
27. Katz Y, Strunk RC (1990) Enhanced synthesis of factor B induced by tumor necrosis factor in human skin fibroblasts is decreased by IL-4. J Immunol 144:4675-4680.
28. Dokter WHA, Esselink MT, Halie MR, Vellenga E (1993) Interleukin-4 inhibits the lipopolysaccharide-induced expression of c-jun and c-fos messenger RNA and activator protein-1 binding activity in human monocytes. Blood 81:337-343.
29. Schwarz EM, Salgame P, Bloom BR (1993) Molecular regulation of human interleukin 2 and T-cell function by interleukin 4. Proc Natl Acad Sci USA 90:7734-7738.
30. Mori N, Shirakawa F, Murakami S, Oda S, Eto S (1993) Inhibitory effect of interleukin 4 on production of interleukin 6 by adult T-cell leukemia cells. Cancer Res 53:4643-4647.
31. Jansen JH, Fibbe WE, Wientjens GJ, Van Damme J, Landegent JE, Willemze R, Kluin-Nelemans JC (1991) Interleukin 4 down-regulates the expression of CD14 and the production of interleukin 6 in acute myeloid leukemia cells. Lymphokine Cytokine Res 10:457-461.
32. Smeland EB, Blomhoff HK, Funderud S, Shalaby MR, Espevik T (1989) Interleukin 4 induces selective production of interleukin 6 from normal human B lymphocytes. J Exp Med 170:1463-1468.
33. Howells G, Pham P, Taylor D, Foxwell B, Feldmann M (1991) Interleukin 4 induces

interleukin 6 production by endothelial cells. Synergy with interferon-γ. Eur J Immunol 21:97-101.
34. Kupper TS, Min K, Sehgal P, Mizutani H, Birchall N, Ray A, May L (1989) Production of IL-6 by keratinocytes. Ann NY Acad Sci 557:454-465.
35. Park LS, Friend D, Sassenfeld HM, Urdal DL (1987) Characterization of the human B cell stimulatory factor 1 receptor. J Exp Med 166:476-488.
36. Obiri NI, Siegel JP, Varricchio F, Puri RK (1994) Expression of high-affinity IL-4 receptors on human melanoma, ovarian and breast carcinoma cells. Clin Exp Immunol 95:148-155.
37. Al Jabaari B, Ladyman HM, Larché M, Sivolapenko GB, Epenetos AA, Ritter MA (1989) Elevated expression of the interleukin 4 receptor in carcinoma: a target for immunotherapy. Br J Cancer 59:910-914.
38. Longley J, Gang Ding T, Cuono C, Durden F, Crooks C, Hufeisen S, Eckert R, Wood GS (1991) Isolation, detection, and amplification of intact mRNA from dermatome strips, epidermal sheets, and sorted epidermal cells. J Invest Dermatol 97:974-979.
39. Fredriksson T, Pettersson U (1978) Severe psoriasis-oral therapy with a new retinoid. Dermatologica 157:238-242.
40. Griffiths CEM, Barker JNWN, Kunkel S, Nickoloff BJ (1991) Modulation of leucocyte adhesion molecules, a T-cell chemotaxin (IL-8) and a regulatory cytokine (TNF-α) in allergic contact dermatitis (rhus dermatitis). Br J Dermatol 124:519-526.
41. Idzerda RL, March CJ, Mosley B, Lyman SD, Van Den Bos T, Gimpel SD, Din WS, Grabstein KH, Widmer MB, Park LS, Cosman D, Beckmann PM (1990) Human interleukin 4 receptor confers biological responsiveness and defines a novel receptor superfamily. J Exp Med 171:861-873.
42. Cox KH, De Leon DV, Angerer LM, Angerer RR (1984) Detection of mRNAs in sea urchin embryos by in situ hybridization using asymmetric RNA probes. Dev Biol 101:485-502.
43. Yokota T, Otsuka T, Mosmann T, Banchereau J, De France T, Blanchard D, De Vries J, Lee F, Arai K (1986) Isolation and characterization of a human interleukin cDNA clone, homologous to mouse B-cell stimulatory factor 1, that expresses B-cell- and T-cell-stimulating activities. Proc Natl Acad Sci USA 83:5894-5898.
44. Spits H, Yssel H, Takebe Y, Arai N, Yokota T, Lee F, Arai K-I, Banchereau J, De Vries JE (1987) Recombinant interleukin 4 promotes the growth of human T cells. J Immunol 139:1142-1147.
45. Chomczynski P, Sacchi N (1987) Single-step method for RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156-159.
46. De Waal Malefyt R, Abrams J, Bennett B, Figdor CG, De Vries JE (1991) Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Exp Med 174:1209-1220.
47. Brenner CA, Tam AW, Nelson PA, Engleman EG, Suzuki N, Fry KE, Larrick JW (1989) Message amplification phenotyping (MAPPing): A technique to simultaneously measure multiple mRNAs from small numbers of cells. Biotechniques 7:1096-1103.
48. Koning H, Baert MRM, De Groot R, Neijens HJ, Savelkoul HFJ (1995) Analysis of cytokine gene expression in stimulated T cells in small children by semi-quantitative PCR. Med Inflamm 4:196-204.
49. Altschul SF, Warren G, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403-410.
50. Ruhl S, Feldman GM, Akahane K, Pluznik DH (1993) Interferon-γ antagonizes interleukin-6-induced expression of Interleukin-4 receptors in murine myeloid cells by a transcriptional mechanism. Blood 82:2641-2648.
51. Ohta Y, Katayama I, Funato T, Yokozeki H, Nishiyama S, Hirano T, Kishimoto T, Nishioka K (1991) In situ expression of messenger RNA of interleukin-1 and interleukin-6 in psoriasis: interleukin-6 involved in formation of psoriatic lesions. Arch Dermatol Res 283:351-356.
52. Scheynius A, Fransson J, Johansson C, Hammar H, Baker B, Fry L, Valdimarsson H (1992) Expression of interferon-gamma receptors in normal and psoriatic skin. J Invest Dermatol 98:255-258.
53. Baker BS, Powles AV, Valdimarsson H, Fry L (1988) An altered response by psoriatic

keratinocytes to gamma interferon. Scand J Immunol 28:735-740.
54. Elder JT, Sartor CI, Boman DK, Benrazavi S, Fisher GJ, Pittelkow MR (1992) Interleukin-6 in psoriasis: expression and mitogenicity studies. Arch Dermatol Res 284:324-332.
55. Fenton MJ, Buras JA, Donnelly RP (1992) IL-4 reciprocally regulates IL-1 and IL-1 receptor antagonist expression in human monocytes. J Immunol 149:1283-1288.
56. Auron PE, Webb AC (1994) Interleukin-1: a gene expression system regulated at multiple levels. Eur Cytokine Netw 5:573-592.
57. Kirnbauer R, Köck A, Neuner P, Förster E, Krutmann J, Urbanski A, Schauer E, Ansel JC, Schwarz T, Luger TA (1991) Regulation of epidermal cell interleukin-6 production by UV light and corticosteroids. J Invest Dermatol 96:484-489.
58. Krueger JG, Ray A, Tamm I, Sehgal PB (1991) Expression and function of interleukin-6 in epithelial cells. J Cell Biochem 45:327-334.
59. Krueger JG, Krane JF, Carter M, Gottlieb AB (1990) Role of growth factors, cytokines, and their receptors in the pathogenesis of psoriasis. J Invest Dermatol 94:135S-140S.
60. Herrmann F, Andreeff M, Gruss HJ, Brach MA, Lubbert M, Mertelsmann R (1991) Interleukin-4 inhibits growth of multiple myelomas by suppressing interleukin-6 expression. Blood 78:2070-2074.
61. Keegan AD, Nelms K, Wang L-M, Pierce JH, Paul WE (1994) Interleukin 4 receptor: signaling mechanisms. Immunol Today 15:423-432.
62. Colgan SP, Resnick MB, Parkos CA, Delp-Archer C, McGuirk D, Bacarra AE, Weller PF, Madara JL (1994) IL-4 directly modulates function of a model human intestinal epithelium. J Immunol 153:2122-2129.

Chapter 4

DERMAL IL-1 AND IL-1-RELATED CYTOKINES AND THEIR RECEPTORS IN PSORIASIS

Dermal IL-1 and IL-1-related cytokines and their receptors in psoriasis

Chapter 4.1

EXPRESSION OF CYTOKINES AND THEIR RECEPTORS BY PSORIATIC FIBROBLASTS. I. Altered IL-6 synthesis.*

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ABSTRACT

IL-6 is overproduced in psoriatic lesions. We investigated the contribution of dermal fibroblasts to the local IL-6 production. Fibroblasts (passageno. 2 to 6) derived from lesional psoriatic (PP) and normal human (NN) skin were used to analyze the secretion of IL-6, and the related cytokines IL-1, IL-8 and TNF-α, and the expression of their corresponding mRNA by bioassays, ELISA and Northern hybridization, respectively. PP fibroblasts cultured under serum-free conditions produced increased amounts of bioactive IL-6. Differences were partly restored in the presence of growth factors or serum. The serum-induced IL-6 production reached a maximum within 24 h after seeding and remained unchanged in PP fibroblasts, whereas comparable amounts of IL-6 were produced only 6 days later in NN fibroblasts. There was a clear expression of IL-6 mRNA in both types of fibroblasts under serum-free conditions. Unexpectedly, fetal calf serum, inactivated fetal calf serum as well as human serum completely inhibited the expression of IL-6 mRNA in all the PP fibroblast cultures investigated. NN fibroblasts were clearly less sensitive to this inhibiting effect of serum. Furthermore, medium supplemented with serum-free component or calcium also repressed IL-6 mRNA expression in PP fibroblasts in contrast to NN fibroblasts. Cycloheximide fully restored the repressing effect of serum indicating that serum induced a labile repressor protein. PP and NN fibroblasts produced negligible amounts of IL-1 and TNF-α, but the production of IL-8, however, was comparable to that of IL-6. Our results show a differently regulated IL-6 synthesis in PP fibroblasts *in vitro*, suggesting an active contribution of dermal fibroblasts to the local IL-6 production in psoriasis.

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INTRODUCTION

Psoriasis is a disease characterized by chronic cutaneous inflammation and increased proliferation of keratinocytes. Cutaneous homeostasis is clearly perturbed in psoriasis. Alterations observed in psoriatic skin include activation of resident and infiltrating cells, expression of inflammatory mediators, their receptors and adhesion molecules, and activation of intracellular signal transducing pathways (1,2). Several lines of investigation favor a contribution of the dermal fibroblast to the pathophysiology of psoriasis. Experiments using athymic nude mice showed that skin biopsies from psoriatic lesions, following transplantation onto these mice, retained the major histologic features of psoriasis (3). Later on, combined grafting studies showed that the classical characteristics of psoriasis only persisted when psoriatic epidermis was transplanted together with psoriatic dermis (4). Moreover, the rates of proliferation of normal and psoriatic human keratinocytes differ *in vivo*, but not in monoculture (5). It thus appeared that psoriatic keratinocytes need to be in contiguity with dermal fibroblasts to maintain epidermal hyperplasia *in vivo*. Several groups observed that the growth of human keratinocyte *in vitro* is supported by the use of fibroblasts or even connective tissue-derived small molecular weight material (5,6). Furthermore, Saiag and colleagues found that upper dermal fibroblasts from psoriatic patients cultured in a collagen lattice induced hyperproliferation in normal epidermal biopsies (7). Reproducibility of this finding depends on the anatomical location of the fibroblast (upper versus deeper dermis), the type of fibroblast culture (fibroblasts incorporated in collagen lattice versus monoculture) and epidermal cell culture (biopsy versus monolayer) used in the fibroblast - keratinocyte co-culture model (5,8,9). All together, studies suggest the involvement of fibroblast-derived cytokines in the pathogenesis of psoriasis. For instance, human dermal fibroblasts are able to produce interleukin 1 (IL-1), IL-6 and IL-8 (10-12), which are not only actively involved in inflammation but are also mitogenic for epidermal cells (13-15). Espinoza and colleagues have indeed shown that psoriatic fibroblasts produce elevated amounts of IL-1β (16).

We previously reported an elevated IL-6 bioactivity in psoriatic suction blister fluids but not in the corresponding serum samples (17). Analysis of IL-6 mRNA of keratinocyte and fibroblast cultures indicated that fibroblasts were the most likely cellular source for this local IL-6 production. Reports on the altered epidermal IL-6 expression in psoriasis are conflicting. Increased epidermal expression of IL-6 protein was detected with immunochemistry and of IL-6 mRNA with in situ hybridization using cultures of keratinocytes as well as skin biopsies from active psoriatic plaques (14,18). However, neither IL-6 protein nor IL-6 bioactivity could be detected in scales from psoriatic lesions and, using in situ hybridization, the increase of IL-6 mRNA in lesional psoriatic epidermis could not be reproduced by others (19,20). Furthermore, the presence of IL-6 mRNA in epidermal keratome biopsies was only obser-

ved with the sensitive polymerase chain reaction (PCR) technique. Contaminating dermal fibroblasts were assumed to be the source of the increased IL-6 expression in lesional psoriatic keratome biopsies (21).

In the present study we therefore investigated the secretion of IL-6 protein and expression of its RNA using early passages of fibroblasts derived from lesional psoriatic (PP) and normal control (NN) skin. The protein and mRNA expression of IL-1, IL-8 and tumor necrosis factor-α (TNF-α) were also studied. Experiments were performed with media supplemented with calcium, growth factors, cytokines and several types of serum.

MATERIALS AND METHODS

Patients and controls

Skin samples were obtained from ten otherwise healthy patients (Outpatient Department of Dermatology, University Hospital Rotterdam-Dijkzigt) with active plaque-type psoriasis after informed consent. Patients remained untreated for at least 3 weeks before entering the study. Ten individuals without history or signs of skin disease, undergoing abdominal or breast plastic surgery (Sint Franciscus Hospital, Rotterdam) served as normal healthy controls. All skin samples were collected after approval of the institutional medical ethical committee (MEC 104.050/SPO/1990/30).

Culturing of fibroblasts

Split-skin specimens from PP and NN skin, including epidermis and superficial dermis, were obtained using a portable dermatome (Davol Inc., Cranston, RI). Epidermis was separated from dermis via trypsinization. Primary cultures of fibroblasts were obtained by plating dermal parts in a 25 cm^2 culture flask (Becton Dickinson, Plymouth, UK). The dermal pieces were allowed to attach after which 2 ml of culture medium was added to the flask. The culture medium consisted of Dulbecco's modification of Eagle's medium (DMEM, Gibco Ltd., Paisley, Scotland) supplemented with 10 % fetal calf serum (FCS, Gibco), 100 U/ml penicillin and 100 µg/ml streptomycin. At confluence, cells were subcultured via trypsinization. After the first passage fibroblast cultures were free of epidermal cells. Contamination with mycoplasma species was excluded by testing antibiotic-free culture supernatants for adenosine phosphorylase activity (Mycotest, Gibco). For experiments, fibroblasts of passageno. 2 to 6 were collected, trypsinization was stopped with 100 µg/ml trypsin inhibitor and cells were extensively rinsed with PBS. Viability was determined by trypan blue exclusion.

Fibroblast-conditioned medium

Fibroblasts were plated in 12-well plates (Costar, Cambridge, MA) at a density of 500 viable cells/cm^2, using 1 ml of one of the following media: 1) a low-calcium (0.15 mM), serum-free medium (medium 1: keratinocyte basal

medium, KBM/unsupplemented MCDB 153, Clonetics, San Diego, CA); 2) medium 1 supplemented with 5 μg/ml insulin, 10 ng/ml human recombinant epidermal growth factor, 0.5 μg/ml hydrocortisone, 0.4 % (v/v) bovine pituitary extract and 50 U/ml gentamicine sulphate (medium 2: keratinocyte growth medium, KGM/supplemented MCDB 153, Clonetics); 3) a high-calcium (1.05 mM), serum-free medium consisting of Ham's F10 (Gibco) and DMEM in a ratio of 1:1 supplemented with antibiotics (medium 3); and 4) medium 3 supplemented with 5 % FCS (medium 4). The effects of different types of serum on the production of cytokines were investigated using another culture system, described below. For kinetic studies, cells and supernatants were collected on 5 consecutive days starting from day 3 after seeding. Each test-condition was tested in triplicates. Supernatants were collected by centrifugation, supplemented with 0.1 % BSA in case of serum-free supernatants and stored at -80 °C. Cells present in the same wells were collected via trypsinization and counted microscopically (Carl Zeiss, Germany).

Bioassays for IL-1, IL-6 and TNF-α

IL-1 bioactivity was measured using a subline of a cloned murine T cell line D10.G4.1, designated D10(N4)M (kindly provided by Dr. S. Hopkins, Manchester, UK) (22). IL-6 bioactivity was measured using a murine hybridoma cell line B9 (kindly donated by Prof. Dr. L. Aarden, Amsterdam, The Netherlands) (23). Proliferation of the cytokine dependent cell lines D10 and B9 was measured via [^{3}H]deoxythymidine incorporation. TNF-α bioactivity was measured using the murine fibroblast cell line WEHI 164.13 (kindly provided by Dr. W. Buurman, Maastricht, The Netherlands) (24). The MTT cytotoxicity assay was used to measure the viability of WEHI cells (25). Recombinant human IL-1β (UBI, Lake Placid, NY), IL-6 (Prof. Dr. L. Aarden) and TNF-α (UBI) served as positive controls for the D10, B9 and WEHI assay, respectively. Cytokine activities of the samples were corrected for background activity of the culture medium and expressed in U/ml or in U/1000 cells, with 1 U/ml corresponding with half-maximal response.

IL-8 ELISA

IL-8 immunoreactivity was measured by enzyme-linked immunosorbent assay (ELISA), as described previously (26). Dr. K. Matsushima (Tochigi-ken, Japan) kindly provided the monoclonal anti-IL-8 IgG_1 (WS4) and the rabbit polyclonal anti-IL-8 antibody (27). Recombinant human IL-8 (Dainippon, Japan) served as a positive control.

Northern hybridization

For RNA analysis fibroblasts were grown on 625 cm^2 culture plates (Gibco) until 60 % confluence was reached. Subsequently, cells were rinsed 2 times with PBS and cultured for 48 h under test-conditions. The test-conditions comprised media 1 to 4 and medium 1 supplemented with calcium

and different types of serum. The differed sera comprised FCS, FCS treated 30 min at 56 °C (inactivated FCS or FCS^-), pooled human AB serum (HS, Red Cross Blood Center, Rotterdam, The Netherlands) and serum-free component (SF-1, Costar) and were tested at 5 % (v/v). Calcium (hydrated calciumchloride) was added to a final concentration of 1.05 mM. Cycloheximide (Sigma) was added to some plates (10 µg/ml) 3 h before cell harvesting. Supernatants were collected and cells were harvested using a rubber policeman (Costar). Total cellular RNA was isolated using the guanidine thiocyanate extraction procedure (28). RNA samples of 25 µg were size fractionated by electrophoresis through 1 % agarose and 17 % formaldehyde gels, transferred to Nytran N nylon membranes (Schleicher and Schuell, Dassel, Germany) (29), and hybridized according to Jeffreys *et al.* with ^{32}P-labeled cDNA probes (30,31). Autoradiography was carried out with Kodak films at -70 °C for 1 to 10 days. Hybridized probes were removed from the nylon membranes according to Sambrook *et al.* (29).

Probes

Probes were derived from cDNA: IL-1α, a 0.6 kb *Eco*RI-*Hin*dIII fragment (Biogen, Basel, Switserland); IL-1β, a 1.3 kb *Pst*I fragment (Genetics Institute, Cambridge, MA); IL-6, a 0.3 kb *Eco*RI-*Hin*dIII fragment (32); IL-8, a 1.3 kb *Eco*RI fragment (33); TNF-α, a 0.6 kb *Eco*RI-*Cla*I frament (Celltech, Berkshire, UK); and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a 0.7 kb *Eco*RI-*Pst*I fragment (34), serving as a control for RNA loading.

Controls

Several cellular controls were used for the expression of cytokines at the protein and mRNA level: a fibroma-derived fibroblast cell line; NN fibroblasts stimulated with 50 ng/ml phorbol myristate acetate (PMA, Sigma Chemie, Bornem, Belgium), 10 µg/ml lipopolysaccharide (LPS, *Escherichia coli* 026:B6, Difco Laboratories, Detroit, MI), 100 U/ml recombinant human IL-1β or 100 U/ml recombinant human TNF-α; and PBMC and the myelomonocytic cell line THP-1 stimulated with LPS. Stimuli were added 14 h before cell harvesting.

Statistical analysis

Bioassay and ELISA results were analyzed with the Wilcoxon Rank Sum Test using STATA™ (Computing Resource Center, Los Angeles, CA).

RESULTS

PP fibroblasts secrete low, but increased amounts of IL-6 compared to NN fibroblasts under serum-free culture conditions

With a low-calcium (0.15 mM), serum-free medium (medium 1: KBM/unsupplemented MCDB 153) PP and NN fibroblasts hardly survived (< 300

viable cells) (Fig 1B). However, low but clearly detectable IL-6 levels were present in the corresponding fibroblast-conditioned media.

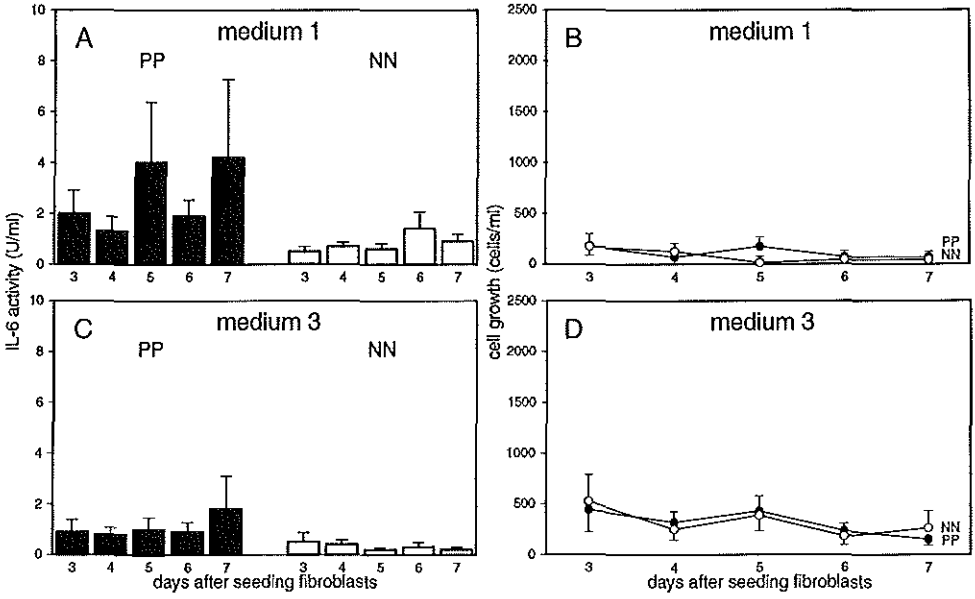

Figure 1. PP fibroblasts secrete low but increased amounts of IL-6 compared to NN fibroblasts under serum-free culture conditions. The IL-6 production (Figs A and C) and cell number (B and D) of PP and NN fibroblasts (both n = 6) were monitored on 5 consecutive days starting from day 3 after seeding using the B9 assay and cell counts, respectively. Cells were seeded at 500/cm^2 in 12-well plates using medium 1 (A and B) and 3 (C and D). See materials and methods section for a description of the media. Closed bars and circles represent PP fibroblasts and open bars and circles NN fibroblasts. Results are given in mean ± SEM.

PP fibroblasts secreted more bioactive IL-6 on each test day (5 consecutive days starting from day 3 after seeding), and on average about three times more IL-6 than NN fibroblasts, as illustrated in Figure 1A. Findings were reproduced with a high-calcium (1.05 mM), serum-free medium (medium 3: a 1:1 ratio of F10:DMEM), although, overall, the IL-6 levels were lower with the latter medium (Fig 1C and D). Fibroma-derived fibroblasts used as a control expressed on average 5 U/ml IL-6, being similar to the values of the PP fibroblasts. It is important to realize that very low numbers of PP fibroblasts are thus able to produce several units IL-6. Differences between PP and NN fibroblasts were more pronounced in the supernatants of larger numbers of fibroblasts (Table III).

The IL-6 production by PP fibroblasts is rapid, high and persisting under serum-stimulated conditions

Using medium 1 supplemented with growth factors, such as insulin, epidermal growth factor and a pituitary extract (medium 2), PP and NN fibroblasts were rescued from death, but did not show a proliferative response (Fig 2B). The IL-6 levels, although higher for PP fibroblasts, increased more substantially for NN fibroblasts (Fig 2A versus Fig 1A). This phenomenon was more marked when serum was used as a stimulus. Medium 4, which is medium 3 containing 5 % FCS, was the only culture medium supporting the proliferation of fibroblasts (Fig 2D). The number of both PP and NN fibroblasts doubled during the test-period of 5 days.

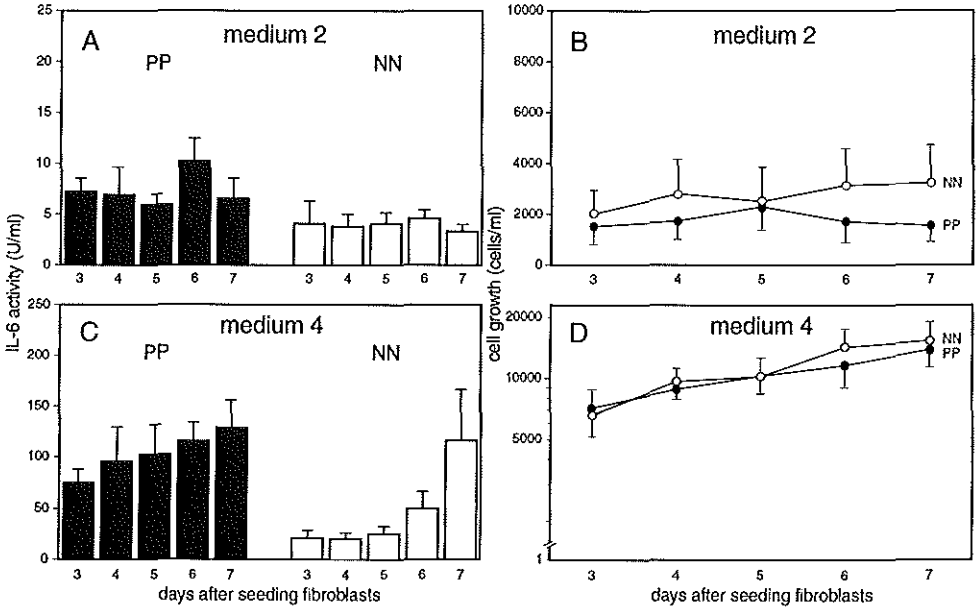

Figure 2. PP fibroblasts rapidly secrete high and persisting amounts of IL-6 under serum-stimulated conditions. See legend to Figure 1 for details. Medium 2 is medium 1 supplemented with growth factors. Medium 4 is medium 3 supplemented with FCS, as described in materials and methods section.

The amounts of bioactive IL-6 present in the supernatants increased about 6 times using NN fibroblasts but only 2 times using PP fibroblasts during this period. The IL-6 production corrected for the corresponding cell numbers reached a plateau level at the beginning of the test (about 10 U/1000 cells) and persisted in PP fibroblasts throughout the test-period, whereas comparable levels of IL-6 were produced only after one week of culture in NN fibroblasts (Table I). The IL-6 production by PP fibroblasts was already maximal at 24 h after seeding. The *de novo* production of IL-6 protein by confluent

fibroblasts, however, checked by changing the medium after 6 days of culture, was not increased in PP fibroblasts when compared to NN fibroblasts (Table II).

Table I. IL-6 production by PP and NN fibroblasts using a serum-containing medium.

Days in culture	Psoriasis U/1000 cells	Normal control U/1000 cells	P value
3	10.2 ± 1.8[a]	3.1 ± 0.7	0.007
4	10.7 ± 4.0	2.1 ± 0.6	0.004
5	11.7 ± 4.9	2.3 ± 0.4	0.010
6	10.4 ± 1.5	3.1 ± 0.7	0.007
7	8.9 ± 1.0	7.3 ± 3.3	0.080

[a] The IL-6 activity present in the supernatants of PP and NN fibroblasts (both n = 6) was monitored on 5 consecutive days starting from day 3 after seeding using the B9 assay. IL-6 activities were corrected for the corresponding cell number and expressed in U/1000 cells. Cells were seeded at 500/cm^2 in 12-well plates using medium 4. Results are given in mean ± SEM. P values < 0.05 were considered statistically significant.

Serum represses the expression of IL-6 mRNA in PP fibroblasts

PP and NN fibroblasts cultured with medium 1 had a clear expression of steady-state IL-6 mRNA levels, as shown in Figure 3. FCS added to medium 1 decreased the IL-6 message to undetectable levels in all the PP fibroblast cultures, but only in half of the NN fibroblast cultures investigated. When using medium 3, or medium 3 supplemented with FCS the expression of IL-6 mRNA was not detectable (not shown). To study the mechanism of the inhibitory process we analyzed the effect of cycloheximide. Results showed that the inhibitory effect of serum components required *de novo* protein synthesis.

Table II. The difference in IL-6 production between PP and NN fibroblasts is lost during culture.

Type of supernatant	Psoriasis U/ml	Normal control U/ml
Day 1	30[a]	7
Accumulated IL-6 at day 7	38	36
Newly synthesized IL-6 at day 7	45	57

[a] The IL-6 production of PP and NN fibroblasts (n = 3) seeded at 500 cells/cm^2 and cultured in medium 4 using 12-well plates was measured with the B9 assay and expressed in U/ml. Supernatants were collected at day 1 and 7 after seeding. Medium was changed 24 h before collecting supernatants at day 7 to analyze the amount of newly synthesized IL-6. Results of representative cultures are given.

Serum and calcium modulate the IL-6 mRNA expression differently in PP and NN fibroblasts

PP and NN fibroblasts responded differently to test-sera such as FCS, inactivated FCS, human serum and a serum-free component, as shown in Figure 4. In PP fibroblasts all types of serum repressed the IL-6 mRNA

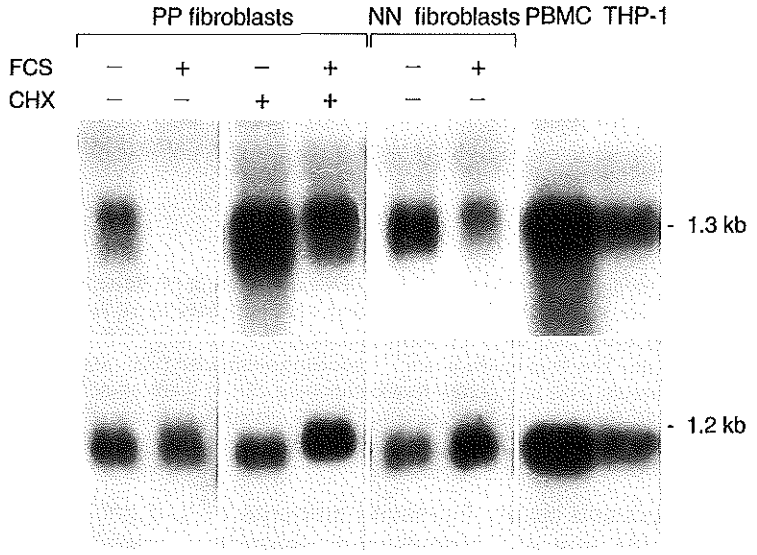

Figure 3. Serum represses the expression of IL-6 mRNA in PP fibroblasts. The expression of steady-state IL-6 mRNA levels in PP and NN fibroblasts (both n = 6) were analyzed by Northern hybridization after two days of culture in medium 1, or medium 1 supplemented with 5 % FCS. Autoradiography, to detect the message for IL-6 (1.3 kb band) and GAPDH (1.2 kb band), was carried out at -70 °C for 3 and 1 days, respectively. Cycloheximide (CHX: 10 µg/ml) was added 3 h before harvesting. PBMC and THP-1 cells were used as controls. See text for details.

expression completely. Unexpectedly, the IL-6 message was also repressed when using the serum-free component as a supplement. However, in NN fibroblasts the repression of IL-6 mRNA expression was only complete in part of the cultures tested with FCS (Fig 3) and inactivated FCS, but always complete with human serum. Moreover, the serum-free component even enhanced the IL-6 message in NN fibroblasts. Calcium, used as supplement, repressed the expression of IL-6 mRNA in PP fibroblasts, whereas the IL-6 message was not affected in NN fibroblasts.

Expression of IL-8 protein and mRNA levels are similar to IL-6

Apart from IL-6, fibroblasts also secrete IL-8, but only negligible amounts of IL-1 and TNF-α using conditions with or without serum. The production of IL-6 and IL-8 using different culture conditions are presented in Table III, and correspond to the same cultures of which RNA data is presented in Figure 3.

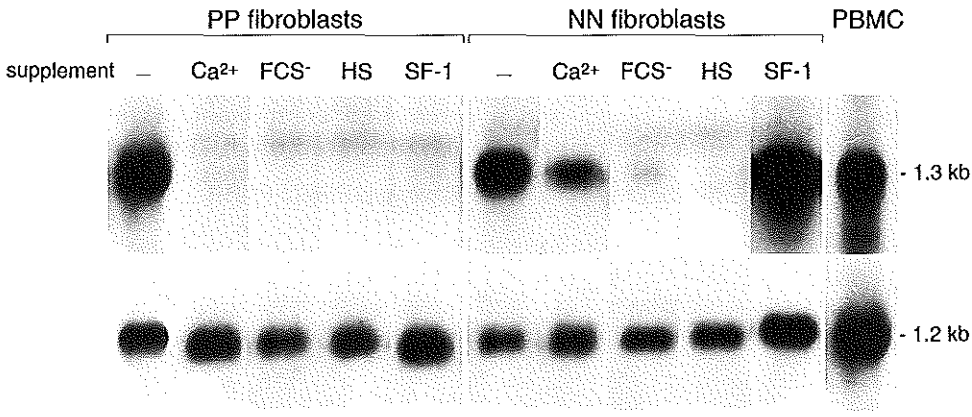

Figure 4. Serum-dependent repression of IL-6 mRNA expression in PP fibroblasts is not restricted to FCS. Steady-state IL-6 mRNA levels in PP and NN fibroblasts (n = 3) were analyzed by Northern hybridization after two days of culture in medium 1 supplemented with calcium or different types of serum. The final calcium (Ca^{2+}) concentration was 1.05 mM. FCS, inactivated FCS (FCS^{-}), human serum (HS) and serum-free component (SF-1) were all added to a final concentration of 5 % (v/v). See legend to Figure 3 for details. Results of representative cultures are given. PBMC were used as a control.

PP fibroblasts produced clearly increased amounts of IL-6 and IL-8 when cultured with both the low and high-calcium serum-free medium. The serum-induced IL-6 production was also elevated in PP fibroblasts after 2 days of culture. This is in line with Figures 1 and 2, and Table I. Differences in the IL-8 production, however, were completely restored by serum. In fact, the kinetics of the serum-induced IL-8 production by PP and NN fibroblasts were

Table III. IL-6 and IL-8 production by PP and NN fibroblasts under different culture conditions.[a]

Medium	Serum	Psoriasis		Normal control	
		IL-6 U/ml	IL-8 pg/ml	IL-6 U/ml	IL-8 pg/ml
1	-	2,961	1,600	289	30
1	+	1,932	940	74	690
3	-	222	310	21	13
3	+[b]	2,171	1,140	194	1,650

[a] The amount of IL-6 and IL-8 present in 48 h supernatants of PP and NN fibroblasts (n = 3) were measured with the B9 assay and IL-8 ELISA, respectively. Media used were medium 1 and 3, and these media supplemented with 5 % FCS. Cells were grown on 625 cm^2 culture plates to semi-confluence before test-media were added. Results are given of a representative PP and NN fibroblast culture.

[b] Medium 3 supplemented with serum is medium 4.

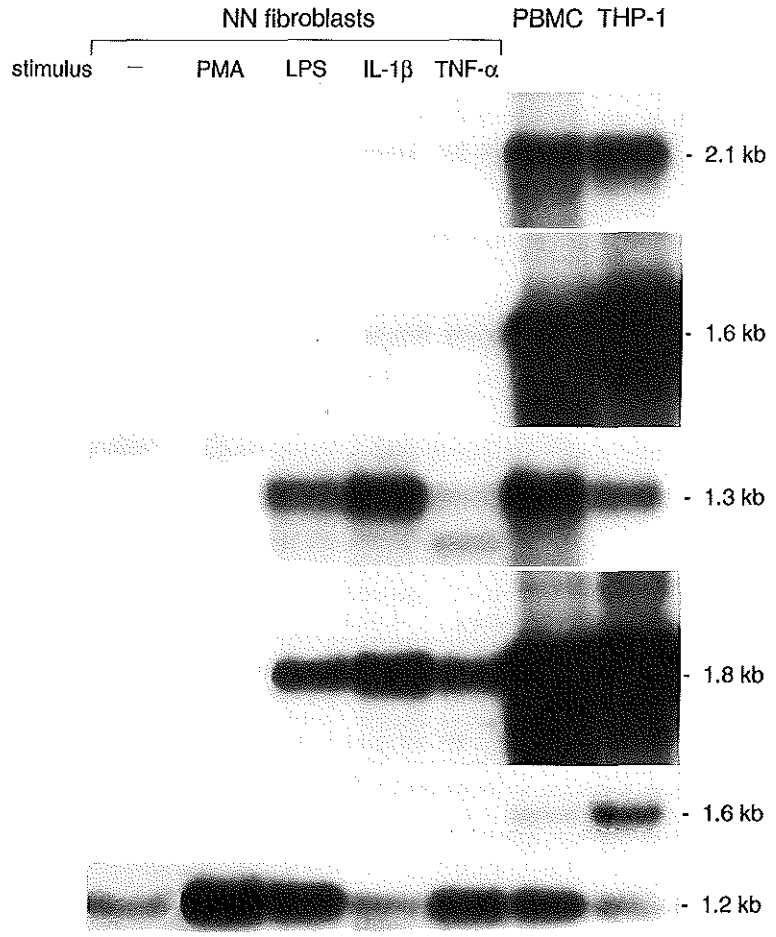

Figure 5. Fibroblasts express IL-6 and IL-8 mRNA, but not IL-1α, IL-1β and TNF-α mRNA. Steady-state levels of IL-1α, IL-1β, IL-6, IL-8 and TNF-α mRNA in NN fibroblasts were analyzed by Northern hybridization after two days of culture in different media with and without stimuli. Autoradiography, to detect the message for IL-1α (2.1 kb band), IL-1β (1.6 kb band), IL-6 (1.3 kb band), IL-8 (1.8 kb band), TNF-α (1.7 kb band) and GAPDH (1.2 kb band), was carried out for 10, 2, 3, 4, 4 and 1 days, respectively. Results of a representative culture are given. Experiment was performed in duplo. PBMC and THP-1 cells were used as controls.

similar (not shown). Fibroblasts did not express IL-1α, IL-1β or TNF-α mRNA. The expression of IL-8 mRNA, however, was similar to that of IL-6 mRNA using the different culture conditions. The use of stimuli like PMA, LPS, recombinant human IL-1β and TNF-α enhanced the expression of IL-6 and IL-8 mRNA, but that of IL-1α, IL-1β and TNF-α mRNA only to a negligible extent (Fig 5). The expression of IL-6 and IL-8 mRNA was most profoundly

enhanced by IL-1β. The corresponding amounts of cytokines present in the conditioned media of the same fibroblast culture are given in Table IV. It is important to note that although the fibroblast IL-8 synthesis is similar to that of IL-6, the differences between PP and NN fibroblasts were most marked for IL-6. In summary, an inventory of our findings on the altered IL-6 synthesis in PP fibroblasts is given in Table V.

Table IV. Cytokine production profile of fibroblasts.

Medium	Stimulus	Cytokines[a]			
		IL-1 U/ml	IL-6 U/ml	IL-8 pg/ml	TNF-α U/ml
1	-	0	210	20	0
1	serum	0	169	45	0
3	-	0	15	2	0
3	serum[b]	0	258	40	0
4	PMA	0	294	53	0
4	LPS	0	13,796	7,900	0
4	IL-1β	0	38,603	1,050	0
4	TNF-α	0	4,523	600	0

[a] The amounts of bioactive IL-1, IL-6 and TNF-α and immunoreactive IL-8 present in 48 h supernatants of NN fibroblasts were measured with the D10, B9, WEHI bioassay and IL-8 ELISA, respectively. IL-1, IL-6 and TNF-α are expressed in U/ml, and IL-8 in pg/ml. Media used were medium 1 and 3, these two media supplemented with 5 % FCS, and medium 4 with stimuli. PMA (50 ng/ml), LPS (10 μg/ml), IL-1β (100 U/ml) or TNF-α (100 U/ml) were added as stimuli to the cells 14 h before collecting supernatants. Results are given of a representative culture. The experiment was performed in duplo. Supernatant of LPS-stimulated PBMC contained 56,000 U/ml IL-1; 28,000 U/ml IL-6; 800 pg/ml IL-8; and 15 U/ml TNF-α.

[b] Medium 3 supplemented with serum is medium 4.

DISCUSSION

The serum-free production of IL-6 and IL-8 by PP fibroblasts was increased when compared to NN fibroblasts (Fig 1 and Table III). The growth factors present in medium 2 enhanced the production of IL-6 (Fig 2) and IL-8 (not shown) most markedly in NN fibroblasts, resulting in a reduced difference in cytokine production between the two types of fibroblasts. Kinetics showed that PP fibroblasts rapidly produced high and persisting levels of IL-6 under serum-stimulating conditions. The production of IL-6, not of IL-8, was significantly enhanced in PP fibroblasts when compared to NN fibroblasts (Table I). Using confluent PP fibroblasts (6 days after seeding), however, we observed that the serum-induced IL-6 (Table II) was similar or even less than their normal counterparts. This may explain why the differences in IL-6 production were lost after a culture-period of one week. It remains possible that PP fibroblasts display an altered post-translational modification of IL-6

Table V. IL-6 synthesis is altered in PP fibroblasts.

	stimulus	Psoriasis	Normal control
IL-6 protein	none	+[a]	-
	FCS	+ +	+
IL-6 mRNA	none	+ +	+ +
	FCS	-	±
	inactivated FCS	-	±
	human serum	-	-
	serum-free component	±	+ +
	calcium	±	+ +
	CHX	+ +	+ +

[a] Summary of the B9 assay and Northern hybridization results, with the expressions of IL-6 protein and mRNA given in symbols: -, absent or low in all experiments; ±, present in part of the experiments; +/+ +, present/clearly present in all experiments. CHX: cycloheximide.

resulting, for example, in a decreased aggregation of IL-6 monomers which express more activity in the B9 assay (35), or in a prolonged half-life of the protein. However, our finding that differences in IL-6 levels are already present in supernatants collected rapidly (24 h) after seeding do not favor an altered half-life of PP fibroblast-derived IL-6 protein.

The IL-6 production of normal human dermal fibroblasts is highly serum-dependent, as already reported for normal human monocytes and keratinocytes (23,36). Normal human cells typically do not express IL-6 in the absence of a stimulatory signal (37). Fibroblasts derived from PP skin, however, are less dependent on external stimuli for their IL-6 production. The enhanced production of IL-6 is not unique for PP fibroblasts. Constitutive production of IL-6, without exogenous stimuli, has already been described for dermal fibroblasts derived from affected skin sites from patients with systemic sclerosis (38), synovial fibroblasts from patients with rheumatic arthritis (39), mesothelioma cell lines (40), and peripheral blood monocytes from psoriatic patients (18). This suggests that overproduction of IL-6 could represent a general feature of affected mesenchymal cells thereby contributing to the maintenance of inflammation.

IL-6 production is regulated at the level of transcription and mRNA stability (41,42). Transcription of the human IL-6 gene is normally enhanced by serum as evidenced by the location of the *c-fos* serum-responsive element homology (*c-fos* SRE) within the IL-6 promoter (43). PP and NN fibroblasts had a clear, but similar expression of steady-state IL-6 mRNA levels using

medium 1 (0.15 mM calcium). This protein-deficient medium may be unable to support the production of a repressor protein of IL-6 mRNA transcription. Unexpectedly, this protein is present in serum-containing media, as explained later on. One also cannot exclude the possibility that medium 1 has a stabilizing effect on IL-6 mRNA. The corresponding IL-6 protein levels, especially of PP fibroblasts (Table III), do not favor, however, inhibition of IL-6 translation. With medium 3 (1.05 mM calcium) the message for IL-6 is not detectable. Normal human keratinocytes, grown in a serum-free medium containing 0.15 mM calcium, did not express detectable IL-6 mRNA levels using Northern hybridization. Low levels of IL-6 mRNA could be induced in these cells by treatment with 1.8 mM calcium (21). This suggests cell-type specific differences in the induction of IL-6 gene expression. Serum reduced the IL-6 message completely in all the PP fibroblast cultures, but only in part of the NN fibroblast cultures investigated. The expression of IL-6 mRNA is, however, reported to be induced in human foreskin, synovial and bone marrow stromal fibroblasts within several hours in response to serum when fibroblasts were made quiescent by serum deprivation and RNA levels were analyzed using blot hybridization with poly(A+) RNA or PCR amplified products (44-46). Apart from technical differences, our findings using human dermal fibroblasts may be attributed to tissue-specific serum-responsiveness of fibroblasts. The repressing effect of serum on the IL-6 mRNA expression in PP fibroblasts could be completely abolished by cycloheximide treatment. This suggests that, at least with medium 1, serum induces a labile protein which represses the levels of IL-6 mRNA.

Experiments were performed to determine whether the inhibiting effect was specific for FCS. In PP fibroblasts the IL-6 mRNA expression was repressed by all test-sera. Complement factors, known to enhance the IL-6 production of stimulated human monocytes (47), did not affect the IL-6 mRNA expression in our experiments, as shown by the use of inactivated serum. Furthermore, the use of human serum showed that a species-specific serum component did not seem to be involved. However, the repression of the IL-6 message in NN fibroblasts was more susceptible to human serum than FCS. Whether the presence of cytokines, their soluble receptors or anti-cytokine autoantibodies in serum contributes to differences in responsiveness between PP and NN fibroblasts remains to be resolved (48,49). The use of a serum-free supplement or calcium repressed the expression of IL-6 mRNA in PP fibroblasts, whereas the levels of IL-6 mRNA were unaffected or even enhanced in NN fibroblasts. Components of serum-free supplement (e.g. albumin, ethanolamines, insulin, transferrin and free fatty acids) and calcium are reported to be inducers of IL-6 production (21,50,51). Thus, on the one hand our findings confirm that serum factors and calcium induce production of IL-6 in normal human dermal fibroblasts and on the other hand indicate that the IL-6 production in PP fibroblasts is regulated differently by serum factors and calcium. Factors present in FCS and human serum as well as several growth factors were reported to induce proliferation in PP fibroblasts

to a higher extent when compared to NN fibroblasts (52,53). However, we and others did not observe differences in cell growth between both types of fibroblasts (5). Our study suggests that IL-6, being anti-mitotic for fibroblasts, is not primarily involved in the regulation of PP fibroblast growth *in vitro*, as is the case for platelet-derived growth factor (PDGF) (16). The altered IL-6 synthesis of multi-passaged PP fibroblasts confirms early studies which demonstrated several inherent abnormalities such as increased production of extracellular matrix components, intracellular signaling and resistance to therapeutics resulting in an hyperactive state of these cells *in vitro* (54-56).

Human fibroblasts are, apart from IL-6, potent producers of IL-8, but do not produce IL-1α, IL-1β or TNF-α even when using potent stimuli like PMA, LPS and the cytokines IL-1β and TNF-α. These cells express negligible amounts of the corresponding mRNA (Table IV and Fig 5). Fibroblasts derived from different tissues display some discrepancies with regard to the expression of IL-1 (57). Espinoza and colleagues showed that anti-IL-1β polyclonal antibody partly neutralized the fibroblast mitogenic activity of supernatants of PP fibroblasts. Their results indicate that the latter assay probably is more sensitive when compared to the D10 assay (16). The production of IL-8 was similar to that of IL-6. However, differences in the serum-induced production of IL-6 protein between PP and NN fibroblasts were specific for this cytokine (Table III). Taken together, our findings show that PP fibroblasts display an altered synthesis of IL-6 *in vitro*, which is regulated differently by serum factors and calcium. These alterations are summarized in Table V. The *in vivo* relevance of IL-6 to skin physiology and pathology has previously been extensively reviewed (58,59). During cutaneous inflammation IL-6 maintains the inflammatory response by attracting leukocytes and activating inflammatory and resident cells via the induction of cytokines and adhesion molecules. Moreover, fibroblast-derived IL-6 may even enhance antigen presentation as IL-6 can induce the expression of the HLA-B7 gene in human fibroblasts (60). Cutaneous IL-6 production is drastically increased during injury, infections, neoplasia and autoimmune diseases. We provide evidence that dermal fibroblasts are an important source of the elevated IL-6 levels in psoriatic lesions. The contribution of IL-6 to the pathogenesis of psoriasis is still obscure. However, the findings that vitamin D3 analogs are an efficient anti-psoriatic therapy and that these substances decrease fibroblast-derived IL-6 and IL-8 levels *in vitro* imply a role of dermal IL-6 and IL-8 in psoriasis (61,62). In our opinion dermal fibroblasts are actively involved in maintaining the inflammatory response in psoriatic lesions.

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REFERENCES

1. Bos JD (1988) The pathomechanisms of psoriasis; the skin immune system and cyclosporine (review). Br J Dermatol 118:141-155.
2. Christophers E, Schubert C (1986) Psoriasis. In Thody AJ, Friedmann PS (eds). Scientific basis of dermatology (a physiological approach). Churchill Livingstone Publisher, London, pp. 151-174.
3. Krueger GG, Manning DD, Malouf J, Ogden B (1975) Long-term maintenance of psoriatic human skin on congenitally athymic (nude) mice. J Invest Dermatol 64:307-312.
4. Fräki JE, Briggaman RA, Lazarus GS (1983) Transplantation of psoriatic skin onto nude mouse. J Invest Dermatol 80:31S-35S.
5. Baden HP, Kubilus J, Macdonald MJ (1981) Normal and psoriatic keratinocytes and fibroblasts compared in culture. J Invest Dermatol 76:53-55.
6. Karasek MA (1972) Dermal factors affecting epidermal cells in vitro. J Invest Dermatol 59:99-101.
7. Saiag P, Coulomb B, Lebreton C, Bell E, Dubertret L (1985) Psoriatic fibroblasts induce hyperproliferation of normal keratinocytes in a skin equivalent model in vitro. Science 230:669-672.
8. Priestley GC, Lord R (1990) Fibroblast-keratinocyte interactions in psoriasis: failure of psoriatic fibroblasts to stimulate keratinocyte proliferation in vitro. Br J Dermatol 123:467-472.
9. Saiag P, Coulomb B, Rowland-Payne CM, Dubertret L, Touraine R (1986) Lasers, dermal fibroblasts and psoriasis. Br J Dermatol 115:744-745.
10. Mauviel A, Temime M, Charron D, Loyau G, Pujol J-P (1991) Induction of interleukin-1β production in human dermal fibroblasts by interleukin-1α and tumor necrosis factor-α. Involvement of protein kinase-dependent and adenylate cyclase-dependent regulatory pathways. J Cell Biochem 47:174-183.
11. Van Damme J, Cayphas S, Opdenakker G, Billiau A, Van Snick J (1987) Interleukin 1 and poly(rI)·poly(rC) induce production of a hybridoma growth factor by human fibroblasts. Eur J Immunol 17:1-7.
12. Larsen CG, Anderson AO, Oppenheim JJ, Matsushima K (1989) Production of interleukin-8 by human dermal fibroblasts and keratinocytes in response to interleukin-1 or tumour necrosis factor. Immunology 68:31-36.
13. Ristow HJ (1987) A major factor contributing to epidermal proliferation in inflammatory skin diseases appears to be interleukin 1 or a related protein. Proc Natl Acad Sci USA 84:1-940-1944.
14. Grossman RM, Krueger J, Yourish D, Granelli-Piperno A, Murphy DP, May LT, Kupper TS, Sehgal PB, Gottlieb AB (1989) Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proc Natl Acad Sci USA 86:6367-6371.
15. Tuschil A, Lam C, Halsberger A, Lindley I (1992) Interleukin-8 stimulates calcium transients and promotes epidermal cell proliferation. J Invest Dermatol 99:294-298.
16. Espinoza LR, Espinoza CG, Cuéllar ML, Scopelitis E, Silveira LH, Grotendorst GR (1994) Fibroblast function in psoriatic arthritis. II. Increased expression of β platelet derived growth factor receptors and increased production of growth factor and cytokines. J Rheumatol 21:1507-1514.
17. Prens EP, Benne K, Van Damme J, Bakkus M, Brakel K, Benner R, Van Joost T (1990) Interleukin 1 and interleukin 6 in psoriasis. J Invest Dermatol 95:121S-124S.
18. Neuner P, Urbanski A, Trautinger F, Möller A, Kirnbauer R, Kapp A, Schöpf E, Schwarz T, Luger TA (1991) Increased IL-6 production by monocytes and keratinocytes in patients with psoriasis. J Invest Dermatol 97:27-33.
19. Gearing AJH, Fincham NJ, Bird CR, Wadhwa M, Meager A, Cartwright JE, Camp RDR (1990) Cytokines in skin lesions of psoriasis. Cytokine 2:68-75.
20. Gillitzer R, Berger R, Mielke V, Muller C, Wolff K, Stingl G (1991) Upper keratinocytes of psoriatic lesions express high levels of NAP-1/IL-8 mRNA in situ. J Invest Dermatol 97:73-

79.
21. Elder JT, Sartor CI, Boman DK, Benrazavi S, Fisher GJ, Pittelkow MR (1992) Interleukin-6 in psoriasis: expression and mitogenicity studies. Arch Dermatol Res 284:324-332.
22. Hopkins SJ, Humphreys M (1989) Simple, sensitive and specific bioassay of interleukin 1. J Immunol Meth 120:271-276.
23. Aarden LA, De Groot ER, Schaap OL, Lansdorp PM (1987) Production of hybridoma growth factor by human monocytes. Eur J Immunol 17:1411-1416.
24. Espevik T, Nissen-Meyer J (1986) A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumor necrosis factor from human monocytes. J Immunol Meth 95:99-105.
25. Mosmann TJ (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Meth 65:55-63.
26. Deleuran B, Iversen L, Kristensen M, Field M, Kragballe K, Thestrup-Pedersen K, Stengaard-Pedersen K (1994) Interleukin-8 secretion and 15-lipoxygenase activity in rheumatoid arthritis: in vitro anti-inflammatory effects by interleukin-4 and interleukin-10, but not by interleukin-1 receptor antagonist protein. Br J Dermatol 33:520-525.
27. Ko Y, Mukaida N, Panyutich A, Voitenok NN, Matsushima K, Kawai T, Kasahara T (1992) Establishment of a sensitive enzyme-linked immunosorbent assay for human interleukin 8. J Immunol Method 149:227-235.
28. Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156-159.
29. Sambrook J, Fritsch EF, Maniatis T (eds) (1989) Molecular cloning: a laboratory manual. Cold spring harbor laboratory press, New York (second edition), Units 7.43-7.50 and 9.58.
30. Jeffreys AJ, Flavell RA (1977) A physical map of the DNA regions flanking the rabbit β-globin gene. Cell 12:429-439.
31. Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6-13.
32. Brakenhoff JPJ, De Groot ER, Evers RF, Pannekoek H, Aarden LA (1987) Molecular cloning and expression of hybridoma growth factor in Escherichia coli. J Immunol 139:4116-4121.
33. Dixit VM, Green S, Sarma V, Holzman L, Wolf FW, O'Rouke K, Ward PA, Prochownik EV, Marks RM (1990) Tumor necrosis factor-α induction of novel gene products in human endothelial cells including a macrophage-specific chemotaxin. J Biol Chem 265:2973-2980.
34. Benham FJ, Hodgkinson S, Davies KE (1984) A glyceraldehyde-3-phosphate dehydrogenase pseudogene on the short arm of the human X chromosomes defines a multigene family. EMBO 3:2635-2640.
35. May LT, Santhanam U, Sehgal PB (1991) On the multimeric nature of natural human interleukin-6. J Biol Chem 266:9950-9955.
36. Kirnbauer R, Köck A, Schwarz T, Urbanski A, Krutmann J, Borth W, Damm D, Shipley G, Ansel JC, Luger TA (1989) IFN-β2, B cell differentiation factor 2, or hybridoma growth factor (IL-6) is expressed and released by human epidermal cells and epidermoid carcinoma cell lines. J Immunol 142:1922-1928.
37. Kishimoto T (1989) The biology of interleukin-6. Blood 74:1-10.
38. Feghali CA, Bost KL, Boulware DW, Levy LS (1992) Mechanisms of pathogenesis in scleroderma I. Overproduction of interleukin 6 by fibroblasts cultured from affected skin sites of patients with scleroderma. J Rheumatol 19:1207-1211.
39. Bucala R, Ritchlin C, Winchester R, Cerami A (1991) Constitutive production of inflammatory and mitogenic cytokines by rheumatoid synovial fibroblasts. J Exp Med 173:569-574.
40. Schmitter D, Lauber B, Ragg B, Stahel RA (1992) Hematopoietic growth factors secreted by seven human pleural mesothelioma cell lines: interleukin-6 production as a common feature. Int J Cancer 51:296-301.
41. Zhang Y, Lin J-X, Vilček J (1990) Interleukin-6 induction by tumor necrosis factor and interleukin-1 in human fibroblasts involves activation of a nuclear factor binding to a kB-like sequence. Mol Cell Biol 10:3818-3823.
42. Elias JA, Lentz V (1990) IL-1 and tumor necrosis factor synergistically stimulate fibroblast IL-6 production and stabilize IL-6 messenger RNA. J Immunol 145:161-166.

43. Ray A, Sassone-Corsi P, Sehgal PB (1989) A multiple cytokine- and second messenger-responsive element in the enhancer of the human interleukin-6 gene: similarities with c-fos gene regulation. Mol Cell Biol 9:5537-5547.
44. Goodman L, Stein GH (1994) Basal and induced amounts of interleukin-6 mRNA decline progressively with age in human fibroblasts. J Biol Chem 269:19250-19255.
45. Guba SC, Sartor CI, Gottschalk LR, Ye-Hu J, Mulligan T, Emerson SG (1992) Bone marrow stromal fibroblasts secrete interleukin 6 and granulocyte-macrophage colony-stimulating factor in the absence of inflammatory stimulation: demonstration by serum-free bioassay, enzyme-linked immunosorbent assay, and reverse transcriptase polymerase chain reaction. Blood 80:1190-1198.
46. Rosenbaum JT, Cugnini R, Tara DC, Hefeneider S, Ansel JC (1992) Production and modulation of interleukin 6 synthesis by synoviocytes derived from patients with arthritic disease. Annal Rheum Dis 51:198-202.
47. Montz H, Koch KC, Zierz R, Gotze O (1991) The role of C5a in interleukin-6 production induced by lipopolysaccharide or interleukin-1. Immunology 74:373-379.
48. Maliszewski CR, Sato TA, Davison B, Jacobs CA, Finkelman FD, Fanslow WC (1994) In vivo biological effects of recombinant soluble interleukin-4 receptor. Proc Soc Exp Biol Med 206:233-237.
49. Hansen MB, Svenson M, Diamant M, Bendtzen K (1993) High-affinity IgG autoantibodies to IL-6 in sera of normal individuals are competitive inhibitors of IL-6 in vitro. Cytokine 5:72-80.
50. Shacter E, Arzadon GK, Williams JA (1993) Stimulation of interleukin-6 and prostaglandin E2 secretion from peritoneal macrophages by polymers of albumin. Blood 82:2853-2864.
51. Schillaci R, Eugui EM, Roldan A (1994) Insulin effect on interleukin 1 (IL-1) and interleukin 6 (IL-6) production by lipopolysaccharide-stimulated human monocytes. Horm Metab Res 26:113-115.
52. Espinoza LR, Aguilar JL, Espinoza CG, Cuéllar ML, Scopelitis E, Silveira LH (1994) Fibroblast function in psoriatic arthritis. I. Alteration of cell kinetics and growth factor responses. J Rheumatol 21:1502-1506.
53. Priestley GC, Adams LW (1985) Mitogenic effects of sera from normal and psoriatic subjects on human skin fibroblasts. Arch Dermatol Res 277:13-15.
54. Priestley GC, Adams LW (1983) Hyperactivity of fibroblasts cultured from psoriatic skin. II. Synthesis of macromolecules. Br J Dermatol 109:157-162.
55. Raynaud F, Evain-Brion D (1991) Protein kinase C activity in normal and psoriatic cells: cultures of fibroblasts and lymphocytes. Br J Dermatol 124:542-546.
56. MacLaughlin JA, Gange W, Taylor D, Smith E, Holick MF (1985) Cultured psoriatic fibroblasts from involved and uninvolved sites have a partial but not absolute resistance to the proliferation-inhibition activity of 1,25-dihydroxyvitamin D3. Proc Natl Acad Sci USA 82:5409-5412.
57. Yamato K, El-Hajjoui Z, Koeffler HP (1991) Expression of hematopoietic growth factor RNAs in human mesenchymal cells from various organs. Leukemia research 15:551-558.
58. Turksen K, Kupper TS, Degenstein L, Williams I, Fuchs E (1992) Interleukin 6: insights to its function in skin by overexpression in transgenic mice. Proc Natl Acad Sci USA 89: 5068-5072.
59. Sehgal PB (1990) Interleukin-6: molecular pathophysiology. J Invest Dermatol 94: 2S-6S.
60. May LT, Helfgott DC, Sehgal PB (1986) Anti-beta-interferon antibodies inhibit the increased expression of HLA-B7 mRNA in tumor necrosis factor-treated human fibroblasts: structural studies of the beta 2 interferon involved. Proc Natl Acad Sci USA 83:8957-8961.
61. Srviastava MD, Deluca H, Ambrus JL (1994) Inhibition of IL-6 and IL-8 production in human fibroblast cell lines by 1,25 (OH)2 vitamin D3 and two of its analogs with lower calcemic activity. Res Commun Chem Pathol Pharmacol 83:145-150.
62. Larsen CG, Kristensen M, Paludan K, Deleuran B, Thomsen MK, Zachariae C, Kragballe K, Matsushima K, Thestrup-Pedersen K (1991) 1,25 $(OH)_2$-D_3 is a potent regulator of interleukin-1 induced interleukin-8 expression and production. Biochem Biophys Res Com 176:1020-1026.

Chapter 4.2

EXPRESSION OF CYTOKINES AND THEIR RECEPTORS BY PSORIATIC FIBROBLASTS. II. Decreased TNF receptor expression.*

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ABSTRACT

Psoriatic fibroblasts produce enhanced amounts of IL-6 *in vitro*. This state of activation may reflect an altered expression of cytokine receptors, involved in auto/paracrine induction of IL-6. Cultures of dermal fibroblasts derived from lesional psoriatic (PP) and normal control (NN) skin were therefore analyzed for their ability to bind biotinylated recombinant human cytokines using flow cytometry. PP and NN fibroblasts bound negligible amounts of IL-1α and IL-1β, but clearly bound IL-4, IL-6 and TNF-α. Serum upregulated the number of NN fibroblasts which bound TNF-α, and to a lesser extent IL-6, but not the number of binding sites per cell. In contrast, this upregulation was significantly less in PP fibroblasts. This was not a result of differences in growth characteristics, receptor occupancy or an inability of stimulated PP fibroblasts to bind TNF-α. Immunocytochemistry of cells grown on slides showed that the TNF receptor type I (TNFRI) was the predominant receptor in NN fibroblasts and was localized to the nucleus and cytoplasm. The expression of TNFRI was clearly decreased in PP fibroblasts, which confirmed the binding studies. A slow and serum-induced shedding of TNFRI was observed, but not of the TNFRII, in both types of fibroblasts. Confluent multipassaged PP fibroblasts display both a decreased TNFR expression as well as an enhanced IL-6 production under serum conditions. These inherent abnormalities of PP fibroblasts imply the involvement of dermal fibroblasts in the maintenance of chronic inflammation in psoriasis.

INTRODUCTION

Human dermal fibroblasts are actively involved in inflammatory processes. These resident cells are not only responsible for the production and mainte-

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nance of the connective tissue matrix, but are also able to activate the overlying epidermal cells and contiguous endothelial cells, as well as to attract and activate infiltrating leukocytes. Their ability to take part in cellular communication is, at least in part, determined by the expression of adhesion molecules, cytokines and cytokine receptors. Human dermal fibroblasts are able to express the major histocompatibility complex (MHC) class I and II molecules (1,2), β_1 integrins and the intercellular adhesion molecule-1 (ICAM-1) (3,4). It has furthermore been reported that fibroblasts produce interleukin 1 (IL-1) isoforms, IL-6, IL-8, monocyte chemotactic protein-1 (MCP-1), leukemia inhibitory factor (LIF), platelet-derived growth factors (PDGFs) and the colony stimulating factors (CSFs) (5-11). Human dermal fibroblasts also express receptors (R) for IL-1, tumor necrosis factor (TNF), the interferons (IFNs), PDGF and the transforming growth factors (TGFs) (12-16). It is important to note that cytokine receptors often reflect the activation state of cells and are involved in the regulation of cytokine activity. Most receptors occur, apart from membrane forms, in soluble forms (17). Soluble receptors either antagonize cytokine activity by scavenging, neutralization and prevention of extravascular escape of cytokines, or agonize cytokine activity by increase of persistence and chaperoning of cytokines (18). The activity of cytokines is therefore, at least in part, the result of both the expression of cytokines as well as their receptors.

Active participation of dermal fibroblasts in the inflammatory mechanisms of psoriasis is evidenced by their hyperactive state *in vitro*. Psoriatic fibroblasts produce elevated amounts of superoxide anion, PDGF-BB and IL-1β, express elevated levels of surface proteases (e.g. exoaminopeptidases and cluster of differentiation (CD)26), protein kinase C activity and PDGFRβ, and show an altered proliferative response to serum factors, growth factors and several therapeutics (19-25). Fibroblasts are furthermore a potential source for the locally produced bioactive IL-6 in psoriasis (26,27). We observed that psoriatic fibroblasts express an increased production of IL-6 *in vitro* (chapter 4.1). IL-6 may link cutaneous inflammation and keratinocyte hyperproliferation in psoriasis. Moreover, in a mouse model, a psoriasiform inflammatory reaction was preceded by an increase in the endogenous IL-6 production in the skin (28). The present study was conducted to investigate whether the enhanced fibroblast-derived IL-6 activity in psoriasis was associated with an altered receptor expression. We therefore analyzed the expression of binding sites for IL-1α, IL-1β, IL-4, IL-6 and TNF-α, and the production of biologically active IL-6, as well as the IL-6-related cytokines IL-1 and TNF-α, of early passages of fibroblast cultures derived from lesional psoriatic (PP) and normal control (NN) skin. Experiments were performed using a low and high-calcium basal medium and stimuli such as cytokines and serum.

MATERIALS AND METHODS

Patients and controls

Ten patients with active plaque-type psoriasis and ten healthy volunteers, undergoing plastic surgery, were studied as described (chapter 4.1).

Culturing of fibroblasts

Split-skin specimens from PP and NN skin were used to establish fibroblast cultures (chapter 4.1). For experiments, fibroblasts of passageno. 2 to 6 were used. Cells were seeded in 175 cm^2 flasks and grown to subconfluence using Dulbecco's modification of Eagle's medium (DMEM, Gibco Ltd., Paisley, Scotland) supplemented with 10 % fetal calf serum (FCS, Gibco), 100 U/ml penicillin and 100 µg/ml streptomycin. Cells were subsequently rinsed twice with PBS and incubated for 24 or 72 h with one of the following test-media: 1) a low-calcium (0.15 mM), serum-free medium (keratinocyte basal medium, KBM/unsupplemented MCDB 153, Clonetics, San Diego, CA); 2) a high-calcium (1.05 mM), serum-free medium consisting of Ham's F10 (Gibco) and DMEM in a ratio of 1:1 supplemented with antibiotics; 3) the high-calcium medium supplemented with 5 % FCS. The stimuli used were 50 ng/ml phorbol myristate acetate (PMA, Sigma Chemie, Bornem, Belgium), 10 µg/ml lipopolysaccharide (LPS, *Escherichia coli* 026:B6, Difco Laboratories, Detroit, MI), 100 U/ml recombinant human IL-1β (UBI, Lake Placid, NY), 100 U/ml TNF-α (UBI) and 100 U/ml IFN-γ (Boehringer Ingelheim, Germany), added the last 14 h of the 24 h incubation with the serum-containing medium. Supernatants were collected by centrifugation and stored at -80 °C.

Quantitation of cytokine binding sites

Fibroblasts were collected (0.025 % trypsin and 0.1 % EDTA), trypsinization was stopped with 100 µg/ml trypsin inhibitor (Sigma), and cells were extensively washed with PBS. Viability was determined by trypan blue exclusion and always exceeded 95 %. The mild trypsinization procedure did not affect the binding of cytokines, as checked by the use of cells collected with EDTA only. The labeling was performed according to the protocol supplied by the manufacturer. In short, 5×10^4 cells were incubated for 1 h at 4 °C with approximately 180 ng biotinylated recombinant human IL-1α, IL-1β, IL-4, IL-6 or TNF-α (all purchased from R&D Systems, Minneapolis, MN) using 12 x 75 mm round bottom, polystyrene tubes (Becton Dickinson). Unbound cytokines were removed by two rinses with PBS containing 0.1 % BSA (washing buffer). Next, cells were incubated for 30 min at 4 °C in the dark with avidin-FITC (R&D Systems), used in combination with IL-1 labeling, or streptavidin-phycoerythrin (Becton Dickinson) used in combination with the other cytokines. Subsequently, cells were rinsed twice, resuspended in washing buffer and used for flow cytometry. Peripheral blood mononuclear cells (PBMC), the U937 promonocytic cell line and human epidermal cells

were used as positive controls for the expression of cytokine binding sites (not shown). Analysis was conducted using the FACScan^C and Lysis^C software programs (Becton Dickinson). The frequency of cells positive for the binding of cytokines was determined using single-parameter fluorescence histograms and corrected for aspecific staining of the second step (background). The results are presented either as histograms or as mean ± SEM percentage of positive cells. The relative number of binding sites per cell was estimated by the relative signal-to-noise values (29).

To correct for the number of receptors already occupied with cytokines *in vitro*, the receptor-bound cytokines were stripped in some experiments before labeling. Briefly, cells were suspended in the first stripping buffer (10 mM $Na_3C_6H_5O_7 \cdot 2H_2O$ and 0.14 M NaCl, pH 4.0) and kept for 20 sec on ice (30). The stripping was stopped by adding medium containing HEPES and BSA. Cells were rinsed twice with washing buffer before incubating the cells with the second stripping buffer (0.1 M glycine-HCl, pH 3.0) for 2 min on ice (12). The stripping was stopped and cells were washed twice before labeling. The efficiency of the procedure was checked by stripping cells after incubation with biotinylated cytokines. This reduced the signal intensity to background levels.

Cell cycle analysis

To analyze the cell cycle, cells were labeled with propidium iodide. Briefly, 5 x 10^4 fibroblasts were fixed with ice-cold 70 % ethanol for 15 min on ice. Subsequently, cells were collected by centrifugation (1000 g, 5 min) and treated with 1 U/ml DNAse-free RNAse A (Boehringer Mannheim, Germany) for 30 min at 37 °C. Finally, cells were centrifuged and resuspended in 100 µl of 50 µg/ml propidium iodide (Becton Dickinson), after which flow cytometry was performed. As a negative control, cells were treated with 100 U/ml DNAse (Sigma Chemical Co., St. Louis, MO) instead of RNAse.

ELISA for soluble TNFR

Soluble TNFR type I (sTNFRI) and TNFRII were measured with sandwich enzyme-linked immunosorbent assays (ELISA) using the monoclonal antibodies MR1-1, MR2-2 and rabbit anti-sTNFR antiserum (31).

Bioassays for IL-1, IL-6 and TNF-α

IL-1, IL-6 and TNF-α activities were measured using the B9, D10 and WEHI assay, respectively, as described elsewhere (32-34).

Immunocytochemistry

PP and NN fibroblasts were grown on slides, fixed with a 1:1 methanol:aceton solution for 15 min at room temperature and stained with the mouse anti-human TNFR monoclonal IgG_1 antibodies htr-9 and utr-1 (Dr. M. Brockhaus, Hoffmann-La Roche, Basel, Switzerland), at 10 µg/ml, using the supersensitive alkaline phosphatase kit (Biogenex, San Ramon, CA) to study

the expression of TNFRI and TNFRII, respectively (35). Cells were counterstained with haematoxylin. The negative controls comprised staining with purified mouse IgG_1 (Becton Dickinson) at 10 μg/ml and omitting the first and second step. Staining with 5B5 (Dako, Glostrup, Denmark), a purified mouse monoclonal IgG_1 antibody directed against human prolyl 4-hydroxylase, which is specificly expressed by fibroblasts, at 2 μg/ml, served as a positive control. Stainings were evaluated using a standard Zeiss 16 microscope (Carl Zeiss, Oberkochen, Germany).

Statistical analysis

Flow cytometry, ELISA and bioassay results were analyzed with the Wilcoxon Rank Sum Test using STATA™ (Computing Resource Center, Los Angeles, CA). Significant differences are indicated by P values smaller than 0.05.

RESULTS

PP and NN fibroblasts clearly bind IL-4, IL-6 and TNF-α, but not IL-1α or IL-1β

Cultures of NN fibroblasts hardly bound biotinylated recombinant human IL-1α or IL-1β, even after stimulation with PMA, LPS, or the recombinant human cytokines IL-1β, TNF-α, or IFN-γ, as shown in Figure 1. Fibroblasts positive for binding with IL-4, IL-6 and TNF-α were clearly detectable and the binding of the former two cytokines was upregulated in stimulated fibroblasts. For instance IL-1β-treated fibroblasts showed a substantially increased binding of IL-6, and IFN-γ-treated fibroblasts displayed increased binding of IL-4. PP fibroblasts also clearly bound IL-4, IL-6 and TNF-α (Fig 2), but negligible amounts of IL-1α and IL-1β (not shown). The binding of IL-4, IL-6 and TNF-α by both types of fibroblasts could be modulated by the use of different culture conditions, as shown in Figure 2. The binding of these cytokines was lower, although with a varying degree, using a high-calcium (1.05 mM), serum-free medium (a 1:1 ratio of F10:DMEM) when compared to a low-calcium (0.15 mM), serum-free medium (KBM/unsupplemented MCDB 153) (second versus first bars, Figure 2). An increased binding was observed using FCS-supplemented (5 % v/v) medium (third versus second bars, Fig 2). The observation that PP fibroblasts were able to bind more IL-4 than NN fibroblasts after a 24 h period under serum-conditions was not statistically significant.

PP fibroblasts express a decreased binding of TNF-α under serum conditions

We observed that the binding of IL-6 and TNF-α by NN fibroblasts, but not the other cytokines, increased when cells were cultured for up to 72 h in the presence of FCS. Serum upregulated the mean percentage of NN fibroblasts

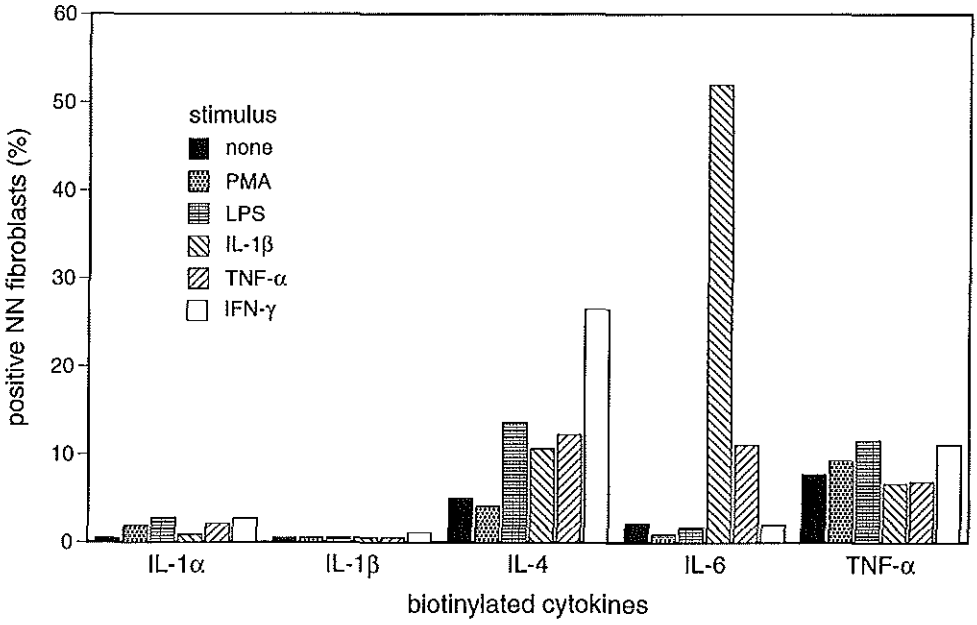

Figure 1. The binding of cytokines by fibroblasts. Subconfluent NN fibroblast cultures were incubated with a high-calcium medium containing 5 % FCS for 24 h and analyzed for their ability to bind the cytokines mentioned on the X-axis using flow cytometry, as described in the materials and methods section. The stimuli used were 50 ng/ml PMA, 10 µg/ml LPS, 100 U/ml IL-1β, 100 U/ml TNF-α and 100 U/ml IFN-γ, added the last 14 h of the 24 h incubation. Results are expressed as the percentage of positive cells after labeling with biotinylated recombinant human cytokines of a representative experiment. The experiment was performed in duplo.

able to bind TNF-α (Fig 3A), but not the number of TNF-α binding sites per cell, as indicated by the relative fluorescence intensities of the same cell samples (Fig 3B). Culturing PP fibroblasts, however, for up to 72 h under serum conditions resulted in a significantly lower number of fibroblasts that bound TNF-α when compared to NN fibroblasts ($P < 0.03$, Fig 3A, and Figs 5A and B). To a lesser extent the same was true for IL-6 (not shown). The increased binding of TNF-α by NN fibroblasts under serum conditions correlated with a decrease in the percentage of cycling cells. The decreased binding of TNF-α by PP fibroblasts, as mentioned above, is, however, not a result of differences in growth characteristics between PP and NN fibroblasts, as checked by cell counts (data not shown) and the percentage of cycling cells (G_2/M phase) (Fig 4). Furthermore, TNF-α binding sites of PP fibroblasts were not occupied with endogenously produced TNF molecules *in vitro*, as evidenced by the observation that fibroblasts did not produce TNF-α and by acid stripping of fibroblasts prior to flow cytometry (Fig 5C). Stimulated PP fibroblasts were also still able to upregulate binding of TNF-α (Fig 5D).

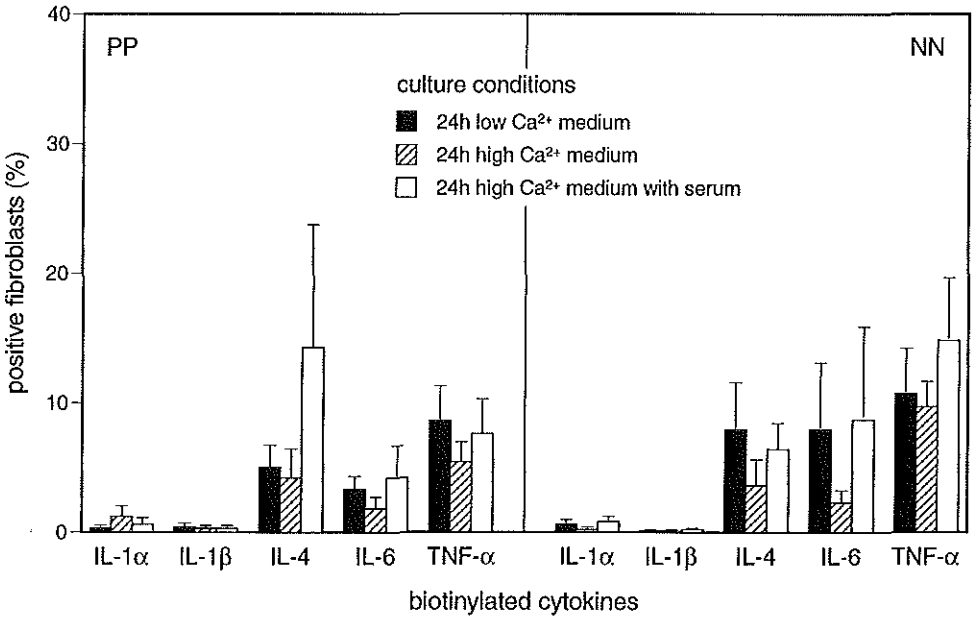

Figure 2. Calcium and serum modulate the binding of cytokines by PP and NN fibroblasts. PP and NN fibroblasts (both n = 10) were incubated with a low or high-calcium (Ca^{2+}), serum-free medium, or a high-calcium medium containing 5 % FCS for 24 h, and analyzed for their ability to bind cytokines. Results are expressed as the mean ± SEM % positive cells.

Shedding of TNFRI does not explain the decreased expression of this receptor in PP fibroblasts

Immunocytochemical staining of cells grown on slides showed that NN fibroblasts cultured under serum conditions clearly express the TNFRI (the 55-kDa receptor form), but only low levels of the TNFRII (the 75-kDa form) (Fig 6). The TNFRI was predominantly localized in the nucleus and to a lesser extent to the cytoplasm. In contrast, PP fibroblasts expressed very low levels of immunoreactive TNFRI.

Fibroblast-conditioned media contained the sTNFRI as well as the sTNFRII, as measured with specific sandwich ELISA (Table I). The shedding of TNFRI was enhanced by serum and was maximal at 72 h. The presence of sTNFRII was, however, almost negligible, which confirmed the immunocytochemical stainings. The amounts of sTNFR present in the supernatants of PP and NN fibroblast cultures were similar and do not explain the differences found for membrane-bound TNFR between PP and NN fibroblasts.

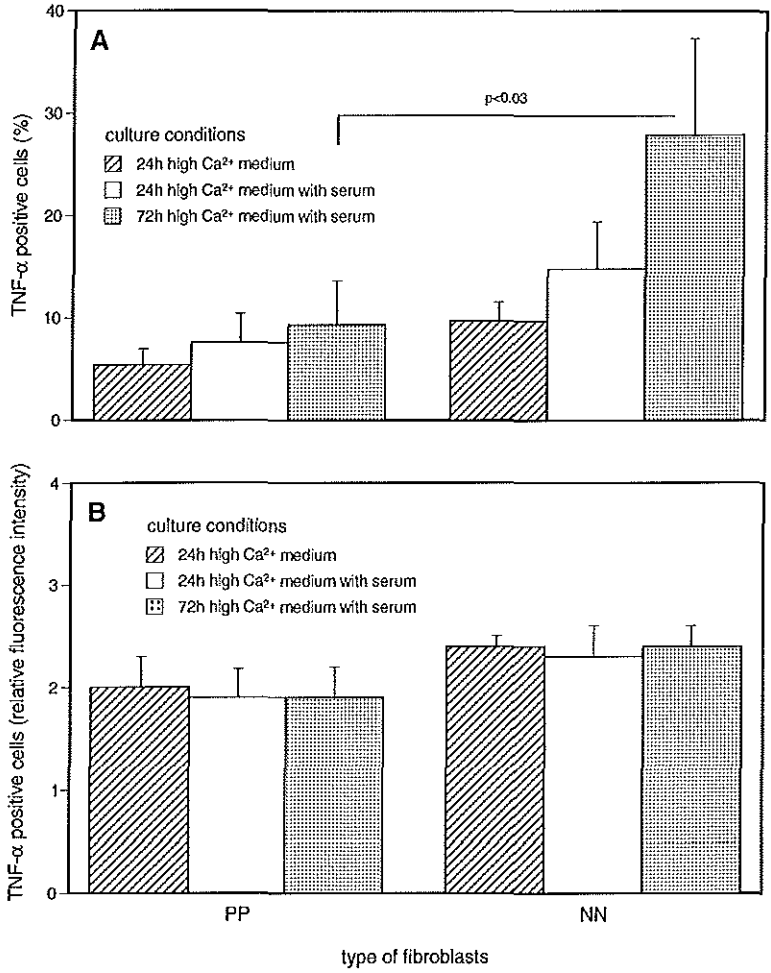

Figure 3. PP fibroblasts express decreased binding of TNF-α under serum conditions. PP and NN fibroblasts (both n = 10) were analyzed using flow cytometry for the percentage of cells which bind TNF-α (Fig A) and the relative number of TNF-α binding sites per cell (B), after culture in a high-calcium (Ca^{2+}), serum-free medium for 24 h, or a high-calcium medium containing 5 % FCS for 24 or 72 h. Percentages of positive cells and the signal to noise ratios are presented as mean ± SEM. The P value indicates a significant difference between PP and NN fibroblasts.

PP fibroblasts co-express decreased TNFR levels and an enhanced IL-6 production

The supernatants of the fibroblast cultures contained substantial amounts of biologically active IL-6 (Table II). Both types of fibroblasts did not produce biologically active IL-1 or TNF-α. The serum-induced increase in IL-6 production correlated with the increase in binding of TNF-α in NN fibroblasts. PP

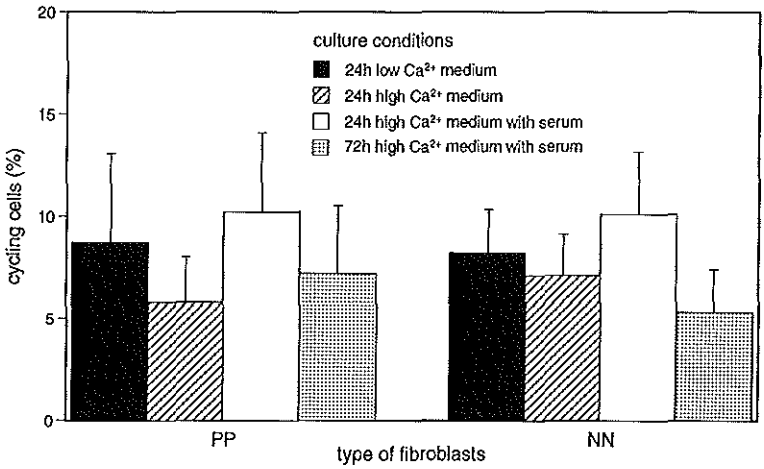

Figure 4. Growth characteristics of PP and NN fibroblasts. Cell cycle analysis was performed on PP and NN fibroblasts (both n = 8) cultured in a low or high-calcium (Ca^{2+}), serum-free medium for 24 h, or a high-calcium medium containing 5 % FCS for 24 or 72 h. Cells were fixed, the DNA was labeled with propidium iodide and analyzed using flow cytometry, as described in the materials and methods section. Results are expressed as the mean ± SEM % cycling cells (G_2/M-phase).

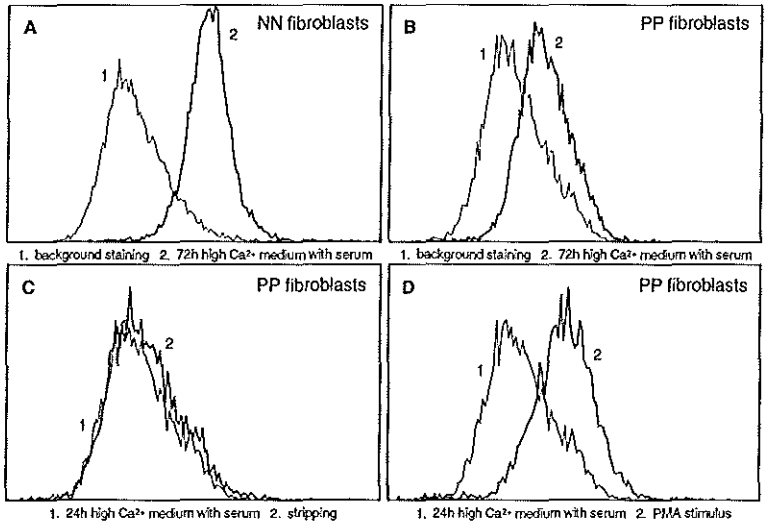

Figure 5. PP fibroblasts do not express occupied TNF-α binding sites and upregulate the binding of TNF-α after stimulation. Fluorescence intensities using biotinylated TNF-α are given of NN (Fig A) and PP fibroblasts (B, C and D) (both n = 3) cultured in a high-calcium medium containing 5 % FCS for 24 or 72 h. Figures A and B illustrate the decreased binding of TNF-α by PP fibroblasts. Figure C shows the effect of acid stripping, and Figure D the effect of a 50 ng/ml PMA stimulus for 14 h on PP fibroblasts. See materials and methods section for details. Shown are a representative NN and PP fibroblast culture.

Table I. Shedding of TNFR by PP and NN fibroblasts.

Culture condition	Psoriasis		Normal control	
	TNFRI pg/ml	TNFRII pg/ml	TNFRI pg/ml	TNFRII pg/ml
24 h high Ca^{2+} medium	8[a]	<5	14	<5
24 h high Ca^{2+} medium with serum	29	8	30	6
72 h high Ca^{2+} medium with serum	120	10	100	7

[a] Supernatants of PP and NN fibroblasts were analyzed for sTNFRI and TNFRII by ELISA. Fibroblasts were cultured in a high-calcium (Ca^{2+}), serum-free medium for 24 h, or a high-calcium medium containing 5 % FCS for 24 or 72 h. Results are given of a representative PP and NN fibroblast culture (both n = 3), and expressed in pg/ml.

fibroblasts, however, expressed an enhanced production of IL-6 together with a decreased binding of TNF-α.

DISCUSSION

This is the first study in which the expression of cytokine receptors on lesional psoriatic dermal fibroblasts was investigated. We observed that PP and NN fibroblasts expressed negligible IL-1 binding sites (Figs 1 and 2). Epidermal cells, used as control cells, were positive for binding of IL-1α and IL-1β which validated our methodology (not shown). Qwarnstrom and colleagues found that cultures of human gingival fibroblasts did express binding sites for both the IL-1α and IL-1β isoform (12). Apart from the possibility that biotinylation of IL-1 affects receptor binding, as Qwarnstromand colleagues used iodinated IL-1, tissue-specific differences cannot be excluded. For example, the production of biologically active IL-1 can be triggered in gingival fibroblasts (36), but not in dermal fibroblasts (chapter 4.1). Our finding of negligible expression of IL-1 binding sites on dermal fibroblasts does not necessarily imply unresponsiveness of these cells to IL-1. Only a few IL-1RI molecules per cell are sufficient for an IL-1 mediated response (37). This is confirmed by our novel finding that IL-1β stimulation of fibroblasts resulted in

Figure 6. PP fibroblasts express decreased levels of TNFRI. See right-handed page. NN (Figs A, C, E and F) and PP fibroblasts (B and D) were stained with the anti-TNFRI antibody htr-9 (A and B) and the anti-TNFRII antibody utr-1 (C and D). Staining of NN fibroblasts with mouse IgG_1 (E) and the antibody 5B5 (F) were used as controls. Cells were grown on slides in a high-calcium medium containing 5 % FCS to confluence, fixed and stained, as described in the materials and methods section. Shown are a representative PP and NN fibroblast culture. The experiment was performed in duplo. Magn 630 X.

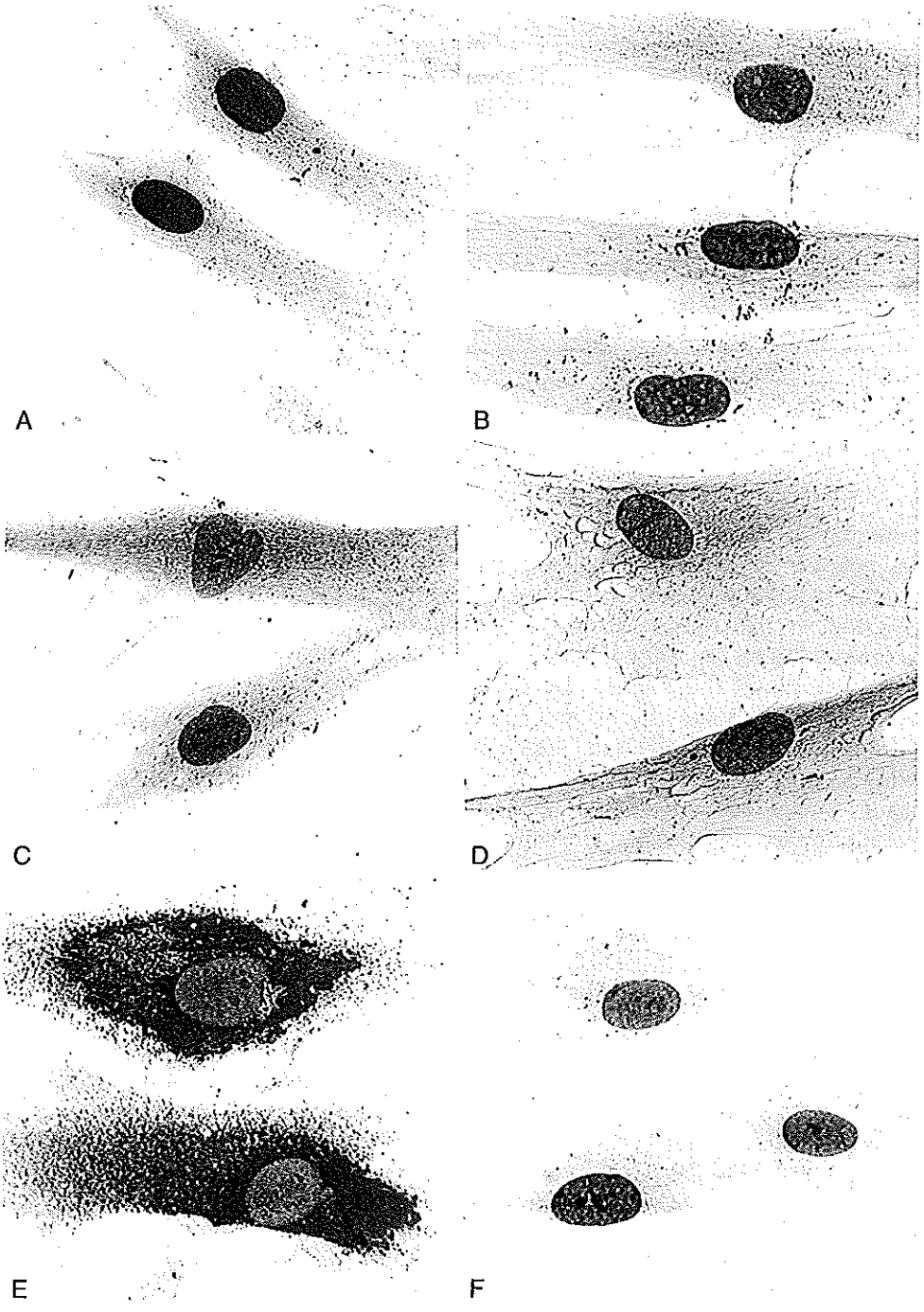
A
B
C
D
E
F

Table II. PP fibroblasts co-express decreased binding of TNF-α and increased IL-6 production.

Culture condition	Psoriasis[a]		Normal control	
	binding TNF-α %	IL-6 production U/10^5 cells	binding TNF-α %	IL-6 production U/10^5 cells
24 h high Ca^{2+} medium	1	33	16	11
24 h high Ca^{2+} medium with serum	0	258	11	6
72 h high Ca^{2+} medium with serum	1	300	40	13

[a] The percentage of PP and NN fibroblasts which bind TNF-α and the corresponding production of IL-6 in U/10^5 cells were analyzed using flow cytometry and the B9 bioassay, respectively. Fibroblasts were cultured in a high-calcium (Ca^{2+}), serum-free medium for 24 h, or a high-calcium medium containing 5 % FCS for 24 or 72 h. The results are shown of a representative PP and NN fibroblast culture (both n = 5).

substantially upregulated binding of IL-6. The IL-4R has been demonstrated on gingival fibroblasts (38), while dermal fibroblasts have been shown to give a biological response to exogenous recombinant human IL-4 and IL-6 (39,40). In this report we provide evidence that human dermal fibroblasts express binding sites for IL-4 and IL-6. Our observation that fibroblasts bind TNF-α confirms the findings by Berman and others (14). The expression of cytokine binding sites by PP and NN fibroblasts was modulated by the use of different culture conditions. Calcium seemed to lower the expression of binding sites for IL-4, IL-6 and TNF-α (Fig 2). However, extracellular calcium, using similar concentrations, enhanced the IL-1R levels in cultured human keratinocytes (41). This probably reflects cell-type specific differences. Addition of FCS resulted in increased binding of IL-4, IL-6 and TNF-α. This increased binding initially parallelled the induction of fibroblast proliferation (third versus second bars, Fig 4), implying enhanced responsiveness to cytokines of fibroblasts starting to proliferate.

Differences in cytokine binding between PP and NN fibroblasts became evident when confluent cultures were analyzed. Culturing of PP fibroblasts for up to 72 h in the presence of serum did not affect the number of cells which bound TNF-α. This is in sharp contrast with the serum-upregulated binding of TNF-α by NN fibroblasts (Fig 3A). The number of TNF-α binding sites per cell, however, did not increase under serum-conditions in either NN or PP fibroblasts (Fig 3B). As the expression of cytokine receptors may directly correlate with the fraction of cells in cycle (42), we analyzed the growth characteristics of PP and NN fibroblasts. It is important to note that studies on the proliferation rates of PP and NN fibroblasts in response to FCS are inconsistent (23,24). Similar proliferation rates for both types of fibroblasts as well as a hyperproliferative state of PP fibroblasts have both been

reported. In our study, however, there were no significant differences in cell counts and the percentage of cycling cells, although the percentage of proliferating cells was somewhat higher in confluent PP fibroblasts (72 h under serum conditions) when compared to NN fibroblasts (Fig 4). Furthermore, the TNF-α binding sites of PP fibroblasts were not occupied with endogenously produced TNF-α (Fig 5C), and PP fibroblasts did also not lose their ability to upregulate the binding of TNF-α (Fig 5D).

TNF-α can bind to two types of TNFR, the TNFRI and TNFRII, which are both active in signal transduction and are responsible for nonredundant TNF activities (43). Most immunomodulatory effects of TNF-α on fibroblasts are mediated by TNFRI. Immunocytochemistry showed that the expression of TNFRI was clearly decreased in PP fibroblasts (Fig 6), which is in line with the binding experiments (Figs 5A and B). These staining results imply that the decreased binding of TNF-α by PP fibroblasts is not due solely to an altered affinity of TNFRs for TNF-α, but may merely reflect a decreased expression of TNFRI. Both types of receptors are shed either by protein kinase C dependent proteolysis (induced by PMA) or by protein kinase C independent proteolysis (induced by IL-1 and IL-4) (44,45). We observed that serum enhanced shedding of TNFRI by both types of fibroblasts, which may be due to serum-induced endogenous protein kinase C activity. The kinetics of the shedding was slow (Table I). A similar slow process of release of sTNFR was also found using PBMC (31). Amounts of sTNFRII in the supernatants were very low but still detectable, being in line with the low expression of this receptor found with immunocytochemistry. Shedding, being similar for both types of fibroblasts, does not explain the decreased TNFR expression by PP fibroblasts. Furthermore, the observation that PP fibroblasts express negligible intracellular levels of immunoreactive TNFR does not support rapid TNFR upregulation and subsequent internalization of the receptors by these cells. Thus, at the moment, we speculate that PP fibroblasts exhibit a deficiency in serum-induced TNFR upregulation. This is in line with other alterations such as an elevated synthesis of extracellular matrix proteins and an increased expression of PDGFRβ, which have been considered as intrinsic phenotypic abnormalities of multi-passaged PP fibroblasts (20,46). PP fibroblasts also express elevated levels of PDGFRβ *in vivo* (47), and show an increased biological response to PDGF *in vitro* (48). Although factors determining the responsiveness to TNF-α are not fully known yet, it has been shown that fibroblasts derived from affected skin from scleroderma patients do not respond to TNF-α in upregulating TNFRII mRNA levels. Scleroderma and normal fibroblasts do not differ at the TNFR protein level, but scleroderma fibroblasts constitutively contain TNFRI and TNFRII mRNA, whereas normal fibroblasts constitutively express mainly TNFRI mRNA (14).

TNF-α has mitogenic activity and many immunomodulatory activities on human dermal fibroblasts (49). For example, TNF-α upregulates the expression of enzymes, cytokines (e.g. IL-1, IL-6 and IL-8), adhesion and MHC molecules, and stimulates chemotaxis. One would expect that the decreased

TNFR expression by PP fibroblasts affects the inflammatory response of these cells to TNF-α. However, several arguments do not favor this hypothesis. First, we only observed a decreased TNFR expression in response to factors present in FCS, not to other stimulating agents (e.g. PMA, Fig 5D). Second, PP fibroblasts co-expressed a decreased binding of TNF-α together with an increased production of IL-6 in contrast to NN fibroblasts (Table II). Third, the degree of receptor density does not always correlate with TNF-α responsiveness (13), and the biological actions of TNF-α are not always preceded by receptor internalization (50). Moreover, using a murine model, subcutaneous injection of a combination of lithium chloride (a drug used to treat manic depressive disease and known to trigger or aggravate psoriasis as a side effect) and TNF-α induced a psoriasiform inflammatory reaction via an increase in the endogenous IL-6 production in the skin (28). Immunohistochemistry of biopsies of human skin also showed that the *in vivo* expression of TNFR is altered in the dermal compartment of psoriatic lesions (51). The decreased TNFR expression by PP fibroblasts demonstrated *in vitro* suggests that dermal fibroblasts are, at least in part, involved in maintaining the *in vivo* milieu characteristic of psoriatic lesions.

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REFERENCES

1. Collins T, Lapierre LA, Fiers W, Strominger JL, Pober JS (1986) Recombinant human tumor necrosis factor increases mRNA levels and surface expression of HLA-A, B antigens in vascular endothelial cells and dermal fibroblasts *in vitro*. Proc Natl Acad Sci USA 83:446-450.
2. Collins T, Korman AJ, Wake CT, Boss JM, Kappes DJ, Fiers W, Ault KA, Gimbrone MA Jr. Strominger JL, Pober JS (1984) Immune interferon activates multiple class II major histocompatibility complex genes and the associated invariant chain gene in human endothelial cells and dermal fibroblasts. Proc Natl Acad Sci USA 81:4917-4921.
3. Ezoe K, Horikoshi T (1993) Tumor necrosis factor-alpha increased the integrin alpha 2 beta 1 expression and cell attachment to type I collagen in human dermal fibroblasts. Biochem Biophys Res Commun 192:281-287.
4. Dustin ML, Rothlein R, Bhan AK, Dinarello CA, Springer TA (1986) Induction by IL-1 and interferon-gamma: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). J Immunol 137:245-254.
5. Mauviel A, Temime M, Charron D, Loyau G, Pujol J-P (1991) Induction of interleukin-1β production in human dermal fibroblasts by interleukin-1α and tumor necrosis factor-α. Involvement of protein kinase-dependent and adenylate cyclase-dependent regulatory pathways. J Cell Biochem 47:174-183.
6. Van Damme J, Cayphas S, Opdenakker G, Billiau A, Van Snick J (1987) Interleukin 1 and poly(rI)·poly(rC) induce production of a hybridoma growth factor by human fibroblasts. Eur J Immunol 17:1-7.
7. Larsen CG, Anderson AO, Oppenheim JJ, Matsushima K (1989) Production of interleukin-8

by human dermal fibroblasts and keratinocytes in response to interleukin-1 or tumour necrosis factor. Immunology 68:31-36.
8. Larsen CG, Zachariae CO, Oppenheim JJ, Matsushima K (1989) Production of monocyte chemotactic and activating factor (MCAF) by human dermal fibroblasts in response to interleukin 1 or tumor necrosis factor. Biochem Biophys Res Commun 160:1403-1408.
9. Lorenzo JA, Jastrzebski SL, Kalinowski JF, Downie E, Korn JH (1994) tumor necrosis factor alpha stimulates production of leukemia inhibitory factor in human dermal fibroblast cultures. Clin Immunol Immunopathol 70:260-265.
10. Raines EW, Dower SK, Ross R (1989) IL-1 mitogenic activity for fibroblast and smooth muscle cells is due to PDGF-AA. Science 243:393-396.
11. Kaushansky K, Lin N, Adamson JW (1988) Interleukin 1 stimulates fibroblasts to synthesize granulocyte-macrophage and granulocyte colony stimulating factors. Mechanism for the hematopoietic response to inflammation. J Clin Invest 81:92-97.
12. Qwarnstrom EE, Page RC, Gillis S, Dower SK (1988) Binding, internalization, and intracellular localization of interleukin-1 beta in human diploid fibroblasts. J Biol Chem 263:8261-8269.
13. Tsujimoto M, Yip YK, Vilček J (1985) Tumor necrosis factor: specific binding and internalization in sensitive and resistant cells. Proc Natl Acad Sci USA 82:7626-7630.
14. Berman B, Wietzerbin J (1992) Tumor necrosis factor-α (TNF-α), interferon-α (IFN-α) and interferon-γ (IFN-γ) receptors on human normal and scleroderma dermal fibroblasts *in vitro*. J Dermatol Sci 3:82-90.
15. Heldin CH, Ek B, Rönnstrand L (1983) Characterization of the receptor for platelet-derived growth factor on human fibroblasts. Demonstration of an intimate relationship with a 185,000-Dalton substrate for the platelet-derived growth factor-stimulated kinase. J Biol Chem 258:10054-10061.
16. Segarini PR, Rosen DM, Seyedin SM (1989) Binding of transforming growth factor-beta to cell surface proteins varies with cell type. Mol Endocrinol 3:261-272.
17. Rose-John S, Heinrich PC (1994) Soluble receptors for cytokines and growth factors: generation and biological function. Biochem J 300:281-290.
18. Debets R, Savelkoul HFJ (1994) Cytokine antagonists and their potential therapeutic use. Immunol Today 15:455-458.
19. Er-raki A, Charveron M, Bonafe JL (1993) Increased superoxide anion production in dermal fibroblasts of psoriatic patients. Skin Pharmacol 6:253-258.
20. Espinoza LR, Espinoza CG, Cuéllar ML, Scopelitis E, Silveira LH, Grotendorst GR (1994) Fibroblast function in psoriatic arthritis. II. Increased expression of beta platelet derived growth factor receptors and increased production of growth factor and cytokines. J Rheumatol 21:1507-1511.
21. Raynaud F, Bauvois B, Gerbaud P, Evain-Brion D (1992) Characterization of specific proteases associated with the surface of human skin fibroblasts, and their modulation in pathology. J Cell Physiol 151:378-385.
22. Raynaud F, Evain-Brion D (1991) Protein kinase C activity in normal and psoriatic cells: cultures of fibroblasts and lymphocytes. Br J Dermatol 124:542-546.
23. Espinoza LR, Aguilar JL, Espinoza CG, Cuéllar ML, Scopelitis E, Silveira LH (1994) Fibroblast function in psoriatic arthritis. I. Alteration of cell kinetics and growth factor responses. J Rheumatol 21:1502-1506.
24. Priestley GC, Adams LW (1985) Mitogenic effects of sera from normal and psoriatic subjects on human skin fibroblasts. Arch Dermatol Res 277:13-15.
25. MacLaughlin JA, Gange W, Taylor D, Smith E, Holick MF (1985) Cultured psoriatic fibroblasts from involved and uninvolved sites have a partial but not absolute resistance to the proliferation-inhibition activity of 1,25-dihydroxyvitamin D3. Proc Natl Acad Sci USA 82:5409-5412.
26. Elder JT, Sartor CI, Boman DK, Benrazavi S, Fisher GJ, Pittelkow MR (1992) Interleukin-6 in psoriasis: expression and mitogenicity studies. Arch Dermatol Res 284:324-332.
27. Prens EP, Benne K, Van Damme J, Bakkus M, Brakel K, Benner R, Van Joost T (1990) Interleukin 1 and interleukin 6 in psoriasis. J Invest Dermatol 95:121S-124S.

28. Beyaert R, Schulze-Osthoff K, Van Roy F, Fiers W (1992) Synergistic induction of interleukin-6 by tumor necrosis factor and lithium chloride in mice: possible role in the triggering and exacerbation of psoriasis by lithium treatment. Eur J Immunol 22:2181-2184.
29. Sharrow SO (1991) Immunofluorescence and cell sorting. In Coligan JE, Kruisbeek AM, Margulies DH, Shevach EM, Strober W (eds) Current protocols in immunology, Greene Publishing and Wiley Interscience, New York, Unit 5.2 and 5.7.
30. Robb RJ, Greene WC, Rusk CM (1984) Low and high affinity cellular receptors for interleukin 2. Implications for the level of Tac antigen. J Exp Med 160:1126-1146.
31. Leeuwenberg JFM, Jeunhomme TMAA, Buurman WA (1994) Slow release of soluble TNF receptors by monocytes *in vitro*. J Immunol 152:4036-4043.
32. Hopkins SJ, Humphreys M (1989) Simple, sensitive and specific bioassay of interleukin 1. J Immunol Meth 120:271-276.
33. Aarden LA, De Groot ER, Schaap OL, Lansdorp PM (1987) Production of hybridoma growth factor by human monocytes. Eur J Immunol 17:1411-1416.
34. Espevik T, Nissen-Meyer J (1986) A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumor necrosis factor from human monocytes. J Immunol Meth 95:99-105.
35. Brockhaus M, Schoenfeld HJ, Schlaeger EJ, Hunziker W, Lesslauer W, Loetscher H (1990) Identification of two types of tumor necrosis factor receptors on human cell lines by monoclonal antibodies. Proc Natl Acad Sci USA 87:3127-3131.
36. Takada H, Mihara J, Morisaki I, Hamada S (1991) Induction of interleukin-1 and -6 in human gingival fibroblast cultures stimulated with bacteroides lipopolysaccharides. Infect Immun 59:295-301.
37. McKean DJ, Podzorski RP, Bell MP, Nilson AE, Huntoon CJ, Slack J, Dower SK, Sims J (1993) Murine T helper cell-2 lymphocytes express type I and type II IL-1 receptors, but only the type I receptor mediates costimulatory activity. J Immunol 151:3500-3510.
38. Park LS, Friend D, Sassenfeld HM, Urdal DL (1987) Characterization of the human B cell stimulatory factor 1 receptor. J Exp Med 166:476-488.
39. Feghali CA, Bost KL, Boulware DW, Levy LS (1992) Human recombinant interleukin-4 induces proliferation and interleukin-6 production by cultured human skin fibroblasts. Clin Immunol Immunopathol 63:182-187.
40. Revel M, Zilberstein A (1987) Interferon-β2 living up to its name. Nature 325:581-582.
41. Blanton RA, Kupper TS, McDougall JK, Dower S (1989) Regulation of interleukin 1 and its receptor in human keratinocytes. Proc Natl Acad Sci USA 86:1273-1277.
42. Dower SK, Qwarnstrom EE, Page RC, Blanton RA, Kupper TS, Raines E, Ross R, Sims JE (1990) Biology of the interleukin-1 receptor. J Invest Dermatol 94:68S-73S.
43. Tartaglia LA, Goeddel DV (1992) Two TNF receptors. Immunol Today 13:151-153.
44. Holtmann H, Wallach D (1987) Down regulation of the receptors for tumor necrosis factor by interleukin 1 and 4 beta-phorbol-12-myristate-13-acetate. J Immunol 139:1161-1167.
45. Taylor DJ (1994) Interleukin-4 (IL-4) induces down-modulation and shedding of the p55 tumour necrosis factor receptor and inhibits TNF alpha's effect on rheumatoid synovial fibroblasts. Rheumatol Int 14:21-25.
46. Priestley GC, Adams LW (1983) Hyperactivity of fibroblasts cultured from psoriatic skin. II. Synthesis of macromolecules. Br J Dermatol 109:157-164.
47. Krane JF, Murphy DP, Gottlieb AB, Carter DM, Hart CE, Krueger JG (1991) Increased dermal expression of platelet-derived growth factor receptors in growth activated skin wounds and psoriasis. J Invest Dermatol 96:983-986.
48. Gu XF, Raynaud F, Evain-Brion D (1988) Increased chemotactic and mitogenic response of psoriatic fibroblasts to platelet-derived growth factor. J Invest Dermatol 91:599-602.
49. Beutler B, Cerami A (1990) Cachectin (tumor necrosis factor): an endogenous mediator of shock and inflammatory response. In Shevach E, Oppenheim JJ (eds) Immunophysiology: the roles of cells and cytokines in immunity and inflammation, University Press, New York, pp 226-236.
50. Hofsli E, Nissen-Meyer J (1989) Recombinant tumour necrosis factor (TNF) fixed to cell monolayers retains it cytotoxic and growth-stimulatory activities. Evidence that internaliza-

tion of TNF is not necessary for induction of biological effects. Scand J Immnuol 29:57-63.
51. Kristensen M, Chu CQ, Eedy DJ, Feldmann M, Brennan FM, Breathnach SM (1993) Localization of tumour necrosis factor-alpha (TNF-alpha) and its receptors in normal and psoriatic skin: epidermal cells express the 55-kD but not the 75-kD TNF receptor. Clin Exp Immunol 94:354-362.

Chapter 5

GENERAL DISCUSSION

General discussion

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GENERAL DISCUSSION

Dysregulated cellular cross talk may initiate and/or mediate psoriasis. Recent reports implicate the pathological relevance of cytokines and their receptors in general, and the IL-1 system in particular, in psoriatic skin (see for review of the literature chapter 1.3). To investigate the interleukin 1 (IL-1) system and IL-1 system-related cytokines in psoriasis in-depth, the present thesis aimed at an integrated study approach which combined several models (i.e. *in vivo*, *ex vivo*, and *in vitro* models) and multiple assays (see chapter 2.2). Studies on the epidermal (chapter 3) and dermal compartment (chapter 4) are discussed in chapters 5.1 and 5.2, respectively. In chapter 5.3, the conclusions are given and the hypothesis-testing is assessed (see for hypothesis chapter 2). Chapter 5.4 presents our current view on the (epi)dermal IL-1 system and IL-1 system-related cytokines in the immunopathogenesis of psoriasis. Finally, future research directions are given in chapter 5.5.

5.1 THE EPIDERMAL IL-1 SYSTEM IN PSORIASIS

The epidermal expression of IL-1 is clearly altered in psoriasis (see Table IV of chapter 1.3.2). As initially available data were inconsistent and often incomplete, the focus of the first experiments was to phenotype the complete epidermal IL-1 system in psoriatic skin. Findings *in vivo* were extended by the characterization of the production of IL-1 and related cytokines by lesional psoriatic epidermal cells *ex vivo* and its modulation by IL-4 . Table I summarizes the findings *in vivo* and *ex vivo* as reported in chapter 3.

IL-1 phenotype of lesional psoriatic epidermis *in vivo* is dominated by antagonists

A thorough analysis of all functionally characterized components of the IL-1 system resulted in a defined phenotype of IL-1 in psoriatic epidermis, given in Table V of chapter 3.1. We have analyzed the expression of multiple members of the IL-1 system both at the RNA and protein level in parallel using semiquantitative techniques, and show for the first time that the expressions of IL-1 and IL-1 receptor (IL-1R) isoforms are coordinatedly altered in lesional psoriatic epidermis.

The *in situ* expression of IL-1α protein was decreased in lesional psoriatic epidermis relative to non-lesional psoriatic and normal control epidermis. This may reflect increased secretion of this cytokine *in vivo*, as suggested by the increased release of IL-1α by lesional psoriatic epidermal cells observed *ex vivo* (chapter 3.2 and Table I). The decrease of IL-1α in lesional psoriatic epidermis was not found using cytosol from freshly isolated epidermal cells

(Table I of chapter 3.3), perhaps as a consequence of the trypsinization procedure to prepare cell suspensions, but was in line with findings on cytosolic keratome extracts (1-3). IL-1α mRNA was not detectable, even with the sensitive reverse transcriptase - polymerase chain reaction (RT-PCR), probably due to its instability (1,4). The *in situ* expressions of IL-1 receptor antagonist (IL-1ra) and IL-1R type II (IL-1RII) proteins were both increased in lesional psoriatic epidermis, being in agreement with findings reported by others (3,5). Furthermore, IL-1ra and IL-1RII both negatively correlated with the expression of IL-1α. This inverse relation is a novel observation and may be inherent to accelerated terminal keratinocyte differentiation in clinically stable psoriatic lesions, as the shift in the ratio between IL-1 agonists (IL-1α) and IL-1 antagonists (IL-1ra and IL-1RII) parallels and may even control keratinocyte differentiation (3,6-8). The increased synthesis of IL-1ra and IL-1RII proteins in lesional psoriatic epidermis may be explained at the level of translation/post-translational modification and transcription, respectively, since intracellular IL-1ra (icIL-1ra) mRNA levels did not differ among psoriatic and normal control epidermal cells, whereas levels of IL-1RII mRNA were increased in lesional psoriatic epidermal cells. Another important observation is the positive correlation between the expressions of IL-1ra and IL-1RII, which suggests an effective inhibition of IL-1 responsiveness in lesional psoriatic epidermis as IL-1ra and IL-1RII can synergistically antagonize IL-1 activity (see chapter 1.1.1 on regulation of IL-1 activity). In fact, it has recently been shown that overexpression of icIL-1ra as well as IL-1RII in human epithelial cells attenuates the response to IL-1 (9,10). Since interferon-γ (IFN-γ) is reported to increase the expression of keratinocyte IL-1RII (11) and is present in increased amounts in lesional psoriatic skin (12), the overexpression of IL-1RII on lesional psoriatic keratinocytes may be mediated by IFN-γ. Expression of IL-1RI was only detectable with RT-PCR, which confirmed the observation that in keratinocytes IL-1RII is the dominant IL-1R isoform (11). IL-1RI mRNA was present to a similar extent in lesional psoriatic and normal control epidermal cells, which underscores our notion that in psoriasis epidermal IL-1 responsiveness is predominantly regulated at the level of IL-1 antagonists.

Freshly isolated lesional psoriatic epidermal cells express clearly increased levels of IL-1β mRNA (chapter 3.1) and cytosolic IL-1β protein (chapter 3.2) when compared to normal control epidermal cells, suggesting increased IL-1β gene transcription by lesional psoriatic epidermal cells. The IL-1β mRNA and protein findings confirmed previous reports with complete skin biopsies (1,12). The *in situ* expression of epidermal IL-1β protein, however, was variabel and mostly not detectable. Nevertheless, a few psoriatic patients revealed suprabasal areas with strong IL-1β immunoreactivity, at least in part derived from neutrophils. These areas were lined by cells positive for both DNA breaks and IL-1β (Fig 7 of chapter 3.3). Epidermal IL-1β greatly affects cutaneous inflammation, as IL-1β induces leukocyte-attracting chemokines, protects leukocytes from apoptosis (13,14) and is required for the generation

of T helper cell type 1 (T_H1)-type cytokines (15) (see also chapter 1.2.2). Therefore, the increased expression of IL-1β in a few patients *in vivo* as well as in lesional psoriatic epidermal cells *ex vivo* (chapter 3.2) favor the contribution of IL-1β to the inflammatory response in psoriasis. The increased expression of IL-1ra and IL-1RII mRNA in freshly isolated lesional psoriatic epidermal cells paralleled those of IL-1β (chapter 3.1), which may reflect an attempt of psoriatic skin to control IL-1β-mediated inflammation.

Apoptosis, the physiological pathway of epidermal cell death *in vivo* (16), was increased in lesional psoriatic epidermal cells (chapter 3.3) and may represent a homeostatic mechanism to counteract the hyperproliferation of psoriatic keratinocytes. Alternatively, apoptosis may be induced by defective cell-matrix interactions (17), and this phenomenon may contribute to psoriatic keratinocyte apoptosis as the expression and adhesive function of integrin receptors are severely altered in these cells (18). Since apoptotic keratinocytes can express autoantigens (19,20) they may be relevant to the pathogenesis of psoriasis (see also chapter 5.4). It is speculated that keratinocyte apoptosis may contribute to the generation of epidermal IL-1β (see below). To substantiate this notion, current investigations focus on whether psoriatic keratinocytes express the IL-1β converting enzyme (ICE).

Taken together, we hypothesize that IL-1 agonists participate in the initiation and/or maintenance of psoriasis. This line of thought is substantiated by the observation that the IL-1α protein levels in non-lesional psoriatic epidermis show a significant inverse correlation with the whole body clinical PASI scores (Spearman's Rank Correlation Coefficient (r_s) = -0.7, n = 8, $P < 0.05$) (Note that the IL-1α levels in lesional psoriatic epidermis were always negligible and do therefore not correlate with PASI scores). In addition, the IL-1β mRNA and protein levels were increased in non-lesional psoriatic skin when compared to normal control skin (1,12). The coordinatedly altered expressions of IL-1(R) isoforms in persistent psoriatic lesions give rise to a phenotype dominated by IL-1 antagonists, possibly as a consequence of feedback mechanisms to IL-1 (see chapter 5.4). One should note that several molecules have recently been cloned which demonstrate a structural homology to IL-1β (i.e. IL-1γ (21)) and IL-1RI (i.e. among others T1/ST2 (22)). Analysis of the expression and function of these potentially new members of the IL-1 system in psoriatic skin may be necessary for a complete understanding of the dysregulation of IL-1 in this skin disease.

The *ex vivo* model: a valid model to monitor epidermal cytokine production

Freshly isolated epidermal cells and non-stimulated, short-term (< 24 h) primary cultures of these cells were introduced as an *ex vivo* model to monitor cytokine production by epidermal cells (chapter 3.2). Epidermal cells *ex vivo* are a valid test model for the following reasons.

First, epidermal cells *ex vivo* closely represent the situation *in vivo* as shown by studies on the expression of DNA fragments (chapter 3.3), cytokine mRNA (chapters 3.1 and 3.4, and ref 23), and cytosolic IL-1 (i.e. cytosolic

IL-1β: chapter 3.2 and ref 1), and production of cytokines (i.e. IL-6: chapter 3.2 and ref 24).
Second, IL-1 production by primary cultures of epidermal cells is not merely based on passive release of preformed IL-1, but cells actively produce, process and secrete mature IL-1 (chapter 3.2).
Third, epidermal cells *ex vivo* are less prone to *in vitro* artefacts than human keratinocyte cultures (i.e. upregulation of cytosolic IL-1β: chapter 3.2 versus ref 25).
Fourth, epidermal cells *ex vivo* show a selective *de novo* synthesis of cytokines (chapter 3.2).
Finally, cytokine production by epidermal cells *ex vivo* can be modulated by treatments *in vivo* (i.e. topical corticosteroids: ref 26) as well as treatments *ex vivo* (i.e. IL-4: chapter 3.4).

Table I. Inventory of *in vivo* and *ex vivo* study results on the epidermal IL-1 system and IL-1 system-related cytokines in psoriasis.[a]

In vivo

IL-1α mRNA -, and (pro-)IL-1α protein ↓ in cytoplasm of basal keratinocytes.
IL-1β mRNA ↑, and IL-1β protein only detectable in suprabasal keratinocytes of a few patients.
IL-1ra mRNA ↔/↑[b], and IL-1ra protein ↑ in cytoplasm of suprabasal keratinocytes.
IL-1RI mRNA ↔, and IL-1RI protein -.
IL-1RII mRNA ↑, and IL-1RII protein ↑ in basal keratinocytes.
IL-4R mRNA ↑, and IL-4R protein ↑[c] in basal and some suprabasal keratinocytes.
The expressions of the individual IL-1(R) isoforms are interrelated.
The IL-4R expression is positively related to those of IL-1ra and IL-1RII.

Ex vivo

Release[d] of (pro-)IL-1α protein ↑, related to necrosis.
De novo synthesis[d] of mature IL-1β protein ↑, related to apoptosis.
Production of IL-6 protein ↑.
Production of TNF-α protein ↔.
IL-4 downregulates the production of IL-6 protein, and to a lesser extent IL-1β.

[a] Symbols: ↓, ↑ and ↔ indicate significantly decreased, increased and similar expression in lesional psoriatic epidermal samples when compared to normal control epidermal samples, respectively; and - indicates not detectable. Note that the *in vivo* expressions of individual IL-1(R) isoforms in non-lesional psoriatic epidermis were similar to those in normal control epidermis.
[b] The first symbol represents icIL-1ra mRNA and the second one secreted IL-1ra (sIL-1ra) mRNA.
[c] Note that the expression of IL-4R protein in non-lesional psoriatic epidermis is also increased when compared to normal control epidermis.
[d] The release and *de novo* synthesis of IL-1 are calculated as described in the materials and methods section of chapter 3.3.

IL-1 production by lesional psoriatic epidermal cells is related to cell death

Using the *ex vivo* model, we show for the first time that lesional psoriatic epidermal cells produce significantly increased amounts of biologically active

IL-1 relative to normal control epidermal cells (chapter 3.2 and ref 26). Moreover, lesional psoriatic epidermal cells produce biologically active IL-1α and IL-1β in a ratio of 3:1, whereas normal control epidermal cells only produce IL-1α. Processing and release of mature, biologically active IL-1 may be a consequence of cell death (27). We noted that lesional psoriatic epidermal cells showed a significantly increased cell death, as measured by trypan blue exclusion, relative to normal control epidermal cells (chapter 3.2), and that this cell death correlated significantly with the percentage of epidermal cell-derived IL-1 released into the medium (i.e. relative release) (chapter 3.3). The relative releases of IL-1α and IL-1β both correlated with the clinical PASI scores (release of IL-1α with PASI: $r_s = 0.9$, $n = 6$, $P < 0.025$; and release of IL-1β with PASI: $r_s = 0.8$, $n = 6$, $P < 0.05$). Both apoptosis and necrosis of monocytic cells is reported to be accompanied by activation of ICE (27,28), which may subsequently activate the calcium-activated neutral protease calpain (CANP: IL-1α processing enzyme) (29), and result in the secretion of mature IL-1α and IL-1β.

In our *ex vivo* model, lesional psoriatic epidermal cells expressed both enhanced apoptosis (chapter 3.3) and necrosis (chapter 3.2). Apoptosis is the principal route of epidermal cell death during short-term culture, as DNA fragmentation clearly preceded loss of plasma membrane integrity. The enhanced production of IL-1β by lesional psoriatic epidermal cells is caused by increased *de novo* synthesis of IL-1β in trypan blue negative cells (i.e. viable/non-necrotic cells) and is related to apoptosis (chapter 3.3). IL-1β processing was observed to precede secretion (Fig 5 of chapter 3.3), as was previously shown for monocytic cells (27), and confirmed the presence of mature IL-1β in cytosolic extracts of lesional psoriatic, but not normal control keratome specimens (2). The finding that IL-1β production by lesional psoriatic epidermal cells *ex vivo* is related to apoptosis is a novel one, and in accordance with the recent report that expression of the anti-apoptotic B cell lymphoma-2 (BCL-2) gene product in the murine keratinocyte cell line PAM-212 is inversely related with expression of IL-1 (30). Necrosis, evident by the release of lactate dehydrogenase activity as a marker for cytosol, may be, at least in part, secondary to apoptosis as apoptotic cells *ex vivo* are not phagocytosed and may ultimately display membrane disintegration (31). Facilitated release of intracellular (pro-)IL-1α, as a consequence of lost membrane integrity by (secondary) necrotic cells, explains the enhanced production of IL-1α by lesional psoriatic epidermal cells (chapter 3.3). Depletion experiments for intra-epidermal leukocytes (chapter 3.2) and the observed ability of the human keratinocyte cell line COLO-16 to secrete functionally active IL-1β (i.e. constituting 25 % of the total IL-1 activity: ref 32), favor keratinocytes as the cell-type responsible for the observed IL-1 production *ex vivo*.

Lesional psoriatic epidermal cells produce increased amounts of IL-6, but not TNF-α

Lesional psoriatic epidermal cells also produced increased amounts of IL-6 (chapter 3.2). The production of this cytokine represents *de novo* synthesis, as IL-6, in contrast to IL-1, is synthesized as a pro-form containing a leader peptide and is secreted via the classic secretory pathway (see chapter 1.1.2). Our finding that short-term culture of lesional psoriatic epidermal cells enhanced both the IL-6 mRNA expression and the IL-6 protein secretion confirmed this notion (chapter 3.4). The *ex vivo* results on IL-6 are in agreement with previous reports on blister fluids and skin biopsies (24,33). However, IL-6 could not be detected in aqueous extracts of the stratum corneum from psoriatic lesions and normal control skin (34).

Lesional psoriatic and normal control epidermal cells were furthermore observed to produce low, but similar amounts of tumor necrosis factor-α (TNF-α) (chapter 3.2). TNF-α production may involve shedding of membrane TNF-α (see chapter 1.1.2). Reports on TNF-α levels in blister fluids and aqueous extracts of the stratum corneum show controversy. In blister fluids either no TNF-α was observed (35) or the amounts of TNF-α were increased in lesional psoriatic skin samples (36). In aqueous scale extracts TNF-α protein and TNF-α activity were either not detectable (35), TNF-α was increased in lesional psoriatic skin but possessed no equivalent biological activity (34), or both TNF-α protein and TNF-α activity were increased in lesional psoriatic skin (37). The extent to which soluble TNFRI (sTNFRI), present in lesional psoriatic skin (37), and metalloproteases, responsible for the shedding of TNF-α and TNFRI (38,39), contribute to these controversies remains to be determined. When interpreting our *ex vivo* results, it is important to realize that these reflect the actual cytokine production by freshly isolated viable epidermal cells, which may account for some of the mentioned discrepancies with *in vivo* results.

Normally, human keratinocytes need a vigorous stimulus to produce substantial amounts of cytokines *in vitro*. Our results, therefore, imply that lesional psoriatic epidermal cells show an activated state *in vivo* and are easily triggered to express an enhanced production of IL-1 and IL-6. Transgenic mice showed that IL-1, but not IL-6, exert direct pro-inflammatory actions and that IL-6 probably acts synergistically with epidermal cell-derived IL-1 in the pathogenesis of psoriasis (5,40).

IL-4R expression by psoriatic epidermal cells is enhanced and downregulates the production of IL-6 protein, and to a lesser extent IL-1β

Psoriatic epidermal cells express enhanced levels of functional IL-4R (chapter 3.4). Analysis of the IL-4R mRNA and protein levels suggests that the increased expression of IL-4R by psoriatic epidermis is due to enhanced transcription of the IL-4R gene. The increased IL-4R expression may be a consequence of the local increase of IL-1 and IL-6 levels in lesional as well as non-lesional psoriatic skin (chapter 4.1, and refs 1,12,24,33,41), as at least

for myeloid cells IL-6 was reported to induce transcription of the IL-4R gene (42). Moreover, the upregulated expression of IL-4R in psoriatic epidermis *in vivo* may reflect a mean to downregulate the local epidermal IL-1 and IL-6 overexpression, as IL-4 inhibits the production of these cytokines by lesional psoriatic epidermal cells *ex vivo* (chapter 3.4). In more detail, IL-4 inhibited the production of IL-6 protein, and to a lesser extent IL-1β, and IL-4 most likely arrested post-transcriptional synthesis of IL-6, since IL-6 mRNA levels were not affected by IL-4 treatment. Lesional psoriatic epidermal cells may even show an altered response to IL-4 relative to normal control epidermal cells, as these latter cells demonstrated decreased levels of both IL-1β and IL-6 mRNA (Fig 5 of chapter 3.4). This observation is in line with the view that psoriatic keratinocytes display an altered responsiveness to cytokines, as has already been demonstrated for IFN-γ and IL-6 (43-45).

The notion that IL-4 may contribute to an effective repression of the epidermal IL-1 system is substantiated by the observation that the IL-4R expression correlated significantly with those of IL-1ra and IL-1RII. Nevertheless, the predominance of T_H1-type cytokines in lesional psoriatic skin *in vivo* (12,46) does not favor IL-4-mediated effects in psoriasis. Studies with lesional skin-derived T cell clones, however, showed that a minority of clones do secrete IL-4 and that keratinocyte proliferation induced by T cell-released cytokines is in part due to IL-4 (46,47). Moreover, recent immunohistochemical analysis indicated that psoriatic skin, although predominantly expressing IFN-γ^+ infiltrating T cells, also express an increased number of IL-4^+ dermal T cells relative to normal skin (not shown). The increase of both the epidermal expression of IL-4R and the inflammatory response in lesional psoriatic, and to a lesser extent non-lesional psoriatic skin, together with the ability of IL-4 to downregulate the inflammatory response (48), stress the relevance of the mentioned effects of IL-4 to the pathogenesis and therapy of psoriasis.

5.2 THE DERMAL IL-1 SYSTEM IN PSORIASIS

Dermal cell - epidermal cell interactions control epidermal homeostasis. The reciprocal induction of plasma membrane-bound and soluble factors such as MHC molecules, adhesion molecules, cytokines, cytokine receptors and extracellular matrix components affect growth, differentiation, state of activation and migration of resident as well as infiltrating cells (see chapter 1.2.1 on fibroblasts). Fibroblast - keratinocyte cocultures showed that keratinocyte-derived IL-1 increased the production of IL-6 by fibroblasts, which in turn is essential in promoting keratinocyte proliferation (49,50). As a feedback response, IL-1α and IL-1β activity, both responsible for the induction of IL-6 production, is downregulated by fibroblasts (49,51).

Fibroblasts are postulated to drive keratinocyte hyperproliferation and the cutaneous inflammatory response in psoriasis as suggested by *in vitro* (i.e coculture of gel-incorporated dermal fibroblasts and keratinocytes) and *in*

vivo experiments (i.e. transplantation studies using immune deficient mice) (see chapter 1.3 on fibroblasts). To study the pathological relevance of fibroblasts to psoriasis, we analyzed the expression of IL-1 and IL-1-related cytokines as well as their receptors by psoriatic fibroblasts *in vitro* (chapter 4).

Fibroblasts contribute to the locally increased IL-6 levels in psoriatic lesions

We observed that human dermal fibroblasts do not produce IL-1α, IL-1β or TNF-α, but are potent producers of IL-6 and IL-8 (chapter 4.1). Normal human fibroblasts among other cell types typically do not express IL-6 or IL-8 in the absence of a stimulatory signal (52,53). Lesional psoriatic fibroblasts, however, showed an increased spontaneous production of IL-6 and IL-8 (which was less dependent on external stimuli such as growth factors or serum). The enhanced production of IL-6 was neither psoriasis nor fibroblast specific, as constitutive IL-6 production has previously been reported for lesional sclerodermal fibroblasts (54) and peripheral blood monocytes from psoriatic patients (55), respectively. This suggests that the increased IL-6 production by lesional psoriatic fibroblasts is secondary to the chronic inflammatory response which characterizes psoriatic lesions. Fibroblast-derived IL-8, measured with enzyme-linked immunosorbent assay (ELISA), probably contributes negligibly to the biologically active IL-8 present in psoriatic lesions, as these cells produce exclusively the less biologically active 77-residue IL-8 form (56).

Serum induced the production of IL-6 and IL-8 protein in normal human dermal fibroblasts, as was previously observed with other cell types (53,57,58). The serum-induced kinetics of IL-6 production by fibroblasts was rapid, high and persistent, and unique for psoriasis. Unexpectedly, lesional psoriatic and normal control fibroblasts expressed similar levels of steady-state IL-6 mRNA levels under serum-free conditions, and serum induced a labile repressor protein of the IL-6 mRNA levels, most profoundly in lesional psoriatic fibroblasts. An overview of the altered IL-6 synthesis in lesional psoriatic fibroblasts is given in Table V of chapter 4.1.

The IL-6 levels are increased locally in psoriatic lesions (33), and contribute to the cutaneous inflammatory response, as for instance IL-6 potentiates the antigen presenting function of human fibroblasts (59). Moreover, anti-psoriatic vitamin D_3 analogs were shown to decrease fibroblast-derived IL-6 levels *in vitro* (60). We conclude that dermal fibroblasts are an important source of the increased IL-6 protein levels in psoriasis.

Lesional psoriatic fibroblasts show a decreased TNFR expression

The enhanced IL-6 protein production in lesional psoriatic fibroblasts may be the result of an altered expression of cytokine receptors, involved in auto/paracrine induction of IL-6. Flow cytometry showed that fibroblasts bound negligible amounts of recombinant human IL-1α and IL-1β, but clearly bound IL-4, IL-6 and TNF-α (chapter 4.2). The lack of IL-1 production

(chapter 4.1) and the negligible expression of IL-1 binding sites on fibroblasts (chapter 4.2) per se do not exclude the possibility that the IL-6 production by lesional psoriatic fibroblasts is induced by IL-1. In fact, Espinoza *et al.* recently showed that lesional psoriatic fibroblasts do produce biologically active IL-1β (61), measured with the use of another, probably more sensitive bioassay than the D10 assay which we used. In addition, only a few IL-1RI molecules per cell are sufficient for an IL-1-mediated response (62), illustrated by the clear IL-1 responsiveness of fibroblasts (Fig 1 of chapter 4.2).

The expression of cytokines, as mentioned above, and their binding sites is modulated by both calcium and serum, probably in a manner specific for dermal fibroblasts, as modulation is different with other cell types or fibroblasts derived from different tissue sources (i.e. bone marrow stroma-derived fibroblasts) (7,63). Serum upregulated the number of normal control fibroblasts which bound TNF-α, and to a lesser extent IL-6. However, culturing lesional psoriatic fibroblasts to confluence under serum-containing conditions did not affect the number of cells which bound TNF-α. Immunocytochemical staining results were in line with the binding experiments and showed that the expression of TNFRI, which mediates most immunomodulatory effects on fibroblasts (64), was clearly decreased in lesional psoriatic fibroblasts. ELISA for sTNFR showed that shedding of the TNFR was not responsible for the decreased TNFR expression by lesional psoriatic fibroblasts (chapter 4.2).

Taken together, we speculate that lesional psoriatic fibroblasts exhibit a deficiency in serum-induced TNFR upregulation. This is in agreement with observations by others that lesional psoriatic fibroblasts display numerous inherent phenotypic abnormalities *in vitro*, all reflecting a hyperactive cellular state, and confirms reports on an altered response of these cells to serum factors, cytokines and therapeutics (see chapter 1.3 on fibroblasts). In analogy, lesional psoriatic fibroblasts express increased levels of functionally active platelet-derived growth factor Rβ (PDGFRβ) (61,65), and lesional sclerodermal fibroblasts show an altered expression of TNFR (66). At the moment, one cannot simply extend our findings on the TNFR expression to the responsiveness to TNF-α, since the degree of receptor density does not always correlate with TNF-α responsiveness (67), and the biological actions of TNF-α are not always preceded by receptor internalization (68).

In total, our studies show that lesional psoriatic fibroblasts co-express decreased TNFR levels and an increased IL-6 production. Interestingly, in mice TNF-α, co-injected subcutaneously with lithium chloride, induced a psoriasis-like inflammatory response via an endogenous local IL-6 production (69). Thus, our results imply, at least partial involvement of dermal fibroblasts in the maintenance of the chronic inflammation in psoriasis.

5.3 CONCLUSIONS

Conclusions drawn from the thesis work are the following.

1) lesional psoriatic epidermis displays an IL-1 antagonist sphere of

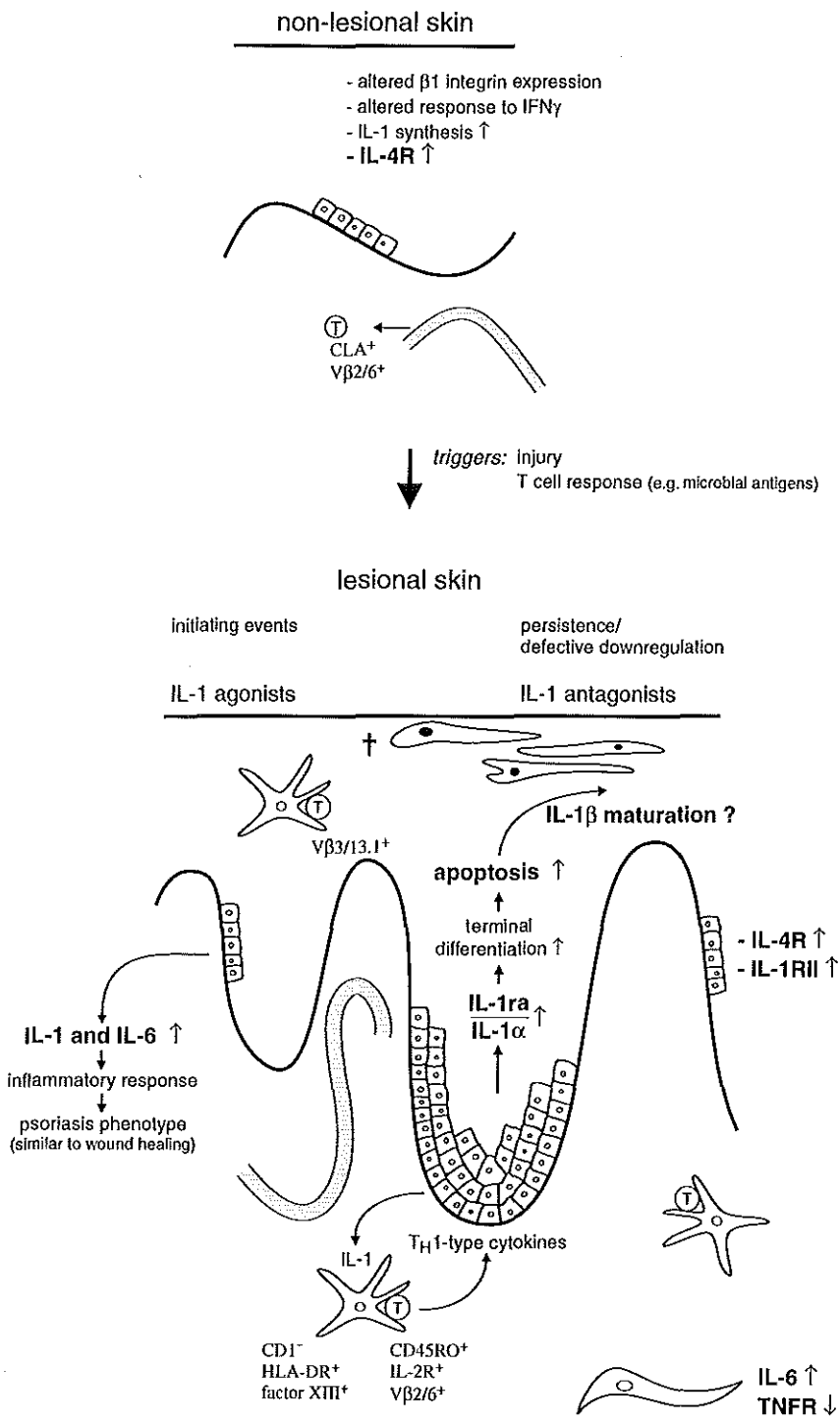
influence *in vivo*;
2) lesional psoriatic epidermal cells secrete elevated amounts of biologically active IL-1α, IL-1β and IL-6 *ex vivo* relative to normal epidermal cells;
3) *de novo* synthesis of IL-1β by lesional psoriatic epidermal cells *ex vivo* is enhanced and related to apoptosis;
4) lesional psoriatic basal keratinocytes *in vivo* express elevated levels of functionally active IL-4R;
5) lesional psoriatic fibroblasts express an enhanced production of IL-6 *in vitro*; and
6) lesional psoriatic fibroblasts express a decreased expression of TNFR under serum-stimulated conditions *in vitro*.

Overall, the first part of the hypothesis is correct, in that the expression of members of the IL-1 system and IL-1 system-related cytokines is, at least in the epidermis, coordinatedly altered.

5.4 CURRENT VIEW ON IL-1 IN THE IMMUNOPATHOGENESIS OF PSORIASIS

A hypothetical scheme that illustrates the pathological relevance of IL-1 to psoriasis is given in Figure 1. This scheme integrates data presented in this thesis (in bold) and data from the literature. It is important to realize that non-lesional psoriatic skin is not normal. Relevant alterations in this type of skin are mentioned in Figure 1 (see chapter 1.3 for references). For instance, keratinocyte stem cells from non-lesional psoriatic skin show enhanced proliferation in response to the cooperative action of IFN-γ, IL-3 and granulocyte macrophage-colony stimulating factor (GM-CSF) when compared to stem cells from normal skin (47). Moreover, injection of IL-2 or IFN-γ in non-lesional psoriatic skin *in vivo* triggers the appearance of lesional skin (70,71). In normal human keratinocytes IFN-γ has already been reported to induce the production of IL-1α (72). In extension of these observations, one could speculate that the secretion of IL-1 by psoriatic keratinocytes, as observed *ex vivo*, is more easily triggered by injury and/or products from activated cutaneous lymphocyte-associated antigen $(CLA)^+$ and variable gene region $(V)\beta2/6^+$ immunosurveilling skin T cells relative to normal keratinocytes. In this context, microbial antigens, acting as superantigens, are good candidates to trigger an initial T cell response (see chapter 1.3.1). At the moment one cannot exclude (auto)antigen(s) in the initiating events of psoriatic lesions. However, a micro-environment dominated by IL-1 agonists and IL-1-

Figure 1. IL-1 in the immunopathogenesis of psoriasis. See right-handed page. The presented scheme integrates data from this thesis (in bold) and recent literature to illustrate the proposed involvement of IL-1 in the initiation and/or maintenance of psoriatic lesions. See chapter 5.4 for details.


non-lesional skin
- altered β1 integrin expression
- altered response to IFNγ
- IL-1 synthesis ↑
- IL-4R ↑
CLA+
Vβ2/6+
triggers: injury
T cell response (e.g. microbial antigens)
lesional skin
initiating events
persistence/
defective downregulation
IL-1 agonists
IL-1 antagonists
Vβ3/13.1+
IL-1β maturation ?
apoptosis ↑
terminal
differentiation ↑
IL-1ra
IL-1α ↑
- IL-4R ↑
- IL-1RII ↑
IL-1 and IL-6 ↑
inflammatory response
psoriasis phenotype
(similar to wound healing)
IL-1
TH1-type cytokines
CD1-
HLA-DR+
factor XIII+
CD45RO+
IL-2R+
Vβ2/6+
IL-6 ↑
TNFR ↓

inducible cytokines such as IL-6, IL-8 and TNF-α may support *an antigen-independent initiation of the inflammatory response* (see chapter 1.2.2), and give rise to the psoriatic phenotype, as is illustrated in Figure 1. In the early developing psoriatic lesion cytokines, being overexpressed by non-lesional psoriatic skin (e.g. IL-1α, IL-1β, IL-6 and IL-8) (see chapter 1.3 on non-lesional psoriatic skin), may initiate endothelial changes (73). It has previously been shown that epidermal alterations precede vascular anomalies (74), which may subsequently lead to inflammatory cell infiltration in psoriatic plaques (75). Data from integrin transgenic mice furthermore support the view that the primary defect is in the keratinocytes (76).

The oligoclonality of T cells in lesional skin (predominant usage of Vβ2/6 and Vβ3/13.1 by dermal and epidermal T cells, respectively) favors *the contribution of antigens or autoantigens to the persistence of psoriatic lesions* (77,78). IL-1α may even prevent the induction of antigen-specific tolerance (79). Sensitization to multiple autoantigens or cryptic epitopes is likely the consequence of chronic inflammation, epidermal hyperplasia, accelerated terminal keratinocyte differentiation and apoptosis (see chapter 1.3.1). It also remains possible that the expression of such neo-epithelial peptides is more easily triggered in psoriatic relative to normal keratinocytes. Binding of (super)antigens and/or autoantigens to immunogenetically determined high-risk human leukocyte antigen (HLA) molecules on antigen presenting cells (i.e. dendrocytes) may subsequently become productive for T cell activation (80). Psoriatic lesions are probably maintained by a self-sustaining cycle of predominantly T_H1-type cytokines derived from dermal dendrocyte-activated memory T cells (81) that stimulate epidermal cells (82), and further epidermal IL-1 potentiation of the T cells (83). Epidermal IL-1β may be involved in the skewing towards the production of T_H1-type cytokines (15). Resident skin cells such as fibroblasts are probably also actively involved in the amplification and/or maintenance phase of psoriasis since these cells show an altered expression of cytokines and their receptors *in vitro* (see chapter 1.3 on fibroblasts and Fig 1). Moreover, the increased production of superoxide anion by lesional psoriatic fibroblasts (84) together with the increased keratinocyte apoptosis in psoriasis (chapter 3.3 and Fig 1) may lead to activation of Langerhans cells and subsequently of naive, antigen-specific T cells (85).

Finally, *downregulatory mechanisms of cutaneous inflammation may be deficient in psoriasis*. These comprise, for example, negative-feedback loops involving the IL-1 system; apoptosis of keratinocytes and effector leukocytes (e.g. clonal deletion of locally activated autoreactive T cells); induction of peripheral tolerance to self-antigens by keratinocytes; and repair of skin tissue which decreases activation of APC. IL-1 is, albeit to varying degrees, involved in every single downregulatory mechanism (see chapter 1.2). The IL-1 antagonist sphere of influence (i.e. increased epidermal expressions of IL-1ra and IL-1RII), favoring a decreased IL-1 responsiveness, possibly reflects a negative-feedback response to IL-1 agonists, perhaps in part

mediated by IL-4 (i.e. increased expression of functional IL-4R). Such an impaired response of lesional psoriatic epidermis to IL-1 may thus, at least in part, be responsible for a deficient downregulation of the psoriatic response.

Why do normal individuals not develop psoriatic lesions? A number of genetically-determined factors, as outlined above, may contribute to the pathogenesis of psoriasis. In short, these factors comprise an altered cytokine responsiveness of non-lesional psoriatic keratinocytes, high-risk HLA molecule binding of antigen(s), and a defective downregulation of the inflammatory response. The polygenic nature of psoriasis suggests that most likely a combination of such factors (as well as perhaps other factors) is involved in the initiation and/or maintenance of the psoriatic phenotype. These factors contribute to the generation of self-sustaining cycles of cytokine-mediated activation of resident and infiltrating cells, of which the IL-1 system is an integral component.

5.5 FUTURE DIRECTIONS

Future work should focus on the primary fault in psoriasis, which in our opinion is restricted to the non-lesional psoriatic keratinocyte. Promising models were recently developed which enable research into the sequence of initiating events resulting in the transition of the non-lesional psoriatic keratinocyte to the lesional psoriatic keratinocyte. For instance, the psoriatic phenotype can be triggered in human non-lesional psoriatic skin transplanted onto mice with severe combined immunodeficiency (86), which may prove to be a valid model to analyse the distorted cytokine responsiveness and release of cytokines. Other relevant models include the β1 integrin transgenic mice (76) and the flaky skin mutant mice (87) (see also chapter 1.3), which may provide means to study single factors that determine susceptibility to develop psoriasis. Definite proof of the pathological role of the IL-1 system (second part of the hypothesis, see chapter 2) may come from a null mutation of one or several members of the IL-1 system in these mice strains.

REFERENCES

1. Cooper KD, Hammerberg C, Baadsgaard O, Elder JT, Chan LS, Sauder DN, Voorhees JJ, Fisher G (1990) IL-1 activity is reduced in psoriatic skin. Decreased IL-1α and increased nonfunctional IL-1β. J Immunol 144:4593-4603.
2. Gruaz D, Didierjean L, Grassi J, Frobert Y, Dayer J-M, Saurat J-H (1989) Interleukin 1 alpha and beta in psoriatic skin: enzymoimmunoassay, immunoblot studies and effect of systemic retinoids. Dermatologica 179:202-206.
3. Hammerberg C, Arend WP, Fisher GJ, Chan LS, Berger AE, Haskill SJ, Voorhees JJ, Cooper KD (1992) Interleukin-1 receptor antagonist in normal and psoriatic epidermis. J Clin Invest 90:571-583.
4. Demczuk S (1988) IL-1/ETAF activity and undetectable IL-1 beta mRNA and minimal IL-1 alpha mRNA levels in normal adult heat-separated epidermis. Biochem Biophys Res Commun 156:463-469.
5. Groves RW, Mizutani H, Kieffer JD, Kupper TS (1995) Inflammatory skin disease in

transgenic mice that express high levels of interleukin 1α in basal epidermis. Proc Natl Acad Sci USA 92:11874-11878.
6. Gruaz-Chatellard D, Baumberger C, Saurat J-H, Dayer J-M (1991) Interleukin 1 receptor antagonist in human epidermis and cultured keratinocytes. FEBS Lett 294:137-140.
7. Blanton RA, Kupper TS, McDougall JK, Dower SK (1989) Regulation of interleukin 1 and its receptor in human keratinocytes. Proc Natl Acad Sci USA 86:1273-1277.
8. Arend WP (1993) Interleukin-1 receptor antagonist. Adv Immunol 54:167-227.
9. Watson JM, Lofquist AK, Rinehart CA, Olsen JC, Makarov SS, Kaufman DG, Haskill JS (1995) The intracellular IL-1 receptor antagonist alters IL-1-inducible gene expression without blocking exogenous signaling by IL-1β. J Immunol 155:4467-4475.
10. Bossù P, Visconti U, Ruggiero P, Macchia G, Muda M, Bertini R, Bizzarri C, Colagrande A, Sabbatini V, Maurizi G, Delgrosso E, Tagliabue A, Boraschi D (1995) Transfected type II interleukin-1 receptor impairs responsiveness of human keratinocytes to interleukin-1. Am J Pathol 147:1852-1861.
11. Groves RW, Giri J, Sims J, Dower S, Kupper TS (1995) Inducible expression of type 2 IL-1 receptors by cultured human keratinocytes. Implications for IL-1-mediated processes in epidermis. J Immunol 154:4065-4072.
12. Uyemura K, Yamamura M, Fivenson DF, Modlin RL, Nickoloff BJ (1993) The cytokine network in lesional and lesion-free psoriatic skin is characterized by a T-helper type 1 cell-mediated response. J Invest Dermatol 101:701-705.
13. Mangan DF, Welch GR, Wahl SM (1991) Lipopolysaccharide, tumor necrosis factor-α, and IL-1β prevent programmed cell death (apoptosis) in human peripheral blood monocytes. J Immunol 146:1541-1546.
14. Colotta F, Re F, Polentarutti N, Sozzani S, Mantovani A (1992) Modulation of granulocyte survival and programmed cell death by cytokines and bacterial products. Blood 80:2012-2020.
15. O'Garra A, Murphy K (1994) Role of cytokines in determining T-lymphocyte function. Curr Opin Immunol 6:458-466.
16. Haake AR, Polakowska RR (1993) Cell death by apoptosis in epidermal biology. J Invest Dermatol 101:107-112.
17. Frisch SM, Francis H (1994) Disruption of epithelial cell-matrix interactions induces apoptosis. J Cell Biol 124:619-626.
18. Pellegrini G, De Luca M, Orecchia G, Balzac F, Cremona O, Savoia P, Cancedda R, Marchisio PC (1992) Expression, topography and function of integrin receptors are severely altered in keratinocytes from involved and uninvolved psoriatic skin. J Clin Invest 89:1783-1795.
19. Grubauer G, Romani N, Kofler H, Stanzl U, Fritsch P, Hintner H (1986) Apoptotic keratin bodies as autoantigen causing the production of IgM-anti-keratin intermediate filament autoantibodies. J Invest Dermatol 87:466-471.
20. Rosen A, Casciola-Rosen L, Ahearn J (1995) Novel packages of viral and self-antigens are generated during apoptosis. J Exp Med 181:1557-1561.
21. Bazan JF, Timans JC, Kastelein RA (1996) A newly defined interleukin-1? Nature 379:591.
22. Sims J, Dower S (1994) Interleukin-1 receptors. Eur Cytokine Netw 5:539-546.
23. Longley J, Gang Ding T, Cuono C, Durden F, Crooks C, Hufeisen S, Eckert R, Wood GS (1991) Isolation, detection, and amplification of intact mRNA from dermatome strips, epidermal sheets, and sorted epidermal cells. J Invest Dermatol 97:974-979.
24. Grossman RM, Krueger J, Yourish D, Granelli-Piperno A, Murphy DP, May LT, Kupper TS, Sehgal PB, Gottlieb AB (1989) Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proc Natl Acad Sci USA 86:6367-6371.
25. Gatto H, Viac J, Charveron M (1991) Levels of immunoreactive interleukin 1a and 1b in basal, supra-basal and cornified epidermal cell populations of normal human epidermis. Comparison with keratinocytes cultured in defined medium. Eur J Dermatol 1:55-59.
26. Debets R, Van Joost T, Benner R, Prens EP (1993) Psoriatic epidermal cells release elevated levels of immunoreactive and biologically active interleukins 1 and 6: modulation

of corticosteroid treatment. In Bernard BA, Schroot B (eds). Pharmacol Skin: From molecular biology to therapeutics. Kärger, Basel, 5:158-166.
27. Perregaux D, Barberia J, Lanzetti AJ, Geoghegan KF, Carty TJ, Gabel CA (1992) IL-1β maturation: evidence that mature cytokine formation can be induced specifically by nigericin. J Immunol 149:1294-1303.
28. Hogquist KA, Nett MA, Unanue ER, Chaplin DD (1991) Interleukin 1 is processed and released during apoptosis. Proc Natl Acad Sci USA 88:8485-8489.
29. Kuida K, Lippke JA, Ku G, Harding MW, Livingston DJ, Su MS-S, Flavell RA (1995) Altered cytokine export and apoptosis in mice deficient in interleukin-1β converting enzyme. Science 267:2000-2003.
30. Marthinuss J, Lawrence L, Seiberg M (1995) Apoptosis in Pam212, an epidermal keratinocyte cell line: a possible role for bcl-2 in epidermal differentiation. Cell Growth Differ 6:239-250.
31. Majno G, Joris I (1995) Apoptosis, oncosis and necrosis. An overview of cell death. Am J Pathol 146:3-15.
32. McKenzie RC, Arsenault TV, Sauder DN, Harley CB (1990) Expression of interleukin-1β in a human keratinocyte cell line. Lymphokine Res 9:391-403.
33. Prens EP, Benne K, Van Damme J, Bakkus M, Brakel K, Benner R, Van Joost T (1990) Interleukin-1 and interleukin-6 in psoriasis. J Invest Dermatol 95:121S-124S.
34. Gearing AJH, Fincham NJ, Bird CR, Wadhwa M, Meager A, Cartwright JE, Camp RDR (1990) Cytokines in skin lesions of psoriasis. Cytokine 2:68-75.
35. Takematsu H, Ohta H, Tagami H (1989) Absence of tumor necrosis factor α in suction blister fluids and stratum corneum from patients with psoriasis. Arch Dermatol Res 281:398-400.
36. Bonifati C, Carducci M, Cordiali Fei P, Trento E, Sacerdoti G, Fazio M, Ameglio F (1994) Correlated increases of tumour necrosis factor-alpha, interleukin-6 and granulocyte monocyte-colony stimulating factor levels in suction blister fluids and sera of psoriatic patients--relationships with disease severity. Clin Exp Dermatol 19:383-387.
37. Ettehadi P, Greaves MW, Wallach D, Aderka D, Camp RD (1994) Elevated tumour necrosis factor-alpha (TNF-alpha) biological activity in psoriatic skin lesions. Clin Exp Immunol 96:146-151.
38. Crowe PD, Walter BN, Mohler KM, Otten-Evans C, Black RA, Ware CF (1995) A metallo- protease inhibitor blocks shedding of the 80-kD TNF receptor and TNF processing in T lymphocytes. J Exp Med 181:1205-1210.
39. Müllberg J, Durie FH, Otten-Evans C, Alderson MR, Rose-John S, Cosman D, Black RA, Mohler KM (1995) A metalloprotease inhibitor blocks shedding of the IL-6 receptor and the p60 TNF receptor. J Immunol 155:5198-5205.
40. Turksen K, Kupper TS, Degenstein L, Williams I, Fuchs E (1992) Interleukin 6: insights to its function in skin by overexpression in transgenic mice. Proc Natl Acad Sci USA 89:5068-5072.
41. Ohta Y, Katayama I, Funato T, Yokozeki H, Nishiyama S, Hirano T, Kishimoto T, Nishioka K (1991) In situ expression of messenger RNA of interleukin-1 and interleukin-6 in psoriasis: interleukin-6 involved in formation of psoriatic lesions. Arch Dermatol Res 283:351-356.
42. Ruhl S, Feldman GM, Akahane K, Pluznik DH (1993) Interferon-γ antagonizes interleukin-6-induced expression of interleukin-4 receptors in murine myeloid cells by a transcriptional mechanism. Blood 82:2641-2648.
43. Baker BS, Powles AV, Valdimarsson H, Fry L (1988) An altered response by psoriatic keratinocytes to gamma interferon. Scand J Immunol 28:735-740.
44. Nickoloff BJ, Mitra RS, Elder JT, Fisher GJ, Voorhees JJ (1989) Decreased growth inhibition by recombinant gamma interferon is associated with increased transforming growth factor-alpha production in keratinocytes cultured from psoriatic lesions. Br J Dermatol 121:161-174.
45. Olaniran AK, Baker BS, Garioch JJ, Powles AV, Fry L (1995) A comparison of the stimulatory effects of cytokines on normal and psoriatic keratinocytes in vitro. Arch Dermatol Res 287:231-236.

46. Schlaak JF, Buslau M, Jochum W, Hermann E, Girndt M, Gallati H, Buschenfelde K-H, Fleischer B (1994) T cells involved in psoriasis vulgaris belong to the Th1 subset. J Invest Dermatol 102:145-149.
47. Bata-Csorgo ZS, Hammerberg C, Voorhees JJ, Cooper KD (1995) Kinetics and regulation of human keratinocyte stem cell growth in short-term primary *ex vivo* culture. Cooperative growth factors from psoriatic lesional T lymphocytes stimulate proliferation among psoriatic uninvolved, but not normal, stem keratinocytes. J Clin Invest 95:317-327.
48. Colgan SP, Resnick MB, Parkos CA, Delp-Archer C, McGuirk D, Bacarra AE, Weller PF, Madara JL (1994) IL-4 directly modulates function of a model human intestinal epithelium. J Immunol 153:2122-2129.
49. Boxman I, Lowik C, Aarden L, Ponec M (1993) Modulation of IL-6 production and IL-1 activity by keratinocyte-fibroblast interaction. J Invest Dermatol 101:316-324.
50. Zhang JZ, Maruyama K, Iwatsuki K, Ono I, Kaneko F (1994) Effects of prostaglandin E1 on human keratinocytes and dermal fibroblasts: a possible mechanism for the healing of skin ulcers. Exp Dermatol 3:164-170.
51. Waelti ER, Inaebnit SP, Rast HP, Hunziker T, Limat A, Braathen LR, Wiesmann U (1992) Co-culture of human keratinocytes on post-mitotic human dermal fibroblast feeder cells: production of large amounts of interleukin 6. J Invest Dermatol 98:805-808.
52. Kishimoto T (1989) The biology of interleukin-6. Blood 74:1-10.
53. Baggiolini M, Dewald B, Moser B (1993) Interleukin-8 and related chemotactic chemokines - CXC and CC chemokines. Adv Immunol 55:97-179.
54. Feghali CA, Bost KL, Boulware DW, Levy LS (1992) Mechanisms of pathogenesis in scleroderma. I. Overproduction of interleukin 6 by fibroblasts cultured from affected skin sites of patients with scleroderma. J Rheumatol 19:1207-1211.
55. Neuner P, Urbanski A, Trautinger F, Möller A, Kirnbauer R, Kapp A, Schöpf E, Schwarz T, Luger TA (1991) Increased IL-6 production by monocytes and keratinocytes in patients with psoriasis. J Invest Dermatol 97:27-33.
56. Schröder JM (1995) Inflammatory mediators and chemoattractants. Clin Dermatol 13:137-150.
57. Kirnbauer R, Köck A, Schwarz T, Urbanski A, Krutmann J, Borth W, Damm D, Shipley G, Ansel JC, Luger TA (1989) IFN-β2, B cell differentiation factor 2, or hybridoma growth factor (IL-6) is expressed and released by human epidermal cells and epidermoid carcinoma cell lines. J Immunol 142:1922-1928.
58. Aarden LA, De Groot ER, Schaap OL, Lansdorp PM (1987) Production of hybridoma growth factor by human monocytes. Eur J Immunol 17:1411-1416.
59. May LT, Helfgott DC, Sehgal PB (1986) Anti-beta-interferon antibodies inhibit the increased expression of HLA-B7 mRNA in tumor necrosis factor-treated human fibroblasts: structural studies of the beta 2 interferon involved. Proc Natl Acad Sci USA 83:8957-8961.
60. Srviastava MD, Deluca H, Ambrus JL (1994) Inhibition of IL-6 and IL-8 production in human fibroblast cell lines by 1,25 (OH)2 vitamin D3 and two of its analogs with lower calcemic activity. Res Commun Chem Pathol Pharmacol 83:145-150.
61. Espinoza LR, Espinoza CG, Cuéllar ML, Scopelitis E, Silveira LH, Grotendorst GR (1994) Fibroblast function in psoriatic arthritis. II. Increased expression of β platelet derived growth factor receptors and increased production of growth factor and cytokines. J Rheumatol 21:1507-1514.
62. McKean DJ, Podzorski RP, Bell MP, Nilson AE, Huntoon CJ, Slack J, Dower SK, Sims J (1993) Murine T helper cell-2 lymphocytes express type I and type II IL-1 receptors, but only the type I receptor mediates costimulatory activity. J Immunol 151:3500-3510.
63. Guba SC, Sartor CI, Gottschalk LR, Ye-Hu J, Mulligan T, Emerson SG (1992) Bone marrow stromal fibroblasts secrete interleukin 6 and granulocyte-macrophage colony-stimulating factor in the absence of inflammatory stimulation: demonstration by serum-free bioassay, enzyme-linked immunosorbent assay, and reverse transcriptase polymerase chain reaction. Blood 80:1190-1198.
64. Tartaglia LA, Goeddel DV (1992) Two TNF receptors. Immunol Today 13:151-153.
65. Gu XF, Raynaud F, Evain-Brion D (1988) Increased chemotactic and mitogenic response of

psoriatic fibroblasts to platelet-derived growth factor. J Invest Dermatol 91:599-602.
66. Berman B, Wietzerbin J (1992) Tumor necrosis factor-α (TNF-α), interferon-α (IFN-α) and interferon-γ (IFN-γ) receptors on human normal and scleroderma dermal fibroblasts in vitro. J Dermatol Sci 3:82-90.
67. Tsujimoto M, Yip YK, Vilček J (1985) Tumor necrosis factor: specific binding and internalization in sensitive and resistant cells. Proc Natl Acad Sci USA 82:7626-7630.
68. Hofsli E, Nissen-Meyer J (1989) Recombinant tumour necrosis factor (TNF) fixed to cell monolayers retains its cytotoxic and growth-stimulatory activities. Evidence that internalization of TNF is not necessary for induction of biological effects. Scand J Immunol 29:57-63.
69. Beyaert R, Schulze-Osthoff K, Van Roy F, Fiers W (1992) Synergistic induction of interleukin-6 by tumor necrosis factor and lithium chloride in mice: possible role in the triggering and exacerbation of psoriasis by lithium treatment. Eur J Immunol 22:2181-2184.
70. Lee RE, Gaspari AA, Lotze MT, Chang AE, Rosenberg SA (1988) Interleukin 2 and psoriasis. Arch Dermatol 124:1811-1815.
71. Fierlbeck G, Rassner G, Muller C (1990) Psoriasis induced at the injection site of recombinant interferon gamma. Results of immunohistologic investigations. Arch Dermatol 126:351-355.
72. Maruyama K, Zhang JZ, Nihei Y, Ono I, Kaneko F (1995) Regulatory effects of anti psoriatic agents on interleukin-1 alpha production by human keratinocytes stimulated with gamma interferon in vitro. Skin Pharmacol 8:41-48.
73. Nickoloff BJ, Mitra RS, Varani J, Dixit VM, Polverini PJ (1994) Aberrant production of interleukin-8 and thrombospondin-1 by psoriatic keratinocytes mediates angiogenesis. Am J Pathol 144:820-828.
74. Parent D, Bernard BA, Desbas C, Heenen M, Darmon MY (1990) Spreading of psoriatic plaques: alteration of epidermal differentiation precedes capillary leakiness and anomalies in vascular morphology. J Invest Dermatol 95:333-340.
75. Brody I (1984) Dermal and epidermal involvement in the evolution of acute eruptive guttate psoriasis vulgaris. J Invest Dermatol 82:465-470.
76. Carroll JM, Romero MR, Watt FW (1995) Suprabasal integrin expression in the epidermis of transgenic mice results in developmental defects and a phenotype resembling psoriasis. Cell 83:957-968.
77. Menssen A, Trommler P, Vollmer S, Schendel D, Albert E, Gürtler L, Riethmüller G, Prinz JC (1995) Evidence for an antigen-specific cellular immune response in skin lesions of patients with psoriasis vulgaris. J Immunol 155: 4078-4083.
78. Chang JCC, Smith LR, Froning KJ, Schwabe BJ, Laxer JA, Caralli LL, Kurland HH, Karasek MA, Wilkinson DI, Carlo DJ, Brostoff SW (1994) $CD8^+$ T cells in psoriatic lesions preferentially use T-cell receptor $V_\beta 3$ and/or $V_\beta 13.1$ genes. Proc Natl Acad Sci USA 91:9282-9286.
79. Nakata Y, Matsuda K, Uzawa A, Nomura M, Akashi M, Suzuki G (1995) Administration of recombinant human IL-1 by staphylococcus enterotoxin B prevents tolerance induction in vivo. J Immunol 155:4231-4235.
80. Elder JT, Henseler T, Christophers E, Voorhees JJ, Nair RP (1994) Of genes and antigenes: the inheritance of psoriasis. J Invest Dermatol 103:150S-153S.
81. Nestle FO, Turka LA, Nickoloff BJ (1994) Characterization of dermal dendritic cells in psoriasis: autostimulation of T lymphocytes and induction of TH-1 type cytokines. J Clin Invest 94:202-209.
82. Prinz JC, Groß B, Vollmer S, Trommler P, Strobel I, Meurer M, Plewig G (1994) T cell clones from psoriatic skin lesions can promote keratinocyte proliferation *in vitro* via secreted products. Eur J Immunol 24:593-598.
83. Chang EY, Hammerberg C, Fisher G, Baadsgaard O, Ellis CN, Voorhees JJ, Cooper KD (1992) T-cell activation is potentiated by lesional psoriatic, but not normal, epidermis. Arch Dermatol 128:1479-1485.
84. Er-raki A, Charveron M, Bonafe JL (1993) Increased superoxide anion production in dermal fibroblasts of psoriatic patients. Skin Pharmacol 6:253-258.
85. Casciola-Rosen LA, Anhalt G, Rosen A (1994) Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratino-

cytes. J Exp Med 179:1317-1330.
86. Boehncke W-H, Dressel D, Zollner TM, Kaufmann R (1996) Pulling the trigger on psoriasis. Nature 379:777.
87. Morita K, Hogan ME, Nanney LB, King LE Jr., Manabe M, Sun TT, Sundberg JP (1995) Cutaneous ultrastructural features of the flaky skin (fsn) mouse mutation. J Dermatol 22:385-395.

SUMMARY

Psoriasis is a common skin disease, which has been well-defined for nearly two centuries, and is featured by accelerated terminal keratinocyte differentiation and leukocyte infiltration. The exact etiology, however, is still unknown. This, together with the notion that the majority of psoriatic patients is not satisfied with the currently available anti-psoriatic therapies, drive fundamental psoriasis research.

Dysregulated communication between resident skin cells and infiltrating leukocytes may initiate and/or mediate psoriasis. The cytokine IL-1 is considered to be an integral component of the cellular immune basis of psoriasis. This is supported by the following:
i) IL-1 may control keratinocyte proliferation and differentiation, as well as cutaneous inflammation; ii) transgenic overexpression of IL-1 in basal keratinocytes induces a psoriasis-like phenotype; and iii) the expression of IL-1 is altered in psoriatic skin.

Human epidermis contains life-threatening amounts of IL-1. The IL-1 activity is, therefore, highly regulated, being the net outcome of interacting IL-1 agonists and antagonists, which together constitute the IL-1 system. **Chapter 1** describes the physiology of the epidermal IL-1 system and the cutaneous inflammatory response, and provides a detailed overview on the cellular immunopathology of psoriasis.

The primary goal of this thesis was to investigate the expression of cytokines and their receptors in psoriatic skin, with special focus on the epidermal IL-1 system. As initially available data were inconsistent and often incomplete, the present thesis aimed at an integrated study approach which combined several models and multiple assays. The hypothesis and the study design are given in **chapter 2.**

The epidermal compartment is dealt with in **chapter 3.** A thorough analysis of the expression of <u>all</u> components of the human IL-1 system, which have been characterized to date, is described in **chapter 3.1** and resulted in a defined phenotype of IL-1 in psoriatic skin. Semiquantitative immunostaining revealed that the expression of IL-1α was significantly decreased in basal keratinocytes of lesional psoriatic epidermis *in vivo* when compared to non-lesional psoriatic and normal control epidermis. The decreased IL-1α expression *in situ* may be a consequence of the increased release of IL-1α by lesional psoriatic epidermal cells as observed *ex vivo* (see below). IL-1ra and IL-1RII were significantly increased in the suprabasal cell layers and basal cell layer, respectively, of lesional psoriatic epidermis, and both correlated negatively with IL-1α. This inverse relation is probably related to accelerated terminal keratinocyte differentiation in psoriatic lesions. Furthermore, a positive correlation was observed between IL-1ra and IL-1RII, which suggests an effective inhibition of IL-1 responsiveness in lesional psoriatic epidermis. In situ hybridization results were consistent with immunostainings. However, IL-1ra mRNA was not increased in lesional psoriatic epidermis,

possibly implying an enhanced post-transcriptional IL-1ra synthesis in lesional psoriatic epidermis. With the use of the more sensitive RT-PCR, a profound increase in IL-1β mRNA expression in freshly isolated lesional psoriatic epidermal cells was observed, which paralleled the mRNA expression of sIL-1ra and IL-1RII. IL-1RI mRNA was clearly present in all epidermal cell samples. Thus, the expressions of IL-1 and IL-1 receptor isoforms are coordinatedly altered in lesional psoriatic epidermis, favoring a local IL-1 antagonist sphere of influence.

Freshly isolated epidermal cells and non-stimulated, short-term primary cultures of these cells were introduced as an *ex vivo* model in **chapter 3.2** to characterize the actual production of IL-1 and related cytokines by epidermal cells. ELISA and bioassay analyses showed that lesional psoriatic epidermal cells released significantly increased amounts of IL-1α, IL-1β and IL-6, but not TNF-α relative to normal control epidermal cells. Membrane-associated IL-1 activity was low and not unique for psoriasis, but cytosolic IL-1β levels were increased in lesional psoriatic epidermal cells. Depletion experiments for intra-epidermal leukocytes pointed towards the resident keratinocyte as the cell-type responsible for the observed IL-1 production *ex vivo*. The production of functionally active IL-1β by lesional psoriatic epidermal cells, but not normal control epidermal cells, is a novel finding which suggests that IL-1β contributes to the inflammatory response in psoriasis.

Further studies, presented in **chapter 3.3**, revealed that the release of IL-1 was significantly correlated with cell death. The increased IL-1β production by lesional psoriatic epidermal cells was caused by increased *de novo* synthesis of IL-1β and was associated with apoptosis. DNA fragmentation, as measured by ELISA and the TUNEL assay, paralleled IL-1β production in epidermal cells *ex vivo*. In addition, a few psoriatic patients demonstrated IL-1β strongly positive suprabasal areas *in situ*, which were lined by cells positive for both DNA breaks and IL-1β. Necrosis, probably secondary to apoptosis, facilitated the release of cytosolic (pro-)IL-1α by lesional psoriatic epidermal cells *ex vivo*. At the moment it is speculated that keratinocyte apoptosis, which we found to be increased in psoriasis, contributes to the generation of epidermal IL-1β.

In **chapter 3.4** the epidermal expression of the IL-4R in psoriasis was analyzed, as its ligand may affect the epidermal IL-1 system. The IL-4R protein and mRNA both localized to the basal and some suprabasal keratinocytes, and their expression was highest in lesional psoriatic epidermis, intermediate in non-lesional psoriatic and lowest in normal control epidermis. Moreover, the epidermal IL-4R was functional in that IL-4 exerted anti-inflammatory effects on epidermal cells via downregulating the production of IL-6, and to a lesser extent IL-1β. An effective repression of the epidermal IL-1 system by IL-4 is furthermore substantiated by the significant correlation between the expression of IL-4R and those of the IL-1 antagonists.

Since dermal cell - epidermal cell interactions control epidermal homeosta-

sis, a second line of investigation, described in **chapter 4**, dealt with the dermal compartment. Fibroblasts have previously been postulated to play a role in the pathology of psoriasis. In **chapter 4.1** the cytokine expression by lesional psoriatic dermal fibroblasts was analyzed *in vitro*. It was observed that fibroblasts did not produce IL-1 or TNF-α, but were potent producers of IL-6 and IL-8. Lesional psoriatic fibroblasts demonstrated an increased production of IL-6 and IL-8 which was less dependent on growth- and serum factors. Moreover, the serum-induced kinetics of IL-6 production was rapid, high and persistent, and unique for psoriasis. Unexpectedly, Northern blot analyses showed that there was a clear expression of IL-6 mRNA in both types of fibroblasts under serum-free conditions, and that calcium and serum inhibited the expression of IL-6 mRNA in all lesional psoriatic fibroblast cultures investigated, in contrast to normal control fibroblast cultures. Taken together, lesional psoriatic fibroblasts demonstrated an altered IL-6 synthesis *in vitro* and this may represent an important source of the locally increased IL-6 levels in psoriatic lesions.

The aberrant regulation of IL-6 synthesis in lesional psoriatic fibroblasts may be the result of an altered expression of cytokine receptors, leading to auto/paracrine induction of IL-6. The cytokine receptor expression by lesional psoriatic fibroblasts was studied in **chapter 4.2**. Flow cytometry showed that fibroblasts only negligibly bound IL-1α and IL-1β, but clearly bound IL-4, IL-6 and TNF-α. Serum upregulated the number of normal control fibroblasts which bound TNF-α, and to a lesser extent IL-6. In contrast, this upregulation was significantly lower in lesional psoriatic fibroblasts. In agreement with the binding experiments, the immunocytochemical expression of TNFRI was clearly decreased in lesional psoriatic fibroblasts. Shedding did not explain the decreased TNFR expression. Thus, lesional psoriatic fibroblasts coordinatedly expressed decreased TNFR levels and an increased IL-6 production, which probably reflects the hyperactive state of these cells *in vivo*.

Studies on the epidermal and dermal compartment are discussed in **chapter 5**. Our current view on IL-1 in the immunopathogenesis of psoriasis is that the secretion of IL-1 by psoriatic keratinocytes, as observed *ex vivo*, is more easily triggered by injury and/or products from activated skin T cells relative to normal keratinocytes. This is consistent with findings relating to an altered cytokine response and increased IL-1 production by non-lesional psoriatic keratinocytes. A micro-environment dominated by IL-1 agonists and related cytokines may initiate the psoriatic phenotype. The inflammatory response, epidermal hyperplasia and apoptosis, characteristic of psoriasis, are probably responsible for sensitization to (auto)antigen(s). Immunogenetically determined high-risk HLA binding of antigens may subsequently become productive for T cell activation. It is believed that psoriatic lesions are maintained by a self-sustaining cycle of predominantly T_H1-type cytokines that stimulate epidermal cells, and further epidermal IL-1 potentiation of T cells. Resident skin cells such as fibroblasts are in our view also actively

involved in the persistence of psoriatic lesions. Finally, downregulatory mechanisms of cutaneous inflammation may be deficient in psoriasis. The predominant expression of IL-1 antagonists by lesional psoriatic epidermis, which points towards a decreased IL-1 responsiveness, possibly reflects a negative-feedback response to IL-1 agonists, perhaps in part mediated by IL-4. Such an impaired IL-1 response may, at least in part, be related to a deficient downregulation of the lesional response. Definite proof of the pathological relevance of the IL-1 system to psoriasis will most likely be provided by recently developed animal models.

SAMENVATTING

Psoriasis is een veel voorkomende huidziekte, welke al bijna twee eeuwen goed gekarakteriseerd is, en gekenmerkt wordt door versnelde terminale keratinocyt differentiatie en leukocyt infiltratie. De exacte ontstaanswijze is echter nog steeds onbekend. Dit, en het feit dat de meerderheid der psoriatische patiënten ontevreden is met de op dit moment voorhanden zijnde anti-psoriatische therapieën, vormen de belangrijkste drijfveren voor basaal psoriasis onderzoek.

Een ontregelde communicatie tussen residente huidcellen en infiltrerende leukocyten leidt mogelijk tot de initiatie en/of het onderhoud van psoriasis. Het cytokine IL-1 wordt beschouwd als een integrale component van de cellulaire immuunbasis van psoriasis. Dit wordt onderbouwd door de volgende argumenten: i) IL-1 heeft het vermogen om de keratinocytproliferatie en -differentiatie, evenals het cutane ontstekingsproces te reguleren; ii) transgene overexpressie van IL-1 in basale keratinocyten induceert een psoriasis-gelijkend fenotype; en iii) de expressie van IL-1 is veranderd in psoriatische huid.

Humane epidermis bevat levensbedreigende hoeveelheden IL-1. De IL-1 activiteit is om die reden sterk gereguleerd, en wordt feitelijk bepaald door de wisselwerking tussen IL-1 agonisten en antagonisten, welke tezamen het IL-1 systeem vormen. **Hoofdstuk 1** beschrijft de fysiologie van het epidermale IL-1 systeem en de cutane ontstekingsreaktie, en biedt een gedetailleerd overzicht van de cellulaire immunopathologie van psoriasis.

De belangrijkste doelstelling van deze dissertatie was het bestuderen van de expressie van cytokinen en hun receptoren in psoriatische huid, met speciale aandacht voor het epidermale IL-1 systeem. Aangezien in eerste instantie de beschikbare onderzoeksgegevens tegenstrijdig en vaak incompleet waren, is het werk van dit proefschrift gebaseerd op een geïntegreerde studie-aanpak gebruikmakend van verschillende modellen en testsystemen. De hypothese en studie-opzet zijn beschreven in **hoofdstuk 2.**

Het epidermale compartiment wordt behandeld in **hoofdstuk 3.** Een uitvoerige analyse van de expressie van alle componenten van het humane IL-1 systeem, welke momenteel gekarakteriseerd zijn, is beschreven in **hoofdstuk 3.1** en resulteerde in een gedefinieerd fenotype van IL-1 in psoriatische huid. Semikwantitatieve immuunkleuring liet zien dat IL-1α significant verlaagd tot expressie komt in basale keratinocyten van lesionaal psoriatische epidermis *in vivo* in vergelijking met niet-lesionaal psoriatische en normale controle epidermis. De verlaagde IL-1α expressie *in situ* is mogelijk het gevolg van de verhoogde uitscheiding van IL-1α door lesionaal psoriatische epidermale cellen zoals waargenomen *ex vivo* (zie beneden). IL-1ra en IL-1RII waren significant verhoogd in respectievelijk suprabasale en basale cellen van lesionaal psoriatische epidermis, en beide bleken negatief gecorreleerd te zijn met IL-1α. Deze inverse relatie is waarschijnlijk impliciet aan de versnelde terminale keratinocyt differentiatie in psoriatische lesies.

Verder bleek dat IL-1ra en IL-1RII onderling positief gecorreleerd waren, welke een effectieve inhibitie suggereert van het vermogen van lesionaal psoriatische epidermis om op IL-1 te reageren. In situ hybridizatie resultaten waren in overeenstemming met de immuunkleuringen. Echter, IL-1ra mRNA was niet verhoogd in lesionaal psoriatische epidermis, indicatief voor een verhoogde post-transcriptionele IL-1ra synthese in lesionaal psoriatische epidermis. Het gebruik van de gevoeliger RT-PCR liet zien dat de IL-1β mRNA expressie in vers geïsoleerde lesionaal psoriatische epidermale cellen sterk verhoogd was, en gelijk op liep met de mRNA expressie van IL-1ra en IL-1RII. IL-1RI mRNA was duidelijk aanwezig in alle epidermale celmonsters. Dus de expressies van IL-1 en IL-1 receptor isovormen zijn op gecoördineerde wijze veranderd in lesionaal psoriatische epidermis, resulterend in een milieu welk lokaal gedomineerd wordt door IL-1 antagonisten.

Vers geïsoleerde epidermale cellen en niet-gestimuleerde, kortdurende primaire kweken van deze cellen werden in **hoofdstuk 3.2** geïntroduceerd als een *ex vivo* model om zo de feitelijke produktie van IL-1 en gerelateerde cytokinen door epidermale cellen te karakteriseren. ELISA en bioassay analyses lieten zien dat lesionaal psoriatische epidermale cellen significant verhoogde hoeveelheden IL-1α, IL-1β, IL-6 en TNF-α uitscheiden in vergelijking met normale controle epidermale cellen. De membraan-geassocieerde IL-1 activiteit was laag en niet uniek voor psoriasis, en de cytosolische IL-1β gehalten waren verhoogd in lesionaal psoriatische epidermale cellen. Depletie experimenten voor intra-epidermale leukocyten gaven aan dat de residente keratinocyt waarschijnlijk het verantwoordelijke cel-type is voor de geobserveerde IL-1 produktie *ex vivo*. De produktie van functioneel actief IL-1β door lesionaal psoriatische epidermale cellen, maar niet door normale controle epidermale cellen, was een nieuwe bevinding welke een rol voor IL-1β suggereert in de ontstekingsreaktie in psoriasis.

Verdere studies, weergegeven in **hoofdstuk 3.3**, lieten een significante correlatie zien tussen de IL-1 produktie en celdood. De verhoogde IL-1β produktie door lesionaal psoriatische epidermale cellen werd veroorzaakt door een verhoogde *nieuwvorming* van IL-1β en was inherent aan apoptose. DNA fragmentatie, gemeten met ELISA en de TUNEL test, liep parallel met de IL-1β produktie in epidermale cellen *ex vivo*. Daarnaast werd in enkele psoriatische patiënten sterke IL-1β immunoreaktiviteit gevonden in suprabasale gebieden *in situ*, welke begrensd werden door cellen die dubbelpositief waren voor DNA fragmenten en IL-1β. Necrose, waarschijnlijk secundair aan apoptose, vergemakkelijkte de uitscheiding van cytosolisch (pro-)IL-1α door lesionaal psoriatische epidermale cellen *ex vivo*. Wij veronderstellen dat keratinocyt apoptose, welke in verhoogde mate werd aangetoond in psoriasis, bijdraagt aan de vorming van epidermaal IL-1β.

In **hoofdstuk 3.4** werd de epidermale expressie van de IL-4R in psoriasis geanalyseerd, aangezien de ligand mogelijk het epidermale IL-1 systeem beïnvloedt. IL-4R eiwit en mRNA waren beide gelokaliseerd in basale en enkele suprabasale keratinocyten, en hun expressie was het hoogst in

lesionaal psoriatische epidermis, intermediair in niet-lesionaal psoriatische epidermis en het laagst in normale controle epidermis. Bovendien bleek de epidermale IL-4R functioneel, aangezien IL-4 anti-ontstekings effecten uitoefende op epidermale cellen via remming van de produktie van IL-6, en in mindere mate IL-1β. Een effectieve repressie van het epidermale IL-1 systeem door IL-4 wordt verder onderbouwd door de significante correlatie gevonden tussen de expressie van IL-4R en die van de IL-1 antagonisten.

Aangezien epidermale homeostase gereguleerd wordt door dermale cel - epidermale cel interacties, richtte een tweede onderzoekslijn, zoals beschreven in **hoofdstuk 4**, zich op het dermale compartiment. Fibroblasten werden al eerder gepostuleerd in de pathologie van psoriasis. In **hoofdstuk 4.1** is de cytokine expressie van lesionaal psoriatische dermale fibroblasten *in vitro* geanalyseerd. Fibroblasten produceerden géén IL-1 of TNF-α, maar waren wel krachtige producenten van IL-6 en IL-8. Lesionaal psoriatische fibroblasten toonden een verhoogde produktie van IL-6 en IL-8, welke minder afhankelijk was van groei- en serumfactoren. Verder was de serum-geïnduceerde kinetiek van de IL-6 produktie snel, hoog en persistent, en uniek voor psoriasis. Northern analyses toonden echter aan dat beide typen fibroblasten een duidelijke IL-6 mRNA expressie hadden onder serum-vrije kweekcondities, en dat calcium en serum de IL-6 mRNA expressie remden in alle bestudeerde lesionaal psoriatische fibroblastkweken, in tegenstelling tot normale controle fibroblastkweken. In z'n totaliteit toonden lesionaal psoriatische fibroblasten een verstoorde IL-6 synthese *in vitro* en vormen zij waarschijnlijk een belangrijke bron voor het lokaal verhoogde IL-6 in psoriatische lesies.

De afwijkende regulatie van de IL-6 synthese in lesionaal psoriatische fibroblasten is mogelijk het gevolg van een veranderde expressie van cytokinereceptoren, resulterend in auto/parakriene inductie van IL-6. De cytokinereceptor expressie van lesionaal psoriatische fibroblasten wordt beschreven in **hoofdstuk 4.2**. Flow cytometrie liet zien dat fibroblasten nauwelijks IL-1α en IL-1β bonden, maar duidelijk IL-4, IL-6 en TNF-α konden binden. Serum verhoogde het aantal normale controle fibroblasten welke TNF-α bonden, en in mindere mate IL-6. Deze opregulatie was echter significant minder in lesionaal psoriatische fibroblasten. In overeenstemming met de bindingsexperimenten was de duidelijke afname in de immunocytochemische expressie van TNFRI in lesionaal psoriatische fibroblasten. Shedding kon de verlaagde TNFR expressie niet verklaren. Dus lesionaal psoriatische fibroblasten toonden zowel een verlaagde TNFR expressie als een verhoogde IL-6 produktie, welke waarschijnlijk de hyperactieve status van deze cellen *in vivo* weerspiegelen.

Studies van het epidermale en dermale compartiment worden bediscussieerd in **hoofdstuk 5**. In onze huidige visie over IL-1 in de immunopathogenese van psoriasis wordt er van uitgegaan dat de secretie van IL-1 door psoriatische keratinocyten, zoals gevonden *ex vivo*, gemakkelijker wordt teweeggebracht door verwonding en/of produkten van geactiveerde T cellen in de huid in vergelijking met normale keratinocyten. Dit is in overeenstem-

ming met bevindingen over een veranderde reaktie op cytokinen en een verhoogde IL-1 produktie van niet-lesionaal psoriatische keratinocyten. Een micro-milieu gedomineerd door IL-1 agonisten en gerelateerde cytokinen initieert mogelijk het psoriatische fenotype. De ontstekingsreaktie, de epidermale hyperplasie en de apoptose, karakteristiek voor psoriasis, zijn waarschijnlijk verantwoordelijk voor sensitizatie tegen (auto)antige(e)n(en). Immunogenetisch bepaalde risicovolle HLA binding van antigenen kan vervolgens tot een produktieve T cel aktivatie leiden. Gedacht wordt dat psoriatische lesies onderhouden worden door een zichzelf stimulerende cyclus van hoofdzakelijk T_H1-type cytokinen welke epidermale cellen stimuleren, en verdere T cel potentiëring door epidermaal IL-1. Residente huidcellen zoals fibroblasten zijn volgens onze kijk ook actief betrokken in de persistentie van psoriatische lesies. Tenslotte kunnen ook mechanismen welke de cutane ontsteking dempen deficiënt zijn in psoriasis. De overheersende expressie van IL-1 antagonisten door lesionaal psoriatische epidermis, welke een verminderde IL-1 response suggereert, is mogelijk het resultaat van een negatieve terugkoppelingsreaktie tegen IL-1 agonisten, wellicht deels gemedieerd door IL-4. Een dergelijke verminderde IL-1 response is, op z'n minst deels, gerelateerd aan een deficiënte terugkoppeling van de lesionale response. Het definitieve bewijs voor de pathologische rol van het IL-1 systeem in psoriasis wordt waarschijnlijk in de nabije toekomst geleverd door recent ontwikkelde proefdiermodellen.

ABBREVIATIONS

AEC : 3-amino-9-ethylcarbazole
APC : antigen presenting cell
BCL : B cell lymphoma
bp : base pairs
CANP : calcium-activated neutral protease calpain
CD : cluster of differentiation
c-fos SRE : *c-fos* serum-responsive element homology
CHX : cycloheximide
CLA : cutaneous lymphocyte-associated antigen
CNTF : ciliary neurotrophic factor
CSF : colony stimulating factor
CT : cardiotrophin
dNTP : deoxynucleotide triphosphate
EC : epidermal cells
EGF : epidermal growth factor
ELISA : enzyme-linked immunosorbent assay
FCS : fetal calf serum
FGF : fibroblast growth factor
GAPDH : glyceraldehyde-3-phosphate dehydrogenase
G-CSF : granulocyte-colony stimulating factor
GM-CSF : granulocyte macrophage-colony stimulating factor
HLA : human leukocyte antigen
HS : human serum
ICAM : intercellular adhesion molecule
ICE : interleukin-1β converting enzyme
icIL-1ra : intracellular interleukin-1 receptor antagonist
IE gene : immediate early response gene
IFN : interferon
IGF : insulin-like growth factor
IL : interleukin
IL-1ra : interleukin-1 receptor antagonist
ISH : in situ hybridization
JAK kinase : Janus kinase
KBM : keratinocyte basal medium
KGF : keratinocyte growth factor
KGM : keratinocyte growth medium
LDH : lactate dehydrogenase
LFA : leukocyte function-associated antigen
LIF : leukemia inhibitory factor
LPS : lipopolysaccharide
Magn : magnification
MAP kinase : mitogen activated protein kinase
MCP : monocyte chemotactic protein
M-CSF : macrophage-colony stimulating factor
MHC : major histocompatibility complex
M_r : relative molecular mass
MTT : 3,(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide
NF : nuclear factor
NGF : nerve growth factor
NN : normal control
OD : optical density
OM : oncostatin M
PASI : psoriasis area and severity index
PBMC : peripheral blood mononuclear cells
PCR : polymerase chain reaction
PDGF : platelet-derived growth factor
PHA : phytohemagglutinin
PMA : phorbol myristate acetate
PN : non-lesional psoriasis
PP : lesional psoriasis
R : receptor
RI : receptor type I
RII : receptor type II
r_s : Spearman's rank correlation coefficient
RT : room temperature
RT-PCR : reverse transcriptase - polymerase chain reaction
SCF : stem cell factor
SDS : sodium dodecyl sulphate
sIL-1ra : secreted interleukin-1 receptor antagonist
sR : soluble receptor
SSC : standard sodium citrate
STAT : signal transducing and activator of transcription factors
TCR : T cell receptor
TGF : transforming growth factor
T_H1 : T helper cell type 1
T_H2 : T helper cell type 2
TNF : tumor necrosis factor
TUNEL : terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling
VCAM : vascular cell adhesion molecule
VEGF : vascular endothelial growth factor
V gene : variable gene region
VLA : very late activation antigen

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That's all folks.

CURRICULUM VITAE

Reno Debets

27 juli 1968	:	Geboren te Maastricht
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1990 - 1991	:	Afstudeerstage 'Ontstekingsmediatoren in psoriasis' Afdeling Immunologie Erasmus Universiteit Rotterdam, Rotterdam Supervisie: Dr. E.P. Prens en Prof. Dr. R. Benner (Rotterdam) en Dr. W.A. Buurman (Maastricht)
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Cursussen and diploma's

- Biostatistiek and medische epidemiologie, Maastricht
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- Wetenschapsfilosofie, Maastricht en Rotterdam
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- In situ hybridizatie, Leiden

Onderwijs activiteiten

- Assistent practicum Histologie voor eerste jaars geneeskunde studenten, Rotterdam
- Assistent practicum Immunologie voor tweede jaars geneeskunde studenten, Rotterdam

PUBLICATIONS

Manuscripts

1. Debets R, Van Joost T, Benner R, Prens EP (1993) Psoriatic epidermal cells release elevated levels of immunoreactive and biologically active interleukins 1 and 6: modulation of corticosteroid treatment. In Bernard BA and Schroot B (eds). Pharmacol Skin: From molecular biology to therapeutics. Kärger, Basel, 5:158-166.

2. Debets R, Savelkoul HFJ (1994) Cytokine antagonists and their potential therapeutic use. Immunol Today 15:455-458.

3. Verburg-van Kemenade BM, Weyts FAA, Debets R, Flik G (1995) Carp macrophages and neutrophilic granulocytes secrete an interleukin-1-like factor. Dev Com Immunol 19:59-70.

4. Prens EP, Debets R, Hegmans JPJJ (1995) T cells in psoriasis. In Van de Kerkhof PCM and Bos JD (Eds). Psoriasis. Clin Dermatol 13:1-15.

5. Debets R, Hegmans JPJJ, Troost RJJ, Benner R, Prens EP (1995) Enhanced production of biologically active interleukin-1α and interleukin-1β by psoriatic epidermal cells *ex vivo*: evidence of increased cytosolic interleukin-1β levels and facilitated interleukin-1 release. Eur J Immunol 25:1624-1630.

6. Debets R, Hegmans JPJJ, Deleuran M, 't Hooft S, Benner R, Prens EP (1996) Expression of cytokines and their receptors by psoriatic fibroblasts. I. Altered IL-6 synthesis. Cytokine 8:70-79.

7. Debets R, Hegmans JPJJ, Buurman WA, Benner R, Prens EP (1996) Expression of cytokines and their receptors by psoriatic fibroblasts. II. Decreased TNF receptor expression. Cytokine 8:80-88.

8. Prens EP, Hegmans JPJJ, Chin A Lien RAM, Troost RJJ, Debets R, Van Joost T, Benner R (1996) Increased expression of interleukin 4 receptors on epidermal cells in psoriasis. Am J Pathol, in press.

9. Van Haarst JMW, Verhoeven GT, De Wit HJ, Hoogsteden HC, Debets R, Drexhage HA. CD1a+ and CD1a- accessory cells from human bronchoalveolar lavage differ in cytokine production and in allostimulatory potential. Manuscript, submitted.

10. Debets R, Hegmans JPJJ, Croughs P, Troost RJJ, Prins J-B, Benner R, Prens EP. The IL-1 system in psoriatic skin. IL-1 antagonist sphere of influence in lesional psoriatic epidermis. Manuscript, submitted.

11. Debets R, Hegmans JPJJ, Benner R, Prens EP. Enhanced apoptosis in psoriatic epidermal cells. IL-1β maturation is related to apoptosis *ex vivo*. Manuscript, submitted.

12. Debets R, Hegmans JPJJ, Croughs P, Mandrusov J, Troost RJJ, Benner R, Prens EP. Psoriatic epidermal cells express elevated levels of functional IL-4 receptors. IL-4 downregulates the IL-1 and IL-6 production *ex vivo*. Manuscript, submitted.

13. Allaerts W, Jeucken PHM, Debets R, Hoefakker S, Claassen E, Drexhage HA. Heterogeneity of pituitary folliculo-stellate cells: implications for interleukin-6 production and accessory function in vitro. Manuscript, submitted.

14. Hoijer MA, Melief M-J, Debets R, Hazenberg MP. Biological activity of peptidoglycan *in vivo* and *in vitro* is decreased after degradation by N-acetyl-muramyl-L-alanine amidase. Manuscript, submitted.

Abstracts

1. Prens EP, Debets R, 't Hooft S, Van Joost T, Benner R (1991) IL-6 production by psoriatic fibroblasts is independent of serum and growth factor(s). J Invest Dermatol 96:1008A.

2. Debets R, Prens EP, 't Hooft S, Hegmans JPJJ, Troost RJJ, Van Joost T, Benner R (1992) Altered regulation of IL-6 synthesis in psoriatic fibroblasts. J Invest Dermatol 98:504.

3. Debets R, Hegmans JPJJ, Prens EP, Troost RJJ, Benner R (1993) Altered IL-1 and IL-6 release by psoriatic epidermal cells and its restoration by topical steroids. J Invest Dermatol 100:213.

4. Debets R, Hegmans JPJJ, Prens EP, Van Joost T, Benner R (1993) Expression of cytokine receptors on psoriatic fibroblasts. J Invest Dermatol 100:472.

5. Debets R, Hegmans JPJJ, Van Joost T, Benner R, Prens EP (1994) Decreased serum-dependence of IL-6 production by psoriatic fibroblasts is associated with decreased TNF receptor expression. J Invest Dermatol 103:427.

6. Debets R, Hegmans JPJJ, Croughs P, Troost RJJ, Benner R, Prens EP (1995) The IL-1 network in lesional psoriatic skin. J Invest Dermatol 105:480.

7. Debets R, Hegmans JPJJ, Croughs P, Mandrusov E, Troost RJJ, Benner R, Prens EP (1995) IL-4 down-regulates the expression of IL-6 in lesional psoriatic epidermal cells *ex vivo*. J Invest Dermatol 105:480.

Other

1. Debets R, Savelkoul HFJ (Eds) (1996) Human Cytokine Protocols. In Walker JM (Series Editor). Methods in Molecular Biology, Humana Press, New York.

2. Prens EP, Debets R (1996) Angiogenesis in psoriasis. J Am Acad Dermatol, in press. (Letter).

Annex

COLOR PLATES

Color plates

Figures 1 to 3 of chapter 3.1.
Figures 4 and 7 of chapter 3.3.
Figures 1 and 2 of chapter 3.4.

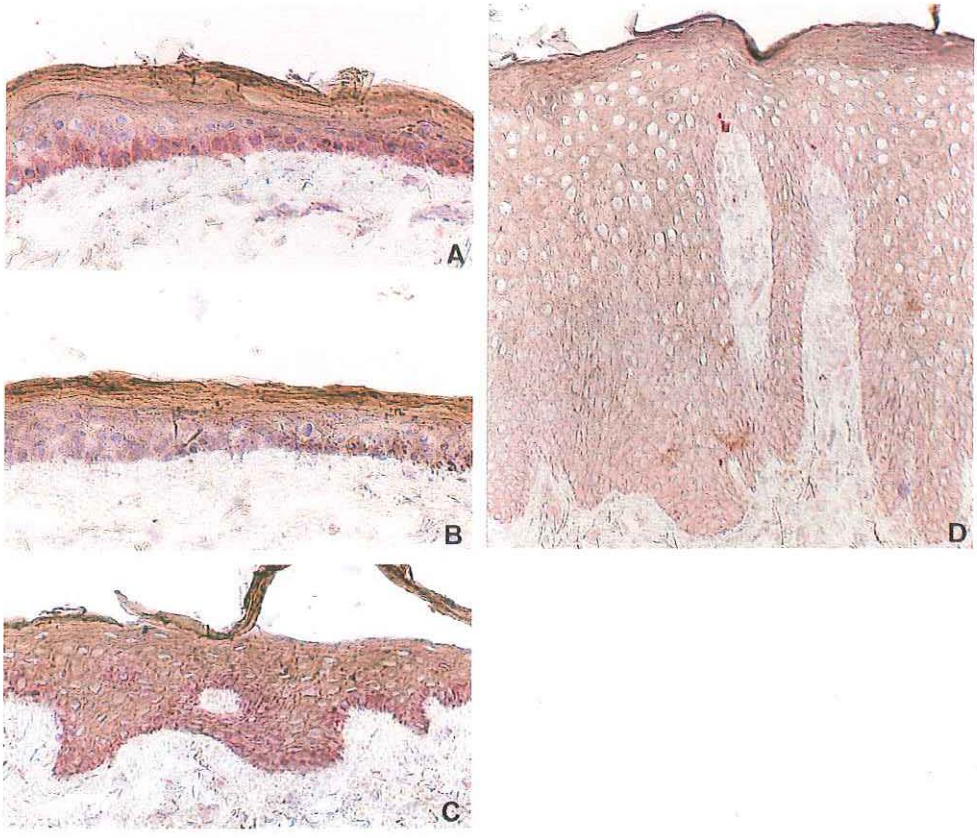

Figure 1 of chapter 3.1. **IL-1α immunoreactivity in psoriatic skin.** Immunostaining of PP (Fig D), PN (C) and NN skin sections (A and B) with 1C12.1 antibody (anti-IL-1α antibody) (A, C and D). Mouse IgG_1 control antibody showed no reactivity (B). Magn 250 X (A-C) and 160 X (D).

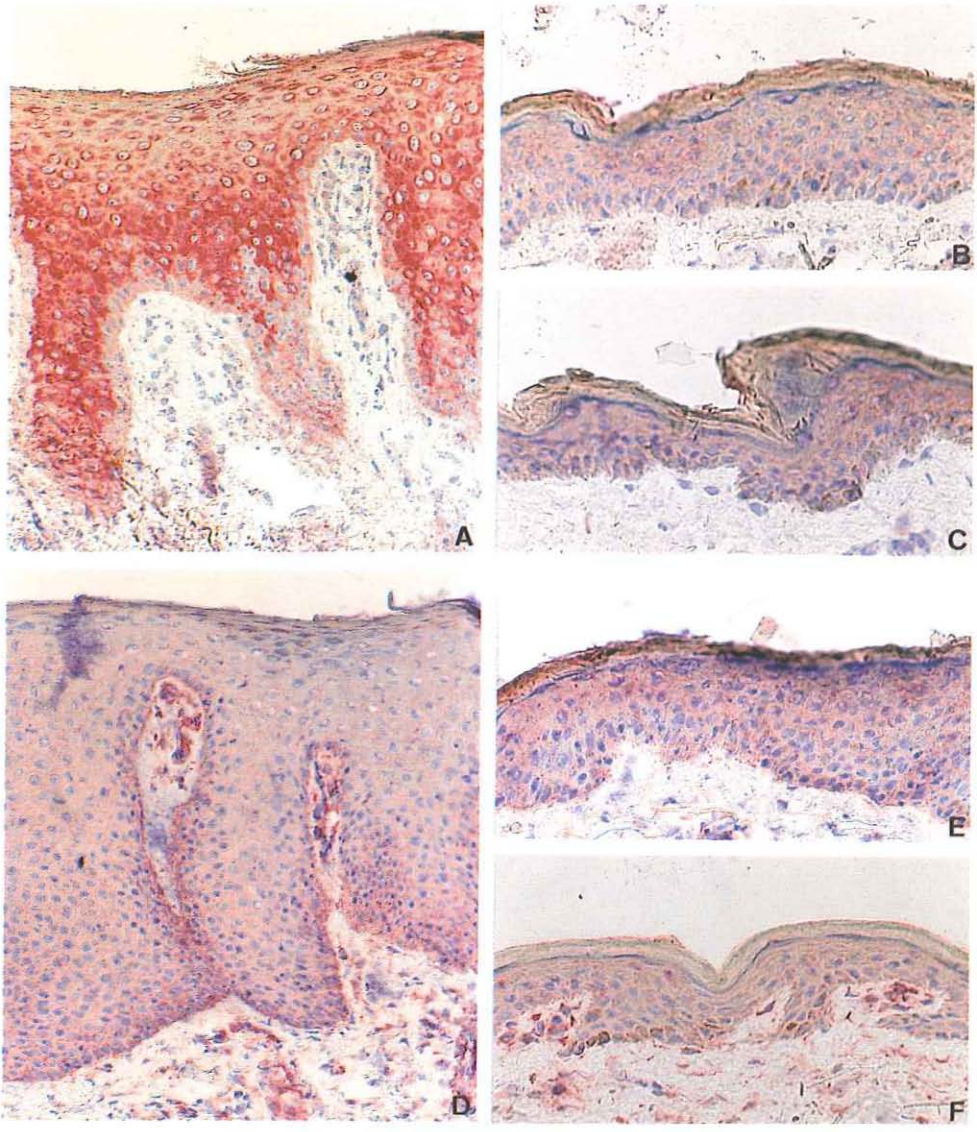

Figure 2 of chapter 3.1. IL-1ra and IL-1RII immunoreactivities in psoriatic skin. Immunostaining of PP (Figs A and D), PN (B and E) and NN skin sections (C and F) with M5 (anti-IL-1ra antibody: A-C) and M2 antibody (anti-IL-1RII antibody: D-F). The immunostainings of IL-1ra and IL-1RII correspond to the same psoriatic patient (Table IV (no. 3) of chapter 3.1). Note that the IL-1RII staining of the PN skin section (E) shows some background just below the stratum corneum, and that the dark brown color in the stratum basale of the NN skin section stained for IL-1RII (F) represents melanin. Magn 160 X (A and D) and 250 X (B, C, E and F).

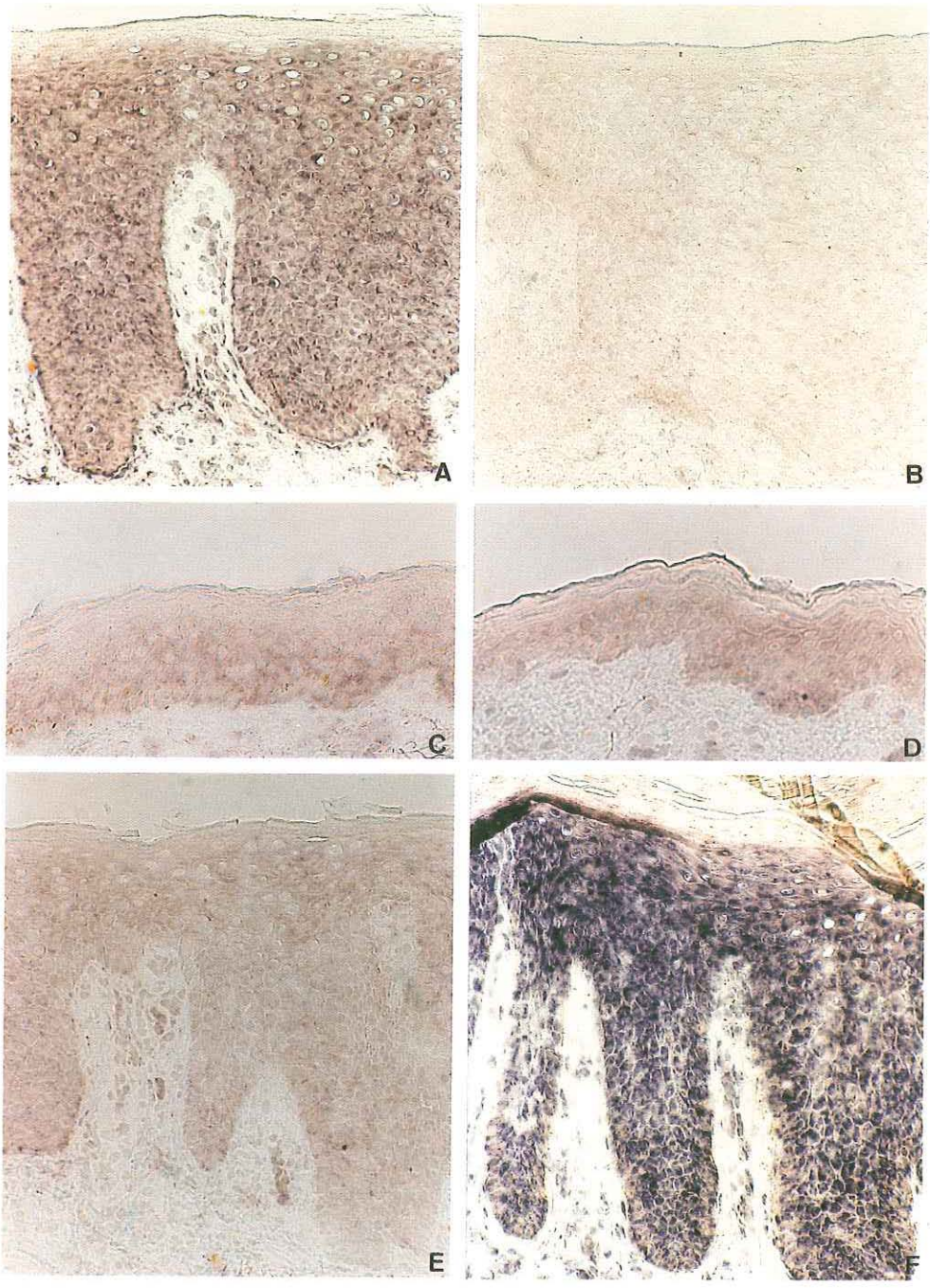

Figure 3 of chapter 3.1. **In situ expression of IL-1ra and IL-1RII mRNA in psoriatic skin.** ISH of IL-1RII mRNA (Figs A-D) and IL-1ra (E) in PP (A, B, E and F), PN (C) and NN skin (D) with digoxigenin-labeled antisense RNA probes (A, C-F). The IL-1RII sense probe gave no hybridization result (B), similar to the hybridization with other sense probes, RNAse treatment prior to hybridization or omission of the probe (not shown). Positive hybridization was observed with the antisense GAPDH probe (F). The presented immunostainings and ISH findings in Figures 2 and 3 of chapter 3.1 correspond to the same psoriatic patient. Magn 160 X (A, B, E and F) and 250 X (C and D).

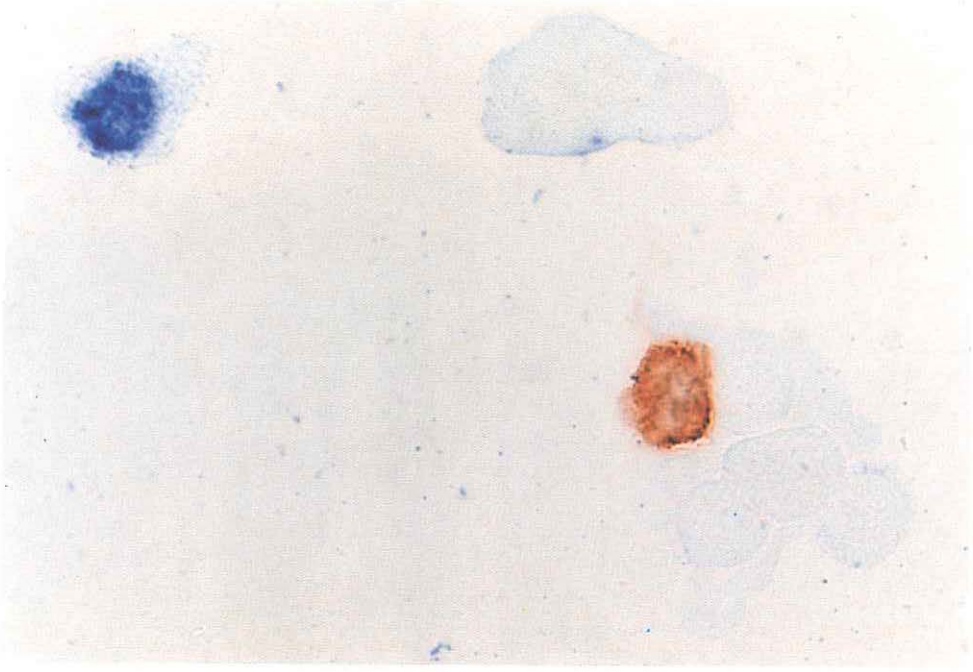

Figure 4 of chapter 3.3. DNA fragmentation by PP EC is not due to intra-epidermal leukocytes. Freshly isolated PP EC were double stained for CD45 (2D1 antibody, using peroxidase-AEC: red color) and DNA termini (TUNEL, using alkaline phosphatase-Fast Blue BB Base: blue color). Results of a representative patient is shown (n = 3). Magn 630 X.

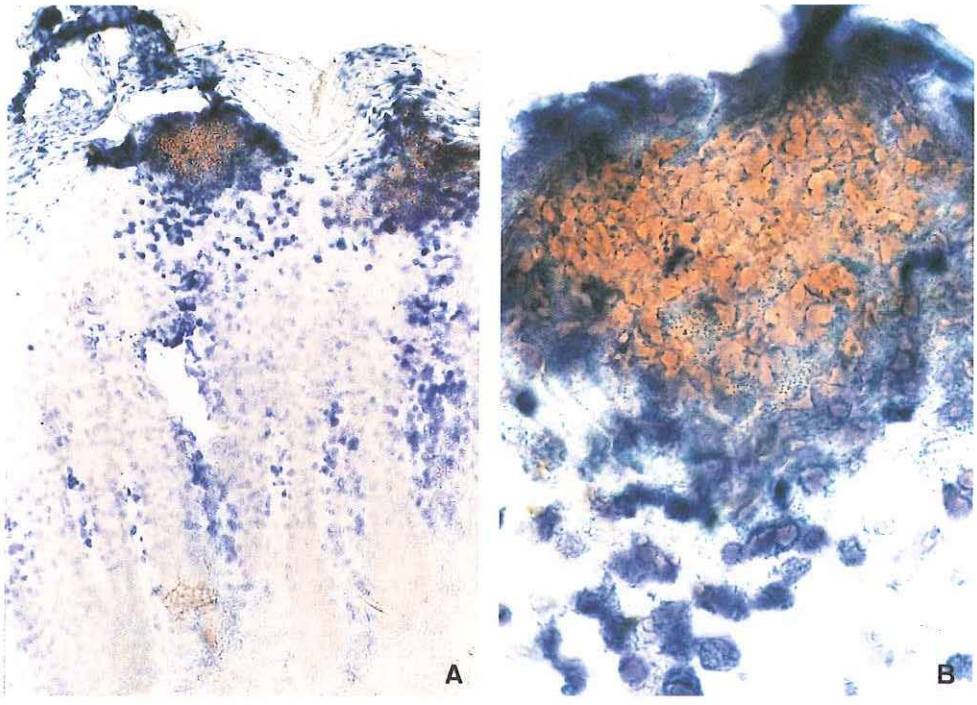

Figure 7 of chapter 3.3. Epidermal IL-1β immunoreactivity co-localizes with TUNEL staining. Sections of PP skin were double stained for IL-1β (Vhp20 antibody, using peroxidase-AEC: red color) and DNA termini (TUNEL, using alkaline phosphatase-Fast Blue BB Base: blue color). Note that only few psoriatic patients expressed a clear epidermal IL-1β immunoreactivity (2 out of 15), of which one is presented. See discussion of chapter 3.3 for details. Magn 110 X (Fig A) and 570 X (B).

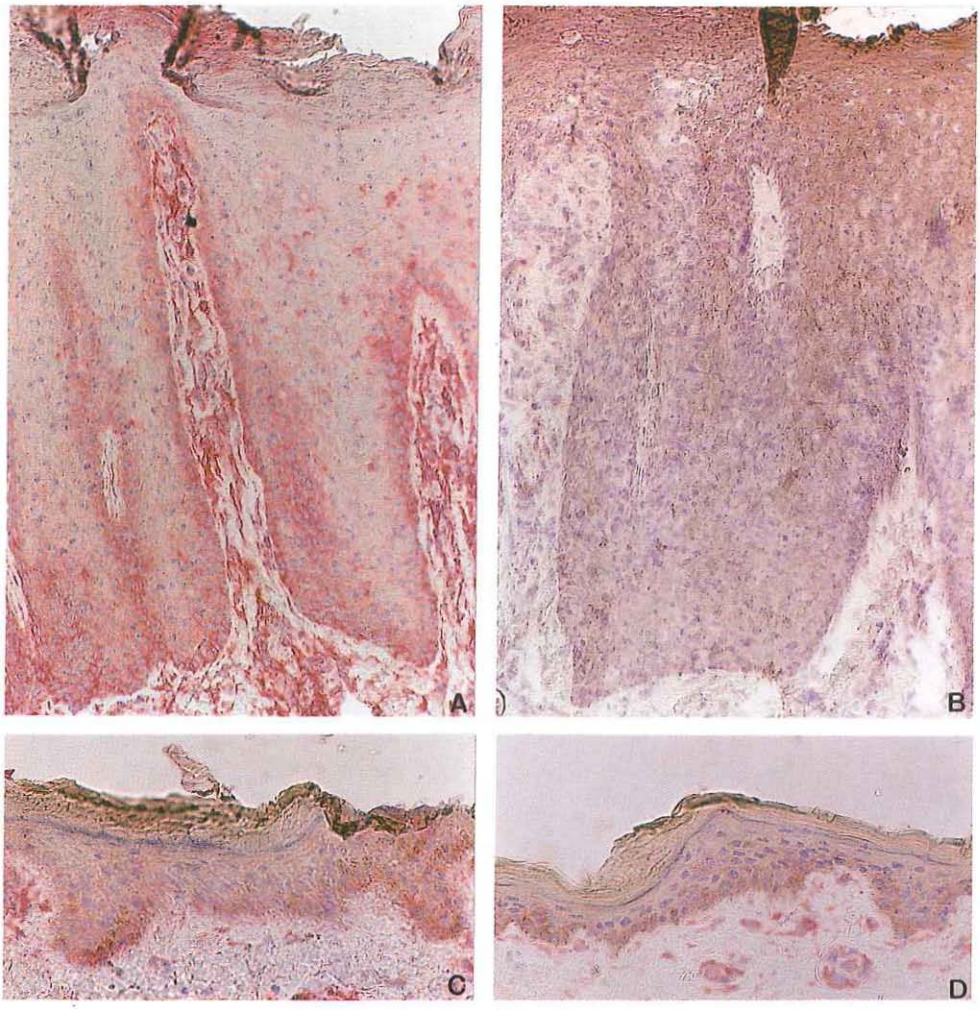

Figure 1 of chapter 3.4. **IL-4R immunoreactivity in psoriatic skin.** Immunohistochemical staining of PP (Figs A and B), PN (C) and NN skin (D) with MR6 antibody (anti-IL-4R antibody: A, C and D). Mouse IgG_1 control showed no reactivity (B). Magn 160 X (A and B) and 250 X (C and D).

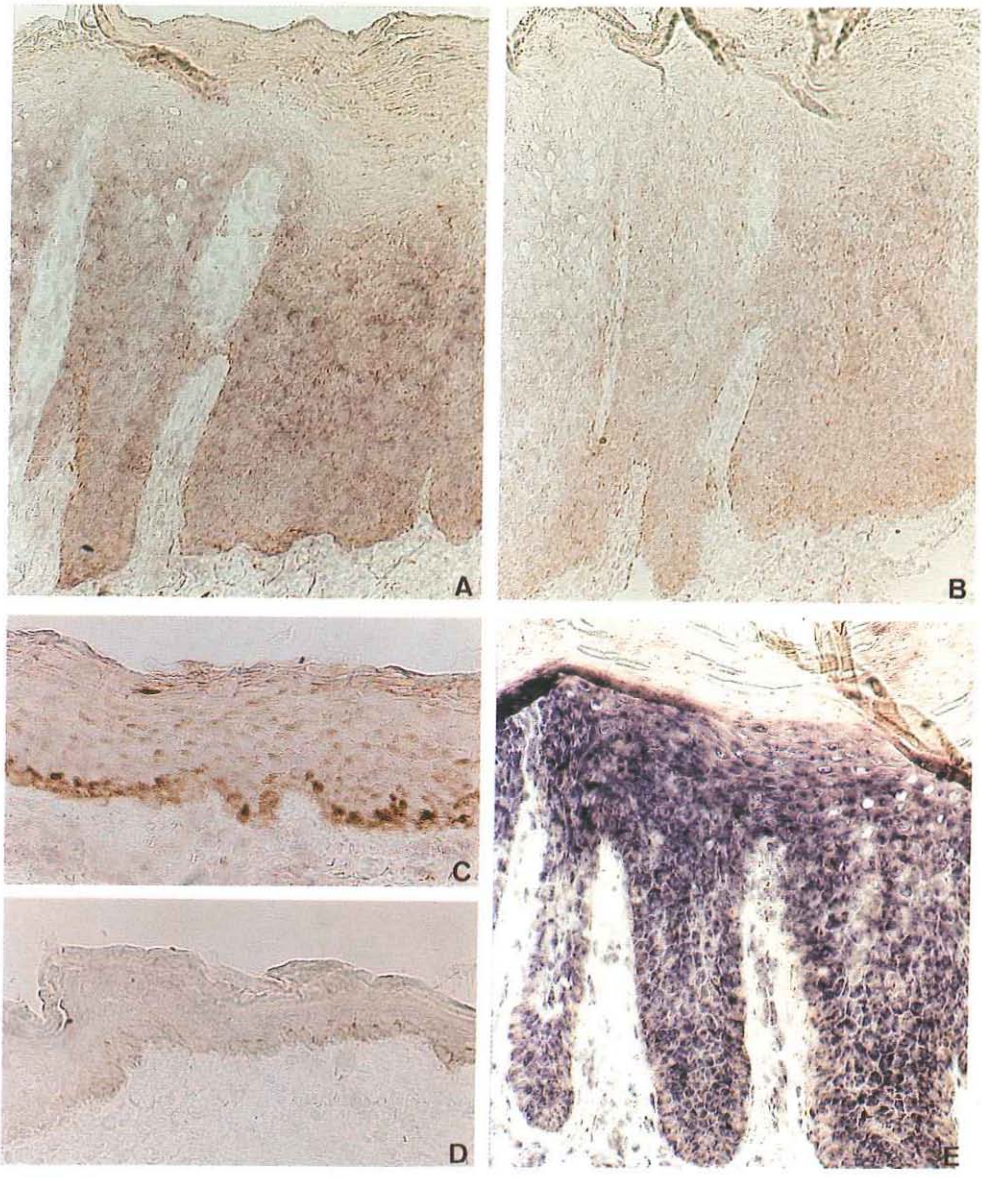

Figure 2 of chapter 3.4. **In situ hybridization of IL-4R mRNA in psoriatic skin.** ISH of IL-4R mRNA in PP (Figs A, B and E), PN (C) and NN skin (D) with the digoxigenin-labeled IL-4R antisense RNA probe (A, C and D). The IL-4R sense probe gave no hybridization signal (B), similar to RNAse treatment prior to hybridization or omitting the probe (not shown). A positive hybridization signal was observed with the GAPDH antisense probe (E). The presented ISH findings and the immunostainings as presented in Figure 1 of chapter 3.4 correspond to the same patient and control. Note that the dark-brown color present in the stratum basale, especially perceptible in the PN skin (C), is melanin, which should not be confused with the blue-purple staining of ISH. Magn 160 X (A, B and E) and 250 X (C and D).