IMMUNOPATHOPHYSIOLOGY OF PSORIASIS

Studies on accessory cells, cytokines and their receptors

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IMMUNOPATHOPHYSIOLOGY OF PSORIASIS

Studies on accessory cells, cytokines and their receptors

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Aan mijn ouders

Aan Trudy, Bas, Erik en Lisette

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Chapter 1

GENERAL ASPECTS OF PSORIASIS

1.1 Brief definition

Psoriasis is a world wide occurring common inflammatory skin disease characterized by epidermal hyperproliferation and clinically by chronic erythematous and scaling papules or plaques that preferentially occur on the elbows, knees, scalp and the flexural regions.

1.2 Historical background

Psoriasis is derived from the Greek word "psora" meaning itch [1]. The first written description of psoriasis-like skin diseases is attributed to Celsus [2]. Galen (133-200 A.D.) introduced the term psoriasis, although initially it also included several other diseases. Up until the eighteenth century, psoriasis was grouped with leprosy which resulted in the same adverse social consequences. To date social isolation remains the main hazard of this disease. In 1841 Hebra postulated psoriasis as a distinct clinical entity approximately 30 years after its first accurate clinical definition and its various manifestations described by Willan [3].

1.3 Epidemiology and genetics

The prevalence of psoriasis varies with race and geographical area. In the Western population this figure is approximately 2% whereas psoriasis is almost absent in certain West-African communities, among North and South American Indians and the Eskimos [4-6]. Psoriasis tends to affect males and females equally.

It has been known for long that psoriasis strongly prevails in certain families. Generally, the following incidences of psoriasis have been observed in siblings: when neither parent has psoriasis ± 7.5%, when one parent has psoriasis ± 15% and when both parents have psoriasis this figure was ± 50%. Autosomal dominant inheritance with incomplete penetration is occasionally observed, but generally inheritance of psoriasis appears to be polygenic [1,7]. HLA typing showed an increased frequency of the HLA class I antigens B13, B17, B27, B37, Cw2 and HLA-Cw6 (relative risk 9 to 22) and of the HLA class II antigens DR6 and DR7 in psoriatic patients as compared with non-psoriatic individuals [1,4,7,8]. Genetic factors appear to be linked with specific clinical manifestations of psoriasis [1,5]. Although not generally applied, some groups discern two types of psoriasis, type I and type II psoriasis. In analogy with autoimmune diabetes, type I psoriasis has an early onset, generally a more severe course, shows frequently positive family history and linkage disequilibrium for HLA antigens Cw6 and DR7. Type II psoriasis has a late onset, less severe course and is associated with HLA Cw2 and B27 [8].

However, the association with several genes and the concordance rate of 65%

in identical twins imply that environmental factors are also involved in psoriasis [7,9].

1.4 Clinical manifestations

The characteristic erythematous, scaling psoriatic lesions begin as small pinpoint papules which expand centrifugally and coalesce. Drop-like "guttate", coin-like "nummular", or even larger "plaque type" lesions may occur. The latter type is the most common form. Linear or zonal psoriasis are sporadically observed. Eventually the whole skin surface may be affected, yielding "erythrodermic psoriasis" which is characterized by prominent, generalized erythema. Finally, annular psoriasis, a ring-shaped psoriatic lesion, is the result of involutions initiating at the center of the lesions [4].

The "pustular types" are manifestations which are thought to be selective forms of psoriasis. They usually occur as localized or as generalized forms. The localized form is a persistent pustular eruption in the hands and the feet and is known as "Andrews-Barber" disease (pustulosis palmoplantaris) [10]. When the lesions are confined to the distal phalanges of the fingers or the toes and are accompanied by tissue necrosis, nail deformation or even bone changes the term "acrodermatitis continua of Hallopeau" is used. The generalized form of pustular psoriasis and its acute variant "Von Zumbusch" disease, in the past, could even be fatal [11,12].

Psoriasis is not always confined to the skin. Common extracutaneous manifestations are "nail psoriasis" and mutilating "psoriatic arthropathy". The involvement of mucous membranes, conjunctivae, alopecia and occasional internal disorders are less common in psoriasis. [13-16].

The clinical course of psoriasis is generally characterized by remissions and exacerbations. Stable lesions which persist for years at a specific site such as the elbow, knee or scalp also occur. The first clinical manifestations usually occur during the second decade but onset in early infancy and very old age have also been described. Observations suggest that the earlier the age of onset of psoriasis, the more severe the disease [3]. Taken together, it may be stated that "each patient has his/her own psoriasis" [17].

1.5 Histopathology

In the established lesion, the most common histological findings are elongated rete ridges, epidermal hyperplasia, absence of the granular layer, a thickened parakeratotic stratum corneum and a dense inflammatory infiltrate in the dermis. Infiltration of neutrophils into the epidermis generally leads to the development of Munro's micro abcesses. Excessive invasion of neutrophils from the tip of dermal papillae into the epidermis and subsequent subcorneal accumulation leads to typical

spongiform pustules of Kogoj observed in pustular psoriasis [5]. Both types of micro abcesses are highly diagnostic for psoriasis [18]. The upward expansion of the dermal papillae is clinically evident from the characteristic pinpoint bleedings when scales are removed (Auspitz sign).

The inflammatory infiltrate is largely confined to the dermis and consists of polymorphonuclear leukocytes, lymphocytes and monocytes/macrophages. The inflammation is most prominent in the active forms of psoriasis [4]. Infiltrating lymphocytes comprise mainly T cells; the ratio of $CD4^+$ and $CD8^+$ immunoregulatory cells is normal in quiescent lesions but may increase during exacerbations [19].

1.6 Pathophysiology

Clinically uninvolved skin is not normal

Uninvolved skin of psoriatic patients histologically forms an intermediate between normal healthy skin and lesional skin. Epidermal keratinocytes of uninvolved psoriatic skin show enhanced proliferation, capillaries are dilated and there is a dermal lymphocytic infiltrate with an equilibrium between different immunoregulatory T cells subsets. The activity of the enzyme phospholipase A_2 (PLA_2), and the arachidonic acid metabolite leukotriene B_4 (LTB_4) are increased in uninvolved psoriatic skin [20,21]. These findings indicate that the whole skin possesses the potential to develop psoriasis [1,20].

Trigger factors in psoriasis

The main trigger responsible for the events resulting in local epidermal hyperplasia and recruitment of leukocytes in psoriatic lesions remains as yet unidentified. Careful time-course studies showed that extravasation and the influx in the epidermis of activated $CD4^+$ T lymphocytes and monocytes were the earliest immunohistological alterations in newly developing psoriatic lesions. In addition, electron microscopically intercellular widening and loss of desmosomes is observed at this stage in the epidermis. Other early signs in the dermis are dilated tortuous capillaries and degranulation of mast cells in the upper dermis. Additional subpopulations of inflammatory cells are observed at later stages [19,21]. The observation that the epidermal and dermal changes are *preceded* by extravasation and subsequent infiltration of $CD4^+$ T lymphocytes into the epidermis suggests that, whatever the trigger factor, T lymphocytes and monocytes are involved in the induction of new lesions [22].

Two types of exogenous trigger factors are discerned, local and systemic ones [5]. The best known local trigger factor is the Koebner phenomenon or isomorphic response. The Koebner reaction occurs in about 25% of psoriatic patients and

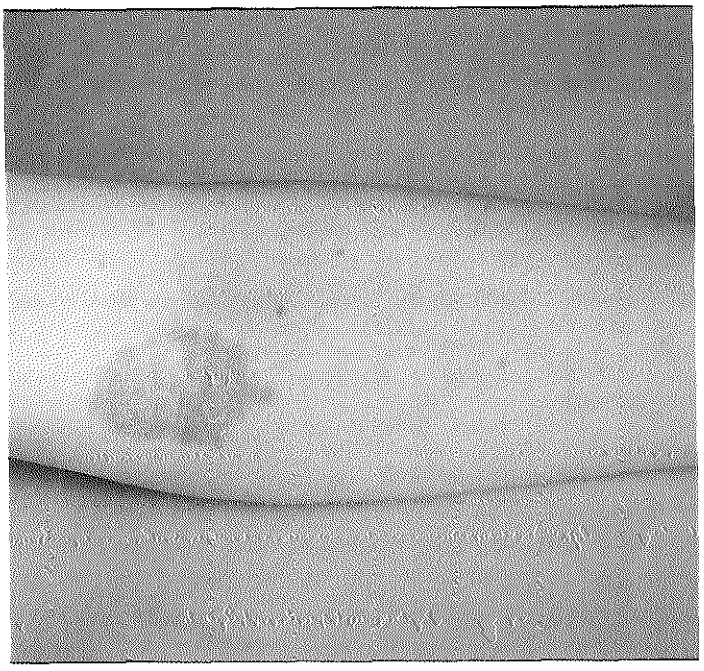

Figure 1. Koebner phenomenon induced in the cubital fossa by repeated venipunctures. An identical lesion was also induced at the contralateral side.

Table 1. Comparison of healthy control and lesional psoriatic epidermis

Skin type	Fgerm (%)	GF (%)	Tcy (h)	Tep (h)	PR (cells/h)
Lesional psoriatic	50	100	40	3.5	5
Healthy control	30	20-40	37	21	0.25
Reference	23	4	23	23	23

Fgerm: Fraction of germinative cells.
GF: Growth fraction or proportion of germinative cells that are actively cycling.
Tcy: Cell-cycle time in hours.
Tep: Turnover time of the epidermis (transit time in hours)
PR: Production-rate of epidermal cells (cells per hour).

comprises the development of typical psoriatic lesions approximately two weeks after any type of skin injury (Fig. 1). Systemic trigger factors include bacterial infections, especially hemolytic streptococci, hormones (e.g. (withdrawal of) systemic corticosteroids and somatotrophic hormone), emotional stress and drugs such as chloroquine (but apparently not hydroxychloroquine), lithium, β-adrenergic blocking agents and indomethacin [1,3]. The mode of action operative in case of a specific trigger factor is largely hypothetical. Promising and attractive molecular evidence on the mechanism of stress as a trigger factor of psoriasis are emerging.

Alterations in the number of cycling keratinocytes in psoriatic lesions

The etiology of psoriasis is still unclear. Crucial roles are played by keratinocytes and the inflammatory infiltrate, since epidermal hyperplasia and a prominent inflammatory infiltrate are characteristic features of the disease. Epidermal hyperplasia in psoriatic lesions is considered to be the result of an increase in the fraction of cycling keratinocytes leading to an increased rate of production of epidermal cells [4,23]. The latter has been shown not to be due to a decreased cell cycle time [24]. The differences in epidermal cell kinetics between healthy control and lesional psoriatic skin are summarized in Table I.

In culture, psoriatic keratinocytes do not exhibit an enhanced proliferative activity as compared with normal keratinocytes. Active lesional keratinocytes even appear to grow at a slower rate than uninvolved psoriatic and normal keratinocytes in culture [25,26].

Altered keratinocyte differentiation

The increased number of proliferating keratinocytes in psoriatic lesions exhibit some ultrastructural alterations such as widened intercellular spaces, abnormal gap-junctions and a marked decrease in tonofilaments [27]. The decrease in keratohyalin granules forms the ultrastructural basis for the absence of the granular layer observed in lesional skin biopsies upon light-microscopic examination [4]. Additional alterations affecting the plasma-membrane of keratinocytes are mainly of a biochemical nature. These include decreased membrane fucopeptides, altered lectin binding properties and an increased membrane N-acetylglucosamine and sialic acid content [28]. These changes presumably reflect modification of the epidermal glycosylation system. Abnormal differentiation of keratinocytes in psoriatic lesions also appears from altered cornification. High molecular weight keratins (67 kD) are either decreased or absent, and low molecular weight keratins (50 kD) are increased as compared with healthy skin. These keratins in psoriatic lesions tend to aggregate and to form abnormal structures. Disturbance in the synthesis of the cornified cell envelope is evident since involucrine, a major component of this envelope, forms much earlier in the maturation pathway. In addition, the membrane-bound transglutaminase which catalyzes the

cross-linking of involucrine, is reduced in active parakeratotic regions [4,27].

Alterations in the levels of epidermal eicosanoids

An increased amount of arachidonic acid and its metabolites are produced in lesional psoriatic epidermis by the increased enzymatic activity of PLA_2 [29]. PLA_2 is normally inhibited by lipocortin, but in psoriatic lesions lipocortin activity is decreased, probably due to an abnormal hyperphosphorylated isoform. Thus increased amounts of LTB_4 (and to a lesser extent LTC_4 and LTD_4), 12- and 15-hydroxyeicosatetraenoic acid (HETE) are formed in lesional skin [30]. LTB_4 and 12-HETE are potent chemotactic agents for neutrophils and LTB_4 is also able to induce keratinocyte proliferation [31]. Prostaglandins do not seem to be involved in the pathophysiology of psoriasis. Platelet activating factor (PAF), a potent chemoattractant, is also increased in psoriatic lesions, again as a result of the increased PLA_2 activity [32]. Increased levels of complement (C_{3c}, C_4, C_5) and derived chemotactic substances (C_{5a}, C_{5a} des arg) are also observed in psoriasis [33].

Alterations in several other important epidermal enzyme activities like decreased cyclic AMP, PKCβ, markedly increased cyclic GMP, PLC, ODC, calmodulin, tyrosine kinase and several proteases, e.g. tissue plasminogen activator, skin-derived anti-leukopeptidase (SKALPs), have also been reported [29,34-40].

Alterations in non-keratinocytes in psoriatic lesions

In psoriatic lesions, besides keratinocytes some other skin cells also display abnormalities, implying that they may be involved in the disease process. These include fibroblasts, endothelial cells, monocytes, sensory neurons, (epi-)dermal dendritic cells and T cells. Especially the relation with emotional stress in the disease process is getting clearer, since increased neuropeptide expression (e.g. Substance P) and increased contact sites between nerve fibers and dermal mast cells is observed in psoriatic lesions [41]. The alterations observed in antigen presenting cells (APC), T lymphocytes and in relevant dermal cells in psoriatic skin are discussed in more detail in the following Chapters.

Experiments using athymic nude mice showed that skin biopsies from psoriatic lesions, following transplantation onto these mice, retained the major histological characteristics of psoriasis [42]. However, later on, combined grafting studies showed that those classical characteristics of psoriasis only persisted when psoriatic epidermis was transplanted together with psoriatic dermis [43]. Psoriatic keratinocytes thus need the contiguity of fibroblasts and/or other dermal cells to maintain epidermal hyperplasia. Clear alterations observed in relevant dermal cells are described below.
Fibroblasts Although data on the role of fibroblasts in the pathogenesis of psoriasis are controversial, it is theoretically possible that dermal fibroblasts may promote hyperproliferation of adjacent epidermal cells. Fibroblasts also display some

membrane-associated enzymatic alterations [44]. Growth, however, of normal adult and neonatal foreskin keratinocytes could not be increased when cultured on a feeder layer of normal or psoriatic fibroblasts [26,45]. In contrast, in a dermal equivalent model, psoriatic fibroblasts were shown to induce hyperproliferation in normal epidermal cells [46].

Monocytes Increased numbers of monocytes, lying in close approximation to the basement mebrane, have been observed in psoriatic lesions. These cells exhibited increased adhesiveness to normal endothelial cells *in vitro* and activate neutrophils via soluble mediators [47,48].

Endothelial cells Psoriatic endothelial cells are clearly activated. They display structural abnormalities which together with increased adhesiveness for leukocytes facilitate trafficking of immune cells [49].

More specific alterations observed in non-keratinocytes from psoriatic lesions are briefly discussed in Chapter 2.

Thus psoriatic epidermal cells show considerable plasma membrane and intracellular biochemical abnormalities, probably as a response to transmembranous signals elicited by cytokines, which are discussed in Chapter 2. The main question remains: which alteration(s) represent(s) the basic defect(s) and which are epiphenomena?

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Chapter 2

SKIN INFLAMMATION IN PSORIASIS

2.1 Psoriasis, an immunological disease

In addition to all previously mentioned alterations in psoriatic skin, the possibility of an ongoing immune response must also be considered. It has been postulated that psoriasis is a disease of keratinocyte proliferation mediated by T lymphocytes and/or pro-inflammatory products [1]. Such an immunologically induced hyperproliferation may be induced by recognition of a putative "psoriasis-related antigen". The occurrence of such an antigen is indicated by the finding that T cells of peripheral blood of psoriatic patients show a clear proliferative response *in vitro* to autologous unpurified epidermal cells from lesional as well as uninvolved skin (Chapter 3). This proliferation of T cells is probably antigen-driven. The putative psoriasis (auto)antigen may be a glycoprotein-product of lesional keratinocytes regarded as a non-self, possibly a MHC class II-bound sialylated peptide [2-4]. Molecular mimicry may also occur, e.g. with streptococcal or mycobacterial antigens. Alternatively, the antigen may be a retroviral product or a response to drug hypersensitivity resulting in the membrane expression of neo-peptides [5,6]. Autoantibodies directed against psoriatic skin antigens, e.g. stratum corneum antibodies, have indeed been reported [7]. Another observation supporting the view of an immunological mechanism in psoriasis is the marked immunosuppressive activity of most effective antipsoriatic treatment modalities. Cyclosporin A, for example inhibits epidermal antigen presenting cell (APC) function, and T cell activation resulting in clear reduction of T cell growth factors (Chapter 7) [8,9].

Pro-inflammatory mediators released by activated T cells may recruit leukocytes and initiate skin inflammation. Interactive signalling between resident and non-resident skin cells (shown in Table I) ensues. We propose the term cutaneous signalling system for this dynamic process. The separate cellular components of this system are described in the next paragraph, while the physiological and pathophysiological role(s) of their products are discussed in paragraphs 2.3 to 2.5.

2.2 Cellular constituents of human skin

Human skin is the largest organ. It is a complex organ composed of the epidermis, the dermis and subcutaneous fat [10,11]. The epidermis is the principal barrier between the host and the environment. The cellular constituents of the epidermis consist of keratinocytes, dendritic (Langerhans) cells (2-5% of total epidermal cells), melanocytes (± 5% of basal epidermal layer), sporadically Merkel cells and TcR-$\alpha\beta^+$ and sporadically TcR-$\gamma\delta^+$ T lymphocytes. Keratinocytes make up about 95% of the cell mass of human epidermis and are responsible for the biochemical and physical integrity of the skin [11]. The antigen-presenting Langerhans cell in the epidermis plays a pivotal role in the immunological branch of the host

defence system [12]. Little is known on the ontogeny, fate and function of Merkel cells and TcR$\gamma\delta^+$ T lymphocytes in human skin [13,14].

The epidermis is separated from the dermis by a basement membrane which is composed of proteoglycans, laminin and (type 4) collagen. The basement membrane does not form a barrier for inflammatory mediators. The dermis comprises a papillary and a reticular compartment which consists mainly of 'ground substance' which is made of collagen, reticulum fibers, elastin and glycosaminoglycans [11]. The cellular constituents of the dermis consists of collagen producing fibroblasts, endothelial cells, mast cells, recirculating T lymphocytes, tissue macrophages and dendritic cells. Also the pilo-sebaceous units, demyelinated nerve fibers and their end-organs (e.g. papil of Vater) reside in this substance. The subcutaneous fat serves mainly as a layer of protection, insulation and as a energy depot for the human body and it comprises fat cells, capillaries and some sweat glands.

Table I. The cellular constituents of the skin immune system (SIS)

	Resident cells	Non-resident cells
Epidermis	Keratinocytes	Dendrocytes*
	Langerhans cells	T lymphocytes*
	Melanocytes	
	Merkel cells	
	TcR$\alpha\beta^+$ T lymphocytes	
	TcR$\gamma\delta^+$ T lymphocytes#	
Dermis	Dendrocytes	Dendrocytes/(LC)*
	Endothelial cells	Granulocytes
	Fibroblasts	NK-cells
	Mast cells	Monocytes*
	Monocytes/tissue macrophages	B lymphocytes
	Neuronal elements	T lymphocytes
	Pilo-sebaceous units	
	Sweat glands	
	T lymphocytes	
Subcutis	Fat cells	
	Sweat glands	

* = recirculating cells.
# = sporadically occurring.

2.3 Cytokines

Cytokines are non-specifically inducible soluble hormone-like polypeptides with immunoregulatory functions. They are of clinical relevance, especially in infection and sepsis, auto-immunity, cancer and transplantation [15]. The term "cytokine" comprises monokines from monocytes, lymphokines from lymphocytes, hemopoietic, transforming and other mitogenic factors, the interferons and tumor necrosis factors.

2.3.1 General aspects

Cytokines were initially believed to be mainly inducible in cells of lympho-hemopoietic origin, like regulatory T cells and macrophages [16]. It is now clear that many other cell types are also able to secrete cytokines under specific circumstances. They differ from endocrine hormones in that hormones are produced in specialized glands, are continuously present in the circulation and serve to maintain homeostasis. In contrast, cytokines are generally not constitutively produced, act over short distances as autocrine and paracrine intercellular signals in local tissues and serve to restore homeostasis. This group of heterogeneous glycoproteins has several more common characteristics. Cytokines are low molecular weight glycoproteins (usually < 25 kD in monomeric form). They are mostly synthesized as larger precursor molecules. They are extremely potent, generally acting in the picomolar range. They interact with high affinity (K_d= 0.01 to 1 nM) with specific cell surface receptors. Cytokine receptors on target cells are usually present in relatively low numbers (less than 10,000 receptors per cell). Cytokines interact in a network or a cascade system by inducing each other, transmodulating cytokine cell surface receptors and by cross-regulation via synergistic, additive or antagonistic interactions on cell functions [17]. Their most characteristic feature is their pleiotropy, which means that cytokines have multiple effects on various cell types, and redundancy, which means that several cytokines can replace each other for specific target cell activities.

Three cytokines and their receptors are specifically addressed in this introductory chapter, because they display overlapping biological properties and fulfill key functions in cutaneous inflammation. These cytokines are interleukin(IL)-1, IL-6 and tumor necrosis factor alpha (TNF-α), which were initially believed to be produced mainly by activated monocytes [18]. Various other types of cells, including keratinocytes and fibroblasts, have now been shown also to produce these cytokines. The biochemical properties and the biological activities of these three cytokines are summarized in Table II.

Table II. Cellular sources, biochemical and biological properties of IL-1α and -β, IL-6 and TNF-α[#]

	IL-1α and -β	IL-6	TNF-α
Producers	Macrophages, keratinocytes, endothelial cells, T cells B cells, astrocytes, microglia, fibroblasts	Macrophages, T cells, B cells, keratinocytes, endothelial cells, fibroblasts, astrocytes, mesangial cells, bone marrow stroma cells	Macrophages, T cells, NK cells
Biological activities	Induction of: PGE_2 synthesis growth of fibroblasts bone resorption ICAM-1 expression fever sleep anorexia synthesis of collagenase growth and differentiation of T and B cells	Induction of: B cell differentiation plasmacytoma growth acute phase protein synthesis T cell activation and differentiation macrophage differentiation hematopoietic stem cell growth maturation of megakaryocytes neural cell differentiation mesangial cell growth myeloid leukemic cell differentiation	Tumoricidal activity Inhibition of lipoprotein lipase Induction of: bone resorption myeloid leukemic cell differentiation procoagulant activity growth and differentiation of B cells ICAM-1 expression

[#] Adapted from 18.

Interleukin-1 (IL-1)

IL-1 is the term for two polypeptides, IL-1α (pI 5.3) and IL-1β (pI 7.2), that possess a broad spectrum of inflammatory, metabolic, physiological, hematopoietic and immunological activities (Table I). IL-1α is predominantly produced by resting human keratinocytes as suggested by the IL-1α to IL-1β ratio of 2 [20,21]. IL-1β, in contrast, is the main form produced by activated monocytes with an IL-1β/IL-1α ratio of 25 to 50 [22]. IL-1α and IL-1β show approximately 46% homology at the DNA level, but the final amino acid homology is only 26% [15]. The IL-1α and IL-1β genes are located on chromosome 2 (on band q13 and q13-q21, respectively, both comprising 7 exons), and are probably derived from a common ancestral gene via duplication.

Both molecules are synthesized as 31 kD precursors (IL-1α: 271 aminoacids (aa), IL-1β: 269 aa) that are proteolytically cleaved to the 17.5 kD mature (153 aa for both molecules) proteins. However, IL-1α and IL-1β differ in the way they are processed and modified after translation. The precursor form of IL-1β is only partly biologically active, whereas the IL-1α precursor is fully biologically active [20]. Two different molecular domains on each IL-1 isoform mediate receptor binding. This implies differential biological activities of the two isoforms [23]. Keratinocytes, certain epithelial

cells and certain cells in the central nervous system are the only cells that constitutively produce IL-1. In keratinocytes, IL-1 is stored in an intracellular pool that is composed of pro-IL-1α and pro-IL-1β. Pro-IL-1β, in contrast to pro-IL-1α, has minimal biological activity, and cannot be enzymatically cleaved efficiently by keratinocytes to the active mature form. Keratinocyte-derived pro-IL-1β, therefore, is probably cleaved by proteases from other resident (epi)dermal cells or inflammatory cells [22]. The latter would imply that in the epidermis, under certain conditions, only IL-1α activity may occur extracellularly [20,24]. Human epidermis thus contains an impressive reservoir of in total 20 to 60 μg IL-1, which is equivalent to 4 to 12 nmol/L IL-1 [25]. Since IL-1 can activate certain cells at concentrations lower than 10 pmol/L, this quantity forms a powerful weapon in the first line of defence of the host. IL-1, however, is safely stored in the keratinocyte and external stimuli are required for the release of this intracellular pool of IL-1.

Two types of IL-1R (IL-1RtI and IL-1RtII) have been identified [26]. The IL-1 receptor type I (IL-1RtI) is expressed on a wide variety of cells, which is in accordance with the pleiotropic functions of IL-1. IL-1RtI is a single 80 kD glycoprotein (gp) with an extracellular domain of 319 amino acids (aa), a trans-membranous part of 21 aa and a cytoplasmic domain of 217 aa [27,28]. The extracellular part of the receptor possesses three immunoglobulin (Ig-)like domains indicating that this receptor belongs to the Ig-superfamily [19,29]. The different receptor families are briefly described in the legend of Fig. 1, which illustrates these cytokine binding proteins. IL-1RtI binds the mature forms of IL-1α and IL1-β and is also able to bind the precursor of IL-1α but not of IL-1β [30]. The second receptor (IL-1RtII), a single 68 kD gp, initially discovered on B cell lines, is also expressed on many cell types [26,31]. Soluble receptor forms are described [32]. Both IL-1 binding receptor-proteins are separate gene products [31]. At present, it is unclear whether the two types of receptors have common or different signalling pathways [33]. The IL-1RtI has the ability to rapidly internalize IL-1, whereas IL-1 bound to type II receptors remains at the cell surface for as long as 60 min and is poorly internalized [34]. Furthermore, the half-life of type II receptors is also shorter than that of type I receptors, 2 versus 5-12 hours [35]. Recently the same has shown that both types of IL-1R are accompanied by different IL-1 processing pathways [36]. It is noteworthy that IL-1 down-regulates the surface expression of its own type 1 receptor [37,38]. In addition, IL-1R expression is up-regulated by phorbol esters, UV irradiation and T cell cytokines [39]. Recently, a third IL-1 isoform is described, the IL-1-receptor antagonist (IL-1ra), which binds to the IL-1R and seems to play a regulatory role in cellular activation [40-42].

Signal transduction via IL-1 generally involves a sequence of four important elements. These are an IL-1R linked GTP-binding protein, cAMP, protein kinase (PK) A and the DNA binding protein NF-κB [43]. IL-1 has been shown to activate a serine kinase that is distinct from PKA and PKC in fibroblasts [33].

Interleukin-6 (IL-6)

IL-6 is a 21-28 kD gp with multifunctional properties. It is produced by both lymphoid and non-lymphoid cells (Table II). The IL-6 gene is located on chromosome 7 (p15-21) and comprises 5 exons. The IL-6 promoter contains three functional domains that are involved in IL-6 induction [15]. These are 1) a 23 bp IL-6 multiresponsive element (MRE), 2) a nuclear factor (NF) IL-6 binding site, an element responsive to IL-6 itself and IL-1, TNFα and lipopolysaccharide (LPS) and 3) a NF-κB binding site, which mediates IL-6 induction by IL-1 and TNFα [19]. IL-6 synthesis involves different (cell-type-specific) proteolysis, glycosylation and post-translational NH_2-terminal processing and phosphorylation. This explains the differences in molecular mass of the mature protein [44,45]. The functional significance of these distinct post-translationally modified forms of IL-6 remains elusive. Moreover, IL-6 complexes may be formed after secretion [46]. The amino acid sequence and the genomic structure of IL-6 show considerable homology with that of granulocyte-colony stimulating factor (G-CSF), which is indicative for similarities in the tertiary structure of these cytokines [18].

The IL-6 receptor (IL-6R) is expressed on lymphoid as well as non-lymphoid cells. IL-6R is composed of an external ligand-binding (340 aa), a transmembranous (29 aa) and an intracellular domain (80 aa). The receptor has one Ig-like domain in the extracellular portion, which is not involved in ligand binding. The remainder of the extracellular part belongs to the cytokine receptor family (Fig. 1). The IL-6 receptor system consists of two functionally different polypeptide chains: a ligand-binding 80 kD IL-6R and a signal transducing 130 kD glycoprotein. Following binding of IL-6, the two proteins become associated at their extracellular segments [47]. The p80 IL-6R may occur in a soluble form, and complexes of IL-6 and IL-6R(p80) may mediate signal transduction and thus exert biological effects. Considering the pleiotropic functions of IL-6, it is conceivable that different gp130 variants or that a third associating molecule exists. At least two signalling pathways are involved in the induction of IL-6 by IL-1 or TNFα. One requires PKC activation and the other involves adenylate cyclase. The latter is especially evident in fibroblasts [19].

Tumor Necrosis Factor Alpha (TNF-α)

TNF-α (cachectin), a 17 kD polypeptide, serves as an endogenous mediator of inflammatory, immune and host defence functions. The genes for TNF-α (and TNF-β) are closely located to the MHC class II region on chromosome 6 (p21.3) and consist of 4 exons [18]. TNF-α is mainly produced by macrophages, T lymphocytes and NK cells (Table II). It is produced as a 233 aa pro-TNF-α form, which is processed to a 157 aa mature non-glycosylated peptide. Secreted TNF-α occurs as a trimeric molecule with each subunit consisting of an anti-parallel β-sandwich. TNF-α and TNF-β display about 30% homology in amino acid sequence and have common biological

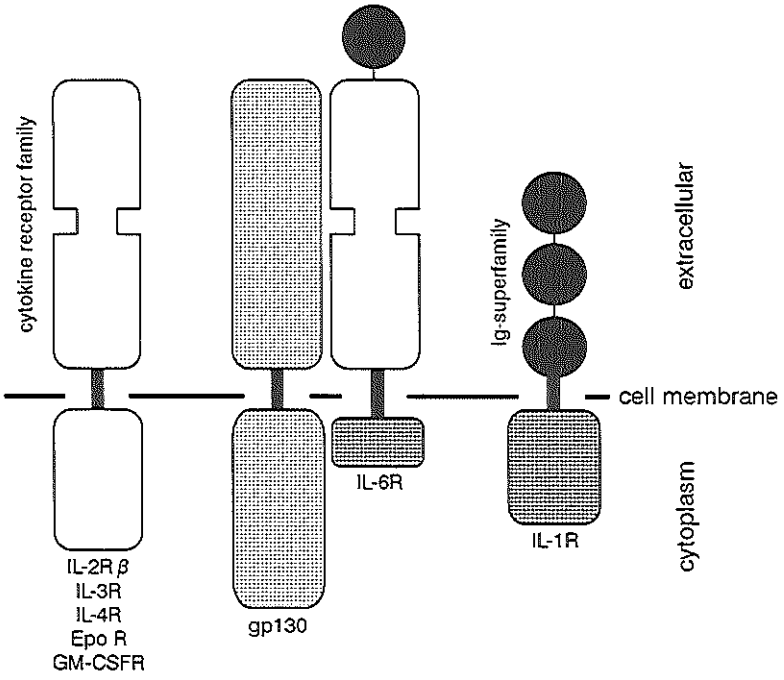

Figure 1. Structural relationship between cytokine receptors (adapted from 19). Most cytokine receptors (TNF-R1 and TNF-R2 excluded) belong to either the Ig-superfamily or the cytokine receptor family. Only the IL-6 receptor is composed of both elements. A third group of cytokine receptors may be formed by the growth factor receptors (e.g. EGF-R) which are characterized by a large cytoplasmic domain with tyrosine-kinase activity [15]. Little is known about signal transduction mechanisms. It is, however, suggested that different target cells possess specific receptor-associating transducing proteins. These may interact with structurally related cytokine receptors. In addition, each type of cytokine receptor may also interact with multiple signal transducers. Such interactions could explain the pleiotropic and redundant effects of cytokines.

activities.

TNF receptors have been found on virtually all cells and tissues. At present, two categories of TNF-binding proteins, a 55-60 kD (TNF-R1) and a 75-80 kD (TNF-R2) receptor have been reported reflecting the two types of TNF-R cDNA cloned. Both receptors bind TNF-α as well as TNF-β and can occur in soluble form [48-50]. Human TNF-R1 and TNF-R2 do neither belong to the cytokine receptor nor to the Ig-receptor-superfamily. The extracellular domains of TNF-R1 and TNF-R2 share 28% identity, which is equivalent to the homology that each of them share with a group of surface proteins including nerve growth factor, Fas antigen, Bp50, Ox40 and CD27. There is a complete absence of homology between the intracellular domains of the two TNF receptors, implying that they utilize distinct signalling pathways [51]. TNF receptors

are continuously internalized and this process is accelerated by the binding of TNF. Cultured normal human keratinocytes, in contrast to the inflammatory infiltrate, express the 55 kD TNF-R only [52].

All three cytokines are generally not constitutively produced, but transiently induced by various stimuli. Inducers and suppressors of these cytokines are shown in Table III, while similar information for the receptors is shown in Table IV. In the cytokine network, IL-1 and TNF-α are considered as primary cytokines in inducing the local production of other cytokines such as IL-2, IL-6, IL-8, IFN-β and CSF. This may explain the pleiotropic effects of these primary cytokines on a variety of cells. IL-1 and TNF-α are able to induce each other. Moreover, IL-1, IL-6 and TNF-α are able to induce their own production [19].

Table III. Inducers and suppressors of IL-1, IL-6 and TNF-α[#]

Cytokine	Inducers	Suppressors
IL-1α/β	Lipopolysaccharide, staphylococcal exotoxins, Klebsiella glycoproteins, viruses, phorbol esters, calcium ionophores, leukotrienes, immune complexes, C3a and C5a, phagocytosis, proteoglycans, collagens types 2 and 9, urate crystals, trauma, adherence, IL-1*, TNF-α, IFN-γ, GM-CSF	Corticosteroids, retinoic acid, cAMP, PGE_2, IFN-γ, IL-2, TGF-β
IL-6	Lipopolysaccharide, mitogens, phorbol esters, calcium ionophores, Staphylococcus aureus, viruses, IL-1, TNF-α, IFN-β, PDGF, UV-irradiation, poly (I)(C), cAMP and cAMP-generating agents	Corticosteroids
TNF-α	Lipopolysaccharide, bacteria, PPD, viruses, immune complexes, phorbol esters, cGMP, IL-1, IL-2, PAF, TNF-α, IFN-γ, GM-CSF	Corticosteroids, CsA, Vit.-D_3 PGE_2, PAF-R antagonist, IL-6, TGF-β

* IL-1α may also down-regulate IL-1 via induction of PGE_2 synthesis.

\# Key references: 18,53-55.

Table IV. Inducers and suppressors of IL-1R, IL-6R and TNF-R[#]

Cytokine receptor	Inducers	Suppressors
IL-1R	PMA, lectins, IL-2: T cells and B cells EBV, Dexamethasone: B cells, MØ PDGF, prostaglandins: FB IFN-γ, UV-irradiation: KC	IL-1: T and B cells RA: KC TGF-β: T cells, FB
IL-6R		IL-1, IL-4
TNF-R	Lectins, IFNs, IL-2, IL-4	PMA, IL-1

[#] Key references: 56-58.

2.3.2 Skin-derived cytokines

The resident and non-resident cell types in normal human skin provide a virtually unlimited local cytokine repertoire. Most cells need an inducing factor to produce and release cytokines. Epidermal cells such as Langerhans cells and keratinocytes are believed to initiate and orchestrate skin inflammation by the release of primary cytokines and via the induction of cytokine(s) and/or cytokine-receptors in adjacent cells. Cytokines derived from melanocytes, dermal T lymphocytes, fibroblasts, monocytes, mast cells and endothelial cells probably fulfill an accessory role in inflammatory processes in the skin [39]. The skin may thus be conceived as the most important peripheral limb of host defence [10]. The cytokines potentially produced by each cell type are listed in Table V.

Table V. Cytokine production capacity of relevant non-keratinocytes in normal human skin[#]

Cell type	IL-1	IL-6	IL-8	TNF-α	IFN-γ	GM-CSF	TGF-α	TGF-β1	bFGF	PDGF	MCP-1
Dendrocytes/ Langerhans cells	+		+								
Melanocytes	+	+	+	+		±		+			
T cells	+	+	+	+	+	+		+			+
Endothelial cells	+	+	+			+		+	+	+	+
Fibroblasts	+	+	+	+		+		+	+	+	+
Mast cells		+	+	±		+					
Tissue macrophages	+	+	+	+		+	+	+	+	+	+

[#] Key references: 53-55,59-66.

Keratinocyte-derived cytokines

Cytokines recently identified in cultured keratinocytes are listed in Table VI. The 'resting' keratinocyte is able to produce only one biologically active cytokine, namely IL-1α [24,25]. External stimuli may result in the release of substantial amounts of e.g. active IL-1α and pro-IL-1β from keratinocytes. For example, skin trauma or UV-exposure will result in the release of active IL-1 and TNF-α and subsequent autocrine and paracrine stimulation of contiguous keratinocytes, Langerhans cells and other cells initiating the "cytokine cascade". It has indeed been shown that cultured keratinocytes were responsive to exogenous IL-1 and TNF-α *in vitro* [39]. The cytokines listed in Table VI are produced mainly by stimulated keratinocytes.

Table VI. Keratinocyte derived cytokines[#]

Cytokines known to be produced	IL-1α and -β; IL-3; IL-6; IL-8; TNF-α; IFN-α and-β1; GM-, G- and M-CSF; TGF-α and -β2; bFGF; PDGF; MCP-1; suppressor-factors; neuropeptides and thymopoietin
Cytokines assumed to be produced	TNFβ; IGF-1 and -2
Cytokines not produced by keratinocytes	IL-2; IL-4; IL-5; IFN-γ

[#] Key references: 39,67,68.

Cytokines produced by Langerhans cells and other cells in the skin

It is assumed that human Langerhans cells may direct and orchestrate the immune response by the configuration of cytokines released at the moment T lymphocytes are activated [69]. For example, Langerhans cells might direct T lymphocytes towards specific differentiation stages such as the Th1 or Th2 subsets depending on the type of antigen recognized, the manner in which that antigen is processed and presented [70]. Skin mast cells and probably trafficking basophils have also been shown to produce cytokines. These include IL-3, IL-4, IL-5, IL-6, IL-8, TNF-α(minute quantities) and GM-CSF [71]. Cytokines like IL-1, IL-2, IL-3, IL-6 and IL-8, released by surrounding cells, also have great influence on mast cells. Cytokines are able to lower the "triggering threshold" or directly stimulate mediator release from mast cells and prime and induce them to synthesize arachidonic acid metabolites [72]. Also cross-linking of the FcεR1 by antigen has been shown to induce the production of cytokines in mast cells [73].

In conclusion, the whole spectrum of cytokines is potentially available in human skin.

2.4 Cutaneous signalling system

In the last decade it has been shown that keratinocytes are not passive bystanders in the immune response of the skin. In contrast, keratinocytes appear to play an active and central role in the first line of defence of the host [74]. The observations that normal keratinocytes have the capacity to produce a broad spectrum of cytokines, compelled Kupper to introduce the activated keratinocyte model [67]. The concept of the activated keratinocyte is essential for the perception of cutaneous inflammation. Briefly, diverse stimuli like contact allergens and UV light may trigger cutaneous inflammation by direct activation of keratinocytes and subsequent production of pro-inflammatory cytokines and expression of adhesion molecules. Via paracrine stimulation in response to keratinocyte-derived cytokines, adjacent keratinocytes, dermal microvascular endothelial cells and fibroblasts are activated and induced to produce other cytokines and to up or down-regulate the expression of cytokine receptors and/or adhesion molecules. This results in selective recruitment of specific immunocompetent cells in the epidermis and the dermis. The ability of keratinocytes to produce an almost complete repertoire of cytokines and immunoregulatory peptides is compatible with the idea that they have an important pathophysiological role in cutaneous inflammation [74].

In further detail, the activated keratinocyte model proposes the following scheme of events. After activation of keratinocytes, several processes ensue. These are 1) as mentioned, *de novo* gene expression of various secondary cytokines such as IL-3, IL-6, IL-8, TNF-α and G(M)-CSF with activating and chemotactic activities on leukocytes and mast cells; 2) (upregulation of) the expression of adhesion molecules on keratinocytes (ICAM-1) and endothelial cells (ICAM-1, ELAM-1, VCAM-1 and probably other as yet unidentified adhesion molecules); 3) keratinocyte proliferation and chemokinesis; 4) fibroblast proliferation, type 4 collagen and collagenase synthesis; and 5) chemotaxis [29,74]. The activation of the dermal vascular endothelium, as indicated by the up-regulation of membrane adhesion molecules, results in increased adhesiveness for different cell types and trapping of inflammatory cells in the capillaries. The composition of the inflammatory infiltrate, for example specific mononuclear cells, is believed to depend on the character and combination of chemotactic cytokines produced by keratinocytes, resident fibroblasts, monocytes, dendrocytes and tissue macrophages. These factors will dictate the net migratory behaviour of the inflammatory cells during the early phases of inflammation. For example, predominant release of IL-1 and IL-8 (cytokines with extreme chemotactic activity for T lymphocytes) by keratinocytes, will encourage directional migration of T lymphocytes towards the epidermis. LFA-1^+ migrated T lymphocytes will adhere to keratinocytes via interactions with the ligand ICAM-1 [74,75]. This type of adhesion may directly induce cytokine release from T lymphocytes and other inflammatory cells,

resulting in further amplification of cutaneous inflammation. The keratinocyte-derived MCP-1, on the other hand, specifically recruits monocytes. The released pro-inflammatory cytokines such as IFN-γ and TNF-α, produced by activated T cells and monocytes, respectively, are able to stimulate the keratinocyte and to maintain the cell in an activated state via the IL-1/IL-1R loop. Indeed, keratinocytes have been shown to possess the appropriate cytokine receptors necessary to respond to paracrine activation [67]. Receptors for IFN-γ, for example, are constitutively expressed in high levels in normal epidermis [76,77]. Other receptors are also present on keratinocytes [52,78,79]. Routes by which keratinocytes can be activated are illustrated in Fig. 2.

IFN-γ and TNF-α also induce ICAM-1 expression on keratinocytes, whereas the expression of HLA-DR on these cells can be induced by IFN-γ only. These molecules

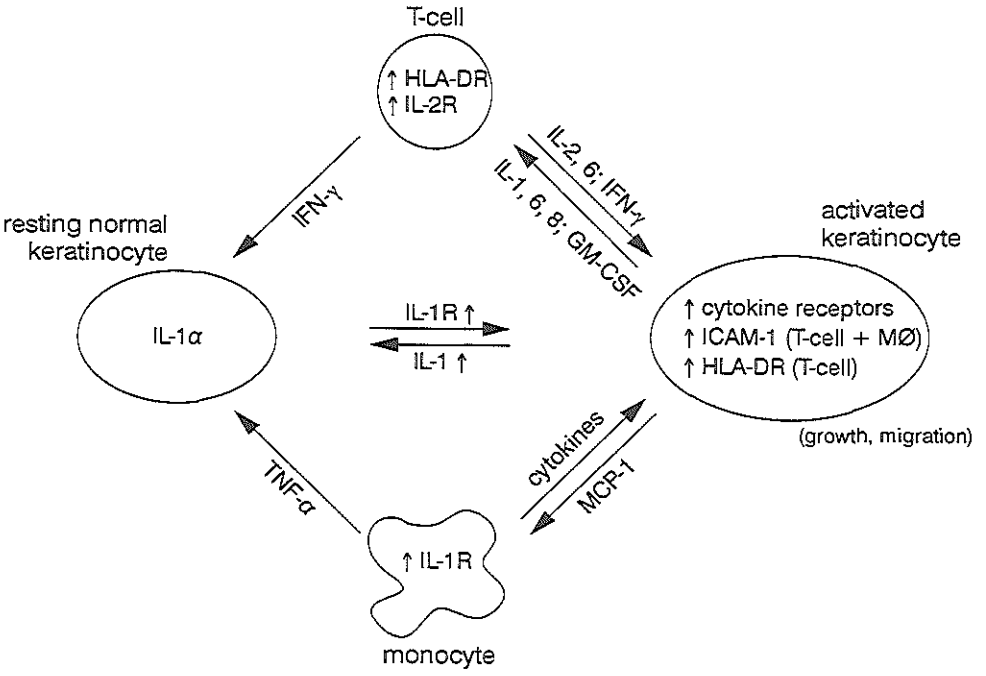

Figure 2. Routes of activation and biological responses of human keratinocytes (adapted from 39). Keratinocytes may be activated in an autocrine or paracrine fashion. IL-1 may be involved in both pathways. Keratinocytes activated in paracrine fashion by T cell cytokines differ from those activated by monocyte-derived cytokines. Conversely, different types of activated leukocytes are also generated upon paracrine activation by keratinocyte-derived cytokines [83].

are important for the evolution, full development and regulation of cutaneous inflammation. The induction of ICAM-1 and HLA-DR are obviously not confined to keratinocytes, but can also be induced on fibroblasts and endothelial cells. IFN-γ is also able to induce the production of secondary chemotactic cytokines in keratinocytes, such as monocyte chemotactic protein-1, MCP-1, IFN-γ-induced protein 10 (γ-IP-10). TNF-α furthermore induces keratinocyte IL-8 [74,75]. MCP-1 and IL-8, both small peptides, are actually considered the most important skin derived chemotactic cytokines. The mentioned molecules belong to different subfamilies (C-X-C-family: IL-8, gro and C-C-family: MCP-1, MIP, RANTES) of a supergene family of chemotactic cytokines [75,80]. Both IFN-γ and TNF-α are able to induce TGF-α production in keratinocytes. TGF-α, IL-1 and IL-6 are potent autocrine growth factors for keratinocytes, ontogenetically designed to fulfill a central role in re-epithelialization during wound healing [81,82].

In the amplification phase of cutaneous inflammation, the recruitment of leukocytes and the interaction between epidermal cells and inflammatory cells is intensified [74,75]. In contrast with initial, antigen-independent activation of keratinocytes, an antigen-dependent component may be involved in the later stages [74]. The decline of activation signals causes the keratinocyte to down-regulate its release of IL-1, the expression of IL-1R and to become refractory to further stimulation. Finally, under normal conditions down-regulatory mechanisms switch off the state of activation, e.g. via loss of keratinocyte ICAM-1 expression. Consequently, cutaneous inflammation is resolved. Restoration of the intracellular pool of active IL-1 renders the keratinocyte responsive again to subsequent trigger factors of cutaneous inflammation [39]. Keratinocytes thus orchestrate skin inflammation by serving as cellular 'signal transducers', capable to transduce exogenous stimuli into production of cytokines, cytokine-receptors, adhesion molecules and chemotactic factors [74].

The above discussed sequence of events represents carefully balanced functional interactions between cellular constituents of the skin immune system and their soluble products. Dysbalances or genetically determined incapacities to produce (or similar abnormalities resulting in overproduction of) certain molecules of this interactive system, underly skin diseases such as psoriasis.

Our concept of the "cutaneous signalling system" emphasizes that not the mere physical or static presence of the cellular constituents of the skin immune system [10], but their functional interactions are of decisive importance for the prevention or development of skin disease.

2.5 Cytokines and their specific receptors in psoriasis

The combination of keratinocyte hyperproliferation with epidermal leukocyte accumulation distinguishes psoriasis from other epidermal hyperplasias. The

pathogenesis of psoriasis is thus linked, at least in part, to processes which regulate leukocyte trafficking and inflammation. As put forward in the previous section, this makes cytokines and their receptors suspects in the pathogenesis of psoriasis. In the last few years alterations regarding the cytokine profile in psoriasis have indeed been reported. These alterations will be discussed briefly. One should keep in mind, however, that the cascade of keratinocyte derived pro-inflammatory cytokines operative in psoriasis, under normal conditions has beneficial effects for the host.

Interleukin-1

Lesional psoriatic cytosolic extracts of keratome specimens contained less IL-1α but increased amounts of IL-1β protein as compared with extracts of uninvolved psoriatic skin or normal skin [84]. IL-1β mRNA is also increased in psoriatic skin extracts [85]. The considerable IL-1α predominance in normal skin is apparently lost in psoriasis due to the down-regulation of IL-1α and up-regulation of IL-1β [20]. Comparable results were obtained with 10,000 g supernatants of normal and psoriatic skin specimens, although the mature form was only present in psoriatic skin [86]. When aqueous extracts of the stratum corneum (scale extracts) were used, it was observed that preferentially mature IL-1α was decreased in psoriatic lesions as compared with normal skin. IL-1β was almost undetectable in both types of scale extracts [87,88]. In addition, suction blister fluids from psoriatic lesions as well as from normal skin contained similarly low levels of IL-1α and no IL-1β. However, IL-1β was over-expressed on the plasma membrane and in the intracellular compartment of epidermal cells [88,89]. Slightly elevated levels of IL-1α as well as IL-1β mRNA were detected in cultured keratinocytes from psoriatic lesions. Lesional fibroblasts, in contrast, expressed almost undetectable levels of IL-1α and IL-1β mRNA [89]. To make the picture still more complex, immunohistological studies by others showed that both normal and psoriatic epidermis expressed more IL-1α than IL-1β. In normal skin, the expression of IL-1α was predominantly intercellular whereas in lesional psoriatic epidermis its distribution was predominantly within the cytoplasm [90].

About the IL-1 bioactivity there is more consensus in the literature. The total IL-1 bioactivity in psoriatic epidermis is considerably decreased as compared with normal skin [91]. IL-1α comprises all the bioactive material [85,87]. The IL-1β in the psoriatic lesion may be of a novel form: it is a processed molecule with negligible bioactivity [20]. The presence of an IL-1 inhibitor in psoriatic skin may explain at least in part the reduced IL-1 bioactivity [85,92]. In psoriatic scale extracts, however, inhibitory activity could not be detected [93]. The decreased IL-1α levels in psoriatic lesions may be due to the elevated IL-6 levels in these lesions, since IL-6 is known to inhibit IL-1 production [94]. Since several enzymatic abnormalities have been reported in psoriatic skin, it may be that the abnormal isoforms of IL-1 are due to improper cleavage or even non-cleavage of IL-1 precursor molecules by defective serine-proteases [95].

Interleukin-1 receptors

It has been postulated that the primary defect in psoriasis may be the dysregulation of keratinocyte IL-1R in the absence of an increase of epidermal IL-1 [25]. Our own investigations revealed a decreased expression of IL-1R on lesional EC. The results on other cytokine receptors on EC in psoriasis are described in detail in Chapter 6.

Interleukin-6

Increased levels of IL-6 were observed in skin from active psoriatic plaques by immunohistochemistry. Strong staining was confined to keratinocytes and inflammatory dermal cells [81,96-98]. Using *in situ* hybridization, abundant IL-6 mRNA was demonstrated in psoriatic epidermis. The increase in IL-6 mRNA was more pronounced in the transitional zone (the border between psoriatic plaques and uninvolved skin) of progressive psoriatic lesions [81,96,99]. In contrast, another investigator, using *in situ* hybridization, failed to detect increased IL-6 mRNA in lesional epidermis [100]. IL-6 protein expression was also detected in a higher percentage of keratinocytes cultured from lesional psoriatic skin relative to cultured keratinocytes from uninvolved psoriatic skin [81]. However, IL-6 mRNA was expressed at very low levels in cultured keratinocytes from both psoriatic lesions and normal skin, whereas cultured lesional fibroblasts showed a clear IL-6 mRNA expression [89]. Our own studies revealed that cultured psoriatic fibroblasts produce increased amounts of bio-active IL-6 as compared with fibroblasts from healthy controls [Debets et al., submitted]. Blister fluids from psoriatic lesions contained elevated levels of bioactive IL-6, while the levels in sera of these patients were undetectable [89]. Another study of patients with severe psoriasis did show elevated serum levels of IL-6, probably derived from activated peripheral blood mononuclear cells [81,96,101]. However, neither IL-6 nor IL-6 activity could be detected in extracts of scales from psoriatic lesions and normal skin [87].

Interleukin-8

On the one hand, immunoreactive IL-8 was detected in suprabasal keratinocytes in psoriatic lesions, whereas IL-8 expression was not found in normal skin [102]. We confirmed these observations [Prens et al., manuscript in press]. On the other hand, others reported a reciprocal relationship between the expression of IL-8 and the disease activity [103]. Biologically active IL-8 was unequivocally shown to be elevated in extracts of psoriatic lesions [87]. High levels of IL-8 mRNA were also detected in lesional epidermis, especially in active plaques [101,16]. Using *in situ* hybridization, IL-8 mRNA was confined to the upper epidermal layers [100]. Since this cytokine specifically attracts neutrophils and T cells it was considered a key cytokine in the maintenance of psoriasis.

Tumor necrosis factor α and β

TNF-α and TNF-β were detected in extracts of scales from psoriatic lesions in contrast to extracts of normal skin. The TNF in these samples possessed no equivalent biological activities [87]. Using immunohistochemical localization TNF-α was identified especially in macrophages in the upper dermis in lesional skin, while there was no detectable TNF in normal skin [102]. Another group did not detect TNF-α, neither in psoriatic nor in normal skin [97]. In another study both TNF-α protein and TNF-α activity were undetectable in both extracts of scales and blister fluids [104]. TNF-α mRNA was also undetectable in psoriatic lesions using *in situ* hybridization [100]. Psoriatic monocytes from untreated patients seemed to activate neutrophils more efficiently, probably via increased TNF-α production [101]. Finally, TNF-α and TNF-β immunoreactivity was not increased in supernatants of stimulated peripheral blood mononuclear cells from psoriatic patients [105].

Interferons

Suction blister fluids from psoriatic plaques, but neither uninvolved skin nor sera from psoriatic patients, contained IFN-γ and some IFN-α activity [106]. Another group could not confirm this finding [107]. Expression of IFN correlated positively with disease activity. IFN-α and IFN-γ were primarily confined to basal keratinocytes, the stratum corneum and dermal mononuclear cells, respectively. Interferons were undetectable in uninvolved skin or normal healthy skin [108]. The IFN-α and IFN-γ proteins were also detected in extracts of psoriatic lesions in contrast to extracts of normal skin. However, no equivalent biological activities could be detected [87,107]. In contrast to normal keratinocytes, psoriatic keratinocytes were indeed found to produce γ-IP-10, possibly as a result of elevated levels of IFN-γ in psoriatic lesions [109].

Interferon receptors. Psoriatic keratinocytes and normal keratinocytes respond differently to IFN-γ. Psoriatic keratinocytes showed a weaker HLA-DR expression and less inhibition of cell growth upon stimulation with IFN-γ. It has been shown that psoriatic KC do not down-modulate their EGF/TGF-αR upon exposure to IFN-γ. Thus possible alterations in the IFN-γR or receptor-signalling mechanism in psoriasis are indicated [75,110]. The IFN-γR was observed only in the basal layers in psoriatic epidermis [76].

Colony stimulating factors

In one study, G-CSF and GM-CSF protein and bioactivity were undetectable in scale extracts of psoriatic lesions and normal skin [87]. However, other investigators did detect increased levels of immunoreactive GM-CSF (but not G-CSF) in lesional scale extracts, while these cytokines were undetectable in blister fluids [111,112]. Psoriatic monocytes appeared to produce increased amounts of GM-CSF [101].

Growth factors

Transforming growth factor (TGF) α. TGF-α is overexpressed in psoriatic epidermis, with the expression primarily confined to the upper stratum spinosum. In uninvolved and normal healthy skin, in contrast, the protein is predominantly expressed in the stratum basale and the lower stratum spinosum [113]. In lesional epidermal extracts increased TGF-α protein and TGF-α mRNA have been detected. TGF-α has also been detected in lesional dermis [102,114].

TGF-β. TGF-β transcripts were not increased in psoriasis and EGF mRNA was even undetectable in both psoriatic and normal skin [114,115]. Psoriatic endothelial cells are less responsive to the down-regulatory effects of TGF-β on the expression of adhesion molecules [116].

Growth factor receptors

Transforming growth factor-α receptors. The natural receptor for TGF-α, the EGF receptor, is overexpressed in psoriatic epidermis, with a distribution similar to that of TGF-α [117]. However, no increase in EGF/TGFα-R mRNA was observed in psoriasis [114].

Insulin-like growth factor receptors. An increased expression of IGF-1R has been observed in lesional psoriatic epidermis [118]. Fibroblasts are a potential source of IGF-1 in this respect [119].

Platelet-derived growth factor receptors. Psoriatic dermal endothelial cells and fibroblasts express an increased number of PDGF-R-β [120].

Other important immunoregulatory cytokines such as IL-2 and IL-4 were undetectable in scale extracts of psoriatic lesions and normal skin [87]. However, others did report elevated levels of IL-2 in lesional extracts but not in suction blister fluids of lesional skin. Increased levels of soluble IL-2R did occur in blister fluids from psoriatic skin [107]. In addition, soluble IL-2R was increased in serum of psoriatic patients [121]. Our group did not detect immunoreactive IL-4 in culture supernatants of psoriatic and normal epidermal cells. Finally, over-expression of proto-oncogenes is probably not involved during the steady-state epidermal hyperplasia in psoriasis [115].

2.6 Aim of the study

The above data show that prodigious alterations occur in the expression of cytokines and their receptors in psoriasis. Although a crucial role for cytokines in the pathogenesis is almost definite, the results do not unequivocally demonstrate a primary cytokine defect in psoriasis. Furthermore, the data described are often contradictory. This may be caused by the use of different skin specimens, the detection of cytokines and receptors at different levels (eg. transcription, protein and

bioactivity) combined with the use of different detection techniques. Despite the fact that data on cytokines in normal and psoriatic skin is far from complete, several alterations in psoriasis are evident:

1) dysregulation of IL-1;
2) increased levels of IL-6, IFN-γ and especially IL-8 and TGF-α;
3) altered responsiveness of keratinocytes to IFN-γ; and finally;
4) clearly increased expression of the EGF/TGF-α receptor.

Taking these findings into account, psoriasis may be considered a multi-factorial immunological disease in genetically predisposed individuals with primary defect(s), probably residing in the cutaneous signalling system. A specifically altered cytokine profile in association with an altered response of the keratinocytes to these cytokines links inflammation with the hyperactive state in the epidermis in psoriasis. It is conceivable that this deviant cutaneous signalling system plays a central role in the pathogenesis of psoriasis.

The aims of our studies were to further delineate the basic abnormalities in skin APC and the cutaneous cytokine network in psoriasis. The experimental work on APC, cytokines and cytokine receptors in the psoriatic skin are described in Chapters 3 to 7. In Chapter 8, the implications of our findings are discussed in the context of the literature data on the altered cytokine network in psoriasis.

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Chapter 3

THE AUTOLOGOUS MIXED EPIDERMAL CELL - T LYMPHOCYTE REACTION IS ELEVATED IN PSORIASIS
A crucial role for epidermal HLA-DR$^+$/CD1a$^-$ antigen presenting cells*

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SUMMARY

The objective of this study was to determine whether epidermal cells (EC) from psoriatic lesions and uninvolved skin could stimulate autologous T lymphocytes in the *in vitro* autologous mixed epidermal cell - T lymphocyte reaction (autologous MECLR). The functional role of antigen-presenting cell (APC) subsets was concurrently determined in this reaction. Mononuclear cells and purified T lymphocytes from peripheral blood of psoriatic patients showed a clear proliferative response to autologous unpurified epidermal cells from involved as well as uninvolved skin. The autologous mixed leukocyte reaction (MLR) was not elevated in psoriatic patients. In healthy controls and contact allergy patients, T lymphocyte proliferation was not observed in the autologous MECLR and in the autologous MLR. The level of proliferation in the autologous MECLR from psoriatic patients correlated to the number of epidermal cells that were added. To exclude the possibility that the observed proliferation in the autologous MECLR in psoriasis was due to the presence of epidermal T lymphocytes that were being stimulated and expanded *in vitro*, the stimulator EC were gamma irradiated (30 Gy) in some experiments. Preincubation of EC with cyclosporin A (CsA) significantly inhibited the autologous MECLR. The CsA-induced inhibition could be neutralized by the addition of fresh untreated EC to these cultures. This indicated that one of the modes of action of CsA in resolving psoriasis is, as some investigators have already shown, via inhibition of epidermal accessory cell function. In the autologous MECLR, APC from psoriatic skin could initiate this reaction, whereas APC from peripheral blood could not. This occurred in an MHC class II restricted fashion. Depletion experiments showed that Langerhans cells ($HLA\text{-}DR^+/CD1a^+$) were not the principal stimulators of autologous T lymphocytes in the MECLR. These results indicated that mainly $HLA\text{-}DR^+/CD1a^-$ epidermal cells from psoriatic patients could stimulate autologous peripheral blood T lymphocytes in an MHC class II restricted fashion.

INTRODUCTION

The etiology of psoriasis is still unclear [1-5]. Some early studies led to the assumption that psoriasis was a genetically determined primary keratinocyte disease with the net result of increased proliferation [3]. Keratinocytes have indeed been observed to produce, *in vivo* and *in vitro*, a wide array of inflammatory mediators and cytokines including those capable of autocrine stimulation of keratinocyte growth [4-9]. However, the main trigger responsible for the cascade of events resulting in local epidermal hyperplasia, and recruitment of leukocytes in psoriatic lesions remains unidentified. It has been demonstrated that intra-epidermal infiltration of $CD4^+$

lymphocytes precedes the epidermal changes in developing psoriatic lesions [10]. Native and recombinant lymphokines can induce, *in vivo* and *in vitro*, the expression on keratinocytes of aberrant molecules such as HLA-DR, gamma IP-10, and ICAM-1, which are normally expressed in psoriatic lesions [7-9]. Supernatants from unstimulated peripheral blood mononuclear cells (PBMC), Con-A stimulated PBMC and allo-stimulated purified T lymphocytes were shown to be able to induce keratinocyte proliferation *in vitro* [11]. An important observation, clearance of severe psoriasis after allogeneic bone marrow transplantation, has recently been reported [12]. These studies indicate that the abnormalities in psoriatic keratinocytes may be triggered by, or are highly dependent on, bone marrow-derived leukocytes and their cytokines. They support the recent hypotheses on immune mechanisms in psoriasis [13-15].

Further evidence for the role of the cellular immune system in psoriasis has been obtained in recent years. Immunophenotyping of the inflammatory infiltrate using cryostat sections showed a marked increase in the number of HLA-DR^+/$CD1a^-$ dendritic cells and a slight increase in HLA-DR^+/$CD1a^+$ Langerhans cells in the epidermis from early psoriatic skin lesions [16,17]. The infiltrate in the dermis at this stage consisted mainly of activated ($CD4^+$/$CD25^+$/HLA-DR^+/VLAα-4^+) helper-inducer or memory T lymphocytes [16-18]. Sera of psoriatic patients also showed signs of T cell activation. These sera were recently shown to contain elevated levels of soluble IL-2 receptor (TAC, [19]). Intra-epidermal $CD4^+$ T lymphocytes were observed in close apposition to HLA-DR^+ dendritic cells, a picture normally seen in contact hypersensitivity (DTH-like) skin reactions [14]. $CD4^+$ T lymphocytes appeared to be bound specifically to dermal capillary endothelia in psoriatic plaques, but not in uninvolved skin from either psoriatic patients or normal individuals [20]. The presence of gamma interferon (IFN-γ) was demonstrated in suction blister fluid from psoriatic plaques, but not in blister fluid from uninvolved skin or in sera from psoriatic patients [21]. Gamma IFN-induced protein (gamma IP-10), HLA-DR and ICAM-1 were expressed on keratinocytes and in the dermal infiltrate in psoriatic plaques [22]. Since keratinocytes are not known to produce IFN-γ, its most likely sources would be intra-epidermal or dermal T lymphocytes [23]. Although beneficial effects have also been reported, induction or exacerbation of psoriasis upon treatment with recombinant INF-γ and INF-α have been well documented [24,25]. Cyclosporin A (CsA), was observed to be effective in clearing psoriasis [26-29]. The therapeutic effect of CsA and other therapeutic regimens in psoriasis was correlated to a decrease or disappearance of HLA--DR^+/$CD1a^-$ cells in the epidermis, and to a decrease in $CD4^+$ T lymphocytes in the epidermal and dermal inflammatory infiltrate [17,18,30]. Considered together, these data suggest an involvement of $CD4^+$ T lymphocytes and HLA-DR^+/$CD1a^-$ dendritic cells in the pathogenesis of psoriasis.

These observations have been corroborated by functional *in vitro* data. An

increased *in vitro* autologous mixed epidermal cell - T lymphocyte reaction autologous (MECLR), induced by unpurified epidermal cells, has recently been described in psoriatic patients [31-33]. In addition to an elevated autologous MECLR, Steinmuller et al also reported on the release of a leukocyte migration-inhibition factor by psoriatic EC, whereas cell-mediated cytotoxicity was not observed in psoriatic patients [32]. We have also observed an increased *in vitro* autologous MECLR in psoriatic patients [34,35].

In the epidermis of normal skin, only one predominant type of APC, the MHC class II positive, Fc and C3 receptor bearing Langerhans cell(s), has been observed [36,37]. Epidermal cell suspensions depleted of Langerhans cells are unable to present antigen to antigen-specific T lymphocytes [38]. Langerhans cells are also the main allostimulatory cells in the allogeneic MECLR [39,40]. Epidermal cell suspensions depleted of HLA-DR^+/$CD1a^+$ Langerhans cells, therefore, do not stimulate allogeneic T cells [40,41]. The functional significance of the two dendritic cell subsets, namely HLA-DR^+/$CD1a^+$ and HLA-DR^+/$CD1a^-$ cells present in involved psoriatic skin, is not yet clear.

In this communication we report the results of *in vitro* studies on the kinetics of the autologous MECLR, the role of epidermal Langerhans cells and other APC subsets, and the role of MHC class II in T cell - APC interaction(s) in psoriasis.

MATERIALS AND METHODS

Patients and controls

Twenty-four otherwise healthy patients (9 men, 15 women, median age 43) with active plaque-type, untreated psoriasis (needing clinical treatment) were tested. The autologous mixed leukocyte reaction (MLR) and the autologous MECLR were performed in all patients. Their PBMC were immunophenotyped using a broad panel of monoclonal antibodies (MoAb). As controls, autologous MLR and autologous MECLR were performed in 24 age- and sex-matched healthy volunteers (individuals without history or signs of skin disease undergoing abdominal plastic surgery) and in patients with active allergic contact dermatitis, mainly to nickel.

Preparation of epidermal cell suspensions

Epidermal sheets from involved and uninvolved skin of psoriatic patients were obtained using the suction blister technique [42]. Raising blisters in psoriatic plaques appeared quite difficult. The time necessary to obtain blisters was approximately 2.5-4 hours; even then, in some cases no blisters were obtained. Single cell suspensions of EC were prepared using standard methods [39]. Briefly, epidermal sheets were floated in a Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS) solution containing 0.25% trypsin (ICN Biochemicals, Cleveland, OH) for 30 min, and gently agitated every 5 min, followed by a 15-min incubation in a 0.025% deoxyribonuclease (DNase; Sigma, St. Louis, MO) solution at room temperature. The epidermal cells were filtered through sterile 100-μm and 30-μm mesh nylon gauzes and resuspended in RPMI 1640 medium supplemented with penicillin, streptomycin, fresh L-glutamine, 20 mM Hepes (RPMI complete medium), 15% heat-inactivated human AB serum, and 0.01% DNase. Cells

were counted using a hemocytometer and their viability was determined by trypan blue exclusion.

Removal of langerhans cells and other HLA-DR$^+$ cells from epidermal cell suspensions

Langerhans cells (HLA-DR$^+$/CD1a$^+$) or HLA-DR$^+$ cells were removed from the EC suspension by an immunomagnetic rosetting technique using anti-CD1a and anti-HLA-DR MoAb and goat anti-mouse-conjugated paramagnetic beads (Dynal, Oslo, Norway) [43]. Briefly, suspended EC were labeled for 30 min at 4°C with optimal dilutions of MoAb, washed three times, incubated with the conjugated magnetic beads, spun down and the pellet incubated for 30 min. The pellet was carefully resuspended and the rosettes were counted using a hemocytometer. The rosetted fraction was concentrated to one side of the tube using a strong magnet. The non-rosetted cells were collected, leaving the rosetted cells adhered to the side of the tube. The latter procedure was repeated twice. In all depletion experiments, the numbers of CD1a$^+$ and HLA-DR$^+$ EC were determined using a two-step immunofluorescence technique. The number of rosetted cells was compared with the fluorescence data before depletion and the efficiency checked again later by immunofluorescent staining and counting under a microscope.

Isolation of peripheral blood mononuclear cells

PBMC were isolated using density gradient centrifugation on Lymphoprep (density 1.077, Nyegaard, Oslo, Norway) [44]. All rinsing, also when isolating other cells, was done in RPMI complete medium containing 1% human AB serum.

Isolation of purified T lymphocytes

T lymphocytes were isolated by a rosetting technique using 2-aminoethylisothiouroniumbromide (AET)-treated sheep red blood cells (SRBC) [45]. The purity of this fraction, determined by immunofluorescence after incubation with anti-CD3(Leu-4) was approximately 95%, whereas 99% was CD2$^+$ (OKT11$^+$). In this fraction, contamination with CD14$^+$ monocytes and CD20$^+$ B lymphocytes averaged 0 - 1% as determined by fluorescence microscopy or FACScan (Becton Dickinson, Sunnyvale, CA).

Isolation and purification of peripheral blood monocytes

Monocytes were purified from the plastic adherent fraction of PBMC, or from the non-rosetting fraction after rosetting the T lymphocytes, using AET-treated SRBC as described by Gaudernack and Bjercke [46].

Monoclonal antibodies and immunophenotyping of cell suspensions

The following MoAb were used: CD1a (66IIC7, Dr. M. van der Rijn, Amsterdam), CD2(OKT11, Ortho Diagnostic Systems, Raritan, NJ), CD3(Leu-4), CD4(Leu-3), CD8(Leu-2), CD16(Leu-11b), CD25(IL-2 receptor), HLA-DR (all from Becton Dickinson), CD20 (B1, Coulter Clone, Hialeah, FL), CD11b (OKM-1, Ortho Diagnostic Systems), transferrin receptor (T9, 66IG10, Dr. M. van der Rijn), CD14 (My3, My4, Dr. J.D. Griffin, Boston, MA), CD15 (VIM-D5, Dr. W. Knapp, Vienna, Austria). The MoAb and antisera used in blocking and panning experiments were dialyzed against complete RPMI medium without serum and stored at -20°C until use. Immunophenotyping was performed using a two-step immunofluorescence technique as described by van Dongen et al [47]. Cells were counted using Standard Zeiss 16 microscopes equipped with a IV FL epi-illumination condensor (Carl Zeiss, Oberkochen, FRG). The percentage of positive cells was calculated by counting the number of cells showing positive fluorescence from a total of 200 - 400 cells, or by FACScan analysis (Becton Dickinson).

Autologous MECLR and MLR

Mixed cultures of unpurified epidermal cells and PBMC or purified T cells were set up in quadruplicate in round-bottomed microtiter plates (Costar, Cambridge, MA). To each well 4×10^4 epidermal cells and $1 - 2 \times 10^5$ PBMC or T cells were added in a final volume of 0.2 ml complete RPMI medium supplemented with 15% human AB serum. The autologous MLR comprised the spontaneous proliferation of separately plated fractions of PBMC and/or of cocultured purified T and autologous non-T cells (monocytes, dendritic cells and B cells). In pilot experiments the stimulatory capacity of 30 Gy irradiated EC and PBMC were compared with that of unirradiated EC and PBMC. As the autologous response using unirradiated EC and PBMC did not differ significantly from that of 30 Gy-irradiated EC and PBMC, only unirradiated EC and PBMC were used in subsequent experiments. On days 6, 7 and 8, the cultures were pulsed with 0.5 μCi = 18.5 kBq tritiated thymidine ([^{3}H]TdR, 185 GBq/mmol, Amersham, Buckinghamshire, UK) and harvested 8 h later using a semi-automatic cell harvester (Skatron, Oslo, Norway) onto glass fiber filters. The amount of [^{3}H]TdR that was incorporated was determined by liquid scintillation counting (Tri-Carb 1500, Packard Instrument Co., Downersgrove, IL). The results were expressed as mean ± SEM disintegrations per min (dpm) of quadruplicate cultures.

Cell suspensions were fixed with 0.01% glutaraldehyde (Grade I, Sigma Chemical Co.) for 30 sec on ice, followed by five rinses with cold complete RPMI medium containing 15% human AB serum.

Blocking of the autologous MECLR with MoAb

In some experiments MoAb specific for monomorphic MHC class II (anti-HLA-DR, Becton Dickinson) were added to the autologous MECLR cultures. In other studies at our laboratory this MoAb effectively blocked MHC class II restricted responses.

Preincubation of EC with Cyclosporin A

The effect of Cyclosporin A (CsA) on the autologous MECLR was studied by incubating EC from psoriatic lesions for 1 h at 37°C with 2.5 μg/ml CsA (pure substance, kindly provided by Sandoz, Basel, Switzerland) in complete medium containing 1% human AB serum. After incubation, the EC were rinsed five times with warm (37°C) complete medium containing 15% human AB serum. The last two rinses did not contain detectable levels of CsA as measured using the standard Sandoz Cyclosporin A radioimmunoassay (CsA-RIA) kit.

Statistical analysis

The results were analyzed with STATA^{TM} (computer program for statistical analysis, Computing Resource Center, Los Angeles, CA). The test used to determine statistical significance is mentioned together with the "p" values in abbreviated form, e.g. the Wilcoxon test (WT), Wilcoxons signed rank sum test (WSRT).

RESULTS

The Autologous MECLR in psoriatic patients and in controls

The PBMC of all psoriatic patients showed a clear proliferative response to autologous unpurified epidermal cells (Table I). The level of the responses did not correlate with the clinical severity of their psoriasis and showed considerable inter-patient variability. Unpurified epidermal cells from psoriatic lesions induced higher proliferation

rates in autologous T lymphocytes than unpurified epidermal cells from uninvolved skin ($p<0.001$, WT). The autologous MLR was normal in psoriatic patients (Table I). Gradual increment in the number of epidermal cells from psoriatic lesions resulted in increased proliferative responses of PBMC, showing maximum T lymphocyte proliferation at 4-8 x 10^4 epidermal cells per well (Fig. 1). To exclude the possibility that the observed proliferation in the autologous MECLR in psoriasis was due to the presence of epidermal T lymphocytes which were being stimulated and expanded *in vitro*, the EC were gamma irradiated (30 Gy) in some experiments. The results of these experiment are shown in Table II. It can be seen that the differences between irradiated and non-irradiated EC were not significant (WSRT).

In the age- and sex-matched control group of healthy volunteers and in patients with allergic contact dermatitis, no significant proliferation was observed in the autologous MECLR, using 4 x 10^4 stimulator EC and 10^5 responder PBMC (Table I).

The immunophenotypic pattern of PBMC samples from psoriatic patients did not differ significantly from those of healthy controls and patients with active allergic con-

Table I. Proliferation rates (mean ± SEM) in the autologous MECLR and in different cell subsets from psoriatic patients, healthy controls, and allergic contact dermatitis (ACD) patients[a]

	EC	T cells	PBMC[b]	EC + PBMC[c]
PsoriasisP[d]	94 ± 6.4 (n=16)	789 ± 132 (n=16)	2,177 ± 455 (n=24)	42,688 ± 6,730 (n=24)
PsoriasisN[d]	81 ± 7.3 (n=8)	579 ± 140 (n=8)	1,987 ± 634 (n=8)	20,369 ± 4,320[e] (n=8)
Controls	89 ± 7.1 (n=16)	621 ± 118 (n=16)	1,907 ± 373 (n=24)	2,178 ± 320 (n=24)
ACD patients	76 ± 6.9 (n=16)	797 ± 214 (n=16)	1,746 ± 420 (n=16)	1,957 ± 401 (n=16)

[a] Unpurified epidermal cells (EC) (4 x 10^4 EC/well), purified T cells (10^5 cells/well) and PBMC (10^5 cells/well) were cultured separately.

[b] The autologous MLR is represented here. PBMC were plated in two separate fractions (total 10^5/well), in this case using unirradiated stimulator PBMC. The results are shown asthe mean ± SEM dpm of the maximum day-[^{3}H]TdR incorporated.

[c] In the autologous MECLR 4 x 10^4 unirradiated EC were cocultured for 6-8 d with 10^5 autologous PBMC.

[d] Psoriasis P = EC from psoriatic plaque; Psoriasis N = EC from uninvolved skin from psoriatic patients.

[e] $p < 0.001$, WT, when compared with MECLR using EC from psoriatic plaques.

tact dermatitis (Table III). The only exception was that most PBMC samples from psoriatic patients contained significantly more transferrin receptor (4.3%; $CD71^+$) and IL-2 receptor (4.4%; $CD25^+$) positive activated T lymphocytes than PBMC samples from healthy controls (0.5% CD71 and 0.2% $CD25^+$; $p<0.001$, WT; Table III). These results may be influenced by the fact that PBMC fractions from three patients contained high numbers (mean 17%) of activated T lymphocytes. If these three patients were excluded, the mean percentage of $CD25^+$ and $CD71^+$ cells in psoriatic patients was 2.9%. Epidermal cells from psoriatic lesions contained more $HLA\text{-}DR^+$ cells than uninvolved and control skin ($p<0.0005$, WT, Table IV).

The roles of skin APC and peripheral blood APC in the autologous MECLR

When purified T lymphocytes from psoriatic patients were cocultured with autologous unpurified EC, lower proliferation rates were observed than after coculturing

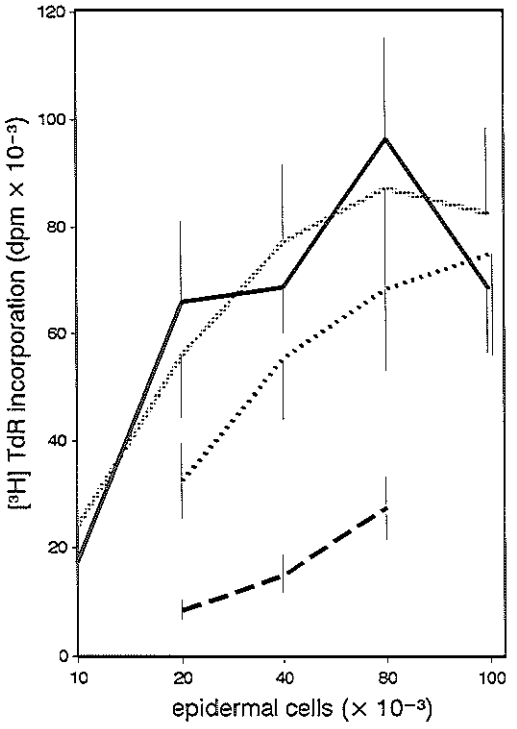

Figure 1. Dose-response curves of the autologous MECLR. Increasing numbers of unpurified epidermal cells from psoriatic lesions were co-cultured with fixed numbers of autologous PBMC (10^5 cells/well). On days 6, 7, and 8 the cultures were pulsed with 0.5 μCi (= 18.5 kBq) [^{3}H]TdR per well and harvested 8 h later. The results are the arithmetic mean ± SEM dpm of quadruplicate cultures on the day of maximum proliferation. Curve, the results of an individual patient.

Table II. The effect of irradiated stimulator EC on the autologous MECLR in psoriatic patients[a]

Patient n=8	EC + PBMC[b]	EC/irr + PBMC	autologous MLR[c]
1	27,801 ± 4,885	24,183 ± 3,619	2,135 ± 1,068
2	22,808 ± 5,000	20,858 ± 1,930	2,858 ± 667
3	18,145 ± 4,202	20,338 ± 3,537	795 ± 317
4	17,102 ± 2,386	22,856 ± 4,637	1,403 ± 216
5	43,041 ± 3,009	38,626 ± 2,156	3,566 ± 691
6	22,052 ± 3,061	22,175 ± 3,551	2,405 ± 1,251
7	20,524 ± 1,661	20,554 ± 1,973	1,643 ± 341
8	16,184 ± 3,286	15,469 ± 724	1,224 ± 356

[a] The results are shown as the mean ± SEM cpm of the maximum [^{3}H]TdR incorporation.
[b] In the autologous MECLR, 4 x 10^4 irradiated and unirradiated EC from psoriatic plaques were cocultured for 6-8 days with 10^5 autologous PBMC. The results obtained using irradiated psoriatic EC are not significantly different from those obtained using unirradiated EC (WSRT).
[c] In the autologous MLR 10^5 PBMC were co-cultured with 10^5 irradiated autologous PBMC.

Table III. Immunophenotyping of PBMC samples from psoriatic patients, controls, and allergic contact dermatitis (ACD) patients[a]

MoAb	Psoriasis (n=24)	Controls (n=60)	ACD (n=24)
CD20	6.1 ± 2.5	7.0 ± 3.7	6.2 ± 3.6
CD3	57.7 ± 8.2	59.1 ± 13.1	61.1 ± 9.6
CD4	38.2 ± 6.6	38.4 ± 10.9	38.1 ± 9.5
CD8	20.5 ± 6.3	20.8 ± 5.4	23.1 ± 5.4
CD4/CD8	2.0 ± 0.9	2.0 ± 0.8	1.7 ± 0.8
CD14	22.6 ± 8.6	18.9 ± 8.5	19.1 ± 7.7
HLA-DR	29.3 ± 8.3	27.5 ± 8.8	27.6 ± 8.1
CD25(IL-2R)	4.4 ± 9.7[b]	0.2 ± 0.2	1.1 ± 0.6
CD71(TFR)[c]	4.3 ± 4.8[b]	0.5 ± 0.5	1.7 ± 1.2

[a] The percentage mean ± SD of monocyte and lymphocyte subsets in PBMC samples from psoriatic patients, healthy controls and ACD patients. Clusters of differentiation (CD) are shown.
[b] $p < 0.001$, WT, when compared to the results in healthy controls.
[c] TFR = transferrin receptor.

unpurified PBMC together with EC (Table V). When the non-T cell fraction (after AET rosetting, APC in Table V) was cocultured with autologous EC no significant prolifera tion occurred (Table V). This demonstrated that T lymphocytes were proliferating in the autologous MECLR. Proliferation of T lymphocytes via back stimulation in the EC suspension was excluded, because irradiation of the EC suspension did not affect the proliferative response in the autologous MECLR (Table III).

The role of peripheral blood and skin APC was studied by eliminating their antigen-processing function by fixing with 0.01% glutaraldehyde for 30 sec. Addition of fixed peripheral blood APC to EC and to purified T lymphocytes resulted in a significantly lower response than when using unfixed peripheral blood APC ($p<0.02$, WSRT). However, when the unpurified fixed EC suspension was used, proliferation was almost completely inhibited, even in the presence of unfixed peripheral blood APC ($p<0.02$, WSRT) (Table V). These results suggest that peripheral blood APC have an accessory rather than an initiating function in the autologous MECLR.

The role of HLA-DR$^+$/CD1a$^+$ and HLA-DR$^+$/CD1a$^-$ epidermal APC in the autologous MECLR

To determine which epidermal APC subset was involved in the activation of T lymphocytes in psoriasis, EC were depleted of HLA-DR$^+$/CD1a$^+$ cells only, or of both HLA-DR$^+$/CD1a$^+$ and HLA-DR$^+$/CD1a$^-$ cells. When the CD1a-depleted EC fraction from psoriastic patients was cocultured with autologous T lymphocytes, a minimal inhibition of the MECLR was observed. In Fig. 2, the paired results of four representative patients are shown. In the remaining patients, paired studies could not be performed owing to insufficient number of EC. However, individual depletion and HLA-DR blocking studies were performed. The results of these studies were in

Table IV. The mean ± SD percentage of HLA-DR$^+$ and CD1a$^+$ cells in EC suspensions from psoriatic patients and controls

	HLA-DR$^+$	CD1a$^+$
Psoriasis P[a] (n = 10)	5.1 ± 1.6[b]%	1.8 ± 0.7%
Psoriasis N[a] (n = 8)	1.9 ± 0.6%	1.6 ± 0.4%
Controls (n = 10)	1.6 ± 0.4%	1.5 ± 0.4%

[a] Psoriasis P = EC from psoriatic plaques; Psoriasis N = EC from uninvolved skin of psoriatic patients.
[b] Significance $p < 0.0005$, WT, when compared to the results in healthy controls.

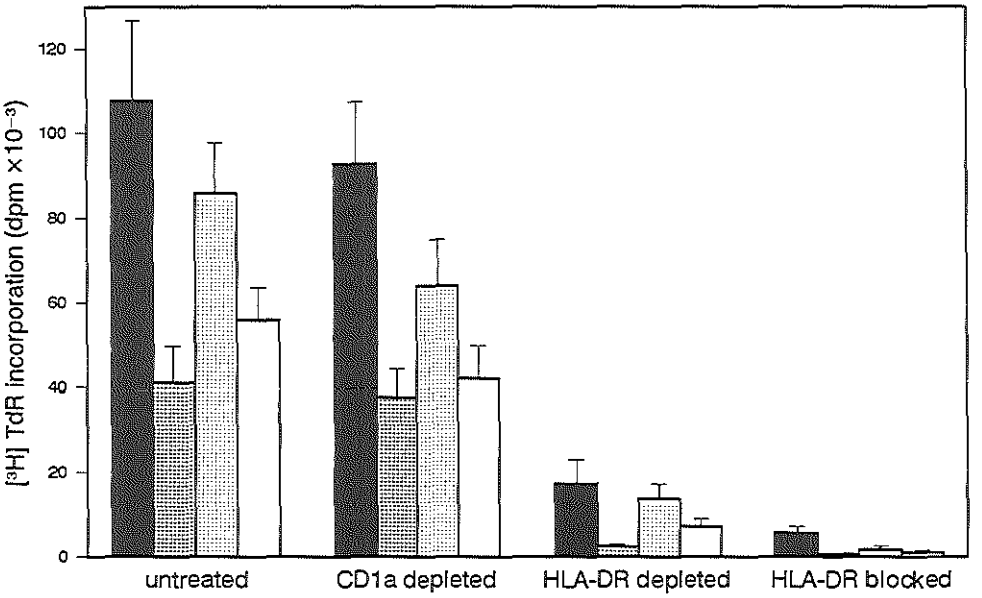

Figure 2. The role of MHC class II positive skin APC subsets in the autologous MECLR. Purified T lymphocytes (10^5 cells/well) were co-cultured with autologous untreated EC, CD1a depleted EC, or CD1a plus HLA-DR-depleted EC, all at a concentration of 4×10^4 EC/well. On days 6, 7, and 8 the cultures were pulsed with 0.5 μCi (= 18.5 kBq) [^{3}H]TdR per well and harvested 8 h later. Blocking was performed by addition of dialyzed anti-HLA-DR MoAb (Becton Dickinson, dilution 1:100) to the cultures. Filled, bold speckled, fine speckled, and blank bars each represents the arithmetic mean ± SEM dpm of quadriplicate cultures on the day of maximum proliferation of an individual patient. Paired results of four representative patients are shown.

agreement with those shown in Fig. 2. Removal of all HLA-DR$^+$ cells from the EC suspension from psoriatic patients resulted in a strong inhibition (mean 80 to 90%) of the autologous MECLR. The same inhibition was observed by the addition of anti-monomorphic HLA-DR MoAb to these cultures (Fig. 2). These results indicate that epidermal HLA-DR$^+$/CD1a$^-$ APC mainly stimulate autologous T lymphocytes in the MECLR, whereas HLA-DR$^+$/CD1a$^+$ APC did weakly or not at all.

Our observation that EC from uninvolved psoriatic skin stimulated autologous T lymphocytes raised the question whether EC from normally appearing skin from psoriatic patients would be able to stimulate in the autologous MECLR. This was studied using PBMC and irradiated EC from an identical twin discordant for psoriasis

Table V. The proliferative activity (mean ± SEM) of *in vitro* (co)cultured peripheral blood cell subsets and EC from psoriatic patients

Co-cultured cell types	Proliferation rate, mean ± SEM in dpm[a] (n = 8)
	basal proliferation rates
EC	127 ± 35
PBMC	2,371 ± 794
T cells	722 ± 102
APC	559 ± 173
EC + APC	831 ± 171
T + APC	1,107 ± 237
	The role(s) of peripheral blood and epidermal APC in autologous MECLR[b]
EC + PBMC	54,024 ± 9,730
EC + T	35,283 ± 13,508
EC + T + APC	61,610 ± 16,690
EC + T + APCfix	32,125 ± 10,819
ECfix + T + APC	1,869 ± 345

[a] Proliferation rates represent the mean ± SEM dpm [^{3}H]TdR incorporation values of quadruplicate cultures of eight patients.

[b] Unpurified EC (4 x 10^4 EC/well) from psoriatic lesions were cocultured with autologous PBMC, purified peripheral blood T cells (10^5 cells/well), or with autologous T cells and non-T cells (APC) (4 x 10^4 cells/well). Glutaraldehyde (0.01%)-fixed APC or EC were also cocultured with purified T cells and unfixed EC or APC. On days 6, 7, and 8 the cultures were pulsed with 0.5 μCi (= 18.5 kBq) [^{3}H]TdR per well and harvested 8 h later.

(women, aged 67, the affected sibling having psoriasis for over 50 years). It appeared that EC from apparently normal skin from the unaffected sibling was able to stimulate autologous PBMC and PBMC from the affected sibling and vice versa (Fig. 3). Although these results were obtained from a single set of twins, they indicated that normal skin from a genetically predisposed individual without the disease had a similar capacity to stimulate proliferation in the MECLR as uninvolved psoriatic skin.

Inhibition of the autologous MECLR by CsA

Preincubation of EC with 2.5 μg CsA per ml resulted in a clear inhibition of the autologous MECLR (n=5, mean = 69 ± 7% SEM, $p < 0.05$, WSRT) (Fig. 4).

Pretreatment with the solvent (ethanol/PBS 1:1000) alone had no effect on the accessory capacity of EC or PBMC (data not shown). To check for the possible

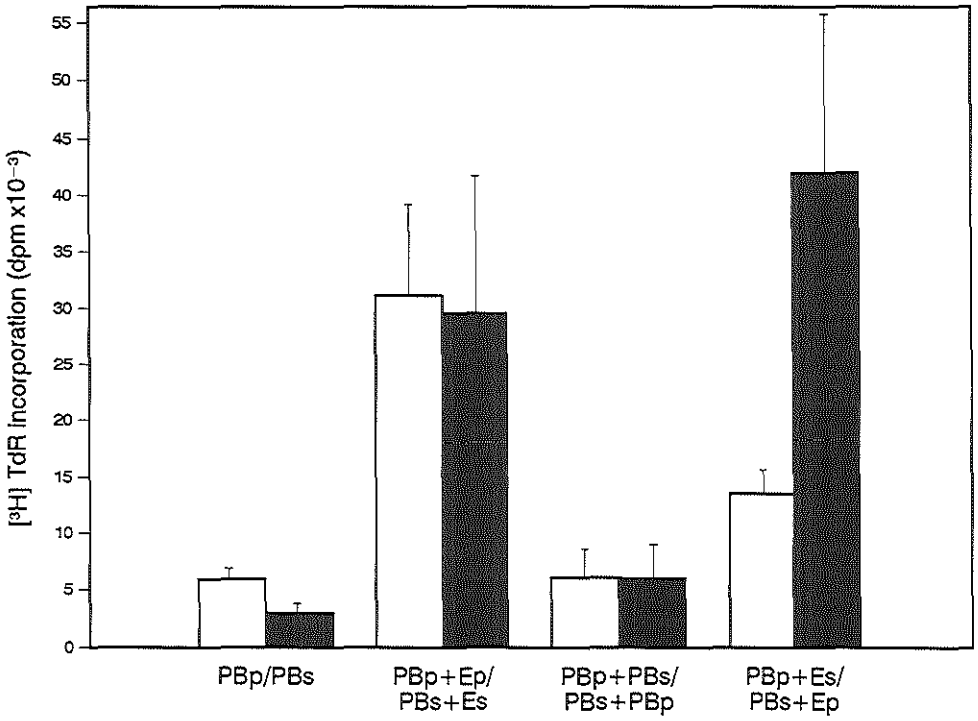

Figure 3. Comparison of the abilities of EC and PBMC from an identical twin discordant for psoriasis to stimulate in a two-way autologous MECLR. Blank bars, the proliferation obtained with responder cells from the psoriatic patient (PBp); solid bars, the proliferation obtained with responder cells from the unaffected twin sister (PBs). Irradiated unpurified EC (from uninvolved skin) and PBMC were cocultured with fixed numbers of autologous responder PBMC (10^5 cells/well) from the psoriatic patient and unaffected sister and vice versa. On days 6, 7, and 8 the cultures were pulsed with 0.5 μCi (= 18.5 kBq) [^{3}H]TdR per well and harvested 8 h later. The results are the arithmetic mean ± SEM dpm of quadruplicate cultures on the day of maximum proliferation.

release of CsA from the preincubated EC, the CsA levels in EC culture supernatants were measured. On days 7 and 8 of culture, low levels i.e., 25 ng CsA per ml, could be measured in three of five culture supernatants. These levels appeared not to interfere with the assay, in that addition of up to 75 ng/ml CsA did not inhibit the autologous MECLR (Table VI) or the ConA response (data not shown). Others have shown that when free CsA was added at the beginning of a MECLR, significant inhibition occurred at concentrations in the 100 ng/ml range [48]. In addition, when EC preincubated with CsA and untreated EC were cocultured together in equal

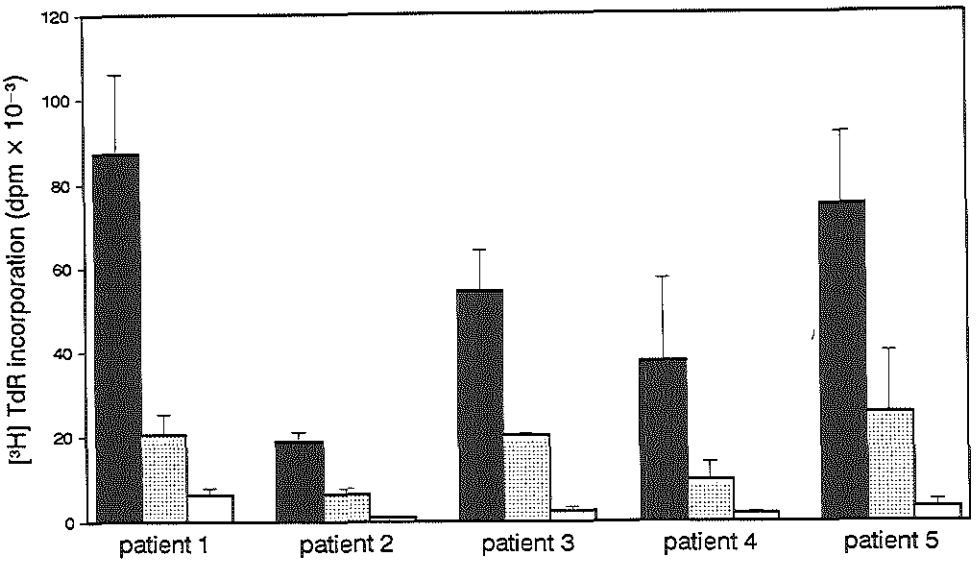

Figure 4. The effect of preincubation of lesional EC with 2.5 μg CsA per ml on the autologous MECLR. Lesional EC were incubated with CsA in RPMI complete medium containing 1% human AB serum for 1 h at 37° C. The cells were washed 5 times with warm RPMI complete medium containing 15% human AB serum. On days 6, 7, and 8 the cultures were pulsed with 0.5 μCi (= 18.5 kBq) [^{3}H]TdR per well and harvested 8 h later. The results are the arithmetic mean ± SEM dpm of quadruplicate cultures on the day of maximum proliferation. The difference between the treated (speckled bars) and untreated (solid bars) is statistically significant ($p < 0.05$, WSRT). Blank bars, the autologous MLR of each patient.

Table VI. The effect of CsA-pretreated EC on the autologous MECLR and neutralization of the inhibitory effects by the addition of fresh EC[a]

	AMECLR	AMECLR/CsA pulse	AMECLR/CsA pulse + Fresh EC
	18,348 ± 3,632[a]	7,828 ± 4,885[a]	21,628 ± 9,503[b]
75 ng CsA	19,882 ± 3,214	nt	nt

[a] Unpurified EC (4×10^4 EC/well) from psoriatic lesions were co-cultured with autologous PBMC (10^5 cells/well). On days 6, 7, and 8 the cultures were pulsed with 0.5 μCi (= 18.5 kBq) [^{3}H]TdR per well and harvested 8 h later.

[b] Proliferation rates represent the mean ± SEM cpm [^{3}H]TdR incorporation of quadruplicate cultures in 5 patients.

numbers (4 x 10^4 each), the CsA-induced inhibition of proliferation in the autologous MECLR was neutralized (Table VI). This suggests that the observed inhibitory effect was caused by the effect of CsA on the accessory function of EC and not by direct inhibition of the proliferation of the co-cultured lymphocytes.

DISCUSSION

The results indicate that in psoriatic patients autologous unpurified EC stimulate T lymphocyte proliferation. Such a reaction could not be demonstrated in the control group of healthy volunteers and in patients with allergic contact dermatitis. Elevated proliferation has been reported in the autologous MECLR in a limited number of healthy individuals [49]. The latter observation cannot be compared to ours, because the number of Langerhans cells (LC) used differed considerably, namely 25 x 10^3 purified LC per well versus approximately 500 - 2000 LC in our experiments. In the autologous MLR, generally high cell numbers are utilized (up to 5 x 10^5 per well) [50,51]. In our study, the number of stimulator EC (2 - 4 x 10^4 per well) was equal to the number of EC used to present antigens such as nickel or tetanus. Using the latter system, low background and high antigen-specific proliferation values are obtained. In the present studies, proliferation in the autologous MECLR was a consistent finding in psoriatic patients, whereas, using identical *in vitro* conditions, it did not occur in a large number of healthy individuals (personal observations). In cutaneous T cell lymphoma, the autologous MECLR was increased when EC from involved skin were used. However, low proliferation values (background) were observed when EC from uninvolved skin were used as stimulators [52].

Recently, it was observed that also after 4 MED UVB irradiation autologous MECLR activity could be demonstrated in which suppressor-inducer and suppressor/cytotoxic T lymphocytes were preferentially activated by HLA-DR^+/$CD1a^-$ epidermal APC [53]. In addition to an elevated autologous MECLR, EC from psoriatic lesions were found to produce leukocyte migration-inhibition factor, whereas these features were not observed in lichen planus [32]. The latter finding seems surprising at first, because lichen planus is known to exhibit some (immuno)histologic characteristics of psoriasis, i.e., a prominent mononuclear inflammatory infiltrate and HLA-DR^+/$CD1a^-$ epidermal cells. These diseases, however, also have fundamental clinical and (immuno)histologic differences. Thus, it is conceivable that both diseases evolve via different pathophysiologic pathways. Based on clinical and experimental observations, it was recently reported whether these two diseases were mutually exclusive [54].

The proliferative response in the autologous MECLR in psoriatic patients depends on the number of unpurified epidermal cells added to the cultures, but is inducible

even by relatively small numbers of autologous EC (10^4 EC, containing ± 500 HLA-DR^+ cells/well). Although it has been shown that psoriatic epidermis contains a considerable number of T lymphocytes, the contribution of these cells to the proliferative response via backstimulation seems negligible (Tables II and V). We calculated an average of one T cell per 10^4 EC using a limiting dilution procedure. This would correspond to four T cells per well (data not shown). Even under optimal culture conditions, such low numbers of T cells would not contribute significantly to the observed high incorporation of [^{3}H]thymidine. The results obtained using gamma-irradiated EC exclude the possibility that the proliferation in the autologous MECLR was due to the presence of epidermal T lymphocytes that were being backstimulated and expanded *in vitro*. However, it may well be that this observation does not strictly reflect the *in vivo* situation. The method used to isolate EC (e.g., trypsin/DNase digestion, mechanical disruption, and sieving) may be responsible for the observed inability of lesional T lymphocytes to participate *in vitro* in the autologous MECLR. The stimulation observed using EC from uninvolved skin also argues against an important role for lesional T lymphocytes in the autologous MECLR in psoriasis, because activated T cells are hardly observed in uninvolved skin. Uninvolved skin of psoriatic patients has been shown to contain small numbers of HLA-DR^+/$CD1a^-$ APC (Table III and [48]). The autologous MECLR induced by EC from uninvolved skin could be blocked by addition of anti-HLA-DR MoAb or by depletion of HLA-DR^+ cells from the EC suspensions (data not shown). The presence of these HLA-DR^+/$CD1a^-$ epidermal APC, in combination with metabolically and biochemically altered keratinocytes, could explain the *in vitro* elicitation of an autologous MECLR by EC from uninvolved skin.

Unpurified EC from psoriatic patients stimulate autologous T lymphocytes in the autologous MECLR and therefore contain the necessary APC to initiate this reaction. The results of the dose-response studies, and depletion and fixation experiments indicate that HLA-DR^+/$CD1a^-$ APC are the principal stimulators in the EC suspensions from psoriatic lesions. The proliferation observed in this system was induced only by autologous unpurified EC and not by autologous peripheral blood APC. Although it is still unclear whether the autologous MLR is exclusively antigen driven, it is clear that MHC class II plays a central role [50]. It might be that epidermal HLA-DR^+ APC present an antigen (putative psoriasis related) to these lymphocytes [13]. The putative antigen is possibly MHC class II associated with X, where X may represent a yet-undefined (probably skin-specific) peptide residing in the peptide-binding groove of the MHC class II molecule [33,50,53,55].

The real specificity of *in vitro* cloned putative autoreactive T cells remains of concern [56]. Non-specific effects induced as a result of cross-reactivity especially with xenogeneic proteins (e.g., FCS components, SRBC components) in the culture medium should be minimized or prevented [56]. The chance of such effects in short term *in vitro* memory - T cell responses as performed by us is negligible, because we

used medium containing human AB serum for culturing and rinsing. The occurrence of such interference in our system is also highly unlikely, because whole PBMC in the autologous MLR, cocultured purified T and non - T cells from psoriatic patients, and cocultured PBMC and EC in controls, showed only background levels of proliferation (Tables I and V). The results of fixation studies suggest that glutaraldehyde fixation inhibited the re-expression of such an antigen in association with MHC class II on the plasma membrane of EC. This possibility cannot be ruled out, because EC were fixed immediately after trypsinization. This means that the antigen is probably trypsin sensitive and that it is re-expressed on the membrane of EC in the initial phase of culturing. Induction of the autologous MECLR by sheer-increased numbers of (bare) HLA-DR molecules occurring in psoriatic lesions is unlikely, as HLA-DR molecules are not affected by trypsin and/or glutaraldehyde treatment. This was confirmed in immunofluorescence studies in which no alteration in HLA-DR expression on PBMC and EC was observed following trypsin and/or glutaraldehyde treatment. Removal of 40 to 90% of the total HLA-DR^+ EC population by depleting HLA-DR^+/$CD1a^+$ LC from the EC resulted only in mild inhibition of the autologous MECLR (Fig. 3). Furthermore, fixation with glutaraldehyde did not inhibit the antigen-presenting function of nickel-pulsed EC (data not shown), indicating that at the concentrations of glutaraldehyde used, HLA-DR molecules remained functional. These results suggest that the stimulatory capacity of epidermal APC is altered in psoriatic lesions. Indeed, epidermal APC from psoriatic lesions have recently been shown to stimulate allogeneic T lymphocytes more strongly than EC from uninvolved psoriatic skin or from healthy individuals [33]. This increased allogeneic stimulation was attributed to an increased number of HLA-DR^+/$CD1a^-$ cells in lesional EC suspensions. In this study, we also observed higher numbers of HLA-DR^+ cells than $CD1a^+$ cells in EC suspensions from psoriatic lesions ($p < 0.0005$, WT; Table IV). We analyzed the role of these two (dendritic) cell subsets in the autologous MECLR by selective depletion of one or both of these APC from the EC suspensions. Our observation that HLA-DR^+/$CD1a^+$ LC were poor stimulators of the autologous MECLR is in sharp contrast to the superior capacity of these cells to present native antigen to specific T lymphocytes or T cell hybridomas [33,57-60]. In the latter system, depletion of HLA-DR^+/$CD1a^+$ cells from fresh EC suspensions resulted in complete inhibition of proliferation. This discrepancy is attributed to the findings that in normal human epidermis the LC is the only population that expresses HLA-DR, whereas in psoriatic lesions also other cell types express HLA-DR [16-18,41]. The current results show that a HLA-DR^+/$CD1a^-$ APC subset indeed plays a crucial role in the autologous MECLR.

The inhibition of the autologous MECLR by CsA is not surprising, as it has been shown that CsA is able to inhibit the antigen-presenting function of APC in other systems [61,62]. It was recently demonstrated that the antigen-presenting capacity in the skin of psoriatic patients undergoing oral CsA therapy is significantly inhibited in

an allogeneic MECLR model [48]. Here we show that *in vitro* treatment of psoriatic EC with CsA also inhibited their capacity to stimulate autologous T lymphocytes. The latter results support the conclusions by Cooper et al that one of the modes of action of CsA in psoriasis occurs via the inhibition of antigen-presenting activity of lesional epidermal APC [48].

Recently, it was reported that cultured LC lost the expression of CD1 and cytoplasmic Birbeck granules, while concurrently the expression of MHC class II was upregulated and powerful accessory functions were acquired. Cultured LC became potent stimulators of both autologous and allogeneic MECLR [57-60,63]. The data on the capacity of fresh and cultured LC to present and process antigen remain controversial [60,62 vs 59]. Streilein et al attempted to clarify this matter by using murine cells, and showed that fresh LC processed and presented native antigen more efficiently than cultured LC, whereas cultured LC appeared equally efficient in presenting preprocessed antigen and superior at stimulating allogeneic T lymphocytes [63]. In the same study [63], it was also shown that cultured LC exhibit a unique feature that was not shared by fresh LC namely an extraordinary capacity to stimulate syngeneic T lymphocytes. The authors considered "fresh and cultured LC as the *in vitro* representatives, respectively, of their *in vivo* counterparts: intraepidermal LC, and LC that have migrated to draining lymph nodes (nodal LC)".

Elevated levels of cytokines and of inflammatory mediators may alter the microenvironment in psoriatic lesions in such a way that *in vivo* lymph node or *in vitro* culture conditions are simulated. Because the phenotype and some functional characteristics of "cultured" LC and HLA-DR$^+$/CD1a$^-$ APC occurring in psoriatic lesions may be similar, it is tempting to speculate that this subset resembles the *in vivo* equivalent of *in vitro* "cultured" LC. It remains to be established whether the HLA-DR$^+$/CD1a$^-$ APC subset in EC in other dermatoses exhibit similar functional characteristics.

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Chapter 4

THE ROLE OF THE LFA-FAMILY OF ADHESION MOLECULES, CD36(OKM5) AND MEMBRANE BOUND IL-1 (mIL-1) IN THE AUTOLOGOUS MECLR IN PSORIASIS*

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SUMMARY

Increased levels of "aberrant" and certain constitutive membrane molecules such as CD36(OKM5), CD54(ICAM-1), γ-IP10, mIL-1 and CD29(4B4) are expressed by epidermal cells (EC) from plaque-type psoriatic lesions. These molecules are involved in important accessory or signalling functions in the immune system. It is not yet clear whether these molecules are only passively expressed or whether they fulfill an important biological role in psoriasis. The first objective of this study was to corroborate and compare the expression of CD29, CD45RA, CD54(ICAM-1), CD36(OKM5) and mIL-1 in cryostat skin sections and in epidermal cell suspensions (ECS) using immunoperoxidase and immunofluorescence techniques. Secondly, the functional relevance of lymphocyte function-associated antigens (and ligands), CD36(OKM5) and mIL-1 was delineated *in vitro* in the autologous mixed epidermal cell - T lymphocyte reaction (MECLR). Their role was studied by blocking with specific MoAb and antisera or, in the case of CD36(OKM5), by depleting CD36(OKM5)$^+$ cells from the ECS prior to culturing. Increased numbers of CD36(OKM5)$^+$ (27 ± 12%), CD54(ICAM-1)$^+$ (32 ± 16%) and mIL-1$^+$ (55 ± 23%) cells were observed in ECS from psoriatic lesions. In ECS from normal skin the numbers of CD36(OKM5)$^+$ and CD54$^+$ cells ranged between 0 and 2% in all cases, while for mIL-1 this figure was 10 ± 3%. MoAb to CD2, CD11a(LFA-1α), CD18(LFA-1β), CD54(ICAM-1) and CD58(LFA-3), and an antiserum to IL-1, inhibited the autologous MECLR in all patients. This indicates that the adhesion molecules and mIL-1 on EC from psoriatic lesions fulfill an accessory role in the autologous MECLR. Depletion of CD36(OKM5)$^+$ cells from psoriatic ECS did not affect the proliferative responses in the autologous MECLR demonstrating that, whereas the expression of CD36(OKM5) in ECS from psoriatic lesions was increased, this molecule was not involved in the stimulation of autologous T lymphocytes in psoriasis. This is in clear contrast to the pivotal role played by UV-induced CD11b(OKM1)$^-$/CD36(OKM5)$^+$ epidermal APC in the autologous MECLR and by CD11b(OKM1)$^-$/CD36(OKM5)$^+$ peripheral blood APC in the autologous MLR. The accessory function of mIL-1 in the autologous MECLR observed in this study illustrates its role in psoriasis and emphasizes the relevance of its presumed dysregulation in this disease.

INTRODUCTION

The autologous MECLR is elevated in psoriasis patients. This is mainly due to the spontaneous stimulation of autologous T lymphocytes in the absence of antigen by HLA-DR$^+$/CD1a$^-$ epidermal APC [1-3]. Cytokines produced by activated lymphocytes have been shown to be mitogenic for keratinocytes and to induce the

increased expression of "aberrant" molecules in psoriatic skin [4-9]. These observations are in line with the important role(s) played by immuno-pathogenetic mechanisms in this disease [7,10-15].

Epidermal cells from psoriatic lesions express increased levels of "aberrant" membrane molecules such as CD36(OKM5), CD54(ICAM-1) and γ-IP10 [16-19]. Increased levels of constitutive molecules such as membrane bound IL-1(mIL-1) and CD29(4B4) have also been reported [20-22]. Recently, meticulous studies showed that normal human epidermis contains very few CD36(OKM5)$^+$ cells [17,23]. Native and recombinant lymphokines such as IFN-γ, IL-1, IL-4, and/or TNF-α, have been shown to induce or upregulate, *in vivo* and *in vitro*, the expression of γ-IP10, HLA-DR, ICAM-1 and/or CD36(OKM5) on keratinocytes [4,5,24]. Whether the "aberrant" molecules are only passively expressed by keratinocytes or fulfilled an important biological role in (inflammatory) skin diseases is unclear and has led to several speculations [16,18,25-28]. The functional significance of these molecules on epidermal cells has been questioned since these molecules are involved in important inter-leukocyte accessory or signalling functions in other *in vivo* and *in vitro* systems.

In the autologous mixed lymphocyte reaction (MLR), CD11b(OKM1)$^-$/CD36 (OKM5)$^+$ monocytes are potent stimulators of autologous T lymphocytes [29]. Epidermal CD11b(OKM1)$^-$/CD36(OKM5)$^+$ APC which are induced in human skin by UV-irradiation show the same distinctive antigen-presenting properties as their counterparts in the peripheral blood [25,26,30]. Apart from being the thrombospondin-receptor, CD36(OKM5) also functions as the receptor for Malaria falciparum [31,32] and as such it plays an important role in the sequestration of this parasite [33,34].

In psoriatic lesions, CD36(OKM5) is intensely expressed on keratinocytes, sporadically on intra-epidermal dendritic cells and consistently on dermal dendritic and endothelial cells. Although the number of intra-epidermal CD36(OKM5)$^+$ dendritic cells appeared to be rather low, it was considered possible that in co-operation with keratinocytes, these cells could stimulate autologous T lymphocytes in the autologous MECLR [35-39]. In addition, keratinocytes can produce different pro-inflammatory cytokines [40]. Therefore, keratinocytes may play a more important role in causing inflammation than previously suspected [7,24,40]. Furthermore, keratinocytes seem to regulate skin inflammation via their ability to modulate cutaneous immunological reactions, e.g. by inducing T cell unresponsiveness or by producing cytokine inhibitors [37,40-42].

Anti-CD11a (LFA-1α) and anti-CD18(LFA-1β) MoAb have been shown to inhibit (allo)antigen-specific and mitogen-induced T cell activation and proliferation [43-45]. The activation of antigen-specific peripheral blood T lymphocytes via epidermal APC is more dependent on adhesion molecules than activation via peripheral blood APC [46]. Epidermal cells from psoriatic lesions express an increased level of ICAM-1 [7,9,47-49]. Generally LFA-1/ICAM-1 are involved in T lymphocyte-endothelial cell

interactions which are necessary for their migration to the sites of inflammation. Such a mechanism has also been demonstrated in psoriasis [8,13,15,50]. Adhesion molecules such as CD11a(LFA-1α), CD18(LFA-1β) and CD54(ICAM-1) thus play a role in the immune response by facilitating antigen-presentation or lymphocytic infiltration [45,51,52]. Both CD2 and LFA-3 are more specifically involved in the regulation of T cell functions. With the exception of NK cell-mediated cytolysis, the patterns of inhibition caused by anti-CD2 and anti-CD58(LFA-3) MoAb were similar to those observed using anti-LFA-1(α and β) MoAb [45,53]. Thus far, the role of CD2, CD11a(LFA-1α), CD18(LFA-1β), CD54(ICAM-1) and CD58(LFA-3) in the activation of autologous T lymphocytes in psoriasis has remained unclear.

Membrane bound IL-1 (mIL-1) fulfills an important accessory role in the activation of autologous T lymphocytes *in vitro* in several (murine) models [54-56]. The function of mIL-1 in humans has also been documented [57-61]. In a previous study, we reported on the increased expression of mIL-1 on keratinocytes from psoriatic lesions [20]. A dysregulation of IL-1 in psoriasis has also been observed by others [62-64]. Blocking of IL-1 (receptor) with natural or recombinant IL-1 inhibitors, analogs and/or antagonists seem promising and their application in clinical trials is planned [65-69]. This prompted us to investigate the possible functional role(s) of mIL-1 in psoriasis.

In this communication, we report the results of our investigations into the expression of certain constitutive and "aberrant" molecules, the LFA-family of adhesion molecules, and especially mIL-1, in cryostat sections and ECS of lesional psoriatic skin. The results were compared with the results of *in vitro* studies on their functional role in the autologous MECLR.

MATERIALS AND METHODS

Patients and controls

Otherwise healthy patients with stable plaque-type, untreated psoriasis were investigated after informed consent had been obtained. The autologous mixed lymphocyte reaction (MLR) and the autologous MECLR were performed in all patients. The number of patients in each experiment is shown in the Tables and Figures (n=x). The epidermal cell suspensions (ECS), cryostat skin sections and PBMC were immunophenotyped using a panel of MoAb. As controls, the MLR, autologous MECLR and *in vitro* blocking studies were performed using ECS and PBMC of patients with patch test-confirmed nickel contact dermatitis and also using nickel and tetanus toxoid as antigens. Cryostat skin sections and ECS from nickel dermatitis patients and healthy volunteers without history or signs of skin disease undergoing abdominal plastic surgery served as controls in the immunophenotyping investigations.

Preparation of ECS

Split-skin specimens from involved skin of psoriasis patients and from surgically excised abdominal skin of controls were obtained using a portable dermatome. In nickel dermatitis patients, blister roofs were obtained using the suction blister technique [70]. Single cell suspensions of EC were

prepared using standard methods [71]. Briefly, the split-skin specimens were rinsed thoroughly with phosphate-buffered saline (PBS), cut in pieces of ± 0.5 cm^2 and floated in Ca^{2+} and Mg^{2+} -free PBS containing 0.0125% trypsin (ICN Biochemicals, Cleveland, OH) and 0.02% EDTA for 45 min at room temperature and in the presence of 0.025% deoxyribonuclease solution (DNase; Sigma, St. Louis, MO) during the last 15 min of the incubation. At this stage, the epidermis was separated from the dermis using fine forceps. The epidermal sheets were cut with scissors and the EC were filtered through sterile 100-μm and 30-μm mesh nylon gauzes and resuspended in RPMI 1640 medium supplemented with penicillin, streptomycin, fresh L-glutamine, 20 mM Hepes (RPMI complete medium), 20% heat-inactivated human AB serum and 0.01% DNase. Cells were counted using a hemocytometer and their viability was determined by trypan blue exclusion.

Removal of CD36(OKM5)$^+$ cells from ECS

CD36(OKM5)$^+$ cells were removed from ECS with an immunomagnetic rosetting technique using anti-CD36(OKM5) MoAb and goat-anti-mouse Ig conjugated paramagnetic beads (Dynal, Oslo, Norway) [72]. Briefly, suspended EC were labeled for 30 min at 4°C with optimal dilutions of MoAb, washed three times, incubated with the conjugated magnetic beads, spun down and the pellet incubated for 30 min. The pellet was carefully resuspended and the rosettes were counted using a hemocytometer. The rosetted fraction was concentrated to one side of the tube using a strong magnet. The non-rosetted cells were collected, leaving the rosetted cells adhered to the side of the tube. The latter procedure was repeated twice. In all depletion experiments, the number of OKM5(CD36)$^+$ EC was assessed using an indirect immunofluorescence technique. The number of rosetted cells was compared with the fluorescence data before depletion and the efficiency of the depletion was checked again later by immunofluorescent staining and counting under a microscope.

Isolation of peripheral blood mononuclear cells

PBMC were isolated using density gradient centrifugation on Lymphoprep (density 1.077, Nyegaard, Oslo, Norway) [73]. All rinsing, also when isolating other cells, was done in RPMI complete medium containing 1% human AB serum; FCS was not used in this study.

Isolation of purified T lymphocytes

T lymphocytes were isolated with a rosetting technique using 2-aminoethylisothiouronium bromide (AET)-treated sheep red blood cells [74]. The purity of this fraction as determined by immunofluorescence after incubation with anti-CD3(Leu4) and FITC-conjugated goat-anti-mouse Ig secondary antibody was found to be approximately 95%, whereas 99% was CD2(OKT11)$^+$ In this fraction, contamination with CD14$^+$ monocytes and CD20$^+$ B lymphocytes ranged from 0 to 1% as determined by fluorescence microscopy or FACscan analysis (Becton Dickinson, Sunnyvale, CA).

Monoclonal antibodies and immunophenotyping of cell suspensions

The following MoAb were used for immunophenotyping the peripheral blood samples of all patients and controls: CD2 (OKT11, Ortho Diagnostic Systems, Raritan, NJ), CD11a(LFA-1α) (CLB-LFA1/2, Dr. R. van Lier, Central Laboratory of the Red Cross Blood Transfusion Service (CLB), Amsterdam), CD18(LFA-1β) (CLB-LFA1/1, Dr. R. van Lier), CD54(ICAM-1) (RR1/1.1.1, Dr. R. Rothlein, Boehringer Ingelheim, Ridgefield, CC), CD58(LFA-3) (TS2/9, Dr. T.S. Springer, Dana Farber Institute, Boston, MA), CD3(Leu-4), CD4(Leu-3), CD5(Leu-1), CD8(Leu-2), CD16(Leu-11b), CD25(IL-2 receptor), HLA-DR (all from Becton Dickinson), CD20 (B1, Coulter Immunology, Hialeah, FL), CD11b(CR3,OKM1) and CD36 (OKM5) (Ortho Diagnostic Systems), CD71 (transferrin receptor, T9, 66IG10, Dr. M. van der

Rijn, Amsterdam), CD14 (My3, My4, Dr. J.D. Griffin, Boston, MA), CD15 (VIM-D5, Dr. W. Knapp, Vienna, Austria), CD29 (4B4, Coulter Immunology), CD45RA (2H4, Coulter Immunology), IL-1β (Cistron Biotechnology, Pine Brook, NJ) and TcR-$\gamma\delta$ (11F2, Dr. J. Borst, The Netherlands Cancer Institute, Amsterdam).

The following MoAb were used for immunophenotyping the ECS and/or cryostat skin sections: CD1a (66IIC7, Dr. M. van der Rijn), CD54(ICAM-1) (RR1/1.1.1, Dr. R. Rothlein), HLA-DR, CD45(HLe-1-FITC-conjugated, Becton Dickinson), CD36(OKM5, Ortho), TcR-$\gamma\delta$ (11F2, Dr. J. Borst), CD29(4B4, Coulter), CD45RA(2H4, Coulter), anti-IL-1β (Cistron).

Immunophenotyping of EC, density gradient isolated PBMC or whole blood samples were performed using indirect immunofluorescence and double-labeling (SimulSET^{R}) immunofluorescence techniques, followed by FACscan analysis. The indirect immunofluorescence technique was performed as described by Van Dongen et al. [75]. CD36(OKM5)/CD45 double-labeling comprised addition of rhodamine labeled goat-anti-mouse IgG to detect OKM5, incubation for 20 min with 10% normal mouse serum followed by incubation with the optimal concentration of HLe1-FITC. Double-labeling was also performed to detect CD3/CD4, CD3/CD8, CD3/CD25 and CD3/HLA-DR double positive cells according to prescribed standard procedures (SimulSET^{TM}). The FACscan data were analyzed using the SimulSET^{C} and Lysis^{C} software programs (Becton Dickinson). Some peripheral blood and all EC samples were counted using Standard Zeiss 16 microscopes equipped with a IV FL epi-illumination condensor (Carl Zeiss, Oberkochen, Germany). The percentage of positive cells was calculated by counting the number of cells showing positive fluorescence from a total of 200 - 400 cells. The results were expressed as the mean percentage ± SD.

Indirect immunoperoxidase staining of cryostat skin sections

Immunohistochemistry was performed on 5 μm thick cryostat sections of OCT embedded biopsy specimens from lesional skin. Indirect immunoperoxidase staining was done with 3 amino-9-ethyl-carbazole (AEC) as the substrate. Briefly, the thoroughly air-dried sections were fixed in acetone for 5 min at room temperature, dried again and rinsed in PBS (pH 7.8) containing 0.1% bovine serum albumin, incubated for 30 min at 37°C with an optimal dilution of the primary MoAb and subsequently with the peroxidase-conjugated secondary rabbit anti-mouse Ig antiserum in the presence of 10% normal human and 10% normal rabbit serum. Finally, the sections were rinsed with distilled water and counter-stained slightly with Mayer's hematoxylin and mounted in Aquamount (BDH, Pool, UK). The controls comprised the use of isotype-control MoAb to an irrelevant epitope and omission of the first and second steps (PBS), and omission of the addition of normal human and rabbit sera.

Autologous MECLR and MLR

Mixed cultures of unpurified EC and PBMC were set-up in quadruplicate in round-bottomed microtiter plates (Costar, Cambridge, MA). To each well, 4×10^4 EC and $1 - 2 \times 10^5$ PBMC were added in a final volume of 0.2 ml complete RPMI medium supplemented with 15% human AB serum. The autologous MLR comprised the spontaneous proliferation of separately plated fractions of PBMC. In a previous study, the stimulatory capacity of 30 Gy irradiated EC and PBMC were compared with that of unirradiated EC and PBMC. Since the autologous response using unirradiated EC and PBMC did not differ significantly, only unirradiated EC and PBMC were used [3]. On days 6, 7 and 8 the cultures were pulsed with 0.5 μCi = 18.5 kBq tritiated thymidine ([^{3}H]TdR, 185 GBq/mmol, Amersham, Buckinghamshire, UK) and harvested 8 hours later using a semi-automatic cell harvester (Skatron, Oslo, Norway) onto glass fiber filters. The amount of [^{3}H]TdR that was incorporated was determined by liquid scintillation counting (β-plate, Pharmacia/LKB, Uppsala, Sweden). The results were expressed as mean ± SEM counts per minute (cpm) of quadruplicate cultures.

Table I. Source and specificity of antibodies used in depletion and blocking experiments

MoAb	Source	Specificity	Isotype	Final concentration
NKI L-07	Netherl. Cancer Inst.	LFA-1α/CD11a	IgG1	10 μg/ml
CLB LFA1/1	Centr. Lab. Blood Trans	LFA-1β/CD18	IgG1	10 μg/ml
OKT11	Ortho Co.	LFA-2/CD2	IgG2a	1 μg/ml
TS 2/9	T.S. Springer	LFA-3/CD58	IgG1	1 μg/ml
RR1/1.1.1	R.R. Rothlein	ICAM-1/CD54	IgG1	1 μ/ml
HLA-DR	Becton Dickinson	–	IgG2a	0.25 μg/ml
OKM5	Ortho Co.	CD36	IgG1	1 μg/ml
Polyclonal antibodies				
Rabbit α-IL-1	J. van Damme*	human IL-1β	–	1/1000
Goat α-IL-6	L. Aarden#	human IL-6	–	1/1000

Normal goat, mouse and rabbit sera, at a dilution of 1/100, were used as controls.

* Ref: Van Damme J, De Ley M, Van Snick J, Dinarello CA, Billau A. J Immunol 139:1867-1872, 1987.

\# Ref: Helle M, Boeije L, de Groot E, de Vos A, Aarden LA. J Imunol Meth 138:47-56, 1991.

Blocking of the autologous MECLR with antibodies

The MoAb used in blocking experiments are summarized in Table I. Sodium azide (NaN_3), a toxic preservative was removed from the MoAb by overnight dialysis at 4°C against an excess of RPMI complete medium. They were then stored at -20°C until use. In some experiments MoAb specific for monomorphic MHC class II (anti-HLA-DR, Becton Dickinson) were added to the mixed epidermal cell - T lymphocyte cultures. In other studies at our laboratory this MoAb effectively blocked MHC class II restricted responses.

The antiserum to IL-1 was raised in a rabbit by immunization with highly purified natural human IL-1β. It contained no preservatives and was shown to neutralize natural as well as recombinant human IL-1β (Dr. J. Van Damme, Leuven, Belgium). The antiserum to IL-6 was raised in a goat by immunization with human recombinant IL-6. It too contained no preservatives and was shown to neutralize natural as well as recombinant human IL-6 (Dr. L. Aarden, CLB, Amsterdam). At our laboratory complete neutralization was observed with these antisera at dilutions of 1/16,000, 2.5 IU/ml rIL-1β and 5 IU rIL-6/ml in the D10N and the B9 bio-assays, respectively.

Positive control antigens in the *in vitro* T lymphocyte proliferation assay

A stock solution containing pure tetanus toxin (exotoxin from Clostridium tetani), without preservatives or aluminum hydroxide, was purchased from Calbiochem/Behring Diagnostics (San Diego, CA) and stored at 4°C. It was used at a final concentration of 3 μl/ml in cultures. Concanavalin A (Con-A) type IV-S was purchased from Sigma Chemical Co. and used at a final concentration of 10 μl/ml in cultures. A stock solution containing 10 mg nickel-sulphate ($NiSO_4.6H_2O$ analytical grade, purity >99%, Merck, Darmstadt, Germany) per ml was prepared in distilled water and stored at -20°C. It was

used in culture at final concentrations of 5, 7.5 and 10 μg/ml. The culture and labeling conditions and other procedures parallelled those used in the autologous MECLR.

Statistical analysis

The results were analyzed with STATA™ (computer program for statistical analysis, Computing Resource Center, Los Angeles, CA). The test used to determine statistical significance is mentioned in the abbreviated form e.g Wilcoxon test (WT) and Wilcoxons signed rank sum test (WSRT), together with the "p" values.

RESULTS

Immunophenotyping of peripheral blood cells

In all psoriasis patients the absolute number of peripheral blood leukocytes, lymphocytes and monocytes fell within the normal range. The peripheral blood of most patients, however, contained an increased fraction of activated ($CD3^+/CD25^+$, $CD3^+/HLA\text{-}DR^+$) T cells as determined by double immunofluorescence staining ($p < 0.01$, WT, not shown). The number of $TcR\gamma\delta^+$ peripheral blood T lymphocytes, $CD5^+$ B cells and of $CD11a^+$, $CD18^+$, ICAM-1^+, LFA-3^+, $OKM1^+$, $OKM5^+$, mIL-$1\beta^+$ peripheral blood leukocytes did not differ significantly from those of controls (not shown).

Immunophenotyping of ECS and immunohistology of cryostat skin sections

In the ECS from psoriatic lesions no $TcR\gamma\delta^+$ cells, but 3.2 ± 0.5% $CD45^+$ cells were observed. In psoriatic ECS, the number of CD36(OKM5)$^+$, CD54(ICAM-1)$^+$ and mIL-$1\beta^+$ cells were clearly increased. Immunofluorescence microscopy showed that lesional psoriatic ECS contained 23 ± 12% CD36(OKM5)$^+$, 31 ± 14% ICAM-1^+ and 57 ± 21% mIL-1^+ cells (Table II). In psoriatic ECS, the number of cells that were positive for both CD45 and CD36(OKM5) was less than 1% in all experiments. Uninvolved ECS

Table II. Mean ± SD percentage of CD36(OKM5)$^+$, CD58(ICAM-1)$^+$ and mIL-1^+ EC in lesional and uninvolved psoriatic skin, and in healthy control skin

	PP*	PN*	NN*
CD36(OKM5)$^+$	23 ± 12%	0.3 ± 0.1%	0.5 ± 0.1%
CD58(ICAM-1)$^+$	31 ± 14%	0.6 ± 0.1%	0.5 ± 0.1%
mIL-1^+	57 ± 21%	12 ± 3%	4 ± 0.5%

* PP= lesional psoriatic skin (n=15); PN= uninvolved psoriatic skin (n=8); NN= healthy control skin (n=10).

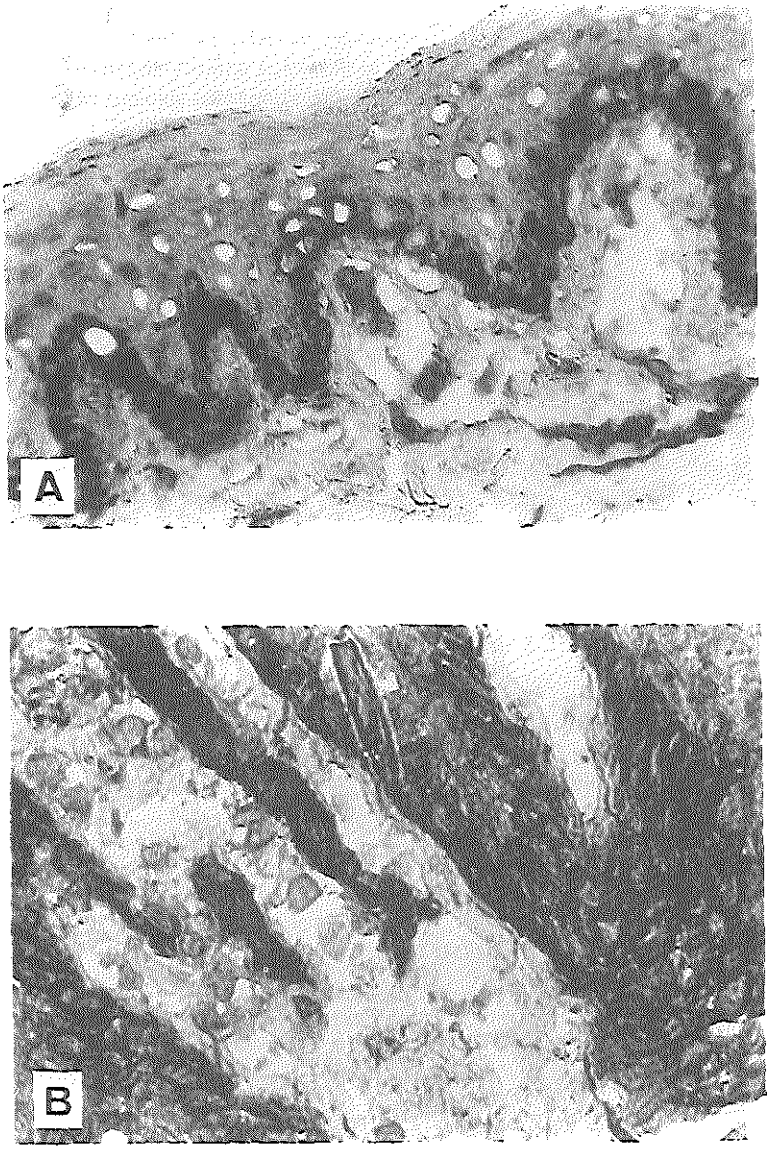

Figure 1. Expression of CD29 in uninvolved (A) and involved (B) psoriatic skin (indirect immunoperoxidase technique, magnification x250 and x400 respectively). Note CD29 expression only on basal cells in A, while in lesional psoriatic epidermis (B) expression on suprabasal cells is also seen.

from psoriasis patients contained 0-2% CD36(OKM5)$^+$ and ICAM-1$^+$ cells, but 12 ± 3% mIL-1$^+$ cells ($p < 0.05$, WT, if compared to normal control skin). In ECS from healthy controls, the number of CD36(OKM5)$^+$ and CD54(ICAM-1)$^+$ EC was negligible (means 0.5 and 0.5, range: 0 to 2%). The number of cells that were positive for mIL-1 was 4 ± 0.5%, which could be upregulated to a maximum of approximately 30% mIL-1$^+$ cells 6 hours after *in vitro* UVB-irradiation (not shown). The results of immunofluorescence and immunohistochemical investigations into the expression of mIL-1 in psoriasis are not shown here since they have already been published separately [20]. The results of microscopical examination of the immunohistochemically stained cryostat sections were as follows.

CD29(4B4). The constitutive expression of CD29 on basal epidermal cells was clearly increased in cryostat sections of psoriatic lesions. It was frequently observed that both single cells and clusters of cells located in the spinous layer were also positive (Figs. 1a and 1b).

CD36(OKM5). There was a characteristic strong intercellular expression of CD36(OKM5) in the upper epidermal layers similar to that reported by others [16]. Cells with a clear dendritic morphology which were CD36(OKM5)$^+$ were sporadically observed intra-epidermally, but in contrast, these cells were frequently observed in the upper papillary dermis. Endothelial cells were also strongly positive.

CD45RA(2H4). Epidermal cells from psoriatic lesions expressed significantly decreased levels of CD45RA as compared with uninvolved psoriatic skin and healthy control skin. In cryostat sections from the latter, the strongest staining was normally observed in the supra-basal layers. Lesional cryostat sections in most cases showed a faint diffuse staining indicating that the characteristic staining pattern in the upper epidermal layers was lost (Figs. 2a and 2b).

CD54(ICAM-1). ICAM-1 was expressed on the keratinocytes overlying the clusters of inflammatory cells in the dermis. Keratinocytes overlying and flanking the rete ridges showed the most pronounced staining. Dermal capillaries and some dermal mononuclear cells were also positive (not shown).

Blocking of the autologous MECLR with MoAb to adhesion molecules

The functional role of adhesion molecules was investigated by adding blocking antibodies to the autologous MECLR. The proliferative responses that were obtained were compared with the proliferative responses of untreated epidermal cells co-cultured with autologous peripheral blood lymphocytes. The results of blocking experiments using MoAb specific for the adhesion molecules CD2(LFA-2/T11), CD11a(LFA-1α), CD18(LFA-1β), CD54(ICAM-1) and CD58(LFA-3) are shown in Fig. 3. It can be seen that all MoAb significantly inhibited the autologous MECLR ($p < 0.0025$, WSRT).

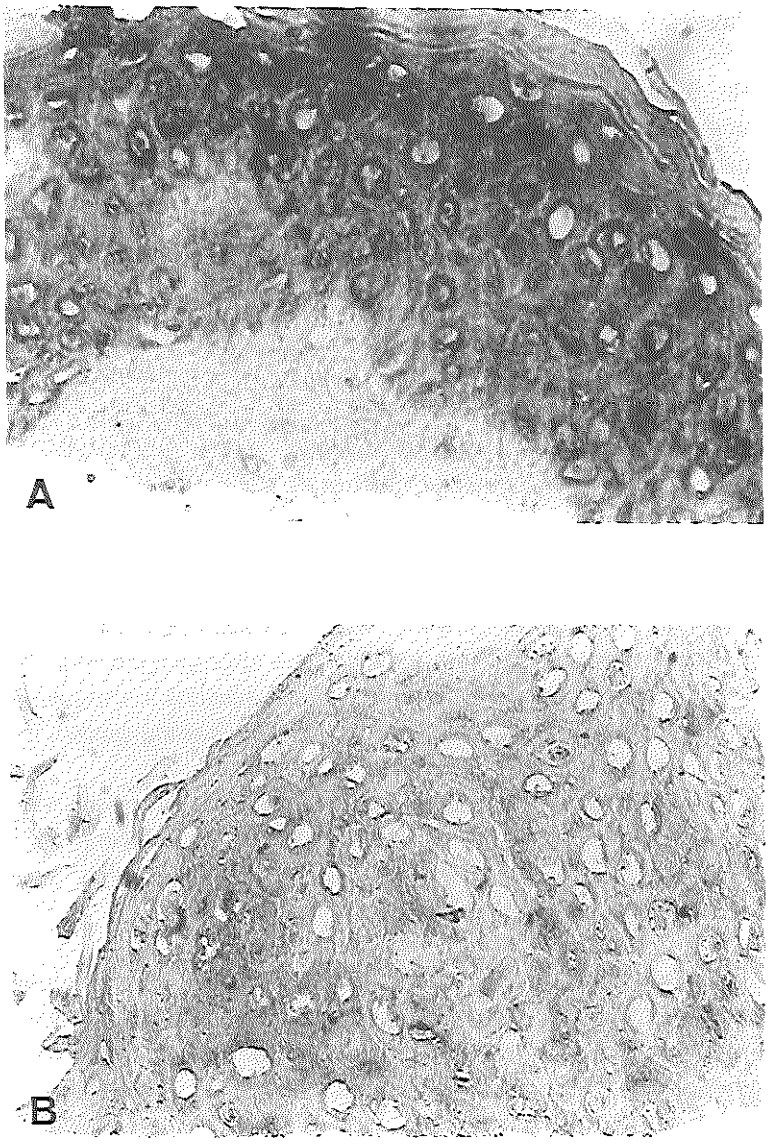

Figure 2. Expression of CD45RA in uninvolved (A) and involved (B) psoriatic skin (indirect immunoperoxidase technique, magnification x400). A diffuse (nuclear, cytoplasmic and weak membrane) staining is observed in A, whereas the expression of CD45RA is reduced in B.

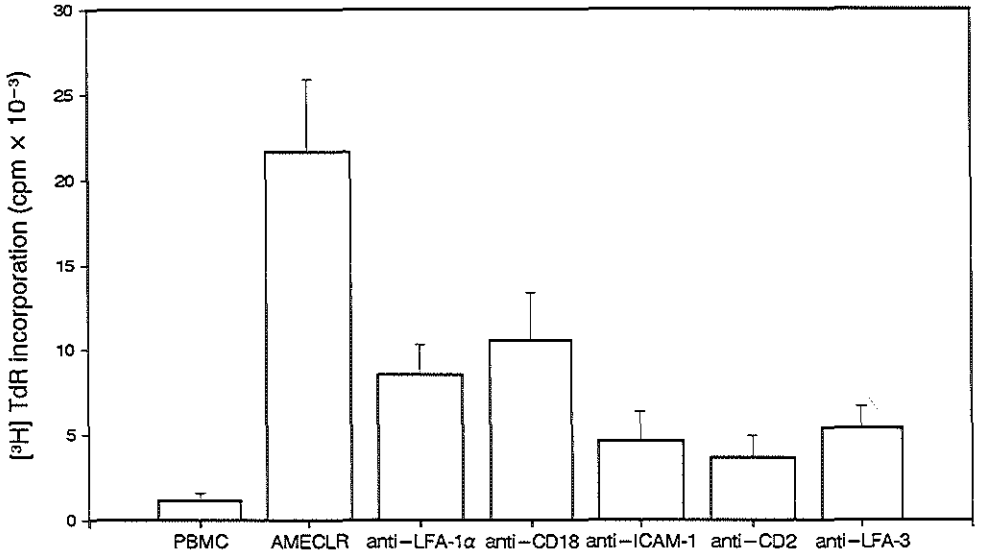

Figure 3. The effect of blocking with anti-LFA MoAb on the autologous MECLR. Each bar represents the mean ± SEM [^{3}H]TdR incorporated in the autologous MLR, MECLR and blocked MECLR from twelve patients. The culture and [^{3}H]TdR labeling procedures are described in the Materials and Methods section. The source and specificity of the MoAb used are shown in Table I. All MoAb significantly inhibited the autologous MECLR ($p < 0.0025$, WSRT).

Blocking of the autologous MECLR with an IL-1-specific antiserum

Since there was an increased expression of mIL-1 on psoriatic EC and since an accessory role of mIL-1 had already been demonstrated in earlier studies, the function of this molecule was investigated by adding an IL-1-specific rabbit antiserum to the autologous MECLR. Prior to adding autologous PBMC, the EC were pre-incubated for 30 min at room temperature with the anti-IL-1 antiserum. As controls 1% normal rabbit serum, a goat IL-6-specific antiserum and anti- HLA-DR MoAb were also added to the autologous MECLR. The IL-1-specific antiserum caused a varied but consistent inhibition (mean 35 ± 14%, $p < 0.002$, WSRT) of the autologous MECLR (Fig. 4). Normal rabbit serum (1%, not shown) or anti-IL-6 antiserum did not affect the outcome of the autologous MECLR, while anti-HLA-DR MoAb significantly inhibited this reaction (91 ± 8%, $p < 0.002$, WSRT) (Fig. 4). Furthermore, as a control, the anti-IL-1 antiserum was added to AET-SRBC-purified peripheral blood T lymphocytes from 8 different patients with proven contact dermatitis to nickel and autologous EC were

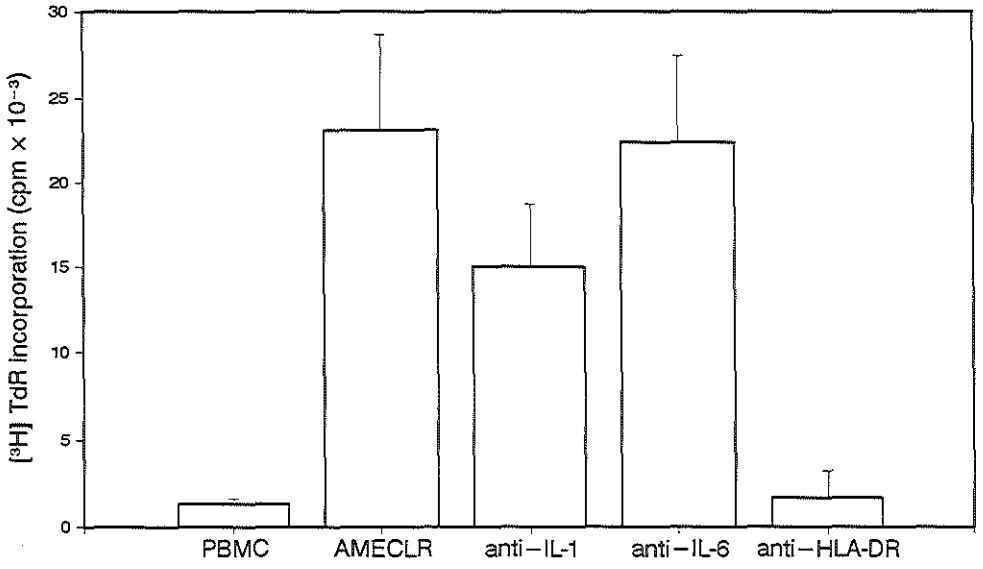

Figure 4. The effect of blocking with anti-IL-1, anti-IL-6 and anti-HLA-DR antibodies on the autologous MECLR. Each bar represents the mean ± SEM [^{3}H]TdR incorporated in the autologous MLR, MECLR and blocked MECLR from fifteen patients. The culture and [^{3}H]TdR labeling procedures are described in the Materials and Methods section. The source and specificity of the MoAb and antisera used are shown in Table I. The anti-HLA-DR MoAb and anti-IL-1 polyclonal antiserum significantly inhibited the autologous MECLR ($p < 0.002$, WSRT).

used as APC to present nickel. In these experiments addition of anti-IL-1 antiserum caused varying effects. In 3 cases a slight inhibition and in 5 cases a slight increase in the proliferative response was observed (not shown). The results in these cases were in clear contrast to the consistent inhibitory effects obtained in the autologous MECLR in psoriasis.

The investigations on depletion or blocking with the MoAb 4B4 and 2H4 were not conducted, since it was not technically possible to prepare a sufficient quantity (one mg) of purified NaN_3-free MoAb, without high financial costs.

Depletion of CD36(OKM5)$^+$ cells from psoriatic ECS

Depletion of CD36(OKM5)$^+$ cells using an immunomagnetic rosetting technique was highly reproducible with an almost complete removal of CD36(OKM5)$^+$ cells from ECS from psoriatic lesions. After depletion, the ECS contained 0-0.5% CD36(OKM5)$^+$

cells as determined by immunofluorescence microscopy. This meant a maximum of 200 CD36(OKM5)$^+$ cells per well with 99% being keratinocytes in the autologous MECLR. As shown in Table III, the depletion of these cells did not significantly affect the autologous MECLR. In 3 cases a slight decrease and in 6 cases various increases in the proliferative responses were observed.

Table III. The effect of depletion of CD36(OKM5)$^+$ epidermal cells on the autologous MECLR (AMECLR) in psoriasis patients

	Proliferation (cpm)*		
Patient	Background	AMECLR	AMECLR-CD36(OKM5) depleted
1.	944 ± 175	24,636 ± 2536	27,351 ± 1605
2.	484 ± 180	16,196 ± 1628	19,029 ± 1254
3.	1773 ± 862	15,369 ± 4759	21,843 ± 6830
4.	613 ± 206	20,940 ± 2132	24,911 ± 2037
5.	1093 ± 223	16,428 ± 1452	14,052 ± 1175
6.	951 ± 317	15,324 ± 1596	18,773 ± 2567
7.	2537 ± 763	37,442 ± 7059	39,190 ± 4872
8.	1319 ± 341	26,635 ± 1755	24,860 ± 2199
9.	1161 ± 128	15,496 ± 1235	12,864 ± 1307

* Figures represent the mean ± SEM [^{3}H]TdR incorporation of quadruplicate cultures. The specificity and the concentrations of MoAb used are shown in Table I.

DISCUSSION

The increased number of activated T lymphocytes in the peripheral blood of psoriasis patients observed in this study is in accordance with that observed in our previous study in which single staining procedures were used [3]. The normal number of TcR$\gamma\delta^+$ cells in the peripheral blood and the absence of these cells in ECS from lesional psoriatic skin exclude an important role of these cells in the pathogenesis of psoriasis.

The results of immunohistochemical studies on cryostat sections confirmed the presence of "aberrant" molecules in psoriatic lesions. An increased expression of CD36(OKM5) and CD54(ICAM-1) has been reported in a number of inflammatory dermatoses [16,18,19,26,76]. The reduced expression of CD45RA on keratinocytes in lesional psoriatic skin to our knowledge has not been previously reported.

In this study an increased number of CD36(OKM5)$^+$ EC was also observed upon immunofluorescence microscopy of EC suspensions. The microscopic appearance of

CD36(OKM5)$^+$ EC was that of medium to large-sized keratinocytes. The low number of CD45$^+$/CD36(OKM5)$^+$ EC as determined by double-labeling, and its distribution in cryostat sections indicated that the majority of the CD36(OKM5)$^+$ cells in lesional skin were not bone marrow-derived and thus were most likely keratinocytes. Although a considerable proportion of psoriatic keratinocytes showed intense expression of CD36(OKM5), the results of depletion experiments indicated that these cells had no accessory function in the autologous MECLR. These results and the results of our previous study on the autologous MECLR in psoriasis suggest that keratinocytes do not fulfill an accessory function in this system. This may be explained by the fact that a limited number of keratinocytes in psoriatic lesions express MHC class II antigens which seem necessary to perform such an accessory function [38,39].

The results of the blocking studies using MoAb to the LFA-family of adhesion molecules indicate a clear functional accessory role of these molecules in the autologous MECLR in psoriasis. This observation was not unexpected since the accessory function of the LFA-family of adhesion molecules has been documented in other *in vitro* systems in which skin APC were used [46,77]. Increased LFA-mediated adherence of peripheral blood lymphocytes on cryostat sections of psoriasis lesions has also been reported [50,78,79]. In this study the MECL cultures to which anti-LFA MoAb were added showed a reduced cell-cell aggregation (few clustered cells). Taken together, the results of this study and our previous study lead to the conclusion that in the autologous MECLR, the LFA-mediated accessory function is probably mediated via HLA-DR$^+$/CD1a$^-$/CD58(LFA-3)$^+$/ICAM-1$^+$ APC which have not yet been characterized further [3]. The role of CD54(ICAM-1)$^+$ keratinocytes in the autologous MECLR seems restricted to co-immobilization of T lymphocytes resulting in cluster formation, facilitating the activation of T lymphocytes by other APC in the epidermis. Simon et al reported on LFA-mediated vigorous stimulation of allogeneic and autologous PBMC by PMA-treated cultured keratinocytes [80]. MHC class I and II antigens appeared not to be involved, while IFN-γ pretreatment abrogated the stimulatory capacity of PMA-treated keratinocytes in this functional *in vitro* system [80]. On the one hand, the *in vitro* induction of ICAM-1 on keratinocytes may have *in vivo* relevance in the antigen-independent initiation phase of inflammation as described by Barker et al [24]. On the other hand, IFN-γ which abrogates keratinocyte stimulation, is generally produced in the effector phase of inflammatory skin conditions and has been shown to induce CD54(ICAM-1) and HLA-DR on keratinocytes *in vivo*. In addition CD54(ICAM-1)$^+$ keratinocytes were co-located with LFA-1$^+$ T cells in inflammatory skin [8,50]. The full implications of this attractive model for *in vivo* skin inflammation, therefore, remain difficult to predict. In any case, it does not apparently operate in the autologous MECLR.

The observed inhibition by the polyclonal antibodies to IL-1, indicates that mIL-1 on psoriatic keratinocytes play an accessory role in autologous MECLR. Since

free/soluble IL-1 was either totally absent or present in very low amounts in the supernatants of psoriatic ECS, it is conceivable that the observed accessory function of IL-1 must have originated from a membrane bound form. There is no definite proof of this as yet, since fixing EC with glutaraldehyde strongly inhibited the autologous MECLR [3]. Membrane bound IL-1 has been demonstrated mainly on monocytes. It has also been demonstrated on keratinocytes and B lymphocytes [54-57,59,81]. This form of IL-1 differs from the free soluble form in its molecular weight (31 kD vs. 17 kD) and is expressed as an intrinsic membrane protein or via lectin-like binding [60]. In human skin, a 52 kD isoform has also been reported [82,83]. The functional relevance of mIL-1 has been well documented in several *in vitro* systems, but its role in chronic inflammatory skin disease is not yet fully understood. Cyclosporin-A, an efficacious anti-psoriatic drug, has been shown to reduce the expression and accessory function of mIL-1 *in vitro* [84]. This observation in conjunction with our results suggest an additional alternative mode of action of CsA in psoriasis.

The accessory function of mIL-1 in the autologous MECLR and its elevated expression on EC from uninvolved psoriatic lesions observed in this study, emphasizes the relevance of (m)IL-1-dysregulation in this disease. Further studies are currently in progress to investigate its precise role in psoriasis.

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Chapter 5

INTERLEUKIN-1 AND INTERLEUKIN-6 IN PSORIASIS*

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SUMMARY

We report on the levels of expression of IL-1 and IL-6 in skin from psoriatic patients. Different approaches were pursued. Initially, the levels of IL-1β and IL-6 were measured in suction blister fluid from lesional and uninvolved skin from psoriatic patients, using a sensitive enzyme-linked immunosorbent assay (ELISA) and bio-assay. Skin sections were also examined for the presence of IL-1 and IL-6 using IL-1β- and IL-6-specific antibodies. Finally, the expression of IL-1 and IL-6 mRNA was determined in cultured keratinocytes (KC) and fibroblasts from psoriatic skin.

Suction blister fluid from lesional and uninvolved psoriatic skin and from skin of healthy individuals did not contain detectable levels (> 100 pg/ml) of IL-1β. Blister fluid from psoriatic lesions contained low but significant levels of IL-6, whereas the serum levels of IL-6 in these patients was undetectable. Using cryostat skin sections and an IL-1β-specific monoclonal antibody (MoAb) in an indirect immunoperoxidase technique, a diffuse staining in the entire epidermis was observed in sections of uninvolved skin from psoriatic patients. In cryostat sections of psoriatic lesions, a faint diffuse staining of the epidermis and a pronounced "dot-like" intracellular staining pattern was observed. On the other hand, the same IL-1β-specific MoAb showed, in an indirect immunofluorescence technique using unfixed epidermal cells (EC), bright membrane staining in EC suspensions from psoriatic lesions. Slightly elevated levels of IL-1β and IL-1α mRNA were observed in cultured KC from psoriatic lesions as compared to normal KC and in the HEp-2 cell line. Very low levels of IL-6 mRNA were expressed in KC from psoriatic lesions and healthy individuals. Fibroblasts from psoriatic lesions expressed extremely low levels of IL-1α and IL-1β, but high levels of IL-6 mRNA.

The results point to a paradoxical situation in psoriatic skin: blister fluid from psoriatic lesions contains no IL-1β, while IL-1β is overexpressed on the plasma membrane and in the intracellular compartment of EC. This finding may help in explaining the observed absence of IL-1 in aqueous extracts of psoriatic scales. Since cultured KC from psoriatic lesions express minimal levels of IL-6 mRNA, dermal fibroblasts, probably together with the inflammatory infiltrate, may represent a major source of IL-6 in psoriatic lesions *in vivo*.

INTRODUCTION

Locally produced pro-inflammatory cytokines are considered to play an important role in the initiation and/or maintenance of inflammatory diseases, e.g. psoriasis [1-3]. In psoriatic lesions there are increased levels of some cytokines and inflammatory mediators, whereas the levels of others are decreased [2-7]. Cytokine-induced products are also observed in psoriatic lesions [8]. Interleukine-1 (IL-1) and, to a

much lesser extent, IL-6 are constitutively produced by EC [9,10]. Certain biologic activities of IL-1, such as T cell and polymorphonuclear leukocyte chemoattraction and stimulation of keratinocyte proliferation, support the involvement of IL-1 in the pathogenesis of psoriasis [1,11-14]. Until recently, however, only limited data on the role of IL-1 and IL-6 in psoriasis were available. This was probably due to the fact that measuring the activity of IL-1 or ETAF (EC-derived thymocyte-activating factor) in skin extracts using bio-assays was rather unreliable and troublesome [2,3]. The widely used IL-1 thymocyte co-mitogenesis (LAF) assay has recently been shown to be unsuitable for distinguishing IL-1 and IL-6 activities [15]. IL-1-like activities were therefore erroneously attributed to IL-6.

Recently, newer methods for the detection of IL-1 and IL-6 have become available [15-18]. In this study we measured IL-1β and IL-6 in suction blister fluid from lesional and uninvolved psoriatic skin using an ELISA technique and a bio-assay. The use of an ELISA technique to measure IL-1β circumvents the possible false negative results that can readily occur in a bio-assay, e.g., due to the presence of IL-1 inhibitors in psoriatic lesions as reported by Fincham et al. [2]. To determine the possible source(s) of IL-1 and IL-6 *in vivo*, cryostat skin sections and EC suspensions from psoriatic lesions were examined for IL-1 and IL-6 using indirect immunoperoxidase and indirect immunofluorescence techniques. Furthermore, the expression of IL-1 and IL-6 mRNA was also determined *in vitro* using cultured KC and cultured fibroblasts (FB) from psoriatic lesions, uninvolved skin, and skin from healthy individuals.

MATERIALS AND METHODS

IL-1β and IL-6 in suction blister fluid

Suction blisters were raised as described by Kiistala and Mustakallio [19]. The contents of the blisters was aspirated using a needle and syringe and stored at -20°C in small aliquots until use. Sera from the patients were stored and assayed in the same way as the control sera. *IL-1β specific ELISA*: IL-1 was measured using an IL-1β-specific ELISA kit (Cistron Biotechnology Corp., Pine Brook, NJ). Using this ELISA system the detection limit, in our hands, was 100 pg IL-1β per ml. *IL-6 Bio-Assay*: IL-6 was measured in a functional bio-assay using IL-6-dependent hybridoma cells as described previously [17,18]. Briefly, $2x10^3$ IL-6-dependent hybridoma cells (C8106H8 or 7TD1) were cultured with serial dilutions of blister fluid and/or serum. After 3 to 4 d, proliferation was measured colorimetrically. A selected spleen cell Con-A supernatant containing 1000 U/ml served as the internal standard.

Immunostaining of skin sections and cell suspensions

An IL-1β-specific MoAb (IgM isotype) was purchased from Cistron Biotechnology Corp. This MoAb was generated by immunizing mice with rHuIL-1β and, according to the specifications of the manufacturer, it should not cross-react with IL-1α, TNF, IL-3, IL-4, IL-6 and none of the interferons. IL-1β specificity and possible cross-reactivity with IL-6 were rechecked in our laboratory by pre-adsorption

tests with recombinant human IL-1β, IL-6, and purified natural human IL-1. One monoclonal and one polyclonal antibody specific to human IL-6 and with IL-6-neutralizing capacity were kindly provided by Dr. M. Helle (CLB, Amsterdam). Standard indirect immunoperoxidase and indirect immunofluorescent staining techniques were used on skin cryostat sections of 10 psoriatic patients and 10 healthy age- and sex-matched control individuals.

Keratinocyte and fibroblast cultures

Keratinocytes from psoriatic patients and controls were cultured in a serum-free medium (KGM, Clonetics, Corp., San Diego, CA). Fibroblasts were cultured in Dulbecco's Modified Eagles Medium (DMEM) supplemented with antibiotics and 5% FCS.

IL-1α, IL-1β and IL-6 cDNA probes and northern blot hybridization

IL-1α, IL-1β and IL-6 mRNA were detected using Northern blot analysis and hybridization with human IL-1α, IL-1β and IL-6 DNA probes, as described by Jeffreys and Flavell [20]. IL-1 (α and β) probes were generously donated by Genetics Institute (Cambridge, MA). The IL-6 probe was kindly provided by Dr. J. Brakenhoff and Dr. L.A. Aarden (CLB, Amsterdam, The Netherlands). Briefly, autoradiograms of Northern blots were prepared by hybridization of 25 μg total RNA on nylon filters with linearized ^{32}P-labeled DNA probes specific for IL-1α (1.7 kb), IL-1β (1.3 kb), and IL-6 (0.3 kb). Total RNA from cultured KC and FB from psoriatic patients, healthy controls, and control cell lines were used. As a control, a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe was used to detect the 1.2 kb GAPDH band in Northern blots.

RESULTS

IL-1 and IL-6 in suction blister fluid

Suction blister fluid from lesional and uninvolved psoriatic skin and from skin of healthy individuals did not contain detectable levels (i.e., > 100 pg/ml) of IL-1β. The

Table I. Levels of IL-6 in Suction Blister fluid[a]

Patient (n=5)	Psoriasis P (U/ml)	Psoriasis N (U/ml)	Control skin (U/ml)
1	50	4	< 3[b]
2	20	5	
3	80	< 3	
4	40	nt	
5	200	12	

[a] Psoriasis P, blister fluid from psoriatic plaques; Psoriasis N, blister fluid from uninvolved skin from psoriatic patients.

[b] Mean value obtained in suction blister fluid from five healthy controls. IL-6 was measured in a bio-assay using IL-6 dependent hybridoma cells.

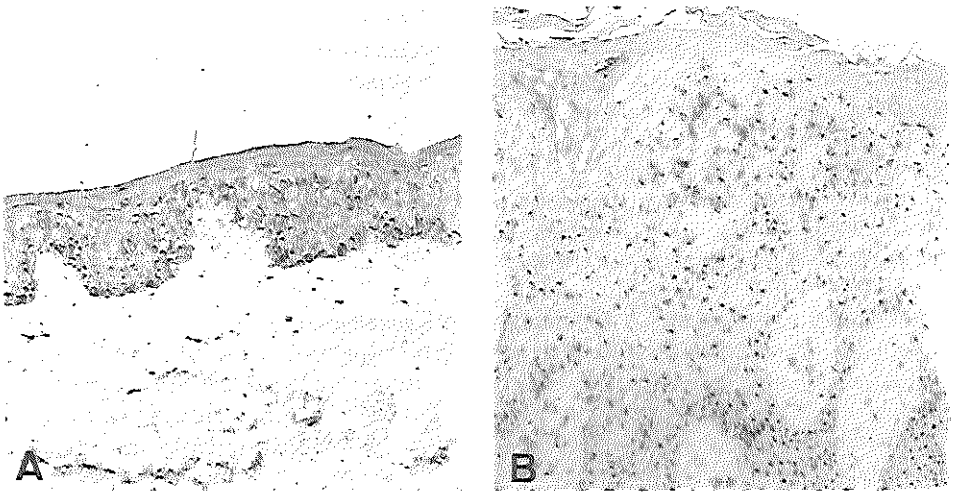

Figure 1. Detection of IL-1β in cryostat sections of uninvolved skin (A) and psoriatic lesions (B) (indirect immunoperoxidase technique; magnification X32 and X320, respectively). Different staining patterns are observed: diffuse staining of the epidermis in uninvolved skin and an intracellular "dot-like" staining pattern in lesional skin.

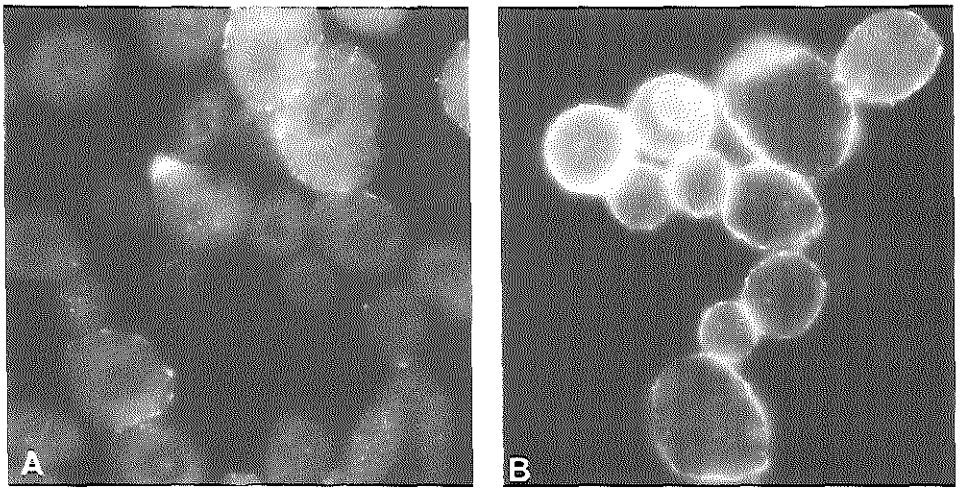

Figure 2. Detection of IL-1β in EC suspensions from uninvolved skin (A) and psoriatic lesions (B) (indirect immunofluorescence technique; magnification X630). Variable basal expression of membrane bound IL-1β (A) in approximately 8 to 12% of EC, bright expression of membrane bound IL-1β (B) in approximately 60 to 80% of EC.

supplied recombinant human IL-1β standard and a supernatant of Con-A stimulated PBMC served as positive controls. Blister fluid from psoriatic lesions contained low but significant levels of IL-6, whereas no significant levels were detected in blister fluid from uninvolved psoriatic skin and skin from healthy controls (Table I). The sera of healthy controls and psoriatic patients had no detectable IL-6 activity.

IL-1 and IL-6 in cryostat skin sections and EC suspensions

Using an IL-1β-specific MoAb in an indirect immunoperoxidase technique on cryostat skin sections, a moderate diffuse staining of the whole epidermis was observed in sections of uninvolved skin from psoriatic patients and, to a lesser extent, in skin sections of healthy controls (Fig. 1A). In cryostat sections of psoriatic lesions, a faint diffuse staining of the epidermis and a pronounced "dot-like" intracellular staining pattern were observed (Fig. 1B). Basal cells were more prominently stained than cells in the other epidermal layers. On the other hand, upon immunofluorescence staining of freshly prepared unfixed EC in suspension, using the same IL-1β-specific MoAb, showed an intense membrane staining of EC from psoriatic lesions (Fig. 2B). In the latter case, approximately 60 - 80% of all EC showed this bright membrane-bound IL-1β expression. In EC suspensions from uninvolved psoriatic and healthy control skin, a variable expression was observed in approximately 10% of the total EC population (Fig. 2A). IL-6 activity, however, was neither detected in skin sections nor in the freshly prepared "suspension-labeled" EC with the monoclonal and polyclonal antibodies used.

IL-1α, IL-1β and IL-6 mRNA expression in cultured KC, FB, and control cell lines

The levels of IL-1β and IL-1α mRNA were slightly increased in cultured KC from psoriatic lesions, as compared to normal KC and the HEp-2 cell line. No significant difference in the constitutive production of IL-1α and IL-1β mRNA was observed (Fig. 3). Keratinocytes from psoriatic lesions and healthy individuals expressed very low levels of IL-6 mRNA. Fibroblasts from psoriasis expressed undetectable levels of IL-1α an IL-1β, but slightly increased levels of IL-6 mRNA (Fig. 3). Addition of recombinant human IFN-γ and IL-1β to some cell lines hardly influenced the expression of IL-1α, IL-1β or IL-6 mRNA.

DISCUSSION

The data presented here show that IL-6 levels are increased in suction blister fluid from lesional psoriatic skin. A similar increase in the level of IL-1 was not observed. IL-6 was undetectable in the sera from psoriatic patients, suggesting that IL-6 is locally produced in psoriatic lesions. A recent study using blister fluid from

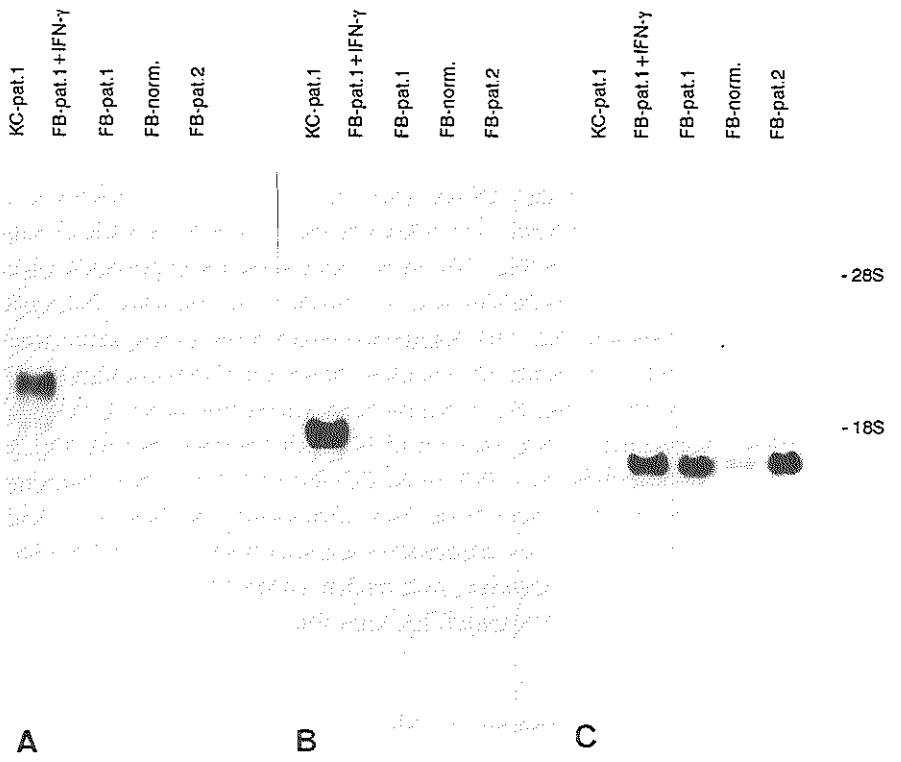

Figure 3. Autoradiogram of Northern blot, using 25 μg total RNA from cultured KC and fibroblasts from psoriatic patients, healthy controls and control cell lines. KC-derived RNA, probed for human IL-1α, IL-1β and IL-6 mRNA, shows increased expression of IL-1α (2.1 kb) **(A)** and IL-1β (1.7 kb) **(B)** and very low levels of IL-6 mRNA (1.3 kb) **(C)**. Significant expression of IL-6 but almost undetectable levels of IL-1 (α and β) message is observed in fibroblast-derived RNA.

pemphigus vulgaris and pemphigoid bullae showed that detection of locally produced IL-1 in blister fluids was possible [20]. It is unclear whether this discrepancy is due to different sensitivities of the assays used or represent different levels of IL-1. Our attempts to detect IL-6 in fixed or unfixed skin sections and EC suspensions using IL-6-specific MoAb were unsuccessful. The finding that minimal levels of IL-6 mRNA were expressed in cultured keratinocytes from psoriatic lesions, whereas dermal fibroblasts expressed increased levels of IL-6, indicate that dermal fibroblasts, probably together

with the inflammatory infiltrate, are the major source of IL-6 in psoriatic lesions *in vivo*.

The expression of IL-1 in psoriatic skin appears to be rather paradoxical: IL-1β is not released at a detectable level in blister fluid from psoriatic lesions, whereas IL-1β is overexpressed on plasma membranes and in the intracellular compartment of EC. The significance of this observation, concerning e.g., the possible biological activity and role, is not yet clear. It was recently shown that aqueous extracts of psoriatic scales contained decreased or undetectable levels of IL-1 activity [2,3]. The observed peculiar expression-site of IL-1β in psoriatic lesions may explain the decreased levels of IL-1 in aqueous extracts of psoriatic scales [2,3]. Our immunohistochemical and fluorescence results on IL-1 and IL-6 in psoriatic skin partially confirm the data of some recent studies [5,7,21,22]. Oxholm et al [5] found increased expression of IL-6, but failed to demonstrate IL-1 in lesional psoriatic skin. Romero et al [7] found increased inter- as well as intracellular expression of IL-1α and IL-1β in psoriatic skin. Increased membrane bound IL-1β [23] and preferential cytoplasmic accumulation of IL-1β [22] have been described in other systems. The differences may be explained by the fact that different antibodies were used by the various investigators. The use of a panel of IL-1 (α and β) and IL-6-specific MoAb or polyclonal antisera for 'in situ' detection of these cytokines, should resolve these discrepancies.

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Chapter 6

INCREASED EXPRESSION OF INTERLEUKIN-4 RECEPTORS AND DECREASED EXPRESSION OF INTERLEUKIN-1 RECEPTORS ON EPIDERMAL CELLS IN PSORIASIS

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SUMMARY

Increased levels of expression of cytokines such as IL-6, IL-8, IFN-γ, TGF-α and TNF-α have been observed in psoriatic lesions. These cytokines may trigger keratinocyte hyperproliferation either directly by serving as growth factors e.g. IL-6, IL-8, or by activation of keratinocytes and the subsequent induction of autocrine growth loops. Proliferation of the psoriatic keratinocytes may be maintained by stimulation of autocrine and paracrine growth by cytokines (such as IL-6, IL-8, TGF-α, Insulin-like Growth Factors and Fibroblast Growth Factors) produced by activated keratinocytes, fibroblasts, endothelial cells and/or the recruited inflammatory cells. The activation and putative autocrine growth of keratinocytes are regulated by a complex process involving interactions of cytokines with their appropriate receptors. Since dysregulation of keratinocyte proliferation is one of the main abnormalities observed in psoriasis, cytokine receptors may play an important role in the pathophysiology of psoriasis. To date, little is known about the expression of cytokine receptors in healthy and diseased skin. Therefore we investigated the expression of the following interleukin-receptors (IL-*n*R): IL-1R, IL-4R, IL-6R and TNF-R in freshly isolated epidermal cell suspensions (ECS) from healthy skin and from untreated lesional psoriatic skin. The results showed that the IL-1R expression was significantly decreased in psoriatic ECS compared with normal control ECS. We propose the provocative hypothesis that, the decreased expression of IL-1R may be the result of increased bio-activity of IL-1 in psoriatic lesions. No differences were observed in the expression of IL-6R and TNF-R in psoriatic and in healthy control ECS. Viable psoriatic ECS, however, expressed significantly increased levels of IL-4R as compared with healthy control ECS. Over-expression of IL-4R occurred while neither unstimulated nor LPS-PMA-stimulated psoriatic ECS, however, from the same patients produced any detectable immunoreactive IL-4. Our results correspond with the observed increased expression of IL-4R on epithelial tumors, and thus imply that over-expression of IL-4R may be a marker of keratinocyte proliferation and activation. The significance of these findings in the context of the pathophysiology of psoriasis is discussed.

INTRODUCTION

Although new data on the expression and regulation of cytokines in psoriatic skin are rapidly accumulating, their exact role in the pathogenesis of psoriasis still remains elusive. In psoriatic lesions increased levels of cytokines such as IL-1β, IL-6, IL-8, IFN-γ, TNF-α and TGF-α have been observed [1-10]. Cytokines may trigger hyperproliferation of keratinocytes either directly by serving as a growth factor (e.g. IL-6 and IL-8), or via activation (e.g. TNF-α and IFN-γ) [11,12]. It has been shown that

Table I. Keratinocyte derived cytokines

Cytokines known to be produced	IL-1α and -β; IL-3; IL-6; IL-8; TNF-α; IFN-α and-β1; GM-, G- and M-CSF; TGF-α and -β2; bFGF; PDGF; MCP-1; suppressor-factors; neuropeptides and thymopoietin
Cytokines assumed to be produced	TNFβ; IGF1 and 2
Cytokines not produced by keratinocytes	IL-2; IL-4; IL-5; IFNγ

activated normal human keratinocytes are able to produce several cytokines which have well-defined immunological functions (Table I). Proliferation of psoriatic keratinocytes may be maintained by stimulation of autocrine and paracrine growth by cytokines or growth factors (IL-6, IL-8, transforming growth factor α (TGF-α), insulin-like growth factors (IGF) and/or fibroblast growth factors (FGF) produced by activated keratinocytes, the inflammatory infiltrate, fibroblasts and/or endothelial cells [13-15]. *In vivo* regulation of keratinocyte activation and autocrine and paracrine growth mechanisms are complex processes involving interactions between cytokines from different cellular sources and their receptors. The question remains whether the characteristic pathophysiological epidermal alterations observed in the epidermis in psoriasis arise spontaneously due to an intrinsic defect in the keratinocytes or whether they result from an immunological event initiated by bone marrow-derived cells [16-20]. Cytokines from activated lymphocytes can activate EC to proliferate and to produce cytokines and also to express "aberrant" molecules [15,19,21-25]. Cytokines produced by activated EC may also affect the function of the epidermal Langerhans cells (LC) and moreover, recruit other types of inflammatory cells to the site of inflammation [24,26,27].

Psoriatic EC were shown to stimulate autologous T lymphocytes *in vitro*. This was mediated via HLA-DR^+/$CD1a^-$ epidermal antigen-presenting cells (APC) resulting in an elevated autologous mixed epidermal cell T-lymphocyte reaction (AMECLR) [20,28-30]. This illustrates the interaction between keratinocytes and bone marrow-derived cells in psoriasis. It may be that bone marrow-derived cells are essential in initiating psoriasis, because severe recalcitrant psoriasis cleared after bone marrow transplantation [17]. In contrast, psoriasis was induced in a recipient of allogeneic (HLA-matched) bone marrow from an individual with psoriasis [31]. In any case, these data emphasize the importance of immuno-pathogenic mechanisms in psoriasis [16,24,32,33].

Investigations on the expression of cytokine receptors in healthy human skin and in psoriasis are scarce [10,34,35]. These investigations in psoriasis mainly concern the

overexpression of the EGF/TGF-α receptor [10,12,19,34]. The ligand for the EGF-R TGF-αR is also over-expressed in psoriatic lesions [9]. Using an immunoperoxidase technique and frozen skin sections, Scheynius et al., observed an altered pattern of the expression of the IFN-γR in psoriatic epidermis as compared with normal human skin [36]. Over-expression of IL-1R on psoriatic keratinocytes has also been reported [37]. The latter and other reports seem to confirm the dysregulation of IL-1 in psoriasis proposed by Cooper et al. (Table II) [1,3,8,38-42]. In studies on cytokines and their receptors in other skin diseases, elevated IL-6R mRNA and increased production of immunoreactive and bioactive IL-6 have been described in Kaposi sarcoma skin lesions and in two Kaposi sarcoma cell-lines [43].

In the present study, the levels of expression of various interleukin-receptors were investigated on freshly isolated EC from involved psoriatic and normal human skin. The results clearly indicate that the interleukin receptor-profile is altered in psoriatic lesions.

Table II. Expression of IL-1 in control skin and in psoriatic epidermis

Type of skin sample	IL-1 Isoform	Healthy control skin mRNA	IR	BA	Psoriatic lesions mRNA	IR	BA	Reference
Extracts of keratome	IL-1α	-	++	++	-/±	↓↓	±/↓#	[1,39,40,70,71]
Specimens	IL-1β	-	±	-	↑↑	↑↑	↓↓*/-	[1,39,40,70,71]
Stratum corneum	IL-1α		+	+		↓	↓	[8,72]
Scale extracts	IL1-β		+	+		↓	-	[8,72]
Suction blister	IL-1α		±			±		[72]
fluid	IL-1β		-			-		[3,72]
Cryostat skin	Il-1α	±	-/++		++	+		[1,3,4,41,70]
sections (IIP/IIF)	IL-1β		+		+	↑		[1,3,41,70]
Epidermal cell	IL-1α		+					[71,73]
suspensions	IL-1β		+		↑↑			[3,73]
Cultured kera-	IL-1α	+	+	+	↑	+	+	[1,3,49,71]
tinocytes (KGM)	IL-1β	+	↑↑	±	↑	↑	±	[1,3,71]

* = increased production and release of non-functional mature or novel inactive pro-IL-1β isoforms.
# = conflicting data from different investigators. Decreased bio-activity is not due to the occurrence of an IL-1 inhibitor in psoriatic lesions [1,42]. IR = immuno-reactive; BA = bioactive; IIP/IIF = indirect immunoperoxidase, indirect immunofluorescence; KGM = keratinocyte growth medium; - = absent (undetectable); ± = detected at low levels; + = detectable; ++ = clearly detectable; ↑/↑↑ = (markedly) increased; ↓/↓↓ = (markedly) decreased.

MATERIALS AND METHODS

Patients and controls

Thirty otherwise healthy patients with stable plaque-type, untreated psoriasis were studied after informed consent. The disease activity, severity and extent were determined for each patient and expressed as PASI scores. Epidermal cell suspensions (ECS) were stained using a panel of biotinylated recombinant human (rHu) cytokines and monoclonal antibodies. ECS from 15 healthy volunteers (individuals without history or signs of skin disease undergoing abdominal or breast plastic surgery) served as controls.

Preparation of epidermal cell suspensions

Split-skin specimens from involved skin of psoriasis patients and from surgically excised abdominal or breast skin from controls were obtained using a portable dermatome. Single cell suspensions of epidermal cells were prepared from the split skin specimens using standard methods [44]. Briefly, the split-skin specimens were rinsed thoroughly with Ca^{2+} and Mg^{2+} free phosphate-buffered saline (PBS) and cut in pieces of ± 0.5 square cm. Subsequently the pieces were floated in PBS containing 0.0625% trypsin (ICN Biochemicals, Cleveland, OH, U.S.A.), 0.32% glucose and 0.1% EDTA for 45 min (at 37°C / 5%CO_2) and during the last 15 min of the incubation in the presence of 0.025% deoxyribonuclease (DNase; Sigma, St. Louis, Missouri, U.S.A.). At this stage the epidermis could be separated from the dermis by fine forceps. The sheets were cut with scissors and the epidermal cells were filtered through sterile 100 μm and 30 μm mesh nylon gauzes, washed twice in RPMI 1640 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 6 mM fresh L-glutamine (RPMI complete medium), 250 μg/ml trypsin inhibitor (derived from egg-white, Sigma). Cells were counted using a hemocytometer. Their viability was determined, directly after isolation by trypan blue exclusion. The viability was checked again during FACScan analysis, using propidium iodide and fluorescein-diacetate. Previous studies showed that cells with positivity for FITC after uptake and hydrolysis of FITC-diacetate are viable cells [45]. Using this priciple, Hansson et al. described an adapted method to study cell proliferation, using carboxy-fluoresceindiacetate, which seemed to have the same level of sensitivity as the conventional [^{3}H]TdR incorporation method [46]. The number of patients studied in a specific experiment is mentioned in the figures and tables ($n=x$).

Monoclonal antibodies, immunofluorescence staining

CD1a (66IIC7, Dr. M. van der Rijn, Amsterdam; and for specific applications: 66IIC7-FITC-conjugated, Dr. T. Godthelp/A.Kleinjan, Rotterdam, The Netherlands), anti-HLA-DR (biotinylated, Becton Dickinson, San Jose, CA, USA), CD45(HLe-1-FITC-conjugated, Becton Dickinson), anti-TNF-R1 (p55, htr-9) and TNF-R2 (p75, utr-1) MoAb (Dr. M. Brockhaus, Hoffmann-LaRoche, Basle, Switzerland), anti-IL-4R MoAb (Mr6, M. Larché, London), and a polyclonal rabbit antiserum to IL-6R (Dr. P.C. Heinrich, Aachen, Germany) were used to examine the composition of the freshly prepared ECS of patients and controls.

ECS were immunophenotyped using direct and indirect immunofluorescence techniques. The immunofluorescence techniques were performed as described by Van Dongen et al. [48]. Some EC samples were counted using Standard Zeiss 16 microscopes equipped with an IV FL epi-illumination condensor (Carl Zeiss, Oberkochen, FRG). The percentage of positive cells was calculated by counting the number of cells showing positive fluorescence from a total of 200 - 400 cells.

Labeling of ECS using biotinylated rHu cytokines

ECS were stained using a panel of biotinylated rHu cytokines. The expression of the following

interleukin-receptors (IL-*n*R) were studied: IL-1R (using rIL-1α), IL-4R, IL-6R and TNF-R. The cells were labeled using biotinylated recombinant human cytokines and avidin-FITC (all purchased from R&D Systems, Minneapolis, MN) or streptavidin-phycoerythrin (Becton Dickinson) as the second step, followed by FACScan analysis. Unbiotinylated rHuIL-1β was generously provided by Glaxo/Biogen (Geneva, Switzerland). IL-4 was obtained from DNAX (Palo Alto, CA, USA) via Dr. H.F.J. Savelkoul (Rotterdam) and IL-6 was obtained from Drs. L.A. Aarden/J. Braakenhof, CLB (Amsterdam). Recombinant HuTNF-α and IL-1α were purchased from Amersham (Buckinghamshire, UK) and Genzyme (Cambridge, MA, USA) respectively.

ECS were labeled with biotinylated rHu cytokines using the protocol supplied by the manufacturer. Briefly, 10^5 EC were incubated for 1 hr at 4°C with specific rHu cytokine solution containing 65 to 300 ng rHu cytokine protein. Unbound cytokine was removed by two rinses. This was followed by incubation with avidin-FITC or strepavidin-phycoerythrin for 30 min at 4°C in the dark. Finally, the cells were rinsed twice and resuspended in FACScan-buffer, and kept on ice till FACScan analysis was performed. The whole procedure of preparation, labeling and analysis of ECS normally required about 4 to 5 hours. The FACScan analysis was conducted using the FACScan^C, Paint a Gate^C and Lysis^C software programs (Becton Dickinson). The results are presented either as histograms (y-axis representing the number of cells, x-axis the fluorescence intensity), or as the mean ± SEM percentage of positive cells. The level of expression of cytokine receptors was calculated and expressed as the difference between EC stained with avidin-FITC or strepavidin-PE alone and EC stained with biotinylated rHu cytokine and (strept)avidin-FITC/PE.

To determine the relative fluorescence intensities of different EC samples, and express them as an indirect value for the number of binding sites per cell, the following formula was used: FSN (fluorescence signal-to-noise ratio) = $10^{[(Ch\#exp. - Ch\#contr.)/Ch\#D]}$, where Ch#exp.= measured mode channel number of experimental sample, Ch#control = measured mode channel number of negative control sample, and Ch#D= number of channels per decade [46].

Controls

The controls comprised the omission of the first step (PBS). The specificity, in case of labeling with rHu cytokines, was checked by pre-incubating ECS with equivalent amounts of unbiotinylated ("cold") rHu-cytokines followed by the above mentioned complete labeling procedure. The latter procedure almost completely reduced the binding of biotinylated rHu cytokines. The sensitivity and specificity were also checked during some time-course studies using stimulated and unstimulated human PBMC from several donors, D10(N4)M, B9, EL-4, U937, KG1a and THP-1 cell lines. In these studies reproducible labeling and fluorescence intensities were obtained. Two stripping-buffers were used consecutively to obtain efficient stripping of receptor-bound cytokines in order to check for *in vivo* binding of cytokines to their receptors [50,51]. Briefly, EC were suspended and kept on ice for 1 min in buffer I (pH 4.0) [50], neutralized by adding Hepes containing medium and rinsed. Next the EC were suspended and kept on ice for 30 sec in buffer II (pH 3.0) [51], neutralized again by adding medium containing Hepes and rinsed twice, followed by the complete labeling procedure.

Measurement of immunoreactive IL-4 in ECS supernatants

Lesional psoriatic and healthy control EC samples were cultured overnight at a concentratrion of 10^6 EC/ml, in complete RPMI$^+$ with 20% human AB-serum and/or in serum-free low calcium medium (KBM, Clonetics, San Diego, CA, USA). In some cases the EC were stimulated with 1 ng/ml LPS and 50 ng/ml PMA. After a culture period of 16 hr, the ECS were spun down at 1000g, the supernatants collected and stored at -80°C until used. When serum-free culture medium was used, 0.5% BSA was

added to the supernatants prior to freezing. All culture media used were also stored and assayed in parallel as blank controls. Immunoreactive IL-4 was measured using a sensitive ELISA (R&D systems). The detection limit, using human recombinant IL-4 as a reference standard, was 4 picograms.

Statistical analysis

The results were analyzed with STATA^{TM} (computer program for statistical analysis, Computing Resource Center, Los Angeles, CA). The test used to determine statistical significance is mentioned in the abbreviated form, e.g Wilcoxon test (WT) and Wilcoxons' signed rank sum test (WSRT), together with the "p" values.

RESULTS

The viability of cells in the total ECS directly after preparation from healthy and psoriatic skin ranged from 70 to 85% as assessed by trypan-blue exclusion. The viability of cells in the total ECS at FACScan analysis ranged from 55% to 75% as assessed by propidium iodide (PI) uptake and FITC-diacetate labeling. Subpopulations

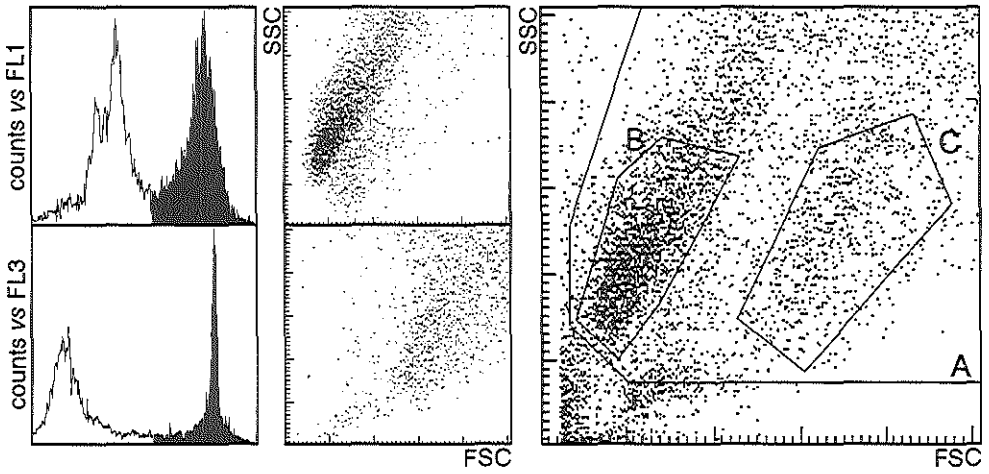

Figure 1. Characteristic forward scatter versus side scatter (FSC/SSC) dot plots of ECS of healthy control and psoriatic skin. *Gate A* comprises total EC excluding cellular debris, *gate B* the viable, $FITC^+/PI^-$ cells, after uptake and processing of FITC-diacetate (*fluorescence signal shown in D*), *gate C* the non-viable, $PI^+/FITC(diacetate)^-$ cells, showing uptake of PI (*fluorescence signal shown in E*). As can be seen these are clearly mutually exclusive populations. Identification of these populations was reproducible in ECS isolated from all patients and controls.

of EC showing uptake of PI and those that were positive for FITC, after uptake and processing of FITC-diacetate, were clearly mutually exclusive populations (Figs. 1b and 1d) compared with (Figs. 1c and 1e). On the basis of the above mentioned findings 3 gates could be established in the forward scatter versus side scatter plots. The first gate comprised total EC excluding the cellular debris (Fig. 1a). The second gate comprised the viable EC (PI^-/FITC-diacetate$^+$) and the third gate the non-viable EC (PI^+/FITC-diacetate$^-$ subpopulation (Fig. 1b and c). Identification of these subpopulations was reproducible in ECS isolated from all patients and controls. The expression of IL-*n*R was determined separately in each EC subpopulation.

Expression of IL-*n*R on freshly isolated ECS

An example of the expression of the fluorescence signal, in this case after incubation with biotinylated rHuIL-4, on FACScan analysis is given in Fig. 2. The expression of most cytokine receptors varied considerably between the various ECS isolated from different psoriatic patients, whereas this variability was less pronounced in EC preparations isolated from healthy controls. To compensate for the variability in psoriatic patients, a relatively large group (n=30) was investigated. The expression of IL-*n*R receptors on EC from psoriatic skin and from healthy controls were compared.

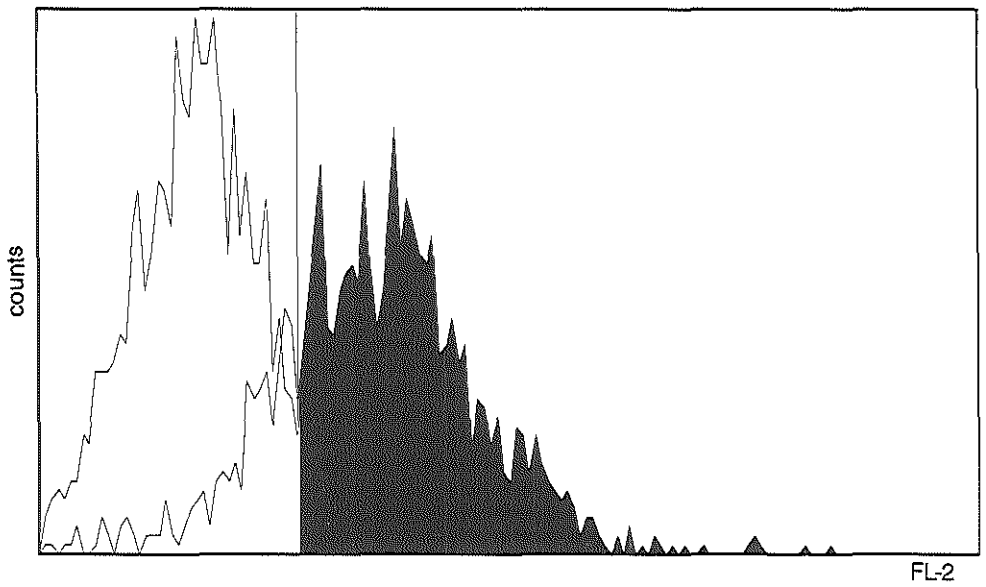

Figure 2. Illustration of interleukin receptor expression on an EC sub-population. It shows the background fluorescence and the expression of IL-4R in viable ECS. This population correspond with the FSS/SSC plot of fraction *B* shown in Fig 1.

Expression of interleukin-1 receptor

As shown in Fig. 3, the number of IL-1R$^+$ cells in ECS was highest in the non-viable EC fraction. This was found in both the psoriatic patients and controls. In ECS from psoriatic patients the number of IL-1R$^+$ cells in the viable fraction was almost half of that in the non-viable fraction, while in healthy control ECS this difference was less clear (a non-viable to viable ratio of 1.9, versus 1.3 in EC from healthy controls). The mean number of IL-1R$^+$ cells in viable psoriatic ECS was 37 ± 7%, the mean number in viable EC from healthy controls was 58 ± 9%, $p < 0.002$, WT. Moreover, the number of IL-1R$^+$ cells in the complete ECS from psoriatic patients was lower than that in the complete ECS from healthy controls, although this difference was not significant. Comparison of the relative fluorescence intensities (relative signal-to-noise values) showed that the number of IL-1 binding sites per cell in the viable ECS from psoriatic patients did not differ significantly from that in the viable ECS from healthy controls (Fig. 5).

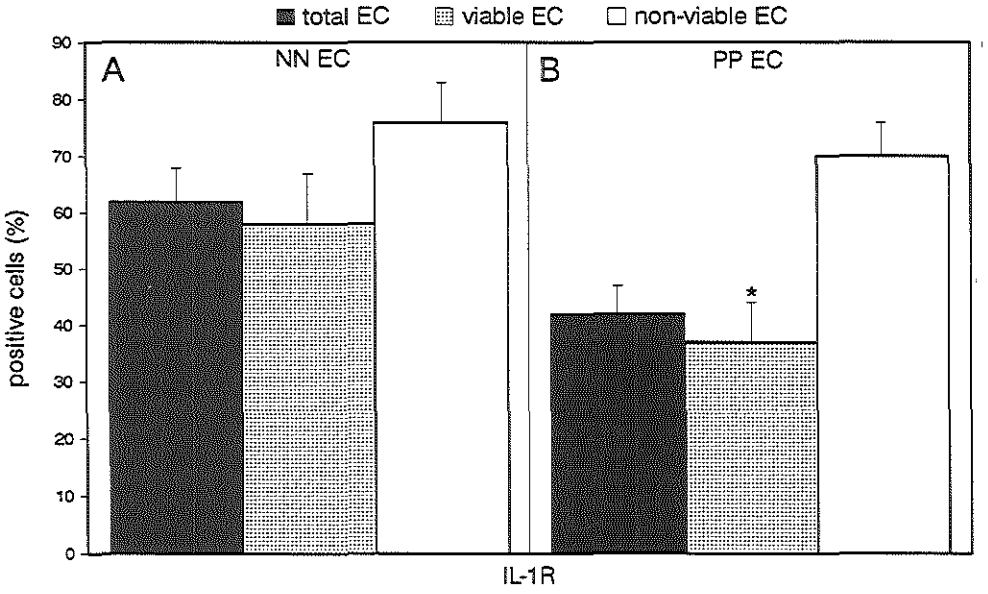

Figure 3. Expression of interleukin-1 receptor in normal and psoriatic EC. In normal and psoriatic ECS the highest number of IL-1R$^+$ cells are seen in the non-viable EC fraction. Viable psoriatic EC showed a markedly decreased number of IL-1R$^+$ cells compared to that in the non-viable fraction. In healthy control ECS this difference was less clear (non-viable to viable ratio of 1.90, versus 1.31 respectively).

Expression of interleukin-4 receptor

A significantly increased number of IL-4R$^+$ cells was observed in the viable fraction of ECS from psoriatic patients as compared with healthy controls (Fig. 4). The number of IL-4R$^+$ cells in the viable fraction of ECS from psoriatic patients was twice the number of that in the viable fraction of ECS from healthy controls (mean value psoriatic ECS is 62% versus 28% in healthy control ECS. The expression of IL-4R$^+$ cells in the total and non-viable fraction of ECS from psoriatic patients did not differ significantly from that of controls. Comparison of the relative signal-to-noise values showed that the number of IL-4 binding sites per cell in the viable fraction of ECS from psoriatic patients was nearly twice that in the viable fraction of ECS from healthy controls ($p < 0.002$, WT, Fig. 5).

Expression of interleukin-6 and TNF receptors

No differences were observed between the number of IL-6R$^+$ cells in the EC fractions from psoriatic patients and healthy controls. The numbers of TNF-αR- cells

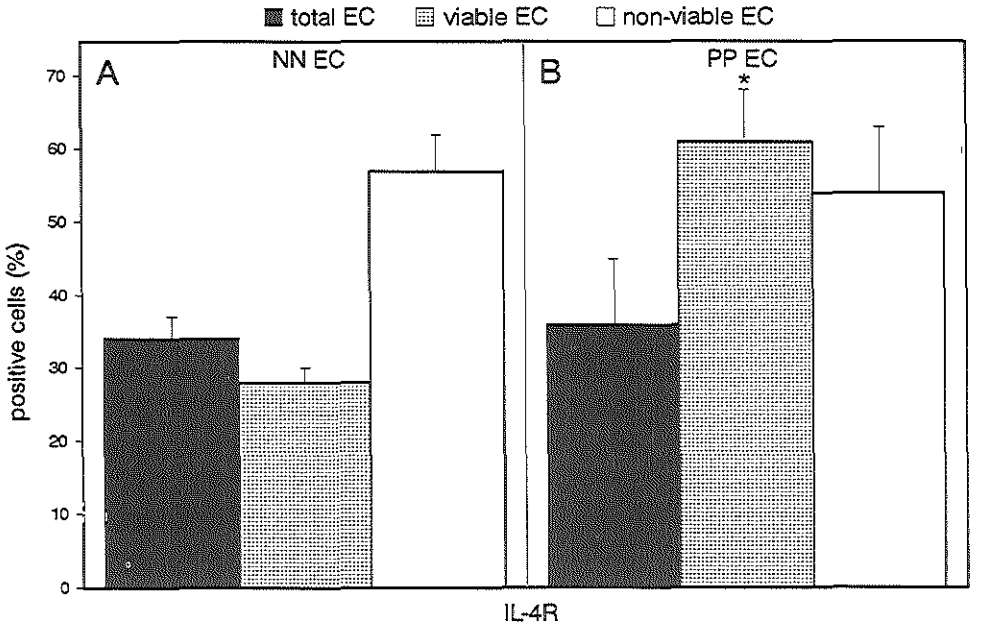

Figure 4. Expression of interleukin-4 receptor in normal and psoriatic ECS. The number of IL-4R$^+$ cells is significantly increased in the viable fraction of psoriatic ECS as compared with viable EC from healthy controls ($p < 0.002$, WT).

in ECS from psoriatics was slightly higher than in ECS from healthy controls. This difference, however, was not statistically significant. The number of IL-6 and TNF binding sites per cell assessed by the relative fluorescence in all fractions also did not differ significantly.

Immunoreactive IL-4 in supernatants of overnight cultured ECS

Supernatants of psoriatic, healthy control and LPS-PMA-stimulated ECS did not contain detectable levels of (immunoreactive) IL-4. In total 10 psoriatic patients and 5 healthy control ECS supernatants were tested. IL-4 was also undetectable in the supernatants of ECS that were cultured in basal medium (not shown).

Correlation between disease activity and IL-R expression

No correlation could be established between the PASI score and the expression of interleukin receptors on psoriatic EC. This is valid for all EC fractions and all interleukin receptors studied.

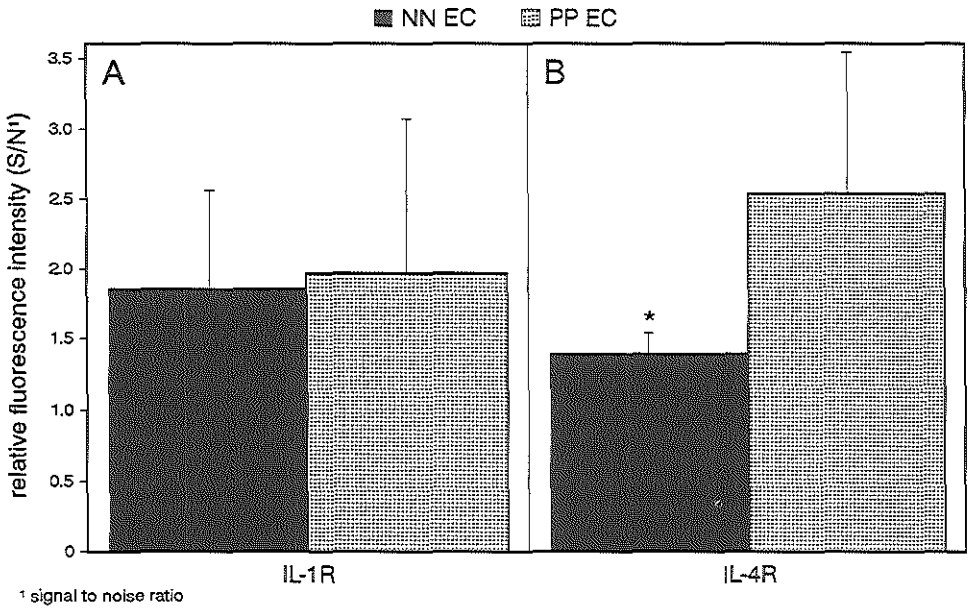

Figure 5. Relative fluorescence intensities (signal to noise ratios = S/N ratio) of IL-1 and IL-4 receptors on freshly isolated viable normal (NN) and psoriatic (PP) EC. The difference between the fluorescence intensities of IL-4R$^+$ psoriatic and healthy control cells is statistically significant ($p < 0.03$, WT; NN $n=10$, PP $n=16$).

DISCUSSION

Results of this study indicate that cycling EC from psoriatic lesions contain a significantly decreased number of IL-1R$^+$ and an increased number of IL-4R$^+$ cells as compared with cycling EC from healthy controls. These differences became evident quite early in the study, in spite of the considerable variation in the number of IL-*n*R$^+$ cells in the ECS from psoriatic patients. The pattern of IL-*n*R expression in ECS from healthy controls was more constant indicating a low inter-individual variation. Major fluctuations in the basal IL-1R expression were also observed in different lots of cultured normal neonatal keratinocytes [52]. The fluctations were observed, however, only in different lots but not from experiment to experiment with keratinocytes from the same individual [52]. The latter caused the investigators to suggest that the fluctuations were donor specific, which is also our impression. For proper interpretation of the results and because data on the expression of IL-*n*R in healthy and diseased human skin are very limited, the number of individuals that were investigated in this study were increased to 30 psoriatic patients and 15 healthy controls. The results presented here portray authentic epidermal expression of IL-R, since we have shown that psoriatic ECS normally only contain in mean 3% CD45$^+$ bone marrow-derived cells (Prens et al., submitted).

Table III. Characteristics of the cytokine receptors studied

Cytokine receptor	Nature and MW (kD)	Glyco-sylation	Number per cell	Affinity	Expression	Soluble form
IL-1RtI	p80	yes	0-2x10^4	K_d= 1 nM	Broad	?
IL-1RtII	p68	yes	0-2x10^4	K_d= 1 nM	B cells neutrophils	yes
IL-4R	p130(p75?)	yes	10^2-3.10^3	K_d= 40-120 pM	Broad	yes
IL-6R	p80	yes	10^2-12.10^3	K_d=10 pM/1 nM	Broad	yes
TNF-R1	p55	yes	10^2-10^4	K_d= 0.5 nM	Broad	yes
TNF-R2	p75	yes	10^2-10^4	K_d=0.1 nM	Broad	yes

kD= kilo Dalton; K_d = dissociation constant; p = protein; IL-1RtI/II = receptor type 1/2.

The original ECS, in initial experiments, was divided into two samples which were processed in parallel in order to check for interference of receptor-bound ligand on EC, which might mask the genuine number of IL-*n*R. Ligand possibly bound to EC *in vivo* was dissociated from the receptors using two different stripping buffers (pH4 and pH3) consecutively prior to incubation with biotinylated rHu-interleukins. The IL-*n*R expression of acid-stripped EC was compared with that of the untreated EC sample. These experiments showed that acid-stripping resulted in only minor shifts in the expression of the different IL-*n*R (results not shown). In contrast, the fluorescence signal of *in vitro* labeled control EC was almost completely removed (not shown).

Table IV. Classification and biological effects of ligand binding on different cytokine receptors

Cytokine receptor	Internalization	Signal transduction	Effects on receptors*
IL-1R, Ig superfamily	yes, rapidly, nuclear localization	via IL-1R-linked G-protein, phospholipase C, Diacyl-glycerol, protein kinases, NF-κB	IL-1Rtl ↓, IL-6R-mRNA↓, TNF-R↓, LH-R ↓, TGF-βR ↑, EGF-R affinity ↓
IL-4R, haematopoietin/ cytokine-receptor super-family	yes, rapidly	Phopho-Inositol-mediated/adenylate cyclase	IL-4R ↑, FcεRII ↑, FcγR1-3↓, blocks generation of high affinity IL-2R in T lymphocytes
IL-6R, Ig-like and haematopoeitin/ cytokine recept or superfamily	yes??	via surface gp130, PKC, adenylate cyclase, and nuclear factor (NF)-IL-6	IL-2R ↑, IL-6R ↑
TNF-R1/2	yes	phospholipase A_2, serine phosphorylation of intracellular 26 kD protein, PKC	TNF-R ↓, IL-2Rα↑

↑ increased.
↓ decreased.
* Only biological effects on other (cytokine) receptors are shown. Every cytokine additionally has a broad spectrum of other relevant biological activities.
LH-R luteinizing hormone receptor.

These observations suggest that *in vivo*, in psoriatic skin lesions and in healthy control skin, native IL-1, IL-4, IL-6 and TNF-α bind to their respective receptors on the EC and are rapidly internalized. The theoretical possibility that the receptors in psoriatic lesions are not occupied due to absence of the ligand is not very likely since e.g. IL-6 and TNF-α are abundantly present in psoriatic lesions [3,38,39]. It thus appears that most interleukin receptors on EC are occupied only for a short period. Since no interference occurred due to occupied IL-*n*R on EC, the results presented here reflect the actual number of IL-*n*R$^+$ EC of a given patient with a certain level of disease activity. Relevant information about the cytokine receptors studied is given in Table III and IV.

The observed dissimilarity in the expression of IL-R in different EC fractions was most apparent and significant for IL-1R and IL-4R. Previous studies have shown that certain receptors may be expressed preferentially by cells of a specific epidermal layer [34,36,53,54]. For example, IFN-γ receptors are expressed throughout the entire epidermis in normal skin, whereas in psoriatic lesions the expression of IFN-γR was confined only to the lower part of the epidermis [36] (Table V). EGF/TGF-αR expression was shown to be predominantly expressed in the basal layer in healthy control skin, whereas in psoriatic skin upper layers showed increased expression of EGF/TGF-αR [34] (Table V). The meaning and implications of these variations in IL-*n*R expression in different EC fractions are still unclear and can only be speculated.

Table V. Membrane expression of different cytokine receptors in control skin and in psoriatic epidermis

Cytokine receptor	Normal skin	Epidermal fraction/layer	Psoriatic skin	Epidermal fraction/layer
IL-1R	++	non-viable > viable(cycling)	↓	non-viable » viable(cycling)
IL-4R	+	all	↑↑	viable(cycling) » non-viable
IL-6R	+	non-viable > viable	+	all
TNF-R	±	all	+	all
EGF/TGF-αR	+	basal/lower spinous	↑↑	all/upper spinous
IFN-γR	+	all	↓	basal and spinous

↑/↑↑ increased / markedly increased.
↓ decreased.
+/++ detectable respectively clearly detectable.
± weakly expressed.

Blanton et al. observed a significantly (9 to 20-fold) increased expression of IL-1R in cultured postconfluent normal neonatal keratinocytes, in which squamous differentation was induced by high Ca^{2+} concentrations (2mM) and PMA stimulation (10 ng/ml) [52]. Extrapolation of these findings to *in vivo* conditions would imply that the high expression of IL-1R in the non-viable EC fraction of healthy and psoriatic skin represents normal increased expression due to squamous differentiation.IL-1R, IL-4R, IL-6R and TNF-R were determined in the present study using biotinylated rHu cytokines. EGF/TGF-R was determined using radioactively labeled EGF, FN-γR was detected using mouse MoAb and indirect immunoperoxidase technique and are data from references number [34, 36].

The decreased number of IL-1R$^+$ cells in the viable fraction of psoriatic ECS could be the result of intrinsicly altered IL-1R turn-over, or down-regulation due to chronic *in vivo* over-exposure to IL-1 in psoriatic lesions. The latter provocative hypothesis is proposed since quite some characteristics of psoriatic lesions coincide with increased IL-1 bio-activity *in vivo*. It has been shown that in psoriatic lesions, like in proliferating cultured keratinocytes, IL-1β mRNA and protein are over-represented, whereas IL-1α is relatively decreased [1,39]. Although, on the one hand, IL-1β seems to lack bio-activity in psoriatic lesions, it may be secreted and converted *in vivo* into a biological active form by aberrant proteases form psoriatic keratinocytes 'in the activated state' or by proteases from contiguous intra-epidermal inflammatory cells [40]. IL-1α, on the other hand, may be decreased due to rapid consumption by psoriatic EC. The proposed hypothesis is conceivable provided regulatory mechanisms operative in EC are identical to that in other cell types where exposure to IL-1 has been shown to result in down-regulation of both types of IL-1R [55]. In addition, manifestations such as the nuclear localization of IL-1 in lesional psoriatic epidermis, overexpression of IL-6 and IL-8 (both IL-1 inducible cytokines), significant neutrophil chemo-attraction (via IL-1-induced IL-8) and increased production of arachidonic acid metabolites by IL-1-primed neutrophils could be regarded as evidence of *in vivo* bio-availability, binding and internalization of IL-1 [1,3,41,56]. Finally, increased EGF-R/TGF-R expression in psoriatic lesions may also be attributed to IL-1, since IL-1 has been shown to decrease the affinity of EGF-R/TGF-R for its ligand [9,57]. However, rapid and probably altered metabolism of IL-1 mRNA, IL-1 protein synthesis and IL-1R expression and internalization in psoriatic lesions does not permit detection of its activity by classical means such as simple tissue/cell extraction procedures followed by Northern or Western blotting, bio-assay or ELISA.

The clinical significance of more insight into regulatory interactions between cytokines and their receptors may be illustrated by the observation that IL-1R antagonists can prevent disease in the animal model. Inhibiton of IL-1R with natural or recombinant IL-1 inhibitors, analogues or antagonists, is currently being investigated in clinical trials e.g. in the field of rheumatoid arthritis [58-62].

The relevance of the elevated IL-4R expression observed in psoriatic EC in this study is unclear. To our knowledge studies on the levels of IL-4 in supernatants of psoriatic ECS have not yet been reported. The presence of this cytokine in psoriatic lesions was theorethically possible since IL-4 is almost exclusively produced by T lymphocytes and activated IL-2R$^+$ T lymphocytes are present in the epidermal layers and in the dermal inflammatory infiltrate in these lesions [63,64]. It was unclear whether they belonged to the T_h1 or T_h2 T lymphocyte sub-class, especially since little is known about the profile of cytokines produced by T lymphocytes *in situ* in psoriatic lesions. However, elevated IL-2, IFN-γ and gamma interferon induced protein-10 (γ-IP-10) levels have been reported in psoriatic lesions [5,6,8,63]. Immunoreactive IL-4 was undetectable in *scale extracts* from healthy control skin and from psoriatic lesions [8]. The latter and the observation that *in vitro* cloned T lymphocytes from psoriatic lesions produced IL-2 and IFN-γ (data on IL-4, IL-5 or IL-10 were, however, not reported), indicate that they may belong to the T_h1 subtype [65]. Thus, our results that supernatants of stimulated and unstimulated *ex vivo* psoriatic ECS, containing T lymphocytes, do not contain IL-4, favor a role for T_h1 T lymphocytes in the pathophysiology of psoriasis.

The biological effect(s) of IL-4 on normal and psoriatic keratinocytes is unknown. It has been reported that IL-4 has the capacity to inhibit the growth of malignant cells and, moreover, that the expression of IL-4R is increased in certain epithelial tumors [66,67]. IL-4 administered subcutaneously in hydrophilic gels around the cutaneous lesions induced by Leishmania major in Balb/c mice caused resolution of the skin lesions [68]. It is noteworthy that chronic cutaneous Leishmaniasis lesions in man are also characterized by epidermal hyperplasia and conspicuous dermal granulomatous inflammation.

Autocrine growth mechanisms are now believed to play an important role in AIDS related Kaposi sarcoma, because a significantly increased expression of immunoreactive and bioactive IL-6 and of IL-6R mRNA were shown in Kaposi sarcoma cell-lines and skin lesions [43]. These observations tempt speculations on a possible relationship between altered proliferation and differentiation involving autocrine growth stimulation as observed in carcinomas, Kaposi sarcoma and psoriasis and the production and expression of cytokines and cytokine receptors. The increased expression of IL-4R may represent a physiological attempt towards negative feedback signalling, since it is known that IL-4 is able to counteract the effects of IL-1, IL-6, IL-8 and TNF-α via down-regulation of their genes at the level of transcription [69]. Levels of the latter cytokines are indeed increased in psoriatic lesions.

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Chapter 7

EFFECTS OF CYCLOSPORIN A ON EPIDERMAL CYTOKINE RECEPTORS IN PSORIASIS. IN VIVO AND IN VITRO STUDIES

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SUMMARY

In the last several years, oral Cyclosporin A (CsA) has become a more frequently utilized treatment modality for severe recalcitrant psoriasis. Although considerable progress has been made in unraveling the mode of action of CsA in psoriasis, certain aspects still remain elusive. Since increased levels of cytokines such as IL-6, IL-8, IFN-γ, TGF-α and TNF-α have been observed in psoriatic lesions, cytokines and their respective receptors are believed to play a crucial role in the pathophysiology of psoriasis. Cytokines are able to exert their biological functions only if their respective receptors are expressed on the target cells. To date, little is known about the influence of CsA on cytokine receptors in healthy and psoriatic skin. Therefore, we investigated the expression of interleukin-receptors (IL-*n*R) such as IL-1R, IL-4R, IL-6R and TNF-R in freshly prepared epidermal cell suspensions (ECS) of psoriatic lesions before and two weeks later during oral CsA treatment. Freshly prepared ECS from healthy control skin were also initially investigated for the expression of interleukin-receptors and investigated again, simultaneously with ECS from psoriatic patients after *in vitro* stimulation with LPS and PMA. During 2 weeks of oral treatment with CsA, a significant increase in the number of TNF-R^+ cells was observed, while the slight increase in the number of IL-$1R^+$ and IL-$6R^+$ cells during treatment was not statistically significant. The significantly increased number of IL-$4R^+$ viable EC from psoriatic lesions remained unaltered during oral CsA treatment.

ECS prepared from healthy skin which were stimulated *in vitro* with LPS and PMA showed a shift towards the 'psoriatic phenotype'. CsA added to ECS from psoriatic lesions and control skin *in vitro* did not appear to have clear effect(s) on the expression of epidermal cytokine receptors. The increase in the number of TNF-R^+ cells during oral treatment with CsA could be due to an undirected inhibition of cytokines from e.g. the inflammatory infiltrate by CsA because this effect could not be reproduced by addition of CsA to ECS *in vitro*. Thus, clinical improvement in psoriasis obtained by oral treatment with CsA is accompanied by minor alterations in the expression of cytokine receptors on EC prepared from psoriatic lesions.

INTRODUCTION

The new anti-psoriatic drug Cyclosporin A (CsA) is gaining more acceptance as an effective treatment modality for severe recalcitrant psoriasis. Recently, this drug has been officially approved and registered for treatment of psoriasis in the Netherlands. CsA has the potential to clear psoriasis via two distinct pathways. The first pathway comprises its immunosuppressive effects on the inflammatory infiltrate. CsA inhibits the transcription of IL-2, IFN-γ, IL-3, IL-4 and TNF-α in T lymphocytes and at higher

concentrations it also inhibits the expression of IL-2 receptor [1-3]. Although initially debated, CsA may inhibit the capacity of Langerhans cells to present antigen and to stimulate T lymphocytes [4-7].

The second pathway comprises a direct effect of CsA on keratinocytes which leads to an improvement in psoriasis [7]. However, the observed inhibition in growth of cultured keratinocytes caused by CsA [8] led to queries on its clinical relevance because the inhibitory effect of CsA was observed only under serum-free culture conditions or at doses exceeding the presently recommended therapeutic dose [7,8]. The inhibitory effect of CsA on the growth does not seem to depend on the inhibition of autocrine growth factors such as TGF-α and IL-6 [9]. Other basic effects of CsA observed were down-regulating the expression of ICAM-1 on keratinocytes and decreased γ-IP10 immunoreactivity in psoriatic plaques [9,10]. Reduced expression of ICAM-1 on papillary endothelial cells caused by CsA is an important mechanism for hindering the recruitment of inflammatory cells into the skin and the subsequent decrease in an ongoing immune response. Although these details have been elucidated, considerable part of the mode of action of CsA in psoriasis remains unclear. The latter obviously coincides with the fact that the understanding of the pathogenesis of psoriasis is still incomplete.

Data on the expression and the regulation of cytokines in psoriatic skin are accumulating rapidly. Their exact role in the pathogenesis of psoriasis, however, remains elusive. In psoriatic lesions, increased levels of expression of immunoreactive cytokines such as IL-1β, IL-6, IL-8, IFN-γ, TNF-α and TGF-α have been observed [11-20]. Cytokines may trigger hyperproliferation of keratinocytes either directly by serving as a growth factor (e.g. IL-6, IL-8), indirectly via activation by TNF-α and IFN-γ or both, (e.g. IL-1) [21, 22]. It has been shown that activated normal human keratinocytes were able to produce several cytokines which have well defined immunological functions [23]. Proliferation of the psoriatic keratinocyte may be maintained via persistent stimulation of autocrine and paracrine growth by cytokines or growth factors such as TGF-α, insulin-like growth factors (IGFs) and/or fibroblast growth factors (FGFs) produced by activated keratinocytes, the cells of the recruited inflammatory infiltrate, fibroblasts and/or endothelial cells [24, 25].

In vivo regulation of keratinocyte activation by autocrine and paracrine growth mechanisms are complex processes involving interactions between cytokines from different cellular sources and their respective receptors. Cytokines from activated lymphocytes can activate epidermal cells (EC) to proliferate and to produce cytokines and also to express "aberrant" molecules [25-31]. Cytokines produced by activated EC may also affect the function of the epidermal Langerhans cell and recruit other types of inflammatory cells to the site of inflammation [32-34]. Available data from the literature on the expression of cytokine mRNA, protein and bioactivity *in vivo* are summarized in Table I.

Table I. Summary on literature data on the *in vivo* expression of cytokines in normal and lesional psoriatic epidermis

Cytokine	Normal Epidermis			Psoriatic Epidermis			Reference
	mRNA	IR	BA	mRNA	IR	BA	Number
IL-1α	-	++	++	↓↓	↓↓	±	[11,14,53,54]
IL-1β	-	±	-	↑↑	↑↑	↓↓/-*	[11,14,53,54]
IL-2		-			+		[55]
IL-3		+			+		[24]
GM-CSF		+			↑↑		[56,57]
MCAF/MCP-1	+	+		+	+		[58]
IL-4		-			-		[Prens, submitted]
IL-6	-	+	±	↑	↑↑	↑↑	[12,14]
IL-8	+	+	+	↑↑	↑↑	↑↑	[18,59]
IFN-γ	-	+	-	+	↑↑	-	[18,55,60]
IFN-α		+	-		+	-	[18]
TNF-α	-	+	-	-	+	-	[59]
TGF-α	+	+		↑↑	↑↑		[19]
TGF-β	+	+		+	+		[61]

* = increased production, but non-functional mature and novel (pro-)IL-1β isoforms.
±/+ = detectable.
++ = easily detectable.
↑/↑↑ = increased/clearly increased expression.
↓/↓↓ = decreased/clearly decreased expression.

Investigations on the expression of cytokine receptors in psoriasis have shown that the EGF/TGF-α receptor was over-expressed [22,29,35,36]. TGF-α, the ligand for the EGF-R, was also over-expressed in psoriatic lesions [19]. Scheynius et al observed an altered pattern in the expression of the IFN-γR in psoriatic epidermis as compared with normal human skin [37]. Over-expression of IL-1R on psoriatic keratinocytes has also been reported [38]. Recently, we observed an increased number of IL-4R$^+$ cells and decreased number of IL-1R$^+$ cells in lesional psoriatic EC suspensions (ECS) [Prens et al, submitted].

We reasoned that clinical improvement of psoriasis due to oral treatment with CSA may be accompanied by alterations in the expression of IL-*n*R in psoriatic epidermis. Therefore, in the present study, *in vivo* and *in vitro* effects of CsA treatment on the expression of interleukin receptors on lesional EC from psoriatic patients and *in vitro* effect(s) of CsA on EC from healthy controls were investigated.

MATERIALS AND METHODS

Patients and controls

Eight patients with recalcitrant psoriasis of long duration participated in a clinical study to investigate the effect(s) of oral CsA treatment. The patients were otherwise healthy and provided informed consent as required by the Medical Ethical Committee of the University Hospital Rotterdam-Dijkzigt. All patients had stable plaque-type psoriasis which had not been treated for at least 4 weeks prior to receiving CsA. The disease activity, severity and extent were calculated for each patient before treatment and every two weeks thereafter and expressed as PASI scores. If the appropriate routine laboratory investigations were normalities, CsA was administered at a dose of 3 to 5 mg/kg body weight/day as reported previously [39,40].

Preparation of epidermal cell suspensions

Split-skin specimens from involved skin of untreated psoriatic patients, psoriatic patients who were treated with CsA and from surgically excised abdominal or breast skin from healthy controls without history or signs of skin disease, were obtained using a portable dermatome. Single cell suspensions of EC were prepared from the split skin specimens using standard methods [41]. Briefly, the split-skin specimens were rinsed thoroughly with Ca^{2+} and Mg^{2+}-free phosphate-buffered saline (PBS), cut in pieces of ± 0.5 cm^2 and floated in PBS containing 0.0625% trypsin (ICN Biochemicals, Cleveland, OI, U.S.A.), 0.32% glucose and 0.1% EDTA for 45 min at 37°C and in the presence of 0.025% deoxyribonuclease (DNase; Sigma, St. Louis, MO, U.S.A.) during the last 15 min of the incubation. At this stage, the epidermis was separated from the dermis using fine forceps. The epidermal sheets were cut with scissors and the EC were filtered through sterile 100-μm and 30-μm mesh nylon gauzes and rinsed twice with RPMI 1640 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 6 mM fresh L-glutamine, 20 mM Hepes (RPMI complete medium) and 250 μg/ml trypsin inhibitor (derived from egg-white, Sigma). Cells were counted using a hemocytometer and their viability was determined by trypan blue exclusion. The viability was checked again during FACScan analysis using propidium iodide and FITC-diacetate. Previous studies showed that cells which were positive for FITC after uptake and hydrolysis of FITC-diacetate or carboxyfluorescein diacetate were viable, proliferating cells [42,43]. The number of patients studied in a specific experiment is mentioned in the figures and tables ($n=x$).

Immunophenotyping

CD1a (66IIC7, Dr. M. van der Rijn, Amsterdam) and CD45 (HLe-1-FITC-conjugated, Becton Dickinson, San Diego, CA) MoAb were used to immunophenotype and to examine the composition of the freshly prepared ECS of patients and controls. ECS were immunophenotyped using direct and indirect immunofluorescence techniques. The indirect immunofluorescence technique was performed as described by Van Dongen et al. [45]. Some EC samples were counted using Standard Zeiss 16 microscopes equipped with a IV FL epi-illumination condensor (Carl Zeiss, Oberkochen, FRG). The percentage of positive cells was calculated by counting the number of cells showing positive fluorescence from a total of 200 - 400 cells.

Labeling of ECS using biotinylated rHu cytokines

ECS were stained using a panel of biotinylated recombinant human (rHu) cytokines. The expression of the following interleukin-receptors (IL-nR) were studied: IL-1R (using rIL-1α), IL-4R, IL-6R and TNF-R. The cells were labeled using biotinylated recombinant human cytokines and avidin-FITC (all purchased from R&D Systems, Minneapolis, MN) or streptavidin-phycoerythrin (Becton Dickinson) as

the second step, followed by FACScan analysis. Unbiotinylated rHuIL-1β was generously provided by Glaxo/Biogen (Geneva, Switzerland). IL-4 was obtained from DNAX (Palo Alto, CA) and IL-6 was obtained from Drs. L.A. Aarden/J. Braakenhof CLB (Amsterdam). Recombinant HuTNF-α and IL-1α were purchased from Amersham (Buckinghamshire, UK) and Genzyme (Cambridge, MA) respectively.

ECS were labeled with biotinylated rHu cytokines using the protocol supplied by the manufacturer. Briefly, 10^5 EC were incubated with specific rHu cytokine solution containing, depending on the specific cytokine used, 65 to 300 ng rHu cytokine protein for 1 hr at 4°C. Unbound cytokine was removed by two rinses. This was followed by incubation with avidin-FITC or streptavidin-phycoerythrin for 30 min at 4°C in the dark. Finally, the cells were rinsed twice and resuspended in FACScan-buffer and kept on ice till FACScan analysis was performed. The whole procedure of preparation, labeling and analysis of ECS normally required about 4 to 5 hours. The FACScan analysis was conducted using the FACScan^C, Paint a Gate^C and Lysis^C software programs (Becton Dickinson). The results are presented either as histograms (y-axis representing the number of cells, x-axis the fluorescence intensity), or as the mean ± SEM percentage of positive cells. The level of expression of cytokine receptors was calculated and expressed as the difference between EC stained with avidin-FITC or streptavidin-PE alone and EC stained with biotinylated rHu cytokine and (strept)avidin-FITC/PE.

To determine the relative fluorescence intensities of different EC samples, and express them as an indirect value for the number of binding sites per cell, the following formula was used: FSN (fluorescence signal-to-noise ratio) = $10^{[(Ch\#exp. - Ch\#contr.)/Ch\#D]}$, where Ch#exp.= measured mode channel number of experimental sample, Ch#control = measured mode channel number of negative control sample, and Ch#D= number of channels per decade [46].

Controls

The controls comprised the use of isotype-control MoAb to an irrelevant epitope and omission of the second step (PBS). The specificity was checked by pre-incubating ECS with equivalent amounts of unbiotinylated ("cold") rHu cytokines followed by the above mentioned complete labeling procedure. The latter procedure almost completely reduced the binding of biotinylated rHu cytokines. The sensitivity and specificity were also checked during the course of the studies using stimulated and unstimulated human PBMC, D10(N4)M, B9, EL-4, U937, KG1a and THP-1 cell lines. In these studies reproducible labeling and fluorescence intensities were obtained. Equally diluted quantities of ethanol, the solvent for CsA, were used as controls for the *in vitro* studies with CsA. The solvent did not exert any adverse effect on EC.

Two stripping-buffers were used consecutively to obtain efficient stripping of receptor-bound cytokines in order to check for *in vivo* binding of cytokines to their receptors [47, 48]. Briefly, EC were suspended and kept on ice for 1 min in buffer I (pH 4.0) [47], neutralized by adding medium containing Hepes and washed. Next the EC were suspended and kept on ice for 30 sec in buffer II (pH 3.0) [48], neutralized again by adding medium containing Hepes, rinsed twice and followed by the complete labeling procedure.

Short-term culture of EC and time-course studies following stimulation with LPS and PMA

Lesional psoriatic and healthy control EC samples were cultured at a concentration of 10^6 EC/ml, in a total volume of 1 ml complete RPMI medium containing 20% human AB serum. Both types of EC samples were either stimulated with 1 ng/ml LPS and 50 ng/ml PMA. In initial experiments, unstimulated EC were also cultured simultaneously. To each culture, 2.5 μg/ml CsA (generously supplied by Sandoz, Basel) was added, whereas cultures to which CsA was not added served as controls. After culturing for 1, 2, 4 and 16 h, the EC were harvested and assayed for the expression of receptors as described

earlier. CsA was always added at least 30 min prior to stimulation with LPS and PMA.

Statistical analysis

The results were analyzed with STATA™ (computer program for statistical analysis, Computing Resource Center, Los Angeles, CA). The test used to determine statistical significance is mentioned in the abbreviated form, e.g two-sample Wilcoxon test (WT) and Wilcoxons signed rank sum test (WSRT), together with the "p" values.

RESULTS

The viability of EC was determined directly after isolation and again during FACScan analysis. Directly after isolation, the viability ranged from 70 to 80% as assessed by trypan blue exclusion. The viability of cells in the total ECS at FACScan analysis ranged from 50% to 75% as assessed by propidium iodide (PI) uptake and FITC-diacetate labeling. As described previously, subpopulations of EC showing uptake of PI ($PI^+/FITC^-$) and those which were positive for FITC after uptake and processing of FITC-diacetate ($PI^-/FITC^+$), were clearly mutually exclusive populations [Prens et al, submitted]. When fresh EC were used, the total ECS (excluding cellular debris), the viable and non-viable EC fractions could be easily gated and separately analyzed. However, culturing clearly changed the physical properties of most viable

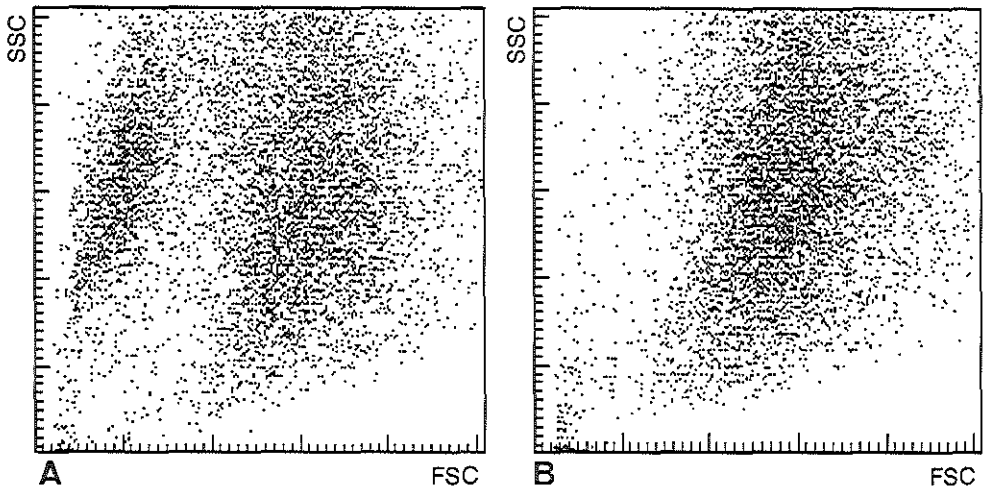

Figure 1. An example of the altered physical properties of cultured EC. In **Fig 1a** a dot plot of a freshly prepared ECS from a psoriatic lesion with distinct EC fractions. **Fig 1b** shows that after a 16 h culture period the original distribution of EC in the dot plot is significantly altered.

EC which resulted in a more dispersed distribution of viable EC in the FSC/SSC dot plots. Thus, it was no longer possible to set a gate for these scatter plots which contained a homogenous population of viable EC (more than 95% viable cells). The latter was also attributable to the fact that the overall viability within the ECS declined to approximately 30% viable cells after culturing for 16 h (Fig. 1).

Expression of interleukin receptors on freshly isolated EC before and after 2 weeks of oral treatment with CsA

Similar to that reported in our previous study (Prens et al., submitted), in this study also no correlation could be established between the expression of cytokine receptors and the disease activity expressed as the PASI score (results not shown). Although skin lesions of all patients showed considerable regression during two weeks of CsA treatment (the improvement ranged from 30% to 60% as compared with the pre-treatment PASI scores), no correlation could be established between the improvement rate and the expression of cytokine receptors.

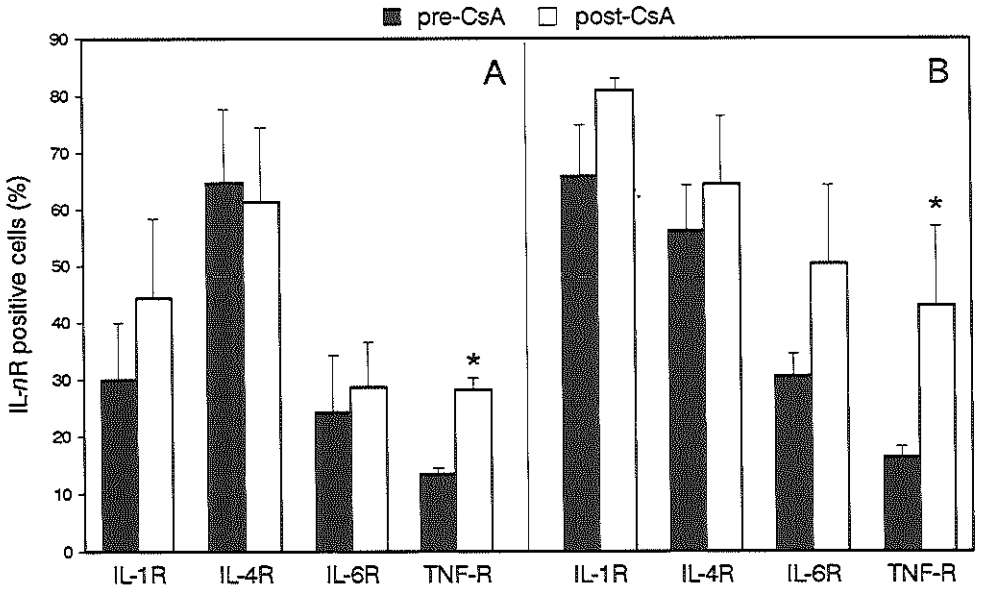

Figure 2. Effects of oral treatment with CsA on the expression of cytokine receptors on freshly prepared ECS from psoriatic lesions. **Fig 2a** shows the mean ± SEM number of IL-nR^+ cells in the viable EC fraction before and after 2 weeks of oral treatment with CsA. **Fig 2b** shows the mean number ± SEM of IL-nR^+ cells in the non-viable EC fraction before and after 2 weeks of treatment. The increase in the number of TNF-R was statistically significant ($p < 0.05$, WSRT, $n = 8$).

Interleukin-1 receptors. The number of IL-1R$^+$ cells in the non-viable EC fraction of ECS from psoriatic patients was as high as the controls, as shown in Figs. 2a and 2b. We recently showed that the number of IL-1R$^+$ cells was significantly decreased in viable fraction of ECS from psoriatic lesions. Before CsA treatment, the mean number of IL-1R$^+$ cells in viable psoriatic ECS was 30 ± 10%. The mean number after treatment was 44 ± 14% (Fig. 2a). In the non-viable EC fraction these figures were 66 ± 9% and 81 ± 2%, respectively (Fig. 2b). The number of IL-1 binding sites per EC did not change significantly in all EC fractions during CsA treatment when the relative fluorescence intensities (fluorescence signal-to-noise (S/N) values) were compared (Fig. 3).

Interleukin-4 receptors. As shown in a recent study [Prens et al, submitted], the viable fractions of psoriatic EC clearly contained more IL-4R$^+$ cells than those of controls. Before CsA treatment, the mean number of IL-4R$^+$ cells in viable psoriatic

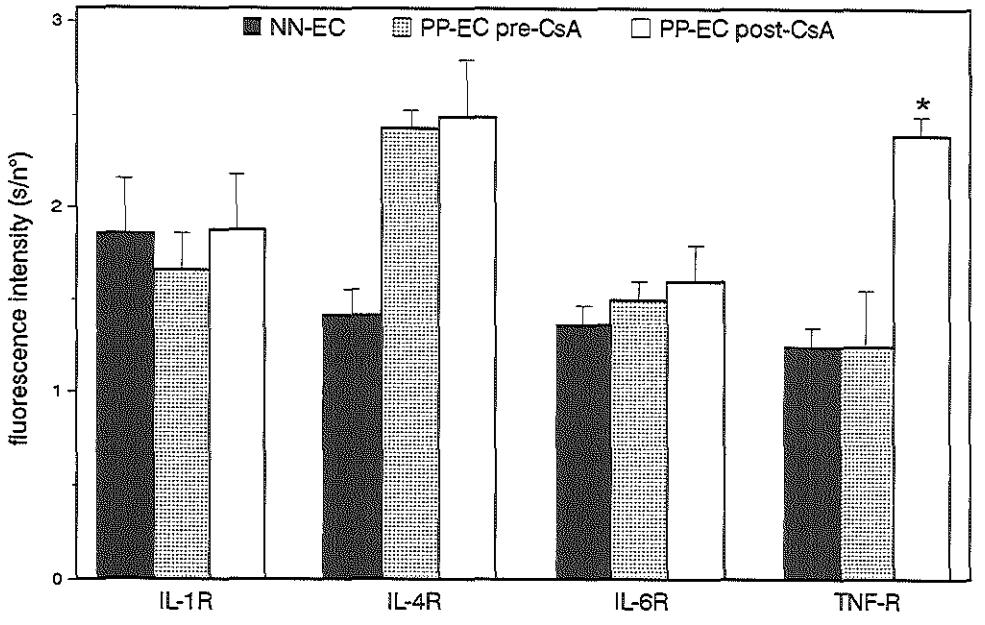

Figure 3. Effects of oral treatment with CsA on the fluorescence intensities (signal to noise-S/N ratio) of cytokine receptors on freshly prepared ECS from psoriatic lesions. Closed bars represent, as a reference, the mean ± SEM S/N ratio of IL-*n*R$^+$ cells from control ECS and of ECS from psoriatic lesions. The ratios before and after 2 weeks of CsA treatment are represented by the speckled and open bars, respectively.

EC was 65 ± 13%. The mean number after treatment was 61 ± 13% (Fig. 2a). In the non-viable EC fraction, these figures were 56 ± 8% and 64 ± 12% respectively (Fig. 2b). These differences were not statistically significant in any EC fraction. The number of IL-4 binding sites per EC did not change significantly in any EC fraction during CsA treatment (Fig. 3).

Interleukin-6 receptors. During treatment with CsA, the number of IL-6R$^+$ EC increased, especially in the non-viable EC fraction. The differences, however, were not statistically significant (Figs. 2a and b). The number of IL-6 binding sites per cell did not differ significantly in any EC fraction (Fig. 3).

TNF receptors. After 2 weeks of CsA treatment, a significant increase in the number of TNF-R$^+$ EC was observed ($p<0.05$, WSRT). This increase was most evident in the non-viable EC fraction (Fig. 2b). Although the number of TNF binding sites per cell had increased after two weeks of treatment with CsA, this increase was not statistically significant. The results on the expression of cytokine receptors in the total ECS were similar to those shown in Figs. 2a and 2b and are, therefore, not shown.

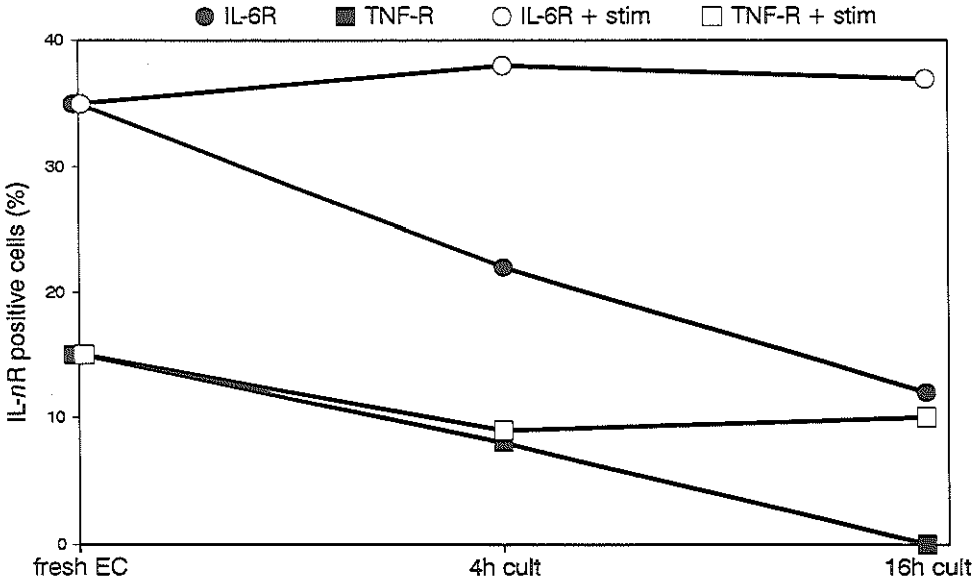

Figure 4. The spontaneous decline in the number of IL-6R$^+$ (circle) cells and TNF-R$^+$ cells (square). Cells were labeled after culturing control EC for 1, 4 and 16 h (closed symbols). Stimulation with 1 ng/ml LPS and 50 ng/ml PMA prevents this decline (open symbols).

In vitro effects of CsA on the expression of interleukin receptors on psoriatic and healthy control EC

Initial time-course experiments showed that the expression of IL-6R and TNF-R on unstimulated EC of psoriatic patients and of controls rapidly declined on *in vitro* culturing (Fig. 4). CsA added *in vitro* to the EC had no effect on this spontaneous decline. The expression of IL-1R and IL-4R remained quite stable under unstimulated conditions. EC stimulated with LPS and PMA showed little or no decrease in the expression of IL-6R and TNF-R. The rapid decline in the number of IL-6R$^+$ and TNF-R$^+$ cells could be due to the transfer of EC from the *in vivo* integument to the *in vitro* milieu. This *in vitro* conditioning was accompanied by a progressive decline in cell viability. Stimulation with LPS of PMA was imperative in making feasible the *in vitro* investigations into the effect of CsA on the expression IL-6- and TNF-receptors on EC. Moreover, in psoriatic lesions EC are in a more or less constant state of activation. Stimulation of EC with LPS and PMA *in vitro* was, thus, regarded as a

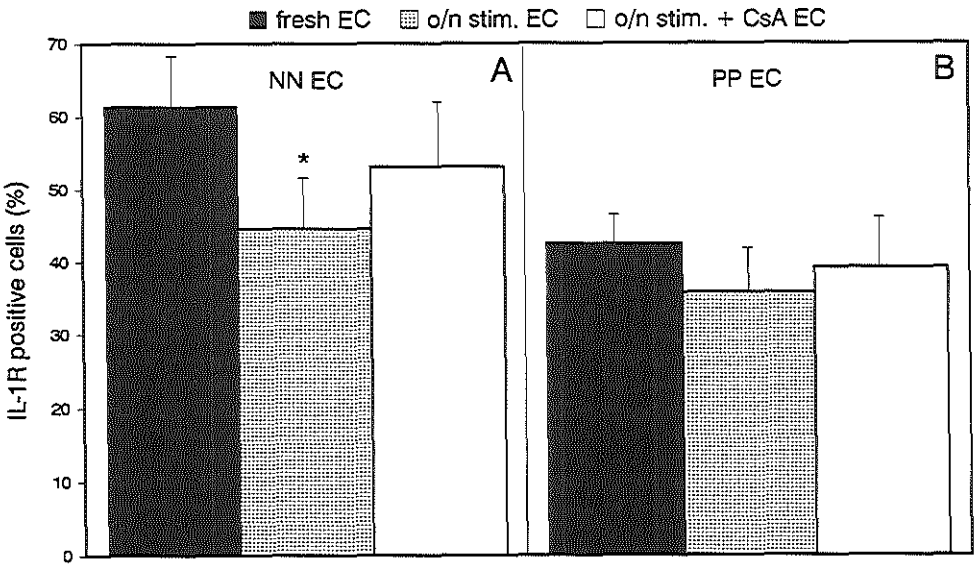

Figure 5. The effect of culturing in the presence of 2.5 μg/ml CsA on the number of IL-1R$^+$ in the total ECS. **Fig 5a** shows the effects of CsA on *in vitro* cultured EC from healthy controls. The decrease in the number of IL-1R$^+$ cells is statistically significant ($p < 0.02$, WSRT, $n = 12$). **Fig 5b** shows that *in vitro* stimulation with LPS and PMA and culturing of psoriatic EC in the presence of CsA has only minor effects ($n = 12$).

means to keep psoriatic EC activated and as a means to activate control EC *in vitro*. The time-course studies showed a merely stabilizing effect of LPS and PMA stimulation on the expression of all IL-*n*R during the first 4 h. Therefore we allowed for the conditioning period *in vitro*, and further measurements were all conducted after culturing for 16 h.

Interleukin-1 receptors. In LPS and PMA stimulated ECS of healthy controls, the number of IL-1R$^+$ EC significantly decreased during the 16 h culture period, as compared with freshly isolated EC ($p < 0.02$, WSRT, Fig. 5), whereas the relative number of IL-1 binding sites per EC did not change significantly. ECS of healthy controls which were stimulated with LPS and PMA and cultured in presence of CsA for 16 h showed a less pronounced decrease in the number of IL-1R$^+$ cells (Fig. 5).

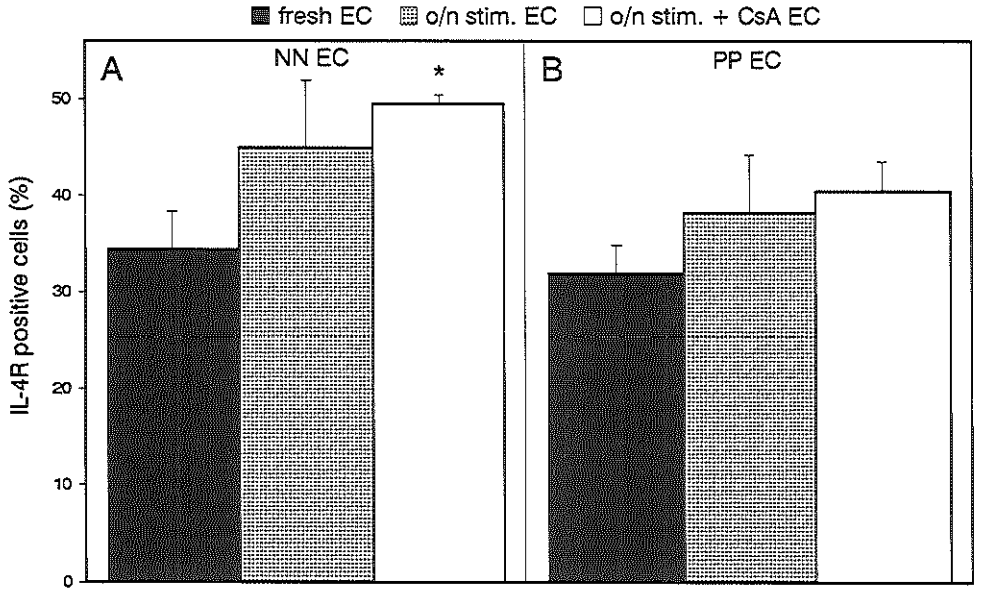

Figure 6. The effect of culturing in the presence of 2.5 μg/ml CsA on the number of IL-4R$^+$ cells in the total ECS. **Fig 6a** shows the effects of CsA on cultured EC from healthy controls. The increase in the number of IL-4R$^+$ cells in ECS cultured in the presence of CsA is statistically significant as compared with the number of IL-4R$^+$ cells in freshly isolated EC ($p < 0.05$, WSRT, $n=12$). **Fig 6b** shows that *in vitro* stimulation with LPS and PMA and culturing of psoriatic EC in the presence of CsA resulted in a slight increase in the number of IL-4R$^+$ cells ($n=12$).

The mean number of IL-1R$^+$ EC and the number of IL-1 binding sites per EC of psoriatic patients did not alter significantly after stimulation with LPS and PMA or in the presence of CsA. The mean percentage of IL-1R$^+$ EC was 42 ± 4% in fresh ECS, 36 ± 6% in stimulated, cultured ECS and 39 ± 7% in ECS stimulated in the presence of CsA.

Interleukin-4 receptors. ECS of healthy controls, stimulated with LPA and PMA showed some increase in the number of IL-4R$^+$ cells as compared with freshly isolated control EC (Fig. 6), whereas the number of IL-4 binding sites per cell did not change significantly during 16 h of culturing (not shown). The number of IL-4R$^+$ EC of healthy controls stimulated with LPS and PMA in the presence of CsA was higher than in untreated stimulated EC (Fig. 6). This increase was statistically significant as compared with the number of IL-4R$^+$ cells in freshly isolated control EC ($p < 0.05$, WSRT, Fig. 6).

In ECS from psoriatic lesions the mean number of IL-4R$^+$ EC slightly increased from 32 ± 3% (fresh EC) to 38 ± 6% (stimulated, cultured EC) to 40 ± 3% (stimulated, cultured EC + CsA). The number of IL-4 binding sites per EC did not alter significantly during culturing with LPS/PMA and after addition of CsA (not shown).

Interleukin-6 receptors. In ECS of healthy controls, the mean number of IL-6R$^+$ EC remained unchanged after LPS and PMA stimulation and culturing for 16 h (21 ± 4%). The number of IL-6 binding sites per cell did not alter during the 16 h culture period. When stimulated with LPS and PMA and cultured in presence of CsA a slight increase in the number of IL-6R$^+$ cells was observed (32 ± 6%).

The mean number of IL-6R$^+$ EC of psoriatic patients did not alter significantly after stimulation with LPS and PMA and after addition of CsA *in vitro* (not shown). The number of IL-6 binding sites per EC slightly decreased when stimulated and cultured in the presence of CsA.

Table II. Summary of the effects of *in vivo* and *in vitro* treatment with CsA on epidermal cytokine receptors

Interleukin Receptor	*In vivo* Oral CsA PP EC	2.5 μg/ml CsA *in vitro* During 16 h NN EC		PP EC	
		LPS/PMA	CsA	LPS/PMA	CsA
IL-1R	↑	↓↓	↓	↓	↔
IL-4R	↔	↑	↑↑	↔	↔
IL-6R	↑	↔	↑	↔	↔
TNF-R	↑↑	↔	↔	↔/↓	↑

↔ = unaltered. ↑/↑↑ = increased / significantly increased. ↓/↓↓ = decreased / significantly decreased. PP = lesional psoratic EC. NN = healthy control EC.

TNF receptors. In ECS of controls and psoriatic patients, no significant changes in the number of TNF-R$^+$ EC and in the number of TNF binding sites per cell were observed during *in vitro* stimulation and after addition of CsA. Although, the number of TNF-R$^+$ cells in ECS of psoriatic patients almost doubled (from 10% to 18%) after the addition of CsA to EC *in vitro*, the difference was not statistically significant. All results are summarized in Table II.

DISCUSSION

We believe that the results obtained in this study favour an authentic expression of IL-*n*R on epidermal cells without any interference by contaminating inflammatory cells, because psoriatic ECS normally contain a negligible number (approximately 3%) of CD45$^+$ bone marrow-derived cells (Prens et al., submitted). In addition, control experiments, using a stripping technique involving two different stripping-buffers consecutively prior to incubation with biotinylated rHu-interleukins showed that there were minor changes in the expression of the different IL-*n*R (results not shown). In contrast, the same stripping technique almost completely eliminated the fluorescence signal of *in vitro* labeled control EC. These results indicate that the actual number of IL-R$^+$ EC were determined because the likely interference caused by IL-R that were occupied on EC was minimal.

Although a clearly increased expression of IL-1R and IL-6R on EC of some patients was observed it failed to reach statistical significance. This could have been due to the varying number of IL-R$^+$ EC. Similar to the observations in our study, major fluctuations in the expression of IL-1R were also observed in different batches of cultured neonatal keratinocytes [49]. These fluctuations were believed to be donor specific [49]. Moreover, in the current study the clinical response to oral CsA treatment also varied considerably from patient to patient. Therefore, we decided to evaluate the effect of CsA treatment modalities on the biological properties of EC, for other further clinical trials only at points of predefined improvement in the PASI score.

The significant increase in the number of TNF-R$^+$ EC was unexpected because the role of TNF in the pathophysiology of psoriasis is poorly documented. TNF-α, a pro-inflammatory cytokine, is active in the earliest stages of inflammatory processes and is able to induce the production of a broad range of other cytokines and inflammatory mediators. Gearing et al demonstrated significant quantities of TNF-α in extracts of scales from psoriatic lesions [18]. The effects of CsA treatment *in vivo* on the expression of TNF-R were only partially reproducible *in vitro*, but failed to reach statistical significance. This suggests that the increase was secondary to the inhibition in the production of inflammatory cytokines by CsA. With the technique that was used in our study, it was not possible to distinguish between the p55 TNF-R1 and p75 TNF-RII.

We showed that freshly prepared EC from psoriatic lesions contained a significantly decreased number of IL-1R$^+$ and an significantly increased number of IL-4R$^+$ cells as compared with viable EC from healthy controls. In this study, it

appeared that EC from healthy controls stimulated with LPS and PMA showed a shift towards the 'psoriatic phenotype' which was characterized by a decreased number of IL-1R$^+$ and an increased number of IL-4R$^+$ cells (Prens et al, submitted). This shift is illustrated in Fig. 7 in which the number of IL-1R$^+$ and IL-4R$^+$ cells in fresh ECS and simulated ECS of controls are compared with those of psoriatic patients. The decreased expression of IL-1R may also be the result of increased IL-1 production and release by EC due to stimulation with LPS and PMA. In other cell types, exposure to IL-1 has been shown to result in down-regulation of the expression of the IL-1R [51, 52]. ECS from untreated psoriatic lesions appeared to release IL-1β because supernatants of the cultured ECS used in this study contained increased levels of immunoreactive IL-1β and bioactive IL-1 when compared with supernatants of ECS from skin of healthy controls (Debets et al, in press). Cooper et al also showed that immunoreacting IL-1B was realsed from biopsy samples of psoriatic lesions [11]. In contrast to our observations, Blanton et al. observed a significantly (9 to 20-fold) increased expression of IL-1R in post-confluent cultured neonatal keratinocytes which had been stimulated with 10 ng/ml PMA [49]. Stimulation with PMA also caused the

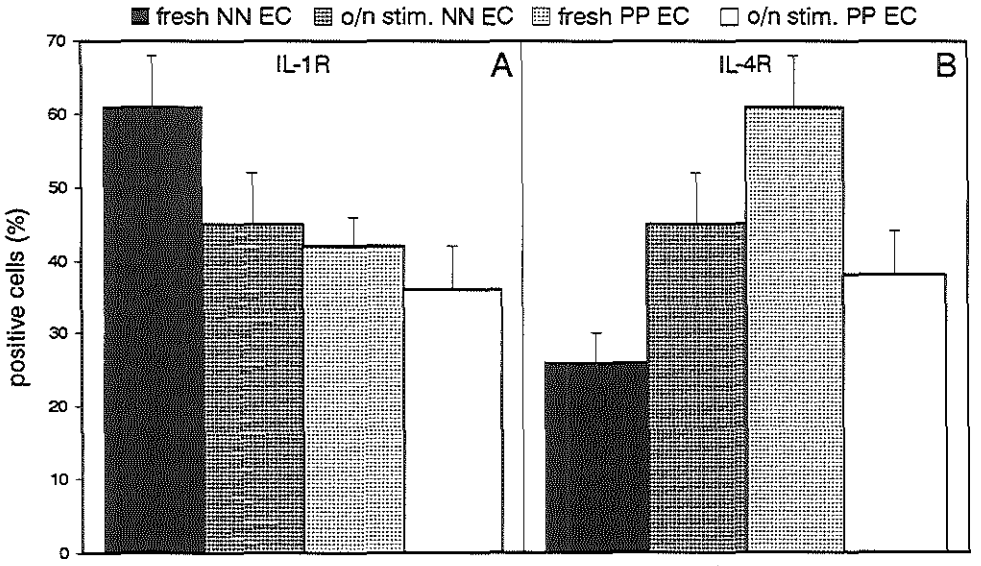

Figure 7. Illustration of the shift towards the 'psoriatic phenotype' of healthy control EC during *in vitro* stimulation with LPS and PMA. **Fig 7a** shows the number of IL-1R$^+$ cells in freshly prepared ECS of healthy controls and ECS from psoriatic lesions after stimulation and culturing for 16 h. **Fig 7b** shows the number of IL-4R on freshly isolated viable EC of healthy controls and EC from psoriatic lesions and after culturing total ECS for 16 h ($n=12$).

release of IL-1 in the culture supernatant (60-fold) and a dramatic increase (>4000-fold) in cellular IL-1α. These discrepancies in the results may be explained by the fact that we used total ECS which also contained different cell types such as Langerhans cells and melanocytes.

With regard to the increase in the expression of IL-4R, time-course experiments showed that the increase in the number of IL-4R$^+$ EC was already evident after 2 to 4 h. This increase was most clear in freshly prepared psoriatic ECS and in the viable EC fraction. Despite the fact that gating of a homogeneous viable population (>95% viable cells) in a flow cytometer was not possible after 16 h of culture, the increase was still observed in the total EC population. In an earlier study we proposed that over-expression of IL-4R could be a manifestation or a marker for keratinocyte proliferation and activation.

In previous studies into the effects of oral CsA in psoriasis patients, striking reductions were observed in the number of activated T lymphocytes in psoriatic lesions. In addition, in a recent study the clear effects of CSA on the immune infiltrate were corroborated [9]. In contrast CsA had no significant effect on markers of keratinocyte growth and activation such as IL-6, and TGF-α. The expression of keratin K16 was also demonstrated [9]. Therefore, it was proposed that the principal effect of CsA treatment in psoriasis patients was in decreasing the activation of immune cells in the inflamatory infiltrate [9].

The results presented here indicate that clinical improvement of psoriasis obtained by oral treatment with CsA was accompanied by, with the exception of TNF-R, minor alterations in the profile of the interleukin receptors in psoriatic lesions during the first two weeks of treatment. These findings indicate that although cytokines and their respective receptors are involved in the pathophysiology of psoriasis, treatment of psoriasis with CsA does not have a prominent effect on the expression of the epidermal cytokine receptors that were investigated. Furthermore, the lack of effect of CsA *in vitro* on the expression of epidermal IL-4R, a marker of epithelial activation and proliferation, seems to confirm the view that clinical improvement of psoriasis obtained by CsA is not primarily achieved via its effect(s) on epidermal cells but rather via its effect(s) on the inflammatory infiltrate.

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Chapter 8

GENERAL DISCUSSION AND CONCLUSIONS

8.1 Interactions of T lymphocytes with accessory cells in psoriasis

Our observations that epidermal cells (EC) from psoriatic lesions and uninvolved skin stimulated autologous T lymphocytes in the *in vitro* autologous mixed epidermal cell - T lymphocyte reaction (autologous MECLR) (Chapter 3) coincided with similar observations reported by three other groups [1-3]. Such an elevated autologous MECLR has also been reported in cutaneous T cell lymphoma and after 4 MED UVB irradiation of normal skin [4,5]. Our subsequent goal was to delineate the functional role of antigen-presenting cell (APC) subsets in this reaction. It was observed that "classical" Langerhans cells (HLA-DR$^+$/CD1a$^+$) were not the principal stimulators of T lymphocytes in the MECLR, but HLA-DR$^+$/CD1a$^-$ APC. Our studies further indicated that keratinocytes did not fulfill an accessory function in this reaction. This may be explained by the fact that a limited number of keratinocytes in psoriatic lesions expressed MHC class II antigens which apparently seem necessary to perform an accessory function (Chapter 3) [6,7].

Several studies indicated that increased levels of "aberrant" and certain constitutive membrane molecules such as CD29(4B4), CD36(OKM5), CD54(ICAM-1), γ-IP10 and mIL-1 on epidermal cells from plaque-type psoriatic lesions [8-15]. The important accessory or signalling functions of these molecules in the immune system spurred us to examine the expression of CD29, CD45RA, ICAM-1, OKM5 and mIL-1 in psoriatic lesions and in healthy skin (Chapter 4) [16-18]. The functional relevance of lymphocyte function-associated antigens (LFA) and ligands, OKM5 and mIL-1 were investigated in detail *in vitro* using the autologous MECLR (Chapter 4). Simultaneous immunohistochemical and immunofluorescence studies confirmed the presence of "aberrant" molecules in psoriatic lesions (Table I). It was shown that adhesion mole-

Table I. Summary of alterations in the mean percentage of CD36(OKM5)$^+$, CD54(ICAM-1)$^+$, mIL-1$^+$, HLA-DR$^+$ and CD1a$^+$ in epidermal cell suspensions (ECS) from lesional (PP), uninvolved psoriatic (PN) and healthy control skin (NN)

Marker	PP	PN	NN
CD36(OKM5)$^+$	23 ± 12%†*	0.3 ± 0.1%	0.5 ± 0.1%
CD54(ICAM-1)$^+$	31 ± 14%*	0.6 ± 0.1%	0.5 ± 0.1%
mIL-1$^+$	57 ± 21%*	12 ± 3%*	4 ± 0.5%
HLA-DR$^+$	5.1 ± 1.6%*	1.9 ± 0.6%	1.6 ± 0.4%
CD1a$^+$	1.8 ± 0.7%	1.6 ± 0.4%	1.5 ± 0.4%

† = Figures represent the mean percentage of positive cells ± SD. (Chapters 3 and 4.)
* = These figures are significantly higher than in uninvolved psoriatic and/or in control ECS.

cules such as CD2(T11), CD11a(LFA-1α), CD18(LFA-1β), ICAM-1, CD58(LFA-3) and mIL-1 on epidermal cells from psoriatic lesions fulfilled an accessory role in the autologous MECLR, whereas OKM5 was not involved. The latter probably reflects passive expression of this molecule on epidermal cells. Taken together, our studies led to the conclusion that in the autologous MECLR the accessory function is probably mediated via HLA-DR$^+$/CD1a$^-$/LFA-3$^+$/ICAM-1$^+$ APC which have not yet been characterized further. The accessory function of mIL-1 in the autologous MECLR illustrated its role in psoriasis and emphasized the relevance of its presumed dysregulation in this disease.

8.2 Cytokines and their specific receptors in psoriasis

Data on the expression and the regulation of cytokines in psoriatic skin have accumulated rapidly. However, the exact role of cytokines in the pathogenesis of psoriasis still remains elusive. In psoriatic lesions, increased expression of cytokines such as IL-1β (mainly immunoreactive), IL-6, IL-8, IFN-γ, TNF-α and TGF-α have been observed [19-28]. Since it was shown that activated normal human keratinocytes were able to produce several pro-inflammatory cytokines, the question as to whether epidermal cells are able to trigger, activate and maintain psoriasis independently of immune cells emerged once again [29-30]. Cytokines may trigger hyperproliferation of keratinocytes either directly by serving as growth factors (e.g. IL-6, IL-8), indirectly via activation (e.g. TNF-α and IFN-γ) or both (e.g. IL-1) [29-32]. Thus, it is conceivable that proliferation of keratinocytes in psoriasis may be maintained by autocrine and paracrine growth mechanisms.

The main goal of investigators examining the role of cytokines in inflammatory skin diseases has been to identify the most important cytokine(s), their cellular source and to suggest the likely sequence of events occurring *in vivo* in the "cytokine cascade". The potential cellular sources of such cytokines in psoriatic lesions are numerous.

We pursued different approaches to investigate cytokines in psoriatic lesions, because it had become clear that transcription, translation and secretion of cytokines were complex processes which were not strictly associated with each other *in vivo*. It was observed that, with the techniques used, IL-1β was undetectable in suction blister fluid from lesional and uninvolved skin from psoriatic patients and from skin of healthy individuals. In contrast, we measured significant levels of IL-6 in suction blister fluid from lesional psoriatic skin, but not in the sera of the corresponding patients (Chapter 5). In skin sections, IL-1β was expressed in a distinctive dot-like pattern. In contrast, immunofluorescence staining of cytocentrifuge preparations of epidermal cell suspensions (ECS) from psoriatic lesions revealed an increased membrane staining. Slightly elevated levels of IL-1 mRNA, preferentially IL-1β mRNA, were also observed

in cultured keratinocytes (KC) from psoriatic lesions, whereas very low levels of IL-6 mRNA were found in KC from psoriatic lesions and healthy skin. Fibroblasts from psoriatic lesions expressed extremely low levels of IL-1α and IL-1β, but high levels of IL-6 mRNA. Therefore, we consider dermal fibroblasts together with the inflammatory infiltrate as the major sources of local IL-6 production in lesional psoriatic skin (Chapter 5).

The paradoxical situation concerning IL-1 in psoriatic skin was also reported by Cooper et al. [33]. Our findings that IL-1β is stored intracellularly and in the plasma membrane of EC, could explain the observed absence of IL-1 in *aqueous* extracts of psoriatic scales [20,21]. Cooper et al. reported further evidence for defects in the regulation of IL-1 in psoriasis, by demonstrating *decreased* levels of IL-1 in psoriatic lesions based on a decreased synthesis of IL-1α and an increased production of non-functional IL-1β [33].

Additional studies at our laboratory showed that viable EC from psoriatic lesions contained a statistically significant decreased number of IL-1R$^+$ cells as compared with viable EC from healthy controls (Chapter 6). Based on this observation and the data mentioned earlier we propose the provocative hypothesis that the decreased expression of IL-1R may be the result of *increased* bio-activity of IL-1 in psoriatic lesions. This proposal is supported by the observations that 1) the expression of the IL-1R was down-regulated upon exposure to IL-1 *in vitro* [34,35], and 2) cultured EC from untreated psoriatic lesions released IL-1β in culture supernatants. The supernatants contained increased levels of immunoreactive IL-1β and increased IL-1 bioactivity as compared with supernatants of cultured ECS from healthy skin [Debets et al., in press].

We further showed that freshly prepared viable EC from psoriatic lesions contained a significantly increased number of IL-4R$^+$ cells as compared with viable EC from healthy controls. Moreover, EC from healthy controls stimulated with LPS and PMA showed a shift towards the "psoriatic phenotype", thus characterized by a decreased number of IL-1R$^+$ cells and an increased number of IL-4R$^+$ cells (Chapter 7). Time-course experiments showed that the increase in the number of IL-4R$^+$ EC was already evident after 2 to 4 h of *in vitro* culturing. Overexpression of IL-4R occurred while neither unstimulated nor LPS/PMA-stimulated psoriatic EC from the same patients or healthy control EC produced any detectable levels of immunoreactive IL-4. These results parallel the observed increased expression of IL-4R on epithelial tumors and indicate that overexpression of IL-4R could be a manifestation of keratinocyte proliferation and activation [36]. It has to be determined if EC upregulate their IL-4R in response to cytokines derived from the inflammatory infiltrate.

The role of TNF in the pathophysiology of psoriasis is not extensively documented. TNF-α, a pro-inflammatory cytokine, is active in the earliest stages of inflammatory processes and is able to induce the production of a broad range of other cytokines

and inflammatory mediators [13,37]. Gearing et al. demonstrated significant quantities of immunoreactive TNF-α in scale extracts from psoriatic lesions [25]. However, several other groups failed to detect TNF-α protein or mRNA in lesional skin (Chapter 2, paragraph 5) [38,39]. In addition, locally and systemically administered TNF-α has been shown to improve psoriasis [40,41]. Normal and psoriatic EC express a similar number of TNF-R, whereas an increased expression of TNF-R on EC from psoriatic lesions were observed during treatment with cyclosporin A (CsA).

No abnormalities in the expression of IL-6R were observed in psoriatic ECS as compared with healthy control ECS (Chapter 6). This was rather unexpected because expression of IL-6 was unequivocally demonstrated to be increased in psoriatic lesions [23,24,32,42]. This again illustrates the complexity of the process of transcription, translation and secretion of cytokines and their specific receptors, and that these steps are not always synchronized *in vivo* [43].

8.3 Mode(s) of action of cyclosporin A in psoriasis

CsA may exert its beneficial effects in clearing psoriasis via two distinct pathways. The first pathway comprises its immunosuppressive effects on the immune cells in the inflammatory infiltrate [44-46]. CsA inhibits the transcription of mRNA for cytokines such as IL-2, IFN-γ, IL-3, IL-4 and TNF-α in T lymphocytes [47-49]. At high concentrations, CsA inhibits the expression of IL-2R and IL-4R as well. The second pathway comprises a direct growth inhibitory effect of CsA on keratinocytes [50]. Other basic effects of CsA observed were down-regulating the expression of ICAM-1 on keratinocytes and on papillary endothelial cells, and decreasing γ-IP10 immunoreactivity in psoriatic plaques [51-53]. Since certain aspects of the mode of action of CsA in psoriasis still remained elusive, we investigated the *in vivo* and *in vitro* effects of treatment with CsA on different immunobiological properties of epidermal cells (Chapters 3 and 7).

Immunohistological studies by others showed striking reductions in the number of activated T lymphocytes in biopsies of psoriatic lesions of patients who had received oral CsA treatment [44-46]. These results were reproducible in our laboratory. Further immunohistological investigations showed that CsA given orally to psoriatic patients had little or no influence on the levels of expression of most cytokines in their lesions. However, a partial but clear reduction in the levels of expression of IL-1β, IL-6 and IL-8 was demonstrated [Prens et al., in press].

Our *in vitro* studies showed that CsA-pulsed EC significantly inhibited the autologous MECLR (Chapter 3). This was not unexpected because it had already been shown that CsA inhibited the capacity of Langerhans cells to present antigen and to stimulate T lymphocytes [46,54]. The antigen presenting capacity in the skin of psoriatic patients under oral CsA therapy was observed to be inhibited significantly

in an allogeneic MECLR model. The results of our investigations supported the conclusions by Cooper et al. that one of the modes of action of CsA in psoriasis occurs via the inhibition of antigen-presenting capacity of lesional epidermal APC [46]. In addition CsA decreased the expression and the accessory function of membrane-bound IL-1 (mIL-1) in the MECLR (Chapter 4) [55].

Further studies showed that clinical improvement of psoriasis after therapy with CsA was accompanied by a significant increase in the number of TNF-R^+ cells only (Chapter 7). This increase was considered secondary to the inhibition of the production of cytokines *in vivo* from the inflammatory infiltrate by CsA, because such an increase was not observed after addition of CsA to ECS *in vitro*.

The expression of the IL-4R, a marker for keratinocyte proliferation and activation, on EC also remained unaltered during *in vitro* culturing in the presence of CsA. These findings corroborate those of a recent study in which the suppressive effects of oral CsA treatment on the immune infiltrate were clearly observed, but there was little or no effect on markers of keratinocyte growth and activation such as IL-6, TGF-α and keratin K16 [51]. Therefore, it is conceivable that the primary effect of CsA in psoriatic patients is to reduce the inflammatory infiltrate and its immunobiological function [51]. This seems to be confirmed by the observation that the potent immunosuppressant FK506, which was recently shown to improve psoriasis, did not have a direct anti-proliferative effect on keratinocytes [56].

From the combined results of our clinical and *in vitro* investigations it can be concluded that evaluation of the effect of clinical treatment modalities on the biological functions of EC should preferentially be performed at points of predefined clinical improvement as expressed in the PASI score rather than at fixed time-points.

8.4 Overall scheme of events of the pathophysiology of psoriasis

In *uninvolved* skin of psoriatic patients basic abnormalities such as slightly enhanced proliferation of keratinocytes and dilated capillaries with a slightly increased ICAM-1 expression predispose to facilitated activation of keratinocytes and of trafficking of peripheral blood leukocytes towards the epidermis [13,57]. These basic abnormalities in clinically normal skin emphasize that the whole skin has the potential to develop psoriasis. Disturbance of the existing, skin disease preventing, equilibrium between $CD4^+$ and $CD8^+$ immunoregulatory T lymphocytes in the dermal lymphocytic infiltrate of uninvolved skin probably accounts for the development of overt skin disease (Fig. 1a) [44].

Psoriasis an immunological disease. The following observations strongly support the view of an initial and an ongoing immune response in psoriasis: 1) the extravasation of $CD4^+$ T lymphocytes and monocytes [58], 2) the subsequent infiltration of $CD4^+$ T lymphocytes into the epidermis preceding the epidermal and dermal changes

in newly developing lesions [58], 3) the predominance of activated (CD4/CD25/CD29/CD45RO/CDw60/HLA-DR)$^+$ T lymphocytes, (CD1a/HLA-DR/factor XIIIa/RFD1)$^+$ dendrocytes and monocytes in fully developed psoriatic lesions [59-61], 4) the stimulation of T lymphocytes in the absence of antigen by autologous epidermal CD1a$^-$ APC [1-3], 5) the association between certain HLA antigens and psoriasis, 6) the usually life-long course of the disease, reflecting immunological memory, 7) the occurrence of spontaneous remissions and exacerbations suggesting imbalances between inducing and suppressive immunoregulatory mechanisms, 8) the acquisition and clearance of psoriasis following bone marrow transplantation [62,63], 9) the induction of psoriasis by local and systemic treatment with IFN-α and IFN-γ [64,65] and 10) the beneficial effects of immunosuppressive agents such as corticosteroids, methotrexate, CsA, FK506 and PUVA [66-68]. Such an immune response may act as a trigger factor and as a maintenance factor for persistent epidermal hyperplasia in psoriasis. Thus, in this view, *triggering* of psoriasis occurs by exaggerated, genetically determined antigen presentation and T cell activation by epidermal APC, whereas *epidermal hyperplasia* in psoriasis reflects either an abnormal response of epidermal cells to or an abnormal production of growth promoting cytokines by the inflammatory infiltrate and/or resident dermal cells. Streptococcal, viral or undefined autoantigens (stratum corneum proteins) have been suggested as the putative sialylated "psoriasis antigen" responsible for the primary activation of CD4$^+$ T cells and consequent initiation of psoriasis [67-70]. Cytokines produced following activation of T cells and indirectly by activated EC recruit other types of inflammatory cells to the site of inflammation and so amplify the inflammatory response [19,25,71-73]. Additionally, the microenvironment of epidermal APC in psoriatic lesions may be affected in such a way that *in vivo* lymph node conditions or *in vitro* culture conditions are simulated resulting in the phenotype and function of the so-called "cultured" Langerhans cells [74]. *In vitro* "cultured" or "mature" Langerhans cells share striking similarities such as the phenotype and functional characteristics with HLA-DR$^+$/CD1a$^-$ APC occurring in psoriatic lesions [75]. The latter subset is thus considered as the *in vivo* equivalent of *in vitro* "cultured" Langerhans cells. It has been shown that cytokines such as TNF-α, IL-3 and GM-CSF have great influence on the functional capacities of *in vitro* cultured Langerhans cells, in that TNF-α sustains the Langerhans cells in their naive state, whereas IL-3 and GM-CSF facilitate the "mature or cultured" state of Langerhans cells [76-79].

Cytokines such as IL-6, IL-8, IFN-γ, TGF-α, GM-CSF and TNF-α, which occur in increased levels in psoriatic lesions, are able to activate and stimulate autocrine and paracrine growth of keratinocytes and so to maintain the psoriatic process. In addition, these cytokines from activated lymphocytes not only activate EC to proliferate and to produce cytokines, but also to express "aberrant" membrane molecules. The cytokines with growth promoting properties, such as IL-1, IL-6, IL-8, TGF-α, and the growth

factors insulin-like growth factors (IGFs) and/or fibroblast growth factors (FGFs) are derived form activated keratinocytes, the inflammatory infiltrate, fibroblasts and/or endothelial cells in psoriatic lesions.

Cytokines are able to exert their biological functions only if appropriate receptors are expressed on the target cells. The implication(s) of the observed overexpression of the EGF/TGF-R, IL-4R and decreased IL-1R in psoriatic lesions remain unclear. Psoriatic KC do not down-regulate their EGF/TGF-αR upon exposure to IFN-γ in contrast to normal KC. We propose that the increased EGF/TGF-R represents, apart from IFN-γ resistance, a feedback mechanism induced by decreased affinity for its ligand due to the increased presence of IL-1 in psoriatic lesions (Chapter 6) [80]. Quite some characteristics of psoriatic lesions indeed correspond with increased IL-1 bio-activity *in vivo*: 1) the nuclear localization of IL-1 in lesional psoriatic epidermis, 2) overexpression of IL-6 and IL-8 (both IL-1 inducible cytokines), 3) significant neutrophil chemo-attraction (via IL-1-induced IL-8, LTB_4) and 4) increased production of arachidonic acid metabolites by IL-1-primed neutrophils could be regarded as evidence of *in vivo* bio-availability, binding and internalization of IL-1.

The decreased number of IL-1R$^+$ cells is in agreement with this and represents an intrinsically altered IL-1R turn-over. Although, on the one hand, IL-1β seems to lack bio-activity in psoriatic lesions, it may be secreted and converted *in vivo* into a biologically active form by aberrant proteases from psoriatic keratinocytes "in the activated state" or by proteases from contiguous intra-epidermal inflammatory cells [81]. IL-1α, on the other hand, may be decreased due to rapid consumption by psoriatic EC. This provocative hypothesis is conceivable provided that regulatory mechanisms operative in EC are identical to that in other cell types where exposure to IL-1 has been shown to result in down-regulation of both types of IL-1R [34,35].

The expression of IL-4R is increased in certain epithelial tumors [36,82]. This increased expression of IL-4R may represent a physiological attempt towards negative feedback signalling, since it is known that IL-4 is able to counter-act the effects of IL-1, IL-6, IL-8 and TNF-α via down-regulation of their genes at the level of transcription [83].

8.5 Conclusions

The fundamental aspects of the pathogenesis of psoriasis concern the primary factors which initiate the cascade of events such as (epi)dermal cell activation, the recruitment of the inflammatory infiltrate and the maintenance of persistent skin inflammation and epidermal hyperplasia. Although the pathogenesis of psoriasis clearly involves multiple factors, the generally almost uniform abnormalities which are observed in psoriatic lesions indicate that inflammation in psoriasis is achieved via a final common pathway. Unravelling this common inflammatory response is not only

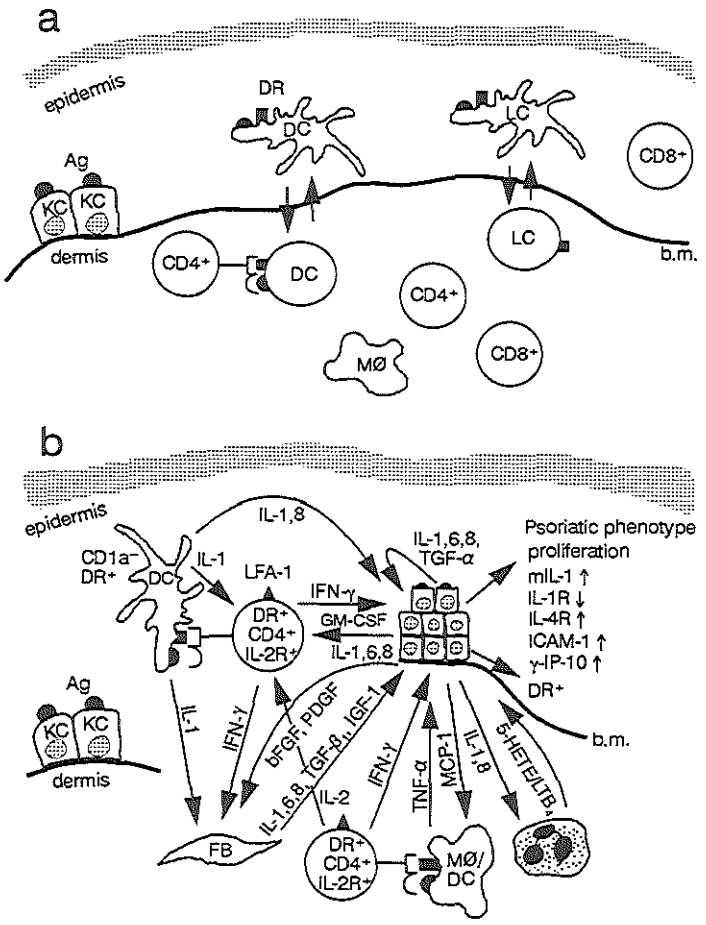

Figure 1. Schematic illustration of events of inflammation in psoriasis. The basal distribution of skin immune cells in uninvolved skin of psoriatic patients is shown in (A). Low levels of the putative psoriasis related antigen may be expressed by epidermal cells. Activation does not occur, probably due to the natural barrier formed by the basement membrane (b.m.) which prevents massive antigen presentation due to the maintenance of (the existing) equilibrium by immunoregulatory T lymphocytes. (B): Disclosure of the putative psoriasis related antigen to lymphocytes, e.g. due to skin wounds or inflammatory processes, leads to antigen presentation and activation of T lymphocytes. The cytokines released in this process may separately or synergistically induce proliferation of keratinocytes in a paracrine fashion. Autocrine proliferation ensues due to cytokines released by the activated KC. The activated KC, T cells, MØ and FB release cytokines which amplify the inflammatory reaction by recruitment of additional cell types. DC = dendritic cell; LC = Langerhans cell; $CD4^+$ = $CD4^+$ T lymphocyte; $CD8^+$ = $CD8^+$ T lymphocyte; MØ = monocyte/macrophage; KC = keratinocyte; FB = fibroblast; ▲ = the putative psoriasis related antigen. ▬ = HLA-DR membrane molecule; ▲ = LFA-1 membrane molecule.

essential for a better understanding of this process, but is also imperative for further investigations into the multiple etiological factors of psoriasis. It should be reminded that the initial sequence of events in psoriasis concerning cellular activation and release of cytokines represents a normal physiological mechanism with also beneficial effects for the host as in (epi)dermal hyperproliferation during wound healing [84]. In psoriasis, however, an non-beneficial outcome is achieved due to altered production and/or cellular responsiveness to the soluble products of inflammation. The clinical significance of more insight into regulatory interactions between cytokines and their receptors may be illustrated by the observation that e.g. IL-1R antagonists can prevent disease in an animal model [85].

In this thesis additional evidence is provided on the altered antigen-presenting characteristics of lesional psoriatic epidermal Langerhans cells, the membrane molecules involved, the increased expression of mIL-1, IL-6 and IL-4R and the decreased expression of IL-1R in psoriasis. Additional modes of action for the therapeutic effect of CsA in psoriasis have also been suggested. Based on the evidence summarized in this thesis it is proposed that the characteristic abnormalities observed in psoriasis arise mainly due to an abnormal immune response of bone marrow-derived cells to an as yet unknown stimulus in combination with an abnormal production or response of keratinocytes to cytokines and other mediators of inflammation.

8.6 References

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Chapter 9

SUMMARY / SAMENVATTING

Psoriasis is a chronic, non-infectious inflammatory skin disease characterized by well defined red scaly skin lesions. About 2% of the Western population suffers with varying severity from this disease. Strictly in medical terms, this disease does not follow a severe course in the majority of the patients. In about 10% of the patients, an inflammation of the joints (psoriasis arthropathica) is observed which strongly resembles rheumatoid arthritis. About 10% of the patients with this particular type of inflammation of the joints are seriously disabled. Appearance of skin, scalp and nails in psoriasis clearly disagrees with the "normal" Western idea of beauty. This may result in psychosocial problems such as social isolation and relational problems. Often, the financial costs of life-long therapy and hospital admissions for clinical treatment with the accompanying loss in productivity are considerable.

Historically, psoriasis has attracted considerable attention from investigators in the field of dermatological research. This led to very detailed knowledge on various aspects of the disease. The red scaly lesions observed in psoriasis appear to develop via hyperproliferation of keratinocytes, accompanied by an inflammatory infiltrate in the dermis. What triggers and maintains this process is unknown. Meanwhile, it is obvious that the inflammatory infiltrate is essential in inducing this disease. An example of a patient being cured of his persistent psoriasis after having received bone marrow transplantation has been described in the literature. A case of a patient without any previous history of psoriasis who developed psoriasis after being transplanted with bone marrow from a psoriatic patient has also been described. However, several major aspects of the pathogenesis of psoriasis still remain elusive. Therefore, it may take many years before a complete insight into the pathogenesis of this disease is to be obtained.

In this thesis, the results of clinical and laboratory investigations are presented on the role of the local immune system in psoriasis. This so-called "skin immune system" comprises bone marrow-derived cells and epidermal cells. In particular, the function of epidermal antigen-presenting cells, the contribution of cytokines and the influence of Cyclosporin A (CsA) were investigated.

A short review on the general aspects of psoriasis, historical background, epidemiology, genetic predisposition, clinical presentation, pathophysiology and pathogenesis is presented in Chapter 1.

The cellular elements which make up the skin and those which play a role in the process of inflammation are discussed in Chapter 2. The cytokines which fulfill a signalling role in inflammation and which induce their production via interactions with cells of the surrounding tissues are summarized and discussed with particular emphasis on psoriasis.

The results of experimental studies demonstrating that antigen-presenting cells from psoriatic lesions were of a different phenotype and were also functionally

different from those of patients without psoriasis are described in Chapter 3. Antigen-presenting cells from psoriatic lesions were also observed to possess an exceptional ability. In the absence of antigen, these cells were able to stimulate peripheral blood lymphocytes of psoriatic patients. It is speculated that this "spontaneous" activation is caused by naturally occurring (auto)antigens present in psoriatic lesions.

In Chapter 4, several molecules are discussed which are involved in the induction, execution and maintenance of inflammatory reactions. In psoriatic patients, several of these molecules occur on antigen-presenting cells in the epidermis and appeared to be involved in the "spontaneous" activation of peripheral blood lymphocytes. Membrane bound interleukin-1, an expression form of which the role in chronic inflammatory diseases has not yet been reported, appeared to fulfill an accessory role in the activation of T lymphocytes in psoriasis.

Investigations on the expression of interleukin-1 and interleukin-6 in psoriatic skin are dealt with in Chapter 5. In earlier publications, the presence of interleukin-1 could not be demonstrated in aqueous extracts of scales from psoriatic lesions. In contrast, from our immunohistochemical and immunofluorescence studies it appeared that interleukin-1 was substantially increased in psoriatic lesions and occurred mainly as membrane bound form on the epidermal cells of these lesions. Interleukin-6 was clearly increased in the suction blister fluid from psoriatic plaques, whereas its level in peripheral blood of psoriatic patients was comparable with that in the peripheral blood of controls.

The results of the investigations into the expression of interleukin receptors on epidermal cells of psoriatic patients are presented in Chapter 6. There was an increased expression of interleukin-4 receptors on the epidermal cells of psoriatic lesions, whereas the expression of interleukin-1 receptors on these cells was decreased.

In Chapter 7, the results are presented of studies on the effects of CsA treatment on the expression of interleukin receptors in the lesions of psoriatic patients. Although clinically a rapid improvement of psoriatic lesions was observed, it appeared that two weeks of treatment with CsA had little effect on the expression of interleukin receptors on EC, except for the TNF receptor.

In conclusion, the investigations described in this thesis show that a specific sub-type of antigen-presenting cell(s) plays a crucial role in the "spontaneous" activation of autologous T cells in psoriasis. This process may not only be an important factor in the development of psoriatic lesions, but also in the maintenance of the disease. The production of certain cytokines was clearly increased in psoriatic plaques. Interleukin-6 and interleukin-8 and interleukin-4 receptor expression were prominently increased. Furthermore, in psoriasis the location, production and regulation of the naturally occurring α and β forms of interleukin-1 were abnor-

mal, while the IL-1R expression was significantly decreased. CsA had no clear effect on most of the interleukin receptors within two weeks of treatment. These observations confirm the view that the clinical improvement of psoriasis obtained with CsA does not primarily occur via its effects on epidermal cells, but rather via its effect on the inflammatory infiltrate.

Psoriasis is een chronische niet-besmettelijke huidziekte gekenmerkt door rode schilferende huidafwijkingen waaraan, in meerdere of mindere mate, ongeveer 2% van de Westerse bevolking lijdt. Deze ziekte heeft in het merendeel van de gevallen in strikt medische zin een "niet ernstig" beloop. Ongeveer 10% van de psoriasis patiënten vertoont een sterk op reuma gelijkende vorm van gewrichtsontsteking (psoriasis arthropathica). Bij ongeveer 10% van de psoriasis patiënten met deze specifieke vorm van gewrichtsontsteking ontstaat vrij ernstige invaliditeit. De verborgen kant van deze huidziekte bevindt zich vooral op het psycho-sociale en economische vlak. De huid-, haar- en nagelverschijnselen van psoriasis zijn duidelijk niet in overeenstemming met die van het "normale" Westerse schoonheidsbeeld. Dit kan resulteren in het ontstaan van psycho-sociale problemen, zoals sociaal isolement en relationele problematiek. De kosten van vaak levenslange therapie en ziekenhuis opnames voor klinische behandeling, met werkverzuim als gevolg, zijn aanzienlijk.

Psoriasis heeft van oudsher veel aandacht getrokken van onderzoekers op dermatologisch gebied. Dit heeft tot vrij veel detaillistische kennis omtrent deze huidziekte geleid. De rode schilferende huidafwijkingen bij psoriasis blijken tot stand te komen door hyperproliferatie van keratinocyten in samenhang met een ontstekingsinfiltraat in de dermis. Wat dit proces losmaakt en onderhoudt, is nog onbekend. Het lijkt inmiddels duidelijk dat het ontstekingsinfiltraat essentieel is voor de inductie van de ziekte. Uit de literatuur is namelijk een voorbeeld bekend van een patiënt die beenmerg transplantatie onderging en daarna genas van zijn hardnekkige psoriasis. Ook is een patiënt beschreven waarbij door transplantatie van beenmerg van een psoriasis patiënt psoriasis werd geïnduceerd, terwijl die persoon voordien nooit aan psoriasis had geleden. Veel is nog onbekend van de pathogenese van psoriasis. Het zal daarom nog wel jaren duren voordat een volledig inzicht is verkregen in de pathogenese van deze huidziekte.

In dit proefschrift worden de resultaten beschreven van patiëntgebonden en laboratorium onderzoek naar het functioneren van het lokale immuunsysteem van de huid bij psoriasis. Dit bestaat uit beenmerg-afkomstige en epidermale cellen. Met name werden epidermale antigeen presenterende cellen, de bijdrage van cytokinen en cytokine-receptoren en de invloed van cyclosporine A (CsA) op het lokale immuunsysteem bij psoriasis bestudeerd.

In Hoofdstuk 1 wordt een kort overzicht gegeven over psoriasis in het algemeen, zoals historische achtergrond, epidemiologie, genetische predispositie, klinische verschijnselen, pathofysiologie en pathogenese.

In Hoofdstuk 2 worden de cellulaire elementen waaruit de huid is opgebouwd en die een belangrijke rol bij ontsteking spelen, besproken. De stoffen die een signaalfunctie vervullen bij ontsteking (cytokinen), en een ontsteking kunnen induceren in samenspel met cellen uit de omliggende weefsels, worden vervolgens

besproken in het algemeen en bij psoriasis in het bijzonder.

In Hoofdstuk 3 worden de resultaten beschreven van experimenten die aantonen dat antigeenpresenterende cellen in de aangedane huid van psoriasis patiënten een afwijkend fenotype hebben. Ook qua functie bleken antigeenpresenterende cellen uit aangedane psoriasis huid met die van personen zonder huidziekte te verschillen. Antigeenpresenterende cellen uit psoriasis plekken bezitten het uitzonderlijke vermogen om lymfocyten uit het perifere bloed van patiënten "spontaan", zonder hulp van antigeen, te stimuleren. Het is mogelijk dat een in psoriasis huid voorkomend natuurlijk (auto)antigeen deze stimulatie veroorzaakt.

In Hoofdstuk 4 worden enkele eiwitten belicht die een belangrijke functie vervullen bij het opwekken, uitvoeren en in stand houden van ontstekingsreacties. Sommige van deze eiwitten, cytokinen genoemd, zijn aanwezig in of op antigeenpresenterende epidermale cellen. Zij blijken ook betrokken te zijn bij de spontane activatie van lymfocyten uit het perifere bloed door epidermale antigeenpresenterende huidcellen van psoriasis patiënten. Membraan gebonden interleukine-1, een expressievorm waarvan de functie bij ziekten gekenmerkt door chronische ontsteking nog niet eerder beschreven is, blijkt bij de activatie van T lymfocyten een ondersteunende rol te vervullen.

In Hoofdstuk 5 wordt de expressie van interleukine-1 en interleukine-6 in psoriasis huid behandeld. In vroegere publikaties kon geen interleukine-1 aangetoond worden in "eelt"-extracten afkomstig van psoriasis plekken. Uit ons immunohistochemisch en immunofluorescentie onderzoek bleek dat interleukine-1 wel degelijk verhoogd aanwezig is in psoriasis laesies. Het interleukine-1 blijkt echter vooral in membraan-gebonden vorm voor te komen op epidermale cellen van psoriasis laesies. Het interleukine-6 bleek duidelijk verhoogd in het blaarvocht van psoriasis plaques, terwijl het voorkomen in het bloed van psoriasis patiënten niet afweek van dat van controle personen zonder deze huidziekte.

In Hoofdstuk 6 worden de resultaten beschreven van onderzoek naar receptoren voor interleukinen op epidermale cellen van psoriasis patiënten en gezonde controle-personen. Epidermale cellen van psoriasis laesies hadden een verhoogde receptor-expressie voor interleukine-4 en een verlaagde expressie van de receptor voor interleukine-1.

In Hoofdstuk 7 worden de resultaten beschreven van onderzoek naar de effecten van CsA op de expressie van interleukine receptoren in de huid van psoriasis patiënten die behandeld werden met dit middel. Hoewel vrij snel klinische verbetering wordt waargenomen, blijkt dat twee weken behandeling met CsA nog weinig verandering veroorzaakt in de expressie van de meeste interleukine receptoren, met uitzondering van de TNF receptor. Deze resultaten bevestigen het vermoeden dat de klinische verbetering van psoriasis door CsA niet via een direct effect op epidermale cellen verloopt, doch voornamelijk via het remmende effect op

het onstekingsinfiltraat.

Concluderend kan gesteld worden dat het onderzoek, dat beschreven wordt in dit proefschrift, heeft aangetoond dat antigeenpresenterende cellen een belangrijke rol vervullen bij de "spontane" activatie van eigen T lymfocyten bij psoriasis. Dit mechanisme kan zowel in een zeer vroege fase bij het ontstaan van psoriasis plekken als bij het instandhouden van psoriasis een belangrijke factor zijn. In psoriasis plekken wordt een aantal cytokinen duidelijk in verhoogde mate geproduceerd. Met name de interleukine-6, interleukine-8 en interleukine-4 receptor-expressie zijn sterk verhoogd. Bij het interleukine-1 is van beide natuurlijke α- en β-vormen de lokalisatie, produktie en regulatie abnormaal, terwijl de interleukine-1 receptor expressie significant verlaagd is. Een geneesmiddel als CsA heeft na twee weken therapie geen duidelijke effecten op de meeste interleukine receptoren op epidermale cellen. Verbetering van psoriasis door CsA verloopt dus blijkbaar niet via een direct effect op epidermale cellen, maar voornamelijk via het remmende effect op het onstekingsinfiltraat.

ABBREVIATIONS

aa : amino acids
ACD : allergic contact dermatitis
AET : (2)-amino-ethylisothiouronium bromide
APC : antigen presenting cell(s)
b.m. : basement membrane
BSA : bovine serum albumin
CD : cluster of differentiation
cpm : counts per minute
CsA : cyclosporin A
DC : dendritic cell(s)
DNA : deoxyribonucleic acid
EC : epidermal cell(s)
ECS : epidermal cell suspension(s)
ELAM : endothelial leukocyte adhesion molecule
ELISA : enzyme-linked immunosorbent assay
FACS : fluorescence activated cell scanner
FB : fibroblast(s)
FCS : fetal calf serum
(b)FGF : (basic) fibroblast growth factor
FITC : fluorescein isothiocyanate
FSC : forward scatter
GAPDH : glyceraldehyde-3-phosphate dehydrogenase
G(M)-CSF: granulocyte (macrophage) colony stimulating factor
gp : glycoprotein
h : hour
HETE : hydroxyeicosatetraenoic acid
HLA : human leucocyte antigen
ICAM-1 : intercellular adhesion molecule-1
IFN : interferon
Ig : immunoglobulin
IGF : insulin-like growth factor
IL : interleukin
IL-*n*R : interleukin receptor
γ-IP-10 : γ-interferon-induced protein 10
KC : keratinocyte(s)
K_d : dissociation constant
kD(a) : kilo Dalton
LC : Langerhans cell(s)
LFA : lymphocyte function-associated antigen(s)
LPS : lipopolysaccharide
LT : leukotriene
MØ : monocyte(s)/macrophage(s)
MCP-1 : monocyte chemotactic protein-1
MHC : major histocompatibility complex
MLR : mixed lymphocyte reaction
MECLR : mixed epidermal cell-T lymphocyte reaction
mIL-1 : membrane-bound IL-1
MoAb : monoclonal antibody/antibodies
MRE : multiresponsive element
MW : molecular weight
NAP : neutrophil activating peptide
NF : nuclear factor
p : protein
PAF : platelet activating factor
PBMC : peripheral blood mononuclear cell(s)
PBS : phosphate buffered saline
PDGF : platelet derived growth factor
PE : phycoerythrin
PG : prostaglandin
PK : protein kinase
pI : isoelectric point
PI : propidium iodide
PLA_2 : phospholipase A_2
PMA : phorbol myristate acetate
PUVA : psoralen ultraviolet A therapy
R : receptor
rHu : recombinant human
rIL : recombinant interleukin
RNA : ribonucleic acid
SKALP : skin-derived antileukopeptidase
SD : standard deviation
SIS : skin immune system
SRBC : sheep red blood cell(s)
SSC : side scatter
TcR : T cell receptor
[^{3}H]TdR : tritiated thymidine
TGF : transforming growth factor
TNF : tumor necrosis factor
UV : ultraviolet
VCAM : vascular cell adhesion molecule
VLA : very late antigen

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CURRICULUM VITAE

De schrijver van dit proefschrift werd op 25 juni 1956 te Willemstad, Curaçao (NA) geboren. In 1974 werd het diploma Atheneum B behaald aan het Maria Immaculata Lyceum te Curaçao. In datzelfde jaar werd begonnen met de studie Geneeskunde aan de Rijksuniversiteit Groningen. Het doctoraal examen Geneeskunde werd in 1979 afgelegd. Van medio 1979 tot en met november 1980 werden de co-assistentschappen doorlopen in het St. Jozef Ziekenhuis en het Geertruiden Ziekenhuis te Deventer (thans Stichting Deventer Ziekenhuizen). Het artsexamen werd in mei 1981 afgelegd aan de Rijksuniversiteit Groningen.

Van eind 1980 tot de aanvang van de opleiding tot dermatoloog werd patiëntgebonden onderzoek verricht en vond de eerste dermatologische scholing plaats in de praktijk van dr. G. Smeenk te Deventer. De opleiding tot Dermatovenereoloog werd genoten van december 1981 tot december 1985 op de afdeling Dermatologie en Venereologie, Academisch Ziekenhuis Dijkzigt te Rotterdam (opleiders: prof. dr. E. Stolz en prof. dr. Th. van Joost). In 1983 werd gedurende 3 maanden onder leiding van prof. dr. F. Camacho-Martinez, te Sevilla, Spanje, voltijds gewerkt aan de verfijning van dermato-chirurgische technieken. In december 1985 vond de inschrijving plaats in het specialisten register als Dermatovenereoloog.

Van januari 1986 tot heden werd, op de afdeling Immunologie, Academisch Ziekenhuis Dijkzigt en Erasmus Universiteit Rotterdam (hoofd: prof. dr. R. Benner), onderzoek verricht naar immunoregulatie van allergische huidziekten en psoriasis. Dit onderzoek vond plaats in samenwerking met prof. dr. Th. van Joost en medewerkers van de afdeling Dermatologie van het Academisch Ziekenhuis Dijkzigt. Vanaf 1991 is hij part-time als Universitair Docent verbonden aan de afdeling Immunologie van de Erasmus Universiteit en het Academisch Ziekenhuis Dijkzigt. Gedurende de onderzoeksperiode werd practicum-onderwijs in de Immunologie gegeven aan derdejaars studenten Geneeskunde en een bijdrage geleverd aan de blokcursus Dermatologie voor co-assistenten. Tevens wordt als gastdocent meegewerkt aan post-graduate onderwijs in de Immunologie aan de Haagse Hogeschool, Hogeschool Brabant en de Erasmus Summer School on Immunology.

Sedert 1986 is hij, eveneens part-time, werkzaam als dermatoloog; aanvankelijk in de praktijk van dr. J.D.R. Peereboom-Wynia, Stichting Bronovo/Nebo te 's-Gravenhage en vervolgens in de Maatschap Huidartsen Midden-Zeeland (collegae dr. J.M.W. Habets en Mw. I. Vermeiden), Ziekenhuis Walcheren te Middelburg/Vlissingen en het Oosterschelde Ziekenhuis te Goes.

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