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CD40 ligation-induced cytokine production in human skin explants is partly mediated via IL-1

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Abstract

CD40 ligation by CD40 ligand$^+$ CD4$^+$ T cells has been claimed to be involved in inflammatory responses in human skin. However, these data are derived from *in vitro* cell culture systems and immunohistochemistry, and the mechanisms involved have not been fully elucidated. We previously observed that cells in intact normal human skin secrete high levels of IL-6 and IL-8 upon stimulation with IL-1β. *In vitro* studies have shown that CD40 ligation on human keratinocytes results in the production of IL-6 and IL-8 as well. We used a novel tissue culture system with intact normal human skin, and show that antibody ligation of CD40 results in the induction of several pro- and anti-inflammatory cytokines. IL-6, IL-8, tumor necrosis factor (TNF)-α, IL-12 and IL-1β were induced upon CD40 ligation and IFN-γ stimulation, while IL-10 could be induced by CD40 ligation alone and was reduced again by the addition of IFN-γ. Since CD40 ligation on monocytes and dendritic cells *in vitro* results in the secretion of IL-1, which is pre-stored in high concentrations in normal human keratinocytes, we subsequently investigated whether CD40 induced IL-6 and IL-8 production in skin is mediated via IL-1. Indeed IL-1 receptor antagonist inhibited the CD40 ligation-induced IL-6 and IL-8 production, while TNF-α and IL-10 production were not affected. These data show that CD40 ligation-induced secretion of IL-6 and IL-8, but not TNF-α and IL-10, is partially mediated via IL-1 and that IL-1 plays a prominent role in the inflammatory response initiated by CD40 ligation in intact human skin.

Introduction

CD40 is a member of the tumor necrosis factor (TNF) receptor family and was first discovered as a 50-kDa surface antigen on B lymphocytes (1). The ligand of CD40 (CD40L or CD154) is a 39-kDa glycoprotein and is functionally expressed on activated CD4$^+$ T cells (2). Research was initially focused on the role of CD40 in the humoral immune response. The importance of CD40 in isotype switching is emphasized by the observation that mutations in the CD40L gene lead to X-linked hyper-IgM syndrome (3,4). CD40 is also expressed by non-B cells and appeared to be a key player in other immune responses as well. Stimulation of CD40 on dendritic cells results in the up-regulation of co-stimulatory molecules like CD80 and CD86 (5). Besides enhancing co-stimulation, CD40 ligation induces the secretion of inflammatory cytokines such as IL-1, TNF-α, IL-6 and IL-8 from monocytes (6). The IL-12-mediated activation of T_h1 cells is promoted via CD40 stimulation on macrophages and dendritic cells which results in IL-12 production by these cells (7,8). Furthermore, CD40 ligation-induced responses are also involved in tumoricidal activity and NO production (9,10).

CD40 expression is not restricted to leukocytes. Expression of CD40 on resting human keratinocytes was first observed by Denfeld *et al.* (11) and was shown to be up-regulated by IFN-γ. Activation of CD40 on keratinocytes by CD40L results in elevated expression of ICAM-1, IL-8, IL-6 and TNF-α (11–13). Ligation of CD40 on human fibroblasts *in vitro* leads to expression of ICAM-1, VCAM-1 and IL-6 production (14). The proliferation of skin cells is influenced by CD40 as well. CD40 triggering on keratinocytes inhibits proliferation and promotes differentiation (12,15). Conversely, CD40 ligation on fibro-

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blasts *in vitro* stimulates their proliferation (14,16). CD40 is also functionally expressed on human epidermal Langerhans cells, and its ligation results in enhancement of viability and up-regulation of ICAM-1 and CD86 expression (17).

The role of CD40–CD40L interactions during inflammation in human skin was studied in *Leishmania* and *Mycobacterium leprae* infections (18,19). Both studies clearly indicate that cognate interactions of $CD40L^+$ $CD4^+$ cutaneous T cells with CD40-expressing antigen-presenting cell from patients suffering from *L. major* or *M. leprae* infection result in the induction of bioactive IL-12 (18,19). Furthermore, CD40 function may also play a role in the disease process of the inflammatory skin disease psoriasis, since up-regulation of CD40 expression in psoriatic lesions has been reported (11). Recently, Mehling *et al.* showed that mice overexpressing CD40L in skin develop dermatitis on the ears, face, tail and/or paws (35). Finally, it has been shown that CD40 ligation plays a role in skin allograft rejection (20) and migration of Langerhans cells from skin to the draining lymph nodes (21,35). It is therefore plausible that CD40 has a function in skin inflammation.

The data on CD40 ligation-induced cytokine expression in human skin presented during recent years were derived from *in vitro* or immunohistochemical staining studies. However, knowledge of the mechanisms underlying CD40 ligation-induced cytokine expression by skin cells in their natural environment is lacking.

Therefore we used a novel culture system (22) to study the role of CD40 ligation in intact human skin. We previously showed that IL-6 and IL-8 production in normal human skin is increased after stimulation with IL-1β (22). It is also known that CD40 ligation on monocytes *in vitro* results in the induction of IL-1 (23) and that high concentrations of IL-1 are pre-stored in normal human keratinocytes (24,25). Therefore we asked whether the CD40-induced IL-6 and IL-8 production in skin is mediated via IL-1.

The data presented here show that CD40 stimulation in intact normal human skin results in the induction of several pro- and anti-inflammatory cytokines, and that CD40 ligation-induced secretion of IL-6 and IL-8, but not TNF-α, IL-10 and IL-1β, is largely mediated via IL-1.

Methods

Skin biopsies

Normal skin biopsies were obtained from six healthy volunteers undergoing breast reduction in the Department of Plastic Surgery of the Sint Franciscus Gasthuis, Rotterdam, The Netherlands. After informed consent, biopsies with an average length of 3 mm were taken with a 3-mm diameter biopsy punch (Stiefel, Leuven, Belgium) and were either snap-frozen in Tissue Tek (Bayer, München, Germany) or cultured (see below). After culture, biopsies were immersed in Tissue Tek (Bayer) and snap-frozen in liquid nitrogen. Biopsies were stored at –80°C until use.

Skin organ culture

Biopsies were cultured as described elsewhere (22). In brief, a 2-mm hole was punched in a Netwell filter (pore size 0.75 μm; Corning Costar, Corning, NY). The biopsy was inserted into the hole (three biopsies per filter) and the filter containing the biopsies was placed in a 12-well plate containing 1 ml medium. Skin biopsies were cultured in IMDM (Gibco/BRL, Paisley, UK) containing 1% heat-inactivated human serum (Sigma, St Louis, MO), 100 U/ml penicillin and 100 μg/ml streptomycin (Biowhittaker, Verviers, Belgium) under special conditions (95% O_2/5% CO_2 at 32°C) in a culture bag. The biopsies were cultured under the following conditions: Biopsies were pre-treated for 24 h in medium containing 1000 U/ml IFN-γ (Boehringer Ingelheim, Alkmaar, The Netherlands) or in medium devoid of IFN-γ. After pre-treatment the filters containing the biopsies were washed in PBS and transferred to a new 12-well plate containing 1 ml medium with or without 1000 U/ml IFN-γ and stimulus. Biopsies were stimulated with an agonistic anti-CD40 mAb (clone 64 mouse anti-human CD40; Tanox Pharma, Amsterdam, The Netherlands; isotype mouse IgG1; concentration 20 μg/ml) alone or antibody in the presence of IL-1 receptor agonist (IL-1ra; Synergen, Denver, CO; concentration 1 μg/ml). Biopsies cultured in the presence of an antibody of the same isotype as the anti-CD40 antibody, but of irrelevant specificity, served as a control. After 3 days of stimulation the supernatants and biopsies were isolated, and stored at –80°C until use.

Cytokine ELISA

Maxisorb ELISA plates (Nunc, Roskilde, Denmark) were coated for 18 h at 4°C with 100 μl of 0.5 μg/ml anti-human IL-6, anti-human IL-8, anti-human TNF-α, anti-human IL-1β or anti-human IL-10 mAb (Biosource, Camarillo, CA) followed by blocking with 0.5% BSA (Sigma) for 2 h at room temperature. 100 μl of the recombinant IL-6, IL-8, TNF-α, IL-1β or IL-10 (Biosource) standard or sample and 50 μl of 0.2 μg/ml biotin-linked anti-human IL-6, anti-human IL-8, anti-human TNF-α, anti-human IL-1β or anti-human IL-10 (Biosource) polyclonal detection antibody were simultaneously added to each well. The standards were diluted in PBS containing 0.5% BSA (Sigma) and 0.1% Tween 20 (Merck). Samples, standards and detection antibodies were incubated for 2 h at room temperature. Cytokines were detected using streptavidin-linked peroxidase (CLB, Amsterdam, The Netherlands) and TMB peroxidase substrate (Kirkegaard & Perry, Gaithersburg, MD). The OD was measured at 450 nm. ELISA readings were performed in duplicate.

Immunohistochemistry

Skin biopsies of three representative donors were snap-frozen in liquid nitrogen, and cryosections were cut using a cryostat (Jung Frigocut 2800 E; Leica, Rijswijk, The Netherlands) and stored in a sealed box containing silica gel at –80°C prior to use. Sections were fixed in acetone for 10 min at room temperature and pre-incubated for 10 min with PBS containing 0.05% Tween 20 (Merck, Whitehouse Station, NJ) at room temperature. Subsequently sections were incubated for 18 h at 4°C with a biotin-linked antibody specific for both hIL-12p40 and hIL-12p70 (C8.6; PharMingen, San Diego, CA; dilution 1:200), followed by incubation with peroxidase-linked avidin (Dako, Carpinteria, CA). Sections by which the antibody was omitted or replaced by an isotype-matched antibody, raised against an irrelevant antigen (keyhole limpet hemocyanin)

Table 1. IFN-γ differentially regulates CD40 ligation-induced cytokine secretion

Cytokine	Additions			
	Without IFN-γ		With IFN-γ	
	None	αCD40	None	αCD40
IL-6 (ng/ml)	4.37 ± 1.08	7.32 ± 1.48[a]	0.63 ± 0.10[b]	6.41 ± 1.23[a]
IL-8 (ng/ml)	14.26 ± 7.16	22.89 ± 9.87[a]	1.71 ± 0.52 [b]	13.92 ± 5.24[a]
TNF-α (pg/ml)	18.46 ± 5.40	84.14 ± 22.66[a]	13.89 ± 2.85	120.68 ± 23.85[a]
IL-1β (pg/ml)	<2.00	2.65 ± 3.62[a]	<2.00	10.83 ± 3.30[a]
IL-10 (pg/ml)	6.03 ± 3.13	34.61 ± 13.27[a]	<1.00	3.59 ± 1.01[a]

Normal skin biopsies were cultured in the presence and absence of IFN-γ, and either or not stimulated with agonistic anti-CD40 mAb. Later, IL-6, IL-8, TNF-α, IL-1β and IL-10 concentrations were measured in the supernatant by ELISA. Data represent the mean ± SEM (pg/ml or ng/ml) of six donors in independent experiments of identical design.

αCD40 = agonistic anti-CD40 mAb.

[a]αCD40 compared to not stimulated: $P < 0.05$.

[b]IFN-γ compared to no IFN-γ: $P < 0.05$.

(mouse IgG1; R & D Systems, Minneapolis, MN), during the first incubation served as a control. 3-Amino-9-ethylcarbazole (Sigma) was used as the chromogen. Staining intensity and numbers of positive cells were reproducibly ranked by two independent observers blinded to treatment using a semi-quantitative scoring scale. Staining intensity scale: – (no staining) to +++ (high staining intensity). Scoring scale for the number of positive cells: – (no positive cells) to +++ (many positive cells) (>25 per image field, magnification ×100).

Statistical analysis

The Wilcoxon signed ranks test was used to determine the significance of differences between treatment and non-treatment. $P < 0.05$ was considered to be significant.

Results

CD40 ligation in normal human skin induces both pro- and anti-inflammatory cytokines

To assess whether CD40 stimulation in human skin results in the elevation of cytokine expression, normal human skin biopsies were cultured in the presence of an agonistic anti-CD40 mAb. Secretion of IL-1β by normal skin biopsies was significantly elevated upon stimulation with the agonistic anti-CD40 mAb. IL-1β was not detectable in supernatants of non-stimulated biopsies or biopsies cultured in the presence of IFN-γ. Secretion of IL-1β was further enhanced by the addition of IFN-γ to the agonistic anti-CD40 mAb compared to stimulation with the mAb alone (Table 1).

Culturing of biopsies in medium alone resulted in spontaneous IL-6 and IL-8 secretion. This IL-6 and IL-8 release was significantly inhibited by the addition of IFN-γ (70–80%, Table 1, $P < 0.05$). The agonistic anti-CD40 mAb alone stimulated IL-6 and IL-8 production. However, in the presence of IFN-γ, the agonistic anti-CD40 mAb was able to prevent the down-regulation of the cytokines induced by IFN-γ alone (Table 1). IL-6 secretion by biopsies cultured in the presence of the agonistic anti-CD40 mAb and IFN-γ showed a 10-fold increase compared to biopsies cultured in the presence of IFN-γ alone (Fig. 1 and Tables 1 and 2). Culturing in the presence of an isotype-matched control antibody and IFN-γ had no effect (unstimulated: 0.63 ± 0.10 pg/ml and isotype-matched control antibody: 1.16 ± 0.34 pg/ml; mean ± SEM, $P = 0.27$).

Similar effects were observed for IL-8 secretion, which showed a 9-fold increase upon stimulation of CD40 in the presence of IFN-γ (Fig. 1 and Tables 1 and 2). Comparable levels of TNF-α were produced by biopsies cultured in medium alone or in the presence of IFN-γ. Addition of the agonistic CD40 mAb alone stimulated TNF-α production 5-fold, while agonist mAb combined with IFN-γ induced a 10-fold higher TNF-α production compared to medium or IFN-γ alone (Tables 1 and 2). Additionally, immunohistochemistry experiments were performed to monitor the effect of CD40 ligation on the expression of IL-12 using an antibody recognizing both IL-12p40 and IL-12p70. These experiments revealed that IL-12p40/p70 expression in the tissue was elevated upon stimulation of CD40 as well (Fig. 2). Biopsies cultured in the presence of agonistic CD40 mAb and IFN-γ displayed a diffuse staining in the epidermis with occasional stronger positive cells with dendritic morphology (probably Langerhans cells). Positively stained infiltrates were observed in the dermis, including cells with macrophage or dendritic morphology. All positively stained cells showed a cytoplasmatic staining pattern. Cells of biopsies cultured in the presence of IFN-γ alone also included IL-12p40/p70$^+$ cells, but numbers of positive cells were markedly lower compared to biopsies in which CD40 was stimulated. Biopsies in which CD40 was stimulated in the absence of IFN-γ displayed staining patterns similar to biopsies cultured in the presence of IFN-γ and agonistic CD40 mAb. No staining was observed when the IL-12p40/p70 antibody was omitted (shown in Fig. 2D) or when this antibody was replaced by an antibody of the same isotype raised against an irrelevant antigen (data not shown). The limited availability of the culture medium did not allow us to monitor the secretion of IL-12p40 or IL-12p70 using ELISA.

CD40 ligation also affected IL-10 secretion. In medium devoid of IFN-γ, IL-10 secretion was spontaneously induced in some skin biopsies (Table 1). Upon CD40 stimulation and in the absence of IFN-γ, IL-10 was significantly increased compared to the amount of IL-10 secreted by unstimulated

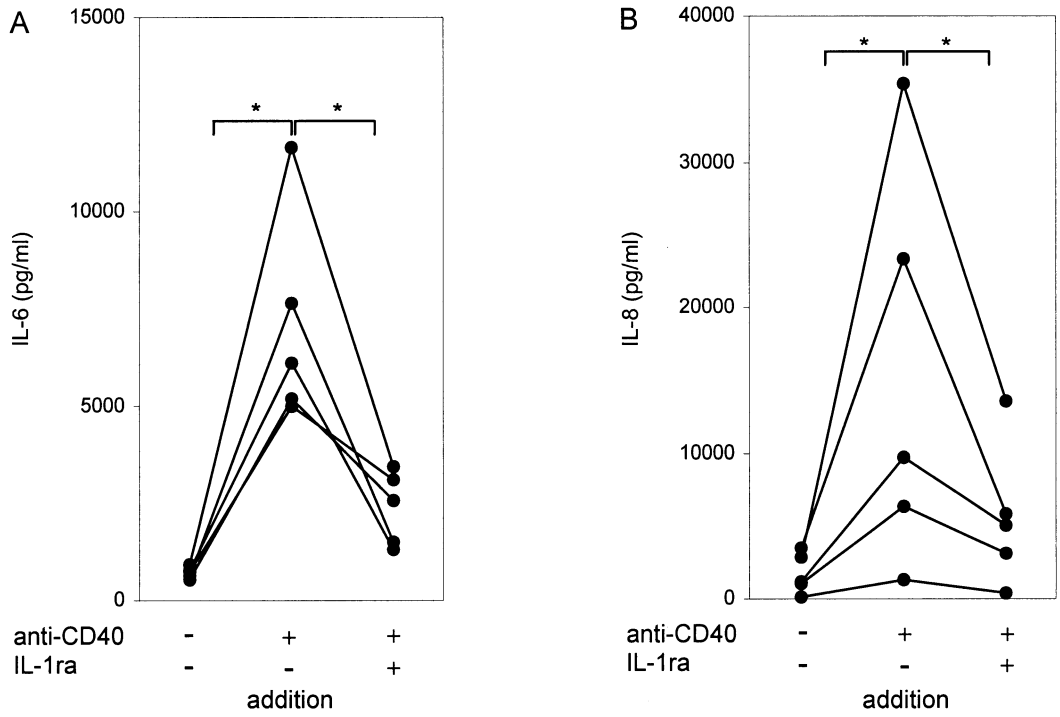

Fig. 1. CD40 ligation in normal human skin biopsies in the presence of IFN-γ stimulates IL-6 and IL-8 production. Normal skin biopsies were cultured for a total of 4 days in the presence of IFN-γ to up-regulate CD40 expression and stimulated with an agonistic anti-CD40 mAb as described in Methods. IL-6 and IL-8 concentrations measured in supernatants of biopsies from five donors. (A) IL-6 secretion. (B) IL-8 secretion. Data of five independent experiments of identical design are presented. *$P < 0.05$.

biopsies (Table 1). Similarly to IL-6 and IL-8, IFN-γ inhibited IL-10 secretion by normal human skin biopsies. For IL-10, however, CD40 stimulation was not able to overcome the inhibitory effect of IFN-γ.

These data show that CD40 ligation in normal human skin explants results in the induction of both pro-inflammatory (IL-1β, IL-6, IL-8, TNF-α and IL-12) and anti-inflammatory (IL-10) cytokines.

CD40 ligation-induced secretion of IL-6 and IL-8, but not TNF-α and IL-10, is mediated via IL-1

To investigate whether IL-1 is involved in CD40 ligation-induced IL-6, IL-8, TNF-α and IL-10 secretion, normal human skin biopsies were stimulated with the CD40 agonistic mAb and IFN-γ in the presence of IL-1ra. Culturing of normal skin biopsies in the presence of IL-1ra and IFN-γ significantly inhibited IL-6 and IL-8 secretion compared with culturing in the presence of IFN-γ alone (Table 2). No effect of IL-1ra on TNF-α and IL-10 secretion in cultures with IFN-γ was observed (Table 2). In the presence of IFN-γ, IL-1ra significantly inhibited CD40 ligation-induced IL-6 and IL-8 secretion by normal human skin biopsies by ~60% ($P < 0.05$, Table 2 and Fig. 1). Conversely, in IFN-γ-containing cultures, CD40 ligation-induced TNF-α secretion was not affected by IL-1ra, which was also observed for IL-10 (Table 1). CD40 ligation-induced IL-1β levels were also influenced by IL-1ra. Culturing of biopsies in the presence of CD40 agonistic mAb in combination with IL-1ra and IFN-γ resulted in an increase of IL-1β secretion ($P < 0.05$, Table 2).

Comparable results were obtained when normal skin biopsies were stimulated with CD40 agonistic mAb in the presence of IL-1ra in cultures devoid of IFN-γ (data not shown).

These data show that CD40 ligation-induced secretion of IL-6 and IL-8, but not TNF-α or IL-10, is mediated to a major extent via IL-1.

Discussion

Recent studies suggest that CD40 ligation plays a key role in inflammatory responses in skin. However, these studies used *in vitro*, *in vivo* or *in situ* analysis. Here, we present functional data on the effects of CD40 ligation on cytokine expression in intact human skin using a recently developed *ex vivo* culture system (22). Our data indicate that CD40 stimulation in intact normal skin results in the induction of pro- and anti-inflammatory cytokines. Furthermore, we demonstrate that CD40 ligation-induced secretion of IL-6 and IL-8, but not TNF-α and IL-10, is partly mediated via IL-1.

CD40 ligation in normal human skin results in secretion of pro- and anti-inflammatory cytokines

First we showed that stimulation of CD40 in intact normal human skin results in the induction of IL-1β secretion. IL-1β induction upon CD40 stimulation has also been demonstrated in monocytes (23), dendritic cells (26) and Langerhans cells (27). It is therefore possible that monocyte-derived dendritic cells or macrophages in skin are the primary source of the IL-1β measured in our system. Although it is known that human

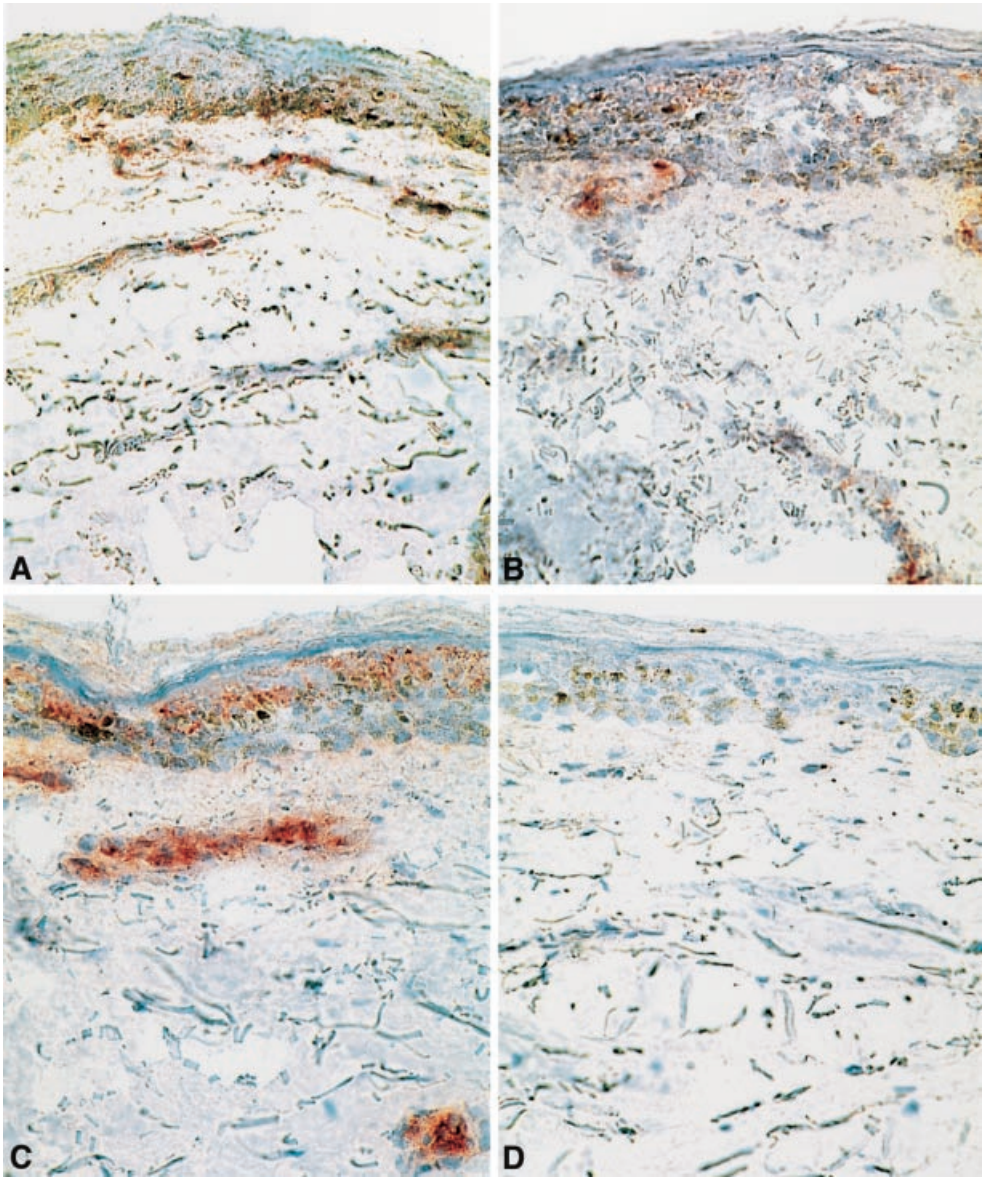

Fig. 2. IL-12p40/p70 expression is elevated upon CD40 stimulation. Acetone-fixed cryostat sections were stained with an IL-12p40/p70-specific mAb as described in Methods. Culture conditions: (a) no stimulation, (b) 1000 U/ml IFN-γ, and (c) 1000 U/ml IFN-γ and anti-CD40 mAb. (a–c) Stained with a mAb specific for IL-12p40/p70. (d) 1000 U/ml IFN-γ and anti-CD40 mAb, omission of primary antibody. Scale bar: 50 μm.

keratinocytes can produce IL-1β, Denfeld *et al*. (11) demonstrated that ligation of CD40 on keratinocytes *in vitro* did not induce IL-1β production. The difference in observation reported by Denfeld *et al*. and our group can be explained by the difference in the system used. We studied the effects of CD40 ligation on all cells present in normal skin, whereas Denfeld *et al*. investigated CD40 ligation on keratinocytes only. Furthermore, the IL-1β concentration in the supernatant of CD40-stimulated keratinocytes might be too low to detect. Finally the difference in observation might suggest that CD40 ligation does not result in up-regulation of IL-1β in keratinocytes and that the IL-1β detected in our system after CD40 stimulation is not keratinocyte derived or that factors induced upon CD40 ligation in cells other than keratinocytes induce IL-1β in skin-resident cells.

Several reports show that CD40 ligation on skin cells *in vitro* induces IL-6, IL-8 and TNF-α expression. CD40 ligation-induced IL-6, IL-8 as well as TNF-α production by human keratinocytes in suspension has been reported (11–13). Fibroblasts are also known to produce IL-6 upon CD40 triggering (14). CD40 stimulation on endothelial cells results in the up-regulation of IL-6, IL-8 and TNF-α as well (28). Our data confirm and extend the data presented by these groups by showing that induction of pro-inflammatory cytokines like IL-6, IL-8, TNF-α and IL-12 upon CD40 stimulation also occurs in intact human skin.

Table 2. CD40 ligation-induced secretion of IL-6 and IL-8, but not TNF-α and IL-10, is mediated via IL-1

Cytokine	Additions			
	None	αCD40 mAb	IL-1ra	αCD40 mAb + IL-1ra
IL-6 (ng/ml)	0.72 ± 0.08	7.11 ± 1.23[a]	0.19 ± 0.03[a]	2.38 ± 0.42[a,b]
IL-8 (ng/ml)	1.72 ± 0.63	15.20 ± 6.23[a]	0.65 ± 0.40[a]	5.56 ± 2.19[a,b]
TNF-α (pg/ml)	16.38 ± 1.70	138.04 ± 20.04[a]	16.94 ± 3.66	129.06 ± 31.36[a]
IL-1β (pg/ml)	<2.00	10.83 ± 3.30[a]	<2.00	26.09 ± 7.34[a,b]
IL-10 (pg/ml)	<1.00	3.65 ± 1.11[a]	<1.00	3.96 ± 1.75[a]

Normal skin biopsies were cultured in the presence of IFN-γ and stimulated with agonistic anti-CD40 mAb alone or antibody in the presence of IL-1ra. Later, IL-6, IL-8, TNF-α, IL-10 and IL-1β concentrations were measured in the supernatant by ELISA. ELISA readings were done in duplicate. Data represent the mean ± SEM (pg/ml or ng/ml) of five donors in independent experiments of identical design.

αCD40 = anti-CD40 mAb.

[a]αCD40, IL-1ra and αCD40 + IL-1ra compared with not stimulated: $P < 0.05$.

[b]αCD40 compared with αCD40 + IL-1ra: $P < 0.05$.

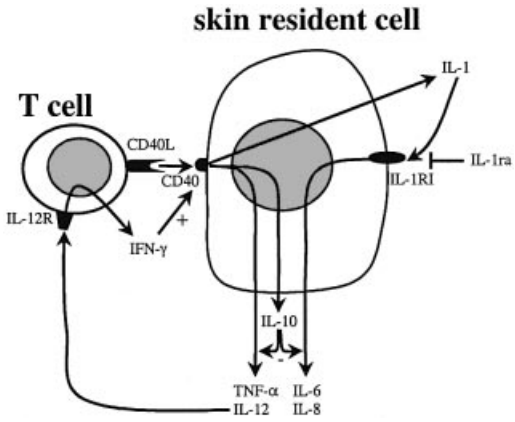

Fig. 3. CD40 ligation-induced expression of IL-6 and IL-8, but not TNF-α and IL-10, is mediated via IL-1. See text for details.

Induction of IL-10 by CD40 stimulation in skin has not been reported yet. Denfeld *et al.* (11) reported that CD40 ligation on keratinocytes did not result in the induction of IL-10. However, CD40 ligation-induced IL-10 expression has been reported in other *in vitro* systems. For example, IL-10 expression in peripheral blood mononuclear cells from patients with Graves disease was up-regulated upon stimulation of CD40 in combination with IL-4 (29) and CD40-mediated IL-10 induction was also observed by dexamethasone pre-treated dendritic cells (30). This suggests that the probable IL-10 source in our system may be monocyte-derived cells like dermal dendritic cells, Langerhans cells or macrophages which are present in normal human skin.

CD40 ligation-induced secretion of IL-6 and IL-8, but not TNF-α and IL-10, is partly mediated via IL-1

Next, we investigated whether IL-1 plays a role in the CD40 ligation-induced cytokine expression. We observed that IL-1ra could inhibit CD40-induced IL-6 and IL-8, but not TNF-α or IL-10 expression. However, IL-6 and IL-8 levels observed after culturing in medium containing CD40 agonistic antibodies and IL-1ra did not equal the levels observed after culturing with IL-1ra alone. This indicates that CD40 ligation-induced IL-6 and IL-8 secretion is also influenced by factors other than IL-1. TNF-α might be a candidate because it has been shown that this cytokine can induce IL-6 and IL-8 secretion by synovial fibroblasts (31), and TNF-α is also elevated upon CD40 stimulation in our system. In contrast to the decreased IL-6, IL-8 and unchanged TNF-α secretion, IL-1β secretion was elevated after stimulation of CD40 in combination with IL-1ra. The explanation for this may be that IL-1β binding to the IL-1 receptor is blocked by IL-1ra in cultures containing both IL-1ra and anti-CD40. Consequently, this IL-1 receptor blockade results in the secretion of IL-1β normally bound to its receptor in cultures lacking IL-1ra.

CD40 ligation in human skin facilitates an inflammatory environment

During our studies we observed that the spontaneous IL-6 and IL-8 secretion in the culture medium was down-regulated upon treatment with IFN-γ. Down-regulation of IL-6 expression by IFN-γ represents a novel observation whereas inhibition of IL-8 expression by IFN-γ in thymic epithelial cells has been reported (32). An explanation for the down-regulation of IL-6 and IL-8 secretion could be that IFN-γ induces expression of IL-6 and IL-8 receptors. Subsequent binding of IL-6 and IL-8 to these receptors could result in a decreased secretion into the medium. Previous studies reported that IL-6 receptor expression by monocytes and intestinal epithelial cells is up-regulated by IFN-γ (33,34). Additionally we showed that the agonistic CD40 mAb was able to prevent the IFN-γ-mediated down-regulation of IL-6 and IL-8 expression, while it was not able to overcome the IFN-γ-mediated down-regulation of IL-10. This suggests that CD40 ligation by activated T cells in skin inhibits the IFN-γ directed repression of pro-inflammatory cytokine production, whereas it does not affect the repression of anti-inflammatory cytokines. Because IL-10 promotes T_h2 development, the IFN-γ-mediated repression of IL-10, even in the presence of CD40 ligation, may be in advantage for the maintenance of the T_h1 balance in inflamed skin. Additionally, an inflammatory environment is promoted via CD40 ligation-induced IL-12 production, even in the absence of IFN-γ.

A model for the CD40-mediated cytokine network in normal human skin

The mechanism of CD40 ligation leading to cytokine expression in normal skin is summarized in the following model (Fig. 3). During inflammation, T cell- or NKT cell-derived IFN-γ elevates CD40 expression. Subsequent CD40 ligation on skin-resident cells by CD40L on activated T cells results in the secretion of IL-1 followed by the IL-1-induced IL-6 and IL-8 production. TNF-α and IL-10 are directly mediated by CD40 ligation or via other factors induced after CD40 ligation. Additionally, CD40 induces IL-12 expression, which subsequently induces IFN-γ expression and consequently promotes an inflammatory environment. Finally, IL-10 has a down-regulating effect on the expression of TNF-α, IL-12, IL-6 and IL-8.

To our knowledge, this is the first study integrating IL-1 and CD40 regulated immune responses in human skin. The data presented provide evidence that IL-1 is involved in CD40-mediated immune responses in the intact human skin. Since both IL-1 and CD40 expression are functionally implicated in inflammatory skin diseases like psoriasis, the present information adds to the understanding of the mechanisms involved in the onset and maintenance of inflammation in skin.

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Abbreviations

CD40L	CD40 ligand
IL-1ra	IL-1 receptor agonist
TNF	tumor necrosis factor

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