

CC Chemokine Ligand 18, An Atopic Dermatitis-Associated and Dendritic Cell-Derived Chemokine, Is Regulated by Staphylococcal Products and Allergen Exposure¹

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Atopic dermatitis is a chronic inflammatory skin disease with a steadily increasing prevalence. Exposure to allergens or bacterial superantigens triggers T and dendritic cell (DC) recruitment and induces atopic skin inflammation. In this study, we report that among all known chemokines CCL18/DC-CK1/PARC represents the most highly expressed ligand in atopic dermatitis. Moreover, CCL18 expression is associated with an atopic dermatitis phenotype when compared with other chronic inflammatory skin diseases. DCs either dispersed within the dermis or clustering at sites showing perivascular infiltrates are abundant sources of CCL18. In vitro, microbial products including LPS, peptidoglycan, and mannan, as well as the T cell-derived activation signal CD40L, induced CCL18 in monocytes. In contrast to monocytes, monocyte-derived, interstitial-type, and Langerhans-type DCs showed a constitutive and abundant expression of CCL18. In comparison to Langerhans cells, interstitial-type DCs produced higher constitutive levels of CCL18. In vivo, topical exposure to the relevant allergen or the superantigen staphylococcal enterotoxin B, resulted in a significant induction of CCL18 in atopic dermatitis patients. Furthermore, in nonatopic NiSO₄-sensitized individuals, only relevant allergen but not irritant exposure resulted in the induction of CCL18. Taken together, findings of the present study demonstrate that CCL18 is associated with an atopy/allergy skin phenotype, and is expressed at the interface between the environment and the host by cells constantly screening foreign Ags. Its regulation by allergen exposure and microbial products suggests an important role for CCL18 in the initiation and amplification of atopic skin inflammation. *The Journal of Immunology*, 2004, 173: 5810–5817.

Atopic dermatitis represents one of the most common chronic inflammatory skin diseases and shows a steadily increasing lifetime prevalence of 10–20% in children and 1–3% in adults (1).

Accumulating clinical and experimental evidence suggests that allergens such as birch or grass pollen (2), as well as house dust mite Ags *Dermatophagoides pteronyssinus* ((Der p) 1 and 2),⁴ play a role in the initiation and amplification of atopic skin inflammation (3). Furthermore, 90% of atopic dermatitis patients show le-

sions colonized with *Staphylococcus aureus* (4) and exhibit a significant reduction of colonization in nonlesional skin (5). Various products of *S. aureus*, such as peptidoglycan (PGN) and superantigens, are known to induce the expression of proinflammatory cytokines in monocytes and dendritic cell (DC) subsets (6). Clinical observations showing an improvement of patients following antistaphylococcal therapy support a role for *S. aureus* in the pathogenesis of atopic dermatitis (7).

Histopathologically, atopic dermatitis skin lesions show an inflammatory infiltrate composed of predominantly CD4⁺ and cutaneous lymphocyte-associated Ag plus memory T cells (8). Skin-selective homing of memory and effector T cells represents an important immunological event in the initiation and amplification of atopic dermatitis lesions. In early phases of the disease, memory T cells with a Th2 phenotype infiltrate the skin at sites of atopic skin inflammation; however, chronic lichenified atopic dermatitis lesions are characterized by the dominance of skin-infiltrating Th1 cells (9).

Chemokines are small, secreted proteins that mediate directional migration and critically regulate organ-specific homing of distinct leukocyte subsets in vivo (10, 11). This superfamily of chemotactic proteins (45 in the human) is thought to be among the first functional protein families completely characterized at the molecular level (10). This offers, for the first time, the opportunity to identify all relevant members associated with physiological or pathological processes.

Although insights into the pathogenesis of atopic dermatitis have evolved over the past several years, the etiology of this disorder remains elusive. In the present study, we systematically analyzed the

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Received for publication May 28, 2004. Accepted for publication August 13, 2004.

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¹ This study was supported by the European Union (QLK4-CT-2001-00366), the German Research Foundation (SFB503), and the Finnish Medical Foundation.

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⁴ Abbreviations used in this paper: Der p, house dust mite Ag *Dermatophagoides pteronyssinus*; PGN, peptidoglycan; DC, dendritic cell; SEB, staphylococcal enterotoxin B; LTA, lipoteichoic acid.

expression of all known chemokines in chronic inflammatory skin diseases and identified *CCL18/DC-CK1/PARC* to be specifically associated with atopic dermatitis. Allergen exposure, as well as staphylococcal products, markedly induced this chemokine in vitro and in vivo, suggesting important chemokine-driven processes during the initiation and amplification of atopic skin inflammation.

Materials and Methods

Patients

Six-millimeter punch biopsies were taken, after obtaining informed consent, from either healthy individuals ($n = 14$) or patients with atopic dermatitis ($n = 15$), psoriasis ($n = 48$), or lupus erythematosus ($n = 9$). The clinical diagnosis of cutaneous lupus erythematosus was confirmed by histological evaluation, serological examinations, and UV-provocation testing. Atopic dermatitis patients were identified according to the criteria defined by Hanifin and Rajka (12). Furthermore, patients suffering from chronic plaque psoriasis in typical locations were enrolled into the study. Normal skin samples were obtained from patients undergoing plastic surgery and reporting no history of atopic disorders or inflammatory skin diseases.

Moreover, atopic dermatitis patients with a history of house dust mite allergy underwent atopic dermatitis patch testing as described previously (13). Briefly, atopy patch tests were performed with house dust mite Ags containing a mix of *Dermatophagoides farinae* and *D. pteronyssinus* species (Chemotechnique Diagnostics, Malmö, Sweden). For staphylococcal enterotoxin B (SEB) patch tests, SEB (Sigma-Aldrich, St. Louis, MO) at $0.226 \mu\text{g}/\mu\text{l}$ in 0.9% NaCl solution was applied in Finn chambers (Epitest, Hyrylä, Finland) on healthy-appearing dorsal skin of atopic dermatitis patients. For the induction of chemical-induced allergic skin inflammation, NiSO_4 at 5% in petrolatum (Epikon, Espoo, Finland) was applied in small Finn Chambers (Epitest) on nonlesional back skin of nickel-sensitized patients ($n = 7$) with no history of atopic dermatitis.

For the induction of chemical-induced irritant skin inflammation, the chemical irritant sodium lauryl sulfate (Merck, Darmstadt, Germany) at 1% in water was applied in large Finn Chambers (Epitest) on nonlesional back skin of Ni-sensitized patients ($n = 7$). The clinical score of patch test lesions was evaluated according to the recommendations of International Contact Dermatitis Research Group (14). Furthermore, the degree of inflammation was histopathologically analyzed and scored by two independent investigators (Table I).

Skin specimens were obtained before and 2, 6, and 48 h after protein allergen, superantigen, hapten, or irritant exposure. These studies were approved by the appropriate local ethics committees.

Cell isolation and cell culture

Human primary epidermal keratinocytes, dermal fibroblasts, and dermal microvascular endothelial cells were purchased from Cambrex (San Diego, CA) and cultured as described (15). Cells were treated with $\text{TNF-}\alpha$ (10 ng/ml), $\text{IL-1}\beta$ (5 ng/ml), $\text{IFN-}\gamma$ (20 ng/ml), and IL-4 (50 ng/ml; R&D Systems, Minneapolis, MN) for 18 h or left untreated. Skin single-cell suspensions were prepared from 6-mm biopsies after mechanical disintegration and enzymatic digestion as described previously (16). Single-cell suspensions of atopic dermatitis skin were stimulated with the house dust mite Ag Der p 2 (0.3 μM ; Allergopharma, Reinbeck, Germany) for 24 h. Furthermore, PBMCs were treated with SEB (1 $\mu\text{g}/\text{ml}$; Sigma-Aldrich).

DC generation and stimulation

Interstitial DCs and Langerhans cells were generated from peripheral blood monocytes as described previously (17, 18). Briefly, mononuclear cells were isolated from peripheral blood and cultured for 6 days in the presence of GM-CSF and IL-4 for the generation of interstitial DC-type cells (17) or

in the presence of GM-CSF, IL-4 , and $\text{TGF-}\beta$ plus $\text{TNF-}\alpha$ during the last 2 days of the culture for the generation of LC-type cells (18). For stimulation experiments, cells were cultivated for 2 days in the presence of either LPS (25 ng/ml), lipoteichoic acid (LTA; 10 $\mu\text{g}/\text{ml}$), PGN (10 $\mu\text{g}/\text{ml}$), SEB (10 ng/ml), or mannan from *Saccharomyces cerevisiae* (10 $\mu\text{g}/\text{ml}$). All reagents were purchased from Sigma-Aldrich. The murine CD40L-transfected fibroblast cell line (CD40L L cells) was kindly provided by Schering-Plough (Dardilly, France), and also used as a stimulator of DC maturation at the ratio of one CD40L L cell per five DCs (19). After 6 and 24 h, cells and supernatants were harvested for further analyses.

Quantitative real-time PCR (TaqMan) analysis

Quantitative real-time PCR analyses were performed as previously described (11, 15). Skin biopsies were homogenized in liquid nitrogen using a Mikro-Dismembrator U (Braun Biotech, San Diego, CA), and RNA was extracted with a TRIzol reagent according to the manufacturer's instructions (Invitrogen Life Technologies, Karlsruhe, Germany). Four micrograms of RNA were treated with DNase I (Boehringer Mannheim, Mannheim, Germany) and reverse transcribed with oligo dT₁₄₋₁₈ (Invitrogen Life Technologies) and random hexamer primers (Promega, Madison, WI) using standard protocols. A total of 25 ng of cDNA were amplified in the presence of 12.5 μl of TaqMan Universal Mastermix (Applied Biosystems, Foster City, CA), 0.625 μl of gene-specific TaqMan probe, 0.5 μl of gene-specific forward and reverse primers, and 0.5 μl of water. As an internal positive control, 0.125 μl of 18S RNA-specific TaqMan probe and 0.125 μl of 18S RNA-specific forward and reverse primers were added to each reaction. Gene-specific probes used FAM as a reporter, whereas probes for the internal positive control (18S rRNA) were associated with the VIC reporter. Alternatively, 25 ng of cDNA were amplified in the presence of target-specific primer combinations and SYBR Green Mastermix (Applied Biosystems, Foster City, CA). Chemokine ligand- and receptor-specific primers and target-specific probes were obtained from Applied Biosystems. Samples underwent the following stages: stage 1, 50°C for 2 min; stage 2, 95°C for 10 min; and stage 3, 95°C for 15 s followed by 60°C for 1 min. Stage 3 was repeated 40 times. Gene-specific PCR products were measured by means of an ABI PRISM 7700 or 5700 Sequence Detection Systems (Applied Biosystems) continuously during 40 cycles. Target gene expression was normalized between different samples based on the values of the expression of the internal positive control (18S rRNA) or ubiquitin.

Immunohistochemistry

For immunohistochemical analyses, skin sections were fixed in acetone and preprocessed with H_2O_2 , followed by an avidin- and biotin-blocking step (Avidin/Biotin Blocking kit; Vector Laboratories, Burlingame, CA). Sections were stained with mAbs against human CCL18 (polyclonal goat IgG; R&D Systems, Minneapolis, MN), or isotype control Ab (Jackson ImmunoResearch Laboratories, West Grove, PA). Development of the staining was performed with a Vectastain ABC kit and an AEC kit (Vector Laboratories). Sections were counterstained with hematoxylin.

ELISA

For the detection of the CCL18 protein, a CCL18-specific ELISA system (Human CCL18 DuoSet; R&D Systems) was used according to the manufacturer's instructions. Dilution series of recombinant human CCL18 protein were used for standard curves (R&D Systems).

Results

Increased expression of CCL18 in the skin is associated with an atopic dermatitis phenotype

The chemokine superfamily represents one of the first protein families completely characterized at the molecular level (10). To identify family members involved in the pathogenesis of chronic inflammatory skin diseases, we performed comprehensive quantitative real-time PCR analyses of skin specimens of healthy individuals ($n = 14$), cutaneous lupus erythematosus patients ($n = 9$), and nonlesional or lesional skin of psoriasis ($n = 48$) and atopic dermatitis ($n = 15$) patients using primer sets for all known human chemokines. Our results indicated that chronic inflammatory skin diseases could be distinguished at the molecular level by their chemokine expression profiles (Fig. 1). In particular, CCL18 was specifically and significantly up-regulated in lesional atopic skin

Table I. Clinical and histological classification of NiSO_4 - or SLS-patch test lesions in Ni-sensitized patients ($n = 7$)

	Clinical Score		Histological Score	
	NiSO_4	SLS	NiSO_4	SLS
+	5	6	1	2
++	1	1	5	3
+++	1	0	1	2

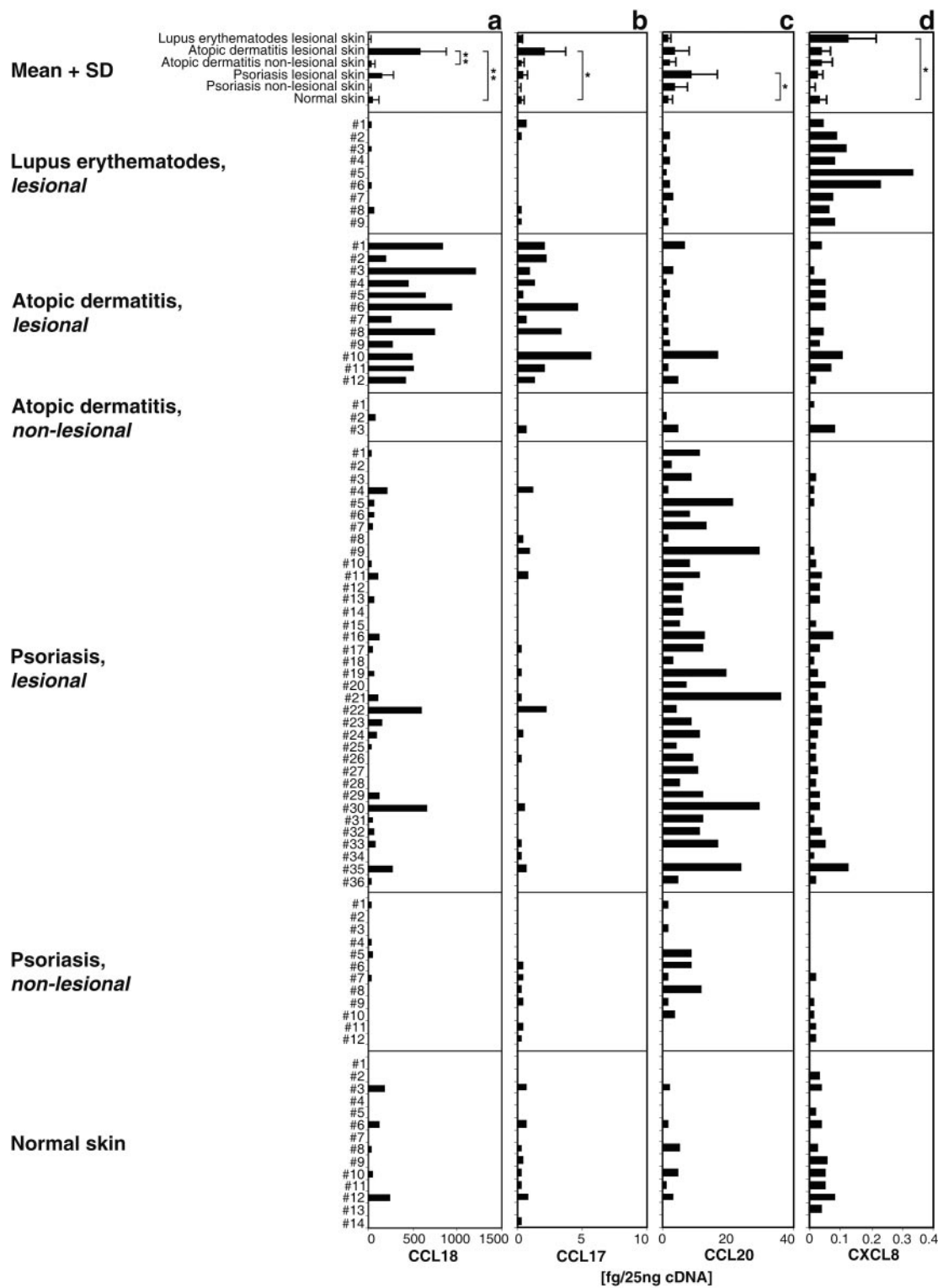


FIGURE 1. CCL18 expression is specifically associated with an atopic dermatitis phenotype. *a–d*, Quantitative real-time PCR analyses of CCL18, CCL17, CCL20, and CXCL8 in normal vs inflamed skin. Values are expressed in femtograms of the target gene in 25 ng of total cDNA. Measurements of individual samples and mean + SD are shown. A Student's *t* test was performed; *, *p* < 0.05; **, *p* < 0.01.

when compared with normal skin or lesional skin of other inflammatory skin diseases (*p* < 0.01; Fig. 1*a*). Among all known chemokines, CCL18 represented the most highly expressed ligand in atopic dermatitis and the absolute amount of CCL18 mRNA in lesional atopic skin was >100-fold higher than that of CCL17/*TARC*, a chemokine previously reported to be associated with an atopic skin phenotype (Fig. 1, *a* and *b*; Ref. 20). Quantitative

real-time PCR analyses demonstrated a significant induction of CXCL8/*IL-8* transcripts in lupus erythematosus patients (*p* < 0.05; Fig. 1*d*); however, no significant association of CXCL8 was shown with a psoriatic phenotype. In contrast to CXCL8, the CC chemokine CCL20/*MIP-3α* was significantly and specifically up-regulated in lesional psoriatic skin (*p* < 0.05; Fig. 1*c*) as it was shown recently (21).

Interstitial DCs and Langerhans cells are the main source of CCL18 in the skin

To identify the anatomical location and define the cellular origin of CCL18, we performed immunohistochemical analyses of skin sections from healthy individuals as well as nonlesional and lesional skin from atopic dermatitis patients using CCL18-specific Abs (Fig. 2). CCL18 was absent in the normal skin of healthy or nonlesional skin of atopic individuals, but abundant expression was observed in lesional atopic dermatitis (Fig. 2, *a–e*). CCL18-positive cells within the dermis exhibited a dendritic morphology (Fig. 2*d*, *inset*) and demonstrated two patterns of distribution. First, CCL18-producing cells were evenly dispersed within the dermal compartment suggesting a network of immunological sentinels (Fig. 2*d*). Second, CCL18-positive cells clustered in perivascular pockets showing a close anatomical association with skin-infiltrating lymphocytes (Fig. 2*e*). Within the epidermis, cells with a dendritic morphology, as well as keratinocytes of the uppermost layers, showed immunoreactivity for CCL18 (Fig. 2, *f–h*).

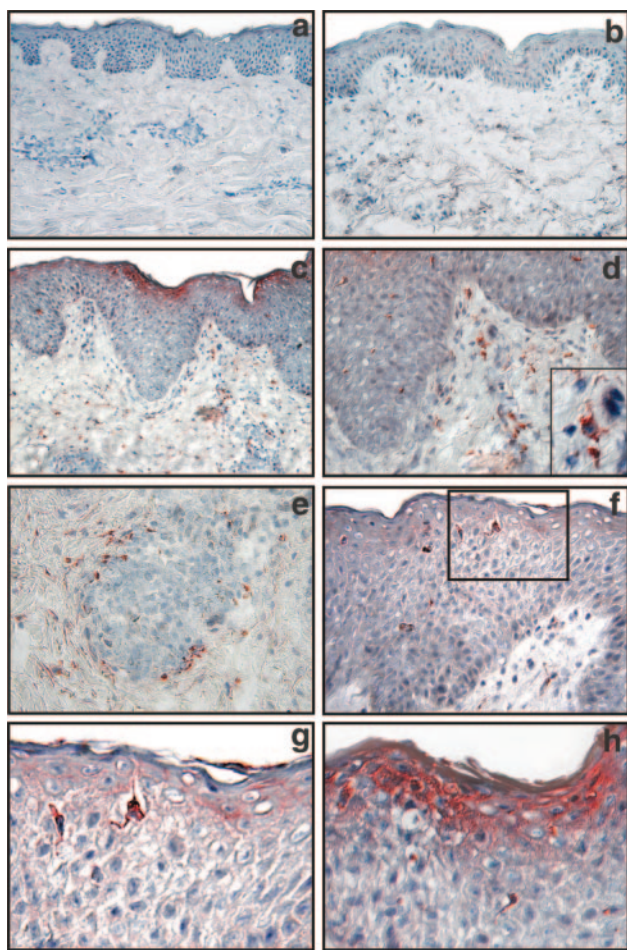


FIGURE 2. Dermal DCs and keratinocytes are the cellular origin of CCL18 expression in atopic skin inflammation. CCL18 expression in normal, nonlesional atopic, and lesional atopic skin. *a*, Absence of CCL18 in the normal skin. *b*, Absence of CCL18 in nonlesional atopic skin. *c* and *d*, Marked expression in inflamed atopic skin and marked expression on cells with dendritic morphology (*d*, *inset*). *e*, CCL18-positive cells clustered in perivascular pockets. *f* and *g*, CCL18-positive cells in the epidermis exhibiting a dendritic morphology. *h*, CCL18-positive staining in the uppermost part of the epidermis. Representative results of 1 of 10 patients are shown. Magnifications were as follows: $\times 250$ (*a–c*), $\times 400$ (*d–f*), and $\times 800$ (*g* and *h*).

Staphylococcal products induce CCL18 production in vitro

Because primary proinflammatory cytokines (i.e., TNF- α and IL-1 β), Th cell-derived effector cytokines (i.e., IL-4 and IFN- γ), and microbial products are known to play a crucial role in the initiation and amplification of atopic skin inflammation (1, 22, 23), we investigated their role in the regulation of CCL18. In vitro, stimulation of cultured human primary keratinocytes and dermal fibroblasts with IFN- γ induced CCL18 expression (Fig. 3*a*). Next to IFN- γ , the type 2 cytokine IL-4 up-regulated the expression of CCL18 transcripts in cultured dermal fibroblasts (Fig. 3*a*). In contrast to structural cells, resting Langerhans-type cells, and especially interstitial-type DCs, expressed CCL18-specific transcripts at significantly higher levels (>100 -fold; Fig. 3, *b* and *c*).

To obtain further insights into the regulation of CCL18, quantitative real-time RT-PCR analyses were performed on monocytes, and monocyte-derived interstitial-type and Langerhans-type DCs after treatment with microbial compounds representing fungi (mannan), Gram-negative (LPS), and Gram-positive (PGN, SEB, and LTA) bacteria, or an activation signal (CD40L) produced by skin-infiltrating memory T cells (Fig. 3, *b* and *c*). LPS, PGN, mannan, and CD40L markedly induced the expression of CCL18 mRNA in monocytes (Fig. 3, *b* and *c*). Analysis of CCL18 protein production in resting and activated monocytes confirmed results obtained at the mRNA level (Fig. 3, *b–d*). In contrast to monocytes, monocyte-derived dendritic cell subsets showed constitutive and abundant expression of CCL18 in vitro (Fig. 3, *b* and *c*). Moreover, PGN stimulation of Langerhans-type DCs resulted in a rapid (6 h) up-regulation of CCL18 transcripts (Fig. 3, *b* and *c*). Analyses of CCL18 protein expression in cell culture supernatants of interstitial-type DCs and Langerhans-type DCs revealed that both dendritic cell subsets produced large amounts of CCL18 protein ranging from 14.25 to 23.19 ng/ 10^6 cells or 0.92 to 2.62 ng/ 10^6 cells, respectively (Fig. 3*d*).

Allergen and superantigen exposure induces CCL18 expression in vivo

Topical exposure of nonlesional skin of atopic dermatitis patients to relevant allergens induces skin lesions mimicking atopic dermatitis, supporting a crucial role for allergens in triggering atopic skin inflammation (12). To obtain further insights into the pathogenesis of atopic skin inflammation, atopic dermatitis patients ($n = 8$) with a history of house dust mite allergy were subjected to atopy patch testing. Before as well as 2, 6, and 48 h after allergen exposure, skin specimens were obtained and analyzed by quantitative real-time RT-PCR or immunohistochemistry (Fig. 4, *a–c*). CCL18 transcripts were significantly induced 48 h after topical exposure to *D. pteronyssinus* and *D. farinae* Ags ($p < 0.05$; Fig. 4*a*). Looking at the individual kinetics of chemokine expression, CCL18 mRNA was induced as early as 2–6 h after house dust mite major allergen exposure in the majority of atopic dermatitis patients (Fig. 4*a*). Confirming the results of quantitative real-time PCR analyses, immunohistochemical evaluation of atopy patch test lesions showed markedly increased numbers of CCL18-positive cells 48 h after house dust mite allergen exposure (Fig. 4, *b* and *c*). To further analyze the capacity of specific allergens to regulate CCL18 expression in atopic skin, single cell suspensions of lesional atopic dermatitis skin were treated with recombinant Der p 2, in vitro. CCL18 mRNA was markedly induced (25-fold) 24 h after Der p 2 stimulation, indicating a role for this chemokine in allergen-specific immune responses (Fig. 4*d*).

Because colonization with superantigen-producing bacteria such as *S. aureus* may trigger atopic dermatitis (23, 24), CCL18 expression was examined in atopic dermatitis patients ($n = 6$) before

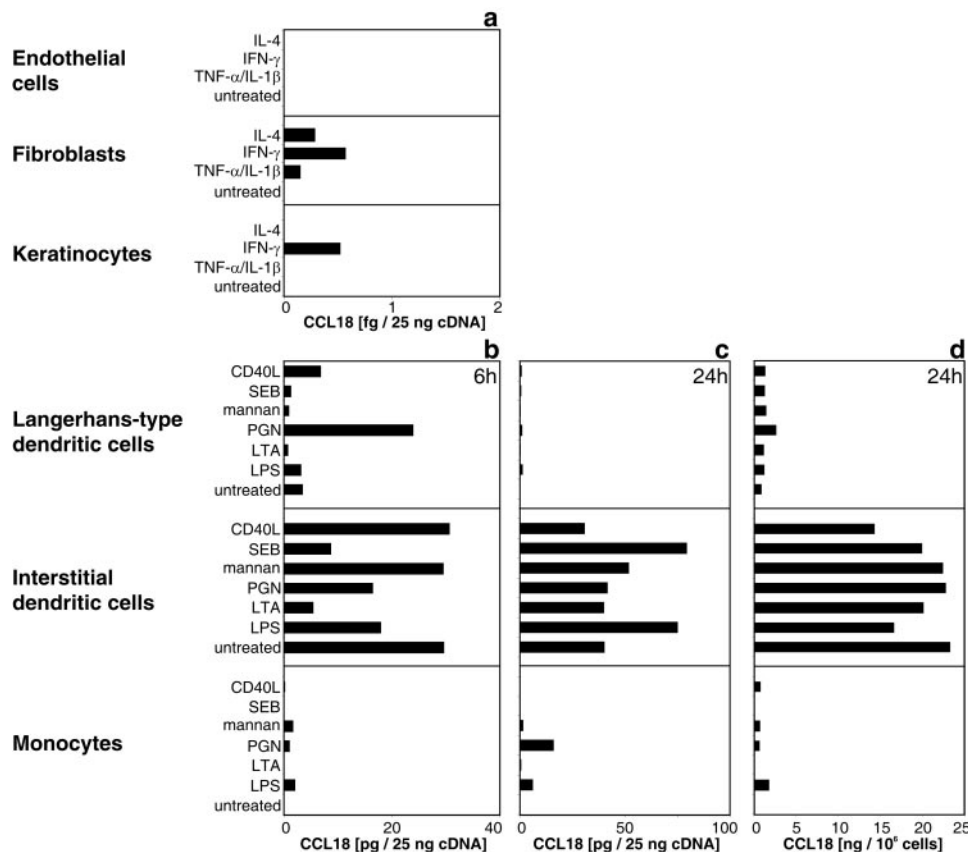


FIGURE 3. T cell effector cytokines and staphylococcal products regulate CCL18. Quantitative real-time PCR and ELISA analyses of CCL18 expression in cellular constituents of the skin. *a*, Analysis of CCL18 in cDNA obtained from cultured human primary keratinocytes, dermal fibroblasts, dermal microvascular endothelial cells treated with medium alone, or with TNF- α /IL-1 β , IFN- γ , or IL-4 for 18 h. Values are expressed in femtograms of the target gene in 25 ng of total cDNA. Representative data from single donors. *b* and *c*, Analysis of CCL18 mRNA in monocytes, or monocyte-derived dendritic cell subsets and Langerhans cells 6 and 24 h after stimulation with LPS, LTA, PGN, mannan, SEB, and CD40L. Values are expressed in picograms of the target gene in 25 ng of total cDNA. *d*, ELISA analysis of CCL18 in supernatants obtained from cultured monocytes, DCs, and Langerhans cells treated with medium alone or with LPS, LTA, PGN, mannan, SEB, and CD40L. Values are expressed in nanograms of CCL18 protein produced by 10⁶ cells.

as well as 2, 6, and 48 h after patch testing with the superantigen SEB. Topical exposure to SEB induced skin lesions clinically and histopathologically, resembling atopic dermatitis, and subsequent quantitative real-time PCR analyses showed that the expression of CCL18 was significantly induced in skin samples 48 h after exposure to SEB ($p < 0.05$; Fig. 4e). Furthermore, superantigen (SEB) stimulation of PBMCs resulted in a rapid (6 h) and marked increase of CCL18 expression in vitro ($p < 0.05$; Fig. 4f), suggesting that skin-infiltrating leukocytes are the major source of superantigen-triggered CCL18 production.

To further investigate the role of CCL18 in nonatopic eczematous skin inflammation, we induced allergic or irritant contact dermatitis using NiSO₄ or SLS epicutaneous patch tests, respectively. For this purpose, Ni-sensitized patients were exposed to the relevant hapten NiSO₄ or to the chemical irritant SLS, skin biopsies were obtained before as well as 2, 6, and 48 h after chemical exposure and subsequently CCL18 expression was determined using real-time PCR and immunohistochemistry (Fig. 5). Although no significant differences in clinical and histological scores between NiSO₄- and SLS patch-tested patients were observed (Table I), a significant induction of CCL18 mRNA and protein was exclusively observed during the induction of allergic skin inflammation ($p < 0.05$; Fig. 5). All Ni-sensitized patients showed a marked induction of CCL18 after exposure with the relevant hapten NiSO₄; however, treatment with the chemical irritant SLS did not regulate the expression of this chemokine (Fig. 5).

Taken together, these findings indicate that relevant protein allergens, haptens, and superantigens, but not unspecific stimuli such as chemical irritants or mechanical injury (data not shown), induce CCL18 production.

Discussion

Chemokines represent a superfamily of small cytokine-like proteins, which mediate directional migration in vitro and control leukocyte trafficking in vivo (10). Recent studies demonstrated that members of this family of chemoattractive proteins play an important role in the organization of innate and adaptive immune responses (25, 26). Today, the chemokine superfamily is thought to be among the first functional protein families completely identified at the molecular level (10). This offers, for the first time, the opportunity to identify all relevant members of a functional protein superfamily involved in physiological or pathophysiological processes.

In the present study, we demonstrate that chronic inflammatory skin diseases display distinct chemokine profiles, which help to distinguish atopic dermatitis, psoriasis, cutaneous lupus erythematosus, and normal skin at the molecular level. One member of the chemokine superfamily, CCL18, was specifically and significantly up-regulated in lesional atopic skin when compared with normal skin or lesional skin of other inflammatory skin diseases. Among all known chemokines, CCL18 represents the most highly expressed ligand in atopic dermatitis and the absolute amount of

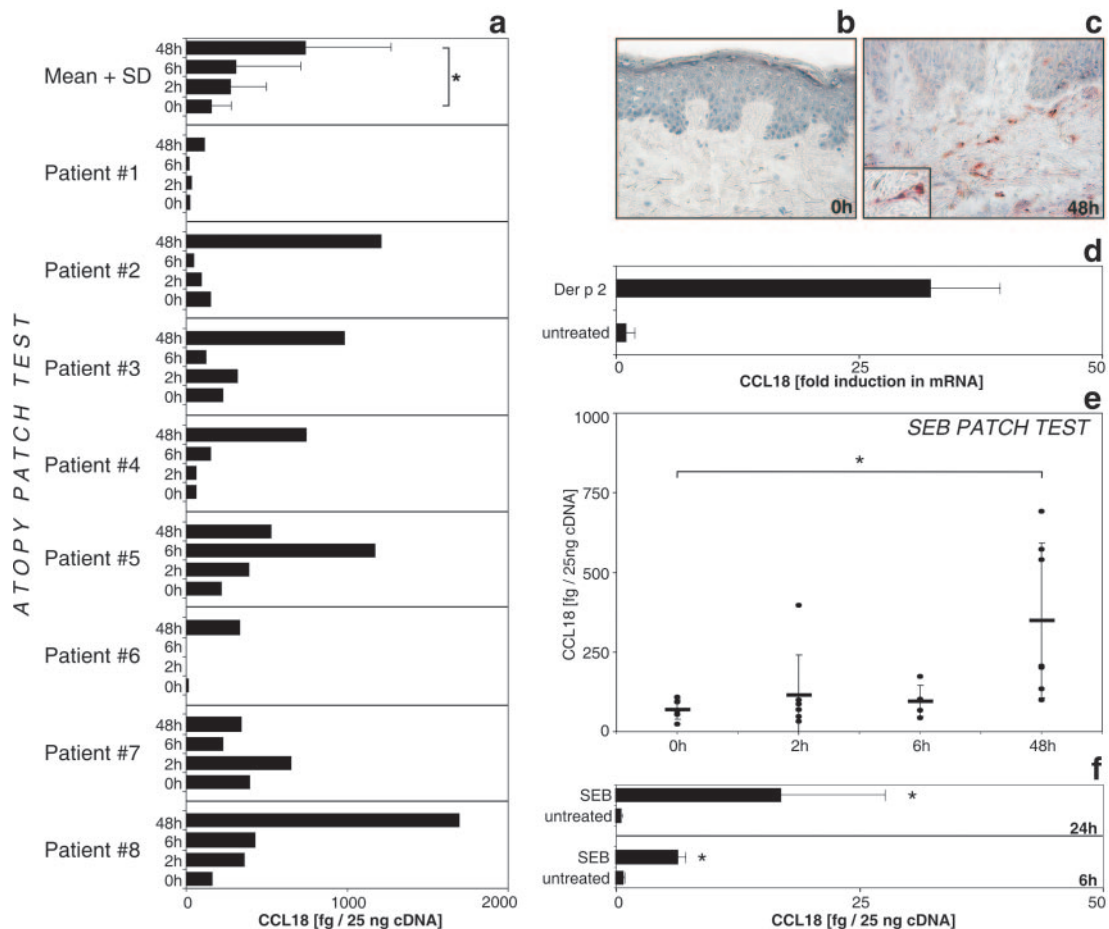


FIGURE 4. Allergen and superantigen exposure induce the expression of CCL18 in vitro and in vivo. *a*, Quantitative real-time PCR analysis of CCL18 in atopic dermatitis patients ($n = 8$) subjected to atopy patch tests with house dust mite allergen. Skin samples were obtained 0, 2, 6, and 48 h after allergen exposure. Values are expressed as femtograms of the target gene in 25 ng of total cDNA. A Student's *t* test was performed, $*, p < 0.05$. *b* and *c*, CCL18 expression before (*b*) and 24 h after (*c*) topical exposure of atopic dermatitis patients with house dust mite allergens; magnification, $\times 250$. *d*, Quantitative real-time PCR analysis of CCL18 expression in single cell suspensions of lesional atopic dermatitis skin 24 h after in vitro stimulation with the house dust mite Ag *Der p 2*. *e*, Quantitative real-time PCR analysis of CCL18 in atopic dermatitis patients ($n = 6$) before and 2, 6, and 48 h after SEB exposure. *f*, Analysis of CCL18 expression in PBMCs 6 and 24 h after treatment with SEB. Values are expressed as femtograms of target gene in 25 ng of total cDNA. A Student's *t* test was performed; $*, p < 0.05$.

CCL18 mRNA in lesional atopic skin was >100 -fold higher than those seen for CCL17, a chemokine previously reported to be associated with an atopic skin phenotype indicating a crucial role for this chemokine in atopic inflammation (20). Expression profiling of chronic inflammatory skin diseases also resulted in an unexpected and surprising observation. Although CXCL8/IL-8 was originally extracted from psoriatic scales (20), and thought to play an important role in the pathogenesis of psoriasis, its expression was not significantly up-regulated in psoriatic skin. In this context, it may be important to note that CXCL8 was recently abandoned as a therapeutic target in psoriasis supporting comprehensive profiling approaches to obtain insights into the etiology of diseases (27). In contrast to CXCL8, the CC chemokine CCL20 was significantly and specifically up-regulated in lesional psoriatic skin as it was shown recently (21). Furthermore, lesional skin of lupus erythematosus patients showed a significant induction of CXCL8 transcripts.

The highly abundant and specific expression of CCL18 in atopic dermatitis suggests an as yet unknown function in this disease. The gene-encoding human CCL18 is located in chromosome 17q11.2. To date, no receptor and no rodent homologue has been identified for this chemokine. However, whole genome scale DNA microar-

ray analyses in a cynomolgus monkey (*Macaca fascicularis*) demonstrated that CCL18 was one of the most strongly up-regulated genes in a monkey asthma model, linking this chemokine with the pathogenesis of atopic diseases (28).

CCL18 was detected in the germinal centers and T cell areas of lymph nodes both representing sites where primary B and T cell responses are initiated (29). Furthermore, CCL18 is chemotactic for T cells (30) suggesting that this chemokine plays a role in the organization of T cell responses. This idea is supported by a recent study demonstrating that human CCL18 enhanced Ag-specific primary CD8⁺ T cell responses when coadministered with malaria vaccines in mice (31). In this study, we report that CCL18 was absent in the normal skin of healthy individuals and in the nonlesional skin of atopic individuals but showed abundant expression in lesional atopic dermatitis. The CCL18-positive cells within the dermis exhibited a dendritic morphology and they were either evenly dispersed within the dermal compartment suggesting a network of immunological sentinels, or clustered in perivascular pockets showing a close anatomical association with skin-infiltrating lymphocytes. Within the epidermis, Langerhans cells and keratinocytes in the uppermost layers showed immunoreactivity for CCL18. Hence, CCL18 is expressed at the interface between the

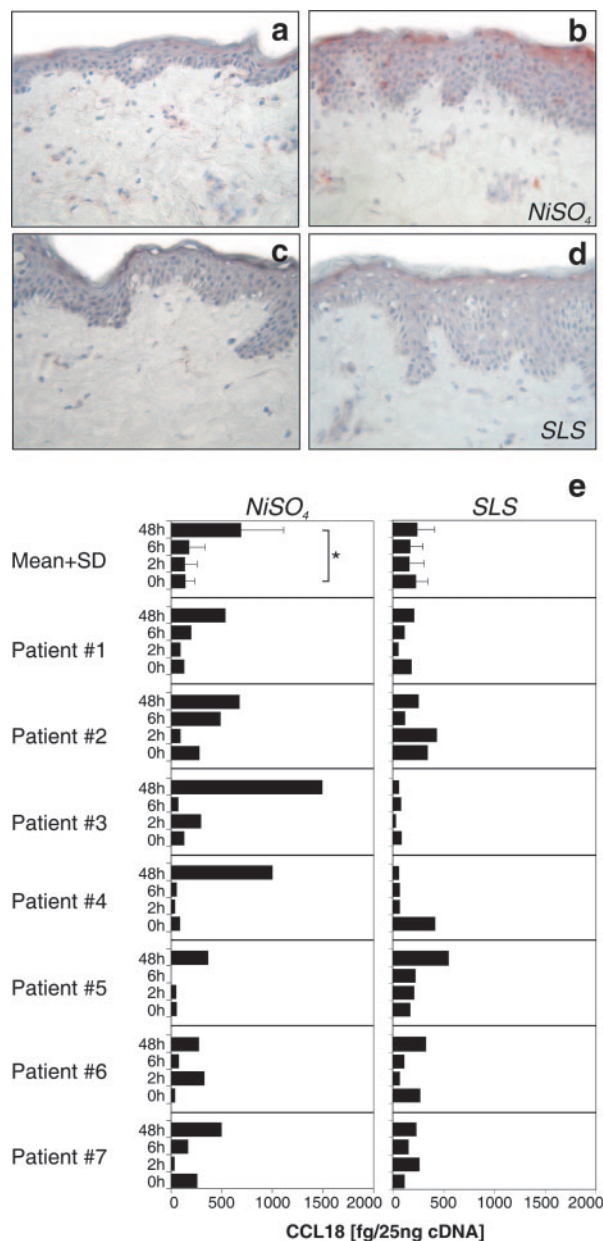


FIGURE 5. Increased CCL18 expression in hapten-induced allergic but not in chemical induced irritant skin inflammation. *a–d*, The detection of CCL18 expression was performed by immunohistochemistry in the skin of nontreated as well as NiSO₄- or SLS-treated Ni-sensitized individuals. *a* and *b*, CCL18 expression is not detected before exposure of Ni-sensitized patients to NiSO₄ (*a*). However, an increased expression of CCL18 is observed 48 h after topical application of NiSO₄ (*b*). *c* and *d*, CCL18 expression before (*c*) and 48 h after (*d*) exposure of Ni-sensitized patients to SLS. Representative results of one of seven patients are shown. Magnification, $\times 400$. *e*, Quantitative real-time PCR analysis of CCL18 expression in Ni-sensitive individuals ($n = 7$) subjected to NiSO₄ or SLS patch test. Skin samples were obtained 0, 2, 6, and 48 h after exposure to NiSO₄ or SLS. Values are expressed as femtograms of target gene in 25 ng of total cDNA. A Student's *t* test was performed; *, $p < 0.05$.

environment and the host and is produced by cells constantly screening foreign Ags.

Clinically, atopic dermatitis patients show an increased frequency of microbial infections and current concepts suggest that staphylococcal products play an important role in the initiation and amplification of atopic skin inflammation (22–24, 32). Indeed,

CCL18 was significantly induced in the skin of atopic dermatitis patients after topical exposure to SEB in vivo. Moreover, superantigen (SEB) stimulation of PBMCs markedly increased CCL18 expression in vitro confirming recent observations by Schutzyer et al. (33).

Although CCL18-producing cells were absent in normal and nonlesional atopic dermatitis skin, monocyte-derived resting interstitial-type and Langerhans-type DCs showed constitutive and abundant expression of CCL18 suggesting that regulation of this chemokine may depend on cell culture conditions. In monocytes, LPS, PGN, mannan and CD40L markedly induced the expression of CCL18. Furthermore, Th-derived effector cytokines and proinflammatory cytokines could induce CCL18 in structural cells of the skin such as keratinocytes and dermal fibroblasts. Although the absolute amount of CCL18 transcripts produced by structural cells of the skin in vitro was low in comparison to Langerhans-type and especially interstitial-type DCs, cytokines released from skin-infiltrating effector T cells may contribute to the abundant expression of CCL18 in atopic skin during acute and chronic phases of the disease (9). Our findings support previous observations that have described abundant expression of CCL18 by immature DCs in vitro (24, 34) and CCL18 production by lung macrophages as well as alveolar epithelial cells in vivo (22) but they substantially extend our understanding about its regulation and pathophysiological role (35).

Topical exposure of nonlesional skin of atopic dermatitis patients to relevant allergens induces skin lesions mimicking atopic dermatitis, supporting a crucial role for allergens in triggering atopic skin inflammation (12). Indeed, CCL18 was significantly induced in skin specimens of atopic dermatitis patients with a history of house dust mite allergy after topical exposure to *D. pteronyssinus* and *D. farinae* Ags, in vivo. Furthermore, treatment of single cell suspensions of lesional atopic dermatitis skin with the recombinant house dust mite allergen Der p 2 markedly induced CCL18, indicating a role for this CC chemokine in allergen-specific immune responses.

This notion is supported by our findings showing the induction of CCL18 during hapten-induced allergic, but not in chemical-induced irritant skin inflammation.

Taken together, findings of the present study suggest a model in which exposure to allergens and microbial products induces CCL18 production in atopic individuals and CCL18 consequently mediates the recruitment of T cells and ensures their encounter with allergen- or superantigen-loaded DCs. Subsequently, effector mediators such as IL-4, IFN- γ , or CD40L derived from activated skin-infiltrating T cells may amplify CCL18 production and atopic skin inflammation.

In conclusion, CCL18 represents a chemokine, which is associated with an atopy/allergy skin phenotype, expressed at the interface between the environment and the host, secreted by cells constantly screening for foreign Ags and regulated by microbial products and allergen exposure. Hence, CCL18 may play an important role in the initiation and amplification of atopic skin inflammation.

Acknowledgments

We thank P. Franken-Kunkel for excellent technical assistance.

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