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IL-4 Enhances Keratinocyte Expression of CXCR3 Agonistic Chemokines

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IFN-induced protein of 10 kDa (IP-10), monokine induced by IFN-γ (Mig), and IFN-inducible T-cell α-chemoattractant (I-TAC) belong to the non-glutamate-leucine-arginine motif (ELR) family and act solely through the CXCR3 receptor for potent attraction of T lymphocytes. In this study, we evaluated the capacity of the T cell-derived cytokines IL-4, IL-10, and IL-17 to modulate IP-10, Mig, and I-TAC in cultured human keratinocytes and CXCR3 expression in T cells from allergic contact dermatitis (ACD). IL-4, but not IL-10 or IL-17, significantly up-regulated IFN-γ- or TNF-α-induced IP-10, Mig, and I-TAC mRNA accumulation in keratinocytes and increased the levels of IP-10 and Mig in keratinocyte supernatants. Immunohistochemistry of skin affected by ACD revealed that >70% of infiltrating cells were reactive for CXCR3 and that CXCR3 staining colocalized in CD4+ and CD8+ T cells. Nickel-specific CD4+ and CD8+ T cell lines established from ACD skin produced IFN-γ and IL-4 and expressed moderate to high levels of CXCR3. Finally, CXCR3 agonistic chemokines released by stimulated keratinocytes triggered calcium mobilization in skin-derived nickel-specific CD4+ T cells and promoted their migration, with supernatant from keratinocyte cultures stimulated with IFN-γ and IL-4 attracting more efficaciously than supernatant from keratinocytes activated with IFN-γ alone. In conclusion, IL-4 exerts a proinflammatory function on keratinocytes by potentiating IFN-γ- and TNF-α induction of IP-10, Mig, and I-TAC, which in turn may determine a prominent recruitment of CXCR3+ T lymphocytes at inflammatory reaction sites. The Journal of Immunology, 2000, 165: 1395–1402.

Chemokines are a vast family of secreted proteins that regulate multiple aspects of inflammatory and immune responses primarily through their chemotactic activity toward subsets of leukocytes (1, 2). The CXC chemokines are divided into two classes depending on the presence of the glutamate-leucine-arginine motif (ELR) in the NH2-terminal domain. IFN-induced protein of 10 kDa (IP-10), monokine induced by IFN-γ (Mig), and IFN-inducible T-cell α-chemoattractant (I-TAC) (3) are all members of the non-ELR CXC class and are induced in a variety of cell types, including endothelial cells, monocytes, fibroblasts, astrocytes, and epithelial cells (3–5). All these chemokines target preferentially memory T cells and NK cells, through a single and shared receptor, the CXCR3 (2, 4, 6).

Keratinocytes are the outermost component of the skin, and they can be activated by diverse factors to produce chemokines important for the recruitment and activation of immune cells, and the formation of the T cell-rich infiltrate that characterizes chronic inflammatory skin diseases such as allergic contact dermatitis (ACD), psoriasis, and atopic dermatitis (7–9). In the skin environment, keratinocytes are considered the major source of IP-10 compared with endothelial cells, monocytes, and fibroblasts (10). The most efficient inducers of IP-10, Mig, and I-TAC synthesis in keratinocytes are IFN-γ and TNF-α (4, 10, 11). During chronic inflammatory diseases, the skin is infiltrated by different T cell subsets. In particular, in the skin affected by ACD, hapten-specific IFN-γ-secreting type 1 CD4+ and CD8+ cells predominate. However, a substantial proportion of IL-4-releasing type 2 lymphocytes and of the newly described Th regulatory cells 1, secreting high amounts of IL-10, are also present (12–14). In addition, both Th1 and Th2 subsets release IL-17, a lymphokine that regulates ICAM-1 expression and chemokine production in keratinocytes and macrophages (7, 15, 16). Although the role of IFN-γ in inducing the non-ELR CXC chemokines in keratinocytes is well established, the contribution of other T cell-derived lymphokines is still unknown.

In this work, we examined the capacity of IL-4, IL-17, and IL-10 to modulate IP-10, Mig, and I-TAC synthesis and release by human keratinocytes and the in vivo expression of CXCR3 receptor in skin affected by ACD as well as in skin-derived nickel-specific CD4+ and CD8+ T cells. Finally, the ability of keratinocyte-derived supernatants to induce intracellular calcium mobilization and migration of nickel-specific CD4+ T cell lines was tested.

Materials and Methods

Patients

Four adult patients with a history of ACD to nickel were selected for this study. Contact allergy was elicited by means of epicutaneous patch testing with 5% NiSO4 in petrolatum. After 24 and 48 h, 4-mm punch biopsies were taken under local anesthesia and either immediately frozen or used to isolate nickel-specific T cell lines. Biopsies from normal skin of healthy individuals were also collected. Skin samples from both allergic and healthy controls were obtained after informed consent.

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3 Abbreviations used in this paper: ELR, glutamate-leucine-arginine motif; ACD, allergic contact dermatitis; IP-10, IFN-induced protein of 10 kDa; I-TAC, IFN-inducible T cell α-chemoattractant; Mig, monokine induced by IFN-γ.
Cytokines and Abs

Human rIFN-γ was obtained from Genzyme (Cambridge, MA). Human IL-17, IL-10, TNF-α, IL-4, and IP-10 and anti-CD3 (49801.111, IgG1), anti-IL-4R (25463.11, IgG2a), and neutralizing anti-IP-10 (33036.211, IgG1) mAbs were purchased from R&D Systems (Abingdon, U.K.). Human M and mouse anti-human IP-10 (4S.B3, IgG2a) and anti-Mig (B8–6, IgG1), FITC-conjugated mouse anti-IFN-γ (4S.B.3, IgG1), rat anti-IL-4 (MP4–25D2, IgG1), anti-CD1a (HI149, IgG), and FITC-conjugated anti-CLA (HECA-452, rat IgM) mAbs, and mouse and rat IgG1 were purchased from BioLegend (San Diego, CA). PE-conjugated anti-CD8 (SK1, IgG1) mAb, and control FITC- or PE-conjugated mouse IgG1 were purchased from Becton Dickinson (San Jose, CA). PE-conjugated anti-TCR γδ (BMA031, IgG2b), FITC-conjugated anti-TCR γδ (IMMU510, IgG1), and anti-CD3 (UCHT-1, IgG1) mAbs were from Immunotech (Marseille, France). Secondary FITC- or PE-conjugated goat anti-mouse IgG were from Dako (Glostrup, Denmark).

Keratinocyte cultures

Primary cultures of human keratinocytes were prepared from plastic surgery skin obtained from normal subjects (n = 3), as previously described (7). In brief, after separation of the epidermis from the dermis with 0.5% dispase (Roche Molecular Systems, Branchburg, NJ), epidermal sheets were treated with 0.25% trypsin (Biochrom, Berlin, Germany), and isolated epidermal cells were seeded (1.2–2 × 10^6 cells/cm^2) on a feeder layer of irradiated 3T3/J2 fibroblasts. Second or third passage keratinocytes were cloned into pCR-TOPO vector (Invitrogen, Carlsbad, CA), and then subcloned by magnetic beads coated with anti-CD4^+ or anti-CD8^+ specific mAb (Dynal, Oslo, Norway) to obtain ≥98% pure CD4^+ or >95% pure CD4^+ T cells, respectively. Purity of the T cell populations was confirmed by flow cytometry analysis. CD4^+ and CD8^+ T cell lines were expanded with autologous PBMC in the presence of 10 μg/ml NiSO_4 (Sigma-Aldrich). Resting T cells were assayed for nickel reactivity after extensive washing to remove IL-2, using autologous PBMC as APC and 10 μg/ml NiSO_4 in 96-well microplates at 37°C, for 4 days and pulsed with 5 μCi/ml [^3]H[jhymidine (Amidine) in the last 16 h. Plates were then harvested onto fiberglass well plates (Packard Instruments, Groningen, The Netherlands). Radioactivity was measured in a Top count (Packard Instruments). Proliferation indexes were measured as the ratio of[^3]H[jhymidine uptake in the presence and the absence of NiSO_4, ranged from 10 to 16 for CD4^+ and 7 to 12 for CD8^+ T cell lines.

Flow cytometry analysis

The immunophenotype of the nickel-specific T cell lines was evaluated by two-color flow cytometry analysis using anti-CLA, anti-CD4, anti-CD8, anti-TCR γδ, and anti-TCR αβ, for analysis of the nickel-specific T-cell lines. The nickel-specific T cell lines were left unstimulated or activated with PMA (10 ng/ml) plus ionomycin (1 μg/ml) for 6 h. Monensin (10 μM; Sigma-Aldrich) and brefeldin A (10 μg/ml; Sigma-Aldrich) were added into the cultures prior to staining to prevent cytokine secretion. T cells were then fixed with 2% paraformaldehyde, permeabilized with 0.5% saponin, and stained with anti-human IFN-γ or rat anti-human IL-4 and analyzed with a FACScan (Becton Dickinson, Mountain View, CA). In control samples, staining was performed using isotype-matched control Ab. To analyze CXCR3 expression after T cell activation, nickel-specific skin-derived CD4^+ lines were activated with autologous adherent monocytes in the presence of 10 μg/ml NiSO_4 and at the indicated time points, cells were fixed, permeabilized or not with 0.5% saponin, and stained with anti-CXCR3 mAb or isotype-matched control Ab, followed by FITC-conjugated goat anti-mouse Ig. CXCR3 detection in T cells was not affected by cell fixation, because a similar staining intensity was measured in cells that were stained with anti-CXCR3 mAb before or after paraformaldehyde fixation (data not shown).

Calcium flux measurements

Intracellular calcium mobilization was measured on skin-derived nickel-specific CD4^+ T cell lines 7-day activated with autologous monocytes plus NiSO_4. An aliquot of T cells (5 × 10^6) was loaded with 8 μM FLUO-3/acetoxymethyl) ester (Molecular Probes, Eugene, OR) and 1 μM/ml Pluronic F-127 for 1 h at 37°C, washed twice with culture medium, and resuspended at 0.3–0.5 × 10^6 cells/400 μl, and basal fluorescence was analyzed in flow cytometry. The calcium flux monitoring was performed on CD4^+ T cells treated with 5 μg/ml NiSO_4 and anti-TCR γδ/anti-CD3 mAbs for 1 h. Finally, cells were washed and re-seeded in un-treated or IFN-γ-treated keratinocyte cultures followed by IP-10, or IP-10 followed by supernatants from IFN-γ-treated keratinocyte cultures.
were expressed 1–3 h after IFN-γ. However, all chemokines (IP-10, I-TAC, and Mig) mRNA were not detected in unstimulated keratinocytes. Induced IP-10, Mig, and I-TAC expression by keratinocytes was measured with a FACScan by 60-s acquisition at a flow rate of 100,000 cells transmigrated in the lower chamber relative to the input number of cells.

Densitometric analysis of mRNA signals. ○, IFN-γ; ▲, IFN-γ + IL-10; ▼, IFN-γ + IL-17; △, IFN-γ + IL-4.

A

B

Transmigration assay

The assay was performed as described (19, 20), with some modifications. In brief, complete RPMI with 0.5% BSA alone and containing rIP-10 (100 ng/ml) or supernatants from keratinocyte cultures untreated or stimulated with IFN-γ or IFN-γ and IL-4 (0.6 ml total quantity) were added to the bottom chamber of 24-well Transwell chambers with uncoated 5-μm-pore polycarbonate filters (Corning Costar, Cambridge, MA). Resting skin-derived nickel-specific CD4+ T cells were resuspended in complete RPMI with 0.5% BSA, and 0.1 ml of cell suspension (10⁶ cells/ml) was added to the top chamber. Transwells (in triplicate for each condition) were then incubated for 1 h at 37°C with 5% CO2. For the blocking experiments, the keratinocyte supernatant and T cell suspension were preincubated respectively with anti-IP-10 (clone 33036.211; 8 μg/ml) or anti-CXCR3 (50 μg/ml) mAb, or control mouse IgG before addition to the top chamber. The number of cells transmigrated in the lower chamber relative to the input was measured with a FACScan by 60-s acquisition at a flow rate of 100 μl/min.

Statistical analysis

Wilcoxon’s signed rank test was used (SigmaStat, Jandel, San Rafael, CA) to compare differences in chemokine release and cell migration. p values ≤ 0.05 were considered significant.

Results

IL-4, but not IL-17 or IL-10, enhances IFN-γ- and TNF-α-induced IP-10, I-TAC, and Mig mRNA expression by keratinocytes

IP-10, I-TAC, and Mig mRNA were not detected in unstimulated keratinocytes by Northern blot analysis. However, all chemokines were expressed 1–3 h after IFN-γ and, more strongly, after IFN-γ and TNF-α treatment. RNA signals peaked at 12 h and slowly decreased thereafter (Figs. 1 and 2). Interestingly, IL-4, but not IL-17 or IL-10, increased IP-10, I-TAC, and Mig mRNA accumulation in keratinocytes induced by IFN-γ alone or IFN-γ plus TNF-α (Figs. 1 and 2). After 12 h, IL-4 augmented IFN-γ-induced IP-10, I-TAC, and Mig mRNA by ~23, 27, and 14%, respectively (Fig. 1B) and by 30% the mRNA for all the three chemokines induced by IFN-γ and TNF-α (Fig. 2B). In addition, the mRNA for these chemokines could also be detected on treatments with IL-4 or TNF-α alone or, more efficiently, with IL-4 and TNF-α together, as assessed by RNase protection assay (data not shown). Consistent with the mRNA data, IL-4 up-regulated IFN-γ- and IFN-γ-TNF-α-induced IP-10 and Mig protein release from keratinocytes (Fig. 3). Keratinocytes could release very large amounts of IP-10 and Mig, reaching up to 3–4 μg/10⁶ cells 72 h after stimulation with IFN-γ, TNF-α, and IL-4. Albeit at low levels, IP-10 and Mig were also induced directly by IL-4 (0.9 ± 0.06 ng/10⁶ cells/48 h; mean ± SD) or TNF-α (1.2 ± 0.09 ng/10⁶ cells/48 h), and to a greater extent by IL-4 plus TNF-α (3.6 ± 0.4 ng/10⁶ cells/48 h). Although IP-10 and Mig mRNA expression showed a similar induction kinetics, IP-10 protein accumulation in the supernatants of activated keratinocytes preceded that of Mig (Fig. 3). IL-4 activity was dose dependent, and it could be completely abolished with a blocking anti-IL-4R mAb (data not shown).

CXCR3 is expressed by T lymphocytes infiltrating ACD skin

Recently, in situ hybridization studies have demonstrated that IP-10, I-TAC, and Mig are expressed in ACD reactions to nickel,
where IP-10 and I-TAC are the most abundant and predominantly expressed by keratinocytes, whereas Mig is found in both the epidermis and dermis (21). In addition to chemokine expression, T lymphocytes infiltrating ACD skin express CXCR3, the cognate receptor for IP-10, I-TAC, and Mig (21). To further characterize the cell types that express CXCR3, double immunostaining of skin biopsies from 24- and 48-h-positive patch test reactions to nickel was performed. More than 70% of infiltrating cells were reactive for CXCR3 (Fig. 4, B and C), that colocalized with the majority of CD4+ and CD8+ cells, but not with CD1a+ dendritic cells (Fig. 4, D and E, and data not shown). Scattered CXCR3+ cells were also identified in normal human skin from healthy individuals in both the epidermis and the dermis (Fig. 4A). CXCR3 staining was stronger along the cell membrane of T cells, but it was variably observed also as cytoplasmic reactivity in T cells and in the intercellular spaces within T cell infiltrates. Furthermore, in ACD lesional skin, but not in normal skin, CXCR3 was expressed by basilar layer keratinocytes, especially in those areas with a heavier inflammatory infiltrate close to the epidermis (Fig. 4B). CXCR3 expression was then examined in nickel-specific CD4+ and CD8+ T cell lines established from ACD lesional skin. These T cell lines were TCR β+ and γδ+, were strictly nickel specific in proliferation assays performed with irradiated autologous PBMC and NiSO₄, and expressed uniformly the skin-homing receptor, cutaneous lymphocyte-associated Ag. The vast majority of resting nickel-specific CD4+ or CD8+ T cells expressed moderate to high levels of CXCR3. On activation, CXCR3 was detected in 50–80% of both CD4+ and CD8+ IFN-γ-producing T cells, in a lower percentage (27–45%) of CD4+ IL-4-producing T lymphocytes, and in about one-half of the small subset of CD8+ T cells synthesizing IL-4 (Fig. 5). Moreover, the staining intensity for CXCR3 on IL-4-producing T cells was lower than on IFN-γ-positive T cells (Fig. 5). A time course analysis of CXCR3 expression on resting and activated CD4+ and CD8+ T cells was also performed. As shown in Fig. 6, resting CD4+ T lines expressed high levels of membrane CXCR3 that decreased 6–48 h after activation and then started to be up-regulated at day 4–5, paralleling data at the mRNA level (data not shown). A similar kinetics of CXCR3 expression was observed for CD8+ T cell lines (data not shown). Staining of resting T cells after cell permeabilization showed a higher fluorescence intensity than in unpermeabilized cells, indicating that part of CXCR3 is intracellular and not expressed on the cell membrane. Of note, the decay kinetics of CXCR3 in permeabilized cells was delayed compared with membrane CXCR3, suggesting that at early time points (6–24 h) after activation substantial...
amount of CXCR3 is present intracellularly (Fig. 6). This finding is consistent with the observation that CXCR3+ T cells in situ were stained both on the cell membrane and in the cytoplasm (Fig. 4).

Superantigen from IFN-γ-treated keratinocytes triggers CXCR3-dependent calcium mobilization in nickel-specific skin-derived CD4+ T cells and promotes their migration

In the following experiments, we tested the biological activity of native keratinocyte-derived CXCR3 ligands on T cell lines established from ACD skin. To this end, supernatants from unstimulated and IFN-γ-stimulated keratinocytes were used on resting skin-derived nickel-specific CD4+ T cell lines in cross-desensitization studies. rIP-10 (100 ng/ml) and conditioned medium from IFN-γ-treated but not from unstimulated keratinocytes induced calcium mobilization in nickel-specific CD4+ T cells (Fig. 7). The responsiveness of CD4+ T cells to IP-10 was lost on prior incubation with supernatants from IFN-γ-treated, but not untreated, keratinocyte cultures (Fig. 7, B and C). Finally, supernatants from IFN-γ-stimulated keratinocytes induced only weak calcium fluxes in T cells that had been previously exposed to rIP-10 (Fig. 7D).

These results strongly suggest that CXCR3 agonists released by activated keratinocytes greatly contribute to trigger functional responses in skin-homing T cells in a CXCR3-dependent manner. To definitively test whether keratinocyte-derived supernatant could promote lymphocyte migration, studies using a Transwell chemotaxis assay were performed. Fig. 8A shows that resting skin-derived nickel-specific CD4+ T cells migrated vigorously in response to supernatant from IFN-γ-stimulated keratinocytes. Preincubation of keratinocyte supernatant or T cells with anti-IP-10 or anti-CXCR3 mAb, respectively, significantly reduced (by 30–50%) the number of migrated cells, suggesting that lymphocyte migration to keratinocyte supernatant was partly IP-10 and CXCR3 dependent. Further inhibition was observed when the two neutralizing mAbs were used in combination (data not shown).

Finally, when supernatants from keratinocytes stimulated with IFN-γ and IL-4 were tested a 2-fold higher migratory response of CD4+ T cells compared with that induced by supernatants from IFN-γ-activated keratinocytes was observed (Fig. 8B). Also in this case preincubation of T cells with anti-CXCR3 partially reduced migration (data not shown).

Discussion

ACD is a common skin disease that is caused by the recruitment into the skin of hapten-specific T lymphocytes which mediate tissue damage through the release of proinflammatory cytokines or
direct cytotoxicity (7, 13, 22). ACD is generally considered as a model of Th1 cell-mediated disorder. However, type 2 cytokines are also represented in ACD skin. In particular, ACD skin expresses IL-4 and a significant portion of nickel-specific T cells, mainly CD4+ cells, release IL-4 (12, 14), but the role of this cytokine in the human disease as well as in mouse contact hypersensitivity is still controversial (23–26). Recently, T regulatory cells have also been isolated from both the blood and lesional skin of ACD patients (14). These cells release large amounts of IL-10 and inhibit in an IL-10-dependent manner the capacity of monocytes and dendritic cells to activate hapten-specific T cells; they thus have an important role in limiting immune responses against haptens. Although once considered just as a target of injury, it is now well established that epidermal keratinocytes are active participants in the generation and amplification of the inflammatory events associated with ACD. Once activated with contact allergen or with cytokines released locally by skin-infiltrating leukocytes, keratinocytes synthesize a wide variety of soluble and membrane mediators involved in the recruitment, retention, and activation of T cells and other leukocytes (7, 9, 27).

In this work, we obtained evidence for a regulatory activity of IL-4 on IP-10, Mig, and I-TAC expression by keratinocytes. In fact, IL-4 significantly enhanced IFN-γ- and TNF-α-induced IP-10, Mig, and I-TAC mRNA accumulation and IP-10 and Mig secretion from keratinocytes. Albeit at low levels, IP-10, Mig, and I-TAC were also induced directly by IL-4 and/or TNF-α. The capacity of IL-4 to induce non-ELR CXC chemokines in keratinocytes extends the proinflammatory functions of this cytokine on keratinocytes that express IL-4R and is consistent with previous studies demonstrating that IL-4 can reinforce the activity of other proinflammatory cytokines in regulating adhesion molecule, cytokine, and ELR CXC chemokine expression by keratinocytes (15). IL-4 potentiation of IFN-γ- and TNF-α-induced chemokine expression has been described also in bone marrow stromal cells (28), whereas in monocytes and macrophages IL-4 appears to inhibit IP-10 induced by IFN-α/γ or LPS (29, 30). In contrast to IL-4,
IL-10 was not capable of modulating chemokine expression by keratinocytes, possibly in relation to the low expression of the IL-10R α-subunit in these cells (our unpublished observations). Also IL-17, a lymphokine expressed in ACD skin and released by nickel-specific CD4+ T cells, was ineffective in regulating IP-10, Mig, and I-TAC, although keratinocytes express IL-17R, and IL-17 could modulate CC and CXC chemokine expression in keratinocytes (7, 15). Other than in ACD, non-ELR chemokines have been shown to be expressed by keratinocytes in situ in other inflammatory skin diseases such as psoriasis and delayed-type hypersensitivity reactions as well as in cutaneous T cell lymphoma, suggesting an important contribution of these chemokines in the formation of the T cell infiltrate in different skin diseases (10, 31, 32).

The functional relevance of CXCR3 agonists released by keratinocytes was confirmed in cross-desensitization studies where supernatants from activated keratinocytes were used on skin-derived nickel-specific CD4+ T cells. In this series of experiments, pre-treatment of T cells with supernatants from activated keratinocytes prevented completely T cell calcium flux induced by IP-10. Conversely, T cells preexposed to IP-10 showed reduced calcium fluxes to keratinocyte-derived supernatants. In parallel, these supernatants elicited a strong T cell migratory response that was partially abolished by neutralization of CXCR3 on T cells or IP-10 in keratinocyte supernatants, suggesting an important contribution of this chemokine receptor/chemokine pair in the recruitment of T cells in the epidermis. In agreement with the finding that IL-4 enhanced IFN-γ-induced production of CXCR3 ligands by keratinocytes, supernatants from keratinocytes activated with IFN-γ and IL-4 were more effective than supernatants from keratinocytes stimulated with only IFN-γ in attracting T cells. On the other hand, the inability of CXCR3 mAb to inhibit completely the T cell migratory response to supernatants from IFN-γ- or IFN-γ/IL-4-stimulated keratinocyte cultures indicates that other non-CXCR3-agonistic chemokines are released by keratinocytes and are capable of attracting T cells (7, 15).

The CXCR3 receptor has been reported to be expressed at higher levels on Th1 compared with Th2 clones, supporting the concept that non-ELR chemokines mobilize preferentially Th1 lymphocytes (33, 34). However, more recent studies have found that CXCR3 is highly expressed on both Th1- and Th2-oriented memory T cell lines as well as in both Th1- and Th2-dominated diseases (35). We found that the vast majority of CD4+ and CD8+ T cells infiltrating ACD skin and nickel-specific CD4+ and CD8+ T cells line established from ACD skin expressed CXCR3. In addition, CXCR3 expression was a feature of both IFN-γ- and IL-4-producing T cells, although CXCR3 was expressed at lower levels in a smaller fraction of the latter cells. These results confirm that CXCR3 is not restricted to type 1 lymphocytes, and suggest that IP-10, Mig, and I-TAC can recruit both IFN-γ- and IL-4-secreting T cells. An interesting observation was that CXCR3 was not expressed exclusively on the T cell surface but also inside the cell both in vitro and in vivo. A similar intracellular localization has been observed for the CXCR4 and CCR2b receptors and is postulated to represent a reservoir that could be rapidly transferred to the plasma membrane (36, 37). The significance of intracellular CXCR3 in T cells is at present unknown. In resting cells, it may represent CXCR3 in transit from the Golgi complex to the cell membrane, whereas the higher levels found in activated cells may derive from receptor-mediated endocytosis of the membrane receptor as TCR-triggered T cells can produce CXCR3 agonistic chemokines (38). Moreover, immunohistochemistry of ACD skin showed that CXCR3 immunoreactivity was also detectable in the intercellular spaces, suggesting that CXCR3, likewise CXCR2 (39), may be shed by T cells and serves additional functions such as chemokine subtraction from binding to the membrane receptor. Finally, CXCR3 expression was not limited to T lymphocytes in ACD skin but extended to basal layer keratinocytes, suggesting new functional roles for CXCR3 in inflammatory disorders. In conclusion, our results reinforce the concept that Th1 and Th2 cell-derived cytokines can efficiently collaborate in promoting and shaping the inflammatory responses during ACD and point to keratinocytes as important active players in the amplification of this immune response.

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References


