

# IL-17 Expression in Human Herpetic Stromal Keratitis: Modulatory Effects on Chemokine Production by Corneal Fibroblasts<sup>1</sup>

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Herpetic stromal keratitis (HSK) is an immunopathologic disease triggered by infection of the cornea with HSV. Key events in HSK involve the interaction between cornea-infiltrating inflammatory cells and resident cells. This interaction, in which macrophages, producing IL-1 and TNF- $\alpha$ , and IFN- $\gamma$ -producing Th1 cells play a crucial role, results in the local secretion of immunomodulatory factors and a major influx of neutrophils causing corneal lesions and blindness. The Th1-derived cytokine IL-17 has been shown to play an important role in several inflammatory diseases characterized by a massive infiltration of neutrophils into inflamed tissue. Here we show that IL-17 is expressed in corneas from patients with HSK and that the IL-17R is constitutively expressed by human corneal fibroblasts (HCF). IL-17 exhibited a strong synergistic effect with TNF- $\alpha$  on the induction of IL-6 and IL-8 secretion by cultured HCF. Secreted IL-8 in these cultures had a strong chemotactic effect on neutrophils. IL-17 also enhanced TNF- $\alpha$ - and IFN- $\gamma$ -induced secretion of macrophage-inflammatory proteins 1 $\alpha$  and 3 $\alpha$ , while inhibiting the induced secretion of RANTES. Furthermore, considerable levels of IFN- $\gamma$ -inducible protein 10 and matrix metalloproteinase 1 were measured in stimulated HCF cultures, while the constitutive secretion of monocyte chemoattractant protein 1 remained unaffected. The data presented suggest that IL-17 may play an important role in the induction and/or perpetuation of the immunopathologic processes in human HSK by modulating the secretion of proinflammatory and neutrophil chemotactic factors by corneal resident fibroblasts. *The Journal of Immunology*, 2002, 169: 5897–5903.

Herpes simplex virus infection of the cornea can lead to the development of a chronic inflammatory disease of the cornea called herpetic stromal keratitis (HSK),<sup>3</sup> a leading cause of nontraumatic blindness in developed countries (1). HSK is considered to result from an immunopathologic process in the cornea involving both innate and adaptive immune responses to the replicating virus (2). Solid support for this viewpoint comes from studies in experimental mouse models. In immunocompetent mice the disease is associated with a biphasic cellular infiltrate in the corneal stroma. In the preclinical phase, when HSV-1 replicates in the corneal epithelium, neutrophils invade the underlying corneal stroma (3). This transient response, triggered by replicating virus (4, 5), is thought to control HSV replication and limit viral spread into peripheral tissues (6, 7). During the second phase, i.e., the clinical phase, a second wave of inflammatory cells, predominantly consisting of neutrophils, infiltrates the corneal stroma (8). An essential factor for the development of HSK is the involvement of IL-2- and IFN- $\gamma$ -secreting CD4<sup>+</sup> T cells, orchestrating the extravasation and activation of neutrophils. These neutrophils are considered to be directly involved in corneal de-

struction (9–11). Additionally, Langerhans cells (LC) and macrophages, secreting the proinflammatory cytokines IL-1 and TNF- $\alpha$ , are essential mediators (12–15).

Extensive studies in murine models of HSK have provided insight into the roles multiple chemokines play in the development of the disease (5, 8, 16, 17). These studies demonstrated the temporal expression of chemokines like IL-8, RANTES, macrophage-inflammatory protein (MIP)-1 $\alpha$ , monocyte chemoattractant protein (MCP)-1, and IFN- $\gamma$ -inducible protein (IP)-10 in affected murine corneas. These chemokines are likely to contribute to the recruitment and activation of lymphocytes, dendritic cells, and neutrophils, initiating the clinical phase of HSK. Although the cellular source of the chemokines involved in HSK remains ill defined, they may be produced by infiltrating inflammatory cells and/or resident corneal cells. Several studies have implied that the interaction with inflammatory cells mainly involves fibroblasts within the corneal stromal layer. The stromal cell layer of the cornea is made up of fibroblasts providing mechanical strength to the cornea by supporting a framework of extracellular matrix. Large numbers of IFN- $\gamma$ - and TNF- $\alpha$ -producing cells have been observed in the stromal layer of HSV-infected murine corneas but not in the epithelial or endothelial layers (18, 19). This corresponds to the location of neutrophils that mainly infiltrate the stromal fibroblast cell layer of the cornea. Similarly, the induction of IL-8, which exhibits neutrophil chemotactic properties, has been shown to be associated with HSV replication in corneal fibroblasts but not in epithelial cells (20).

Evidence is accumulating that activated tissue resident cells, including fibroblasts, are involved in modulating local immune responses by expressing adhesion molecules and secreting regulatory factors like cytokines and chemokines. Macrophage-derived cytokines like IL-1 and TNF- $\alpha$ , and Th1 cytokines like IFN- $\gamma$ , have been shown to activate mesenchymal cells (21–26). Interestingly, another recently described Th1 cytokine, IL-17, can exert

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<sup>3</sup> Abbreviations used in this paper: HSK, herpetic stromal keratitis; MIP, macrophage-inflammatory protein; MCP, monocyte chemoattractant protein; MMP, matrix metalloproteinase; IP, IFN- $\gamma$ -inducible protein; HCF, human corneal fibroblast; LC, Langerhans cell; TCL, T cell line; PMN, polymorph mononuclear cell.

strong synergistic and/or antagonistic effects with the aforementioned proinflammatory cytokines. These effects include the modulation of MHC and adhesion molecules and the induction of a variety of cytokines and chemokines by mesenchymal cells (21–24, 27–30). This modulatory effect of IL-17 could therefore have an indirect but profound effect on the recruitment and activation of different inflammatory cells into the corneal stroma during human HSK. To address this issue, we analyzed the induction of the cytokine IL-6, various chemokines, and matrix metalloproteinase (MMP)-1 by human corneal fibroblasts (HCF) upon stimulation with IL-1 $\beta$ , TNF- $\alpha$ , and IFN- $\gamma$ . The combinative effect of IL-17 with these cytokines on HCF activation was emphasized.

Our data show that IL-17 is expressed in corneas of HSK patients and has a regulatory effect on the induction of immunomodulatory factors by cytokine-stimulated HCF. These results indicate that the interaction of IL-17 with HCF may play a significant role in the initiation and perpetuation of the inflammatory processes in human HSK.

## Materials and Methods

### *Cytokines and mAbs*

Human recombinant IL-1 $\beta$ , IL-17, TNF- $\alpha$ , and IFN- $\gamma$  were obtained from PepróTech (London, U.K.). For blocking experiments, neutralizing mAbs directed to human IL-17 (R&D Systems, Abingdon, U.K.), IL-8, and isotype-matched control mAbs were used (PepróTech).

### *Human intracorneal T cell lines (TCL) and HCF cultures*

The generation of HSV-specific TCL used in this study, obtained from affected corneas of two HSK patients, has been described previously (31). To determine IL-17 mRNA expression, TCL were either left unstimulated or stimulated with PMA (50 ng/ml) and ionomycin (500 ng/ml). Primary HCF cultures were generated from three individual donor corneas that had been rejected for transplantation use due to low endothelial cell counts, and from one transplanted HSK-diseased cornea. The central part of each cornea was finely minced and digested with collagenase essentially as described elsewhere (31). Adherent cells were cultured in six-well plates in medium consisting of a 1:1 ratio of DMEM and Ham F12 nutrient mixture (Life Technologies, Breda, The Netherlands) supplemented with 10% FCS and antibiotics. HCF cultures, with a fibroblast-like morphology, were grown in bulk in 162-cm<sup>2</sup> flasks and cryopreserved. All cells stained positive for vimentin by immunofluorescence staining, whereas almost no reactivity was found for both the acidic and basic subfamilies of cytokeratin (data not shown), indicating that HCF cultures were not contaminated with corneal epithelial or endothelial cells. Third- or fourth-passage corneal fibroblasts were used in all experiments. For cytokine stimulation experiments, HCF from donor corneas were grown in medium of DMEM and Ham F12 with 10% FCS and antibiotics. At confluence, medium was replaced with a serum-free medium consisting of DMEM supplemented with 2 mM L-glutamine, 20  $\mu$ g/ml cholesterol, and 0.3% BSA (all obtained from Sigma-Aldrich, St. Louis, MO). HCF were left for 5 days on serum-free medium before stimulation with cytokines. Serum-free medium was used to maintain a more native biosynthetic phenotype and appearance (32) and to reduce background levels of cytokine and chemokine production (data not shown). Stimulatory cytokines were added to the HCF cultures and supernatants were collected after 48 h. All tested stimulatory conditions were repeated in at least eight individual experiments unless stated otherwise. For blocking experiments, the stimulating mixture of cytokines was preincubated with a neutralizing anti-IL-17 mAb (10  $\mu$ g/ml) at room temperature 30 min before addition to the cell culture. Inhibition of cytokine and chemokine expression was induced by pretreatment of HCF cultures with 10 ng/ml of the anti-inflammatory drug dexamethasone (Sigma-Aldrich) for 2 h.

### *RNA isolation and RT-PCR analyses*

Total cellular RNA was extracted from human HSK corneas, cultured HCF, or intracorneal TCL with TRIzol reagent (Life Technologies) according to the manufacturer's protocol. The cornea buttons analyzed were obtained from three patients with a fulminant HSV-induced necrotizing stromal keratitis after therapeutic penetrating keratoplasty. HSK classification and disease status were defined on the basis of clinical criteria (33). None of the patients were treated with steroids. For RT-PCR analyses, total RNA was converted into cDNA using oligo(dT) and reverse transcriptase.

The following synthetic oligonucleotides were used for PCR amplification: for IL-17 amplification, primers 5'-ATCTCCACCGCAATGAGGAC-3' and 5'-GTGGACAATCGGGGTGACAC-3' (232-bp amplicon); for IL-17R, primers 5'-CTAAACTGCACGGTCAAGAAT-3' and 5'-CTGAGCTCATGCATGGCGTGG-3' (456-bp amplicon). As an internal control for the amount of cDNA, the GAPDH gene was amplified with primers 5'-GGTGAAGGTCGGAGTCAACG-3' and 5'-CAAAGTTGTCATGGATGACC-3' (496-bp amplicon). Amplification was performed in standard PCR buffer with 1 U AmpliTaq Gold DNA polymerase (PerkinElmer, Groningen, The Netherlands), 200  $\mu$ M dNTPs, and 25 pmol of each primer in a total volume of 50  $\mu$ l. DNA amplification was started with a 5-min incubation at 95°C, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min. The final extension was at 72°C for 7 min. PCR products were separated on a 2% agarose gel and visualized by ethidium bromide staining. Southern blotting and labeling with a specific <sup>32</sup>P-labeled oligonucleotide probe was performed to confirm the specificity of the amplicons.

### *ELISA*

Culture supernatants from corneal fibroblasts stimulated for 48 h, referred to as conditioned medium, were harvested and cleared of cellular debris by centrifugation. Secretion levels of IL-6, IL-8, RANTES, MIP-3 $\alpha$ , IP-10, and MMP-1 were measured with commercially available ELISA kits (R&D Systems). MIP-1 $\alpha$  content was measured in an ELISA using a coating mAb, recombinant human MIP-1 $\alpha$  as a standard, and an HRP-conjugated detection mAb (R&D Systems). The amount of MCP-1 in culture supernatants was determined using a commercially available ELISA kit obtained from BioSource (Nivelles, Belgium). The detection limits of the ELISA were as follows: IL-6, <0.7 pg/ml; IL-8, <10 pg/ml; RANTES, <8 pg/ml; MIP-3 $\alpha$ , <0.9 pg/ml; IP-10, <4.5 pg/ml; MMP-1, 21 pg/ml; MIP-1 $\alpha$ , <10 pg/ml; and MCP-1, <20 pg/ml.

### *Neutrophil chemotaxis*

Polymorph mononuclear cells (PMN) were isolated from fresh peripheral blood of healthy, adult volunteers using Polymorph Prep (Nycomed, Oslo, Norway) and residual erythrocytes were lysed. To analyze the chemotactic activity of conditioned medium of HCF, PMN were brought to a final concentration of  $2 \times 10^6$  cells/ml in serum-free medium. The chemotaxis assay was performed in a 24-well Transwell system (Costar, Badhoevedorp, The Netherlands). The bottom wells of the chamber were filled with 100  $\mu$ l of recombinant human IL-8 (10 ng/ml) as a positive control for neutrophil chemotaxis, the control solution, or conditioned medium from stimulated HCF cultures, diluted 1/10 in serum-free medium. The upper wells, holding a polycarbonate filter with a pore size of 3  $\mu$ m, were placed on top and filled with 150  $\mu$ l of neutrophil suspension. The Transwell system was incubated in humidified air with 5% CO<sub>2</sub> at 37°C for 45 min, and the number of cells that had migrated through the filter into the bottom well were counted. Inhibition of IL-8-mediated neutrophil recruitment involved a preincubation of the conditioned medium with 10  $\mu$ g/ml anti-IL-8 mAb 30 min before the experiment at room temperature. As a control, conditioned medium was incubated under similar conditions with an irrelevant isotype-matched control mAb.

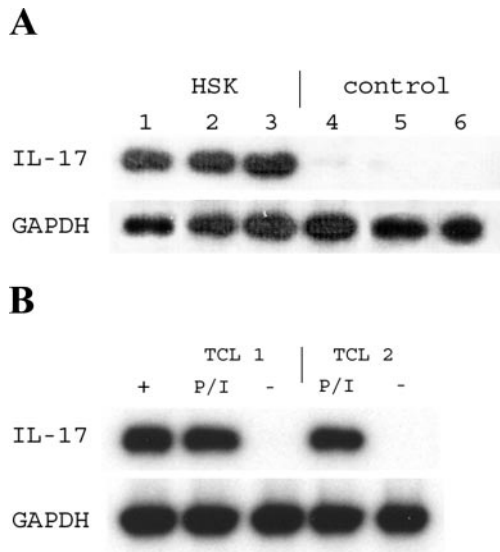
### *Statistical analysis*

Results are expressed as the mean  $\pm$  SD. The statistical significance of the modulatory effect of IL-17 on cytokine-treated was determined by the Mann-Whitney *U* test. Differences resulting in *p* values <0.05 were considered to be statistically significant.

## Results

### *IL-17 expression in corneas and intracorneal TCL obtained from HSK patients*

Whereas the normal healthy cornea is essentially devoid of T cells, HSK is considered an immunopathologic disease orchestrated by corneal-infiltrating CD4<sup>+</sup> Th1 cells secreting IL-2 and IFN- $\gamma$  (9–11). To assess the possible role of the Th1 cytokine IL-17 in human HSK, the expression of IL-17 mRNA was determined in corneas from three patients with fulminant HSK who underwent corneal transplantation to restore sight. IL-17 transcripts could be detected in all HSK corneas, while no IL-17 was detected in control corneas (Fig. 1A). Mitogenic stimulation of two intracorneal TCL generated from corneas of two other HSK patients induced

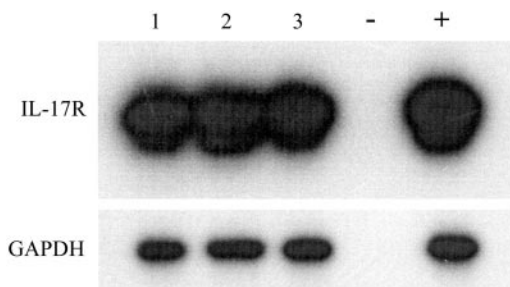


**FIGURE 1.** Expression of IL-17 mRNA expression in human HSK corneas and intraocular TCL analyzed by RT-PCR and Southern blotting. *A*, RNA was isolated from corneas from three patients transplanted during active HSK (lanes 1–3) and from three healthy donor control corneas (lanes 4–6). *B*, HSV-1-specific intracorneal TCL from two other HSK patients were cultured and RNA from a total of 10<sup>6</sup> mitogen-stimulated (P/I) or nonstimulated (–) cells was extracted for analysis. +, Positive control on RNA from mitogen-stimulated PBMC.

IL-17 mRNA expression. This demonstrates that corneal-infiltrating T cells, at least in part, are able to express IL-17 mRNA upon activation (Fig. 1*B*).

*HCF constitutively express the IL-17R*

The keratogenic properties of Th1 cytokines in HSK may in part be due to their modulatory effect on corneal resident cells. Corneal-infiltrating T cells are mainly observed in the corneal stroma, suggesting an interaction between T cells and corneal fibroblasts by means of direct cell-cell contact or soluble factors like IL-17. To evaluate whether corneal fibroblasts express the IL-17R, RT-PCR was performed on RNA extracted from three primary HCF cultures. Two primary HCF cultures had been generated from two healthy donor corneas and one from a transplanted HSK diseased cornea. RT-PCR analysis showed that HCF expressed IL-17R mRNA (Fig. 2).



**FIGURE 2.** Corneal stromal fibroblasts constitutively express IL-17R mRNA. Corneal fibroblasts were cultured from two individual healthy donor corneas (lanes 1 and 2) and one transplanted non-HSK cornea (lane 3). RNA was isolated from 10<sup>6</sup> cells from each culture and assayed for IL-17R mRNA expression by RT-PCR and Southern blotting. –, Water control; +, positive control.

*Synergistic effect of IL-17 on TNF-α-induced IL-6 and IL-8 secretion by HCF*

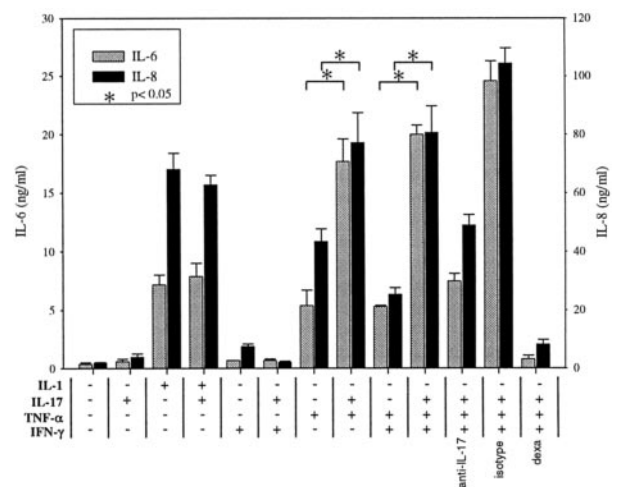
Studies in HSK mouse models have shown a strong induction of IL-6 and IL-8 after HSV-1 infection of the cornea (5, 20). By mimicking the inflammatory situation in the corneal stroma during HSK development in vitro, we determined the modulatory effect of IL-17 (100 ng/ml) on IL-1β (100 ng/ml), TNF-α (50 ng/ml), and IFN-γ (100 U/ml) to induce secretion of IL-6 and IL-8 by HCF. Optimal concentrations of stimulatory cytokines had been determined in preliminary experiments (data not shown).

HCF showed a low level of background secretion of both IL-6 and IL-8, while stimulation with IL-17 or IFN-γ alone or in combination did not show any considerable increase in either IL-6 or IL-8 levels (Fig. 3). However, stimulation of HCF with IL-1β or TNF-α did have a clear stimulatory effect on the secretion of both factors. Although IL-17 itself had no effect, incubation of HCF with combinations of stimulating cytokines showed that it had a synergistic effect on the TNF-α- and TNF-α/IFN-γ-induced secretion of both IL-6 and IL-8. This synergistic effect was almost completely neutralized by preincubating the stimulating cytokine mixture with an anti-IL-17 mAb, but not with an isotype-matched control mAb (Fig. 3). Furthermore, a 2-h pretreatment of HCF with dexamethasone, an immunosuppressive drug commonly used in treating human HSK, almost completely abolished the induced secretion of IL-6 and IL-8 (Fig. 3).

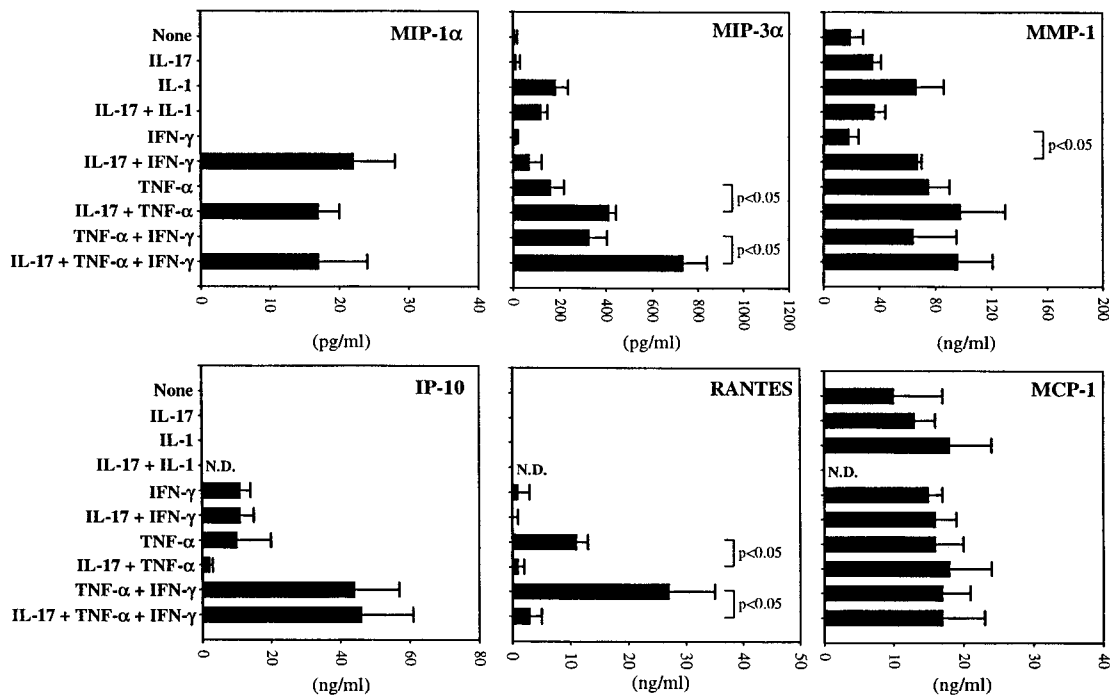
*Modulatory effect of IL-17 on HCF secretion of chemokines and MMP-1*

In addition to IL-6 and IL-8, several other chemokines are expressed in affected corneas and are assumed to play a critical role in the development of HSK (5, 8). Because HCF are a probable cellular source for these chemokines, we also analyzed the effects of stimulation with IL-1β, TNF-α, IL-17, and IFN-γ on chemokine production (including MCP-1, RANTES, MIP-1α, MIP-3α, and IP-10) and MMP-1 by HCF.

The observation by Tumphey et al. (34) that MIP-1α knockout mice fail to develop HSK suggests that this chemokine is a key



**FIGURE 3.** IL-6 and IL-8 secretion by stimulated HCF. Cultured HCF were stimulated for 48 h with the cytokines indicated and IL-6 and IL-8 levels in conditioned medium were determined by ELISA. Cytokine mixtures were preincubated 30 min before an anti-IL-17 mAb or an isotype-matched control mAb (“isotype”) was added to the cell cultures to block IL-17 activity. “Dexa” indicates a 2-h preincubation of the cells with dexamethasone (10 ng/ml). Results are expressed as the mean ± SD of four separate experiments. The statistical significance of the modulatory effect of IL-17 on cytokine-treated was determined by the Mann-Whitney *U* test.



**FIGURE 4.** Chemokine and MMP-1 secretion by stimulated HCF. Cultured HCF were stimulated for 48 h with the indicated cytokines and chemokine and MMP-1 levels in conditioned medium were determined by standard ELISA. Results are expressed as the mean  $\pm$  SD of four separate experiments. The statistical significance of the modulatory effect of IL-17 on cytokine-treated was determined by the Mann-Whitney *U* test. N.D., Not done.

factor in the development of the disease. MIP-1 $\alpha$  secretion by HCF, although in low amounts (up to 30 pg/ml), was observed solely upon costimulation of IL-17 with TNF- $\alpha$ , IFN- $\gamma$ , or both cytokines combined (Fig. 4). Whereas MIP-1 $\alpha$  is mainly chemotactic for neutrophils, MIP-3 $\alpha$  is a powerful chemoattractant for LC precursors (35). Both IL-1 $\beta$  and TNF- $\alpha$  induced the secretion of MIP-3 $\alpha$  by HCF, whereas neither IL-17 nor IFN- $\gamma$  stimulated MIP-3 $\alpha$  secretion (Fig. 4). IL-17 showed a clear synergistic effect with TNF- $\alpha$  and TNF- $\alpha$ /IFN- $\gamma$  on MIP-3 $\alpha$  secretion by HCF, but not with IL-1 $\beta$  or IFN- $\gamma$ .

Intracorneal expression of RANTES and IP-10 is restricted to the preclinical phase of HSK. Both these chemokines are known to induce T cell migration (36). Secretion of RANTES could only be induced by TNF- $\alpha$ , whereas IP-10 production was augmented by TNF- $\alpha$  and IFN- $\gamma$  (Fig. 4). Stimulation of HCF with TNF- $\alpha$  and IFN- $\gamma$  combined had a synergistic effect on the secretion of both RANTES and IP-10. IL-17 showed a remarkable inhibition on the secretion of RANTES by HCF upon stimulation with TNF- $\alpha$  and TNF- $\alpha$ /IFN- $\gamma$ , while IP-10 production was only slightly inhibited by IL-17 (Fig. 4).

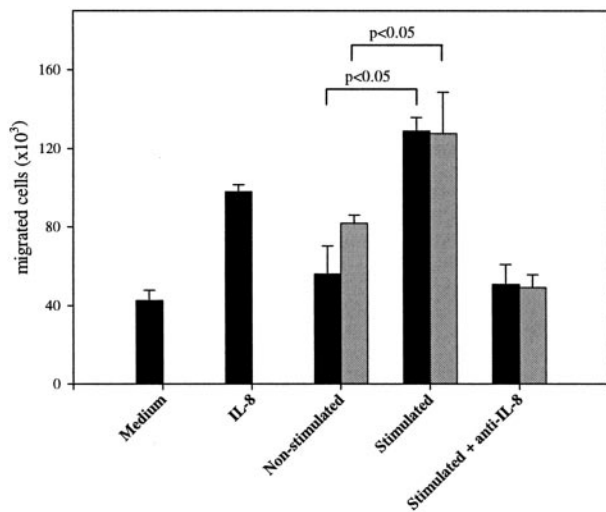
Although the role of MCP-1 is not clear at this point, it has been reported to play a role in CD4<sup>+</sup> T cell recruitment and appears to be rapidly up-regulated and persistently expressed in HSV-1-infected corneas (5). In our experiments, HCF constitutively secreted MCP-1, and no evident changes were observed by incubation with stimulatory cytokines (Fig. 4). Although MMP-1 is not a chemokine, it was included in this study because MMP-1 is known to degrade fibrillar collagens (37), an essential component of the corneal stroma. HCF showed a constitutive secretion of MMP-1, which was not affected by IFN- $\gamma$ . Compared with the mild induction by IL-17, IL-1 $\beta$  and TNF- $\alpha$  induced a strong enhancement of MMP-1 secretion. Interestingly, IL-17 had a synergistic effect with IFN- $\gamma$  on MMP-1 secretion while showing a slight inhibitory effect in combination with IL-1 $\beta$  or TNF- $\alpha$  (Fig. 4).

#### *HCF induce neutrophil recruitment through IL-8 release*

Chemokines expressed within HSV-1-infected corneas are believed to induce the corneal infiltration of neutrophils involved in the development of corneal lesions (3, 5, 8, 16, 17). To test the PMN chemotactic properties of chemokines secreted by HCF, conditioned medium from control and cytokine-stimulated HCF cultures were tested in a neutrophil chemotaxis assay. Conditioned medium from HCF stimulated with the combination of IL-17, TNF- $\alpha$ , and IFN- $\gamma$  was used because it contained the highest levels of IL-8 and MIP-1 $\alpha$  (see Figs. 3 and 4), both potent PMN chemoattractants. rIL-8 (10 ng/ml) was used as a positive control, while fresh serum-free medium served as background control. Compared with the medium control, conditioned medium from stimulated HCF induced a strong migration of PMN. This effect could be blocked by pretreatment of the conditioned medium with a neutralizing anti-IL-8 mAb 30 min before the experiment (Fig. 5). Conditioned medium from nonstimulated HCF caused a mild migration of PMN, probably due to a low level of background production of IL-8 in these cultures (see Fig. 3).

## Discussion

HSK is an HSV-induced corneal disease characterized by complex interactions between infiltrating inflammatory cells and corneal resident cells. These cellular interactions are assumed to result in the induction and perpetuation of a chronic inflammatory process resulting in corneal damage (2, 3, 7, 8). Stimulation of mesenchymal cells with proinflammatory cytokines, of macrophage/monocyte (IL-1 $\beta$  and TNF- $\alpha$ ) or Th1 cell (IFN- $\gamma$ ) origin, has previously been shown to activate and modulate the secretion of chemokines (21–26), suggesting a role of resident cells such as fibroblasts in the recruitment and activation of inflammatory cells to sites of inflammation. Furthermore, recent observations have shown that



**FIGURE 5.** Conditioned medium from stimulated HCF cultures induces neutrophil migration. Serum-free medium served as the background control and recombinant human IL-8 (10 ng/ml) was used as a positive control for neutrophil migration. “Nonstimulated” indicates conditioned medium from cells incubated with serum-free medium alone; stimulated conditioned medium was obtained from cells stimulated with IL-17, TNF- $\alpha$ , and IFN- $\gamma$  simultaneously. Blocking neutrophil migration was achieved by a 30-min preincubation of the conditioned medium with an anti-IL-8 mAb (10  $\mu$ g/ml). Filled and shaded bars represent conditioned media from two different stimulation experiments (mean  $\pm$  SD) and are representative of four individual experiments. The statistical significance of the different chemotactic activity of cytokine-stimulated vs nonstimulated HCF culture conditioned media was determined by the Mann-Whitney *U* test.

activation of resident cells can be heavily modulated by the Th1-derived cytokine IL-17 (21–24, 27–30). Accordingly, the potential interactive role of IL-17 and corneal fibroblasts in the induction and perpetuation of inflammatory processes in human HSK was hypothesized.

The expression and pathogenic properties of IL-1 $\beta$ , TNF- $\alpha$ , and IFN- $\gamma$  in HSK have been extensively studied in the experimental HSK mouse model. These proinflammatory cytokines probably do not have a direct immunopathologic effect by recruiting and stimulating inflammatory cells, but rather exert their effects through interaction with corneal resident cells in an autocrine or paracrine fashion. IL-17 is secreted by activated CD4<sup>+</sup> T cells, mainly Th0 and Th1 (27), and exhibits pleiotropic biological activities on various human tissue resident cells including fibroblasts. IL-17 shares mainly properties with IL-1 $\beta$  and TNF- $\alpha$  in that these three cytokines activate the transcription factor NF- $\kappa$ B in a variety of cell types and all stimulate secretion of proinflammatory factors in mesenchymal cells (27). The present study shows IL-17 expression in affected corneas of patients with fulminant HSK, whereas no IL-17 expression was detectable in control corneas. Mitogenic stimulation of intracorneal TCL obtained from corneas of HSK patients induced IL-17 mRNA expression. The HCF constitutively expressed the IL-17R, consistent with its broad tissue distribution (38).

IL-17 indirectly stimulates granulopoiesis both in vitro (27) and in vivo (39), and has also previously been marked in playing an important role in the pathogenesis of other immune-mediated diseases associated with a massive infiltration of neutrophils into inflamed tissues (40–45). The functional relevance of IL-17 signaling in host defense has recently been demonstrated in an experimental model of *Klebsiella pneumoniae* lung infection in IL-17R knockout mice (46). IL-17R signaling appeared to be critical for the local induction of G-CSF and MIP-2 and subsequent PMN recruitment into the alveolar space. The murine C-X-C chemokines MIP-2 and KC, ho-

mologs of human IL-8, have also been associated with the development of experimental murine HSK (5). Neutralization of MIP-2 decreased corneal PMN infiltration and significantly reduced corneal pathology, demonstrating that MIP-2 is the major chemokine that attracts PMN into the HSV-1-infected cornea in mice (17). Interestingly, in our experiments, conditioned medium from HCF stimulated with the combination of TNF- $\alpha$ , IFN- $\gamma$ , and IL-17 induced chemotaxis of PMN in vitro (Fig. 5). Despite the presence of multiple other chemokines in these conditioned media, this chemotactic activity on PMN was mainly induced by IL-8, because preincubation of conditioned medium with a neutralizing IL-8 mAb almost completely abrogated PMN migration.

The data presented here demonstrate that cytokine-stimulated HCF secrete chemokines associated with HSK. Chemokine secretion was differentially regulated by Th1 cell- and macrophage/monocyte-derived cytokines. Compared with IFN- $\gamma$  and IL-17, IL-1 $\beta$  and TNF- $\alpha$  were more effective in stimulating HCF to secrete IL-6, IL-8, MIP-3 $\alpha$ , and MMP-1. Whereas simultaneous treatment with TNF- $\alpha$  and IFN- $\gamma$  induced significant secretion of RANTES and IP-10 by HCF, no detectable amounts of MIP-1 $\alpha$  were observed upon stimulation with these cytokines. However, neither Th1 cell-derived nor macrophage/monocyte-derived cytokine seemed to significantly alter the constitutive expression of MCP-1.

The induced secretion of IL-6 and IL-8 by HCF stimulated with either IL-1 $\beta$  or TNF- $\alpha$  was consistent with observations previously described (47, 48). Similarly, induction of IL-6 secretion upon stimulation with IL-1 $\beta$  or TNF- $\alpha$  has also recently been observed in myofibroblast cells (49). In addition, such cells do not secrete any MIP-1 $\alpha$  upon stimulation with IL-1 $\beta$  or TNF- $\alpha$  (26), comparable with our results. However, in comparison with another report on chemokine production by HCF (50), we observed some contradiction in the induction of RANTES and MCP-1 secretion by treatment of HCF with IL-1 or TNF- $\alpha$ . In this report stimulation of HCF with IL-1 $\alpha$  or TNF- $\alpha$  resulted in increased levels of RANTES and MCP-1 production. In contrast, stimulation of HCF in our experiments with IL-1 $\beta$  did not induce secretion of detectable amounts of RANTES, nor did IL-1 $\beta$  or TNF- $\alpha$  significantly alter MCP-1 secretion. Although the different effects of IL-1 $\alpha$  and IL-1 $\beta$  on HCF are understandable, the differential effect of TNF- $\alpha$  on MCP-1 production might be related to the usage of serum in cell cultures that might contain costimulatory factors that were absent in our experiments. In a recent report about rheumatoid arthritis in man, RANTES and MCP-1 have been shown to induce IL-6 and IL-8 (51). These chemokines thus appear not to solely play a role in inflammatory cell migration, but also to be involved in the activation of synoviocytes. Consequently, the secretion of the chemokines detected in conditioned medium of cytokine-stimulated HCF may only in part be directly induced by the cytokines added.

As in human HSK corneas, IL-17 expression has been observed in other inflamed tissues, such as the lungs in asthma (52) and the synovium in arthritis (53). IL-17 exerts a modulatory effect on IL-1-, TNF- $\alpha$ -, and IFN- $\gamma$ -induced chemokine secretion by human mesenchymal cells (21–24, 27–30). Furthermore, IL-17 has been described to stimulate the secretion of IL-1 $\beta$  and TNF- $\alpha$  by macrophages (54). In our experiments, IL-17 had a synergistic effect on TNF- $\alpha$ -induced HCF secretion of IL-6, IL-8, and MIP-3 $\alpha$ , which was similar to observations by others (23, 25, 27, 52, 55). Additionally, a synergistic effect was observed for IL-17 with TNF- $\alpha$  and IFN- $\gamma$  on the induction of MIP-1 $\alpha$ , whereas, similar to findings in keratinocytes (21), IL-17 antagonized TNF- $\alpha$ - and/or IFN- $\gamma$ -induced secretion of RANTES. However, in contrast with some of these reports (21, 27), no synergistic effect of IL-17 with

IL-1 $\beta$  or IFN- $\gamma$  was observed, which might be a cell type-specific phenomenon.

As its name suggests, IP-10 is known to be induced by IFN- $\gamma$ , as was also observed in our experiments. Strikingly, IP-10 secretion by HCF was strongly increased upon costimulation with both IFN- $\gamma$  and TNF- $\alpha$ , which is in accordance with a previous report (56). Whereas a previous report (57) showed no significant stimulatory effect of IL-17 on IP-10 induction in keratinocytes, our results showed a slight inhibitory effect of IL-17 on IP-10 secretion by HCF. Secretion of IL-6 and IL-8, induced by HCF stimulation with a combination of IL-17, TNF- $\alpha$ , and IFN- $\gamma$ —resulting in the highest secretion levels (Fig. 3)—was inhibited by pretreatment with an anti-IL-17 mAb to levels similar to those of TNF- $\alpha$  plus IFN- $\gamma$ , demonstrating the specific modulatory effect of IL-17. Glucocorticoids such as dexamethasone are widely used in the treatment of corneal inflammatory diseases. The data presented here show that secretion of IL-6 and IL-8 by cytokine-stimulated HCF can be completely blocked by pretreatment with dexamethasone. This is compatible with the idea that the beneficial effects of glucocorticoids in clinical practice are due to inhibition of exaggerated cytokine production. This effect of glucocorticoids is mediated by their antagonistic action on transcription factors such as NF- $\kappa$ B, which are required for cytokine transcription (58).

The collection of chemokines found to be secreted by HCF upon cytokine stimulation exhibits a broad range of leukocyte-recruiting and -activating potentials. These activities include the migration and activation of T and NK cells (e.g., RANTES, MCP-1, MIP-3 $\alpha$ , and IP-10), LC (e.g., MIP-3 $\alpha$ ), macrophages/monocytes (e.g., MCP-1), and PMN (e.g., IL-8 and MIP-1 $\alpha$ ). Studies on expression profiles of different chemokines in the HSK mouse models have shown that most proinflammatory cytokines and chemokines are expressed throughout both the preclinical and clinical phase of HSK (8). Exceptions to this are the chemokine MIP-1 $\alpha$ , which was detected only during the clinical phase, and the chemokines IP-10 and RANTES, which can only be detected early after HSV-1 infection of the cornea, but not during the clinical phase of the disease (8). Remarkably, in the present study IL-17 exerted a strong stimulatory signal on the induction of IL-8, MIP-1 $\alpha$ , and MIP-3 $\alpha$ , whereas it inhibited the secretion of RANTES and, to a lesser extent, IP-10. This IL-17-specific inhibitory effect on RANTES and IP-10 secretion might reflect a down-regulatory signal by activated T cells to limit the recruitment of additional T lymphocytes, and corresponds with the transient detection of these chemokines in the cornea early after infection. In contrast, increased secretion of IL-8 and MIP-1 $\alpha$  would create an inflammatory environment in the corneal stroma with a high potential for PMN extravasation and activation, where they directly or indirectly cause tissue damage. Although MMP-1 is not a chemokine, its secretion in the corneal stroma might explain some clinical aspects of HSK. As described previously, IL-1 and TNF- $\alpha$  augmented secretion of MMP-1 in human fibroblasts (59). MMP-1 degrades structural type I and type II collagen, and its production in the corneal stroma during HSK might thus contribute to the development of corneal lesions. Accordingly, IL-17 has previously been implicated in synovium matrix destruction in rheumatoid arthritis by similar modes of action (60).

In conclusion, our study shows the ability of cytokine-stimulated HCF to secrete inflammatory mediators that are potentially involved in the immunopathogenesis of HSK. The Th1 cytokine IL-17, expressed in human HSK corneas, modulated these responses in a synergistic or antagonistic fashion. We speculate that during the development of HSK, in addition to corneal-infiltrating cells, corneal fibroblasts are an important cellular source of proinflammatory cytokines and chemokines. Among these is IL-8,

which primarily mediates the extravasation and activation of pathogenic PMN into the cornea. Furthermore, our results suggest that IL-17 might be an important player in the immune activation of HCF. Future studies in murine models might elucidate any potential role of IL-17 in the inflammatory processes leading to HSK.

## References

- Kaye, S. B., K. Baker, R. Bonshek, H. Maseruka, E. Grinfeld, A. Tullo, D. L. Easty, and C. A. Hart. 2000. Human herpes viruses in the cornea. *Br. J. Ophthalmol.* 84:563.
- Hendricks, R. L. 1999. Immunopathogenesis of viral ocular infections. *Chem. Immunol.* 73:120.
- Thomas, J., S. Gangappa, S. Kanangat, and B. T. Rouse. 1997. On the essential involvement of neutrophils in the immunopathologic disease: Herpetic stromal keratitis. *J. Immunol.* 158:1383.
- Babu, J. S., J. Thomas, S. Kanangat, L. A. Morrison, D. M. Knipe, and B. T. Rouse. 1995. Viral replication is required for induction of ocular immunopathology by herpes simplex. *J. Virol.* 70:101.
- Thomas, J., S. Kanangat, and B. T. Rouse. 1998. Herpes simplex virus replication-induced expression of chemokines and proinflammatory cytokines in the eye: implications in herpetic stromal keratitis. *J. Interferon Cytokine Res.* 18:681.
- Tumpey, T. M., S. H. Chen, and J. E. Oakes. 1996. Neutrophil mediated suppression of virus replication after herpes simplex virus type 1 infection of the murine cornea. *J. Virol.* 70:898.
- Shimeld, C., J. L. Whiteland, S. M. Nicholls, D. L. Easty, and T. J. Hill. 1996. Immune cell infiltration in corneas of mice with recurrent herpes simplex virus disease. *J. Gen. Virol.* 71:397.
- Kumaraguru, U., I. Davis, and B. T. Rouse. 1999. Chemokines and ocular pathology caused by corneal infection with herpes simplex virus. *J. Neurovirol.* 5:42.
- Niemialtowski, M. G., and B. T. Rouse. 1992. Predominance of Th1 cells in ocular tissue during herpetic stromal keratitis. *J. Immunol.* 149:3035.
- Hendricks, R. L., T. M. Tumpey, and A. Finnegan. 1992. IFN- $\gamma$  and IL-2 are protective in the skin but pathologic in the corneas of HSV-1-infected mice. *J. Immunol.* 149:3023.
- Streilein, J. W., M. R. Dana, and B. R. Ksander. 1997. Immunity causing blindness: five different paths to herpes stromal keratitis. *Immunol. Today* 18:443.
- Hendricks, R. L., M. Janowicz, and T. M. Tumpey. 1992. Critical role of corneal Langerhans cells in the CD4- but not CD8-mediated immunopathology in herpes simplex virus-1-infected mouse corneas. *J. Immunol.* 148:2522.
- Chen, H., and R. L. Hendricks. 1989. B7 costimulatory requirements of T cells at an inflammatory site. *J. Immunol.* 160:5045.
- Bauer, D., S. Mrzyk, N. van Rooijen, K. P. Steuhl, and A. Heiligenhaus. 2000. Macrophage-depletion influences the course of murine HSV-1 keratitis. *Curr. Eye Res.* 20:45.
- Keadle, T. L., N. Usui, K. A. Laycock, J. K. Miller, J. S. Pepose, and P. M. Stuart. 2000. IL-1 and TNF- $\alpha$  are important factors in the pathogenesis of murine recurrent herpetic stromal keratitis. *Invest. Ophthalmol. Vis. Sci.* 41:96.
- Tumpey, T. M., H. Cheng, X. T. Yan, J. E. Oakes, and R. N. Lausch. 1998. Chemokine synthesis in the HSV infected cornea and its suppression by interleukin-10. *J. Leukocyte Biol.* 63:486.
- Yan, X. T., T. M. Tumpey, S. L. Kunkel, J. E. Oakes, and R. N. Lausch. 1998. Role of MIP-2 in neutrophil migration and tissue injury in the herpes simplex virus-1-infected cornea. *Invest. Ophthalmol. Vis. Sci.* 39:1854.
- Daheshia, M., S. Kanangat, and B. T. Rouse. 1998. Production of key molecules by ocular neutrophils early after herpetic infection of the cornea. *Exp. Eye Res.* 67:619.
- Stumpf, T. H., C. Shimeld, D. L. Easty, and T. J. Hill. 2001. Cytokines production in a murine model of recurrent herpetic stromal keratitis. *Invest. Ophthalmol. Vis. Sci.* 42:372.
- Oakes, J. E., C. A. Monteiro, C. L. Cubitt, and R. N. Lausch. 1993. Induction of interleukin-8 gene expression is associated with herpes simplex virus infection of human corneal keratocytes but not human corneal epithelial cells. *J. Virol.* 67:4777.
- Albanesi, C., A. Cavani, and G. Girolomoni. 1999. IL-17 is produced by nickel-specific T lymphocytes and regulates CD54 expression and chemokine production in human keratinocytes: synergistic or antagonist effect with IFN- $\gamma$  and TNF- $\alpha$ . *J. Immunol.* 162:494.
- Andoh, A., H. Takaya, J. Makino, H. Sato, S. Bamba, Y. Araki, K. Hata, M. Shimada, T. Okuno, Y. Fujiyama, and T. Bamba. 2001. Cooperation of interleukin-17 and interferon- $\gamma$  on chemokine secretion in human fetal intestinal epithelial cells. *Clin. Exp. Immunol.* 125:56.
- Chabaud, M., G. Page, and P. Miossec. 2001. Enhancing effect of IL-1, IL-17, and TNF- $\alpha$  on macrophage inflammatory protein-3 $\alpha$  production in rheumatoid arthritis: regulation by soluble receptors and Th2 cytokines. *J. Immunol.* 167:6015.
- Albanesi, C., C. Scarponi, A. Cavani, M. Federici, F. Nasorri, and G. Girolomoni. 2000. Interleukin-17 is produced by both Th1 and Th2 lymphocytes, and modulates interferon- $\gamma$ - and interleukin-4 induced activation of human keratinocytes. *J. Invest. Dermatol.* 115:81.
- Katz, Y., O. Nadvig, and Y. Beer. 2001. Interleukin-17 enhances tumor necrosis factor  $\alpha$ -induced synthesis of interleukins 1, 6, and 8 in skin and synovial fibroblasts: a possible role as a "fine-tuning cytokine" in inflammation processes. *Arthritis Rheum.* 44:2176.

26. Andoh, A., H. Takaya, T. Saotome, M. Shimada, K. Hata, Y. Araki, F. Nakamura, Y. Shintani, Y. Fujiyama, and T. Bamba. 2000. Cytokine regulation of chemokine (IL-8, MCP-1, and RANTES) gene expression in human pancreatic periacinar myofibroblasts. *Gastroenterology* 119:211.
27. Fossiez, B., O. Djossou, P. Chomarat, L. Flores-Romo, S. Ait-Yahia, C. Maat, J. Pin, P. Garrone, E. Garcia, S. Saeland, et al. 1996. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. *J. Exp. Med.* 183:2593.
28. Teunissen, M. B. M., C. W. Koomen, R. de Waal Malefyt, E. A. Wierenga, and J. D. Bos. 1998. Interleukin-17 and interferon- $\gamma$  synergize in the enhancement of proinflammatory cytokine production by human keratinocytes. *J. Invest. Dermatol.* 111:645.
29. Witowski, J., K. Pawlaczyk, A. Breborowicz, A. Scheuren, M. Kuzlan-Pawlaczyk, J. Wisniewska, A. Polubinska, H. Friess, G. M. Gahl, U. Frei, and A. Jorres. 2000. IL-17 stimulates intraperitoneal neutrophil infiltration through the release of GRO $\alpha$  chemokine from mesothelial cells. *J. Immunol.* 165:5814.
30. Woltman, A. M., S. de Haij, J. G. Boonstra, S. J. Gobin, M. R. Daha, and C. van Kooten. 2000. Interleukin-17 and CD40-ligand synergistically enhance cytokine and chemokine production by renal epithelial cells. *J. Am. Soc. Nephrol.* 11:2044.
31. Verjans, G. M. G. M., L. Remeijer, R. S. van Binnendijk, J. G. Cornelissen, H. J. Volker-Dieben, S. G. Baarsma, and A. D. M. E. Osterhaus. 1998. Identification and characterization of herpes simplex virus-specific CD4<sup>+</sup> T cells in corneas of herpetic stromal keratitis patients. *J. Infect. Dis.* 177:484.
32. Beales M. P., J. L. Funderburgh, J. V. Jester, and J. R. Hassell. 1999. Proteoglycan synthesis by bovine keratocytes and corneal fibroblasts: maintenance of the keratocyte phenotype in culture. *Invest. Ophthalmol. Vis. Sci.* 40:1658.
33. Holland, E. J., and G. S. Schwartz. 1999. Classification of herpes simplex virus keratitis. *Cornea* 18:144.
34. Tumpey, T. M., H. Cheng, D. N. Cook, O. Smithies, J. E. Oakes, and R. N. Lausch. 1998. Absence of macrophage inflammatory protein-1 $\alpha$  prevents the development of blinding herpes stromal keratitis. *J. Virol.* 72:3705.
35. Dieu-Nosjean, M. C., C. Massacrier, B. Homey, B. Vanbervliet, J. J. Pin, A. Vicari, S. Lebecque, C. Dezutter-Dambuyant, D. Schmitt, A. Zlotnik, and C. Caux. 2000. Macrophage inflammatory protein 3 $\alpha$  is expressed at inflamed epithelial surfaces and is the most potent chemokine known in attracting Langerhans cell precursors. *J. Exp. Med.* 192:705.
36. Ward, S. G., and J. Westwick. 1998. Chemokines: understanding their role in T-lymphocyte biology. *Biochem. J.* 333:457.
37. Nagase, H. 1997. Activation mechanisms of matrix metalloproteinases. *Biol. Chem.* 378:151.
38. Yao, Z., M. K. Spriggs, J. M. Derry, L. Strockbine, L. S. Park, T. VandenBos, J. D. Zappone, S. L. Painter, and R. J. Armitage. 1997. Molecular characterization of the human interleukin (IL)-17 receptor. *Cytokine* 9:794.
39. Schwarzenberger, P., V. La Russa, A. Müller, P. Ye, W. Huang, A. Zieske, S. Nelson, G. J. Bagby, D. Stoltz, R. L. Mynatt, et al. 1998. IL-17 stimulates granulopoiesis in mice: use of an alternate, novel gene therapy-derived method for in vivo evaluation of cytokines. *J. Immunol.* 161:6383.
40. Fossiez, F. J., J. Banchereau, R. Murray, C. Van Kooten, P. Garrone, and S. Lebecque. 1998. Interleukin-17. *Int. Rev. Immunol.* 16:541.
41. Laan, M., Z. Cui, H. Hoshino, J. Lötvall, M. Sjöstrand, D. C. Gruenert, B. Skoogh, and A. Lindén. 1999. Neutrophil recruitment by human IL-17 via C-X-C chemokine release in the airways. *J. Immunol.* 162:2347.
42. Lindén, A., H. Hoshino, and M. Laan. 2000. Airway neutrophils and interleukin-17. *Eur. Respir. J.* 15:973.
43. Luzzi, F., T. Parrello, G. Monteleone, L. Sebkova, M. Romano, R. Zarrilli, M. Imeneo, and F. Pallone. 2000. Up-regulation of IL-17 is associated with bioactive IL-8 expression in *Helicobacter pylori*-infected human gastric mucosa. *J. Immunol.* 165:5332.
44. Baggiolini, M. 1998. Chemokines and leukocyte traffic. *Nature* 392:565.
45. Lubberts, E., L. A. Joosten, B. Oppers, L. van den Bersselaar, C. J. Coenen-de Roo, J. K. Kolls, P. Schwarzenberger, F. A. van de Loo, and W. B. van den Berg. 2001. IL-1-independent role of IL-17 in synovial inflammation and joint destruction during collagen-induced arthritis. *J. Immunol.* 167:1004.
46. Ye, P., F. H. Rodriguez, S. Kanaly, K. L. Stocking, J. Schurr, P. Schwarzenberger, P. Oliver, W. Huang, P. Zhang, J. Zhang, et al. 2001. Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense. *J. Exp. Med.* 194:519.
47. Cubitt, C. L., Q. Tang, C. A. Monteiro, R. N. Lausch, and J. E. Oakes. 1993. IL-8 gene expression in cultures of human corneal epithelial cells and keratocytes. *Invest. Ophthalmol. Vis. Sci.* 34:3199.
48. Cubitt, C. L., R. N. Lausch, and J. E. Oakes. 1995. Differences in interleukin-6 gene expression between cultured human corneal epithelial cells and keratocytes. *Invest. Ophthalmol. Vis. Sci.* 36:330.
49. Shimada, M., A. Andoh, K. Hata, K. Tasaki, Y. Araki, Y. Fujiyama, and T. Bamba. 2002. IL-6 secretion by human pancreatic periacinar myofibroblasts in response to inflammatory mediators. *J. Immunol.* 168:861.
50. Tran, M. T., M. Tellaetxe-Isusi, V. Elnor, R. M. Strieter, R. N. Lausch, and J. E. Oakes. 1996. Proinflammatory cytokines induce RANTES and MCP-1 synthesis in human corneal keratocytes but not in corneal epithelial cells:  $\beta$ -chemokine synthesis in corneal cells. *Invest. Ophthalmol. Vis. Sci.* 37:987.
51. Nanki, T., K. Nagasaka, K. Hayashida, Y. Saita, and N. Miyasaka. 2001. Chemokines regulate IL-6 and IL-8 production by fibroblast-like synoviocytes from patients with rheumatoid arthritis. *J. Immunol.* 167:5381.
52. Molet, S., Q. Hamid, F. Davoine, E. Nutku, R. Taha, N. Page, R. Olivenstein, J. Elias, and J. Chakir. 2001. IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. *J. Allergy Clin. Immunol.* 108:430.
53. Chabaud, M., J. M. Durand, N. Buchs, F. Fossiez, G. Page, L. Frappart, and P. Miossec. 1999. Human interleukin-17: a T cell-derived proinflammatory cytokine produced by the rheumatoid synovium. *Arthritis Rheum.* 42:963.
54. Jovanovic, D. V., J. A. Di Battista, J. Martel-Pelletier, F. C. Jolicœur, Y. He, M. Zhang, F. Mineau, and J. P. Pelletier. 1998. IL-17 stimulates the production and expression of proinflammatory cytokines, IL-1 $\beta$  and TNF- $\alpha$ , by human macrophages. *J. Immunol.* 160:3513.
55. Shimada, M., A. Andoh, K. Hata, K. Tasaki, Y. Araki, Y. Fujiyama, and T. Bamba. 2002. IL-6 secretion by human pancreatic periacinar myofibroblasts in response to inflammatory mediators. *J. Immunol.* 168:861.
56. Ohmori, Y., and T. A. Hamilton. 1995. The interferon-stimulated response element and a  $\kappa$ B site mediate synergistic induction of murine IP-10 gene transcription by IFN- $\gamma$  and TNF- $\alpha$ . *J. Immunol.* 154:5235.
57. Albanesi, C., C. Scarponi, S. Sebastiani, A. Cavani, M. Federici, O. De Pita, P. Puddu, and G. Girolomoni. 2000. IL-4 enhances keratinocyte expression of CXCR3 agonistic chemokines. *J. Immunol.* 165:1395.
58. Almawi, W. Y., and O. K. Melemedjian. 2002. Molecular mechanisms of glucocorticoid antiproliferative effects: antagonism of transcription factor activity by glucocorticoid receptor. *J. Leukocyte Biol.* 71:9.
59. Meller, D., D. Q. Li, and S. C. Tseng. 2000. Regulation of collagenase, stromelysin, and gelatinase B in human conjunctival and conjunctivochalasis fibroblasts by interleukin-1 $\beta$  and tumor necrosis factor- $\alpha$ . *Invest. Ophthalmol. Vis. Sci.* 41:2922.
60. Chabaud, M., P. Garnero, J.-M. Dayer, P.-A. Guerne, F. Fossiez, and P. Miossec. 2000. Contribution of interleukin 17 to synovium matrix destruction in rheumatoid arthritis. *Cytokine* 14:1092.