Role of Enhanced Expression of m-CSF in Conjunctiva Affected by Cicatricial Pemphigoid

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PURPOSE. Local proliferation of macrophages has been reported to augment the inflammatory response in various human and experimental diseases. Macrophage accumulation in the sub-mucosa is also an important feature in the pathogenesis of ocular cicatricial pemphigoid (OCP). In the present study, the role of local proliferation of macrophages in conjunctiva affected by OCP and the relationship between local proliferation of macrophages and expression of macrophage-colony-stimulating factor (m-CSF) in such conjunctiva were examined.

METHODS. Biopsy specimens from the conjunctiva of 10 untreated patients with active OCP and from 5 normal subjects were studied for the expression of m-CSF, macrophages, and proliferating cell nuclear antigen (PCNA), a cell cycle protein, by immunohistochemistry. Dual staining for CD68 (a cell surface marker for macrophages) and PCNA was also performed to identify proliferating macrophages. In addition, fibroblasts isolated from conjunctiva of normal individuals and from patients with OCP were studied for the expression of m-CSF by immunostaining and real-time PCR. To identify the factors that induce m-CSF in conjunctival fibroblasts, the fibroblasts were incubated with different concentrations of interleukin (IL)-1α and tumor necrosis factor (TNF)-α, and the levels of m-CSF mRNA were determined by real-time PCR and the amount of m-CSF produced was determined by enzyme-linked immunosorbent assay (ELISA).

RESULTS. Normal conjunctiva showed weak expression of m-CSF in the conjunctival epithelial cells and stroma. Conjunctival expression of m-CSF protein was significantly (P < 0.0001) increased in conjunctival biopsy specimens from patients with OCP, m-CSF was detected in the infiltrating macrophages, stromal cells (presumably fibroblasts), and conjunctival epithelial cells. Compared with normal control conjunctival tissue, a 1.2-fold increase in the expression of mRNA for m-CSF was detected by real-time PCR in the conjunctival tissue obtained from patients with OCP. Increased expression of m-CSF correlated significantly (P < 0.0004) with an increased stromal accumulation of macrophages in conjunctival biopsy specimens of patients with OCP. A number of these accumulated macrophages (CD68-positive) were found to be proliferating (PCNA-positive). In addition, fibroblasts isolated and cultured from conjunctiva of patients with OCP showed significantly increased (1.7-fold) expression of m-CSF compared with normal conjunctival fibroblasts. When conjunctival fibroblasts were treated with IL-1α or TNF-α, real-time PCR and ELISA detected an increased level of m-CSF.

CONCLUSIONS. An increased expression of m-CSF was observed in conjunctiva from patients with active OCP. There was a positive correlation between expression of m-CSF and accumulation of macrophages in conjunctival biopsy sections obtained from patients with OCP. Increased expression of m-CSF, mainly by conjunctival fibroblasts and infiltrating inflammatory cells, may play an important role in the regulation of local proliferation of macrophages in OCP. In the conjunctiva of patients with OCP, this process could augment or enhance the local inflammatory response and tissue injury consequent to it.

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Recent studies have highlighted the mechanism of macrophage accumulation, especially in regard to their local proliferation during various immunoinflammatory processes. Accumulation of macrophages in the conjunctiva is an important event in OCP, and macrophage-derived cytokines and growth factors, including platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β1, could be actively involved in the subsequent conjunctival injuries and in the irreversible conjunctival scarring.

Local proliferation of macrophages has been identified in various organ-specific injuries, including those occurring in the kidney. Most important, the degree of local proliferation of macrophages has been found to be closely associated with the severity of tissue injuries.6–7 Local increase in proliferation of macrophages has been detected in severe renal injuries, including acute renal allograft rejection and crescentic nephritis, and after subtotal nephrectomy.5–10 Local proliferation of macrophages has also been implicated in the pathogenesis of various immune-mediated and autoimmune diseases, such as glomerulonephritis and lupus nephritis. Increased levels of macrophage-colony stimulating factor (m-CSF) in the liver, spleen, and kidney were associated with increased infiltration of macrophages in these organs in lupus-prone mouse strains (NZB/W and MRL.1pr/lpr).11 Local proliferation of macrophages aggravates macrophage-mediated renal injury in human glomerulonephritis and lupus nephritis.12 Moss and Hamilton13 have shown an elevated level of CSFs in the synovial fluid of patients with rheumatoid arthritis and suggest that proliferation of a subpopulation of human peripheral blood monocytes in the presence of CSFs may contribute to the inflammatory process in rheumatoid arthritis. In a separate study, systemic administration of m-CSF or granulocyte macrophage-colony-stimulating factor (GM-CSF) in the acute methylated bovine serum albumin–induced murine arthritis model resulted in an increased accumulation of locally dividing macrophages and in exacerbation of arthritis.7 Recently, it has been shown that CSF could induce in vitro proliferation of a subpopulation of human peripheral blood monocytes.14 Local macrophage proliferation was concomitantly associated with enhanced expression of TNF-α in carbon tetrachloride–induced chronic liver injury in rats, and this cytokine-induced inflammatory response has...
been thought to be associated with the development of subsequent liver fibrosis. Similarly, increased numbers of alveolar macrophages, caused by local proliferation, appear to be involved in the chronic inflammation of pulmonary fibrosis. m-CSF and GM-CSF produced by lung fibroblasts are thought to regulate local proliferation of alveolar macrophages during chronic inflammatory-fibrogenic lung disorders.

Although it is known that local proliferation of macrophages can intensify the inflammatory responses and subsequent tissue injuries in various tissues and organs, similar studies have not been performed in patients with OCP. In this study, local proliferation of macrophages in biopsy specimens from the conjunctiva of patients with OCP was studied. Because m-CSF regulates monocyte/macrophage survival and proliferation, we also studied its possible role in local macrophage proliferation in biopsy specimens of conjunctiva from patients with OCP. In addition, the effects of IL-1α and TNF-α on the induction of m-CSF by conjunctival fibroblasts were studied.

**Materials and Methods**

**Conjunctival Specimens**

Samples of the conjunctiva were obtained from 10 patients with active, untreated OCP. The diagnosis of OCP was based on clinical presentation, histology, and direct immunofluorescence of the conjunctiva demonstrating IgG and C3 at the basement membrane zone. Biopsy specimens of conjunctiva from five patients who were undergoing routine cataract surgery were used as the control. The study followed the guideline of the Declaration of Helsinki for research involving human subjects.

**Isolation of Fibroblasts from Conjunctiva**

 Conjunctival fibroblasts were isolated from normal conjunctiva and from OCP conjunctiva, as described elsewhere. Briefly, conjunctival biopsy specimens were cut into explants of approximately $2 \times 2 \text{ mm}^2$; placed in tissue culture dishes, and covered with Dulbecco’s modified Eagle’s medium (DMEM; Mediatech, Inc., Herndon, VA), containing fetal bovine serum (FBS; Mediatech, Inc.), gentamicin, and amphotericin B; and incubated overnight at 37°C in 95% humidified incubator with 5% CO$_2$. Twenty-four hours later, more DMEM was added, and the medium was changed three times weekly thereafter. The isolated fibroblasts were subcultured with 0.1% trypsin and 0.02% EDTA in Ca$^2+$- and Mg$^2+$-free minimum essential medium (MEM) containing penicillin, streptomycin (Atlanta Biologicals, Norcross, GA), until the cells reached 80% to 90% confluence. Fibroblasts from control and OCP conjunctiva were also grown on slides, fixed with methanol, and used for immunostaining. In addition, RNA isolated from conjunctivae of normal control subjects and from patients with OCP were used in real-time PCR studies.

**Immunohistochemistry**

Immunohistochemistry was performed on paraffin-embedded and on frozen sections of conjunctiva, as described previously. Briefly, tissue sections were blocked with either 10% goat serum or 10% rabbit serum for 1 hour and then incubated overnight at 4°C with the following primary antibodies: rabbit anti-human m-CSF (Leinco Technologies, St. Louis, MO), mouse anti-human CD68 (Dako, Glostrup, Denmark), and mouse anti-human PCNA (Dako). After a wash in phosphate-buffered saline (PBS), the sections were treated with a secondary antibody for 15 minutes (biotinylated goat anti-mouse IgG for CD68 and PCNA; biotinylated goat anti-rabbit IgG for m-CSF), washed with PBS, and incubated further with streptavidin-peroxidase. The reaction products were developed with a mixture of 3,3'-diaminobenzine-4 HCl (DAB) and H$_2$O$_2$. Both the secondary antibodies and streptavidin-peroxidase solution were from a kit (Histostain; Nichirei Co., Tokyo, Japan). Normal mouse serum was used as a negative control for mouse anti-human CD68 and mouse anti-human PCNA antibodies, and normal rabbit serum was used as a negative control for rabbit anti-human m-CSF antibody. Conjunctival fibroblasts isolated from normal and OCP-affected conjunctiva were grown on glass slides, fixed with methanol, and immunostained for m-CSF, as described earlier. To quantitate the number of m-CSF and CD68-positive macrophages and PCNA-positive proliferating cells, immunostained cells were counted randomly in at least five different fields (magnification, ×20) in each biopsy section with the aid of a light microscope (Nikon Corp., Tokyo, Japan). The number of cells in each field from all controls and all patients with OCP was expressed as the mean ± SEM.

**Double Immunostaining**

Double immunostaining was performed to colocalize m-CSF/CD68 and CD68/PCNA as described previously. Briefly, m-CSF or PCNA was initially stained by an alkaline phosphatase method and developed with 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (BCIP-NBT), which produced dark purple staining. Then, the m-CSF- or PCNA-stained sections of conjunctiva were counterstained for CD68 by a peroxidase method and visualized with H$_2$O$_2$ and 3-amino-9-ethylcarbazole (AEC), producing an intense red stain.

**Effects of IL-1 and TNF-α on the Expression of m-CSF by Conjunctival Fibroblasts**

The conjunctival fibroblasts were subcultured and kept in the serum-free medium for 24 hours and then were treated with various concentrations (1, 10 and 100 ng/mL) of IL-1α or TNF-α (R&D Systems, Minneapolis, MN) for 6 hours. Total RNA was extracted from the IL-1α- or TNF-α-treated fibroblasts, and the level of m-CSF was determined by real-time PCR.

**Real-Time PCR**

Total RNA isolated from conjunctival tissues and from fibroblasts of control and OCP conjunctiva was used to detect the relative expression of m-CSF mRNA. The principle of real-time quantitative PCR has been described elsewhere. The quantification of transcription of real-time PCR takes advantage of the 5’ nucleotide activity of DNA polymerase (AmpliTaq Gold; PE-Applied Biosystems, Foster City, CA). Total RNA was extracted from conjunctival tissues and conjunctival fibroblasts with an RNA isolation kit (Qiagen, Valencia, CA). The primers and probe used for detecting m-RNA for m-CSF are as follows: forward, TGC AGC GGC TGA TTG ACA; reverse, TTC AAC TGT TCC TGG TCT ACA AAC TC; probe (TaqMan; PE-Applied Biosystems); FAM (reporter dye)-TCA GAT GGA GAC CTC GTG CCA AAT TAC ATT-TAMRA (quencher dye). Each PCR reaction contained equivalent amounts of total RNA. Real-time PCR was performed in duplicate with a kit used according to the manufacturer’s recommendation (TaqMan One-Step RT-PCR Master Mix Reagents; PE-Applied Biosystems). All the reactions were controlled by standards (nontemplate control and standard positive control). Without extracting the total RNA from conjunctival fibroblasts, we routinely used DNase to prevent DNA contamination. When real-time PCR was performed without addition of reverse transcriptase, no PCR product was detected, either for m-CSF or the housekeeping gene. Amplified PCR products were sequenced (at the core facility of Harvard Institute of Medicine, Boston, MA), and showed a complete homology with the corresponding sequence of the human m-CSF gene. The quantity of mRNA was calculated by normalizing the threshold cycle level ($C_T$) of m-CSF to the $C_T$ of the housekeeping genes 18S ribosomal RNA or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of the same RNA probe, according to the following formula: the mean 18S or GAPDH $C_T$ of each multiplex PCR was performed in duplicate) was subtracted from the mean m-CSF $C_T$ level. This result represents the change in $C_T$ ($\Delta C_T$). This $\Delta C_T$ is specific and can be compared with the $\Delta C_{\text{control}}$ of a calibration sample (for example, control conjunctiva or control conjunctival fibroblasts). The subtrac-
tion of control \( \Delta C_T \) from the \( \Delta C_T \) of OCP samples or fibroblasts is referred to as \( \Delta\Delta C_T \). The relative quantification of expression of m-CSF in conjunctival tissues and fibroblasts isolated from the conjunctiva of patients with OCP (in comparison with that in the control) was determined by \( 2^{-\Delta\Delta C_T} \). For all the probes, the quencher dye was 6-carboxy-tetramethyl rhodamine (TAMRA), and the reporter dye was 6-carboxy fluorescein (FAM) for m-CSF and VIC for 18S and GAPDH.

**Enzyme-Linked Immunosorbertent Assay**

The conjunctival fibroblasts were subcultured and kept in the serum-free medium for 24 hours and were treated with various concentrations (1, 10, or 100 ng/mL) of IL-1\( \beta \) or TNF-\( \alpha \) (R&D Systems) for 24 hours. The supernatant was collected and the level of m-CSF was determined by an ELISA kit (R&D Systems), according to the manufacturer’s protocol.

**Statistical Analysis**

Data were expressed as the mean \( \pm \) SEM. Differences between groups were examined for statistical significance using the \( t \) test or one-way ANOVA. Correlations were examined by linear regression analysis. \( P < 0.05 \) denoted the presence of a statistically significant difference.

**RESULTS**

**Immunostaining**

**Macrophage Colony-Stimulating Factor.** The expression of m-CSF protein was weakly and sparsely detected in the conjunctival epithelium and stromal cells in the control conjunctival sections (Fig. 1A). In contrast, expression of m-CSF increased (control: 3.86 \( \pm \) 0.43; OCP: 26.66 \( \pm \) 1.16; \( P < 0.0001 \)) in conjunctival sections from patients with OCP (Figs. 1B, 1C). The m-CSF-positive cells in conjunctival sections from patients with OCP were found to be macrophages, stromal fibroblasts, and conjunctival epithelial cells. When the conjunctival sections were incubated with rabbit serum instead of polyclonal m-CSF antibody, no specific staining was detected. Moreover, compared with control conjunctival tissue, a 1.2-fold increase in the expression of m-CSF was detected in the conjunctival tissue obtained from patients with OCP, by quantitative real-time PCR.

**Macrophages.** Conjunctival accumulation of CD68-positive macrophages was detected by immunohistochemistry, using a monoclonal antibody. Scattered presence of macrophages was seen in the control conjunctival sections (Fig. 1C). In contrast, a higher number of CD68-positive macrophages was always observed in conjunctival sections from patients with OCP (Fig 1D). The number of CD68-positive macrophages was significantly increased (control: 4.42 \( \pm \) 0.42; OCP: 28.61 \( \pm \) 1.49; \( P < 0.0001 \)) in conjunctival sections obtained from patients with OCP, when compared with control conjunctiva (Fig 2B). When the conjunctival sections were incubated with mouse serum instead of CD68 antibody, no specific staining was detected.

**Proliferating Cell Nuclear Antigen.** Scattered PCNA-positive cells were detected in the conjunctival epithelial cells in control conjunctival sections (Fig. 1E). In contrast, a higher number of PCNA-positive cells was present in conjunctival sections from patients with OCP (Fig. 1F). Those cells were found to be macrophages, stromal fibroblasts, and conjunctival epithelial cells. The number of PCNA-positive cells was significantly increased (control: 2.35 \( \pm \) 0.21; OCP: 22.35 \( \pm \) 0.83, \( P < 0.0001 \)) in conjunctival sections obtained from patients with OCP, compared with control conjunctiva (Fig. 2C). When the conjunctival sections were incubated with mouse serum instead of monoclonal PCNA antibody, no specific staining was detected.

**Proliferating Macrophages.** To identify proliferating macrophages in conjunctival sections, CD68-positive macrophages were counterstained for the cell-cycle protein PCNA. A significant number of accumulated macrophages were PCNA posi-

**FIGURE 1.** Immunostaining for m-CSF (A, B), CD68 (C, D), and PCNA (E, F) in conjunctival sections obtained from control individuals (A, C, E) and patients with OCP (B, D, F). Compared with expression in conjunctival sections obtained from a normal individual (A), there was an increased expression of m-CSF in epithelial cells and stromal cells (arrows) in a conjunctival section of a patient with OCP (B). Compared with a scattered presence of macrophages in a conjunctival section obtained from a normal individual (C), there was an increased infiltration of CD68-positive macrophages (arrows) in the conjunctival section obtained from a patient with OCP (D). Occasional PCNA-positive epithelial cells (arrows) were present in a conjunctival section obtained from a normal individual (E), whereas an increased number of PCNA-positive cells (arrows) were present in the conjunctival section of a patient with OCP (F). Original magnification, \( \times 40 \).
tive, indicating the presence of proliferating macrophages in the sections of conjunctiva obtained from patients with OCP.

**Correlation between Increased Expression of m-CSF and Increased Accumulation of Macrophages**

To examine the relationship between the expression of m-CSF and accumulated macrophages, we counted the number of m-CSF–positive cells and CD68-positive macrophages in the conjunctival sections obtained from patients with OCP and performed a correlation analysis. As shown in Figure 2D, a positive correlation between the expression of m-CSF and accumulation of macrophages was found in the conjunctiva of patients with OCP ($r = 0.4718$, $P < 0.0004$).

**Expression of m-CSF in Fibroblasts**

Fibroblasts isolated from conjunctiva of normal individuals and patients with OCP were studied by immunostaining and real-time PCR, to clarify the possible role of these fibroblasts in the expression of m-CSF. Compared with the fibroblasts isolated from control conjunctiva (Fig. 3A), increased cytoplasmic immunostaining for m-CSF was seen in fibroblasts isolated from conjunctiva of patients with OCP (Fig. 3B). A similar expression pattern was also noted by real-time PCR analysis of total RNA extracted from fibroblasts. Compared with the fibroblasts

**FIGURE 2.** Mean (±SEM) number of m-CSF–positive cells (A), CD68-positive macrophages (B), and PCNA-positive cells (C) in each field in conjunctival sections obtained from all control individuals and all patients with OCP. Correlation between the expression of m-CSF and accumulation of macrophages in conjunctiva of patients with OCP is shown in (D). Compared with control conjunctiva, in conjunctival sections from patients with OCP there were significant increases in cells expressing m-CSF (A; control, $3.86 ± 0.43$ cells; OCP, $26.66 ± 1.16$ cells; $P < 0.0001$), accumulation of CD68-positive macrophages (B; control, $4.42 ± 0.42$; OCP, $28.61 ± 1.49$; $P < 0.0001$), and PCNA-positive cells (C; control, $2.35 ± 0.21$; OCP, $22.35 ± 0.83$; $P < 0.0001$). A positive correlation between the increased expression of m-CSF and increased accumulation of macrophages was found in the conjunctiva of patients with OCP (D; $r = 0.4718$, $P < 0.0004$).

**FIGURE 3.** Immunostaining of m-CSF in fibroblasts isolated from conjunctiva of a control individual (A) and a patient with OCP (B), showing increased cytoplasmic expression of m-CSF in fibroblasts isolated from conjunctiva of the patient with OCP. Original magnification: (A) $\times 20$; (B) $\times 40$. 

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[Image of graphs and figures showing data and correlation analyses.]
Data are expressed as mean picograms per milliliter m-CSF ± SEM. Note that compared with the control, a significant ($P < 0.001$) increase in the level of m-CSF was detected in the supernatant of IL-1α- or TNF-α–treated fibroblasts. $\star P < 0.001$ vs. control.

**TABLE 1.** Level of m-CSF in Control and TNF-α– and IL-α–Treated Cells

<table>
<thead>
<tr>
<th>Dose</th>
<th>0 ng/mL</th>
<th>1 ng/mL</th>
<th>10 ng/mL</th>
<th>100 ng/mL</th>
</tr>
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<tbody>
<tr>
<td>TNF-α</td>
<td>74.6 ± 11.6</td>
<td>594.4 ± 32.9*</td>
<td>803.3 ± 81.9*</td>
<td>911.8 ± 16.9*</td>
</tr>
<tr>
<td>IL-1α</td>
<td>80.3 ± 37.3</td>
<td>1300 ± 852.7*</td>
<td>1762 ± 88*</td>
<td>1097 ± 58.9*</td>
</tr>
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</table>

Because m-CSF is the single major factor that helps in the growth and proliferation of monocytes/macrophages, an elevated level of m-CSF in conjunctiva of patients with OCP probably plays a significant role in the local proliferation of macrophages in the earlier stages of the disease process. Moreover, m-CSF not only plays a role in local macrophage proliferation but also is a chemotactic factor for monocytes. Thus, in addition to its role in local macrophage proliferation in conjunctiva affected by OCP, m-CSF may also promote recruitment of macrophages into the conjunctiva through a chemotactic mechanism. In our in vitro culture system, fibroblasts isolated from conjunctiva of patients with OCP produced significantly increased (approximately 1.7-fold) amounts of m-CSF. Thus, fibroblasts may also play an important role in the recruitment and local proliferation of macrophages in the conjunctiva of patients with OCP.

**Effects of IL-1α and TNF-α on the Expression of m-CSF in Fibroblasts**

When isolated control conjunctival fibroblasts were treated with various concentrations (1, 10, or 100 ng) of IL-1α or TNF-α (Table 1), an increased level of m-CSF was detected in the supernatant. Similarly, increased expression of m-CSF was detected by quantitative real-time PCR analysis in both IL-1α- and TNF-α–treated conjunctival fibroblasts (Fig. 4).

**DISCUSSION**

m-CSF plays an important role(s) in the recruitment and local proliferation of macrophages in various human and experimental diseases. Changes in the expression of m-CSF have been hypothesized to be involved in several immunoinflammatory injuries that subsequently lead to scarring. In this preliminary study, we investigated the expression of m-CSF in conjunctiva obtained from patients with OCP, in an attempt to elucidate its role(s) in the recruitment and local proliferation of macrophages. The results show an association of m-CSF with macrophage accumulation in conjunctiva from patients with OCP. Our results indicate that the expression of m-CSF was increased in conjunctiva from patients with OCP and further show that such expression was closely associated with the increased stromal accumulation and proliferation of macrophages.

m-CSF, also known as CSF-1, regulates the survival, proliferation, and differentiation of mononuclear phagocytes. Numerous studies have shown a close association between the expression of m-CSF and local proliferation of macrophages in experimental and human diseases. For instance, in MRL/lpr/lpr mice, the onset of renal injury has been associated with increased renal expression of m-CSF, followed by the accumulation of macrophages in the kidney. In a recent study in MRL/lpr mice, m-CSF was stably transfected into tubular epithelial cells, which were then implanted under the renal capsule. This resulted in accumulation and/or proliferation of macrophages in the kidney.

The major finding of the present study is that the expression of m-CSF was increased in the conjunctival tissue obtained from patients in whom OCP was in its earlier stages, and this increased expression of m-CSF correlated with the increased conjunctival accumulation of macrophages. We have shown not only an increased expression of m-CSF in conjunctiva of patients with OCP, but also have shown a positive correlation in the expression of m-CSF and accumulation of macrophages.

**FIGURE 4.** The conjunctival fibroblasts were treated with various concentrations of either TNF-α or IL-1α (1, 10, or 100 ng/mL) for 6 hours, and the induction of m-CSF was determined at the mRNA level by quantitative real-time PCR. Real-time PCR was always performed in duplicate, and the mean relative expression of m-CSF in TNF-α– and IL-1α–treated fibroblasts, in comparison with untreated fibroblasts is shown. Expression of m-CSF was increased in both TNF-α– and IL-1α–treated fibroblasts.
cycloheximide inhibited both the basal and IL-1α-induced production of m-CSF, suggesting a requirement for de novo RNA and protein synthesis.55

In our study, induction of m-CSF was noted in TNF-α- and IL-1α-treated cells. The mechanism by which TNF-α or IL-1 regulates the induction of m-CSF was not studied. However, earlier studies have shown that TNF-α and IL-1 exert their functions through activation of nuclear transcription factor NF-κB.36 Although the role of NF-κB in TNF-α- and IL-1-induced m-CSF expression by conjunctival fibroblasts is not yet known, NF-κB has been shown to be a transcriptional regulator of m-CSF in various other cell lines.39,40 Further studies are underway to determine the role of NF-κB in the IL-1α- and TNF-α-induced increased expression and production of m-CSF by conjunctival fibroblasts in patients with OCP.

Several studies have examined the expression of m-CSF in various human and experimental diseases, and an increased expression of m-CSF and local proliferation of macrophages was seen during the disease process. For example, local proliferation of alveolar macrophages under the influence of m-CSF and GM-CSF is involved during chronic inflammation and pulmonary fibrosis.16 More important, a similar association between increased expression of m-CSF and increased accumulation of macrophages was seen in various human renal diseases, and these locally proliferating macrophages appeared to intensify the inflammatory responses and subsequent tissue injuries in the kidney.15 Because m-CSF plays a crucial role(s) in growth and proliferation of monocytes/macrophages,28,30,41 it is thought that increased levels of m-CSF in conjunctival sections may have a key regulatory role for macrophage recruitment as well as local proliferation in conjunctiva of patients with OCP.

A local change of microenvironment may explain why the lesions in OCP are not generalized but rather are confined to the conjunctiva. Conjunctival fibroblast-secreted products, including m-CSF, may change the local microenvironment, and thus could eventually facilitate and/or intensify the local immunoinflammatory responses and subsequent conjunctival fibrosis. Understanding the precise cellular and molecular events that alter the local microenvironment in OCP conjunctiva may facilitate the development of organ-specific therapeutic strategies to treat and/or prevent the progression of irreversible conjunctival scarring in OCP.

In conclusion, the expression and production of m-CSF is increased in the conjunctiva of patients with OCP. Increased expression of m-CSF may be associated with recruitment and accumulation of macrophages and thereby could enhance inflammatory responses. It is possible that macrophage-derived fibrogenic factors contribute to the irreversible scarring that occurs in conjunctiva of patients with OCP.

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