

# Inhibition of Matrix Metalloproteinase-3 Synthesis in Human Conjunctival Fibroblasts by Interleukin-4 or Interleukin-13

Ken Fukuda,<sup>1,2</sup> Youichiro Fujitsu,<sup>1</sup> Naoki Kumagai,<sup>1</sup> and Teruo Nishida<sup>1</sup>

**PURPOSE.** Fibroproliferative lesions of the conjunctiva known as giant papillae are a characteristic of vernal keratoconjunctivitis (VKC). The abundance of T helper 2 (Th2) cells and cytokines is increased in the giant papillae and tear fluid of individuals with VKC, and the Th2 cytokines interleukin (IL)-4 and IL-13 each stimulate the production of extracellular matrix (ECM) proteins by conjunctival fibroblasts. The role of Th2 cytokines in the development of giant papillae was further examined by determination of the effects of these molecules on the production by conjunctival fibroblasts of matrix metalloproteinase (MMP)-3, a key enzyme in ECM degradation.

**METHODS.** The amount of MMP-3 released into the culture medium by human conjunctival fibroblasts was determined by enzyme-linked immunosorbent assay, and the intracellular abundance of MMP-3 mRNA was quantitated by reverse transcription and real-time polymerase chain reaction analysis. Signaling by the transcription factors NF- $\kappa$ B and AP-1 was evaluated by immunoblot and immunofluorescence analyses.

**RESULTS.** Of the Th2 cytokines tested, only IL-4 and -13 inhibited both the basal and IL-1 $\beta$ -induced release of MMP-3 by conjunctival fibroblasts. These effects of IL-4 and -13 were inhibited by neutralizing antibodies to the IL-4 receptor complex. IL-4 and -13 also each reduced the basal abundance, as well as inhibited the IL-1 $\beta$ -induced upregulation, of MMP-3 mRNA in these cells. Neither IL-4 nor -13 affected the IL-1 $\beta$ -induced activation of NF- $\kappa$ B or the AP-1 component c-Jun.

**CONCLUSIONS.** IL-4 and -13 each inhibit MMP-3 synthesis in human conjunctival fibroblasts, suggesting that these Th2 cytokines may contribute to the excessive deposition of ECM in giant papillae by preventing matrix degradation mediated by this enzyme. (*Invest Ophthalmol Vis Sci.* 2006;47:2857-2864) DOI:10.1167/iovs.05-1261

Vernal keratoconjunctivitis (VKC) is a severe and chronic allergic disease of the eye that is associated with pronounced infiltration of inflammatory cells, including eosinophils, mast cells, and lymphocytes. Infiltrated T lymphocytes in individuals with VKC comprise mostly T helper 2 (Th2) cells, which release Th2

cytokines such as interleukin (IL)-4, -5, -9, -10, and -13.<sup>1</sup> The concentrations of Th2 cytokines are thus increased in ocular tissue and tear fluid of VKC patients,<sup>2,3</sup> as are those of proinflammatory cytokines, such as tumor necrosis factor (TNF)- $\alpha$  and IL-1, as well as those of several growth factors.<sup>4,5</sup>

Fibroproliferative lesions of the conjunctiva, most notably giant papillae that develop at the upper tarsal conjunctiva, are characteristic of VKC. These lesions are a consequence of chronic allergic inflammation, forming as a result of excessive deposition of extracellular matrix (ECM), overgrowth of conjunctival resident fibroblasts, and infiltration of inflammatory cells. ECM proteins whose abundance is increased in giant papillae include collagen types I, III, and V as well as fibronectin, tenascin, and laminin.<sup>6-8</sup> Under normal conditions, resident fibroblasts maintain tissue integrity by both synthesizing and degrading ECM proteins, the latter process being achieved by the release of matrix-degrading enzymes such as matrix metalloproteinases (MMPs). Disturbance of this balance between the synthesis and degradation of ECM proteins underlies tissue remodeling in various pathologic conditions. If degradation exceeds synthesis, the loss of ECM proteins results in breakdown of the affected tissue. If synthesis exceeds degradation, excessive deposition of ECM proteins results in hyperplasia or fibrosis, such as those apparent in the giant papillae of VKC.

We and others have shown that Th2 cytokines probably play an important role in the excessive deposition of ECM in giant papillae. Conjunctival fibroblasts thus express receptors for IL-4 and -13 on their surface,<sup>9,10</sup> and these cytokines stimulate the production by these cells of ECM proteins such as collagen types I and III and fibronectin.<sup>10</sup> MMPs are a family of zinc-dependent proteinases that possess a high affinity for ECM components and play a fundamental role in tissue remodeling. Changes in the expression profiles and activation state of MMPs have been identified in giant papillae and tear fluid of individuals with VKC.<sup>11-13</sup> MMPs can be broadly categorized on the basis of their substrate specificity and structure into subgroups such as the interstitial collagenases, gelatinases, stromelysins, and membrane-type MMPs. MMP-3 (stromelysin-1) possesses a broad substrate specificity, being capable of degrading proteoglycans, laminin, fibronectin, and collagens. It is also capable of activating the precursor (pro) forms of other MMPs, including MMP-1, -2, -8, -9, and -13, as well as inactivating plasminogen activator inhibitor I.<sup>14,15</sup> MMP-3 is produced by tissue-resident cells, including fibroblasts and endothelial cells, as well as by inflammatory cells, such as macrophages and neutrophils, in response to the proinflammatory cytokines IL-1 or TNF- $\alpha$ . This enzyme plays an important role in the tissue destruction associated with the chronic inflammation of rheumatoid arthritis and periodontitis.<sup>16,17</sup> In addition, MMP-3 contributes to physiological tissue remodeling as well as to other pathologic processes including cancer, atherosclerosis, and conjunctivochalasis.<sup>18-20</sup>

The concentration of the proinflammatory cytokine IL-1 $\beta$  in tear fluid is increased in patients with tarsal VKC, compared with that in healthy individuals,<sup>4</sup> and the IL-1 receptor antagonist suppresses allergic inflammation in a mouse model of

From the Departments of <sup>1</sup>Biomolecular Recognition and Ophthalmology and <sup>2</sup>Ocular Pathophysiology, Yamaguchi University School of Medicine, Yamaguchi, Japan.

Supported by a Grand-in-Aid for Young Scientists (B) 16791050 from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Submitted for publication September 23, 2005; revised February 15, 2006; accepted May 22, 2006.

Disclosure: **K. Fukuda**, None; **Y. Fujitsu**, None; **N. Kumagai**, None; **T. Nishida**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Ken Fukuda, Department of Biomolecular Recognition and Ophthalmology, Yamaguchi University School of Medicine, 1-1-1 Minami-Kogushi, Ube City, Yamaguchi 755-8505, Japan; k.fukuda@yamaguchi-u.ac.jp.

allergic conjunctivitis, suggesting that IL-1 may play a role in VKC as well as in the pathogenesis of ocular allergy in general.<sup>4,21</sup> In addition, IL-1 $\beta$  is one of the most efficacious inducers of MMP-1 and -3 expression in fibroblasts and contributes to the destruction of various tissues, including the cornea, *in vivo*.<sup>22</sup> The increased production of IL-1 at sites of allergic inflammation is thus thought to promote ECM degradation as a result of upregulation of MMP expression. In giant papillae, however, deposition of ECM proteins is increased, suggesting that other factors may inhibit IL-1-induced MMP expression. We have therefore now investigated whether Th2 cytokines affect MMP-3 synthesis by human conjunctival fibroblasts cultured in the absence or presence of IL-1 $\beta$ .

## METHODS

### Materials

Eagle's minimum essential medium (MEM), fetal bovine serum, and phosphate-buffered saline (PBS) were obtained from Invitrogen-Gibco (Grand Island, NY). All materials used for cell culture were endotoxin minimized. Tissue culture dishes were from Greiner Bio-One (Kems-muenster, Austria) and eight-well chamber slides were from Nalge Nunc (Naperville, IL). Rabbit polyclonal antibodies to the p65 subunit of nuclear factor (NF)- $\kappa$ B were from Santa Cruz Biotechnology (Santa Cruz, CA), horseradish peroxidase-conjugated goat antibodies to mouse or rabbit immunoglobulin G (IgG) were from Chemicon (Temecula, CA), and Alexa Fluor 488-conjugated goat antibodies to rabbit IgG were from Invitrogen (Eugene, OR). Rabbit polyclonal antibodies to I $\kappa$ B- $\alpha$  and to phosphorylated c-Jun as well as a mouse monoclonal antibody to phosphorylated I $\kappa$ B- $\alpha$  were from Cell Signaling (Beverly, MA). A mouse monoclonal antibody (IgG2A) to the human IL-4 receptor, normal mouse IgG2A, human recombinant cytokines, as well as enzyme-linked immunosorbent assay (ELISA) kits for MMP-3 and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) were from R&D Systems (Minneapolis, MN). Antifade mounting medium (Vectashield) was from Vector Laboratories (Burlingame, CA). PCR reagents (QuantiTect SYBR Green) were from Qiagen (Hilden, Germany). Protein assay reagents (Micro BCA) were obtained from Pierce (Rockford, IL), and enhanced chemiluminescence reagents were from GE Healthcare (Piscataway, NJ). PGE<sub>2</sub> was from BIOMOL Research Laboratories (Plymouth Meeting, PA).

### Isolation and Culture of Human Conjunctival Fibroblasts

Three normal human conjunctivas were obtained and they were processed separately, after informed consent was obtained from individuals undergoing eyelid or strabismus surgery. The human tissue was used in strict accordance with the basic principles of the Declaration of Helsinki. Conjunctival fibroblasts were isolated and cultured as described previously.<sup>9,10</sup>

### ELISAs for MMP-3 and PGE<sub>2</sub>

Cells were plated in 24-well plates ( $3 \times 10^4$  cells per well) and cultured at 37°C first for 3 days in MEM supplemented with 10% fetal bovine serum and then for 24 hours in serum-free MEM. They were then incubated for various times in serum-free MEM supplemented with cytokines or PGE<sub>2</sub>. Cells were exposed to IL-1 $\beta$  and to Th2 cytokines or PGE<sub>2</sub> at the same time. The culture medium was collected from each well and centrifuged at 120g for 5 minutes at 4°C. The resultant supernatants were frozen at -80°C until assayed for MMP-3 or PGE<sub>2</sub> by ELISA, with measurement of absorbance at 450 nm. The limits of detection of the assays were 9 pg/mL for MMP-3 and 13.4 pg/mL for PGE<sub>2</sub>. The concentrations of MMP-3 and PGE<sub>2</sub> in culture supernatants were normalized by cell number and expressed as nanograms per  $1 \times 10^6$  cells.

### Quantitative RT-PCR Analysis

Cells were cultured for 24 hours in serum-free MEM and then incubated for 12 hours in serum-free MEM supplemented with cytokines. They were then washed with PBS, and total RNA was extracted (MagNa Pure; Roche Molecular Biochemicals, Mannheim, Germany). The RNA was subjected to reverse transcription (RT) with a kit (Promega, Madison, WI), and the resultant cDNA was subjected to real-time polymerase chain reaction (PCR) analysis by rapid cycling in glass capillaries with a thermocycler (LightCycler; Roche Molecular Biochemicals), as described previously.<sup>10,23</sup> The amount of MMP-3 mRNA was normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. The sequences of the PCR primers for MMP-3 were 5'-GGCACAAATATGGGCACITTA-3' (forward) and 5'-CCGGCAAGATACAGATTCAC-3' (reverse); those for GAPDH were as described previously.<sup>23</sup>

### Gelatin Zymography

Gelatin zymography of culture supernatants was performed as described previously.<sup>12</sup> In brief, culture supernatants were mixed with nonreducing SDS sample buffer (125 mM Tris-HCl [pH 6.8], 20% glycerol, 2% SDS, and 0.002% bromophenol blue) and fractionated by SDS-polyacrylamide gel electrophoresis at 4°C on a 10% gel containing 0.1% gelatin. The gel was then washed in 2.5% Triton X-100 for 1 hour, to promote recovery of protease activity, before incubation for 18 hours at 37°C in a reaction buffer containing 50 mM Tris-HCl (pH 7.5), 5 mM CaCl<sub>2</sub>, and 1% Triton X-100. The gel was finally stained with Coomassie brilliant blue.

### Immunoblot Analysis of I $\kappa$ B- $\alpha$ and c-Jun

The phosphorylation and degradation of I $\kappa$ B- $\alpha$  and the phosphorylation of c-Jun in conjunctival fibroblasts were examined by immunoblot analysis, as described previously.<sup>23</sup> In brief, after incubation at 37°C, first for 24 hours in serum-free MEM and then for 30 minutes with MEM containing cytokines, the cells were washed twice with PBS, lysed with radioimmunoprecipitation buffer, and assayed for protein concentration. Cell lysates (10  $\mu$ g of protein) were subjected to SDS-polyacrylamide gel electrophoresis on a 10% gel under reducing conditions, and the separated proteins were transferred electrophoretically to a nitrocellulose membrane. After blocking of nonspecific sites, the membrane was incubated with antibodies to I $\kappa$ B- $\alpha$ , to phosphorylated I $\kappa$ B- $\alpha$ , or to phosphorylated c-Jun, and immune complexes were then detected with horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence reagents.

### Immunofluorescence Staining for NF- $\kappa$ B and Phosphorylated c-Jun

Immunostaining for NF- $\kappa$ B and phosphorylated c-Jun in conjunctival fibroblasts was performed as described previously.<sup>23</sup> In brief, cell monolayers grown on eight-well chamber slides were incubated at 37°C, first for 24 hours in serum-free MEM and then for 30 minutes with MEM-containing cytokines. The cells were then washed twice with PBS, fixed with 4% paraformaldehyde in PBS, and washed an additional three times with PBS before permeabilization with 100% methanol for 6 minutes at -20°C. Nonspecific adsorption of antibodies was blocked by incubation for 30 minutes with PBS containing 3% bovine serum albumin, and the cells were then incubated for 1 hour at room temperature with antibodies to the p65 subunit of NF- $\kappa$ B or to phosphorylated c-Jun, washed, and incubated for 30 minutes at room temperature with Alexa Fluor 488-conjugated secondary antibodies (1:1000 dilution in PBS containing 3% bovine serum albumin). The cells were finally washed, mounted in mounting medium, and examined with a fluorescence microscope (Axiovert; Carl Zeiss Meditec GmbH, München-Hallbergmoos, Germany).

### Statistical Analysis

Quantitative data are expressed as the mean  $\pm$  SEM for four separate experiments. Differences were evaluated by analysis of variance fol-

lowed by Fisher's protected least significant difference test.  $P < 0.05$  was considered statistically significant.

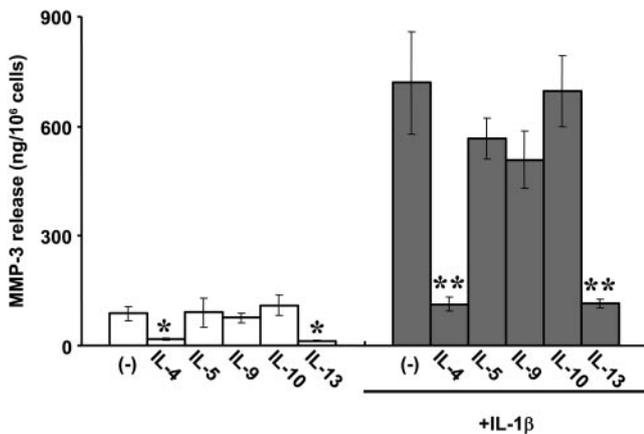
**RESULTS**

**Inhibition of MMP-3 Release from Human Conjunctival Fibroblasts by IL-4 or -13**

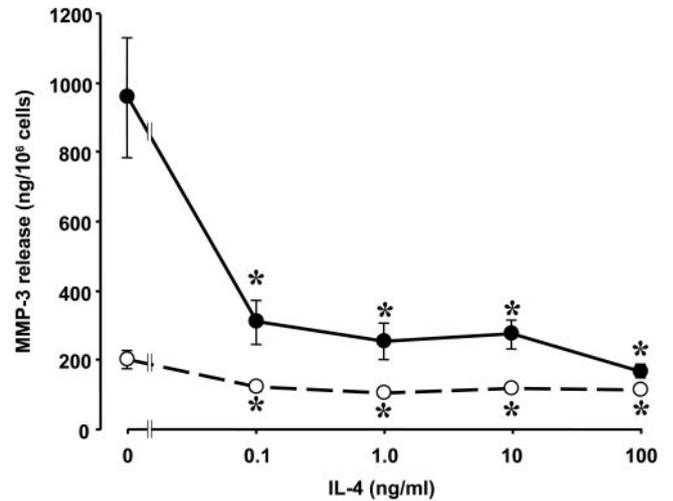
We first investigated the effects of Th2 cytokines on the release of MMP-3 from human conjunctival fibroblasts stimulated with the proinflammatory cytokine IL-1 $\beta$ . The cells were cultured for 24 hours with the Th2 cytokines IL-4, -5, -9, -10, or -13 (each at 10 ng/mL), in the absence or presence of IL-1 $\beta$  (1 ng/mL), and the amount of MMP-3 in the culture supernatant was then determined by ELISA. Conjunctival fibroblasts constitutively released small amounts of MMP-3 into the culture medium (Fig. 1). IL-4 and -13, but not IL-5, -9, and -10, inhibited the basal release of MMP-3 by these cells. IL-1 $\beta$  stimulated the release of MMP-3 from conjunctival fibroblasts, as described previously.<sup>20</sup> IL-4 and -13, but not IL-5, -9, and -10, also markedly inhibited the stimulatory effect of IL-1 $\beta$  on MMP-3 release.

We next examined the concentration dependence of the effects of IL-4 and -13 on the release of MMP-3 by conjunctival fibroblasts. The cells were cultured for 24 hours with various concentrations of IL-4 or -13 in the absence or presence of IL-1 $\beta$  (1 ng/mL), and the amount of MMP-3 in the culture supernatant was then determined. IL-4 inhibited the basal level of MMP-3 release in a concentration-dependent manner; this effect was statistically significant at IL-4 concentrations of  $\geq 0.1$  ng/mL (Fig. 2). The inhibitory effect of IL-4 on IL-1 $\beta$ -induced MMP-3 release was also concentration dependent and significant at  $\geq 0.1$  ng/mL. Similarly, IL-13 inhibited both basal and IL-1 $\beta$ -induced MMP-3 release by conjunctival fibroblasts in a concentration-dependent manner. These effects were statistically significant at IL-13 concentrations of 10 and 1 ng/mL, respectively (Fig. 3).

We then examined the time course of MMP-3 release by conjunctival fibroblasts incubated with the combination of IL-1 $\beta$  (1 ng/mL) and either IL-4 (10 ng/mL) or IL-13 (10 ng/mL). The release of MMP-3 from IL-1 $\beta$ -stimulated conjunctival fibroblasts increased in a time-dependent manner for up to 48 hours (Fig. 4). The presence of either IL-4 or -13 resulted in almost



**FIGURE 1.** Effects of Th2 cytokines on MMP-3 release by human conjunctival fibroblasts. Cells were incubated for 24 hours with the indicated Th2 cytokines (each at 10 ng/mL) in the absence (□) or presence (■) of IL-1 $\beta$  (1 ng/mL). The concentration of MMP-3 in the culture supernatant was then determined by ELISA. Data are expressed as nanograms of MMP-3 released per  $1 \times 10^6$  cells and are means  $\pm$  SEM of results of four separate experiments. \* $P < 0.05$ , \*\* $P < 0.01$  versus the corresponding value for cells incubated in the absence of Th2 cytokines.

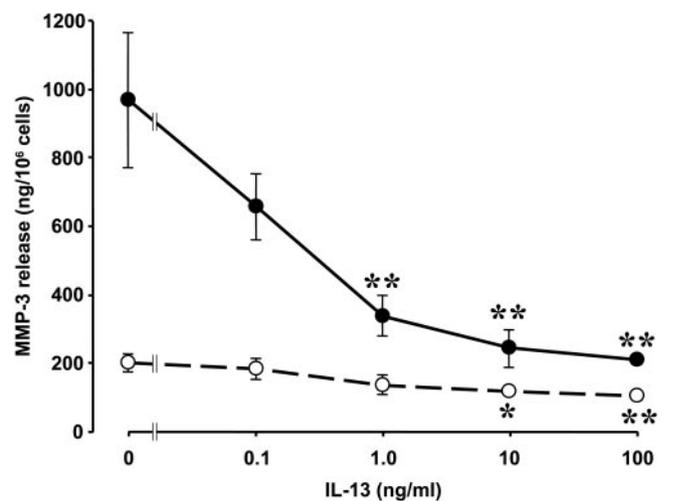


**FIGURE 2.** Concentration dependence of the inhibitory effect of IL-4 on MMP-3 release by conjunctival fibroblasts. Cells were incubated for 24 hours with the indicated concentrations of IL-4 in the absence (○) or presence (●) of IL-1 $\beta$  (1 ng/mL). The concentration of MMP-3 in the culture supernatant was then measured. Data are the mean  $\pm$  SEM of results of four separate experiments. \* $P < 0.01$  versus the corresponding value for cells incubated in the absence of IL-4.

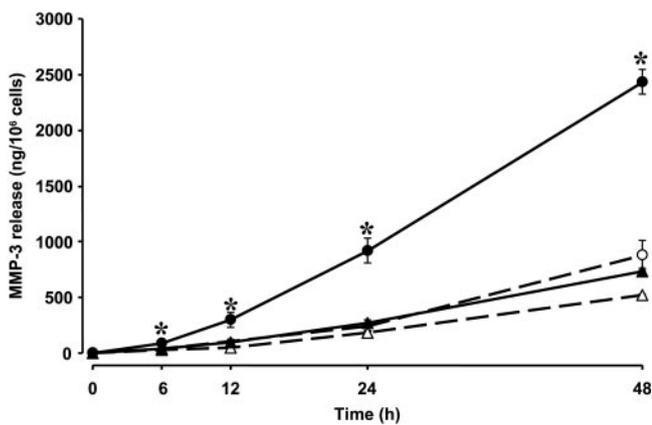
complete inhibition of the stimulatory effect of IL-1 $\beta$  on MMP-3 release at all time points examined.

**Inhibition of the Effects of IL-4 and -13 on MMP-3 Release by Antibodies to the IL-4 Receptor**

To confirm that the inhibitory actions of IL-4 and -13 on MMP-3 release were mediated by the IL-4 receptor complex, we examined the effect of pretreatment of conjunctival fibroblasts with neutralizing antibodies to this complex. Cells were pretreated for 1 hour with the neutralizing antibodies or with control mouse IgG2A (each at 10  $\mu$ g/mL) and were then incubated for 24 hours in the additional presence of cytokines. Pretreatment of cells with the neutralizing antibodies to the IL-4 receptor complex, but not



**FIGURE 3.** Concentration dependence of the inhibitory effect of IL-13 on MMP-3 release by conjunctival fibroblasts. Cells were incubated for 24 hours with the indicated concentrations of IL-13 in the absence (○) or presence (●) of IL-1 $\beta$  (1 ng/mL). The concentration of MMP-3 in the culture supernatant was then measured. Data are mean  $\pm$  SEM of results of four separate experiments. \* $P < 0.05$ , \*\* $P < 0.01$  versus the corresponding value for cells incubated in the absence of IL-13.

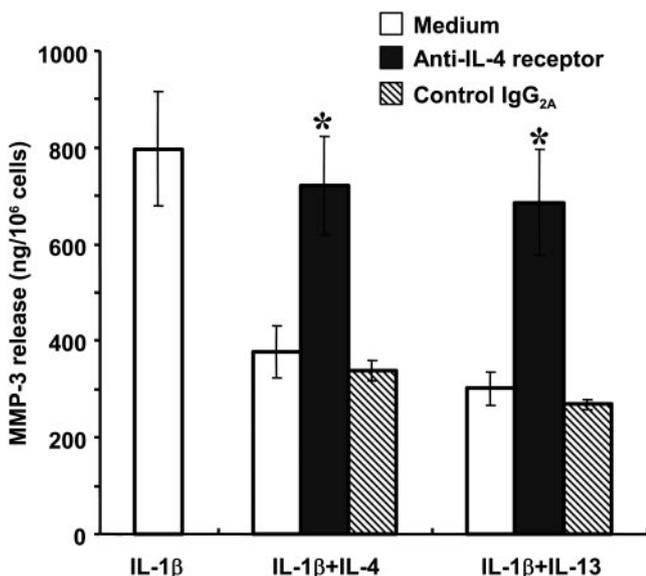


**FIGURE 4.** Time course of MMP-3 release from conjunctival fibroblasts. Cells were cultured for the indicated times in the absence of cytokines (○) or in the presence of IL-1β (1 ng/mL) either without (●) or with IL-4 (△) or IL-13 (▲), each at a concentration of 10 ng/mL. Culture supernatants were then assayed for MMP-3. Data are the mean ± SEM of results of four separate experiments. \**P* < 0.001 versus the corresponding time point for cells incubated in the absence of cytokines.

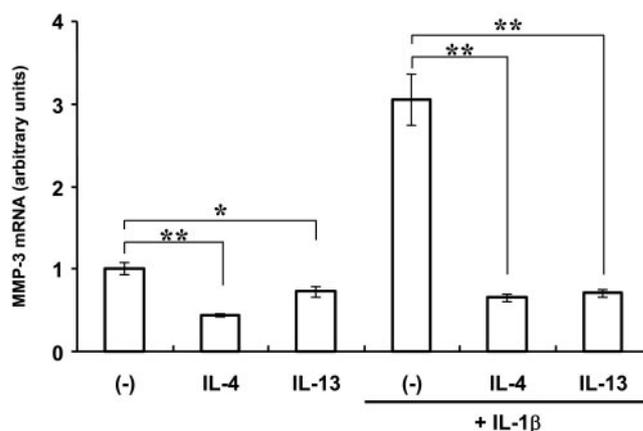
that with control IgG, resulted in inhibition of the suppressive effect of IL-4 or -13 on IL-1β-induced MMP-3 release (Fig. 5). These results thus indicate that the inhibitory effect of IL-4 or -13 on MMP-3 release by human conjunctival fibroblasts is mediated by the IL-4 receptor complex.

**Downregulation of MMP-3 mRNA by IL-4 and -13**

We next investigated the effects of cytokines on the abundance of MMP-3 mRNA in human conjunctival fibroblasts. Cells were cultured with IL-4 or -13 for 12 hours in the absence or



**FIGURE 5.** Effect of neutralizing antibodies to the IL-4 receptor complex on the inhibition by IL-4 or -13 of MMP-3 release from conjunctival fibroblasts. Cells were incubated first for 1 hour in the absence or presence of antibodies to the IL-4 receptor complex or normal mouse IgG<sub>2A</sub>, each at 10 μg/mL, and then for 24 hours in the additional presence of IL-1β (1 ng/mL), IL-4 (10 ng/mL), or IL-13 (10 ng/mL), as indicated. Culture supernatants were then assayed for MMP-3. Data are the mean ± SEM of results of four separate experiments. \**P* < 0.01 versus the corresponding value for cells incubated in the absence of antibodies.



**FIGURE 6.** Downregulation of MMP-3 mRNA in human conjunctival fibroblasts by Th2 cytokines. Cells were incubated for 12 hours in the presence of the indicated combinations of IL-4 (10 ng/mL), IL-13 (10 ng/mL), and IL-1β (1 ng/mL), after which cell lysates were assayed for MMP-3 mRNA by RT and real-time PCR analysis. Data were normalized on the basis of the abundance of GAPDH mRNA, are expressed in arbitrary units, and are the mean ± SEM of results of four experiments. \**P* < 0.05, \*\**P* < 0.01 versus the corresponding value for cells incubated in the absence of IL-4 or -13.

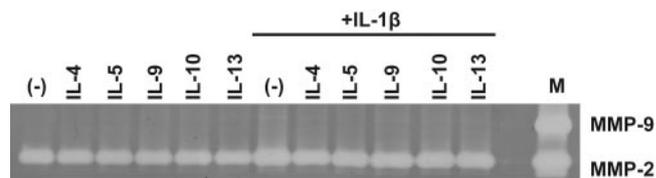
presence of IL-1β, after which the amount of MMP-3 mRNA in cell lysates was assayed by RT and real-time PCR analysis. IL-4 and -13 each reduced the basal amount of MMP-3 mRNA in these cells (Fig. 6). Whereas IL-1β induced an approximately threefold increase in the amount of MMP-3 mRNA, this effect was prevented by IL-4 and -13.

**Lack of Effect of Th2 Cytokines on the Release of MMP-2 and -9**

We investigated whether Th2 cytokines regulate the expression of the gelatinases MMP-2 and -9 in human conjunctival fibroblasts. The cells were cultured for 48 hours with the Th2 cytokines IL-4, -5, -9, -10, or -13, in the absence or presence of IL-1β, and the amounts of MMP-2 and -9 in culture supernatants were then evaluated by gelatin zymography (Fig. 7). Conjunctival fibroblasts constitutively released MMP-2 into the culture medium, but none of the Th2 cytokines examined affected the basal release of this gelatinase. IL-1β induced a small increase in the release of MMP-2, but this action of IL-1β was also not affected by Th2 cytokines. The gelatinolytic band at 92 kDa corresponding to MMP-9 was virtually undetectable for the culture supernatants of cells incubated under any of the conditions tested.

**Lack of Effect of IL-4 or -13 on IL-1β-Induced Activation of NF-κB and AP-1**

Signaling pathways mediated by the transcription factors NF-κB and activator protein (AP)-1 are implicated in induction of



**FIGURE 7.** Lack of effect of Th2 cytokines on the release of MMP-2 and -9 by conjunctival fibroblasts. Cells were incubated for 48 hours with the indicated Th2 cytokines (each at 10 ng/mL) in the absence or presence of IL-1β (1 ng/mL). The amounts of MMP-2 and -9 in culture supernatants were then evaluated by gelatin zymography. Lane M: MMP-2 and -9 standards.

MMP-3 gene expression in several cell types.<sup>24,25</sup> To elucidate the mechanism of action of IL-4 and -13 in human conjunctival fibroblasts, we examined the activation of these transcription factors in these cells. Cells were incubated with IL-4 or -13 in the absence or presence of IL-1 $\beta$  for 30 minutes, after which the phosphorylation and degradation of the NF- $\kappa$ B inhibitor I $\kappa$ B- $\alpha$  were examined by immunoblot analysis. IL-4 or -13 alone had no effect on the phosphorylation state or abundance of I $\kappa$ B- $\alpha$  (Fig. 8A). In contrast, IL-1 $\beta$  induced both the phosphorylation and degradation of I $\kappa$ B- $\alpha$ . These effects of IL-1 $\beta$  were not influenced by IL-4 or -13. We also examined the effects of these cytokines on the subcellular localization of the p65 subunit of NF- $\kappa$ B by immunofluorescence analysis. Under basal conditions, NF- $\kappa$ B immunofluorescence was located predominantly in the cytoplasm of conjunctival fibroblasts (Fig. 8B). No immunofluorescence was apparent in cells stained with normal

rabbit IgG as a negative control (data not shown). Treatment of the cells with IL-4 or -13 alone did not affect the subcellular localization of NF- $\kappa$ B (Figs. 8C, 8D). However, IL-1 $\beta$  induced translocation of NF- $\kappa$ B from the cytoplasm to the nucleus of conjunctival fibroblasts (Fig. 8E). This action of IL-1 $\beta$  was not affected by IL-4 or -13 (Figs. 8F, 8G). These results thus indicate that IL-1 $\beta$  activated the NF- $\kappa$ B signaling pathway in human conjunctival fibroblasts and that this action of IL-1 $\beta$  was not affected by IL-4 or -13.

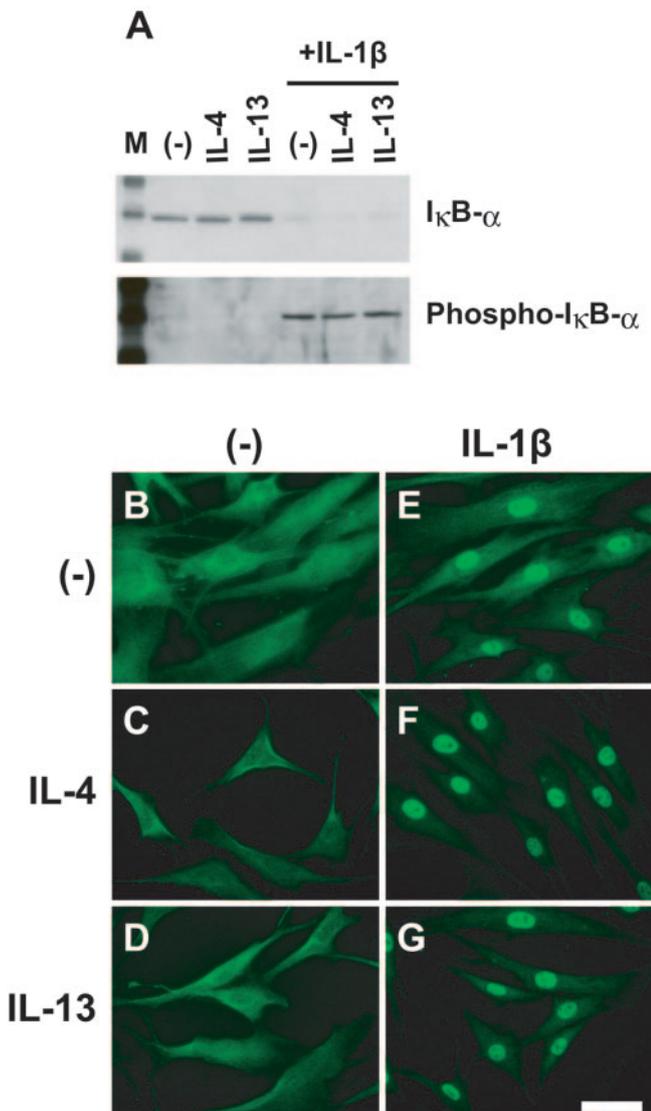
We examined the effects of cytokines on the abundance of the phosphorylated form of the AP-1 component c-Jun. Immunoblot analysis revealed that IL-1 $\beta$  induced the phosphorylation of c-Jun, but that IL-4 or -13 had no effect on the abundance of phosphorylated c-Jun in the absence or presence of IL-1 $\beta$  (Fig. 9A). Immunofluorescence analysis also revealed that phosphorylated c-Jun was virtually undetectable in conjunctival fibroblasts incubated in the absence or presence of IL-4 or -13 alone (Figs. 9B-D). Stimulation of cells with IL-1 $\beta$  resulted in a marked increase in the extent of nuclear immunofluorescence corresponding to phosphorylated c-Jun, and this effect was not influenced by IL-4 or -13 (Figs. 9E-G). These results thus indicate that IL-1 $\beta$  activates the AP-1 signaling pathway in human conjunctival fibroblasts and that this action of IL-1 $\beta$  is not affected by IL-4 or -13.

#### Exclusion of a Role for PGE<sub>2</sub> in Inhibition of MMP-3 Release by IL-4 or -13

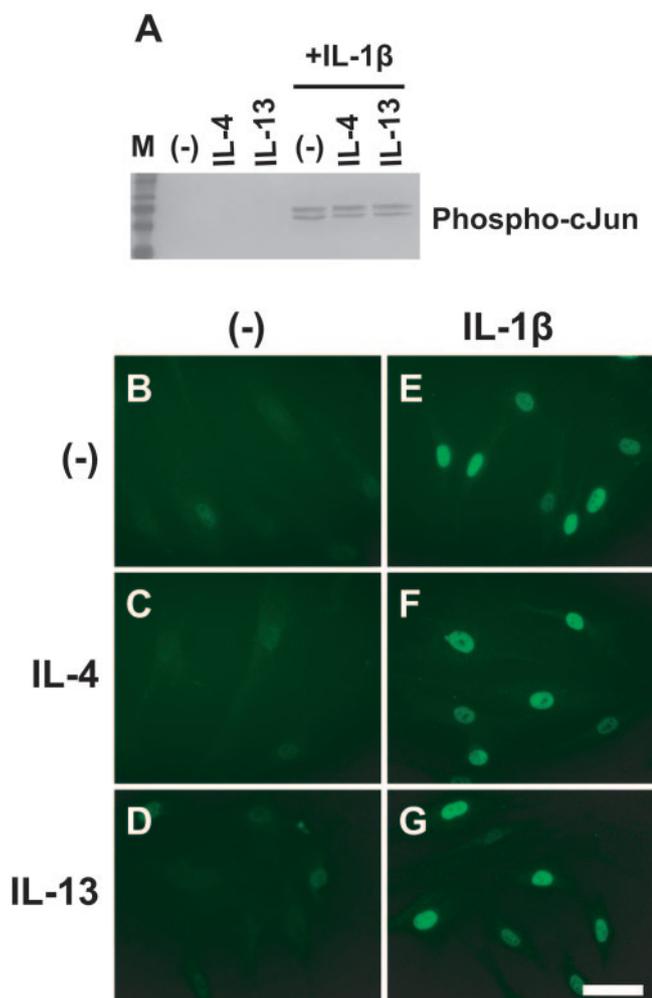
Some studies have suggested that IL-1-induced production of PGE<sub>2</sub> contributes to the stimulatory effect of this cytokine on MMP-3 expression in several types of fibroblasts and that the inhibitory effect of IL-4 on IL-1-induced gene expression is mediated at the level of PGE<sub>2</sub> production.<sup>26-29</sup> We therefore examined whether the inhibitory effects of IL-4 and -13 on MMP-3 release in human conjunctival fibroblasts are also mediated at the level of PGE<sub>2</sub>. We first examined the effects of these cytokines on PGE<sub>2</sub> synthesis. The cells were cultured for 24 hours with IL-4 or -13, in the absence or presence of IL-1 $\beta$ , and the amount of PGE<sub>2</sub> in the culture supernatant was then determined by ELISA. Conjunctival fibroblasts constitutively released small amounts of PGE<sub>2</sub> into the culture medium (Fig. 10A). IL-1 $\beta$  stimulated the release of PGE<sub>2</sub> from these cells, but this effect was not inhibited by IL-4 or -13. We also examined the effect of exogenous PGE<sub>2</sub> on MMP-3 release from conjunctival fibroblasts. The addition of PGE<sub>2</sub> together with IL-1 $\beta$  and either IL-4 or -13 had no effect on the ability of the Th2 cytokines to inhibit IL-1 $\beta$ -induced MMP-3 release (Fig. 10B). These results suggest that the inhibitory effects of IL-4 and -13 on IL-1 $\beta$ -induced MMP-3 expression in human conjunctival fibroblasts are not mediated by suppression of IL-1 $\beta$ -induced production of PGE<sub>2</sub>.

#### DISCUSSION

We have shown that the Th2 cytokines IL-4 and -13 each inhibited basal and IL-1 $\beta$ -induced MMP-3 expression at both the protein and mRNA levels in human conjunctival fibroblasts. Such inhibitory effects were not observed with the Th2 cytokines IL-5, -9, or -10. The effects of IL-4 and -13 were concentration dependent and were mediated by the IL-4 receptor complex expressed on the surface of the cells. We previously demonstrated that IL-4 and -13 also stimulate the synthesis of ECM proteins, including collagen types I and III and fibronectin, in human conjunctival fibroblasts.<sup>10</sup> Together, these observations suggest that IL-4 and -13 may play important roles in the excessive deposition of ECM that contributes to the development of giant papillae in individuals with VKC.



**FIGURE 8.** Effects of cytokines on the NF- $\kappa$ B signaling pathway in conjunctival fibroblasts. (A) Cells were incubated for 30 minutes with IL-4 or -13 (each at 10 ng/mL) in the absence or presence of IL-1 $\beta$  (1 ng/mL), as indicated, after which cell lysates were subjected to immunoblot analysis with antibodies to I $\kappa$ B- $\alpha$  or to phosphorylated I $\kappa$ B- $\alpha$ . Lane M: molecular size standards. (B-G) Cells incubated as in (A) were fixed and subjected to immunofluorescence analysis with antibodies to the p65 subunit of NF- $\kappa$ B. All data are representative results of three independent experiments.

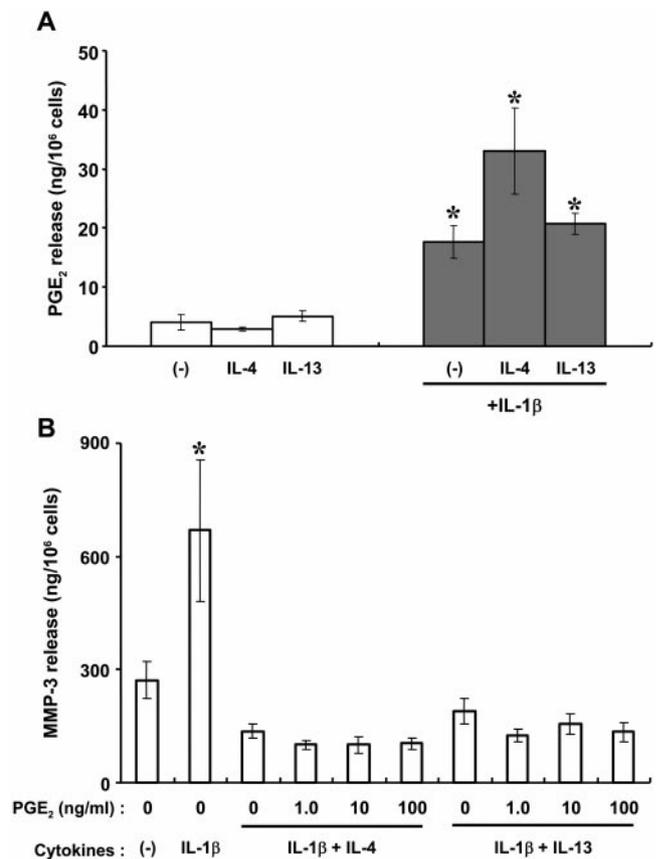


**FIGURE 9.** Effects of cytokines on the AP-1 signaling pathway in conjunctival fibroblasts. (A) Cells were incubated for 30 minutes with IL-4 or -13 (each at 10 ng/mL) in the absence or presence of IL-1β (1 ng/mL), after which cell lysates were subjected to immunoblot analysis with antibodies to phosphorylated c-Jun. (B–G) Cells incubated as in (A) were fixed and subjected to immunofluorescence analysis with antibodies to phosphorylated c-Jun. All data are representative of three independent experiments.

Both IL-4 and -13 have been shown to inhibit the release of the interstitial collagenase MMP-1, but not that of the gelatinase MMP-9, in conjunctival fibroblasts.<sup>30</sup> Conversely, these cytokines stimulate the release from conjunctival fibroblasts of tissue inhibitor of metalloproteinase (TIMP)-1,<sup>30</sup> which inhibits the activities of interstitial collagenases, gelatinases, and stromelysins but not those of bacterial collagenases. Pathologic turnover of ECM is thought to result from an imbalance in the activities of TIMPs and MMPs.<sup>31</sup> IL-4 also induces the synthesis of TIMP-2 in dermal fibroblasts by triggering a signaling pathway mediated by the p38 isoform of mitogen-activated protein kinase.<sup>32</sup> We have also found that IL-4 and -13 each increase the release of TIMP-2 from human conjunctival fibroblasts.<sup>33</sup> Together with the results of our present study, these various observations demonstrate that IL-4 and -13 inhibit the release of MMP-1 and -3, but increase that of the inhibitors TIMP-1 and -2, from conjunctival fibroblasts. These cytokines are thus implicated as inhibitors of matrix degradation in the conjunctiva. The degradation-promoting effects of proinflammatory cytokines such as IL-1 and TNF-α may thus be counteracted by the actions of IL-4 and -13.

We have shown that human conjunctival fibroblasts express IL-4Rα, -2Rγc, -13Rα1, and -13Rα2 chains of the IL-4 receptor complex<sup>9,10</sup> and that IL-4 stimulates the proliferation of these cells by acting at these receptors.<sup>10</sup> We have also demonstrated that, among Th2 cytokines, IL-4 and -13 (but not IL-5, -9, or -10) protects these cells from apoptotic cell death by activating signaling by phosphoinositide 3-kinase and the protein kinase Akt.<sup>9</sup> The mitogenic and antiapoptotic effects of IL-4 and -13 in conjunctival fibroblasts may result in the overgrowth of these cells. IL-4 and -13 may thus contribute to both the excessive deposition of ECM and the overgrowth of conjunctival fibroblasts that give rise to giant papillae in individuals with VKC.

The ECM in giant papillae not only serves as a structural framework but also plays a role in signaling to infiltrating inflammatory cells such as eosinophils, mast cells, and T cells. Attachment of these cells to the ECM promotes their proliferation or prolongs their survival.<sup>34–36</sup> The ECM also regulates the expression of inflammatory cytokines, growth factors, adhesion molecules, and enzymes as well as the production of oxygen radicals by these cells.<sup>37–41</sup> In addition, the ECM serves as a reservoir for cytokines and growth factors that bind to its components, with the ECM stabilizing or increasing the local concentration of these molecules.<sup>42,43</sup> The ECM is thus



**FIGURE 10.** Effects of cytokines on PGE<sub>2</sub> release and effect of PGE<sub>2</sub> on MMP-3 release by human conjunctival fibroblasts. (A) Cells were incubated for 24 hours with IL-4 or -13 (each at 10 ng/mL) in the absence (□) or presence (■) of IL-1β (1 ng/mL). The concentration of PGE<sub>2</sub> in the culture supernatant was then determined by ELISA. (B) Cells were incubated for 24 hours with IL-4 or -13 (each at 10 ng/mL), IL-1β (1 ng/mL), and various concentrations of PGE<sub>2</sub>, as indicated. The concentration of MMP-3 in the culture supernatant was then determined by ELISA. Data are expressed as nanograms of PGE<sub>2</sub> (A) or MMP-3 (B) released per 1 × 10<sup>6</sup> cells and are the mean ± SEM of results of four separate experiments. \*P < 0.01 versus the corresponding value for cells incubated in the absence of cytokines.

thought to contribute to the persistence and activation of infiltrating inflammatory cells during conjunctival allergic inflammation.

Various cytokines and growth factors, including Th2 cytokines, accumulate in giant papillae or tear fluid of individuals with VKC. Among such molecules enriched in giant papillae, proinflammatory cytokines such as IL-1 and TNF- $\alpha$  are able to induce the degradation of ECM proteins by upregulating the expression of MMPs in conjunctival and corneal fibroblasts.<sup>20</sup> Consistent with previous observations,<sup>20</sup> we found that IL-1 $\beta$  stimulates the expression of MMP-3 by conjunctival fibroblasts. This effect of IL-1 $\beta$  was abolished, however, in the presence of IL-4 or -13. The transcription factor AP-1 plays an important role in regulation of the MMP-3 gene by a variety of cytokines, including IL-1.<sup>25</sup> However, although AP-1 is essential for upregulation of MMP-3 expression, it is not sufficient, with NF- $\kappa$ B activity also being required for transcriptional activation of the MMP-3 gene in several cell types, including fibroblasts.<sup>44</sup> A consensus NF- $\kappa$ B binding site has not been identified in the MMP-3 gene promoter,<sup>45</sup> suggesting that NF- $\kappa$ B may regulate the expression of this gene via a different binding site or through interaction with other transcription factors. Indeed, NF- $\kappa$ B has been shown to potentiate transcription from promoters that lack a consensus NF- $\kappa$ B-binding site through interaction with AP-1.<sup>46</sup> The upregulation of integrin expression in fibroblasts by NF- $\kappa$ B also occurs in the absence of a consensus NF- $\kappa$ B promoter element.<sup>47</sup> In the present study, we therefore examined the effects of IL-4 and -13 on the activation of NF- $\kappa$ B and AP-1 in conjunctival fibroblasts. Whereas IL-1 $\beta$  activated both AP-1 and NF- $\kappa$ B, however, IL-4 and -13 did not interfere with these effects. These results are consistent with previous observations showing that the inhibitory effect of IL-4 on IL-1-induced MMP-3 expression is not mediated by inhibition of signaling by NF- $\kappa$ B or AP-1 in human gingival fibroblasts.<sup>48</sup> IL-4 has been shown to affect gene expression by interfering with the DNA-binding activity of AP-1 and NF- $\kappa$ B in monocytes but not in fibroblasts.<sup>49</sup> Although our present data do not address whether the inhibition of MMP-3 expression by IL-4 or -13 is mediated at the level of gene transcription, they are consistent with this notion. MMP-3 expression is regulated primarily at the transcriptional level, and IL-4 inhibits transcription from the MMP-3 gene promoter in transiently transfected human foreskin fibroblasts.<sup>50</sup> The alternative explanation, that the inhibition of MMP-3 expression by IL-4 or -13 is mediated by a decrease in mRNA stability, cannot be excluded, however.

The effects of both IL-4 and -13 on gene expression are generally mediated through activation of signal transducer and activator of transcription 6 (STAT6).<sup>51</sup> Indeed, we have shown that these cytokines activate STAT6 in human conjunctival fibroblasts (Fukuda K, unpublished data, 2005). STAT6 induces transcription through interaction with the coactivators p300 or CBP.<sup>52</sup> NF- $\kappa$ B and AP-1, as well as other transcription factors, also require p300 or CBP for their transactivation activity, and this activity is inhibited by factors such as STATs that compete for the coactivators.<sup>53-56</sup> IL-4 and -13 may thus inhibit transactivation of the MMP-3 gene by depriving AP-1 and NF- $\kappa$ B of coactivators. The precise mechanism by which these Th2 cytokines inhibit the IL-1 $\beta$ -induced expression of MMP-3 in conjunctival fibroblasts remains to be determined.

Previous studies have shown that IL-4 inhibits the IL-1-induced production of PGE<sub>2</sub> in several types of fibroblasts, and this effect has been linked to the suppressive effect of IL-4 on gene expression.<sup>26-29</sup> Other studies do not support a role for prostaglandins in the IL-1-induced expression of MMP-3, however.<sup>48,50</sup> Our present results showing that IL-1 $\beta$ -induced PGE<sub>2</sub> production was not inhibited by IL-4 or -13 and that exogenous PGE<sub>2</sub> did not override the inhibitory effects of IL-4 and -13 on MMP-3 release in conjunctival fibroblasts indicate

that the effects of these Th2 cytokines are not mediated at the level of PGE<sub>2</sub> in our system.

In conclusion, we have shown that IL-4 and -13 each inhibit the production of the matrix-degrading enzyme MMP-3 in human conjunctival fibroblasts. These two cytokines also stimulate the synthesis of ECM proteins and TIMPs by these cells.<sup>10,30,33</sup> The increased synthesis and the decreased degradation of ECM proteins that result from exposure of conjunctival fibroblasts to IL-4 or -13 likely play central roles in the excessive deposition of ECM in the giant papillae of individuals with VKC.

### Acknowledgments

The authors thank Nami Tsuchimochi, Yukari Mizuno, Kumiko Hara, and the staff of Yamaguchi University Center for Gene Research for technical assistance.

### References

- Maggi E, Biswas P, Del Prete G, et al. Accumulation of Th-2-like helper T cells in the conjunctiva of patients with vernal conjunctivitis. *J Immunol.* 1991;146:1169-1174.
- Fujishima H, Takeuchi T, Shinozaki N, Saito I, Tsubota K. Measurement of IL-4 in tears of patients with seasonal allergic conjunctivitis and vernal keratoconjunctivitis. *Clin Exp Immunol.* 1995;102:395-398.
- Uchio E, Ono SY, Ikezawa Z, Ohno S. Tear levels of interferon-gamma, interleukin (IL) -2, IL-4 and IL-5 in patients with vernal keratoconjunctivitis, atopic keratoconjunctivitis and allergic conjunctivitis. *Clin Exp Allergy.* 2000;30:103-109.
- Leonardi A, Borghesan F, DePaoli M, Plebani M, Secchi AG. Procollagens and inflammatory cytokine concentrations in tarsal and limbal vernal keratoconjunctivitis. *Exp Eye Res.* 1998;67:105-112.
- Leonardi A, Brun P, Tavolato M, et al. Growth factors and collagen distribution in vernal keratoconjunctivitis. *Invest Ophthalmol Vis Sci.* 2000;41:4175-4181.
- Abu el-Asrar AM, Geboes K, al-Kharashi SA, et al. An immunohistochemical study of collagens in trachoma and vernal keratoconjunctivitis. *Eye.* 1998;12:1001-1006.
- Abu El-Asrar AM, Meersschaert A, Al-Kharashi SA, Missotten L, Geboes K. Immuno-histochemical evaluation of conjunctival remodelling in vernal keratoconjunctivitis. *Eye.* 2003;17:767-771.
- Leonardi A, Abatangelo G, Cortivo R, Secchi AG. Collagen types I and III in giant papillae of vernal keratoconjunctivitis. *Br J Ophthalmol.* 1995;79:482-485.
- Fujitsu Y, Fukuda K, Kimura K, et al. Protection of human conjunctival fibroblasts from NO-induced apoptosis by interleukin-4 or interleukin-13. *Invest Ophthalmol Vis Sci.* 2005;46:797-802.
- Fujitsu Y, Fukuda K, Kumagai N, Nishida T. IL-4-induced cell proliferation and production of extracellular matrix proteins in human conjunctival fibroblasts. *Exp Eye Res.* 2003;76:107-114.
- Abu El-Asrar AM, Van Aelst I, Al-Mansouri S, et al. Gelatinase B in vernal keratoconjunctivitis. *Arch Ophthalmol.* 2001;119:1505-1511.
- Kumagai N, Yamamoto K, Fukuda K, et al. Active matrix metalloproteinases in the tear fluid of individuals with vernal keratoconjunctivitis. *J Allergy Clin Immunol.* 2002;110:489-491.
- Leonardi A, Brun P, Abatangelo G, Plebani M, Secchi AG. Tear levels and activity of matrix metalloproteinase (MMP)-1 and MMP-9 in vernal keratoconjunctivitis. *Invest Ophthalmol Vis Sci.* 2003;44:3052-3058.
- Lijnen HR, Arza B, Van Hoef B, Collen D, Declerck PJ. Inactivation of plasminogen activator inhibitor-1 by specific proteolysis with stromelysin-1 (MMP-3). *J Biol Chem.* 2000;275:37645-37650.
- Murphy G, Cockett MI, Stephens PE, Smith BJ, Docherty AJ. Stromelysin is an activator of procollagenase: a study with natural and recombinant enzymes. *Biochem J.* 1987;248:265-268.
- Tuter G, Kurtis B, Serdar M, et al. Effects of phase I periodontal treatment on gingival crevicular fluid levels of matrix metalloproteinase-3 and tissue inhibitor of metalloproteinase-1. *J Clin Periodontol.* 2005;32:1011-1015.

17. van Meurs J, van Lent P, Holthuysen A, et al. Active matrix metalloproteinases are present in cartilage during immune complex-mediated arthritis: a pivotal role for stromelysin-1 in cartilage destruction. *J Immunol.* 1999;163:5633-5639.
18. Beaudoux JL, Giral P, Bruckert E, et al. Serum matrix metalloproteinase-3 and tissue inhibitor of metalloproteinases-1 as potential markers of carotid atherosclerosis in infraclinical hyperlipidemia. *Atherosclerosis.* 2003;169:139-146.
19. Nikkola J, Vihinen P, Vlaykova T, et al. High expression levels of collagenase-1 and stromelysin-1 correlate with shorter disease-free survival in human metastatic melanoma. *Int J Cancer.* 2002;97:432-438.
20. Meller D, Li DQ, Tseng SC. 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.* 2000;41:2922-2929.
21. Keane-Myers AM, Miyazaki D, Liu G, et al. Prevention of allergic eye disease by treatment with IL-1 receptor antagonist. *Invest Ophthalmol Vis Sci.* 1999;40:3041-3046.
22. Xue ML, Wakefield D, Willcox MD, et al. Regulation of MMPs and TIMPs by IL-1 $\beta$  during corneal ulceration and infection. *Invest Ophthalmol Vis Sci.* 2003;44:2020-2025.
23. Fukuda K, Kumagai N, Yamamoto K, et al. Potentiation of lipopolysaccharide-induced chemokine and adhesion molecule expression in corneal fibroblasts by soluble CD14 or LPS-binding protein. *Invest Ophthalmol Vis Sci.* 2005;46:3095-3101.
24. Bond M, Chase AJ, Baker AH, Newby AC. Inhibition of transcription factor NF- $\kappa$ B reduces matrix metalloproteinase-1, -3 and -9 production by vascular smooth muscle cells. *Cardiovasc Res.* 2001;50:556-565.
25. Quinones S, Buttice G, Kurkinen M. Promoter elements in the transcriptional activation of the human stromelysin-1 gene by the inflammatory cytokine, interleukin 1. *Biochem J.* 1994;302:471-477.
26. Noguchi K, Shitashige M, Watanabe H, Murota S, Ishikawa I. Interleukin-4 and interferon- $\gamma$  inhibit prostaglandin production by interleukin-1 $\beta$ -stimulated human periodontal ligament fibroblasts. *Inflammation.* 1999;23:1-13.
27. Domeij H, Yucel-Lindberg T, Modeer T. Signal pathways involved in the production of MMP-1 and MMP-3 in human gingival fibroblasts. *Eur J Oral Sci.* 2002;110:302-306.
28. Hayashi Y, Kobayashi M, Kuwata H, et al. Interferon- $\gamma$  and interleukin 4 inhibit interleukin 1 $\beta$ -induced delayed prostaglandin E<sub>2</sub> generation through suppression of cyclooxygenase-2 expression in human fibroblasts. *Cytokine.* 2000;12:603-612.
29. Sugiyama E, Taki H, Kuroda A, et al. Interleukin-4 inhibits prostaglandin E<sub>2</sub> production by freshly prepared adherent rheumatoid synovial cells via inhibition of biosynthesis and gene expression of cyclo-oxygenase II but not of cyclo-oxygenase I. *Ann Rheum Dis.* 1996;55:375-382.
30. Leonardi A, Cortivo R, Fregona I, et al. Effects of Th2 cytokines on expression of collagen, MMP-1, and TIMP-1 in conjunctival fibroblasts. *Invest Ophthalmol Vis Sci.* 2003;44:183-189.
31. Dean DD, Martel-Pelletier J, Pelletier JP, Howell DS, Woessner JF Jr. Evidence for metalloproteinase and metalloproteinase inhibitor imbalance in human osteoarthritic cartilage. *J Clin Invest.* 1989;84:678-685.
32. Ihn H, Yamane K, Asano Y, Kubo M, Tamaki K. IL-4 up-regulates the expression of tissue inhibitor of metalloproteinase-2 in dermal fibroblasts via the p38 mitogen-activated protein kinase dependent pathway. *J Immunol.* 2002;168:1895-1902.
33. Fukuda K, Kumagai N, Fujitsu Y, Nishida T. Fibroblasts as local immune modulators in ocular allergic disease. *Allergol Int.* 2006;55:121-129.
34. Anwar AR, Moqbel R, Walsh GM, Kay AB, Wardlaw AJ. Adhesion to fibronectin prolongs eosinophil survival. *J Exp Med.* 1993;177:839-843.
35. Bianchine PJ, Burd PR, Metcalfe DD. IL-3-dependent mast cells attach to plate-bound vitronectin: demonstration of augmented proliferation in response to signals transduced via cell surface vitronectin receptors. *J Immunol.* 1992;149:3665-3671.
36. Walsh GM, Wardlaw AJ. Dexamethasone inhibits prolonged survival and autocrine granulocyte-macrophage colony-stimulating factor production by human eosinophils cultured on laminin or tissue fibronectin. *J Allergy Clin Immunol.* 1997;100:208-215.
37. Higashimoto I, Chihara J, Kakazu T, et al. Adhesion to fibronectin augments eosinophil radical oxygen products. *Int Arch Allergy Immunol.* 1995;108(suppl 1):48-49.
38. Higashimoto I, Chihara J, Kawabata M, Nakajima S, Osame M. Adhesion to fibronectin regulates expression of intercellular adhesion molecule-1 on eosinophilic cells. *Int Arch Allergy Immunol.* 1999;120(suppl 1):34-37.
39. Kruger-Krasagakes S, Grutzkau A, Krasagakis K, Hoffmann S, Henz BM. Adhesion of human mast cells to extracellular matrix provides a co-stimulatory signal for cytokine production. *Immunology.* 1999;98:253-257.
40. Munoz NM, Rabe KF, Neeley SP, et al. Eosinophil VLA-4 binding to fibronectin augments bronchial narrowing through 5-lipoxygenase activation. *Am J Physiol.* 1996;270:L587-L594.
41. Vaday GG, Lider O. Extracellular matrix moieties, cytokines, and enzymes: dynamic effects on immune cell behavior and inflammation. *J Leukoc Biol.* 2000;67:149-159.
42. Roberts R, Gallagher J, Spooncer E, et al. Heparan sulphate bound growth factors: a mechanism for stromal cell mediated haemopoiesis. *Nature.* 1988;332:376-378.
43. Yamaguchi Y, Mann DM, Ruoslahti E. Negative regulation of transforming growth factor-beta by the proteoglycan decorin. *Nature.* 1990;346:281-284.
44. Bond M, Baker AH, Newby AC. Nuclear factor  $\kappa$ B activity is essential for matrix metalloproteinase-1 and -3 upregulation in rabbit dermal fibroblasts. *Biochem Biophys Res Commun.* 1999;264:561-567.
45. Frisch SM, Ruley HE. Transcription from the stromelysin promoter is induced by interleukin-1 and repressed by dexamethasone. *J Biol Chem.* 1987;262:16300-16304.
46. Stein B, Baldwin AS Jr, Ballard DW, et al. Cross-coupling of the NF- $\kappa$ B p65 and Fos/Jun transcription factors produces potentiated biological function. *EMBO J.* 1993;12:3879-3891.
47. Xu J, Zutter MM, Santoro SA, Clark RA. A three-dimensional collagen lattice activates NF- $\kappa$ B in human fibroblasts: role in integrin  $\alpha_2$  gene expression and tissue remodeling. *J Cell Biol.* 1998;140:709-719.
48. Jenkins K, Javadi M, Borghaei RC. Interleukin-4 suppresses IL-1-induced expression of matrix metalloproteinase-3 in human gingival fibroblasts. *J Periodontol.* 2004;75:283-291.
49. Donnelly RP, Crofford LJ, Freeman SL, et al. Tissue-specific regulation of IL-6 production by IL-4: differential effects of IL-4 on nuclear factor- $\kappa$ B activity in monocytes and fibroblasts. *J Immunol.* 1993;151:5603-5612.
50. Borghaei RC, Rawlings PL Jr, Mochan E. Interleukin-4 suppression of interleukin-1-induced transcription of collagenase (MMP-1) and stromelysin 1 (MMP-3) in human synovial fibroblasts. *Arthritis Rheum.* 1998;41:1398-1406.
51. Fukuda K, Fujitsu Y, Kumagai N, Nishida T. Characterization of the interleukin-4 receptor complex in human corneal fibroblasts. *Invest Ophthalmol Vis Sci.* 2002;43:183-188.
52. Gingras S, Simard J, Groner B, Pfitzner E. p300/CBP is required for transcriptional induction by interleukin-4 and interacts with Stat6. *Nucleic Acids Res.* 1999;27:2722-2729.
53. Smits PH, de Wit L, van der Eb AJ, Zantema A. The adenovirus E1A-associated 300 kDa adaptor protein counteracts the inhibition of the collagenase promoter by E1A and represses transformation. *Oncogene.* 1996;12:1529-1535.
54. Wadgaonkar R, Phelps KM, Haque Z, et al. CREB-binding protein is a nuclear integrator of nuclear factor- $\kappa$ B and p53 signaling. *J Biol Chem.* 1999;274:1879-1882.
55. Sheppard KA, Rose DW, Haque ZK, et al. Transcriptional activation by NF- $\kappa$ B requires multiple coactivators. *Mol Cell Biol.* 1999;19:6367-6378.
56. Horvai AE, Xu L, Korzus E, et al. Nuclear integration of JAK/STAT and Ras/AP-1 signaling by CBP and p300. *Proc Natl Acad Sci USA.* 1997;94:1074-1079.