

IL-4 and IL-13 Regulation of ICAM-1 Expression and Eosinophil Recruitment in *Onchocerca volvulus* Keratitis

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PURPOSE. The presence of eosinophilic granulocytes in ocular tissue is a hallmark of the host response to environmental and parasite allergens. Using a mouse model of *Onchocerca volvulus*-mediated keratitis (river blindness), the present study examined the role of the cytokines interleukin (IL)-4 and IL-13 in regulating recruitment of eosinophils to the cornea through expression of intercellular cell adhesion molecule (ICAM)-1.

METHODS. C57BL/6 mice received an intrastromal injection of recombinant IL-4 and IL-13 (rIL-4 and IL-13) or were immunized by subcutaneous injection prior to receiving an intrastromal injection of a soluble *O. volvulus* extract. Expression of ICAM-1 and recruitment of eosinophils to the cornea were monitored by immunohistochemistry.

RESULTS. Expression of ICAM-1 was elevated after injection of rIL-4 or IL-13 together with recombinant tumor necrosis factor (rTNF)- α . Conversely, expression of ICAM-1 in *O. volvulus*-mediated keratitis was significantly reduced after subconjunctival injection of a monoclonal antibody (mAb) to IL-4 or IL-13. In addition, combined in vivo neutralization of IL-4 and IL-13 inhibited recruitment of eosinophils, but not of neutrophils, to the corneal stroma.

CONCLUSIONS. These findings demonstrate that expression of ICAM-1 and recruitment of eosinophils to the cornea are tightly regulated by IL-4 and IL-13, and indicate that these cytokines are a potential target for immune intervention in ocular allergy and parasitic infections of the eye. (*Invest Ophthalmol Vis Sci* 2002;43:2992-2997)

A characteristic feature of the host response to environmental and parasite allergens is the presence of eosinophilic granulocytes in affected tissues. Eosinophils are also associated with ocular allergy, including conjunctivitis and keratoconjunctivitis,¹ animal models of allergic conjunctivitis,^{2,3} and parasite infections of the eye, including ocular onchocerciasis (river blindness).⁴

The cytotoxic effect of eosinophils is due primarily to cationic proteins in secondary granules, especially eosinophil ma-

ior basic protein (MBP).^{5,6} Trocme et al.^{7,8} demonstrated that eosinophils and eosinophil granule proteins are present in conjunctival tissue from atopic individuals and that eosinophil MBP has a direct cytotoxic effect on corneal epithelial cells that can inhibit corneal wound healing.^{9,10}

Eosinophil recruitment from blood vessels into ocular tissues requires expression of adhesion molecules on vascular endothelial cells.⁶ For example, recruitment of eosinophils is associated with an upregulation of intercellular adhesion molecule (ICAM)-1 in vernal keratoconjunctivitis.¹¹ Our previous studies using a mouse model of parasite allergen-induced keratitis demonstrated that recruitment of eosinophils to the cornea is also mediated by ICAM-1 on limbal vessels.¹² The rationale for the present study was to determine the role of interleukin (IL)-4 and IL-13 in *Onchocerca volvulus*-mediated keratitis in regulating expression of ICAM-1 and infiltration of eosinophils into the corneal stroma. Both cytokines are closely linked in humans on chromosome 5 and in mice on chromosome 11, and they show approximately 30% amino acid sequence homology.¹³⁻¹⁵ These cytokines are produced by type 2 T helper (Th2) cells and mast cells in response to parasite antigens in *O. volvulus*-mediated keratitis.^{16,17} Furthermore, receptors for IL-4 and IL-13 are expressed on vascular endothelial cells in vitro, and adhesion molecule expression can be regulated by recombinant IL-4.¹⁸⁻²⁰ In the present study, we used recombinant IL-4 and IL-13, IL-4 gene knockout mice, and local cytokine neutralization to determine the role of these cytokines in expression of ICAM-1 in vivo and on recruitment of eosinophils to the cornea.

MATERIALS AND METHODS

Preparation of *O. volvulus* Antigens

O. volvulus worms were recovered from subcutaneous nodules that had been surgically removed from infected patients and sent to us from Cameroon. Parasites were recovered after digestion with collagenase (Sigma, St. Louis, MO), as previously described,¹⁶ and homogenized in HBSS with a mortar and pestle. Insoluble material was removed by centrifugation, and the concentration of protein was determined by standard methods (Bio-Rad, Richmond, CA). Antigen was then aliquoted and stored at -80°C until use.

Immunization and Intracorneal Injections of *O. volvulus* Antigens

C57BL/6 mice and IL-4 gene knockout mice on a C57BL/6 background were obtained from The Jackson Laboratory (Bar Harbor, ME). Animals were given three weekly subcutaneous (SC) immunizations with 10 μg *O. volvulus* antigens in a 1:1 ratio with adjuvant containing 10% squalene (Aldrich Chemical, Milwaukee, WI), 0.4% Tween 80 (Fisher, Fair Lawn, NJ), and 1% pluronic acid (BASF, Parsippany, NJ).¹⁶

For injection into the corneal stroma, mice were anesthetized by intraperitoneal injection of 200 μL of a 1.2% solution of 2,2,2-tribromoethanol (TBE; Aldrich Chemical) containing 2.5% 2-methyl-2-butanol (tertiary amyl alcohol; Aldrich) dissolved in dH_2O .²¹ The corneal

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epithelial layer was scarified with a 30-gauge needle, and 10 μg of *O. volvulus* antigens in 5 μL were injected into the corneal stroma with a 33-gauge needle attached to a syringe (Hamilton, Reno, NV). Animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Injections of Anti-IL-4 mAb and Soluble IL-13 Receptor α 2-Fc Fusion Protein into the Subconjunctival Space

Antibody injected into the subconjunctival pocket has been shown to diffuse into the corneal stroma.^{22,23} To block the activity of IL-4 on resident corneal cells and on the vascular endothelium, 25 μg anti-IL-4 mAb (rat IgG1, 11B11; BD PharMingen, San Diego, CA) in 5 μL of sterile HBSS were injected into the subconjunctival space of the one eye, and 25 μg of control rat IgG (Sigma) was injected into the subconjunctival space of the contralateral eye. To block the activity of IL-13 on resident corneal cells and on the vascular endothelium, 25 μg of soluble IL-13 receptor α 2-Fc fusion protein (sIL-13R α 2-Fc; mA25.IgG2am; kindly provided by Deborah Donaldson, Genetics Institute, Wyeth Ayerst Inc., Andover, MA) in 5 μL sterile HBSS were injected into the subconjunctival space of the one eye, and control human IgG (mIgG2am; also provided by Deborah Donaldson) was injected into the subconjunctival space of the contralateral eye. For neutralization of both IL-4 and IL-13, 25 μg anti-IL-4 mAb and 25 μg sIL-13R α 2-Fc in 5 μL sterile HBSS was injected into the subconjunctival space of one eye; 25 μg control rat IgG and 25 μg control human IgG were injected into the subconjunctival space of the contralateral eye. These subconjunctival injections were given 1 hour before intrastromal injection of parasite antigens using a 33-gauge needle attached to a syringe (Hamilton). Additional subconjunctival injections were given 24 and 48 hours later. In previous studies, we found that subconjunctival Ab injections has no apparent effect on the contralateral eye.¹²

Injections of rIL-4, rIL-13, and rTNF- α into the Corneal Stroma

For injection of recombinant cytokines into the corneal stroma, naïve mice were anesthetized by intraperitoneal injection of 200 μL TBE. The corneal epithelial layer was scarified with a 30-gauge needle, and 5 ng rIL-4 (BD PharMingen), rIL-13 (rCHO mIL-13; provided by Deborah Donaldson), and/or TNF- α (BD PharMingen) in 5 μL were injected into the corneal stroma with a 33-gauge needle attached to a syringe (Hamilton).

Detection of Neutrophils and Eosinophils

For immunohistochemical staining, enucleated eyes were snap-frozen in liquid nitrogen, stored at -70°C , and 5 μm sections were air dried overnight and stored at -20°C . Sections were fixed for 10 minutes in acetone at -20°C . Slides were air dried and rehydrated in PBS (pH 7.4). Primary Abs were diluted in PBS containing 1% fetal calf serum and incubated for 2 hours at room temperature. Five micrometer sections were immunostained with rabbit antisera to eosinophil major basic protein (MBP; kindly provided by Jamie Lee, Mayo Clinic, Scottsdale, AZ), diluted 1:5000 to detect eosinophils, as described previously.^{25,26} FITC-conjugated goat anti-rabbit IgG (Caltag Laboratories, Burlingame, CA), diluted 1:200, was used as a secondary Ab and incubated for 45 minutes. Neutrophils were detected using the rat mAb NIMP-R/14 (kindly supplied by Achim Hoerauf, Bernhard Nocht Institute of Tropical Medicine, Hamburg, Germany) diluted 1:100, followed by FITC-conjugated rabbit anti-rat IgG (Caltag Laboratories) diluted 1:200 for 45 minutes. Stained sections were washed in PBS and coverslipped with Vectashield Mounting Medium (Vector Laboratories, Burlingame, CA) to inhibit quenching. Positively stained cells were examined by fluorescence microscopy. Additional sections were stained with modified Harris' hematoxylin (Richard-Allen, Kalamazoo, MI) and examined by bright field and fluorescence microscopy. Total eosinophils per 5- μm section were determined by direct counting. Because neutrophil infil-

tration into the cornea was heavy, neutrophil numbers were determined by capturing three representative images of the peripheral and central cornea at a magnification of 200 \times , with a digital camera (model DC330; DAGE-MTI Inc., Michigan City, IN) and image management software (Scion Image Software, ver. 1.62c; National Institutes of Health, Bethesda, MD, modified by Scion Corp, Frederick, MD). The average number was used as a data point for one cornea.

Evaluation of Staining Intensity

For immunostaining of ICAM-1, sections were prepared as described earlier and stained with rat mAb against ICAM-1 (KAT-1; Caltag Laboratories). Primary Abs were diluted in PBS containing 1% fetal calf serum and incubated for 2 hours at room temperature. FITC anti-rat IgG diluted 1:200 was used as a secondary Ab and incubated for 45 minutes. Stained sections were washed in PBS and coverslipped with mounting medium (Vectashield; Vector Laboratories) to inhibit quenching.

Evaluation of expression of FITC-stained adhesion molecules on limbal vessels was based on the method for quantifying platelet-endothelial cell adhesion molecule (PECAM-1) expression, as previously described.^{12,24} After immunostaining for ICAM-1, images of limbal vessels were captured as described earlier for neutrophils. To evaluate the relative fluorescence intensity, the mean brightness value of the green channel of stained vessel walls from 5- μm corneal sections were determined with image-analysis software (Photoshop 5.0; Adobe Systems Inc., Mountain View, CA) by using the lasso function to highlight the vessel wall. Three stained vessels, each taken from three separate sections of the same cornea, were analyzed. The background reading in unstained areas of the cornea was subtracted from these values, and the mean \pm SE of the fluorescence intensity for each vessel was estimated. Data were then presented as the mean fluorescence intensity.

Statistics

Statistical significance was determined with a paired Student's *t*-test, except for experiments involving IL-4 gene knockout mice, which used an unpaired Student's *t*-test (Prism software; GraphPad, San Diego, CA). $P < 0.05$ was considered to be significant.

RESULTS

Regulation of Expression of ICAM-1 on Limbal Vessels by rIL-4 and IL-13

Receptors for IL-4 and IL-13 have been shown to be expressed on vascular endothelial cells *in vitro*.¹⁸⁻²⁰ To determine whether IL-4 and IL-13 regulate expression of ICAM-1 on limbal vascular endothelial cells, we injected these cytokines into normal mouse corneas in the presence or absence of TNF- α and monitored ICAM-1 by immunohistochemistry. Evaluation of staining intensity was performed by digitized image analysis, as described in the Methods section. After 6 hours, eyes were enucleated, and 5- μm corneal sections were immunostained with mAb for ICAM-1.

Figure 1 shows that expression of ICAM-1 was elevated on the limbal vessels after injection of HBSS compared with expression in naïve corneas, suggesting that trauma alone increases expression of ICAM-1. Injection of rIL-4 and/or IL-13 or rTNF- α alone did not increase expression of ICAM-1 compared with HBSS-challenged eyes.

In contrast, the combination of rTNF- α and either rIL-4 or IL-13 induced a significant increase in expression of ICAM-1 compared with corneas that received saline injections ($P = 0.003$) or cytokines in the absence of rTNF- α ($P = 0.008$). Combined injection of rIL-4 and IL-13 with rTNF- α did not increase expression of ICAM-1 above that of rIL-4 and rTNF- α or rIL-13 and rTNF- α . These data indicate that IL-4 and IL-13 in

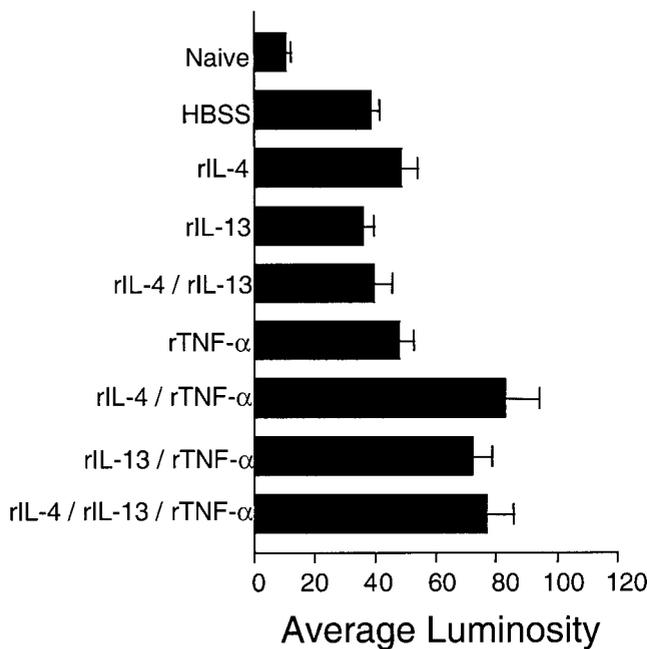


FIGURE 1. Effect of rIL-4, rIL-13, and rTNF- α on expression of ICAM-1 in the limbal vessels. C57BL/6 mice received an intrastromal injection of recombinant cytokines. After 6 hours, eyes were snap frozen, 5- μ m sections were immunostained with mAb against ICAM-1, and limbal vessels were visualized with FITC-labeled anti-rat IgG. Relative expression of ICAM-1 was assessed by image analysis as described in Methods. Data represent mean fluorescence intensity \pm SEM of six eyes per cytokine combination. $P = 0.017$ for TNF- α versus TNF- α IL-4, $P = 0.013$ for TNF- α versus TNF- α IL-13, and $P = 0.022$ for TNF- α versus TNF- α , IL-4, and IL-13.

the presence of TNF- α significantly increase expression of ICAM-1 on limbal vessels.

The Effect of Endogenous IL-4 and IL-13 on Expression of ICAM-1

To determine the effect of endogenous IL-4 and IL-13 on expression of ICAM-1 on limbal vessels, C57BL/6 mice were immunized subcutaneously and *O. volvulus* antigens were injected into the corneal stroma. One hour before injection of the *O. volvulus* antigens, anti-IL-4 mAb, sIL-13R α 2-Fc, or both were injected into the subconjunctival space of one eye. Control rat IgG, human IgG, or both, were injected into the subconjunctival space of the contralateral eye. Subsequent subconjunctival treatments were administered at 24 and 48 hours after intrastromal challenge. After 24 or 72 hours, eyes were enucleated, and expression of ICAM-1 on limbal vessels was determined by immunohistochemistry.

As shown in Figure 2A, expression of ICAM-1 was significantly reduced after subconjunctival injection of anti-IL-4 at 24 and 72 hours after intrastromal challenge compared with the contralateral eyes treated with the control rat IgG ($P = 0.004$ and 0.01, at 24 and 72 hours, respectively). Similarly, sIL-13R α 2-Fc significantly reduced expression of ICAM-1 at 24 hours ($P = 0.01$) but not at 72 hours (Fig. 2B). Combined injection of anti-IL-4 and sIL-13R α 2-Fc significantly reduced expression of ICAM-1 at both time points ($P = 0.009$ and 0.02, at 24 and 72 hours, respectively), but no additive effect was noted (Fig. 2C). This last observation supports our findings from Figure 1, which suggest that coinjection of rIL-4 and IL-13 does not increase limbal expression of ICAM-1 in a cumulative fashion.

As an additional experimental approach to examining the role of IL-4 in limbal expression of ICAM-1, IL-4 gene knockout mice (C57BL/6 background) were immunized subcutaneously and *O. volvulus* antigens were injected intrastromally. Expression of ICAM-1 on limbal vessels was then determined in comparison with immunocompetent C57BL/6 control mice. Figure 2D demonstrates that in IL-4-deficient mice, expression of ICAM-1 was significantly impaired compared with control animals ($P < 0.0001$ and 0.0003, at 24 and 72 hours, respectively). Representative images are shown in Figure 3.

Taken together with the observations on neutralization of IL-4 and IL-13, these data indicate that IL-4 has a more important role than IL-13 in regulating expression of ICAM-1 in the limbus.

The Effect of Endogenous IL-4 and IL-13 on Recruitment of Eosinophils into the Cornea

The acute phase of the inflammatory response in *O. volvulus*-mediated keratitis is characterized by a biphasic granulocyte infiltration, with neutrophils predominating in the first 24 hours after injection of *O. volvulus* antigens, and eosinophils predominating after 72 hours.^{26,28} Our previous studies also showed that gene expression of IL-4 and IL-13 is elevated after injection of *O. volvulus* antigens.^{16,17} To determine the effect

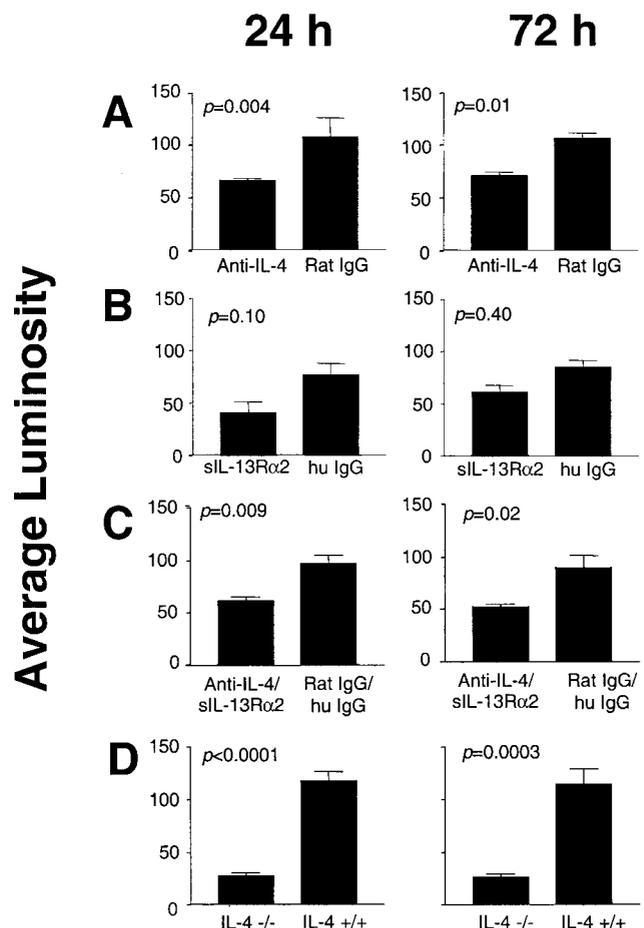


FIGURE 2. Effect of IL-4 and IL-13 on ICAM-1 expression. C57BL/6 mice and IL-4^{-/-} mice were immunized and injected with parasite antigens as described in the Methods. After 24 hours and 72 hours, eyes were removed, snap-frozen, and corneal sections were immunostained with Ab to ICAM-1. Average luminosity was calculated as described in Methods. Data represent mean and SEM of 5 mice per group. The experiment was repeated with similar results.

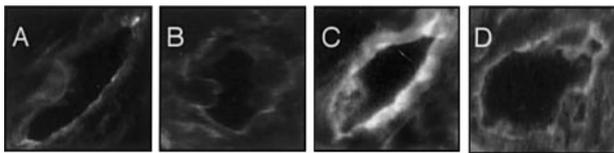


FIGURE 3. Expression of ICAM-1 in the limbal vessels of C57BL/6 and IL-4^{-/-} mice. Representative C57BL/6 and IL-4^{-/-} corneas were immunostained with anti-ICAM-1 mAb. Limbal vessels were visualized by fluorescence microscopy after incubation with secondary FITC-labeled anti-rat IgG. Sections are shown from eyes enucleated 24 hours after injection of parasite antigens. (A) Naïve C57BL/6 mouse. (B) IL-4^{-/-} mouse after intrastromal injection of *O. volvulus* antigens. (C) C57BL/6 mouse after injection of *O. volvulus* antigens and treatment with control rat IgG and human IgG. (D) C57BL/6 mouse after injection of *O. volvulus* antigens and treatment with anti-IL-4 mAb and siL-13Rα2-Fc. Original magnification, ×400.

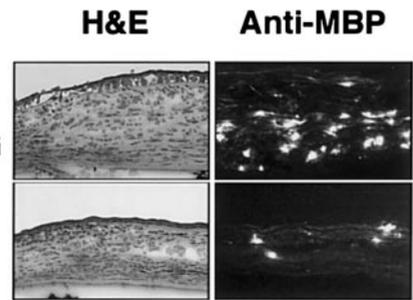


FIGURE 5. Eosinophils in the corneal stroma of C57BL/6 mice. Immunized mice received intrastromal injection of *O. volvulus* antigens and subconjunctival injection of anti-IL-4 mAb and siL-13Rα2-Fc. Representative corneas were immunostained with anti-MBP mAb. Eosinophils were visualized by bright-field (left) or fluorescence (right) microscopy after incubation with secondary FITC anti-rat IgG. Sections are from the central region of the cornea and show the external epithelium, stroma, and corneal endothelium. Original magnification, ×200.

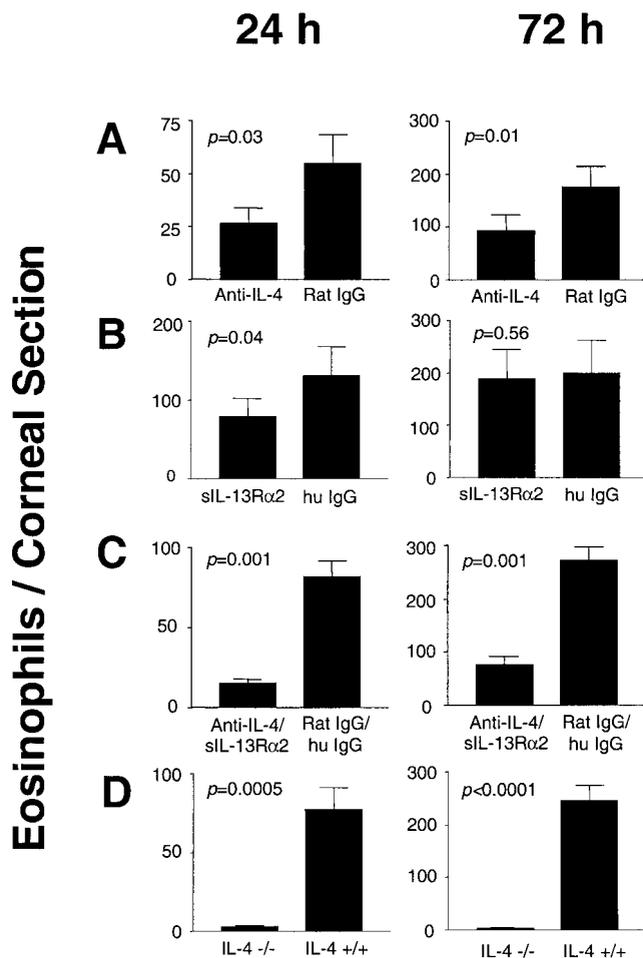


FIGURE 4. Effect of IL-4 and IL-13 ablation on recruitment of eosinophils to the cornea. C57BL/6 and IL-4^{-/-} mice were immunized with subcutaneous injection of *O. volvulus* antigens and subsequently the same parasite antigens were injected into the corneal stroma. IL-4 and IL-13 depletion in C57BL/6 mice was performed as described in the legend to Figure 2. After 24 or 72 hours, eyes were enucleated, and sections were immunostained with mAb against the eosinophil-specific granule MBP. Infiltration of eosinophils into the cornea after (A) local neutralization of IL-4, (B) local neutralization of IL-13, (C) conneutralization of IL-4 and IL-13, and (D) intrastromal challenge in IL-4^{-/-} C57BL/6 mice. Results are the mean ± SEM of five eyes per group. These data are representative of two identical experiments.

of IL-4 and IL-13 on recruitment of eosinophils to the cornea, immunized C57BL/6 mice received intrastromal injection of *O. volvulus* antigens after subconjunctival injection with anti-IL-4 and/or siL-13Rα2-Fc. Subsequent subconjunctival treatments were administered at 24 and 48 hours after intrastromal challenge. After 24 or 72 hours, eyes were removed and 5-μm sections were immunostained with mAb to eosinophil major basic protein (MBP).

As shown in Figure 4A, recruitment of eosinophils to the corneal stroma in anti-IL-4-treated mice at 24 and 72 hours was significantly reduced compared with control eyes treated with rat IgG ($P = 0.03$ and 0.01 , at 24 and 72 hours, respectively). Similarly, siL-13Rα2-Fc inhibited recruitment of eosinophils to the cornea at 24 hours ($P = 0.04$), but not at 72 hours, compared with control eyes treated with human IgG (Fig. 4B). These findings are consistent with the effect of IL-13 ablation on expression of ICAM-1, described earlier. Figure 4C shows that when both IL-4 and IL-13 were neutralized, there was a highly significant reduction in recruitment of eosinophils at 24 and 72 hours ($P = 0.001$).

These findings are supported by the effect of IL-4 deficiency on recruitment of eosinophils to the cornea. As shown in Figure 4D, the number of eosinophils in the corneas of IL-4 gene knockout mice was reduced compared with the number in eyes of immunocompetent C57BL/6 mice. Representative images are shown in Figure 5. These observations agree with previous findings on IL-4-deficient mice on a 129Sv x C57BL/6 background.¹⁷

Taken together with the data presented in Figures 2 and 3, these findings are consistent with the notion that IL-4 and IL-13 mediate recruitment of eosinophils to the cornea by upregulating expression of ICAM-1.

The Effect of Endogenous IL-4 and IL-13 on Recruitment of Neutrophils into the Cornea

To determine whether IL-4 and IL-13 also regulate neutrophil recruitment into the cornea in *O. volvulus*-mediated keratitis, C57BL/6 mice were immunized and received an intrastromal injection of *O. volvulus* antigens, and an injection of anti-IL-4 and/or siL-13Rα2-Fc, as described earlier. After 24 or 72 hours, corneal sections were immunostained for neutrophils.

In contrast to eosinophils, the number of neutrophils in the cornea was not significantly different from the control number at either time point after neutralization of IL-4, IL-13, or both (Fig. 6).

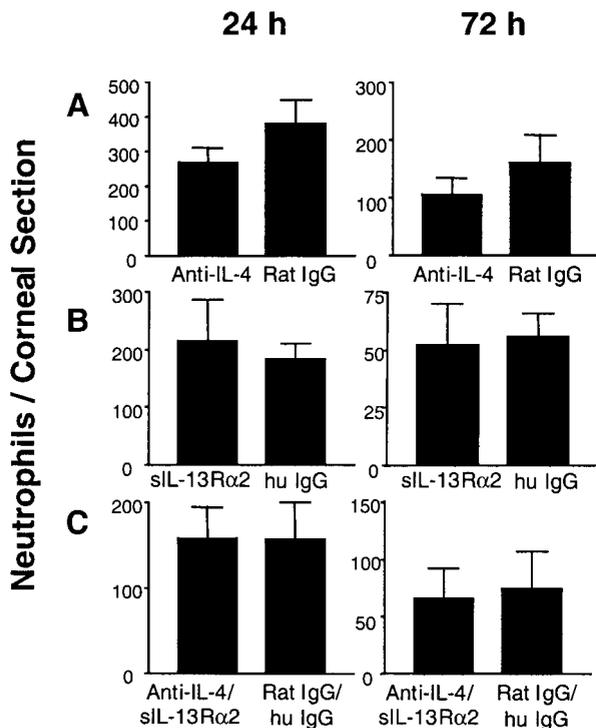


FIGURE 6. Effect of IL-4 and IL-13 ablation on recruitment of neutrophils to the cornea. C57BL/6 mice were injected subconjunctivally with anti-IL-4 mAb and/or sIL-13R α 2-Fc before intrastromal injection of parasite antigens, as described in the legend to Figure 2. Mice were killed either 24 or 72 hours after intracorneal injection, eyes were enucleated, and sections were immunostained with mAb (NIMP-R/14) against neutrophils. Infiltration of neutrophils into the cornea after (A) local neutralization of IL-4, (B) local neutralization of IL-13, and (C) neutralization of IL-4 and IL-13. Results are mean \pm SEM of five eyes per group. $P > 0.05$ in all cases. The data are representative of two identical experiments.

DISCUSSION

Although IL-4 and IL-13 are structurally and functionally related, they have distinct effects in allergic and parasitic diseases. For example, IL-13 receptor antagonists can block symptoms of allergic asthma and parasite-induced disease, even in the presence of IL-4,²⁹⁻³¹ and can block fibrosis in parasite-infected animals.³²

The structural basis for the overlapping functions of IL-4 and IL-13 is a shared receptor subunit, IL-4R α .³³ IL-4 binds to 4R α , and this complex interacts with IL-13R α to yield a high-affinity receptor that mediates signal transduction through a JAK2/Stat6 signaling pathway.^{34,35} Similarly, IL-13 initially binds to IL-13R α and then interacts with IL-4R α to form a functional receptor that activates a similar intracellular signaling pathway.³⁴

In the present study, we used two complementary approaches to assess the role of IL-4 and IL-13 on the expression of ICAM-1 in the limbal vessels. First, we demonstrated that intrastromal injection of recombinant murine IL-4 and IL-13 increases expression of ICAM-1 on limbal vessels; and secondly, we showed that neutralization of IL-4 and IL-13 activity by Ab treatment or receptor antagonists significantly reduced expression of ICAM-1 on limbal vessels in *O. volvulus*-induced keratitis, and inhibited recruitment of eosinophils to the cornea.

Our findings indicate that IL-4 has a more important regulatory role than IL-13 on expression of ICAM-1 in vivo and on

recruitment of eosinophils to the cornea. Although neutralization of IL-4 and IL-13 had a similar effect on expression of ICAM-1 and recruitment of eosinophils 24 hours after intrastromal injection, only the anti-IL-4-treated eyes demonstrated a significant reduction in these parameters at 72 hours. Furthermore, when both cytokines were neutralized, there was an additive effect on recruitment of eosinophils at 24 hours, but not at 72 hours, when the effect on recruitment of eosinophils was similar to that of IL-4 neutralization alone. Although we have yet to determine the basis for this observation, it is possible that there was a difference in regulation of the expression of IL-4 and IL-13 receptors on vascular endothelial cells at these time points.

We also found that there was no additive effect of rIL-4 and IL-13 on expression of ICAM-1 compared with that of injection of individual cytokines. However, we observed that rTNF- α injected with either rIL-4 or -13 was essential to induction of a significant increase in expression of ICAM-1, and that rIL-4 and IL-13 in this case had similar effects. Our finding that TNF- α is also essential for expression of ICAM-1 is similar to reports that eotaxin production by corneal fibroblasts in vitro also requires TNF- α .^{27,36} The mechanistic relationship between TNF- α , IL-4, and IL-13 has yet to be determined in *O. volvulus* keratitis. However, TNF- α upregulates IL-4 and IL-13 receptor expression on vascular endothelial cells in vitro,³⁵ and a similar mechanism may have been involved in the current study.

Although we have yet to determine the basis for the observed difference in effectiveness of IL-13 versus IL-4 neutralization at 72 hours, it is likely to be related to the imbalance of IL-4 and -13 receptor expression on vascular endothelial cells. As baseline expression of the IL-13 binding subunit on vascular endothelial cells is three times higher than that of IL-4R α ,^{35,37} indicating that these cells in limbal vessels may be more sensitive to low levels of IL-13.

Our findings differed from those of an earlier report in which expression of ICAM-1 on corneal epithelial cell cultures was regulated by IFN- γ and TNF- α , but not by IL-4.³⁸ Although yet to be resolved, this discrepancy may be due to the differential effect of regulatory cytokines on epithelial cells versus vascular endothelial cells or to differences between in vivo and in vitro experimental approaches.

Regarding neutrophil infiltration, the present study supports our previous observations that ICAM-1 has no significant effect on neutrophil recruitment to the cornea in our model of *O. volvulus*-mediated keratitis. Despite significant reductions in expression of ICAM-1 after neutralization of either IL-4 or IL-13, the number of neutrophils in the corneal stroma remained unchanged. Although neutrophils express leukocyte function-association antigen (LFA)-1, which is a ligand for ICAM-1, and although ICAM-1 has been shown to mediate migration of neutrophils to other tissues, including the skin and peritoneal cavity, we have shown that PECAM-1 is the primary cellular adhesion molecule that governs recruitment of neutrophils to the cornea in *O. volvulus*-mediated keratitis.¹² In a manner similar to IL-4 and IL-13 regulation of ICAM-1, IFN- γ was demonstrated by Tang and Hendricks²⁴ to regulate expression of PECAM-1 on limbal vessels in murine herpes simplex keratitis.

We have previously demonstrated that CD4⁺ T cells infiltrate the cornea in *O. volvulus* keratitis, and that *O. volvulus* antigens induce a predominant Th2 response, with elevated production of IL-4, IL-5, and IL-13,^{16,17} and CD4⁺ T cells are the most likely source of IL-4 and IL-13. The current findings add an important element to our understanding of the immunopathology of *O. volvulus*-mediated keratitis by demonstrating that a major function of IL-4 and IL-13 is regulation of ICAM-1, which then mediates infiltration of eosinophils to the corneal stroma.

In conclusion, we have demonstrated that blockade of IL-4 and IL-13-receptor interactions in vivo has a significant effect on infiltration of eosinophils into the cornea by inhibiting expression of ICAM-1 on limbal vessels. Furthermore, the pronounced effect of local neutralization of IL-4 and IL-13 by subconjunctival administration of Ab or receptor antagonist indicates that this may be a feasible approach to immunotherapy in ocular allergy, parasitic infections of the eye, or other disease processes in which these cytokines regulate infiltration of eosinophils into inflamed tissues.

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