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Distinct Roles for PECAM-1, ICAM-1, and VCAM-1 in Recruitment of Neutrophils and Eosinophils to the Cornea in Ocular Onchocerciasis (River Blindness)¹

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Infiltration of granulocytes into the transparent mammalian cornea can result in loss of corneal clarity and severe visual impairment. Since the cornea is an avascular tissue, recruitment of granulocytes such as neutrophils and eosinophils into the corneal stroma is initiated from peripheral (limbal) vessels. To determine the role of vascular adhesion molecules in this process, expression of platelet endothelial cell adhesion molecule 1 (PECAM-1), ICAM-1, and VCAM-1 on limbal vessels was determined in a murine model of ocular onchocerciasis in which Ags from the parasitic worm Onchocerca volvulus are injected into the corneal stroma. Expression of each of these molecules was elevated after injection of parasite Ags; however, PECAM-1 and ICAM-1 expression remained elevated from 12 h after injection until 7 days, whereas VCAM-1 expression was more transient, with peak expression at 72 h. Subconjunctival injection of Ab to PECAM-1 significantly inhibited neutrophil recruitment to the cornea compared with eyes injected with control Ab (p = 0.012). Consistent with this finding, corneal opacification was significantly diminished (p < 0.0001). There was no significant reduction in eosinophils. Conversely, subconjunctival injection of Ab to ICAM-1 did not impair neutrophil recruitment, but significantly inhibited eosinophil recruitment (p = 0.0032). Injection of Ab to VCAM-1 did not significantly inhibit infiltration of either cell type to the cornea. Taken together, these results demonstrate important regulatory roles for PECAM-1 and ICAM-1 in recruitment of neutrophils and eosinophils, respectively, to the cornea, and may indicate a selective approach to immune intervention. The Journal of Immunology, 2001, 166: 6795–6801.

Expression of adhesion molecules on vascular endothelial cells is essential for regulating leukocyte trafficking from blood vessels into tissues during an inflammatory response (1). Leukocytes first attach to vascular endothelial cells by low-affinity interactions with P- and E-selectin. Continued inflammatory stimuli in the tissue induce higher affinity interactions mediated by members of the Ig superfamily on vascular endothelial cells, including platelet endothelial cell adhesion molecule 1 (PECAM-1)⁴ (CD31), ICAM-1 (CD54), and VCAM-1 (CD106).

Our studies have focused on determining the molecular mechanisms underlying recruitment of neutrophils and eosinophils into the mammalian cornea in ocular onchocerciasis (river blindness). Using a murine model in which animals are immunized and injected with Ags extracted from the parasitic worm Onchocerca volvulus, we demonstrated that neutrophils and eosinophils infiltrate the cornea and induce severe corneal opacification and neovascularization (2, 3). Since the cornea is an avascular tissue, cellular infiltration originates from limbal vessels in the peripheral region of the cornea, and progresses toward the central cornea where the initial stimulus (injection of parasite Ags) is induced. Because the severity of the clinical response appears to be determined by the number of granulocytes that infiltrate the corneal stroma, identification of essential mediators of recruitment could indicate approaches to immunotherapy. Neutrophil recruitment to the cornea is dependent on expression of the murine IL-8 receptor CXC chemokine receptor 2 (4), whereas eosinophil recruitment is partially dependent on expression of eotaxin (5). Recruitment of both cell types to the cornea is also dependent on development of T cell and Ab responses (3, 6, 7).

Since extravasation from limbal vessels to the corneal stroma is likely to be a critical early event in recruitment of these cells, we examined the relative expression of PECAM-1, ICAM-1, and VCAM-1 on limbal vessels, and determined the role of these adhesion molecules in recruitment of neutrophils and eosinophils to the cornea.

Materials and Methods

Preparation of O. volvulus Ags

O. volvulus worms were recovered from s.c. nodules that had been surgically removed from infected patients in Cameroon and kindly sent to us by Dr. Janet Bradley (University of Nottingham, Nottingham, U.K.). Parasites were recovered after digestion with collagenase (Sigma, St. Louis, MO) as described elsewhere (3), and homogenized in HBSS using a mortar and pestle. Insoluble material was removed by centrifugation, and the concentration of protein was determined by standard methods (Bio-Rad, Richmond, CA).

Immunization and intracorneal injections of O. volvulus Ags

C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME), and mice deficient in ICAM-1 on a C57BL/6 background (8) were obtained from Dr. Dan Bullard (University of Alabama, Birmingham, AL). Animals were given three weekly s.c. immunizations with 10 μg of O. volvulus Ags in a 1:1 ratio with adjuvant containing 10% squalene (Aldrich, Milwaukee, WI), 0.4% Tween 80 (Fisher, Fair Lawn, NJ), and 1% pluronic acid (BASF Bioresearch, Parsippany, NJ) (3).
For injection into the corneal stroma, mice were anesthetized by i.p. injection of 200 μl of a 1.2% solution of 2,2,2-tribromoethanol (Aldrich) containing 2.5% 2-methyl-2-butanol (tertary amyl alcohol; Aldrich) dissolved in distilled H2O (9). The corneal epithelial layer was scarified using a 30-gauge needle, and 10 μg of O. volvulus Ags in 5 μl was injected into the corneal stroma using a 33-gauge needle attached to a Hamilton syringe (Hamilton, Reno, NV). All procedures were in accordance with guidelines determined by the Association for Research in Vision and Ophthalmology.

Corneal opacification was graded by slit-lamp examination and evaluated as described previously (3): 0, no pathology, cornea is completely transparent; 1, slight opacity, cornea has discrete areas of opacity, but is mostly transparent; 2, moderate opacity, cornea is mostly opaque, but underlying iris is still visible; and 3, severe opacity, cornea is completely opaque, iris is not visible.

Injections of mAb to PECAM-1, ICAM-1, and VCAM-1 into the subconjunctival space

IgG injected into the conjunctiva diffuses into the corneal stroma (10, 11). To block the activity of adhesion molecules on the vascular endothelium, 25 μg of anti-PECAM-1 (rat IgG2a, MEC 13.3; PharMingen, San Diego, CA), anti-ICAM-1 (rat IgG2a, KAT-1; Caltag Laboratories, Burlingame, CA), or anti-VCAM-1 (rat IgG1, M/K-2; Caltag Laboratories) Ab in 5 μl was injected into the subconjunctival space of the right eye, and 25 μg of control rat IgG (Sigma) was injected into the left subconjunctival space. Injections were performed using a 33-gauge needle attached to a Hamilton syringe. Subconjunctival injections were repeated 24 and 48 h later.

To detect in vivo-injected Abs binding to the limbal vascular endothelium, eyes were snap-frozen and sections were incubated directly with FITC-conjugated anti-rat IgG (Caltag Laboratories). Positively stained vessels were visualized by fluorescence microscopy.

Detection of neutrophils and eosinophils

Enucleated eyes were fixed overnight in 10% formaldehyde (Sigma) in distilled H2O, processed by standard methods, and embedded in paraffin. Sections (5 μm) were immunostained with rabbit antisera to major basic protein to detect eosinophils as described previously (6, 12). Biotinylated goat anti-rabbit Ig (Dako, Carpinteria, CA) was used as the secondary Ab. Neutrophils were detected using the rat mAb NIMP-R14 diluted 1/100, followed by biotinylated rabbit anti-rat Ig (BioGenex, San Ramon, CA). Sections were then incubated with alkaline phosphatase-conjugated streptavidin (Dako), and positively stained cells were visualized using Vector Laboratories substrate containing levamisole (Vector Laboratories, Burlingame, CA). Sections were counterstained with modified Harris’ hematoxylin (Richard-Allen, Kalamazoo, MI) and examined by bright-field and fluorescence microscopy. Total neutrophils and eosinophils per section were determined by direct counting.

For immunostaining of vascular adhesion molecules, 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 min in −20°C acetone. Slides were air dried and rehydrated in PBS (pH 7.4). Adjacent sections on the same slide were stained with rat mAb against either PECAM-1 (MEC 13.3), ICAM-1 (KAT-1), or VCAM-1 (M/K-2). Primary Abs were diluted 1:100 in PBS containing 1% FCS and incubated for 2 h at room temperature. FITC-conjugated anti-rat IgG (H + L; Caltag Laboratories) diluted 1/100 was used as a secondary Ab and incubated for 45 min. Stained sections were washed in PBS and coverslipped with Vectashield Mounting Medium (Vector Laboratories) to inhibit quenching.

Evaluation of staining intensity

Evaluation of expression of FITC-stained adhesion molecules on limbal vessels was based on the method described by Tang and Hendricks (13) for detection of PECAM. After immunostaining for PECAM-1, ICAM-1, and VCAM-1, images of limbal vessels were captured using a digital camera model DXM-1200 (Nikon, Metro City, IN) and Scion Image Software (version 1.62c; National Institutes of Health, Bethesda, MD, modified by Scion Corp.). To evaluate the relative fluorescence intensity, the mean brightness value of the green channel of the three most intensely stained areas of the vessel was determined using Adobe Photoshop 5.0 (Adobe Systems, San Jose, CA) with a set 400 pixel square area. Four vessels from each eye were analyzed, the background reading in unstained areas of the cornea was subtracted from these values, and the mean ± SE of the fluorescence intensity for each vessel was estimated. Data are then presented as the percent maximum value for each Ab.

Statistics

Statistical significance was determined using an unpaired Student’s t test (Prism GraphPad Software, San Diego, CA). A value of p < 0.05 was considered to be significant.

Results

Differential expression of PECAM-1, ICAM-1, and VCAM-1 in O. volvulus keratitis

O. volvulus keratitis was induced in mice after s.c. immunization and subsequent injection of parasite Ags into the corneal stroma. To determine the relative expression of PECAM-1, ICAM-1, and VCAM-1 on limbal vessels, C57BL/6 mice were sacrificed either immediately after injection of O. volvulus Ags into the corneal stroma (0 h) or after 12 h, 24 h, 72 h, or 7 days (168 h). Eyes were enucleated, and 5-μm sections were immunostained with mAb for PECAM-1, ICAM-1, and VCAM-1.

Fig. 1A (upper panels) shows representative corneas from each time point and demonstrates expression of PECAM-1, ICAM-1, and VCAM-1 on adjacent sections of the same limbal vessels. Although expression of each of these molecules was elevated after intrastromal injection, there were at least two distinct features: first, PECAM-1 was expressed immediately after injection (0 h), whereas ICAM-1 and VCAM-1 were not detected. Second, compared with PECAM-1 and ICAM-1, VCAM-1 expression was transient, being elevated at 72 h after injection, and decreasing by day 7. Corneas from naive mice reacted with Ab to PECAM-1 at a similar level to 0 h and did not react with Ab to ICAM-1 or VCAM-1 (data not shown).

Fig. 1B shows the mean fluorescence intensity of PECAM-1, ICAM-1, and VCAM-1 as described in Materials and Methods. When compared with expression at 0 h, PECAM-1 expression was significantly elevated at 24 h, 72 h, and 7 days (p < 0.05), but not at 12 h. ICAM-1 expression was significantly increased at each time point compared with 0 h, and VCAM-1 expression was significantly elevated 12, 24, and 72 h after intrastromal injection, but not after 7 days.

Neutrophil recruitment to the cornea is dependent on expression of PECAM-1

To determine whether PECAM-1 expression has a role in neutrophil or eosinophil migration to the cornea in O. volvulus keratitis, anti-PECAM-1 mAb was injected into the subconjunctival space of immunized C57BL/6 mice before injection of O. volvulus Ags into the corneal stroma. Control rat IgG was injected into the subconjunctival space of the contralateral eye. Mice were sacrificed 24 or 72 h later, corneas were immunostained to detect neutrophils and eosinophils, and the number of cells in the corneal stroma was determined by direct counting.

Ab injected into the subconjunctival space has been shown to modulate immune and inflammatory responses in the cornea (10, 11). To determine whether subconjunctival injection of rat Ab to PECAM-1 reacted with limbal vessels, eyes were removed 24 or 72 h after intrastromal injection, and corneas were stained directly with secondary FITC anti-rat IgG. As shown in Fig. 2, upper panels, anti-PECAM-1 bound to limbal vessels, whereas no Ab was detected after injection of control rat IgG.

Our previous studies demonstrated that neutrophils and eosinophils can be readily distinguished by immunohistochemical analysis and showed that these cells infiltrate the cornea in a biphasic manner, with neutrophils predominant in the first 24 h after intrastromal injection and being replaced by eosinophils after 72 h (4, 6, 12).

Consistent with this observation, neutrophil recruitment was significantly impaired 24 h after injection (p = 0.012; Fig. 2). To
determine whether PECAM-1 is also important for eosinophil infiltration, mice were injected with anti-PECAM-1 24 and 48 h later, and the number of eosinophils at 72 h was determined by direct counting. In contrast to neutrophils, eosinophil migration to the cornea was not inhibited after injection of anti-PECAM-1 (Fig. 2, lower panel). Neutrophils were not detected at 72 h in either group, indicating that the PECAM-1 blockade was not simply delaying infiltration of these cells.

PECAM-1 is essential for development of corneal opacification in O. volvulus keratitis

Since the presence of neutrophils and eosinophils is associated with loss of corneal clarity in O. volvulus keratitis, we also examined the effect of blockade of these adhesion molecules on the development of corneal opacification. As shown in Fig. 3, corneas of control mice develop significant opacification scores by day 1, coinciding with neutrophil infiltration. Consistent with decreased neutrophil emigration to the cornea in anti-PECAM-1-injected eyes, we found that corneal opacification was significantly reduced after PECAM-1 blockade compared with control corneas ($p < 0.0001$). There was no significant difference on day 3, when eosinophils were the predominant cell type present.

Eosinophil recruitment to the cornea is impaired after ICAM-1 blockade

To determine the effect of ICAM-1 blockade on neutrophil and eosinophil recruitment to the cornea, anti-ICAM-1 mAb was injected into the subconjunctival space before intrastromal injection of parasite Ags and normal rat IgG was injected into contralateral eyes. Animals were sacrificed 24 or 72 h later, and the number of neutrophils and eosinophils in the cornea was assessed. As shown in Fig. 4, upper panels, anti-ICAM-1 Ab bound to limbal vessels after subconjunctival injection. In contrast to PECAM-1, ICAM-1 blockade had no effect on neutrophil infiltration (Fig. 4, center panel). However, eosinophil recruitment was significantly inhibited compared with control eyes ($p = 0.0032$, Fig. 4, lower panel).

As a second experimental approach to determine the role of ICAM-1 in neutrophil and eosinophil recruitment to the cornea, ICAM-1$^{-/-}$ mice were immunized s.c. and injected intrastromally with O. volvulus Ags. The number of neutrophils and eosinophils in the cornea was then determined in comparison to immunocompetent C57BL/6 mice.

Although neutrophil recruitment in ICAM-1$^{-/-}$ mice was not significantly different from control animals (C57BL/6: 1047 ±
similar results.

FIGURE 2. The role of PECAM-1 in neutrophil and eosinophil recruitment to the cornea. C57BL/6 mice were immunized s.c. and injected into the corneal stroma with O. volvulus Ags. Animals were also injected into the subconjunctival space with rat mAb against PECAM-1 or control rat IgG before and after (24- and 48-h) intrastromal injection of parasite Ags. Upper panel, Corneal sections were stained directly with secondary FITC-conjugated anti-rat IgG after 24 h (original magnification, ×400). Note that the limbal vessels are detected after injection of anti-PECAM-1, but not control rat IgG. Center panel, The number of neutrophils per corneal section on day 1; lower panel, eosinophil numbers after 72 h. Note that neutrophil recruitment was impaired significantly in anti-PECAM-1-treated eyes, whereas eosinophil emigration was not affected. Results are mean ± SEM of five mice per group. These experiments were repeated twice with similar results.

141.2, n = 5; ICAM-1−/−: 93.0 ± 21.64, n = 5; p = 0.0465). There was no significant difference in corneal opacification after blockade of ICAM-1 or in ICAM-1−/− mice (data not shown). This finding may be due to incomplete blockade of eosinophil infiltration to the cornea, as there were 62% fewer eosinophils in the cornea after subconjunctival injection of anti-ICAM-1 and 80.7% fewer eosinophils in ICAM-1−/− mice.

Taken together with the observations on local ICAM-1 blockade, these data indicate that ICAM-1 expressed on the limbal vessels has a selective role in eosinophil transmigration to the corneal stroma.

VCAM-1 blockade does not inhibit neutrophil or eosinophil recruitment

To determine whether VCAM-1 expression contributes to neutrophil and eosinophil emigration to the corneal stroma, mAb against VCAM-1 were injected into the subconjunctival space of immunized C57Bl/6 before intrastromal injection of O. volvulus Ags. Eyes were enucleated 24 or 72 h after injection of helminth Ags, and the number of neutrophils and eosinophils in the corneal stroma was determined as described earlier.

FIGURE 4. The role of ICAM-1 in neutrophil and eosinophil recruitment to the cornea. C57BL/6 mice were injected subconjunctivally with mAb against ICAM-1 before intrastromal injection of parasite Ags as described in the legend to Fig. 2. Mice were sacrificed either on day 1 or day 3 after intracorneal injection and eyes were enucleated, processed, and sections were stained with FITC anti-rat IgG or Ab to neutrophils and eosinophils. FITC anti-rat IgG bound to anti-ICAM-1, but not control rat IgG-injected eyes (shown 72 h after injection of parasite Ags; upper panels, original magnification ×400). Neutrophil and eosinophil numbers were determined by direct counting; shown are neutrophil numbers per corneal section on day 1 (center panel) and eosinophil numbers on day 3 (lower panel). Neutrophil recruitment was not affected in anti-ICAM-1-treated eyes (p = 0.6598), whereas eosinophil emigration was significantly inhibited (p = 0.0416). Results are mean ± SEM of five mice per group. These data are representative of three repeat experiments.

Discussion

The transparent nature of the mammalian cornea is due to the highly organized arrangement of collagen fibrils and to a tightly
regulated level of hydration (14), which is maintained by the resident cells. These include epithelial cells, which are the external physical and hydration barrier, stromal fibroblasts (keratocytes), which produce the collagen and proteoglycans that form the matrix of the corneal stroma, and corneal endothelial cells, which maintain the hydration level of the cornea by pumping H₂O from the stroma to the anterior chamber (14).

If the cornea is subjected to trauma or infection, leukocytes infiltrate from limbal vessels toward the site of injury. Although inflammatory cells are important in host defense, they can also have a cytotoxic effect on resident cells in the cornea, resulting in loss of corneal transparency, visual impairment, and possibly blindness. Furthermore, although anti-inflammatory mechanisms are in place in the eye to maintain immune privilege, including expression of Fas ligand (15), intense inflammatory responses that cause clinical damage can still occur.

In onchocerciasis (river blindness), heavy invasion of parasitic worms into the cornea induces peripheral opacification, which progresses centrally to obscure the entire cornea (16). Epidemiological evidence indicates that corneal inflammation is initiated after death and degeneration of the worms and subsequent release of somatic Ags into the corneal stroma (17). In the murine model of ocular onchocerciasis described here, the inflammatory response is initiated after *O. volvulus* Ags are injected directly into the corneal stroma of sensitized animals.

Results obtained using this model are consistent with the proposed sequence of events outlined in Fig. 6. Subcutaneous immunization with *O. volvulus* Ags stimulates a predominant Th2 response, with elevated IL-4, IL-5, eosinophilia, and a parasitic-specific Ab, especially IgG1 (3, 6). After injection of parasite Ags into the cornea, neutrophils and eosinophils are recruited to the stroma where degranulation and release of cytotoxic mediators such as eosinophil cationic proteins disrupts the normal function of the cornea, leading to loss of corneal clarity and visual impairment. Ab appears to contribute by forming immune complexes that mediate degranulation (3).

By identifying vascular adhesion molecules that contribute to the process of recruitment of neutrophils and eosinophils to the cornea, results from the current study add important information to our understanding of the pathogenesis of *O. volvulus* keratitis. The finding that PECAM-1 is important in neutrophil extravasation adds to recent observations demonstrating elevated macrophage-inflammatory protein 2 and KC and an essential role for expression of chemokine receptor CXCR3 chemokine receptor 2 in recruitment of neutrophils to the cornea and development of corneal opacification (4). Similarly, the finding that ICAM-1 expression is important in eosinophil infiltration to the cornea adds to previous reports demonstrating that eotaxin production and P-selectin expression contribute to eosinophil recruitment to this site (5, 18).

These studies show significant but partial effects on cellular infiltration, consistent with the notion of a multistep process (19). Taken together with the observations that blockade of each of these interactions is selective for either neutrophils or eosinophils, these studies indicate that a combined blockade of vascular adhesion molecule interactions and chemokine receptor interactions could have a complete and specific effect on infiltration of these cells to the cornea.

Our findings that PECAM-1 mediates neutrophil extravasation from limbal vessels are consistent with those of Tang and Hendricks (13), who demonstrated that PECAM-1 expression mediates neutrophil recruitment to the cornea in herpes simplex keratitis and that both are regulated by IFN-γ. PECAM-1 is also important in neutrophil infiltration to other tissues, including the lungs and peritoneal cavity (20–23). Furthermore, anti-PECAM-1 Ab can block intradermal accumulation of neutrophils to human skin transplanted onto SCID mice (23, 24). In addition to neutrophils, PECAM-1 is expressed on monocytes, T cells, and platelets. To the best of our knowledge, PECAM-1 has not been shown to directly mediate transmigration of eosinophils, although in vitro studies with IL-4-stimulated HUVECs indicate that CD31-mediated interactions with eosinophils (25).

In contrast to ICAM-1 and VCAM-1, PECAM-1 mediates leukocyte passage through the basement membrane and has no apparent effect on rolling or adhesion (26, 27). However, PECAM-1

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**FIGURE 5.** The role of VCAM-1 in neutrophil and eosinophil recruitment to the cornea. C57BL/6 mice were immunized and injected subconjunctivally with mAb against VCAM-1 (M/K-2) into the right eye or rat IgG into the left eye as control as described above. FITC-conjugated anti-rat IgG bound to corneal vessels after injection of anti-VCAM-1 Ab, whereas no stain was seen in control rat IgG (upper panels, original magnification ×400). Neutrophil and eosinophil numbers were obtained by direct counting, and shown as neutrophil numbers per corneal section on day 1 (middle panel) and eosinophil numbers on day 3 (lower panel). Neither neutrophil (p = 0.735) nor eosinophil (p = 0.487) recruitment was significantly affected in anti-VCAM-1-treated corneas in comparison to rat IgG injection. Results are mean ± SEM of five mice per group and are representative of three repeat experiments.

**FIGURE 6.** The role of vascular adhesion molecules in a proposed sequence of events leading to *O. volvulus* corneal inflammation (see Discussion).
mediates leukocyte transmigration across the endothelial cell layer and passage through the basement membrane, interactions that are dependent on the amino terminal and the membrane proximal regions of the extracellular domain of PECAM-1, respectively (28). Transendothelial migration is also dependent on homophilic interactions between PECAM-1 on leukocytes and PECAM-1 on endothelial cells (22, 24, 26, 28). As neutrophils express PECAM-1 (22) it is likely that homophilic interactions are involved in neutrophil recruitment to the cornea and that mAb to PECAM-1 binds neutrophils and vascular endothelium, leading to blockade of this interaction.

Neutrophils also express LFA-1, which is a ligand for ICAM-1, and ICAM-1 interactions mediate neutrophil migration to other tissues, including the skin and peritoneal cavity (8, 29). Interestingly, in the murine model of Pseudomonas aeruginosa keratitis, ICAM-1-deficient mice had significantly fewer neutrophils in the cornea and anterior chamber (30). The differences between those findings and results of the current study, where neither injection of anti-ICAM-1 nor ICAM-1 deficiency had any significant effect on neutrophil recruitment to the cornea, have yet to be determined.

In contrast to neutrophils, the role of ICAM-1 expression in eosinophil extravasation is consistent with expression of LFA-1 ligand on eosinophils (31, 32) and with reports in which ICAM-1 blockade inhibits the migration of eosinophils to the skin and the airways (33, 34).

A surprising finding was that VCAM-1 blockade had no effect on eosinophil recruitment to the cornea, despite elevated expression coinciding with maximal eosinophil numbers in the cornea. Either VCAM-1 has no role in this experimental model or other adhesion molecules such as ICAM-1 and P-selectin compensate when VCAM-1 activity is blocked. The absence of an effect is not likely to be due to ineffectiveness of the Ab, as M/K-2 inhibits cardiac allograft rejection (35). Future studies will examine the role of VCAM-1 in the context of ICAM-1 and P-selectin depletion.

Eosinophils express very late Ag 4 and eosinophil transmigration across HUVECs is dependent on VCAM-1, especially after exposure to IL-4 (36, 37). VCAM-1 blockade also inhibits IL-4-dependent eosinophil accumulation in the dermis (38). IL-4 is elevated in the cornea in Onchocerca volvulus keratitis (33, 34), and local production of IL-4 may explain the difference between our findings and those of Zhu and Dana (29), who demonstrated expression of ICAM-1 and PECAM-1 on limbal vessels, but did not show VCAM-1 expression. Future studies will examine the role of IL-4 and other immunoregulatory cytokines on expression of adhesion molecules on limbal vessels.

In conclusion, results of this study demonstrate a role for PE-

CAM-1 and ICAM-1 in recruitment of neutrophils and eosino-

phils, respectively, to the corneal stroma. Blockade of vascular adhesion molecule interactions along with chemokine receptor interactions may indicate a feasible approach to immunotherapy in corneal disease, since granulocyte infiltration can be inhibited without affecting systemic responses.

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