IMPORTANCE

We have identified at least two proinflammatory cytokines expressed locally that are involved in the genesis of lymphatic vessels in the normally avascular cornea in response to HSV-1 infection. This finding provides the basis to target IL-6 and TNF-α as additional proangiogenic factors in the cornea during the development of herpetic stromal keratitis as a means to alleviate further neovascularization and tissue pathology associated with the host immune response to the pathogen.

Tumor Necrosis Factor Alpha and Interleukin-6 Facilitate Corneal Lymphangiogenesis in Response to Herpes Simplex Virus 1 Infection

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ABSTRACT

Herpes simplex virus 1 (HSV-1) is a common human pathogen of clinical significance due to its association with vision impairment and encephalitis. In a mouse model of ocular neovascularization, we have previously shown that HSV-1 elicits the genesis of lymphatic vessels into the cornea proper through epithelial cell expression of vascular endothelial growth factor A (VEGFA) dependent upon expression of VEGFR2 during acute infection. We hypothesized that other factors may be involved in lymphangiogenesis, with proinflammatory cytokines as the leading candidates. In the absence of infection or inflammation, intrastromal administration of tumor necrosis factor alpha (TNF-α) coupled with VEGFA elicited lymphatic vessel genesis significantly above either factor alone as well as a vehicle control. Consistent with this observation, anti-TNF-α antibody (Ab) blocked HSV-1-mediated corneal lymphangiogenesis within the first 5 days postinfection. However, TNF-α-deficient (TNF-α−/−) mice displayed a level of corneal vessel growth similar to that shown by wild-type (WT) controls. To investigate the likely redundant nature of cytokines, PCR array analysis of HSV-1-infected TNF-α−/− mice was conducted, and it revealed several factors elevated above those found in HSV-1-infected WT mice, including interleukin-1β (IL-1β), platelet-derived growth factor, angiopoietin 2, insulin-like growth factor 2, and IL-6. Subconjunctival administration of neutralizing Ab to IL-6 blocked lymphangiogenesis in TNF-α−/− mice. Whereas the cornea levels of IL-6 were significantly reduced, there was no appreciable change in the level of IL-1β or other proangiogenic factors analyzed. Collectively, the results suggest in addition to VEGFA, TNF-α and IL-6 promote and likely synergize with VEGFA in corneal lymphangiogenesis during acute HSV-1 infection.
also the robust immune response to the pathogen, which is facilitated by the presence of blood and lymphatic vessels. Identification of factors that promote corneal neovascularization may lead to therapeutic targets for the treatment of HSK.

Recent studies have investigated the mechanisms by which HSV-1 induces lymphangiogenesis in the cornea during early acute infection (10, 11). Following HSV-1 infection of corneal epithelial cells, the HSV-1-encoded transcription factor ICP4 binds the promoter region of VEGFA and drives expression of the proangiogenic growth factor (11). This leads to extensive lymphangiogenesis in the corneal tissue (10, 11). While it has been established that VEGFA plays a critical role during HSV-1-induced corneal lymphangiogenesis, other factors may also contribute. TNF-α is an important inflammatory antiviral cytokine, and recent studies have indicated a possible role for it in the promotion of lymphatic vessel development in other models. For example, a previous study reported that TNF-α can positively influence lymphangiogenesis in the tracheas of Mycoplasma pulmonis-infected mice (12). In a mouse model of ocular surface scarring, topical response to HSV-1.

Furthermore, in the absence of TNF-α/H9251/H11002/H11022/H9262, HSV-1 infection. Our results demonstrate that TNF-α deficiency (TNFR1/H9251/H11002/H11022/H9262), tumor necrosis factor receptor 1 (TNFR1/H9251/H11002/H11022/H9262) mice were purchased from The Jackson Laboratory and Charles River and housed in Dean A. McGee Eye Institute’s animal facility. All mice were age and sex matched. Animal treatment was consistent with the National Institutes of Health guidelines on the care and use of laboratory animals. All experimental procedures were approved by the University of Oklahoma Health Sciences Center Institutional Animal and Care Use Committee. HSV-1 McKrae was propagated in Vero cells and maintained at a stock concentration of 10⁶ PFU/ml. Anesthetized mice were infected with HSV-1 by applying 3 μl of phosphate-buffered saline (PBS) containing 10,000 PFU of virus onto the scarified mouse cornea. For plaque assays, at various times postinfection (p.i.), tissue and cells were isolated, suspended in 500 μl of RPMI medium, and homogenized with a Tissue Miser (Fisher Scientific) for 20 to 30 s. The medium was clarified of cellular debris by centrifugation for 60 s at 10,000 g. Infectious content in the clarified supernatant was then evaluated by plaque assay.

**VEGFA and TNF-α treatment.** WT mice were infected intrastromally with 1 μl (10 ng) of recombinant VEGFA (Prospec), TNF-α (Prospec), a combined treatment of VEGFA and TNF-α, or PBS. Specifically, a glass needle was inserted into the stromal layer of the cornea and 1 μl of sterile PBS (Gibco) with or without VEGFA and/or TNF-α was introduced using a gas-powered microinjection system (MDH) under an ophthalmic surgical microscope (Carl Zeiss, Inc.). Injections were performed every day for 7 days.

**Anti-TNF-α and anti-IL-6 Ab treatment.** WT mice were infected with 10⁶ PFU of HSV-1. On days 0, 2, and 4 p.i., mice were injected subconjunctivally with 10 μl (10 μg) of isotype control (IgG) or anti-TNF-α Ab (R&D; AB-410-NA). TNF-α/H9251/H11002/H11022/H9262/−/− mice were infected with 10,000 PFU of HSV-1. On days 0, 2, and 4 p.i., mice were injected subconjunctivally with 10 μl (1 μg) of isotype control (IgG1) or anti-IL-6 Ab (Biolegend; clone MP5-20F3).

**Bone marrow chimeras.** Bone marrow chimeras were created by irradiating WT and TNF-α/H9251/H11002/H11022/H9262/−/− mice with two 600-rad doses of gamma irradiation spaced 4 h apart. Irradiated mice were then retro-orbitally injected with 3 × 10⁶ bone marrow cells from WT or TNF-α/H9251/H11002/H11022/H9262/−/− mice. The injected bone marrow cells were allowed 10 weeks to reconstitute the hematopoietic compartment. The success of the chimerism was verified by flow cytometry on circulating leukocytes and found to be consistently >95% successful. Following confirmation of the chimerism, the animals were infected with 10⁶ of HSV-1 PFU/eye and assessed for neovascularization.

**Immunohistochemical staining and confocal imaging.** Corneas were fixed for 30 min with 4% paraformaldehyde at 4°C and subsequently washed three times with 1% Triton X-100 in PBS (PBST). Samples were then blocked overnight with Fc block (BD Pharmingen) at 4°C and washed with PBST. After the final wash, rabbit anti-mouse LYVE-1 (Abcam) and hamster anti-mouse CD31 (Millipore) antibodies were incubated overnight at 4°C. Samples were washed again and incubated overnight at 4°C with secondary antibodies conjugated to DyLight549 (Jackson Immunology) and Alexa Fluor 488 (Abcam). After three washes, 4,6-diamidino-2-phenylindole (DAPI; Vector Laboratories) staining of nuclei was performed and corneas were mounted. All cornea images were obtained with a 10X objective using an Olympus IX81-FV500 epifluorescent/confocal laser scanning microscope (Olympus). Images were analyzed with Fluoview software (Olympus). Lymphatic vessel area was defined as the percentage of LYVE-1-positive pixels divided by the total number of pixels per field of view.

**Affymetrix QuantieGene 2.0 Plex assay.** At the desired time p.i., animals were anesthetized and transcardially perfused with 10 ml of PBS. Corneas were removed and processed according to the manufacturer’s protocol for fresh or frozen tissues (QuantieGene sample processing kit). Target hybridization and signal amplification were performed according to the manufacturer’s protocol for fresh or frozen tissues (QuantieGene 2.0 Plex assay). Signal was detected using a Luminex 100 instrument (Bio-Rad) and reported as median fluorescence intensity and is proportional to the number of target RNA molecules present in the sample. Gene expression fold changes were determined by first identifying the normalized value for each sample gene of interest divided by the geometric mean for the housekeeping genes. Next, the fold change was determined by dividing the normalized value for HSV-1-infected corneas by the normalized value for the uninfected corneas. Each sample was assayed using technical duplicates and biological triplicates. Target genes included interleukin 6 (IL-6), platelet-derived growth factor B polypeptide (PDGFb), IL-1β, pros-pero-related homebox 1 (PRX1), hepatocyte growth factor (HGF), matrix metalloproteinases 2 and 9 (MMP2 and MMP9), angiopoietins 1 and 2 (ANGPT1 and ANGPT2), IL-17F, lymphotokines A and B (LTA and LTβ), chemokine (C-C motif) ligand 21b (serine) (CCL21A), notch gene homolog 1 (NOTCH1), fibroblast growth factor 2 (FGF2), TNF-α, VEGFA and VEGFC, insulin-like growth factor 2 (IGF2), hypoxanthine guanine phosphoribosyltransferase 1, actin beta cytoplasmic, and polymerase RNA II.

**Suspension array.** At the desired time p.i., corneas were removed from exsanguinated mice and assayed for the detection of interleukin-6, interleukin-1β, hepatocyte growth factor, vascular endothelial growth factor A, and vascular endothelial growth factor C using a Milliplex map kit (Millipore). Signal was detected using a Luminex 100 instrument (Bio-Rad). The weight of each tissue was used to normalize the amount of cytokine or chemokine per milligram of tissue weight.

**Statistical analysis.** The statistical module Prism version 5.0 (GraphPad Software, Inc., San Diego, CA) was used to perform unpaired two-tailed Student’s t test with group sizes of two or analysis of variance (ANOVA) with Tukey’s t test for larger group sizes.

**RESULTS**

Previous studies have indicated that TNF-α promotes hemangiogenesis and lymphangiogenesis in the mouse trachea under inflammatory and pathogenic conditions (12). However, the con-
tribution of this cytokine to corneal lymphangiogenesis under noninflammatory or inflammatory conditions such as an infection has not been described. In comparison, VEGFA is a well-characterized growth factor known to induce hemangiogenesis. Recently, we have shown that VEGFA can also induce corneal lymphangiogenesis following HSV-1 infection (10). To investigate whether VEGFA or TNF-α is sufficient to promote corneal lymphangiogenesis in the absence of infection, recombinant VEGFA or TNF-α was injected intrastromally into mouse corneas. Following a 7-day course of treatment, the corneas were harvested and evaluated for lymphatic vessel area (LYVE-1) and the number of lymphatic vessel sprouts (A). Injection of TNF-α alone or VEGF-A/TNF-α led to a significant increase in the number of lymphatic sprouts (B). Injection of VEGFA and TNF-α led to a significant increase in lymphatic vessel area (C). The vehicle consisted of PBS used to reconstitute the VEGFA and TNF-α. Results are from two independent experiments with 2 or 3 mice per group per experiment. Bars represent SEMs. ***, P < 0.0001 as determined by ANOVA and Tukey’s post hoc analysis.

Since our results indicate that TNF-α functions in conjunction with VEGFA as a prolymphangiogenic growth factor in the mouse cornea, we next investigated the contribution of TNF-α during HSV-1-induced corneal lymphangiogenesis by comparing WT and TNF-α−/− mice. Specifically, mice infected with HSV-1 were euthanized 5 days p.i., and the corneas were processed for whole-mount staining of blood (CD31) and lymphatic (LYVE-1) vessels as well as viral burden. WT and TNF-α−/− mouse corneas

FIG 1 Recombinant TNF-α induces corneal lymphangiogenesis. Recombinant VEGFA and/or TNF-α (10 ng) was injected intrastromally into WT mouse corneas at 24-h intervals for 7 days. Following treatment, corneas were harvested and evaluated via confocal microscopy for lymphatic vessel area (LYVE-1) and the number of lymphatic vessel sprouts (A). Injection of TNF-α alone or VEGF-A/TNF-α led to a significant increase in the number of lymphatic sprouts (B). Injection of VEGFA and TNF-α led to a significant increase in lymphatic vessel area (C). The vehicle consisted of PBS used to reconstitute the VEGFA and TNF-α. Results are from two independent experiments with 2 or 3 mice per group per experiment. Bars represent SEMs. ***, P < 0.0001 as determined by ANOVA and Tukey’s post hoc analysis.

FIG 2 Neutralization of TNF-α impairs HSV-1-induced corneal lymphangiogenesis. HSV-1-infected WT mice were treated with neutralizing antibodies (10 μg) to TNF-α or isotype control via subconjunctival injections. At 5 days p.i., corneas were harvested and assessed for lymphangiogenesis. Treatment with anti-TNF-α led to a significant decrease in HSV-1-induced lymphatic vessel area (A and B) but had no effect on select proangiogenic factors (C). Results are from two independent experiments with 2 or 3 mice per group per experiment. Bars represent SEMs. ***, P < 0.0001 as determined by Student’s t test.
revealed similar levels of HSV-1-induced neovascularization (lymphangiogenesis and hemangiogenesis) (Fig. 3A and B). As noted previously (14), TNF-α−/− mice displayed a slight, though not significant, increase in viral load in the cornea (Fig. 3C). Similar to TNF-α−/− mice, mice deficient in the receptors for TNF-α (TNFR1−/− and TNFR2−/−), and WT (C57BL/6) mice were infected with HSV-1 (10⁴ PFU/eye) and processed 5 days postinfection (p.i.) for whole-mount staining of blood (CD31) and lymphatic (LYVE-1) vessels. HSV-1 induced similar levels of corneal lymphangiogenesis in C57BL/6 mice and TNF-α−/− mice (A and B). There was a modest but insignificant difference in viral titer recovered from C57BL/6 and TNF-α−/− mouse corneas (C). HSV-1 induced similar levels of corneal lymphangiogenesis in C57BL/6 mice, TNFR1−/− mice, and TNFR2−/− mice (D and E). Results are from two independent experiments with 2 or 3 mice per group per experiment. Bars represent SEMs.

FIG 3 HSV-1-infected TNF-α-deficient and TNF-α receptor-deficient mice display levels of lymphangiogenesis similar to those of WT mice. TNF-α−/− mice (TNF-α−/−/−/−), mice deficient in the receptors for TNF-α (TNFR1−/− and TNFR2−/−/−), and WT (C57BL/6) mice were infected with HSV-1 (10⁴ PFU/eye) and processed 5 days postinfection (p.i.) for whole-mount staining of blood (CD31) and lymphatic (LYVE-1) vessels. HSV-1 induced similar levels of corneal lymphangiogenesis in C57BL/6 mice and TNF-α−/− mice (A and B). There was a modest but insignificant difference in viral titer recovered from C57BL/6 and TNF-α−/− mouse corneas (C). HSV-1 induced similar levels of corneal lymphangiogenesis in C57BL/6 mice, TNFR1−/− mice, and TNFR2−/− mice (D and E). Results are from two independent experiments with 2 or 3 mice per group per experiment. Bars represent SEMs.

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Due to the lack of a phenotype in the TNF-α−/− mice and the redundant nature of some cytokines, we hypothesized that there may be additional proangiogenic molecules compensating for the loss of TNF-α. To address this possibility, the gene expression levels of 20 growth factors, cytokines, and matrix metalloproteinases associated with angiogenesis (IL-6, PDGF, IL-1β, PROX1, HGF, MMP2, ANGPT2, IL-17F, ANGPT1, LTA, CCL21α, NOTCH1, LTβ, FGF2, TNF, VEGFC, FLT1, MMP9, VEGFA, and IGF2) were assessed in WT and TNF-α−/− mouse corneas follow-
ing HSV-1 infection. Of the factors evaluated, only IGF-2, IL-6, IL-1β, PDGF, and ANGPT2 transcripts were elevated in the TNF-α−/− mouse corneas compared to the WT group (Table 1). A previous bioinformatics-based analysis of global transcriptional responses in corneal epithelial cells following HSV-1 infection revealed a critical role for IL-6 in the expression of over 20 cytokines, including PDGF, IL-8, and VEGFA, suggesting that IL-6 may directly or indirectly influence angiogenesis (15). To determine if IL-6 was compensating for the loss of TNF-α in our model, HSV-1-infected TNF-α−/− mice were treated with neutralizing IL-6 Ab and evaluated for corneal lymphangiogenesis. Neutralization of IL-6 in the corneas of TNF-α−/− mice significantly impaired HSV-1-induced lymphangiogenesis (Fig. 4). As expected, treatment of TNF-α−/− mice with anti-IL-6 Ab significantly reduced IL-6 protein levels in the cornea (Fig. 5). However, the administration of anti-IL-6 Ab had no significant effect on the levels of other proangiogenic factors, such as IL-1β, HGF, VEGFA, or VEGFC, suggesting that the impaired lymphangiogenic response was not due to a loss of these factors (Fig. 5).

### DISCUSSION

The current treatment for patients suffering from ocular HSV-1 infection includes acyclovir with or without the inclusion of steroids to inhibit virus replication and suppress unwarranted inflammation. However, even with this treatment many patients suffer from HSK, which underscores a need to identify additional therapeutic targets. Corneal neovascularization is a significant contributing factor to the development of HSK. The goal of the current study was to identify other soluble factors or cytokines expressed during acute HSV-1 infection of the cornea that contribute to neovascularization and, specifically, corneal lymphangiogenesis. There are a number of cytokines that have been detected in the corneas of HSV-1-infected mice, including TNF-α during acute infection or following reactivation of latent virus (16–19). Furthermore, TNF-α has previously been reported to contribute to hemangiogenesis in the cornea following TNF-α pellet implantation (20, 21). Whereas neither recombinant VEGFA nor TNF-α alone significantly increased lymphangiogenesis in the cornea following local administration, together the factors elicited corneal lymphatic vessel genesis. Of interest, TNF-α alone or in combination with VEGFA induced significant sprouting from limbal lymphatics. In adults, lymphatic vessel growth appears to follow the same mechanism as blood vessel growth, with new vessel generation occurring via sprouting from existing vessels because the reservoir of angioblasts has been exhausted (1).

Consistent with this observation, HSV-1-infected WT mice treated with neutralizing Ab to TNF-α displayed a complete lack of response to HSV-1 in terms of corneal lymphangiogenesis. To further characterize the role of TNF-α in corneal lymphangiogenesis, we investigated whether the effect was mediated through TNFR p55 (R1) or TNFR p75 (R2) pathways. Both TNFR1 and TNFR2 have been found to be involved in ocular neovascularization coupled with angiopoietin or fibroblast growth factor (22, 23). However, the absence of either of these receptors had no significant detriment to corneal lymphangiogenesis in response to HSV-1 infection. These results are not terribly surprising since TNFR1 induction of angiogenesis involves macrophages and TNFR2 influence is under a state of hypoxia, neither of which has been found to function during acute HSV-1-mediated corneal lymphangiogenesis (10, 11). As these results did not provide a signaling conduit on which to further pursue the observation, we

### TABLE 1 Comparison of proangiogenic gene expression in WT and TNF-α−/− mouse corneas following HSV-1 infection

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control</th>
<th>TNF-α−/−</th>
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<tr>
<td>IL-6</td>
<td>16.77 ± 8.3</td>
<td>40.54 ± 9.5</td>
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<tr>
<td>IL-1β</td>
<td>12.36 ± 5.5</td>
<td>78.0 ± 21.2</td>
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<tr>
<td>PDGF</td>
<td>0.73 ± 0.1</td>
<td>1.32 ± 0.1</td>
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<tr>
<td>ANGPT2</td>
<td>1.30 ± 0.1</td>
<td>3.84 ± 0.8*</td>
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<tr>
<td>PROX1</td>
<td>0.66 ± 0.1</td>
<td>0.78 ± 0.1</td>
</tr>
<tr>
<td>HGF</td>
<td>0.52 ± 0.1</td>
<td>1.52 ± 0.5</td>
</tr>
<tr>
<td>MMP2</td>
<td>1.80 ± 0.2</td>
<td>2.8 ± 0.5</td>
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<tr>
<td>IL-17F</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.4</td>
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<td>ANGPT1</td>
<td>0.70 ± 0.1</td>
<td>1.40 ± 0.4</td>
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<td>LTA</td>
<td>0.76 ± 0.1</td>
<td>1.20 ± 0.2</td>
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<tr>
<td>CCL21A</td>
<td>2.17 ± 0.7</td>
<td>3.5 ± 0.7</td>
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<tr>
<td>NOTCH1</td>
<td>1.43 ± 0.1</td>
<td>0.97 ± 0.1*</td>
</tr>
<tr>
<td>LTB</td>
<td>0.92 ± 0.1</td>
<td>2.20 ± 0.6</td>
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<tr>
<td>FGFR2</td>
<td>1.15 ± 0.1</td>
<td>2.20 ± 0.6</td>
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<tr>
<td>TNF-α</td>
<td>9.30 ± 2.9</td>
<td>0.9 ± 0.2*</td>
</tr>
<tr>
<td>VEGFC</td>
<td>1.61 ± 0.4</td>
<td>2.85 ± 0.5</td>
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<tr>
<td>MMP9</td>
<td>1.20 ± 0.3</td>
<td>3.30 ± 1.2</td>
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<tr>
<td>VEGFA</td>
<td>2.02 ± 0.8</td>
<td>3.23 ± 0.4</td>
</tr>
<tr>
<td>IFG2</td>
<td>0.75 ± 0.11</td>
<td>1.04 ± 0.1*</td>
</tr>
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*Corneas from HSV-1-infected WT and TNF-α−/− mice were processed for gene expression analysis of 19 target genes associated with angiogenesis using Affymetrix QuantiGene 2.0 Plex assays. Results are from two independent experiments with 2 or 3 mice per group for a total of 5 or 6 mice/group. There was no difference in the expression of genes (except TNF-α) between uninfected WT and TNF-α−/− mice. *, P < 0.05 for comparison of infected WT to TNF-α−/− mice.
investigated the ligand of these receptors, TNF-α. Incorporating studies using TNF-α−/− mice, our results were not consistent with our findings using neutralizing antibody in that there was no difference in lymphangiogenesis in response to virus infection between WT and TNF-α−/− mice. Moreover, TNF-α−/− mouse chimeric studies did not yield tangible results either. As compensatory mechanisms have previously been noted in cases of cytokine or cytokine receptor gene deficiency (24–26), we interpreted the results to suggest that the acquisition of a WT phenotype in the TNF-α−/− mice might be due to redundant proangiogenic factors. PCR array analysis identified two cytokines that were likely candidates, IL-6 and IL-1β, as they were elevated in the TNF-α−/− compared to WT mouse corneas following HSV-1 infection. Corneal epithelial cells are known to produce several proinflammatory cytokines following infection with HSV-1, including TNF-α, IL-6, IFN-β, and IL-8 (27). Bioinformatic pathway analysis of transcriptional events in HSV-1-infected corneal epithelial cells revealed that IL-6 is a critical regulatory element for the induction of several proinflammatory cytokines and proangiogenic factors, including VEGFA (15). IL-6 is highly expressed in the cornea during HSV reactivation (30, 31). In the present study, the administration of IL-6 neutralizing Ab into TNF-α−/− mice significantly impaired HSV-1-induced lymphangiogenesis in comparison to that in isotypic control Ab-treated mice. As this treatment did not significantly alter the protein expression of other proangiogenic factors, including IL-1β, VEGFA, or VEGFC, the data implicate IL-6 as a critical compensatory cytokine that promotes lymphangiogenesis in the absence of TNF-α. Alternatively, the anti-IL-6 neutralizing Ab may have downregulated VEGFR2 expression and thus negated the VEGFA/VEGFR2-mediated lymphangiogenesis event.

Our data indicate that similar to VEGFA, TNF-α can function as a prolymphangiogenic growth factor in the cornea under both noninfectious and inflammatory/infectious conditions. However, it is only after the combination of TNF-α with VEGFA applied intrastromally that the genesis of lymphatic vessels into the cornea of a naive mouse becomes significant. Based on these results and the published results of others identifying proangiogenic molecules (1), it is likely that more than one signaling pathway is involved in the induction process. One possibility is the contribution of distinct cell populations to the complex prolymphangiogenic process or in the timing of expression of proangiogenic factors. Specifically, we have previously reported that resident epithelial cells are the primary source of VEGFA that elicits lymphangiogenesis during the first 5 to 7 days after HSV-1 infection (10, 11). However, we have not excluded the possibility that other cells may respond to the TNF-α/IL-6 axis in driving neovascularization, which may include some leukocyte populations that reside in or infiltrate into the inflamed cornea. Previous studies have indicated that TNF-α regulates Langerhans cell migration from the limbal region of the cornea to the central region following inflammation (32). Inflammatory corneal neovascularization can also lead to epithelial and stromal damage, subsequently increasing IL-1 and ICAM 1 expression (33). Increased ICAM 1 expression on limbal vessels allows for increased infiltration of inflammatory cells such as neutrophils that collectively facilitate HSK in mice (33). In summary, we have identified two additional cytokines, TNF-α and IL-6, that are involved in the genesis of lymphatic vessels in the cornea following HSV-1 infection. We propose these molecules to be additional candidates to target in the pathogenic process of neovascularization.

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REFERENCES


