CXCL1-Deficient Mice Are Highly Sensitive to *Pseudomonas aeruginosa* but Not Herpes Simplex Virus Type 1 Corneal Infection

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PURPOSE. To determine the role of the chemokine CXCL1 on leukocyte recruitment, cytokine production and host resistance during HSV-1 and *Pseudomonas aeruginosa* infection.

METHODS. Viral titer and bacterial load were compared following infection of wild-type (WT) and CXCL1^{-/-} mice. Corneal leukocyte recruitment was determined using flow cytometry. Cytokine levels were assessed by luminex-based suspension arrays. Hematoxylin and eosin (H&E) staining, confocal microscopy, and optical coherence tomography (OCT) were used to visualize leukocyte recruitment and corneal thickening.

RESULTS. HSV-1-infected WT and CXCL1^{-/-} mice possessed similar viral titers in the cornea during late acute infection. Flow cytometry analysis detected similar leukocyte levels in the cornea following infection as well. By comparison, there was a significant increase in *P. aeruginosa* recovered from CXCL1^{-/-} corneas as compared with WT mice. Imaging analysis and histochemical staining revealed impaired leukocyte recruitment to the central cornea and earlier corneal thickening in CXCL1^{-/-} mice. IFN- γ , CCL2, and CCL5 protein levels were similar between WT and CXCL1^{-/-} corneas following HSV-1 or *P. aeruginosa* infection. However, CXCL2 levels were significantly reduced in the CXCL1^{-/-} corneas following either infection.

CONCLUSIONS. The absence of CXCL1 and CXCL2 expression significantly impairs the ability of the host to control *P. aeruginosa* replication through the recruitment of leukocytes to the central cornea. In contrast, CXCL1, CXCL2, and the cells they recruit, are not required for HSV-1 clearance during acute infection. (*Invest Ophthalmol Vis Sci.* 2012;53:6785–6792) DOI:10.1167/iovs.12-10400

Herpes simplex virus type-1 (HSV-1) is among the most successful of human pathogens with a seroprevalence rate between 50% and 80%.¹ Although treatable, infection is lifelong due to the virus establishing a latent infection in sensory neurons, thereby “hiding” from the host immune response.² Upon reactivation, the virus replicates and travels

by anterograde transport to the primary site of infection or other epithelial surfaces fed by the infected sensory nerve fibers.² While viral reactivation is more commonly associated with orolabial lesions, the virus can also be transported to the cornea, resulting in recurring bouts of inflammatory keratitis.

During an acute ocular HSV-1 infection, it is the initial recruitment of activated monocytes to the cornea that reduces viral replication, which is followed by additional innate immune cells (e.g., NK cells) and the adaptive immune response that ultimately clears the virus.³ Furthermore, this process is initiated by the expression of several chemokines. Using endpoint PCR, early studies identified several chemokines that were induced following ocular HSV-1 infection including CXCL1, CXCL2, CXCL10, CCL2, and CCL4.⁴ Moreover, analysis of leukocyte infiltrate following HSV-1 infection revealed polymorphonuclear leukocytes (granulocytes) to be one of the first and most abundant immune cells recruited to the cornea.⁵ Following HSV-1 infection, granulocytes such as neutrophils are recruited in two waves.⁶ The first wave peaks at 48 hours post-infection (p.i.) whereas the second wave begins 8 days p.i. and also includes the infiltration of adaptive immune cells such as CD4⁺ T cells.

In the mouse, two chemokines, CXCL1 and CXCL2, have been identified as neutrophil chemoattractants and are thought to be functional homologues of human IL-8.^{7–9} Early studies employed an anti-Gr-1 antibody (RB6-8C5) to deplete neutrophils and study their role during acute HSV-1 infection. The results revealed a significant reduction in CXCL2 levels following infection, whereas CXCL1 levels were unaffected, suggesting corneal cells may be the primary source for CXCL1 production and both corneal cells and infiltrating leukocytes produce the majority of CXCL2.¹⁰ CXCL1 and CXCL2 target cells through the cognate receptor, CXCR2. Balb/c mice lacking this receptor display minimal Gr-1⁺ cell recruitment to the cornea proper during the first 7 days following HSV-1 infection.¹¹ These results implied that CXCR2 is the primary receptor on Gr-1⁺ cells that responds to CXCL1/CXCL2 expression within the cornea. Furthermore, administration of neutralizing antibody to CXCL2 at the time of HSV-1 infection was found to significantly reduce corneal opacity and leukocyte infiltration, whereas treatment with CXCL1 neutralizing antibody had no significant impact.¹⁰ These results suggested CXCL2 is essential for leukocyte recruitment. However, a recent study using CXCL1-deficient (CXCL1^{-/-}) mice revealed a critical role for this chemokine during adenoviral keratitis.¹² In that study, both CXCL1^{-/-} and CXCR2-deficient (CXCR2^{-/-}) mice exhibited a delay in neutrophil infiltration in response to adenovirus infection.¹²

Overall, these initial studies suggested Gr-1⁺ cells including neutrophils were the primary innate immune cells responsible for viral resistance during acute infection. In contrast, a recent study revealed that mAb RB6-8C5 also binds to some populations of dendritic cells, macrophages, monocytes, and

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Supported by NIH/NEI Grant RO1 EY021238, NEI Core Grant EY12190, RPB Senior Investigator Award (DJJC), and PHF Presidential Professor Award (DJJC).

Submitted for publication June 14, 2012; revised August 7, 2012; accepted August 25, 2012.

Disclosure: K.M. Bryant-Hudson, None; D.J.J. Carr, None

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FIGURE 1. HSV-1 viral titer. Scarified WT and CXCL1^{-/-} mouse corneas were infected with 10^3 PFU HSV-1/cornea. Corneas were harvested at 1, 3, and 7 days p.i. and HSV-1 viral titers were assessed by plaque assay. This figure summarizes 3 to 4 experiments/time point ($n = 9-11$ /group). Error bars represent SEM. * $P < 0.05$.

T cells.¹³ Moreover, the recent use of mAb 1A8 (anti-Ly6G) to induce neutropenia has suggested neutrophils do not play a major role in viral clearance during an intranasal or zosteriform model of HSV-1 infection.^{14,15} Therefore, the role of neutrophils in clearing virus from the eye remains ambiguous.

In the current study, the contribution of neutrophils and CXC chemokines was investigated following ocular infection with HSV-1 and *Pseudomonas aeruginosa*. Similar to the above studies regarding HSV-1 corneal infection, the expression of CXC chemokines including CXCL1 and CXCL2 has been investigated during *P. aeruginosa* ocular infection. Results indicated CXCL1 protein levels are lower compared with CXCL2, and peaked at 12 hours p.i., whereas CXCL2 levels continued to increase during the tested time period (12 hours to day 5 p.i.).¹⁶ The kinetics of such expression suggested CXCL1 may be important for the initial recruitment of neutrophils to the cornea, while CXCL2 may regulate the sustained neutrophil response at later time points.¹⁶

To address some of the above issues, CXCL1^{-/-} mice were infected at the corneal surface with HSV-1 or *P. aeruginosa*. Following infection, pathogen burden and leukocyte recruitment (NK cells, neutrophils, inflammatory monocytes, and macrophages) to the cornea were assessed. In addition, protein levels of several critical chemokines induced following ocular infection were measured. Our results reveal CXCL1 and CXCL2 expression is not required for control of HSV-1 infection in the cornea. However, loss of CXCL1 and CXCL2 significantly increased the number of viable bacteria found following infection. Our data suggest this may be due to a skewed recruitment of leukocytes to the site of infection.

MATERIALS AND METHODS

Animals

C57BL/6 (WT) mice were obtained from The Jackson Laboratory and maintained at Dean McGee Eye Institute. CXCL1-deficient mice (CXCL1^{-/-}) were obtained from Dr. Sergio Lira at the Mount Sinai Medical Center, New York. Animal treatment was consistent with the National Institutes of Health Guidelines on the Care and Use of Laboratory Animals. Authors adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All procedures were approved by the University of Oklahoma Health Sciences Center and Dean McGee Eye Institute Institutional Animal and Care Use Committees.

Virus and Bacteria

HSV-1 (strain McKrae) was grown and maintained as previously described.¹⁷ *P. aeruginosa* (strain PA01) was obtained from Dr. Michelle Callegan (University of Oklahoma Health Sciences Center) and cultured in Luria Broth (LB) media.

Corneal Infection

Male and female C57BL/6 or CXCL1^{-/-} mice (6–10 weeks of age) were anesthetized by intraperitoneal (IP) injection with xylazine (6.6

FIGURE 2. Infiltrating leukocytes following HSV-1 infection. Scarified WT and CXCL1^{-/-} mouse corneas were infected with 10^3 PFU HSV-1/cornea. Corneas were harvested at 3 and 7 days p.i. and processed for flow cytometry. This figure summarizes 2 to 3 experiments/time point ($n = 4-8$ /group). Each bar represents mean $\pm$ SEM.

mg/kg) and ketamine (100 mg/kg) followed by scarification of the cornea using a 25 5/8-gauge needle. For HSV-1 infections, the tear film was then blotted, and the cornea was topically inoculated with 10^3 plaque-forming units (PFU) of HSV-1 in 3 μ L of RPMI-1640 medium. For *P. aeruginosa* infections, the bacteria were grown to OD_{600nm} 1.0 at 37°C. Cultures were subsequently diluted into sterile PBS to a final concentration of 10^4 CFU of *P. aeruginosa* in 3 μ L of sterile PBS. HSV-1 viral titers were determined in the cornea at indicated times p.i. by plaque assay as previously described.¹⁸ *P. aeruginosa* levels were determined in the designated tissues at indicated times p.i. following homogenization of tissue in sterile PBS and plating of serially diluted sample on agar plates. Colony forming units (CFU) were determined for each sample.

Flow Cytometry

At the indicated time p.i., mice were exsanguinated, and the corneas (without the iris and limbus) were removed, processed, labeled with Abs, and analyzed using a flow cytometer (Coulter Epics XL; Beckman Coulter, Brea, CA) with the absolute number of cells residing in the indicated tissue determined as previously described.¹⁹ The following Abs were used for the identification of cell populations; anti-CD3 (clone 1742), anti-NK1.1 (clone PK136), anti-CD45 (clone 30-F11), anti-F4/80 (clone MCA497FA), and anti-Gr1 (clone RB6-8C5).

Suspension Array and ELISA

At the indicated time p.i., cornea were removed from the exsanguinated mice and assayed for the detection of CXCL1, CCL2, CCL5, and IFN- γ using a suspension array system (Bio-Rad). CXCL2 levels were determined by ELISA according to the manufacturer's instructions (mouse CXCL2/MIP-2 Quantikine immunoassay; R&D Systems, Minneapolis, MN).

Immunofluorescence Staining and Analysis of Neutrophil Influx

Corneas were prepared and stained for specific antigens as described previously.²⁰ Images were analyzed with imaging software (MetaMorph Imaging Suite v7.0; MDS Analytical Technologies, Sunnyvale, CA), with the positive area defined as the percentage of Ly6G-positive pixel area per field of view. Each HSV-1 lesion was centered in a 1024×1024 pixel area obtained with a 20 $\times$ objective. All images were captured and analyzed under identical image acquisition settings. Each cornea contained 1 to 2 lesions. The sources of antibodies used are the following: rabbit anti-HSV-1 (DACO); Dylight 549 donkey anti-rabbit (Jackson ImmunoResearch Laboratories, West Grove, PA); and FITC Rat anti-Ly6G (BD Pharmingen, San Jose, CA). Images were taken using an epifluorescence/confocal laser-scanning microscope (IX81-FV500; Olympus, Center Valley, PA).

Histopathology and OCT

For histopathological analysis, eyes were fixed in 4% paraformaldehyde and embedded in paraffin. Sections (5- μ m thick) were cut, deparaffinized, and stained with Hematoxylin and eosin (H&E). Eyes were assessed at regular intervals following infection using OCT (Biophtigen, Triangle Park, NC) for changes in corneal thickness. Images were acquired using spectral domain OCT software (InVivoVue Clinic; Biophtigen).

CXCL5 Neutralization

Neutralizing antibody to murine CXCL5 was purchased from R&D Systems. Infected mice were given subconjunctival injections of 5 μ g of neutralizing antibody to CXCL5 or control goat IgG in 10 μ L of PBS. The anti-CXCL5 antibody 50% neutralizing dose (ND₅₀) is 1 to 5 μ g/mL for 0.2 μ g/mL of recombinant murine CXCL5.

FIGURE 3. Ly6G⁺ neutrophil recruitment to HSV-1 lesions. WT and CXCL1^{-/-} mice were infected with 10^3 PFU HSV-1/cornea. Corneas were harvested at 3 days p.i., processed for confocal microscopy, and stained for HSV-1 antigen (red) and Ly6G expression (green). (A) Representative corneas stained for HSV-1 antigen (red) and Ly6G expression (green). (B) Ly6G positive pixel area per field of view. Each bar represents mean $\pm$ SEM. ($n = 6$ /group). Scale bar, 100 μ m.

Statistics

The statistical module prism was used to perform unpaired two-tailed Student's *t*-test and ANOVA with Tukey's *t*-test. All error bars represent the standard errors of the means. A *P* value < 0.05 was considered significant when comparing C57BL/6 with CXCL1^{-/-} mice.

RESULTS

Cornea Viral Titer and PMN Recruitment following HSV Infection

To assess the contribution of CXCL1 during acute ocular HSV-1 infection, WT and CXCL1^{-/-} mice were infected with 10^3 PFU HSV-1, and the corneas were removed at days 3 and 7 p.i. and assayed for infectious virus by plaque assay (Fig. 1). The results revealed a significant reduction in viral titer at day 3 p.i. for the CXCL1^{-/-} mice; however, by day 7 p.i., the viral burden was similar to WT. The discrepancy in the viral load comparing day

FIGURE 4. Viable *P. aeruginosa* following ocular infection. WT and CXCL1^{-/-} mice were infected with 10⁴ CFU *P. aeruginosa*/cornea. At 6, 12, and 24 hours p.i. corneas were harvested and CFU was determined by serial dilution and plating on agar plates. This figure summarizes 2 to 3 experiments ($n = 6$ –12/group/time point). Each bar represents mean $\pm$ SEM. * $P < 0.05$.

3 and day 7 p.i. time points may be due to an initial loss in virus replication as a result of an unforeseen unique phenotype of the CXCL1^{-/-} mice. Therefore, viral titer was also assessed at day 1 p.i. However, the data revealed similar viral loads for WT and CXCL1^{-/-} mice at the time point (Fig. 1).

Since there was a temporal reduction in HSV-1 titer in the cornea of CXCL1^{-/-} mice at day 3 p.i., we next determined whether the loss of this chemokine contributed to changes in the viral load reflected by leukocyte recruitment including NK cells (CD3⁺ NK1.1⁺), inflammatory monocytes (F480⁺ Gr1⁺), macrophages (F480⁺ Gr1⁻), and neutrophils (F480⁻ Gr1⁺) to the cornea at days 3 and 7 p.i. Cell counts from WT and CXCL1^{-/-} mouse corneas revealed no significant differences (Fig. 2).

While flow cytometry allowed us to measure infiltration of cells into the cornea, it did not address cell topography relative to HSV-1. In our model of ocular HSV-1, lesions localize to the central region of the cornea. Following infection and expression of chemokines, leukocytes extravasate from the vascularized limbus region in the peripheral cornea to sites of active infection. To address neutrophil recruitment to such areas, infected corneas were harvested at day 3 p.i. and examined by confocal microscopy for the expression of HSV-1 antigen and Ly6G, a marker specific for granulocytes. Examination of infected cornea lesions revealed a high level of variability among sample groups (Fig. 3A). Some WT cornea lesions exhibited intense Ly6G staining while others displayed minimal neutrophil recruitment. In contrast, many of the CXCL1^{-/-} mouse cornea lesions displayed little to no

FIGURE 5. Infiltrating leukocytes following *P. aeruginosa* infection. Scarified WT and CXCL1^{-/-} mouse corneas were infected with 10⁴ CFU *P. aeruginosa*/cornea. Corneas were harvested at 6, 12, and 24 hours p.i. and processed for flow cytometry. This figure summarizes two experiments ($n = 3$ –6 corneas/group). Each bar represents mean $\pm$ SEM.

FIGURE 6. Histopathology of HSV-1 and *P. aeruginosa* infected corneas. Scarified WT and CXCL1^{-/-} mouse corneas were infected with 10³ PFU HSV-1/cornea or 10⁴ CFU *P. aeruginosa*/cornea. Corneas were harvested and stained with H&E. (A) HSV-1 infected corneas day 3 p.i. (B) *P. aeruginosa* infected corneas 12 hours p.i. (C) *P. aeruginosa* infected corneas 24 hours p.i. Top panel shows entire cornea. Middle and lower panels show a close up of the central and peripheral cornea. Asterisk indicates site of epithelial loss. Images are representative of two experiments, four corneas per group/time point.

neutrophil recruitment with less variability as compared with corneas from WT mice (Fig. 3A). However, quantitative analysis of areas positive for Ly6G expression revealed no significant difference ($P = 0.2174$) between WT and CXCL1^{-/-} corneas (Fig. 3B).

Bacterial Clearance following *P. aeruginosa* Infection

Previous studies have shown the rapid influx of neutrophils following bacterial infection is critical for controlling pathogen replication.^{16,21} Therefore, *P. aeruginosa* was chosen as a well-characterized bacterial ocular pathogen. Scarified WT and CXCL1^{-/-} mouse corneas were infected with 10⁴ CFU *P. aeruginosa*, and the corneas were removed at 6, 12, and 24 hours p.i. and assayed for viable bacteria. As anticipated, loss of CXCL1 resulted in a significant increase in the number of viable bacteria found in the cornea at 12 and 24 hours p.i. (Fig. 4).

To investigate the mechanism by which this occurs, the number of infiltrating leukocytes including NK cells, inflammatory monocytes, macrophages, and neutrophils was assessed in the cornea at 6, 12, and 24 hours p.i. Unexpectedly, there was no numerical change of leukocytes infiltrating the cornea following a *P. aeruginosa* infection comparing WT with CXCL1^{-/-} mice (Fig. 5).

Histopathology and OCT

Based upon the flow cytometry data, tissue sections were analyzed to further evaluate tissue pathology following infection. The eyes of HSV-1-infected WT and CXCL1^{-/-} mice revealed little cellular infiltration into the cornea in comparison with bacterial-infected samples (Fig. 6A). *P. aeruginosa*

infected corneas revealed similar levels of cellular infiltration at 6 hours (data not shown) and 12 hours p.i. (Fig. 6B). However, at 24 hours p.i., mice infected with *P. aeruginosa* exhibited a dense cellular infiltrate in the cornea and anterior chamber. H&E staining of *P. aeruginosa*-infected CXCL1^{-/-} mice revealed loss in corneal integrity as a result of the “melting” of the epithelial layer in the central cornea (Fig. 6C). Furthermore, leukocyte recruitment to the central cornea of CXCL1^{-/-} mice was marginalized either by the loss of the epithelium or a lack in chemoattractant signaling due to the absence of CXCL1 (Fig. 6C, asterisk). By comparison, WT mice exhibited cellular infiltration to both the peripheral and central cornea with the central corneal epithelium remaining intact. Since H&E staining of *P. aeruginosa*-infected CXCL1^{-/-} corneas revealed a skewed pattern of cell recruitment, we chose to further evaluate the timing of corneal thickening using OCT. OCT analysis of WT and CXCL1^{-/-} mouse corneas was performed at 0, 6, 9, 12, 18, and 24 hours p.i. Results indicated CXCL1^{-/-} mouse corneas appear to thicken earlier than WT following infection, with noticeable edema observed as early as 6 hours p.i. (Fig. 7, arrows). Morphological changes did not occur in WT corneas until 12 hours p.i. (Fig. 7, asterisk). We conclude from the analysis that CXCL1 expression maintains structural integrity of the cornea reducing bacterial burden through appropriate recruitment of phagocytic granulocytes/monocytes and thus, reducing the level of proteases secreted by the colonizing bacteria.

Chemokine Expression following Ocular Infection

The ability of CXCL1^{-/-} mice to clear an ocular infection varied depending on the pathogen (Figs. 1, 3) suggesting different pathogens may elicit different chemokine responses and

FIGURE 7. OCT images of *P. aeruginosa*-infected corneas. Scarified WT and CXCL1^{-/-} mouse corneas were infected with 10⁴ CFU *P. aeruginosa*/cornea. Mice were assessed at regular intervals for corneal thickening via OCT. Arrows indicate corneal thickening at 6 hours p.i. in CXCL1^{-/-} mice compared with WT. Asterisk indicates corneal thickening in WT mice at 12 hours p.i. Four corneas per group/time point were analyzed.

subsequently, different leukocyte recruitment patterns. Therefore, the expression of select chemokines and IFN- γ following either HSV-1 or *P. aeruginosa* infection was next assessed. IFN- γ , CCL2, CCL5, CXCL1, and CXCL2 are expressed in the cornea following ocular infection and are believed to be important for efficient clearance of the virus through the recruitment of innate immune cells and proper activation of the adaptive immune response.²² Luminex-based suspension array analysis of corneal tissue revealed similar levels of IFN- γ , CCL2, and CCL5 between WT and CXCL1^{-/-} mice following either viral or bacterial infection (data not shown). CXCL1 levels were highly expressed following *P. aeruginosa* corneal infection in WT mice (Fig. 8A). Another chemoattractant molecule for granulocytes, CXCL2, was reduced in the corneas of *P. aeruginosa*

(Fig. 8B) and HSV-1-infected (Fig. 8C) CXCL1^{-/-} mice. Since loss of CXCL1 and reduced expression of CXCL2 did not impact viral clearance, the role of another granulocyte chemoattractant CXC chemokine, CXCL5, was evaluated during HSV-1 infection. The administration of neutralizing antibody to CXCL5 resulted in a modest but insignificant increase in virus recovered from the cornea of WT (Fig. 9A) and CXCL1^{-/-} (Fig. 9B) mice compared with animals treated with the isotype control.

DISCUSSION

Several studies investigating the role of innate immune cells during acute ocular infection have suggested that neutrophils are the primary recruited cells that control pathogen replication through the application of RB6-8C5 antibody.^{10,11,23,24} However, RB6-8C5 recognizes both Ly6G and Ly6C antigens, molecules expressed by neutrophils, dendritic cells, and subpopulations of lymphocytes and monocytes.¹³ Therefore, the contribution of neutrophils to viral resistance remains unclear. In the present study, we chose to investigate the role of CXCL1 in pathogen clearance, comparing acute HSV-1 and *P. aeruginosa* corneal infections.

HSV-1-infected WT and CXCL1^{-/-} mice exhibited similar viral burden at day 7 p.i., indicating CXCL1 is not required to facilitate viral clearance. This observation is consistent with previous studies indicating CXCL2 is the primary chemoattractant for neutrophils, the cell type originally thought to be critical for viral clearance.¹⁰ CXCL2 protein levels were also assessed in CXCL1^{-/-} mice following HSV-1 infection. We expected these levels to be similar or increased compared with WT mice as a means to compensate for the loss of CXCL1. However, the absence of CXCL1 corresponded with a similar loss in CXCL2 protein expression. Similar results were reported using CXCL1^{-/-} mice following an adenovirus ocular infection.¹²

During early acute infection, the loss of CXCL1 appears to play a protective role, since CXCL1^{-/-} mice have lower viral titers as compared with WT animals. However, this protection is transient as viral titers quickly increase to levels similar to those seen in WT HSV-1 infected mice (Fig. 1).

Assessment of cornea leukocytes from WT and CXCL1^{-/-} mice following HSV-1 infection revealed similar numbers of NK cells, neutrophils, inflammatory monocytes, and macrophages. Given the absence of CXCL1 and lower levels of CXCL2 in the cornea of HSV-1 infected, CXCL1^{-/-} mice, we conclude these chemokines are not the principal mediators for recruitment of neutrophils to the cornea. While loss of CXCL1 and CXCL2 did not impact total leukocyte recruitment to the cornea, there was a reduced number of neutrophils localized to the HSV-1 lesions. The reduction was not significant, suggesting redundant signals may exist. Moreover, neutralization of CXCL5 in HSV-1-infected WT and CXCL1^{-/-} mice did not alter viral clearance, suggesting CXCL1, CXCL2, and CXCL5 are not required for host resistance during HSV-1. Other mechanisms such as the activation of complement and expression of C5a may recruit neutrophils in the absence of CXCL1 and CXCL2.²⁵ Recent studies investigating viral clearance using intranasal and zosteriform models of HSV-1 infection have found that anti-Ly6G-induced neutropenia does not influence viral replication, whereas mice treated with anti-Gr-1 antibodies suffered from increased viral loads.^{14,15} Yet, another study reported NK cell depletion using anti-ASGM-1 antibody resulted in a delay in HSV-1 clearance during ocular infection.²⁶ Recently, we have found activated monocytes (F4/80⁺, Gr-1⁺, Ly-6C⁺, Ly-6G⁻) are associated with viral surveillance in the cornea during the initial (i.e., first 48 hours p.i.) challenge of the virus.²⁷

FIGURE 8. Chemokine expression following ocular infection. (A, B) WT and CXCL1^{-/-} mice infected with 10^4 CFU *P. aeruginosa*/cornea. (C) WT and CXCL1^{-/-} mice infected with 10^5 PFU HSV-1/cornea. HSV-1 infected corneas (60 hours p.i.) and *P. aeruginosa* infected corneas (18 hours p.i.) were harvested and processed for suspension array or ELISA. This figure summarizes three experiments ($n = 9$ /group). Each bar represents mean $\pm$ SEM. * $P < 0.05$.

Furthermore, treatment of HSV-1-infected mice with anti-Ly6G did not impact viral titers in the cornea.²⁷ Taken together, such findings provide evidence to suggest NK cells and activated monocytes/macrophages are critical for viral clearance during acute HSV-1 infection.

Unlike what was observed during ocular HSV-1 infection, CXCL1^{-/-} mice harbored significantly more viable bacteria than WT mice. Such mice also produced significantly less CXCL2 compared with their WT counterparts. H&E staining of CXCL1^{-/-} *P. aeruginosa* infected corneas revealed a skewed leukocyte recruitment pattern with cells compartmentalized to the peripheral cornea. These data suggest CXCL1 and/or CXCL2 are not required for recruitment of leukocytes into the peripheral cornea. However, these chemokines may be critical for directional migration to the central cornea. Along with aberrant leukocyte movement in the cornea, CXCL1^{-/-} mice displayed increased corneal edema as observed by OCT imaging. These results are similar to what was noted following infection of CXCR2^{-/-} mice suggesting CXCL1/CXCL2 signaling via this receptor is required for localization of leukocytes in response to the bacteria.²⁸

Delayed infiltration of neutrophils to the site of infection may alter their ability to phagocytose bacteria resulting in increased replication in the central cornea. Tissue pathology

following *P. aeruginosa* infection is due to both pathogen and host factors.²⁹ The bacteria can secrete a plethora of proteases and toxins capable of inducing host cell death and tissue damage. Moreover, bacterial factors such as LPS and flagella can activate toll-like receptors, inducing cornea epithelial cells and resident macrophages to produce inflammatory cytokines and chemokines.³⁰

From a diagnostic standpoint, in our study, the use of OCT was a more sensitive means of detecting tissue pathology earlier in comparison with immunohistochemical means. Specifically, changes in apparent corneal thickness were detected within the first 6 hours p.i. in CXCL1^{-/-} mice by OCT while tissue pathology was not detected at 12 hours p.i. by H&E staining. Although this is the first such comparison, it is tempting to speculate OCT imaging will become a standard diagnostic tool for corneal infections leading to a better predictor for treatment and outcome.

This study is the first to compare the contribution of CXC chemokines during ocular infection with HSV-1 and *P. aeruginosa*. Our results demonstrate that CXCL1, CXCL2, and CXCL5 are not required for viral clearance but are instrumental in the control of *P. aeruginosa* infection as a result of proper stoichiometric signaling and placement of leukocytes into the central cornea.

FIGURE 9. HSV-1 viral titer following neutralization of CXCL5. Mice were infected with 10^5 PFU HSV-1/cornea. (A) WT mice. (B) CXCL1^{-/-} mice. At day 0 and 2 p.i., mice were administered neutralizing antibody to CXCL5 or control IgG. Corneas were harvested at day 3 p.i. and HSV-1 viral titers were assessed by plaque assay. This figure summarizes two experiments ($n = 6$ /group). Error bars represent SEM.

Acknowledgments

The authors would like to thank Lindsey Linthicum for her technical help.

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