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CD4+ T Cell Migration into the Cornea is Reduced in CXCL9 Deficient but not CXCL10 Deficient Mice following Herpes Simplex Virus Type 1 Infection[1]

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Abstract

The role of CXCL9 and CXCL10 in the ocular immune response to herpes simplex virus type 1 (HSV-1) infection was investigated using mice deficient in either CXCL9 or CXCL10. CXCL10 but not CXCL9 deficient mice showed an increase in sensitivity to ocular virus infection as measured by an elevation in virus titer recovered in the tear film and corneal tissue. The increase in virus was associated with an increase in the expression of the chemokine CCL2 but no significant change in the infiltration of $CD4^+$ T cells or NK cells into the corneal stroma. In contrast, a significant reduction in $CD4^+$ T cell infiltration into the cornea was found in CXCL9 deficient mice following HSV-1 infection consistent with the absence of CXCL9 expression and reduction in expression of other chemokines including CCL3, CCL5, CXCL1, and CXCL10. Collectively, the results suggest a non-redundant role for CXCL9 and CXCL10 in response to ocular HSV-1 infection in terms of controlling virus replication and recruitment of $CD4^+$ T cells into the cornea.

Keywords

HSV-1; chemokines; CXCL9; CXCL10; knockout mice; cornea; T lymphocyte

1. Introduction

Herpes simplex virus type 1 (HSV-1) infection of the eye induces an inflammatory response consisting of infiltrating leukocytes and the production of cytokines within the infected tissue including the cornea [1,2]. Although PMNs are one of the initial cells that traffic to the cornea [3], $CD4^+$ and $CD8^+$ T lymphocytes are thought to be key components in the development of lesions associated with herpetic stromal keratitis [4–7]. Th2 cytokines exacerbate HSV-1 infection whereas Th1 cytokines are principally produced by those cells infiltrating the infected tissue and contribute towards resistance to virus replication and spread [8–10]. Chemokines

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expressed locally are likely involved in the recruitment of leukocytes into the site of infection with CCL2 and CXCL10 expressed or up-regulated significantly within the first 36 hr post infection relative to other chemokines including CCL3, CCL5, CXCL1, and CXCL9 [11].

The role of CXCL10 within the cornea of mice infected with HSV-1 has been investigated using neutralizing antibody. Specifically, mice administered anti-CXCL10 Ab show a significant reduction in corneal inflammation with a rise in virus titer recovered from the cornea and TG following infection with HSV-1 [12]. However, these mice survive to a greater extent in comparison to control antibody-treated animals [13]. Consistent with these results, CXCR3 deficient mice have an elevated virus titer recovered from the TG and brain stem along with an increase in survival following infection [14]. Taken together, the results suggest CXCR3 and CXCL10 are involved in the control of HSV-1 replication and spread during acute ocular HSV-1 infection.

However, the impact of CXCL10 on lymphocyte recruitment to the site of ocular infection is currently unknown. Previous studies have reported positive [15,16] and negative [17,18] consequences linked to CXCL10 or CXCR3 expression during infection or autoimmune disease implying the role of CXCR3 and its ligands may be tissue- and pathogen-specific.

The present study investigated the role of two CXCR3 ligands, CXCL9 and CXCL10, during the course of acute ocular HSV-1 infection. Both CXCL9 and CXCL10 are expressed or up-regulated in the cornea and TG in response to corneal infection with HSV-1 [12]. Since T cells are recruited to the inflammatory site during acute ocular HSV-1 infection [4–7] and CXCL9 and CXCL10 are associated with the recruitment of activated T cells during inflammatory processes [19–24], questions remain as to the temporal nature of expression, redundancy in recruitment of T cells, and the impact of expression on the production of other cytokines and chemokines in the inflamed tissue following infection. To address these questions, mice deficient in CXCL9 (CXCL9−/−) or CXCL10 (CXCL10−/−) were compared to wild type (WT) controls for resistance to infection and the local host immune response following placement of the virus onto the cornea.

2. Results and Discussion

2.1 Virus titers in WT, CXCL9−/−, and CXCL10−/− mice

Mice infected with HSV-1 were assayed at times post infection (pi) for virus load in the eye. Whereas no differences were found with the cornea in the first 5 days pi, by day 7 pi CXCL10 −/− mice possessed significantly more HSV-1 in comparison to WT or CXCL9−/− mice (Fig. 1). In a similar fashion, virus shedding in the tear film was elevated at day 7 pi but not earlier time points surveyed in the CXCL10−/− mice compared to WT or CXCL9−/− mice. Further time points were not assessed since the majority of CXCL10−/− mice succumb to the infection between day 7 and day 9 pi. Since virus replication was unchanged in the CXCL10−/− mice until day 7 pi, the results imply the adaptive rather than innate immune response is most likely affected by the loss of CXCL10 expression.

2.2 $CD4^+$ T cell infiltration into the cornea is reduced in CXCL9−/− mice

Since increases in virus shedding in the tear film and virus titer within the cornea were observed in CXCL10 deficient mice by day 7 pi, inflammation was assessed at this time point in all groups of mice measuring cytokine/chemokine expression and T lymphocyte infiltration. In measuring a battery of chemokines including CCL2, CCL3, CCL5, CXCL1, CXCL9, and CXCL10, with the exception of CXCL10, only CCL2 levels were modified (elevated) in the cornea of the CXCL10−/− mice in comparison to either the WT or CXCL9−/− mice (Table 1). In contrast, there was a noticeable reduction in CCL3, CCL5, CXCL1, and CXCL10 expression

in the cornea of CXCL9−/− mice in comparison to WT and CXCL10−/− mice (Table 1). Likewise, IL-1β, IL-6, and TNF-α levels were not detectable or were significantly lower in the cornea of CXCL9−/− mice compared to the other two groups (Table 1). The expression of these cytokines/chemokines is in response to infection since uninfected mouse corneas contain significantly lower levels of the assayed analytes [11].

CXCL9 and CXCL10 recruit T cells to sites of inflammation thru CXCR3 [25], and T cells infiltrate the cornea during the latter stages of acute HSV-1 infection [1]. Therefore, T cell trafficking into the cornea was investigated following virus infection. There were no detectable $CD3^+$ T cells in the cornea of uninfected mice and less than 10 cells in the peripheral cornea proximal to the ciliary body at day 3 pi (data not shown). By day 7 pi, $CD3^+CD4^+$ T cells were readily detectable in both the central and peripheral cornea in all groups of mice (Fig. 2). A reduction in the recruitment of $CD4^+$ T cells to the peripheral and central cornea was found in CXCL9−/− mice compared to the WT controls (Fig. 2). Unlike the results in CXCL9−/− mice, $CD4^+$ T cell infiltration into the cornea of CXCL10−/− mice was remarkably similar to WT mice (Fig. 2). $CD8^+$ T cells are also known to infiltrate the cornea following virus infection depending on the strain of HSV-1 and mouse host employed for the study [6,26]. Moreover, in the absence of $CD4^+$ T cells, $CD8^+$ T cells have been found to infiltrate the cornea and elicit transient immunopathology within the stroma following HSV-1 infection [27]. Therefore, it was possible that $CD8^+$ T cells would compensate for the reduction in $CD4^+$ T cells found in the cornea of CXCL9−/− mice following HSV-1 infection. However, a similar pattern of $CD3^+CD4^-$ T cell infiltration was observed in the CXCL9−/− mice compared to WT mice (Fig. 3). Taken together, the results reflect a deficiency of T cell trafficking into the cornea of CXCL9−/− mice following ocular HSV-1 infection.

Since changes in the trafficking of $CD4^+$ T cells did not explain the increased sensitivity of the CXCL10−/− mice to HSV-1 infection, NK cell recruitment was investigated day 7 pi. NK cells are thought to contribute to stromal keratitis [28], control HSV-1 infection [29,30], and respond to a CXCL10 chemical gradient through the receptor CXCR3 [31]. However, similar numbers of NK cells were found residing in the cornea comparing HSV-1-infected WT (225 ± 70 cells/cornea pair), CXCL9−/− (260 ± 90 cells/cornea pair), and CXCL10−/− (190 ± 90/cornea pair) mice.

In this study, the local infiltration of NK and T cells into the cornea following infection does not reflect host control of the virus. Specifically, CXCL10−/− mice possess significantly more HSV-1 in the cornea by day 7 pi compared to WT or CXCL9−/− mice and yet, displayed similar levels of NK and T cell recruitment compared to WT mice. The deficiency in the control of virus replication by CXCL10−/− mice may reside within the nervous system. CXCL10−/− mice possess significantly more HSV-1 in the innervating sensory ganglia for the cornea, the trigeminal ganglia (TG) within the first 72 hr of infection [Wuest et al., manuscript in preparation]. This inability to control HSV-1 replication may translate into an increase in virus found in the cornea during the latter stages of the acute infection. To this end, zosteriform spread of the virus from the TG back to the eye including the tear film, eye proper, and periocular tissue is noted only in virus that is capable of retrograde transport to the TG during acute infection [32].

A reduction in chemokine expression in the cornea of HSV-1-infected CXCL9−/−mice correlates with the drop in T cell recruitment. As activated T cells not only express the receptor for CXCR3 but also express CCR1, CCR2, and CCR5 for the ligands CCL3 and CCL5 among others, the drop in T cell recruitment into the cornea of the CXCL9−/−mice is consistent with the absence or significant reduction in CXCL9, CCL3, and CCL5 levels found in the tissue in response to HSV-1. The results also suggest chemokine expression in the cornea is not driven by levels of antigen alone since CXCL9−/− mice possessed similar titers of HSV-1 compared

to WT mice and yet, showed dramatically reduced cytokine/chemokine levels. Such results are consistent with a recent finding in which highly resistant IFN-α1 transgenic mice display nearly identical levels of chemokines including CCL3, CCL5, CXCL9, and CXCL10 expressed in the cornea as their WT counterparts in response to ocular HSV-1 infection [33].

Both resident corneal cells and infiltrating leukocytes generate the chemokines detected in the present study including CXCL9 and CXCL10 [11,34–37]. Out of the six chemokines investigated, only CCL2 levels were associated with sensitivity to infection since CXCL10−/− mice possessed significantly more virus and expressed elevated levels of CCL2 in the cornea in comparison to the less sensitive WT or CXCL9−/− mice. CCL2 has been linked to increased pathogenesis and mortality in mice infected with HSV-1 [38] perhaps thru a T_H1 to T_H2 switch [39]. However, we have not been able to detect IL-4 and rarely detect IL-10 at the protein level in the corneas of these mice. Moreover, the pattern of IFN-γ expression in the cornea of WT, CXCL9−/−, or CXCL10−/− mice does not support the change or reduction of T_H1 cytokine expression as an explanation for the increased sensitivity of CXCL10−/− mice to ocular HSV-1 infection.

The present results are inconsistent with a previous study in which the administration of anti-CXCL10 antibody to mice resulted in a reduction in edema and infiltrating mononuclear cells in the cornea in comparison to isotype antibody-treated mice following corneal infection with HSV-1 [12]. In the antibody depletion study, ICR mice which are more sensitive to HSV-1 compared to the C57BL/6 background used in the current study were employed. Likewise, the use of the anti-CXCL10 antibody did not neutralize 100% of the targeted chemokine but rather, reduced the level detectable in the infected tissue. The contribution of CXCL10 by resident cells in comparison to hematopoietic cells recruited to the inflamed tissue or residing in the organized lymphoid organs on maturation and trafficking of effector T and NK cells in response to ocular HSV-1 infection is currently unknown. Obviously more work is warranted evident by the inconsistent results in the current study using CXCL10−/− mice and the previous study incorporating neutralizing antibody to CXCL10 [12]. However, the discrepancy between these two studies is not unique since experimental models of multiple sclerosis show inconsistent results using anti-CXCL10 antibody versus CXCL10−/− mice [40,41].

Collectively, the current findings underscore the unique contribution CXCL9 and CXCL10 maintain in the cornea following ocular HSV-1 infection. The absence of CXCL10 renders the host sensitive to virus replication whereas CXCL9 deficiency has a significant impact on T cell recruitment to the cornea. The results emphasize the non-redundant nature of these CXCR3 ligands and the need to further study their role in the host in response to ocular infection.

3. Materials and Methods

3.1 Mice

Wild type (WT) male and female C57BL/6 (The Jackson Laboratory, Bar Harbor, ME), CXCL9−/− [44], and CXCL10−/− [22] mice (6–12 weeks of age) were housed in sterile cages under pathogen free conditions. CXCL9−/− mice were backcrossed onto a C57BL/6 background 8 generations and CXCL10−/− mice were backcrossed 9 generations. All mice were used in accordance with a detailed protocol approved by the Institutional Animal Care and Use Committee of The University of Oklahoma Health Sciences Center and the Dean A. McGee Eye Institute.

3.2 HSV-1 infection

The corneas of anesthetized mice were scarified with a 25 gauge needle prior to infection with 1,000 plaque forming units (PFU) of HSV-1 (McKrae strain) in a volume of 3 μl of RPMI 1640

medium. Mice were anesthetized and perfused with PBS (pH 7.4) at times post infection. The corneas were removed and processed for cytokine/chemokine content by suspension array or ELISA or prepared for whole mount. Alternatively, the corneas were removed and homogenized in RPMI-1640 containing 10% FBS. Following homogenization, the supernatant was clarified (10,000 x g, 1 min) and subsequently assessed for virus content by plaque assay [12]. Uninfected mice served as controls.

3.3 Virus and cells

The McKrae strain of HSV-1 originally obtained from Bryan Gebhardt (LSU Health Sciences Center, New Orleans, LA) was propagated in Vero cells and maintained at −80° C at a concentration of 2 x 10^8 PFU/ml. Frozen stocks were diluted in RPMI 1640 medium immediately prior to use. Plaque assays using African green monkey kidney fibroblasts (Vero cells, ATCC CCL-81, American Type Tissue Culture Collection, Manassas, VA) were performed in RPMI-1640 medium supplemented with 10% FBS (Invitrogen, Calsbad, CA), gentamicin (Invitrogen), and antibiotic-antimycotic solution (Invitrogen) at 37° C, 5% CO_2, and 95% humidity as previously described [45].

3.4 ELISA

The detection of CCL2, CCL3, CCL5, CXCL1, IL-1β, IL-6, TNF-α, and IFN-γ were performed using a suspension array system (Bio-Rad, Hercules, CA) with a sensitivity of 1–2 pg/tissue for each targeted analyte. The corneas were removed from infected mice following perfusion at day 7 pi and homogenized in T-PER tissue protein extraction reagent (Pierce Chemical Co., Rockford, IL). Each sample was analyzed in duplicate along with known amounts of cytokine/chemokine provided by the kits to generate a standard curve used to determine the quantity of each cytokine/chemokine in a given sample. CXCL9 and CXCL10 were detected using commercially available kits (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions with a sensitivity of 25–35 pg/cornea. In this case, corneal samples were prepared as previously described [12]. The amount of cytokine/chemokine measured was normalized to the total wet weight of each cornea.

3.5 Whole mount preparation

The corneas from infected and uninfected perfused mice were removed from isolated eyes using a dissecting microscope (Fisher) and placed into 1.5 ml microcentrifuge tubes containing 4% paraformaldehyde (Sigma Chemical Co., St. Louis, MO) in PBS. After a 20 min incubation period at room temperature, the corneas were washed 5X with 1.0 ml PBS containing 1% Triton X-100 (Sigma) for 10 min/wash. Following the last wash, corneas were soaked in 100 μl of PBS-BGEN (1X PBS containing 3% BSA, 0.25% gelatin, 5 mM EDTA, and 0.025% nonidet 40) and 1 μl of anti-mouse CD16/32 (BD Pharmingen) for 60 min at room temperature. Following the incubation period, 5 μl of rat serum (Vector Laboratories, Burlingame, CA) was added and the samples were incubated overnight at 4° C. Next, the solution was removed, and the corneas were incubated with 2 μl of alexa fluor 647 conjugated anti-mouse CD3 (clone 17A2, BD Pharmingen) and either FITC conjugated anti-mouse CD4 or CD8 in 100 μl PBS-BGEN overnight at 4° C. The following day, the solution was removed, and the corneas were washed 5X in PBS containing 1% BSA and 0.1% Triton X-100 for 15 min/wash. The corneas were then soaked in Vectashield mounting medium containing DAPI (Vector Laboratories) overnight at 4° C and subsequently mounted on slides for subsequent analysis by confocal microscopy.

3.6 Confocal microscopy

Corneas (n=3/group/experiment) were imaged using an Olympus IX81-FV500 epifluorescence/confocal laser-scanning microscope with a UApo 40x water immersion lens.

Samples were excited with 405, 488, 546, and 633 nm wavelength lasers (Olympus, Melville, NY). Scanning images were taken with a step size of 2 μm in the *z*-axis and image analysis was performed using FLUOVIEW software (Olympus). $CD3^+CD4^+$ and $CD3^+CD4^-$ T cells were enumerated from 6 corneas/group/experiment (3–4 experiments/group) of mice consisting of central and peripheral sections.

3.7 Preparation of cornea samples for detection of NK cells by flow cytometry

Corneal buttons from saline-perfused, HSV-1-infected WT, CXCL9−/−, and CXCL10−/− mice were removed 7 days following infection. The corneas were cut into small (1–2 mm segments) pieces and exposed to 1 mg/ml collagenase type I (Sigma Chemical Co., St. Louis, MO) for 60 minute triturating the sample every 20 minutes. Single cell suspensions were washed twice (300 x g, 5 min/wash) in PBS and then incubated on ice for 15 min with 4 μl anti-mouse Fc block (BD Pharmingen, San Diego, CA) in a total volume of 50 μl PBS-1% BSA. Following the incubation, the cells were centrifuged (300 x g, 5 min) and resuspended in 5% normal rat serum (Jackson Immuno Research Inc., West Grove, PA) for an additional 15 min on ice. Cells were then triple labeled with 6 μl containing 2 μl FITC-conjugated anti-mouse CD3, 2 μl phycoerythrin-Cy5-conjugated anti-CD45 (clone 30-F11), and 2 μl PE-conjugated anti-mouse NK1.1 and incubated in the dark on ice for 30 min. Following the incubation period, the cells were washed 3 times with PBS-1% BSA (300 x g, 5 min/wash) and resuspended in PBS containing 1% paraformaldehyde. After overnight fixation at 4° C in the dark, cells were pelleted (300 x g, 5 min) and resuspended in PBS-1% BSA. Immediately before analysis, CountBright absolute counting beads (Invitrogen, Eugene, OR) were added (28,000 beads/sample). Cell suspensions were gated on $CD45^{high}$ labeled cells, and the percentage of NK cells ($NK1.1^+CD3^-$) were determined at this gate setting. A second gate was established to count the number of beads that passed through during the run (500 sec). The absolute number of NK cells was determined by calculating the number of input beads/28,000 X the percent NK cells in the $CD45^{high}$-gated sample.

3.8 Statistics

One-way ANOVA and Tukey's post hoc *t*-test were used to determine significance ($p<.05$) of differences between WT, CXCL9−/−, and CXCL10−/− mice for each parameter under measure. All statistical analyses were carried out using the GBSTAT program (Dynamic Microsystems, Silver Springs, MD).

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References

1. Niemialtowski MG, Rouse BT. Phenotypic and functional studies on ocular T cells during herpetic infections of the eye. J Immunol 1992;148:1864–1870. [PubMed: 1347309]
2. He J, Ichimura H, Iida T, Minami M, Kobayashi K, Kita M, Sotozono C, Tagawa YI, Iwakura Y, Imanishi J. Kinetics of cytokine production in the cornea and trigeminal ganglion of C57Bl/6 mice after corneal HSV-1 infection. J Interferon Cytokine Res 1999;19:609–615. [PubMed: 10433361]
3. Tang Q, Hendricks RL. Interferon γ regulates platelet endothelial cell adhesion molecule 1 expression and neutrophil infiltration into herpes simplex virus- infected mouse corneas. J Exp Med 1996;184:1435–1447. [PubMed: 8879215]
4. Hendricks RL, Janowicz M, Tumpey TM. Critical role of corneal Langerhans cells in the CD4- but not CD8-mediated immunopathology in herpes simplex virus-1-infected mouse cornea. J Immunol 1992;148:2522–2529. [PubMed: 1313845]
5. Manickan E, Rouse BT. Roles of different T-cell subsets in control of herpes simplex virus infection determined by using T-cell-deficient mouse models. J Virol 1995;69:8178–8179. [PubMed: 7494346]

6. Ghiasi H, Cai S, Perng GC, Nesburn AB, Wechsler SL. Both CD4+ and CD8+ T cells are involved in protection against HSV-1 induced corneal scarring. Br J Ophthalmol 2000;84:408–412. [PubMed: 10729300]
7. Stuart PM, Summers B, Morris JE, Morrison LA, Leib DA. $CD8^+$ T cells control corneal disease following ocular infection with herpes simplex virus type 1. J Gen Virol 2004;85:2055–2063. [PubMed: 15218191]
8. Geiger KD, Nash TC, Sawyer S, Krahl T, Patstone G, Reed JC, Krajewski S, Dalton D, Buchmeier MJ, Sarvetnick N. Interferon-γ protects against herpes simplex virus type 1-mediated neuronal death. Virology 1997;238:189–197. [PubMed: 9400592]
9. Ghiasi H, Cai S, Slanina SM, Perng GC, Nesburn AB, Wechsler SL. The role of interleukin (IL-2) and IL-4 in herpes simplex virus type 1 ocular replication and eye disease. J Infect Dis 1999;179:1086–1093. [PubMed: 10191208]
10. Lekstrom-Himes JA, LeBlanc RA, Pesnicak L, Godleski M, Straus SE. Gamma interferon impedes the establishment of herpes simplex virus type 1 latent infection but has not impact on its maintenance or reactivation in mice. J Virol 2000;74:6680–6683. [PubMed: 10864685]
11. Carr DJJ, Tomanek L. Herpes simplex virus and the chemokines that mediate the inflammation. Curr Top Microbiol Immunol 2006;303:47–65. [PubMed: 16570856]
12. Carr DJJ, Chodosh J, Ash J, Lane TE. Effect of anti-CXCL10 monoclonal antibody on herpes simplex virus type 1 keratitis and retinal infection. J Virol 2003;77:10037–10046. [PubMed: 12941914]
13. Wickham S, Ash J, Lane TE, Carr DJJ. Consequences of CXCL10 and IL-6 induction by the murine IFN-α1 transgene in ocular herpes simplex virus type 1 infection. Immunologic Res 2004;30:191–200.
14. Wickham S, Lu B, Ash J, Carr DJJ. Chemokine receptor deficiency is associated with increased chemokine expression in the peripheral and central nervous systems and increased resistance to herpetic encephalitis. J Neuroimmunol 2005;162:51–59. [PubMed: 15833359]
15. Hildebrandt GC, Corrion LA, Olkiewicz KM, Lu B, Lowler K, Duffner UA, Moore BB, Kuziel WA, Liu C, Cooke KR. Blockade of CXCR3 receptor:ligand interactions reduces leukocyte recruitment to the lung and the severity of experimental idiopathic pneuomonia syndrome. J Immunol 2004;173:2050–2059. [PubMed: 15265940]
16. Trifilo MJ, Montalto-Morrison C, Stiles LN, Hurst KR, Hardinson JL, Manning JE, Masters PS, Lane TE. CXC chemokine ligand 10 controls viral infection in the central nervous system: Evidence for a role in innate immune response through recruitment and activation of natural killer cells. J Virol 2004;78:585–594. [PubMed: 14694090]
17. Frigerio S, Junt T, Lu B, Gerard C, Zumsteg U, Holländer GA, Piali L. β cells are responsible for CXCR3-mediated T-cell infiltration in insulitis. Nature Med 2002;8:1414–1420. [PubMed: 12415259]
18. Christen U, McGavern DB, Luster AD, von Herrath MG, Oldstone MBA. Among CXCR3 chemokines, IFN-γ-inducible protein of 10 kDa (CXC chemokine ligand (CXCL) 10) but not monokine induced by IFN-γ (CXCL9) imprints a pattern for the subsequent development of autoimmune disease. J Immunol 2003;171:6838–6845. [PubMed: 14662890]
19. Liu MT, Chen BP, Oertel P, Buchmeier MJ, Armstrong D, Hamilton TA, Lane TE. Cutting Edge: The T cell chemoattractant IFN-inducible protein 10 is essential in host defense against viral-induced neurologic disease. J Immunol 2000;165:2327–2330. [PubMed: 10946253]
20. Liu MT, Armstrong D, Hamilton TA, Lane TE. Expression of Mig (monokine induced by interferon-γ) is important in T lymphocyte recruitment and host defense following viral infection of the central nervous system. J Immunol 2001;166:1790–1795. [PubMed: 11160225]
21. Padovan E, Spagnoli GC, Ferrantini M, Heberer M. IFN-alpha2a induces IP-10/CXCL10 and MIG/CXCL9 production in monocyte-derived dendritic cells and enhances their capacity to attract and stimulate CD8+ effector T cells. J Leukoc Biol 2002;71:669–676. [PubMed: 11927654]
22. Dufour JH, Dziejman M, Liu MT, Leung JH, Lane TE, Luster AD. IFN-γinducible protein 10 (IP-10; CXCL10)-deficient mice reveal a role for IP-10 in effector T cell generation and trafficking. J Immunol 2002;168:3195–3204. [PubMed: 11907072]

23. Trifilo MJ, Lane TE. Adenovirus-mediated expression of CXCL10 in the central nervous system results in T-cell recruitment and limited neuropathology. J Neurovirol 2003;9:315–324. [PubMed: 12775415]
24. Whiting D, Hsieh G, Yun JJ, Banerji A, Yao W, Fishbein MC, Belperio J, Strieter RM, Bonavida B, Ardehali A. Chemokine monokine induced by IFN- γ/CXC chemokine ligand 9 stimulates T lymphocyte proliferation and effector cytokine production. J Immunol 2004;172:7417–7424. [PubMed: 15187119]
25. Farber JM. Mig and IP-10, CXC chemokines that target lymphocytes. J Leukoc Biol 1997;61:246–257. [PubMed: 9060447]
26. Newell CK, Martin S, Sendele D, Mercadal CM, Rouse BT. Herpes simplex virus-induced stromal keratitis: role of T-lymphocyte subsets in immunopathology. J Virol 1989;67:769–775. [PubMed: 2536102]
27. Lepisto AJ, Frank GM, Xu M, Stuart PM, Hendricks RL. CD8 T cells mediate transient herpes stromal keratitis in CD4-deficient mice. Invest Ophthalomol Vis Sci 2006;47:3400–3409.
28. Tamesis RR, Messmer EM, Rice BA, Dutt JE, Foster CS. The role of natural killer cells in the development of herpes simplex virus type 1 induced stromal keratitis in mice. Eye 1994;8:298–306. [PubMed: 7958034]
29. Rager-Zisman B, Quan PC, Rosner M, Moller JR, Bloom BR. Role of NK cells in protection of mice against herpes simplex virus-1 infection. J Immunol 1987;138:884–888. [PubMed: 3805719]
30. Pereira RA, Scalzo A, Simmons A. Cutting edge: A NK complex-linked locus governs acute versus latent herpes simplex virus infection of neurons. J Immunol 2001;166:5869–5873. [PubMed: 11342599]
31. Martin-Fontecha A, Thomsen LL, Brett S, Gerard C, Lipp M, Lanzavecchia A, Sallusto F. Induced recruitment of NK cells to lymph nodes provides IFN-γ for T_H1 priming. Nature Immunol 2004;5:1260–1265. [PubMed: 15531883]
32. Summers BC, Margolis TP, Leib DA. Herpes simplex virus type 1 corneal infection results in periocular disease by zosteriform spread. J Virol 2001;75:5069–5075. [PubMed: 11333887]
33. Carr DJJ, Campbell IL. Herpes simplex virus type 1 induction of chemokine production is unrelated to virus load in the cornea but not in the nervous system. Viral Immunol. 2006in press
34. Carr DJJ, Ash J, Lane TE, Kuziel WA. Abnormal immune response of CCR5-deficient mice to ocular infection with herpes simplex virus type 1. J Gen Virol 2006;87:489–499. [PubMed: 16476970]
35. Gasperini S, Marchi M, Calzetti F, Laudanna C, Vicentini L, Olsen H, Murphy M, Liao F, Farber J, Cassatella MA. J Immunol 1999;162:4928–4937. [PubMed: 10202039]
36. McInnis KA, Britain A, Lausch RN, Oakes JE. Synthesis of α-chemokines IP-10, I-TAC, and MIG are differentially regulated in human corneal keratocytes. Invest Ophthalmol Vis Sci 2005;46:1668–1674. [PubMed: 15851567]
37. Wuest T, Austin BA, Uematsu S, Thapa M, Akira S, Carr DJJ. Intact TLR9 and type I interferon signaling pathways are required to augment HSV-1 induced corneal CXCL9 and CXCL10. J Neuroimmunol 2006;179:46–52. [PubMed: 16884784]
38. Nakajima H, Kobayashi M, Pollard RB, Suzuki F. Monocyte chemoattractant protein-1 enhances HSV-induced encephalomyelitis by stimulating Th2 responses. J Leukoc Biol 2001;70:374–380. [PubMed: 11527986]
39. Kurt-Jones EA, Chan M, Zhou S, Wang J, Reed G, Bronson R, Arnold MM, Knipe DM, Finberg RW. Herpes simplex virus 1 interaction with toll-like receptor 2 contributes to lethal encephalitis. Proc Natl Acad Sci USA 2004;101:13315–1320.
40. Fife BT, Kennedy KJ, Paniagua MC, Lukacs NW, Kunkel SL, Luster AD, Karpus WJ. CXCL10 (IFN-gamma-inducible protein-10) control of encephalitogenic CD4+ T cell accumulation in the central nervous system during experimental autoimmune encephalomyelitis. J Immunol 2001;166:7617–7624. [PubMed: 11390519]
41. Klein RS, Izikson L, Means T, Gibson HD, Lin E, Sobel RA, Weiner HL, Luster AD. IFN-inducible protein 10/CXC chemokine ligand 10-independent induction of experimental autoimmune encephalomyelitis. J Immunol 2004;172:550–559. [PubMed: 14688366]

43. Tsunoda I, Lane TE, Blackett J, Fujinami RS. Distinct roles for IP-10/CXCL10 in three animal models, Theiler's virus infection, EAE, and MHV infection, for multiple sclerosis: implication of differing roles for IP-10. Mult Scler 2004;10:26–34. [PubMed: 14760949]

44. Park MK, Amichay D, Love P, Wick E, Liao F, Grinberg A, Rabin RL, Zhang HH, Gebeyehu S, Wright TM, Iwasaki A, Weng Y, DeMartino JA, Elkins KL, Farber JM. The CXC chemokine murine monokine induced by IFN-γ (CXC chemokine ligand 9) is made by APCs, targets lymphocytes including activated B cells, and supports antibody responses to a bacterial pathogen in vivo. J Immunol 2002;169:1433–1443. [PubMed: 12133969]

45. Halford WP, Gebhardt BM, Carr DJJ. Persistent cytokine expression in the trigeminal ganglion latently infected with herpes simplex virus type 1. J Immunol 1996;157:3542–3549. [PubMed: 8871654]

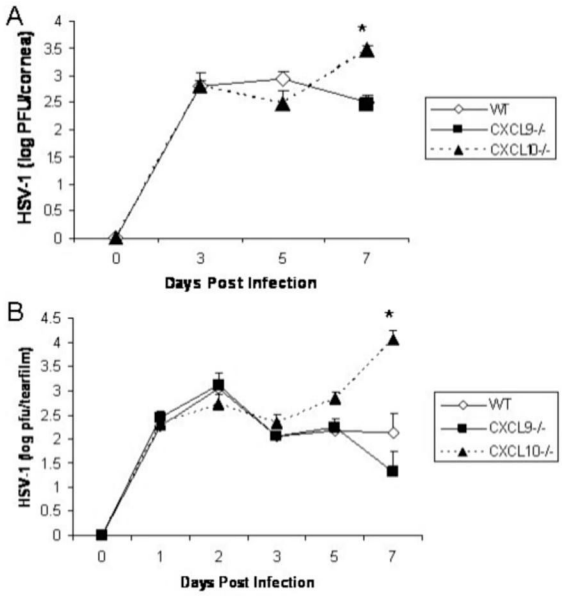

Fig. 1.

CXCL10 possess elevated levels of HSV-1 following ocular infection. (A) Wild type (WT) (n=11), CXCL9 deficient (CXCL9−/−) (n=6), and CXCL10 deficient (CXCL10−/−) (n=11) mice were infected with HSV-1 (1,000 pfu/eye). At day 3, 5, or 7 post infection, the corneas were removed and homogenized in RPMI-1640 medium. The homogenized tissue was clarified (10,000xg, 1 min) and the supernatant was evaluated for virus content by plaque assay using Vero cell monolayers. The results are shown in mean log virus ± SEM summarizing 2 (for CXCL9−/−) or 3 (for WT and CXCL10−/−) experiments with 3–4 mice/group/time point. *p<.05 comparing CXCL10−/− mice to the WT and CXCL9−/− mice. It should be noted earlier time points were not conducted for CXCL9−/− mice. (B) Eye swabs were taken at the indicated time points for each genotype (n = 8–14/group/time point) and assayed for virus content by plaque assay. Each point represents the mean log virus ± SEM, *p<.05 comparing the CXCL10 −/− to the other two groups.

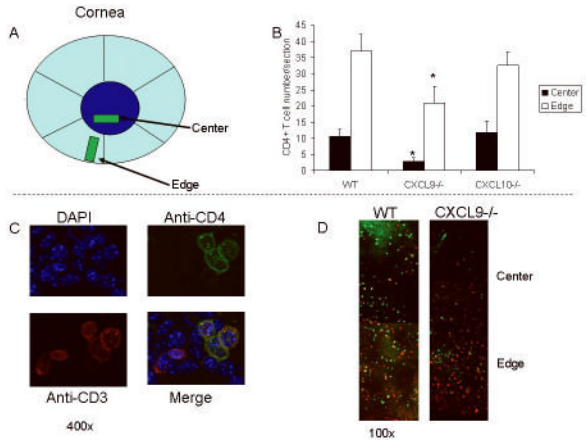

Fig. 2.

$CD4^+$ T cell infiltration into the cornea following ocular HSV-1 infection. Wild type (WT, n=12), CXCL9 deficient (CXCL9−/−, n=6), and CXCL10 deficient (CXCL10−/−, n=12) mice were infected with HSV-1 (1,000 pfu/eye). Seven days post infection, the mice were anesthetized, perfused with PBS, and the corneas were removed and processed for the detection of $CD4^+$ T cells by confocal microscopy. (A) Random sections from the edge and center of the cornea were chosen from the flat mounts for each cornea. (B) $CD4^+$ T were enumerated from 12–18 corneas/group and summarized as mean number ± SEM/section as shown in the figure. *$p < .05$ comparing WT to CXCL9−/− mice. (C) A representative figure showing $CD3^+CD4^+$ T cells is shown. (D) A representative image showing the center and peripheral sections from WT and CXCL9 deficient corneas.

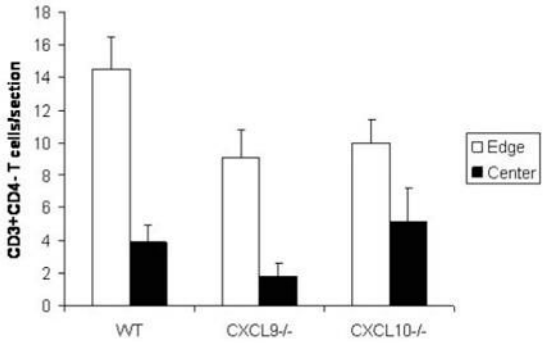

Fig. 3.

$CD3^+CD4^-$ T cell infiltration into the cornea following ocular HSV-1 infection. Wild type (WT, n=12), CXCL9 deficient (CXCL9−/−, n=6), and CXCL10 deficient (CXCL10−/−, n=12) mice were infected with HSV-1 (1,000 pfu/eye). Seven days post infection, the mice were anesthetized, perfused with PBS, and the corneas were removed and processed for the detection of $CD3^+CD4^-$ T cells by confocal microscopy. Random sections from the edge and center of the cornea were chosen from the flat mounts for each cornea. The results are shown as mean number ± SEM/section, n = 24–42 (peripheral) or n = 12–22 (center)/group.

Table 1

Cytokine and Chemokine Expression in the Cornea Following HSV-1 Infection[a]

Group	IL-1β	IL-6	IFN-γ	TNF-α	CCL2	CCL3	CCL5	CXCL1	CXCL9	CXCL10
WT	31.0 ± 7.4[b]	14.9 ± 4.6	2.1 ± 1.1	2.7 ± 1.1	16.2 ± 4.6	35.2 ± 4.7	12.7 ± 2.1	31.7 ± 9.4	47.4 ± 12.9	193 ± 29
CXCL9−/−	0.5 ± 0.5[*]	0 ± 0[*]	0.1 ± 0.1	0 ± 0	11.3 ± 8.6	4.9 ± 1.1[*]	5.7 ± 1.3[*]	2.5 ± 1.0[*]	0 ± 0[*]	114 ± 20[▽]
CXCL10−/−	25.2 ± 8.6	12.0 ± 3.9	0.9 ± 0.3	1.4 ± 1.0	96.0 ± 25[*]	50.3 ± 13	19.3 ± 3.8	31.0 ± 7.4	39.4 ± 7.9	0 ± 0[*]

[a] Wild type (WT), CXCL9 deficient (CXCL9−/−), and CXCL10 deficient (CXCL10−/−) mice (n=6–15/group) were infected with HSV-1 (1,000 pfu/eye). Seven days post infection, the mice were anesthetized and perfused with 20 ml of PBS (pH=7.4). The eyes were removed and the corneas were isolated from the remainder of the eye under a dissecting microscope. The corneas were weighed and homogenized in 0.5 ml of T-PER tissue protein extraction reagent. Each sample was assessed in duplicate for analyte content using a Bioplex suspension array system and reagents according to the manufacturer's instructions (Bio-Rad). It should be noted the content of CXCL9 and CXCL10 were measured as previously described [12].

[b] Numbers represent the mean ± SEM in pg/mg cornea.

[*] p<.05 comparing the CXCL9−/− to WT and CXCL10−/− mice,

[▽] p=.058 comparing the CXCL9−/− to WT group.