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This information is current as of August 9, 2022.

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J Immunol 2008; 181:7985-7993; ;
doi: 10.4049/jimmunol.181.11.7985
http://www.jimmunol.org/content/181/11/7985

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The American Association of Immunologists, Inc.,
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Print ISSN: 0022-1767 Online ISSN: 1550-6606.

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Dysregulation of CXCR3 Signaling due to CXCL10 Deficiency Impairs the Antiviral Response to Herpes Simplex Virus 1 Infection[1]

Todd R. Wuest* and Daniel J. J. Carr[2]*†

The chemokine, CXCL10, chemotactic for NK cells, activated T cells, and dendritic cells is highly expressed during viral infections, including HSV-1. The importance of this chemokine to the control of HSV-1 infection was tested using mice deficient in CXCL10 ($CXCL10^{-/-}$). Following corneal infection, HSV-1 viral titers were elevated in the nervous system of $CXCL10^{-/-}$ mice, which correlated with defects in leukocyte recruitment including dendritic cells, NK cells, and HSV-1-specific $CD8^+$ T cells to the brain stem. In the absence of NK cells and HSV-1-specific $CD8^+$ T cells in wild-type (WT) or $CXCL10^{-/-}$ mice, similar levels of virus were recovered in the nervous system, suggesting these cells are responsible for the observed defects in the control of viral replication in $CXCL10^{-/-}$ mice. Leukocyte mobilization was also compared between WT, $CXCL10^{-/-}$, and mice deficient in the only known receptor for CXCL10, CXCR3 ($CXCR3^{-/-}$). NK cell mobilization was comparably reduced in both $CXCL10^{-/-}$ and $CXCR3^{-/-}$ mice relative to WT animals. However, the reduction in mobilization of HSV-1-specific $CD8^+$ T cells in $CXCL10^{-/-}$ was not observed in $CXCR3^{-/-}$ mice following HSV-1 infection. The defect was not the result of an alternative receptor for CXCL10, as Ag-specific $CD8^+$ T cell recruitment was not reduced in mice which were deficient in both CXCL10 and CXCR3. Thus, CXCL10 deficiency results in reduced mobilization of HSV-1-specific $CD8^+$ T cells as a result of dysregulation of CXCR3 signaling. *The Journal of Immunology,* **2008, 181: 7985–7993.**

Herpes simplex virus type 1 is one of the most widespread and prevalent human infections. Although infection is typically of minor consequence for most individuals, fatal encephalitis due to HSV-1 can occur. In fact, herpes simplex encephalitis is the most common cause of sporadic viral encephalitis in the United States and even with early treatment mortality is between 20 and 30% (1, 2). A variety of factors are known to predispose individuals to herpes simplex encephalitis, particularly pharmacologic or genetic immune deficiency, as well as HIV infection, highlighting the importance of a coordinated immune response to HSV-1.

Successful control of HSV-1 replication requires the mobilization of leukocytes to sites of infection (3–10). Chemokines are a family of small, basically charged proteins which regulate leukocyte migration and functional activity. Although some members of the chemokine family are constitutively expressed, others are dramatically up-regulated in response to inflammatory stimuli (11, 12). The up-regulated expression of inflammatory chemokines and their affinity for negatively charged components of the extracellular matrix allow for the establishment of concentration gradients. Thus, ligation of G protein-coupled chemokine receptors by ligand leads leukocytes along these gradients toward foci of inflammation (11, 12).

Selective expression of chemokines and receptors allows for correspondingly selective leukocyte recruitment depending on the inflammatory stimulus (13). For instance, the chemokine receptors CCR3 and CCR4 are associated with Th2 responses, while CXCR3 and CCR5 ligands are associated with recruitment of Th1-polarized activated T cells as well as NK cells (11–13). The immune response to HSV-1 is of a Th1 etiology with activated $CD4^+$ and $CD8^+$ T cells, as well as NK cells, playing dominant roles in the control of HSV-1 replication (4, 14–16). The chemokine receptor CXCR3 is highly up-regulated in these leukocyte subsets as is the expression of CXCR3 agonists at sites of HSV-1 replication (14, 17–20). CXCR3 deficiency results in suppressed immune responses to a variety of viral pathogens including dengue, West Nile, mouse hepatitis, and lymphocytic choriomeningitis viruses (LCMV)[3] (21–25).

Our group has previously shown that HSV-1 viral burdens are elevated in CXCR3-deficient ($CXCR3^{-/-}$) relative to wild-type (WT) C57BL/6 animals (17). Only three chemokines are known to be high-affinity ligands of this receptor, CXCL9, CXCL10, and CXCL11. Of these, only CXCL9 and CXCL10 are likely to play a role in C57BL/6 mice due to a frame shift mutation within the coding sequence for CXCL11 which results in the introduction of a premature termination codon (26). CXCL10 is only known to signal through CXCR3 and CXCL10 exerts a critical role during several viral infections which is not compensated by CXCL9 expression (21, 23, 24, 27). In addition, CXCL10 is among the earliest and most highly expressed chemokines during HSV-1 infection, suggesting that it may play a pivotal role in the coordinated immune response to HSV-1.

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Received for publication August 23, 2007. Accepted for publication October 1, 2008.

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[1] Principal support for this work is by National Institutes of Health R01 AI067309 (to D.J.J.C.) and a Research to Prevent Blindness Stein Research Professorship (to D.J.J.C.). Additional support includes P20 RR017703 and NEI Core Grant EY12190.

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[3] Abbreviations used in this paper: LCMV, lymphocytic choriomeningitis virus; PI, postinfection; gB, glycoprotein B; TG, trigeminal ganglia; BS, brain stem; DC, dendritic cell; WT, wild type.

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We previously reported a protective effect from CXCR3 deficiency with regard to mortality during HSV-1 infection, despite elevated viral titers (17). We have found that CXCL10$^{-/-}$ mice also exhibit elevated viral burdens. In fact, viral burdens in CXCL10$^{-/-}$ animals significantly exceed those observed in both WT and CXCR3$^{-/-}$ mice associated with elevated mortality of CXCL10$^{-/-}$ animals. This perplexing disparity between the phenotypes of CXCL10$^{-/-}$ and CXCR3$^{-/-}$ mice has also been reported to occur during dengue virus infection as CXCL10$^{-/-}$ mice exhibit increased mortality relative to both WT and CXCR3$^{-/-}$ mice (21).

We report that CXCL10$^{-/-}$ animals display impaired NK and HSV-1-specific CD8$^+$ T cell recruitment and that impaired mobilization of these two populations is responsible for the heightened sensitivity of CXCL10$^{-/-}$ animals with respect to HSV-1 infection. However, although NK cell mobilization was apparently CXCR3 dependent, CXCR3$^{-/-}$ mice did not exhibit defects in HSV-1-specific CD8$^+$ T cell recruitment. Intriguingly, we found the contrast in HSV-1-specific CD8$^+$ T cell recruitment comparing CXCL10$^{-/-}$ and CXCR3$^{-/-}$ animals is likely not the result of an alternate receptor for CXCL10. Rather, dysregulation of CXCR3 signaling due to the absence of CXCL10 results in impaired Ag-specific CD8$^+$ T cell mobilization. This outcome suggests a possible mechanism for disparate sensitivities between CXCR3$^{-/-}$ and CXCL10$^{-/-}$ mice and highlights ill-defined complexities in the CXCL10/CXCR3 chemokine signaling axis.

Materials and Methods

Mice

Animal treatment was consistent with the National Institutes of Health Guidelines on the Care and Use of Laboratory Animals. All procedures were approved by the University of Oklahoma Health Sciences Center and Dean A. McGee Eye Institute Institutional Animal Care and Use Committee. WT C57BL/6 mice were obtained from The Jackson Laboratory. CXCL10-deficient (CXCL10$^{-/-}$) (28), CXCR3-deficient (CXCR3$^{-/-}$) (29), and CXCL9-deficient (CXCL9$^{-/-}$) (30) mice were generated as previously described and backcrossed to the C57BL/6 background for a minimum of nine generations. CXCR3 and CXCL10 doubly deficient mice were generated by crossing CXCR3$^{-/-}$ and CXCL10$^{-/-}$ mice. For all experiments, excluding those using CXCR3/CXCL10 doubly deficient mice, mice used were male. For experiments using CXCR3/CXCL10-deficient mice, both female and male mice were used, and all mice used were age and sex matched with all animals housed under specific pathogen-free conditions. No differences in viral burdens or leukocyte infiltration to HSV-1-infected tissues were observed between male and female mice.

Virus and cells

HSV-1 strain McKrae was originally obtained from B. Gebhardt (Louisiana State University Health Sciences Center, New Orleans, LA) and was propagated on Vero cells. Virus stock was maintained at −80°C at a concentration of 3.15×10^9 PFU/ml, then diluted to 3.33×10^2 PFU/μl immediately before use. Vero cells were maintained in RPMI 1640 medium supplemented with 10% FBS, gentamicin, and antibiotic-antimycotic solution (Invitrogen Life Technologies) at 37°C, 5% CO_2, and 95% humidity.

HSV-1 infection and viral titers

The corneas of anesthetized mice were scarified with a 25-gauge needle, tear films were removed using a chemwipe, and 1000 PFU of HSV-1 was applied to the cornea. At indicated times postinfection (PI), mice were anesthetized and exsanguinated and tissues were harvested. Viral titers within tissues were determined by homogenizing the tissue in RPMI 1640 and clarifying the supernatant (12,000 × *g*, 2 min), then assaying the supernatant by plaque assay on Vero cells.

Flow cytometry

Surface markers were analyzed using anti-mouse F4/80 (Serotec), CD8, CD11c, CD3, CD4, CD8, B220, NK1.1, GR1 conjugated with either FITC or PE (BD Biosciences) and CD8$^+$ T cells specific for the HSV-1 glycoprotein B (gB)-derived epitope SSIEFARL were enumerated using MHC class I tetramer labeled with PE (Baylor University College of Medicine). To analyze leukocytes within trigeminal ganglia (TG) and brain stem (BS), tissues were disrupted with a Wheatley Dounce homogenizer (Fisher Scientific) in RPMI 1640 and the suspension was passed through a 70-μm nylon cell strainer (Fisher Scientific). Lymph node cells were filtered by passing cells through a 70-μm cell strainer. Splenocytes were isolated by extruding splenic contents followed by osmotic lysis of erythrocytes using 0.84% NH_4Cl (Sigma-Aldrich). Samples were blocked with 2 μl of FcBlock anti-mouse CD16/32 (BD Biosciences) and incubated for 15 min. This was followed by the addition of 2 μl of rat serum (Jackson ImmunoResearch Laboratories). After a 15-min incubation, 2 μl of the indicated labeled Abs or 0.5 μl of H-2K^b tetramer reagent, as well as PE-Cy5 rat anti-mouse CD45 (BD Biosciences) was added and incubated. Samples from BS and TG were washed three times while lymph node and spleen cells were washed two times, with 1.0% BSA in 1× PBS. The single-cell suspensions were fixed overnight in 1.0% paraformaldehyde, then resuspended in 1.0% BSA in PBS for subsequent analysis by flow cytometry. Events were gated by forward and side scatter, as well as by high expression of CD45 Ag to distinguish events from resident CD45^{low} microglia. CD45 expression was determined using PE-Cy5-labeled anti-mouse CD45, which we found to be of sufficient intensity and Stokes shift to allow discrimination of labeled cells from autofluorescent CNS tissue. To determine the absolute counts of leukocytes in the BS and TG, suspensions of these tissues included Countbright fluorescent counting beads (Invitrogen Life Technologies), and the absolute count of leukocytes was extrapolated from the ratio of the count of the gated population to the count of the counting beads present at a known concentration.

To determine the number of CD8$^+$ T cells specific for the HSV-1 gB-derived peptide SSIEFARL in the TG and BS of infected mice, CD45$^+$ cells were isolated using a CD45$^+$ cell magnetic microbead isolation kit (Miltenyi Biotec). CD45$^+$ cells were stained as described above, and the number of SSIEFARL- specific CD8$^+$ T cells was extrapolated from the absolute count of CD8$^+$ T cells and the percentage of CD8$^+$ T cells stained with SSIEFARL tetramer.

Chemokine and cytokine analysis

Tissues were suspended in T-Per protein extraction reagent (Pierce Biotechnology) with 1× Calbiochem protease inhibitor mixture and homogenized using a Tissuemiser (Fisher Scientific). Supernatants were clarified by centrifugation (12,000 × *g*, 2 min). Supernatants were assayed for CXCL9, CXCL10, IFN-γ, and IL-4 concentration by ELISA (R&D Systems). Concentrations of CCL2, CCL3, CCL4, IL-1β, and IL-6 were determined by Bio-Plex suspension array (Bio-Rad). For analysis of IFN-γ expression following Ag recognition, lymph node cells were harvested and one million cells in 1.0 ml of RPMI 1640 were plated on 24-well plates and treated with either vehicle or 20 μg/ml SSIEFARL peptide. After incubation for 72 h, supernatants were harvested and assayed for IFN-γ levels by ELISA.

Real-time RT-PCR

RNA was extracted using Ultraspec total RNA isolation reagent according to the manufacturer's instruction (Biotecx). First-strand cDNA was synthesized using avian myeloblastosis virus reverse transcriptase (Promega) with oligo(dT) primers (Promega) according to the manufacturer's instructions with a cycle of 42°C for 45 min, followed by 10 min at 85°C. Quantitative real-time PCR was performed with an iCycler IQ system (Bio-Rad) with a cycle of 3 min at 95°C followed by 40 cycles of 95°C for 30 s, followed by 55°C for 30 s. Primers used were as follows: β-actin forward, 5′CTTCTACAATGAGCTGCGTGTG3′ and β-actin reverse, 5′TTGAAG GTCTCAAACATGATCTGG3′; ISG54 forward, 5′TCTGATTCTGAGGC CTTGCA3′ and ISG54 reverse 5′TGCTGACCTCCTCCATTCTC3′.

TG cultures

Dissociated TG cultures were established by aseptically dissecting TG and suspending the tissues in HBSS (Invitrogen Life Technologies), then incubating with 1.0 mg/ml collagenase types IV and IX (Sigma-Aldrich). The TG were triturated every 10 min for 1 h, washed, and suspended in DMEM (Invitrogen) with 10.0 ng/ml nerve growth factor (Collaborative Biomedical Products), 10% FBS, 1× antibiotic/antimycotic, and 0.2% gentamicin (Invitrogen Life Technologies). The suspension was plated on 24-well microtiter plates (ISC BioExpress) coated with laminin (Trevigen) and type I collagen (Sigma-Aldrich), such that roughly one TG was plated per well. The cultures were allowed to establish themselves for 7 days before infection.

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NK and $CD8^+$ T cell depletion using asialo-GM1 Ab

Volumes of 40-μl rabbit Ab to asialo-GM1 (Cedarlane Laboratories) or control Ab (Jackson ImmunoResearch Laboratories) were injected i.p. both 1 day before and 3 days PI with HSV-1. Depletion of NK and $CD8^+$ T cells was verified in the draining lymph nodes, spleens, TG, and BS by flow cytometry.

Statistics

All statistical analysis was performed using GBStat software (Dynamic Microsystems). Comparisons between two variables were analyzed using Student's *t* test. Comparisons between three or more variables were analyzed using ANOVA and Tukey's *t* test. Survival studies were analyzed by Mann-Whitney *U* rank order test.

Results

HSV-1 viral titers and mortality are elevated in $CXCL10^{-/-}$ mice

To determine whether CXCL10 has a role in the control of HSV-1 infection, WT and $CXCL10^{-/-}$ mice were infected with HSV-1 and TG and BS were removed at days 3, 5, and 7 PI and assayed for infectious virus by plaque assay. Viral titers were significantly elevated in $CXCL10^{-/-}$ mice at days 5 and 7 PI in both the TG and BS (Fig. 1, *A* and *B*). In addition, survival in $CXCL10^{-/-}$ mice was significantly reduced over the duration of the study (Fig. 1*C*). Of the three known high-affinity agonists of CXCR3; CXCL9, CXCL10, and CXCL11, only CXCL9 and CXCL10 are likely to play a role in C57BL/6 mice. The gene for CXCL11 is affected by a frame shift mutation which results in the introduction of a premature stop codon (26). We did not observe any significant difference in HSV-1 viral titers between WT animals and those deficient in another CXCR3 agonist, CXCL9, in either the TG or BS at day 7 PI (data not shown). Thus, CXCL10 is apparently the dominant CXCR3 ligand in this model.

We hypothesized the role CXCL10 has in controlling HSV-1 infection is as a chemoattractant of leukocyte effector populations to the site of infection. CXCL10 is known to induce the chemotaxis of NK, $CD8^+$ T cells, and $CD4^+$ T cells, all of which are known to participate in HSV-1 surveillance (4, 5, 8, 10, 22–24, 28, 31). To address the possibility that CXCL10 exerts an antiviral effect independent of recruited leukocytes, disassociated TG cultures were established from uninfected WT and $CXCL10^{-/-}$ mice and infected with HSV-1, which has been shown previously to induce the expression of CXCL10 in TG cultures (32). There was no significant difference between WT and $CXCL10^{-/-}$ TG cultures (Fig. 1*D*), suggesting that the antiviral effect of CXCL10 is likely dependent on leukocyte populations infiltrating the nervous system following infection.

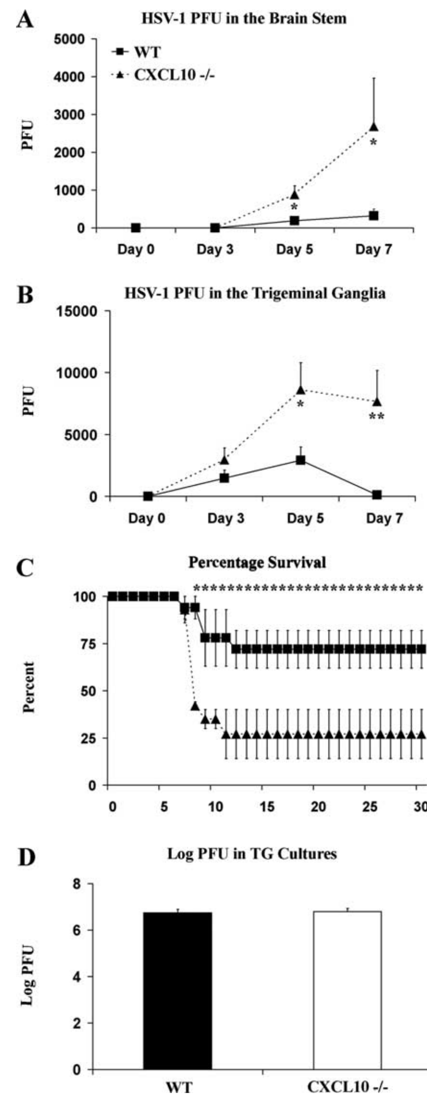

FIGURE 1. HSV-1 viral titers are elevated in CXCL10-deficient mice. WT and $CXCL10^{-/-}$ mice were infected with 1000 PFU of HSV-1 via ocular inoculation. At the indicated time points PI, tissues were harvested and assayed for HSV-1 viral burdens via plaque assay. *A*, Viral titer within the BS. *B*, Viral titer within the TG. *C*, Mortality following HSV-1 infection in WT and $CXCL10^{-/-}$ mice. This figure summarizes three experiments ($n = 15$ for WT and 12 for $CXCL10^{-/-}$; $**$, $p < 0.01$ and $*$, $p < 0.05$ comparing WT to $CXCL10^{-/-}$ mice. *D*, Dissociated TG cultures with $1–3 \times 10^5$ cells/well were established and infected with HSV-1 (multiplicity of infection of 0.01). Supernatants were assayed by plaque assay at 24 h PI. Each point or bar represents the mean ± SEM.

CXCL10 is among the earliest and most highly expressed cytokines following HSV-1 infection

Cytokine levels in WT and $CXCL10^{-/-}$ mice were assayed to determine the temporal regulation of the cytokine milieu and the impact of CXCL10 deficiency on cytokine expression in the TG and BS following infection (Fig. 2). Of the cytokines assayed, CXCL10 was the most highly expressed with the exception of CCL2 within the TG (Fig. 2*A*) and BS (Fig. 2B*)* by day 7 PI. CXCL10 expression was up-regulated by ~40-fold over basal levels in the BS of WT mice as early as day 5 PI (Fig. 2*B*). This time point is the earliest point at which infectious HSV-1 is detectable in the BS. CXCL10 expression remains elevated through day 7 PI in both the BS and TG, suggesting that CXCL10 expression may play a role in the initiation of both innate and adaptive immune responses. Importantly, CXCL10 expression was substantially higher than that of CXCL9 in the TG and BS at all time points assayed. This observation is consistent with experiments performed with $CXCL9^{-/-}$ mice which showed no increase in mortality or HSV-1 viral burdens relative to WT mice (data not shown). This result, the low expression of CXCL9, and apparent absence of CXCL11 in C57BL/6 mice provide further evidence that CXCL10 is the dominant CXCR3 ligand in this model.

TG from $CXCL10^{-/-}$ mice had significantly elevated levels of CCL2 and CXCL1 compared with WT animals at both days 5 and 7 PI as well as IFN-γ (1714 ± 347 vs 5894 ± 176 for WT vs $CXCL10^{-/-}$, respectively) at day 7 PI (Fig. 2*A*). However, these differences were most notable at day 7 PI, a time at which viral titers in the TG of WT animals have declined from their peak to nearly undetectable levels. We have previously reported CCL2 expression within the nervous system is correlated with viral burden (33). Thus, elevated levels CCL2, CXCL1, and IFN-γ within the

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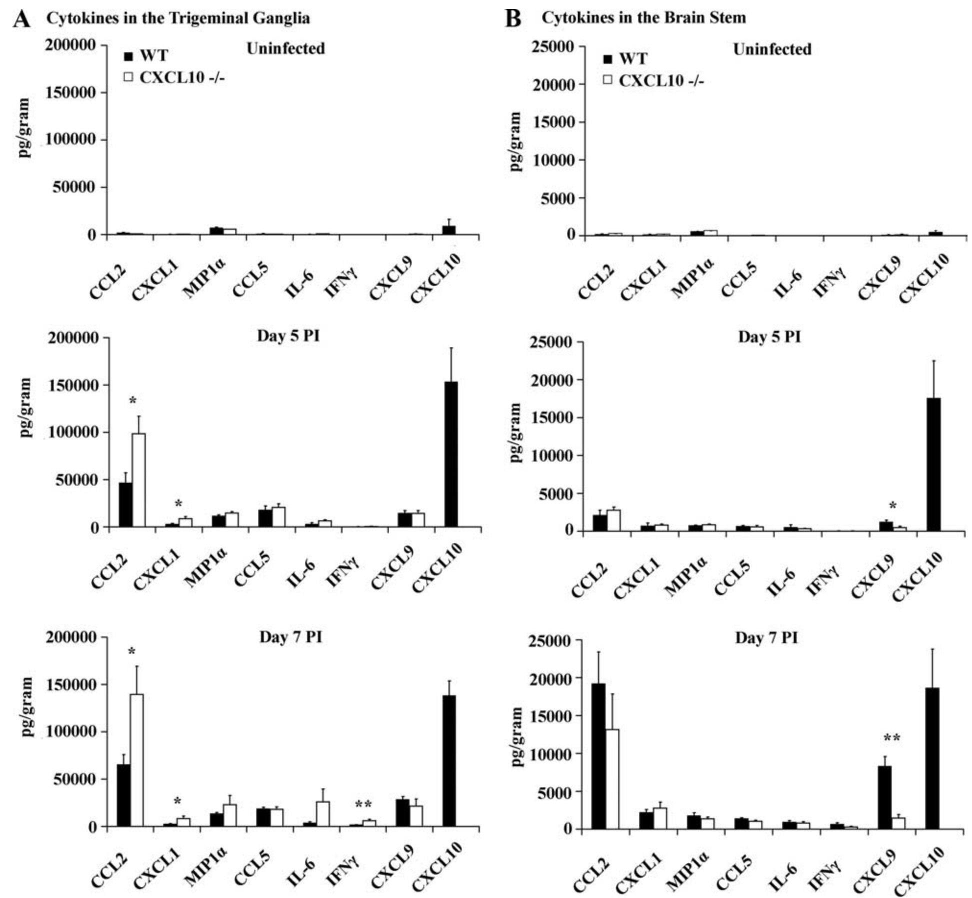

FIGURE 2. Cytokine expression in the TG and BS. Samples were homogenized and supernatants were assayed for the indicated cytokine by suspension bead array or ELISA in the TG (*A*) and BS (*B*). Baseline cytokine levels were determined using uninfected mice ($n = 3$/analyte). Chemokine/cytokine levels following infection are a summary of two to four experiments ($n = 4$–14 samples/analyte). Each bar represents the mean ± SEM expressed in pg/g tissue. **, $p < 0.01$ and *, $p < 0.05$ comparing WT to CXCL10$^{-/-}$ mice.

TG of CXCL10$^{-/-}$ mice may simply be the result of elevated viral burdens.

In comparison to that found in the TG, CXCL10 deficiency had little impact on the cytokine profile in the BS following infection. Of the cytokines assayed, only levels of CXCL9 were significantly different between WT and CXCL10$^{-/-}$ mice with reduced levels at both days 5 and 7 PI in CXCL10$^{-/-}$ mice (Fig. 2*B*). The reduction in CXCL9 expression in CXCL10$^{-/-}$ animals suggests a possible explanation for the lack of redundancy between CXCL9 and CXCL10. CXCL9 expression is driven primarily by IFN-γ (34). Although there was a trend of elevated IFN-γ levels in the BS of WT mice, this result was not statistically significant (Fig. 2*B*, WT = 648 ± 177, CXCL10$^{-/-}$ = 286 ± 106 pg/g, $p > 0.05$). Overall, CXCL10 deficiency appeared to have only a modest influence on cytokine expression in the BS, suggesting that differences in leukocyte recruitment to the BS between WT and CXCL10$^{-/-}$ animals are likely to be the direct result of CXCL10 expression.

Leukocyte mobilization to the nervous system following HSV-1 infection

Studies were performed to address whether CXCL10 deficiency is associated with reduced mobilization of leukocytes to HSV-1-infected tissues as well as to compare CXCL10$^{-/-}$ mice to CXCR3$^{-/-}$ mice. WT, CXCL10$^{-/-}$, and CXCR3$^{-/-}$ mice were infected with HSV-1 and followed over a 7-day time course (Fig. 3). Cell counts of CD4$^+$ T cells, CD8$^+$ T cells, conventional dendritic cells (DC, B220$^-$CD11c$^+$), neutrophils (GR1$^+$F480$^-$), or macrophages (F480$^+$Gr1$^{+/-}$) were not modified by the absence of either CXCL10 or CXCR3 in the TG (Fig. 3*A*). In H-2K^b-restricted C57BL/6 mice, CD8$^+$ T cells are overwhelmingly specific for a particular immunodominant epitope derived from HSV-1 gB, SSIEFARL (35, 36). The mobilization of CD8$^+$ T cells specific for this epitope was not affected by the absence of CXCL10 or CXCR3 in the TG as indicated by quantitative analysis using MHC class I tetrameric reagent (Fig. 3*A*). However, there was a significantly delayed infiltration of NK cells (NK1.1$^+$CD3$^-$) and plasmacytoid DCs (B220$^+$CD11c$^+$) between WT and CXCL10$^{-/-}$ mice (Fig. 3*A*). The absolute cell numbers of these subpopulations were comparable between CXCL10$^{-/-}$ and CXCR3$^{-/-}$ mice, suggesting that mobilization of NK cells and plasmacytoid DCs is CXCR3 dependent and CXCL10 is the primary CXCR3 ligand responsible.

Similar to TG, BS were evaluated for leukocyte recruitment at times PI. Leukocyte populations in the BS modified by the absence of CXCL10 or CXCR3 were diverse and could be divided into three categories; cells impacted exclusively by CXCR3 deficiency, cells modified by CXCL10 deficiency through CXCR3-dependent signaling, and cells modified by CXCL10 deficiency in an apparently CXCR3-independent manner. In the first category, cells dependent on CXCR3 for mobilization to the BS yet not dependent on CXCL10, neutrophils, and macrophages were significantly reduced in the BS by day 7 PI in CXCR3$^{-/-}$ mice, in comparison to both WT and CXCL10$^{-/-}$ mice which were nearly equivalent (Fig. 3*B*). Conventional DCs and NK cells exhibited delayed or reduced recruitment in CXCL10$^{-/-}$ mice and comparable deficiency in CXCR3$^{-/-}$ animals (Fig. 3*B*). Although infiltration of plasmacytoid DCs or conventional DCs was delayed in CXCL10$^{-/-}$ and CXCR3$^{-/-}$ animals, infiltration of plasmacytoid DCs showed a more pronounced dependence on CXCR3. Although the loss of CXCL10 could apparently be compensated for by other CXCR3 or other chemokine receptor ligands with regard

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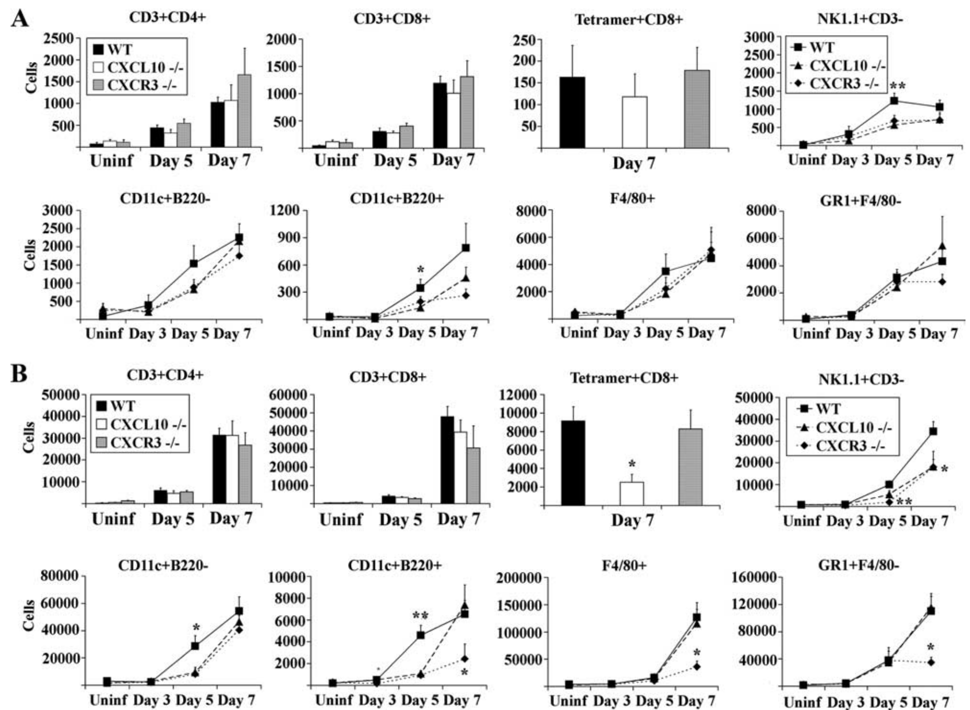

FIGURE 3. Leukocyte mobilization to HSV-1-infected tissues. Leukocytes recruited to the TG (*A*) and BS (*B*) were quantified by flow cytometry following infection. TG and BS from WT, CXCL10$^{-/-}$, and CXCR3$^{-/-}$ animals were homogenized to a single- cell suspension and the resultant populations were phenotypically defined. The results are a summary of two experiments for uninfected mice ($n = 4$/group). For the day 5 and 7 PI time points, the results are a summary of three to five experiments ($n = 6$–10/group). Each point or bar represents the mean ± SEM. **, $p < 0.01$ and *, $p < 0.05$ comparing WT to gene-deficient mice or CXCL10$^{-/-}$ or CXCR3$^{-/-}$ to the other two groups. Uninfect, Uninfected.

to eventual recruitment of conventional DCs, CXCR3 deficiency resulted in significantly reduced numbers of infiltrating plasmacytoid DCs up to the end point of the study (Fig. 3*B*). Dependence on both CXCR3 and CXCL10 was observed regarding NK cells, with numbers equally reduced in CXCL10$^{-/-}$ and CXCR3$^{-/-}$ mice suggesting that CXCL10 is the dominant CXCR3 ligand involved in NK cell mobilization (Fig. 3*B*).

The influence of CXCL10 and CXCR3 deficiency on the recruitment of HSV-1 gB-specific CD8$^+$ T cells to the BS was unexpected. Absolute counts of HSV-1-specific CD8$^+$ T cells were reduced in CXCL10$^{-/-}$ but not in CXCR3$^{-/-}$ animals (Fig. 3*B*). This result appears to suggest a CXCR3-independent influence on HSV-1-specific CD8$^+$ T cell mobilization through CXCL10 expression. Furthermore, this CXCL10-dependent, CXCR3-independent effect was restricted to Ag-specific CD8$^+$ T cells as total CD8$^+$ T cell recruitment to the BS was unaffected by the absence of either CXCL10 or CXCR3 (Fig. 3*B*). This phenomenon was tissue restricted as well, because it was not observed within the TG (Fig. 3*A*).

NK and HSV-1-specific CD8$^+$ T cells are the primary effectors cells affected by the absence of CXCL10

Our studies of leukocyte mobilization into HSV-1- infected tissues indicated that several populations are affected by the absence of CXCL10, including DCs, NK cells, and HSV-1- specific CD8$^+$ T cells. Plasmacytoid DCs express high levels of IFN-α (37–41), which has been shown to restrict HSV-1 replication (42, 43). Measurement of type I IFN by ELISA is relatively insensitive (37); therefore, an indirect measure of type I IFN activity was undertaken by measuring the IFN-stimulated gene ISG54 expression by real-time RT-PCR (37). However, we did not observe differences between WT and CXCL10$^{-/-}$ animals for ISG54 mRNA levels relative to the housekeeping gene β-actin in the BS at either days 5 or 7 PI (data not shown), suggesting differences in BS-localized type I IFN expression is an unlikely explanation for the immunological defects observed in CXCL10$^{-/-}$ mice.

Previous studies have demonstrated that both NK cell and CD8$^+$ T cell populations impact on the control of HSV-1 infection with varying importance depending on the infected tissue and time point (3, 4, 5, 9). To assess the importance of these effector populations in the current model, animals were administered anti-asialo-GM1 Ab. Administration of anti-asialo-GM1 results in depletion of both NK cells and HSV gB-specific CD8$^+$ T cells (Fig. 4, *A–C*). No obvious impact on other populations (CD45^{high} and CD4$^+$CD3$^+$, B220$^+$CD11c$^+$, B220$^-$CD11c$^+$, F480$^+$, or GR1$^+$F480$^-$ or CD45^{low}F4/80$^+$ microglia) in the spleen, lymph nodes, TG, or BS was observed (data not shown). Treatment with anti-asialo-GM1 Ab led to a reduction in the difference in viral titers between WT and CXCL10$^{-/-}$ in both the BS (Fig. 4*D*) and TG (Fig. 4*E*), suggesting the impact of CXCL10 on control of HSV-1 is dependent on recruitment of these effector populations.

CXCL10 is not required for the generation of HSV-1-specific CD8$^+$ T cells

To determine whether the selective deficiency in the recruitment of HSV-1-specific CD8$^+$ T cells to the BS of CXCL10$^{-/-}$ mice was a result of a defect in the generation of Ag-specific CD8$^+$ T cells, cells from the draining lymph nodes were analyzed by flow cytometry. In our hands, HSV-1-specific CD8$^+$ T cells are first reliably detectable at day 5 PI in the draining mandibular lymph nodes and mobilize to the CNS between days 5 and 7 PI. There was no significant difference between WT, CXCL10$^{-/-}$, or CXCR3$^{-/-}$ mice in the numbers of HSV-1-specific CD8$^+$ T cells at either day 5 or day 7 PI (Fig. 5*A*). Previous studies have indicated that B220$^+$CD11c$^+$ cell recruitment to inflamed lymph nodes is CXCR3 dependent (44), and these cells play a role in the generation of anti-HSV-1 CTL responses (45). Furthermore, another study indicated that DC-derived CXCL10 contributes to the retention of CD4$^+$ T cells in lymph nodes (46). However, we observed no significant differences among WT, CXCL10$^{-/-}$, or CXCR3$^{-/-}$ mice for total CD3$^+$CD4$^+$ T, CD3$^+$CD8$^+$ T, B220$^+$CD11c$^+$, or B220$^-$CD11c$^+$ cells (Fig. 5*A*) nor for F4/80$^+$ or GR1$^+$F480$^-$ cells (data not shown) in the draining lymph nodes at any time point.

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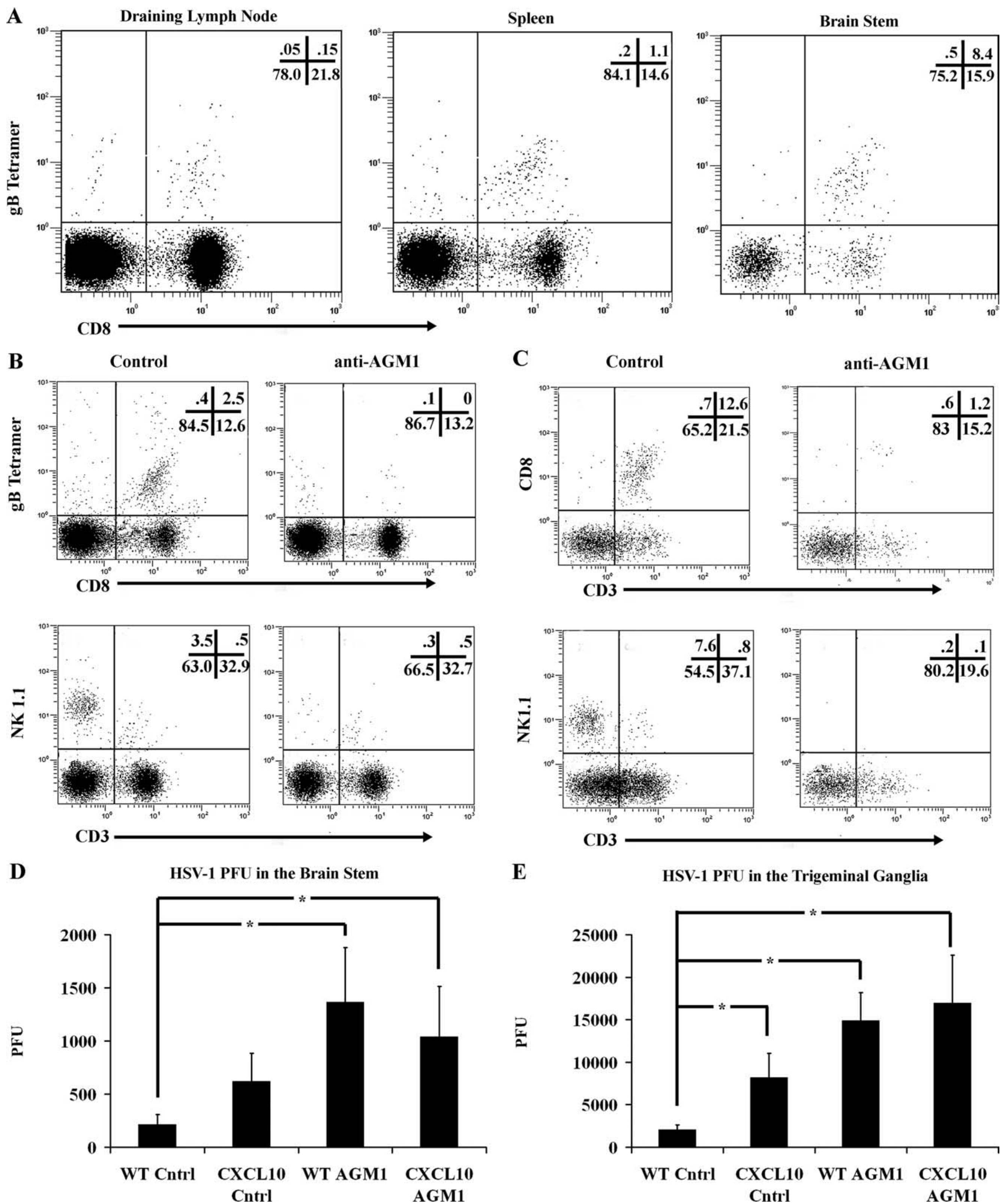

FIGURE 4. Reduced NK and CD8$^+$ T cells responses are critical for loss of virus surveillance in WT and CXCL10$^{-/-}$ mice. *A*, Representative flow diagrams of lymph node, spleen, and BS leukocytes stained for CD8 and expression of the TCR specific for the HSV-1 gB-derived epitope SSIEFARL, as indicated by staining with tetramer. Mice were treated i.p. with either anti-asialo-GM1 (anti-AGM1) or control (Cntrl) Ab 1 day before and 3 days afterHSV-1 infection. Representative flow diagrams for spleen cells (*B*) and BS (*C*) samples at day 7 PI examined for NK cell, CD8$^+$ T cell, and HSV-1-specific CD8$^+$ T cells labeled with the gB-derived peptide SSIEFARL, as indicated by staining with the PE-labeled H-2K^b SSIEFARL tetramer. Treatment with anti- asialo-GM1 was found to result in >95% reduction in the absolute counts of NK cells and HSV-1 gB-specific CD8$^+$ T cells in the spleen (*B*), BS (*C*), lymph node (data not shown), and TG (data not shown). HSV-1 viral titers are shown for BS (*D*) and TG (*E*) at day 7 PI. This figure is a summary of two experiments (n = 4/group) with the bars displayed as mean PFU/tissue ± SEM. *, $p < 0.05$ comparing WT to CXCL10 control, anti-asialo-GM1 WT-treated, and anti-asialo-GM1 CXCL10$^{-/-}$-treated mice.

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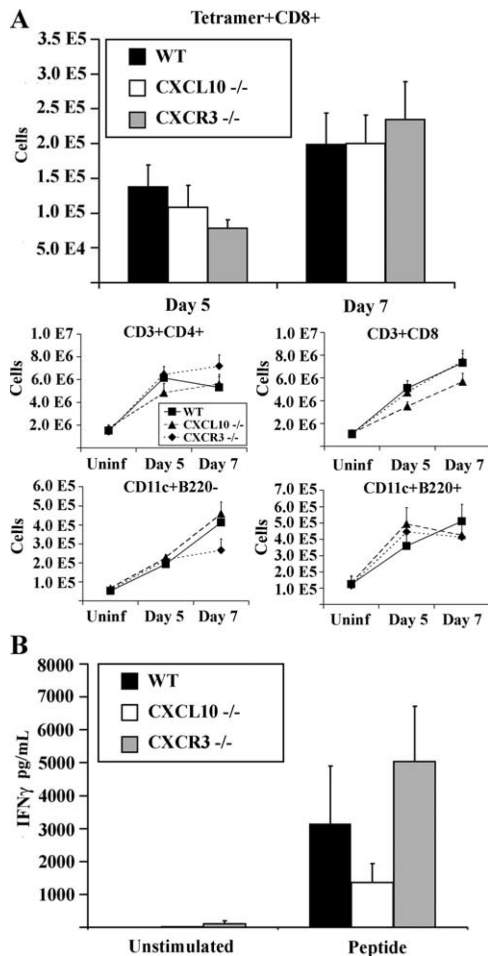

FIGURE 5. CXCL10 does not play a role in the generation of HSV-1-specific $CD8^+$ T cells during virus infection. *A*, Absolute counts of HSV-1 gB-specific $CD8^+$ T cells (Tetramer$^+$CD8$^+$) or other phenotypically defined leukocyte population in the draining mandibular lymph nodes obtained at day 5 or day 7 PI. Uninfected lymph nodes (Uninf) served as controls. The results are taken from two experiments for uninfected mice ($n = 4$ mice) and three to four experiments ($n = 6–8$ mice) for the day 5 and 7 time points. *B*, IFN-γ expression following Ag recognition. Cells taken from the draining lymph nodes of HSV-1-infected mice were stimulated with SSIEFARL peptide (20 μg/ml) and assayed for IFN-γ levels by ELISA 72 h after stimulation. The results are a summary of three experiments ($n = 6$ samples/group). Each point/bar represents the mean ± SEM.

Although there was no apparent defect in the generation of HSV-1-specific $CD8^+$ T cells based on flow cytometric analysis, the function of HSV-1-specific $CD8^+$ T cells may be altered in the absence of CXCL10. Earlier work has demonstrated a decrease in cytokine production by T cells following Ag recognition in CXCL10$^{-/-}$ animals (28). The only known receptor for CXCL10, CXCR3, associates with and utilizes some components of the TCR during CXCL10/CXCR3 signaling (47).

Thus, CXCL10 deficiency could theoretically impact cytokine production following Ag recognition in a CXCR3-dependent fashion. To address this possibility, cells from the lymph nodes of WT, CXCL10$^{-/-}$, and CXCR3$^{-/-}$ animals were harvested and evaluated for production of IFN-γ following Ag recognition. Measurement of IFN-γ levels in culture supernatants following stimulation suggested a trend of reduced IFN-γ production in CXCL10$^{-/-}$ but not CXCR3$^{-/-}$ mice. However, the difference did not reach statistical significance ($p > 0.05$).

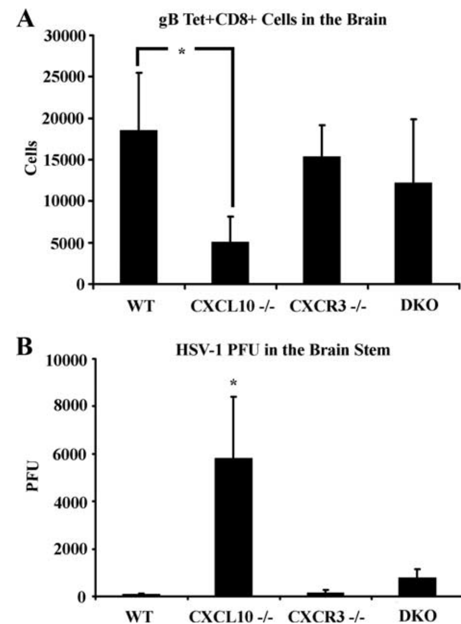

FIGURE 6. The $CD8^+$ T cell response to HSV-1 is dependent on CXCR3. *A*, The absolute number of HSV-1 gB-specific $CD8^+$ cells in the BS of WT, CXCL10$^{-/-}$, CXCR3$^{-/-}$, and CXCL10/CXCR3 double-deficient mice (DKO) were quantified by flow cytometry at day 7 PI. The results are shown as mean ± SEM and represent $n = 7$ per group conducted twice with similar outcomes. *B*, HSV-1 viral titers in the BS of WT, CXCL10$^{-/-}$, CXCR3$^{-/-}$, and DKO mice at day 7 PI. The bar represents the mean ± SEM obtained from four experiments ($n = 9$/group), *, $p < 0.05$ comparing the WT to CXCL10$^{-/-}$ mice.

CXCL10 operates solely through CXCR3 in the mobilization of HSV-1-specific $CD8^+$ T cells to the BS

CXCL10$^{-/-}$ animals exhibit reduced recruitment of gB-specific $CD8^+$ T cells to the BS compared with WT and CXCR3$^{-/-}$ mice. The simplest explanation of this result would be the existence of another receptor for CXCL10. Consistent with this notion, another receptor for the CXCR3 ligand CXCL11 was recently reported (48). To determine whether a receptor for CXCL10 in addition to CXCR3 influenced HSV-1-specific $CD8^+$ T cell mobilization to the BS, CXCL10 and CXCR3 double-deficient mice were generated. CXCL10 deficiency did not impact the recruitment of HSV-1-specific $CD8^+$ T cells to the BS (Fig. 6*A*) or HSV-1 viral titer in the BS (Fig. 6*B*) in the absence of CXCR3. This suggests that the reduced recruitment of HSV-1-specific $CD8^+$ T cells observed in CXCL10$^{-/-}$ mice was not the result of the loss of chemokine signaling between CXCL10 and a receptor other than CXCR3. Rather, CXCR3 is the dominant receptor for CXCL10 involved in the recruitment of HSV-1-specific $CD8^+$ T cells with the loss of CXCL10 resulting in dysregulation of normal CXCR3 signaling.

Discussion

Our findings demonstrate that the chemokine CXCL10 is critical for mobilization of both the innate and adaptive branches of the immune system. CXCL10 is highly expressed at sites of HSV-1 replication and is essential for the control of HSV-1 infection, predominantly through NK and $CD8^+$ T cell-mediated activities. These populations contribute to the control of virus replication through both cytotoxic activity against virally infected cells as well as the production of effector cytokines such as IFN-γ (4, 5, 7–9, 16). We have not observed any significant differences in the capabilities of splenic NK cells isolated from WT, CXCL10$^{-/-}$, and CXCR3$^{-/-}$ animals to lyse Yac-1 cells (T. R. Wuest and D. J. J.

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Carr, unpublished observation). Likewise, no differences were observed between WT and CXCL10$^{-/-}$ mice in CD8$^+$ T cell cytolytic activity against HSV-2-infected target cells (49) nor did we find a significant difference in IFN-γ production in CD8$^+$ T cells isolated from CXCL10$^{-/-}$ animals following stimulation using the HSV-1 gB-derived peptide SSIEFARL. Similarly, CD8$^+$ T cells isolated from CXCL10$^{-/-}$ mice infected with LCMV exhibit normal cytolytic activity toward EL-4 target cells labeled with LCMV-derived peptide (22). Thus, the enhanced susceptibility of CXCL10$^{-/-}$ mice to HSV-1 infection would appear to be limited to effector cell recruitment.

NK and CD8$^+$ T cells exist in a variety of subsets with functionality that is broadly correlated with chemokine receptor expression (50). For example, NK cell subsets can be categorized on the basis of having either high cytokine expression or cytolytic activity. Although CD56^{bright}, high cytokine-expressing NK cells are typically CCR7 positive, CD56^{dim} highly cytotoxic NK cells are predominantly CCR7 negative (50). High cytotoxic activity has been correlated with CXCR1 expression on CD8$^+$ T cells (51). These observations lead to the obvious question of how chemokine deficiency may adversely affect the selective recruitment of specific subsets of leukocyte populations. Given the complex and layered behavior of feedback in biological systems, there is not a simple answer. CXCL10$^{-/-}$ mice have elevated expression of the CXCR1 ligand CXCL1 which may result in the aberrant recruitment of highly cytotoxic subsets of CD8$^+$ T cells to the TG, possibly aggravating injury.

CXCL10 deficiency resulted in significantly reduced or delayed infiltration of several leukocyte populations into HSV-1-infected tissue. CXCL10$^{-/-}$ and CXCR3$^{-/-}$ animals exhibited comparable phenotypes indicating the CXCR3 dependence of CXCL10 signaling in the recruitment of NK1.1$^+$CD3$^-$, B220$^-$CD11c$^+$, and B220$^+$CD11c$^+$ populations. However, the absolute number of Ag-specific CD8$^+$ T cells in the BS was significantly reduced in CXCL10$^{-/-}$ mice. Yet, this population was not modified by CXCR3 deficiency. One possible explanation for the observation of an impaired CD8$^+$ T cell response in CXCL10$^{-/-}$ but not CXCR3$^{-/-}$ mice is the existence of another receptor for CXCL10. Soejima and Rollins (52) provided evidence of the existence of such an alternate receptor for CXCL10 expressed on epithelial and endothelial cells. Alternatively, the antagonistic function of CXCL10 toward CCR3 (53) or another chemokine receptor could influence the CD8$^+$ T cell response. However, the absence of any defect in the recruitment of gB-specific CD8$^+$ T cells in CXCL10/CXCR3 double-deficient mice indicates the dominant signaling pathway for CXCL10 is through CXCR3. Therefore, the particular immunological defects observed in the CD8$^+$ T cell response in CXCL10$^{-/-}$ mice are a consequence of dysregulation of CXCR3 signaling as a result of CXCL10 deficiency.

Historically, chemokines have been viewed strictly as chemoattractant molecules, but their accepted influence on leukocyte mobilization and functional activity has expanded with time. Both chemokines and their receptors are influenced by homo- and heterodimerization and chemokine signaling pathways are affected by chemokine concentration, as well as cross-talk with other signaling pathways such as the use of ITAM and ITIM signaling components during CXCR3 signaling (47, 54, 55). These dynamic interactions allow flexible integration of chemokine-driven responses and CXCL10 has been shown to mediate chemoattraction, chemorepulsion, as well as modulation of responses to other chemokines (10, 47, 55–60). As with CXCR4, CXCR3 utilizes TCR components for signal transduction (47, 56). CXCR3 ligands differentially signal through this pathway (4) and structural components of CXCR3 required for signaling in response to CXCL9, CXCL10, and CXCL11 differ considerably (61–68). Thus, the absence of a particular CXCR3 ligand may directly affect T cell function in a CXCR3-dependent fashion with a variety of possible outcomes.

In summary, our results demonstrate that CXCL10 has a nonredundant role in the chemoattraction of leukocytes involved in the control of HSV-1 replication within the peripheral and CNS. The absence of CXCL10 is correlated with reduced recruitment of NK and HSV-1-specific CD8$^+$ T cells and elevated viral burdens. Animals deficient in CXCL10 and CXCR3 displayed equivalently impaired NK cell recruitment, indicating that CXCL10 is the dominant CXCR3 ligand for NK cells in this model. Our results implicate the presence of previously unexplored complexities in T cell recruitment by CXCR3 ligands. These observations suggest the possibility of unanticipated adverse consequences following pharmacologic modulation of chemokine receptor signaling that may profoundly affect immune responses. Future studies will be required to further delineate the mechanism by which aberrant CXCR3 signaling affects T cell recruitment and function under inflammatory conditions including infection.

Acknowledgments

We thank Gabriel Nguyen for technical assistance. We acknowledge Drs. Joshua Farber, Bao Lu, and Andrew Luster for generously providing CXCL9$^{-/-}$, CXCR3$^{-/-}$, and CXCL10$^{-/-}$ breeder mice, respectively.

Disclosures

The authors have no financial conflict of interest.

References

1. Kennedy, P. G. 2005. Viral encephalitis. *J. Neurol.* 252: 268–272.
2. Kennedy, P. G., and A. Chaudhuri. 2002. Herpes simplex encephalitis. *J. Neurol. Neurosurg. Psychiatry* 73: 237–238.
3. Maghazachi, A. A., B. S. Skalhegg, B. Rolstad, and A. Al-Aoukaty. 1997. Interferon- inducible protein-10 and lymphotactin induce the chemotaxis and mobilization of intracellular calcium in natural killer cells through pertussis toxin-sensitive and -insensitive heterotrimeric G-proteins. *FASEB J.* 11: 765–774.
4. Ghiasi, H., S. Cai, G. C. Perng, A. B. Nesburn, and S. L. Wechsler. 2000. The role of natural killer cells in protection of mice against death and corneal scarring following ocular HSV-1 infection. *Antiviral Res.* 45: 33–45.
5. Reading, P. C., P. G. Whitney, D. P. Barr, M. J. Smyth, and A. G. Brooks. 2006. NK cells contribute to the early clearance of HSV-1 from the lung but cannot control replication in the central nervous system following intranasal infection. *Eur. J. Immunol.* 36: 897–905.
6. Kassim, S. H., N. K. Rajasagi, X. Zhao, R. Chervenak, and S. R. Jennings. 2006. In vivo ablation of CD11c-positive dendritic cells increases susceptibility to herpes simplex virus type 1 infection and diminishes NK and T-cell responses. *J. Virol.* 80: 3985–3993.
7. Mossman, K. L., and A. A. Ashkar. 2005. Herpesviruses and the innate immune response. *Viral Immunol.* 18: 267–281.
8. Manickan, E., and B. T. Rouse. 1995. Roles of different T-cell subsets in control of herpes simplex virus infection determined by using T-cell-deficient mouse-models. *J. Virol.* 69: 8178–8179.
9. Simmons, A., and D. C. Tscharke. 1992. Anti-CD8 impairs clearance of herpes simplex virus from the nervous system: implications for the fate of virally infected neurons. *J. Exp. Med.* 175: 1337–1344.
10. Ghiasi, H., S. Cai, G. C. Perng, A. B. Nesburn, and S. L. Wechsler. 2000. Both CD4$^+$ and CD8$^+$ T cells are involved in protection against HSV-1 induced corneal scarring. *Br. J. Ophthalmol.* 84: 408–412.
11. Zlotnik, A., and O. Yoshie. 2000. Chemokines: a new classification system and their role in immunity. *Immunity* 12: 121–127.
12. Baggiolini, M. 1998. Chemokines and leukocyte traffic. *Nature* 392: 565–568.
13. Ward, S. G., K. Bacon, and J. Westwick. 1998. Chemokines and T lymphocytes: more than an attraction. *Immunity* 9: 1–11.
14. Carr, D. J., and L. Tomanek. 2006. Herpes simplex virus and the chemokines that mediate the inflammation. *Curr. Top. Microbiol. Immunol.* 303: 47–65.
15. Allan, R., J. Waithman, S. Bedoui, C. Jones, J. Villadangos, Y. Zhan, A. Lew, K. Shortman, W. Heath, and F. Carbone. 2006. Migratory dendritic cells transfer antigen to a lymph node-resident dendritic cell population for efficient CTL priming. *Immunity* 25: 153–162.
16. Khanna, K., A. Lepisto, V. Decman, and R. Hendricks. 2004. Immune control of herpes simplex virus during latency. *Curr. Opin. Immunol.* 16: 463–469.
17. Wickham, S., B. Lu, J. Ash, and D. J. Carr. 2005. Chemokine receptor deficiency is associated with increased chemokine expression in the peripheral and central nervous systems and increased resistance to herpetic encephalitis. *J. Neuroimmunol.* 162: 51–59.

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18. Barbi, J., S. Oghumu, C. Lezama-Davila, and A. Satoskar. 2007. IFN-γ and STAT1 are required for efficient induction of CXC chemokine receptor 3 (CXCR3) on CD4+ but not CD8+ T cells. *Blood* 110: 2215–2216.
19. Cook, W., M. Kramer, R. Walker, T. Burwell, H. Holman, D. Coen, and D. Knipe. 2004. Persistent expression of chemokine and chemokine receptor RNAs at primary and latent sites of herpes simplex virus 1 infection. *Virol. J.* 1: 5.
20. O'Garra, A., L. McEvoy, and A. Zlotnik. 1998. T-cell subsets: chemokine receptors guide the way. *Curr. Biol.* 8: R646–R649.
21. Hsieh, M. F., S. L. Lai, J. P. Chen, J. M. Sung, Y. L. Lin, B. A. Wu-Hsieh, C. Gerard, A. D. Luster, and F. Liao. 2006. Both CXCR3 and CXCL10/IFN-inducible protein 10 are required for resistance to primary infection by dengue virus. *J. Immunol.* 77: 1855–1863.
22. Christensen, J. E., C. de Lemos, T. Moos, J. P. Christensen, and A.R. Thomsen. 2006. CXCL10 is the key ligand for CXCR3 on CD8+ effector T cells involved in immune surveillance of the lymphocytic choriomeningitis virus infected central nervous system. *J. Immunol.* 176: 4235–4243.
23. Klein, R. S., E. Lin, B. Zhang, A. D. Luster, J. Tollett, M. A. Samuel, M. Engle, and M. S. Diamond. 2005. Neuronal CXCL10 directs CD8+ T-cell recruitment and control of West Nile virus encephalitis. *J. Virol.* 79: 11457–11466.
24. Trifilo, M. J., C. Montalto-Morrison, L. N. Stiles, K. R. Hurst, J. L. Hardison, J. E. Manning, P. S. Masters, and T. E. Lane. 2004. 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.* 78: 585–594.
25. Stiles, L. N., M. P. Hosking, R. A. Edwards, R. M. Strieter, and T. E. Lane. 2006. Differential roles for CXCR3 in CD4+ and CD8+ T cell trafficking following viral infection of the CNS. *Eur. J. Immunol.* 36: 613–622.
26. Carr, D. J., T. Wuest, and J. J. Ash. 2008. An increase in herpes simplex virus type 1 in the anterior segment of the eye is linked to a deficiency in NK cell infiltration in mice deficient in CXCR3. *J. Interferon Cytokine Res.* 28: 245–251.
27. Zhang, B., Y. K. Chan, B. Lu, M. S. Diamond, and R. S. Klein. 2008. CXCR3 mediates region-specific antiviral T cell trafficking within the central nervous system during West Nile virus encephalitis. *J. Immunol.* 180: 2641–2649.
28. Dufour, J. H., M. Dziejman, M. T. Liu, J. H. Leung, T. E. Lane, and A. D. Luster. 2002. IFN-γ-inducible protein 10 (IP-10; CXCL10)-deficient mice reveal a role for IP-10 in effector T cell generation and trafficking. *J. Immunol.* 168: 3195–3204.
29. Hancock, W. W., B. Lu, W. Gao, V. Csizmadia, K. Faia, J. A. King, S. T. Smiley, M. Ling, N. P. Gerard, and C. Gerard. 2000. Requirement of the chemokine receptor CXCR3 for acute allograft rejection. *J. Exp. Med.* 192: 1515–1520.
30. Park, M. K., D. Amichay, P. Love, E. Wick, F. Liao, A. Grinberg, R. L. Rabin, H. H. Zhang, S. Gebeyehu, T. M. Wright, et al. 2002. 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.* 169: 1433–1439.
31. Saudemont, A., N. Jouy, D. Hetuin, and B. Quesnel. 2005. NK cells that are activated by CXCL10 can kill dormant tumor cells that resist CTL-mediated lysis and can express B7–H1 that stimulates T cells. *Blood* 105: 2428–2435.
32. Austin, B. A., and D. J. Carr. 2007. Herpes simplex virus type 1 suppresses type I interferon-induced chemokine production in trigeminal ganglion cell cultures. In *Chemokine Research Trends.* L. Grinwald, ed. Nova Science Publishers, Hauppauge, NY, pp. 43–60.
33. Carr, D. J., and I. L. Campbell. 2006. Herpes simplex virus type 1 induction of chemokine production is unrelated to viral load in the cornea but not in the nervous system. *Viral Immunol.* 19: 741–746.
34. Whiting, D., G. Hsieh, J. J. Yun, A. Banerji, W. Yao, M. Fishbein, J. Belperio, R. Strieter, B. Bonavida, and A. Ardehali. 2004. Chemokine monokine induced by IFN-γ/CXC chemokine ligand 9 stimulates T lymphocyte proliferation and effector cytokine production. *J. Immunol.* 172: 7417–7424.
35. Hanke, T., F. L. Graham, K. L. Rosenthal, and D. C. Johnson. 1991. Identification of an immunodominant cytotoxic T-lymphocyte recognition site in glycoprotein B of herpes simplex virus by using recombinant adenovirus vectors and synthetic peptides. *J. Virol.* 65: 1177–11786.
36. Cose, S. C., J. M. Kelly, and F. R. Carbone. 1995. Characterization of diverse primary herpes simplex virus type 1 gB-specific cytotoxic T-cell response showing a preferential Vβ bias. *J. Virol.* 69: 5849–5852.
37. Poast, J., H. M. Seidel, M. D. Hendricks, J. A. Haslam, H. B. Levy, and S. Baron. 2002. PolyI:C induction of the interferon system in mice: an initial study of four detection methods. *J. Interferon Cytokine Res.* 22: 1035–1040.
38. Ronnblom, L., U. Ramstedt, and G. V. Alm. 1983. Properties of human natural interferon-producing cells stimulated by tumor cell lines. *Eur. J. Immunol.* 13: 471–476.
39. Perussia, B., V. Fanning, and G. Trinchieri. 1985. A leukocyte subset bearing HLA- DR antigens is responsible for in vitro α interferon production in response to viruses. *Nat. Immun. Cell Growth Regul.* 4: 120–137.
40. Siegal, F., N. Kadowaki, M. Shodell, P. A. Fitzgerald-Bocarsly, K. Shah, S. Ho, S. Antonenko, and Y. J. Liu. 1999. The nature of the principal type 1 interferon-producing cells in human blood. *Science* 284: 1835–1837.
41. Fitzgerald-Bocarsly, P., J. Dai, and S. Singh. 2008. Plasmacytoid dendritic cells and type I IFN: 50 years of convergent history. *Cytokine Growth Factor Rev.* 19: 3–19.
42. Noisakran, S., and D. J. Carr. 2001. Type I interferons and herpes simplex virus infection: a naked DNA approach as a therapeutic option? *Immunol. Res.* 24: 1–11.
43. Jouanguya, E., S. Zhanga, A. Chapgiera, V. Sancho-Shimizua, A. Puela, C. Picarda, S. Boisson-Dupuisa, L. Abela, and J. L. Casanova. 2007. Human primary immunodeficiencies of type I interferons. *Biochimie* 8: 878–883.
44. Yoneyama, H., K. Matsuno, Y. Zhang, T. Nishiwaki, M. Kitabatake, S. Ueha, S. Narumi, S. Morikawa, T. Ezaki, B. Lu, et al. 2004. Evidence for recruitment of plasmacytoid dendritic cell precursors to inflamed lymph nodes through high endothelial venules. *Int. Immunol.* 16: 915–928.
45. Yoneyama, H., K. Matsuno, E. Toda, T. Nishiwaki, N. Matsuo, A. Nakano, S. Narumi, B. Lu, C. Gerard, S. Ishikawa, and K. Matsushima. 2005. Plasmacytoid DCs help lymph node DCs to induce anti-HSV CTLs. *J. Exp. Med.* 202: 425–435.
46. Yoneyama, H., S. Narumi, Y. Zhang, M. Murai, M. Baggiolini, A. Lanzavecchia, T. Ichida, H. Asakura, and K. Matsushima. 2002. Pivotal role of dendritic cell-derived CXCL10 in the retention of T helper cell 1 lymphocytes in secondary lymph nodes. *J. Exp. Med.* 195: 1257–1266.
47. Dar, W. A., and S. J. Knechtle. 2007. CXCR3-mediated T-cell chemotaxis involves ZAP-70 and is regulated by signalling through the T-cell receptor. *Immunology* 120: 467–485.
48. Burns, J. M., B. C. Summers, Y. Wang, A. Melikian, R. Berahovich, Z. Miao, M. E. Penfold, M. J. Sunshine, D. R. Littman, C. J. Kuo, et al. 2006. A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. *J. Exp. Med.* 203: 2201–2213.
49. Thapa, M., R. Welner, R. Pelayo, and D. J. Carr. 2008. CXCL9 and CXCL10 expression are critical for control of genital herpes simplex virus type 2 infection through mobilization of HSV-specific CTL and NK cells to the nervous system. *J. Immunol.* 180: 1098–1106.
50. Wuest, T., and D. J. Carr. 2008. The role of chemokines during herpes simplex virus-1 infection. *Front. Biosci.* 13: 4862–4872.
51. Hess, C., T. Means, P. Autissier, T. Woodberry, M. Altfeld, M. Addo, N. Frahm, C. Brander, B. Walker, and A. D. Luster. 2004. IL-8 responsiveness defines a subset of CD8 T cells poised to kill. *Blood* 104: 3463–3471.
52. Soejima, K., and B. J. Rollins. 2001. A functional IFN-γ-inducible protein- 10/CXCL10-specific receptor expressed by epithelial and endothelial cells that is neither CXCR3 nor glycosaminoglycan. *J. Immunol.* 167: 6576–6582.
53. Loetscher, P., A. Pellegrino, J. H. Gong, I. Mattioli, M. Loetscher, G. Bardi, M. Baggiolini, and I. Clark-Lewis. 2001. The ligands of CXC chemokine receptor 3, I-TAC, Mig, and IP10, are natural antagonists for CCR3. *J. Biol. Chem.* 276: 2986–2991.
54. Inngjerdingen, M., B. Rolstad, and J. C. Ryan. 2003. Activating and inhibitory Ly49 receptors modulate NK cell chemotaxis to CXC chemokine ligand (CXCL) 10 and CXCL12. *J. Immunol.* 171: 2889–2895.
55. Vanbervliet, B., N. Bendriss-Vermare, C. Massacrier, B. Homey, O. de Bouteiller, G. Brière, F. Trinchieri, and C. Caux. 2003. The inducible CXCR3 ligands control plasmacytoid dendritic cell responsiveness to the constitutive chemokine stromal cell-derived factor 1 (SDF-1)/CXCL12. *J. Exp. Med.* 198: 823–830.
56. Kumar, A., T. D. Humphreys, K. N. Kremer, P. S. Bramati, L. Bradfield, C. E. Edgar, and K. E. Hedin. 2006. CXCR4 physically associates with the T cell receptor to signal in T cells. *Immunity* 25: 213–224.
57. Campanella, G. S., J. Grimm, L. A. Manice, R. A. Colvin, B. D. Medoff, G. R. Wojtkiewicz, R. Weissleder, and A. D. Luster. 2006. Oligomerization of CXCL10 is necessary for endothelial cell presentation and in vivo activity. *J. Immunol.* 177: 6991–6998.
58. Tharp, W. G., R. Yadav, D. Irimia, A. Upadhyaya, A. Samadani, O. Hurtado, S. Y. Liu, S. Munisamy, D. M. Brainard, M. J. Mahon, et al. 2006. Neutrophil chemorepulsion in defined interleukin-8 gradients in vitro and in vivo. *J. Leukocyte Biol.* 79: 539–554.
59. Poznansky, M. C., I. T. Olszak, R. Foxall, R. H. Evans, A. D. Luster, and D. T. Scadden. 2000. Active movement of T cells away from a chemokine. *Nat. Med.* 6: 543–548.
60. Weng, Y., S. J. Siciliano, K. E. Waldburger, A. Sirotina-Meisher, M. J. Staruch, B. L. Daugherty, S. L. Gould, M. S. Springer, and J. A. DeMartino. 1998. Binding and functional properties of recombinant and endogenous CXCR3 chemokine receptors. *J. Biol. Chem.* 273: 18288–18291.
61. Cox, M. A., C. H. Jenh, W. Gonsiorek, J. Fine, S. Narula, P. Zavodny, and R. Ripkin. 2001. Human interferon-inducible 10-kDa protein and human interferon-inducible T cell α chemoattractant are allotopic ligands for human CXCR3: differential binding to receptor states. *Mol. Pharmacol.* 59: 707–715.
62. Dagan-Berger, M., R. Feniger-Barish, S. Avniel, H. Wald, E. Galun, V. Grabovsky, R. Alon, A. Nagler, A. Ben-Baruch, and A. Peled. 2006. Role of CXCR3 carboxyl terminus and third intracellular loop in receptor-mediated migration, adhesion and internalization in response to CXCL11. *Blood* 107: 3821–3831.
63. Colvin, R. A., G. S. Campanella, J. Sun, and A. D. Luster. 2004. Intracellular domains of CXCR3 that mediate CXCL9, CXCL10, and CXCL11 function. *J. Biol. Chem.* 279: 30219–30227.
64. Booth, V., D. W. Keizer, M. B. Kamphuis, I. Clark-Lewis, and B. D. Sykes. 2002. The CXCR3 binding chemokine IP-10/CXCL10: structure and receptor interactions. *Biochemistry* 41: 10418–10425.
65. Booth, V., I. Clark-Lewis, and B. D. Sykes. 2004. NMR structure of CXCR3 binding chemokine CXCL11 (ITAC). *Protein Sci.* 13: 2022–2028.
66. Xanthou, G., T. J. Williams, and J. E. Pease. 2003. Molecular characterization of the chemokine receptor CXCR3: evidence for the involvement of distinct extracellular domains in a multi-step model of ligand binding and receptor activation. *Eur. J. Immunol.* 33: 2927–2936.
67. Liao, F., R. L. Rabin, J. R. Yannelli, L. G. Koniaris, P. Vanguri, and J. M. Farber. 1995. Human Mig chemokine: biochemical and functional characterization. *J. Exp. Med.* 182: 1301–1314.
68. Van den Steen, P. E., S. J. Husson, P. Proost, J. Van Damme, and G. Opdenakker. 2005. Carboxyterminal cleavage of the chemokines MIG and IP-10 by gelatinase B and neutrophil collagenase. *Biochem. Biophys. Res. Commun.* 310: 889–896.

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