Chemokines and Chemokine Receptors Critical to Host Resistance Following Genital Herpes Simplex Virus Type 2 (HSV-2) Infection

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Abstract: HSV-2 is a highly successful human pathogen with a remarkable ability to elude immune detection or counter the innate and adaptive immune response through the production of viral-encoded proteins. In response to infection, resident cells secrete soluble factors including chemokines that mobilize and guide leukocytes including T and NK cells, neutrophils, and monocytes to sites of infection. While there is built-in redundancy within the system, chemokines signal through specific membrane-bound receptors that act as antennae detailing a chemical pathway that will provide a means to locate and eliminate the viral insult. Within the central nervous system (CNS), the temporal and spatial expression of chemokines relative to leukocyte mobilization in response to HSV-2 infection has not been elucidated. This paper will review some of the chemokine/chemokine receptor candidates that appear critical to the host in viral resistance and clearance from the CNS and peripheral tissue using murine models of genital HSV-2 infection.

Keywords: Genital HSV-2, chemokines, chemokine receptors, leukocytes, CNS.

HSV-2 BIOLOGY

HSV-2 is a large (150-200nm) double-stranded, DNA virus and a member of the subfamily α-Herpesviridae [1, 2]. A mature virus contains a core, an icosahedral capsid, a tegument in which additional viral proteins reside, and an outer envelope [1]. An approximately 150,000 base pair linear DNA is packaged inside the capsid that encodes at least 80 different viral genes [1]. The outer membrane envelope contains viral glycoproteins (e.g. gB, gC, gD and heterodimer gH/gL) involved in a multistep viral entry into the target cell [3, 4]. The viral entry begins with interaction of gB and gC to heparin sulfate proteoglycans present on the surface of target cells followed by gD binding to other surface receptors including herpes virus entry mediator A and nectin-1α [2, 4]. The viral envelope subsequently fuses with the target cell membrane which requires gD, gB and the heterodimer gH/gL [2]. Following viral entry, local replication commences with a sequential cascade of viral lytic gene transcription/translation and subsequent packaging of unit length viral DNA into empty viral capsids [5-7]. The capsid then acquires viral tegument and glycoproteins followed by the envelopment of the capsid on the underside of the nuclear membrane [6]. After successful packaging of infectious virions, the virus is released thru the rupture of the cell membrane where it will enter sensory nerve endings in the basal aspect of epithelium and via retrograde transport traffic to sacral ganglia in the CNS [7, 8]. One of the hallmark features of HSV-2 is the establishment of latency in a subpopulation of neurons following acute infection of sensory ganglia [9, 10]. In normal adults, it can cause recurrent infection at the original portal of entry as well as adjacent sites after reactivation [11].

HSV-2 INFECTION OF THE CNS

HSV-2 is one of the most common causes of genital ulcer disease in humans that can result in fatal encephalitis and meningitis [8, 10, 12]. Approximately forty to sixty million individuals are infected with HSV-2 in the United States only, with nearly six to eight hundred thousand clinical cases annually [13]. In the immunocompromised patient as well as newborns, the virus can elicit severe and often fatal CNS infection [14-16]. Fortunately, even though a relatively large percentage of the population is seropositive for HSV-2 only a small percentage is subjected to life-threatening complications [2]. However, the increasing prevalence of genital herpes in young adults and co-incidence of HSV-2 with HIV/AIDS are indicative of a major public health impact [17, 18]. To underscore this point, recent evidence suggest the acquisition of HIV and other microbial pathogens increases significantly in HSV-2-infected individuals [17, 19]. Consequently, the need to identify host factors that contribute to resistance against HSV-2 infection/reactivation is tantamount toward the development of an effective prophylactic/therapeutic vaccine.

IMMUNE RESPONSE TO GENITAL HSV-2 INFECTION IN A MOUSE MODEL

The development of a mouse model for genital HSV-2 infection has contributed significantly to understanding the role of both innate and adaptive immune responses to infection [20, 21]. During initial infection of the genital mucosal surface, virus replicates primarily in the epithelium where the pattern recognition receptor (PRR) expressing epithelial cells or innate immune cells including dendritic cells (DCs)
and natural killer (NK) cells recognize viral-associated structures (dsDNA CpG motifs, dsRNA, etc.) and induce the secretion of anti-viral cytokines (type I interferons, IFNs) and chemokines [10]. PRR includes germ-line encoded Toll-like receptors (TLRs) which are expressed on epithelial cells, resident DCs and NK cells [10, 22]. Of the 12 TLRs found in the mouse, two endosome associated receptors TLR3 and TLR9 are involved in protection against genital HSV-2 infection [23-26]. TLR3 and TLR9 recognize viral nucleic acids i.e. dsRNA and dsDNA (CpG motifs) respectively [22]. The recognition of ligands by TLR3 induces nuclear factor-kappa B (NF-kB) and interferon regulatory factor 3 (IRF3) pathways whereas TLR9 induces NF-kB through the myeloid differentiation factor (MyD88)-dependent pathway resulting in expression of interferons and pro-inflammatory cytokines [22]. The activation of DC through TLR9 has been shown to induce IFN-α in response to HSV-2 infection [27]. However, TLR9 is not required for chemokine expression or up-regulation in the vaginal tissue following HSV-2 infection [28]. The absence of chemokine induction by TLR9 in the genital tract was not due to a lack of local expression [24, 29]. These studies implicate a possible role of TLR3 and/or other pattern recognition receptors for local expression of chemokines in response to genital HSV-2 infection in mice.

Recent studies have identified a number of chemokine/cytokine products in the vaginal mucosa following genital HSV-2 infection [30, 31]. The chemokine expression in the vagina influences the mobilization and activation of innate immune cells including polymorphonuclear cells (PMNs), macrophages, and NK cells which play vital role to limit initial viral replication and facilitate adaptive immune response [7, 10]. In addition to chemokines, the importance of the innate immune response including NK cells, NKT cells, and the production of type I IFN (α/β interferon), IL-12, IL-15 and IL-18 in suppressing HSV-2 replication and virus-mediated mortality have been demonstrated using neutralizing antibodies or gene disrupted mice [32-34]. NK cells monitor HSV-2 infection operating through cytokytic processes and possibly through the production of IFN-γ [31]. DCs are crucial for the development of an adaptive immune response to virus infection [7, 10]. DCs encounter viral antigens at the site of infection, migrate to the draining lymph nodes and mature with upregulation of co stimulatory molecules and secretion of cytokines [7]. DCs present viral peptides to T lymphocytes in the context of MHC molecules and stimulate the generation of virus-specific effector T lymphocytes in the lymphoid organs [7, 10] that are critical in suppressing viral replication [35-38]. The role of CD8+ T cells includes the production of anti-viral cytokines including IFN-γ and TNF-α as well as cytolytic activity mediated by perforin/granzyme and Fas/FasL pathways to limit virus replication and spread [8, 35, 37-39].

Recent data suggests HSV-2 trafficking to the CNS is greatly influenced by the innate immune response generated at the primary site of infection [30]. Specifically, the inability of CXCL10 deficient mice (CXCL10−/−) to mobilize NK cells to vaginal tissue to the same extent as wild type (WT, C57BL/6) animals is associated with early CNS virus entry. The outcome includes increased mortality, higher viral loads, and severe inflammation in the CNS during acute infection [30]. A schematic representation of genital HSV-2 infection in a mouse model and the ensuing immune response is summarized in Fig. (1).

**CHEMOKINES IN THE CNS DURING HSV-2 INFECTION**

Chemokines are small (8-17-kd molecular mass) secreted peptides constitutively produced or induced after viral infection [40, 41]. Currently, approximately 50 chemokines and 20 chemokine receptors have been reported [41]. Based on the first two paired and conserved cysteine molecules at the amino-terminal end, chemokines have been classified into four groups: CXC type chemokines, CC type chemokines, CX3C chemokines (one member) and C chemokines (two members) [40]. Similarly, chemokine receptors are G-protein coupled, seven-transmembrane receptors classified according to the chemokine(s) they bind [41]. Many chemokines are redundant in function and/or promiscuous in binding multiple receptors, but selectivity in tissue expression and targeting specific effector cells for recruitment may ultimately influence the inflammatory response of the host and overall outcome of the infection. Upon receptor binding, the G-protein complex dissociates into two subunits Gα and Gβγ [42]. The former subunit induces the activation of phosphatidylinositol 3-kinase (PI3-kinase) while another subunit induces phospholipase C, protein kinase C, and Ca2+ influx [42]. In addition, mitogen activated protein (MAP) kinase and Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling are also involved in chemokine-receptor signaling [43].

Apart from their participation in mediating chemotaxis, T cell differentiation, cell cycle, and angiogenesis [42], chemokines are also involved in several inflammatory events including viral infection and autoimmune disease [44]. Their critical role in genital HSV-2 infection can well be anticipated, and studies have provided evidence of chemokine and pro-inflammatory cytokine production in the CNS following infection with HSV-2 [30, 31, 45-47]. However, most of the studies have focused on the mobilization of T and B cells into the vagina or mobilization of cells between vaginal epithelium and the draining lymph nodes during infection. The expression of chemokines in the nervous system and the temporal or spatial relationship they have with lymphocyte mobilization have not been elucidated.

**CXCL1**

Keratinocyte-derived chemokine (KC, CXCL1) is an ELR (Glu-Leu-Arg) containing CXC type chemokine and a functional homologue of IL-8 [48, 49]. The epithelial cells of female genital mucosa are capable of producing CXCL1 in culture in response to HSV-2 infection [50]. CXCL1 expression is up-regulated in the vagina and CNS of mice during acute HSV-2 infection [30]. The chemokine specifically targets neutrophils through the receptor CXC-R2 promoting chemotaxis and activation of neutrophils [51, 52]. Neutrophils are the early and pre-dominant innate immune cells that infiltrate the genital tract and contribute toward the resolution of HSV-2 [7, 10, 53, 54]. Currently, the induction of CXCL1 in HSV-2-infected tissue is unknown but the presence of NF-κB binding site within the CXCL1 promoter suggests the potential involvement of PRR and/or pro-inflammatory molecules in CXCL1 induction [55-59]. An earlier study found neither IL-6, IFN-γ, nor MAP kinase
pathways up-regulate CXCL1 production by astrocytes in culture in response to Théler’s murine encephalomyelitis virus (TMEV) infection [49]. However, IL-1α and TNF-α have been found to up-regulate CXCL1 production by astrocytes [49]. TNF-α alone may not be responsible for CXCL1 induction within the CNS since anti-TNF-α antibody administered in HSV-2 infected mice does not significantly reduce expression of CXCL1 within the spinal cord or brain stem [Thapa and Carr, manuscript submitted]. Collectively, CXCL1 expressed locally within the genitalia is likely involved in the initial immune response through the recruitment of PMNs, a potent, innate immune component to HSV-2 infection.

CXCL9

Monokine induced by interferon-γ (MIG, CXCL9) is a non-ELR containing CXC-type chemokine that specifically binds the CXCR3 receptor expressed on activated T cells, NK cells, monocytes, DCs, and B cells [60-64]. The strong induction of CXCL9 in the CNS in response to viral pathogens including HSV-2, lymphocytic choriomeningitis virus, murine hepatitis virus and vaccinia virus [30, 65-67] supports a central role for this chemokine participation in the CNS immune response.

CXCL9 deficient (CXCL9−/−) mice are sensitive to HSV-2 infection compared to WT animals, but less so than CXCL10−/− mice based on viral titer, inflammation, and mortality. Compared to CXCL10 expression, CXCL9 expression is delayed in the vagina but rapidly elevated in the draining lymph nodes (Inguinal/Iliac lymph nodes, I/ILN) following infection [30]. By comparison, CXCL10 levels gradually increase post infection but do not reach the same level achieved by CXCL9 in the draining lymph nodes [30]. Taken together, it would appear the early chemokine response is segregated based on tissue expression negating redundancy. In addition, CXCL9−/− mice compensate for the loss of CXCL9 with CXCL10 levels similar to WT mice. Even though CXCL9−/− mice express appreciable levels of CXCL10, the loss of CXCL9 in the spinal cord results in a reduction in NK cell and virus-specific CD8⁺ T cell mobilization and cytolytic activity along with an elevation in viral

Fig. (1). A schematic representation of acute genital HSV-2 infection and the ensuing immune response in a mouse. Following inoculation, virus infects the mucosa of the vagina and local replication occurs in the epithelial cells. Recognition of virus by epithelial cells, DCs and NK cells in the vagina induces secretion of soluble factors including type 1 interferon which in turn induces the secretion of chemokines/cytokines including CXCL9 and CXCL10. Chemokines recruit PMNs, macrophages, and NK cells to the vagina. T cells are mobilized later to the site upon induction of the adaptive immune response in the organized lymphoid organs. After successful replication in the vagina, virus enters sensory nerve endings in the basal aspect of the epithelium and traffics to sacral ganglia in the CNS. Virus undergoes a second lytic replication in the sensory ganglion and further travels to the brain. The lytic replication in the CNS induces inflammatory responses with local production of chemokine/cytokine products including CXCL9, CXCL10, & TNF-α. The chemokine/cytokine storm mediates the infiltration of PMNs, macrophages, NK cells and effector T cells to the CNS.
CXCL10 deficient (CXCL10−/−) mice are more susceptible to infection compared to CXCL9−/− or WT mice based on viral titer, mortality and expression of select chemokines including CXCL1, CCL2, CCL3 and CCL5 [30]. The expression of these chemokines in the CNS is consistent with other virus infections including vesicular stomatitis virus, lymphocytic choriomeningitis, and mouse hepatitis virus [68-70]. Collectively, the outcome of a deficiency in CXCL9 expression is to some degree compensated by the expression of CXCL10 which preserves the recruitment capabilities for NK cells. However, CD8+ effector T cells mobilization to the CNS is attenuated and associated with an elevation of virus replication and spread. In addition to effector cell trafficking to the CNS, other factors are likely involved in viral surveillance (e.g., type I interferons) within the spinal cord and brain requiring further analysis.

CXCL10

Interferon inducible protein-10 (IP-10, CXCL10) is another non-ELR containing CXC type chemokine that specifically binds the CXCR3 receptor expressed on activated T cells and facilitates the recruitment of virus-specific T cells into the CNS promoting a Th1 response [60, 62, 71, 72]. Similar to CXCL9, the strong induction of CXCL10 in the CNS in response to many viral pathogens including HSV-2, murine hepatitis virus, murine CMV, dengue virus, and HIV as well as Toxoplasma gondii [30, 73-77] underscores the importance of this chemokine in the local immune response.

In a recent report, it was found even though both CXCL9 and CXCL10 operate through the same receptor, CXCR3, CXCL10 deficient (CXCL10−/−) mice are more susceptible to infection compared to CXCL9−/− or WT mice based on viral titer, mortality and clinical presentation following HSV-2 infection (Fig. 2a-c) [30]. The increase in susceptibility may, in part, be due to a failure to fully compensate for the loss of CXCL10 with CXCL9 levels. This relationship correlates with a reduction in NK cell mobilization to the vagina and early detection of HSV-2 in the spinal cord of CXCL10−/− mice [30].

The uncontrolled expression of chemokines and other soluble inflammatory factors promotes excessive infiltration of inflammatory leukocytes into the CNS adversely affecting the host ultimately resulting in exaggerated immunopathology [30, 63]. A case in point, TNF-α is the sole cytokine found to be elevated in the nervous system of HSV-2-infected CXCL10−/− mice [30]. As TNF-α is neurotoxic [78], it is predicted the increase in CNS TNF-α levels in these mice may be the primary contributing factor in the higher mortality rate in comparison to WT or CXCL9−/− mice. In fact, a recent study found neutralization of TNF-α in HSV-2-infected CXCL10−/− mice offset the elevated mortality rate of these mice despite increased CNS viral titers [Thapa and Carr, manuscript submitted].

CCL2

Monocyte chemoattractant protein-1 (MCP-1, CCL2) is a C-C type chemokine often found associated with inflammatory CNS disorders, lung infections, and viral infections including HSV-2 [30, 79-81]. Using in situ hybridization techniques, CCL2 was found to be induced rapidly in the infected brain of mice in a restricted fashion both by endothelial and parenchymal microglial cells as well as by infiltrating cells [47]. CCL2 can attract macrophages, monocytes and T cells that express the CCR2 receptor [82]. The association of CCL2 and CCR2 in neuropathogenesis has also been demonstrated in other disease models including experimental autoimmune encephalomyelitis (EAE) [83]. In addition, resistance of CCR2 deficient mice to EAE [83] correlates with impairment of macrophage recruitment to the CNS and provides support for this chemokine/receptor system as a critical component in the development of CNS inflammatory processes. Previous studies have found CCL2 expression is regulated by NF-kB as NF-kB binding sites are located 2700 bp upstream of the transcriptional start site for CCL2 [84, 85]. In addition, TNF-α induces CCL2 transcription in vitro as well as in vivo [86-88]. A recent study found neutralization of TNF-α reduces the expression of CCL2 in the CNS of HSV-2-infected CXCL10−/− mice following genital infection with HSV-2 [Thapa and Carr, manuscript submitted]. This observation suggests the expression of CCL2 in CNS of HSV-2-infected mice may be TNF-α regulated most probably through an NF-kB-dependent pathway.

CCL3

Macrophage inflammatory protein-1α (MIP-1α, CCL3) is another C-C type chemokine mainly produced by macrophages, DCs, neutrophils, astrocytes, and fibroblasts [43, 89, 90]. The chemokine signals primarily through CCR1 and CCR5 expressed by monocytes, T cells, NK cells, neutro-
phils and DCs [91, 92]. During HSV-2 infection, CCL3 is elevated in the infected tissue of mice [30]. The induction of CCL3 has been ascribed to regulation by NF-κB dependent pathways rather than interferon regulatory factors since there is no ISRE element in the CCL3 promoter [41, 93, 94]. In addition to NF-κB, the presence of a CD28RE element in close proximity to an AP-1 site suggests additional regulatory factors may be activated following virus infection [41]. There is evidence CCL3 drives Th1 development through IFN-γ production [95]. To this end, the application of CCL3 cDNA in a DNA vaccine induces antigen-specific Th1 type cell-mediated responses through production of IFN-γ and IL-2 which protects vaccinated animals against HSV-2 infection [95]. In addition, CCL3 has been shown to possess anti-HIV-1 activity by binding to co-receptors of the virus [44]. However, CCL3 has also been linked to immunopathology associated with viral and bacterial infections as well as EAE due to its major role in the recruitment of mononuclear cells [96, 97]. These data suggest a dichotomy in CCL3 function depending on the model under study. As TNF-α has been described as one of the inducing factors for CCL3 [58], the elevated CCL3 levels in CNS of chemokine/receptor deficient mice (CXCL9−/−, CXCL10−/−, CCR5−/−) mice following genital HSV-2 infection might be TNF-α-mediated [30, 31].

CCL5

Regulated upon activation, normal T cell expressed and secreted (RANTES, CCL5) signals through four different receptors CCR1, CCR3, CCR4 and CCR5 which are expressed by T cells, NK cells, monocytes, basophils and memory T cells [98-100]. Previous studies suggest NF-κB, MAP kinase and IRF3 are involved in regulating CCL5 expression during viral infection [101-104]. Relative to HSV-2, expression of CCL5 by macrophage is regulated by both NF-κB and IRF3 [104]. An NF-κB binding site resides in the CCL5 promoter at -30 position relative to the transcriptional start site [105]. It has also been reported that TNF-α enhances CCL5 expression through an NF-κB-dependent pathway [106]. In comparing mice vaccinated with constructs containing CCL2, CCL3 or CCL5 along with HSV-2 gD DNA, one study reported the CCL5 transgene possessed greater efficacy compared to CCL2 or CCL3 in response to HSV-2 infection as measured by reduced morbidity and mortality [100]. CCL5 supported the recruitment of memory CD4+ T cells as well as Th1 differentiation [100]. Following genital HSV-2 infection, CCL5 expression significantly increases in vaginal tissue as well as the spinal cord and brain stem [30]. The absence of CCL5 has not been addressed in the genital HSV-2 mouse model. However, the application of anti-CCL5 antibody to mouse hepatitis virus-infected mice suggests CCL5 expression promotes inflammation and has an overall detrimental impact on CNS infection [107].

CXCR3

CXCR3 is a G-protein coupled receptor specific for CXC-type chemokines CXCL9, CXCL10 and CXCL11 [62, 108, 109]. CXCR3 is preferentially expressed on activated T cells, NK cells and macrophages [109, 110]. Previous findings suggest the expression of both CXCR3 ligands CXCL9 and CXCL10 are required to mobilize virus-specific CD8+ T cells to the CNS as well as generate an effective immune response to genital HSV-2 infection [30]. Moreover, studies have implicated the importance of CXCR3 expression on plasmacytoid dendritic cell (pDC) migration and CTL function within the lymph node [111]. These observations suggest CXCR3 signaling may be critical for the generation of CTL effector function in lymph nodes and as a consequence, play an important role in controlling HSV-2 infection.

The importance of CXCR3 signaling in the recruitment of T cells has been demonstrated in neuro-inflammatory diseases such as multiple sclerosis, EAE, and viral infections of the CNS [110, 112, 113]. We are currently investigating the role of CXCR3 in the host response to genital HSV-2 infection. Preliminary studies with CXCR3 deficient (CXCR3−/−) mice suggest they are highly sensitive to HSV-2 infection compared to WT controls as determined by HSV-2 titers and mortality [Thapa and Carr, unpublished result]. In contrast to reduced mobilization of effector cells which are observed in the absence of CXCL9 and CXCL10 expression, HSV-2-infected CXCR3−/− mice mobilize HSV-specific CD8+ T cells to infected tissue normally but are found to have a defect at the level of CTL effector function. It is anticipated, CXCR3 expression is required for appropriate activation (education) of effector cells rather than recruitment/mobilization in the genital HSV-2 mouse model.

CCR5

CCR5 is a chemokine receptor mainly expressed on T cells, NK cells, macrophages and DCs and interacts with its ligands CCL3, CCL4 and CCL5 [114-116]. Previously, it was reported the absence of CCR5 had no significant impact on T cell mobilization [31]. However, NK cell infiltration was diminished and a reduction in NK cell levels was associated with a rise in tissue-associated HSV-2 titers [31]. Similarly, the role of CCR5 has been implicated in resistance to intraperitoneal infection with HSV-2 impacting on NK cell recruitment and activity at the site of infection [114]. Likewise, the significance of CCR5 mobilization of NK cells in response to an unrelated pathogen, Toxoplasma gondii has also been described [116]. Our results are in agreement with observations reporting no difference in T cell trafficking to the CNS following an intracranial infection with lymphocytic choriomeningitis virus [117] or the role of CCR5 on NK cell recruitment to the CNS following Toxoplasma gondii infection [116]. Taken together, CCR5 appears to be a necessary receptor within the nervous system to control genital HSV-2 infection in mice primarily through NK cell trafficking.

EFFECOR CELLS FROM LYMPH NODE VS SPLEEN: WHICH IS MORE PROTECTIVE?

Lymph nodes and spleen are organized secondary lymphoid organs critical to the development of the adaptive immune response [118]. Both lymph nodes and spleen trap antigen and antigen presenting cells from sites of infection and provide a advantageous microenvironment to present antigens to circulating lymphocytes [118]. Lymphocytes are known to migrate to draining lymph nodes through lymphatic vessels [119], and chemokines are found to attract lymphocytes from the blood as they enter lymph nodes through high endothelial venules [120]. However, the spleen has no direct association with the lymphatic system and collects antigens from blood [118]. An earlier study indicated
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Fig. (3). Effector cells from lymph nodes suppress HSV-2 spread in vivo. WT (C57BL/6) mice (n = 3) were infected with HSV-2 (2,000 PFU/vagina) and exsanguinated on day 7 p.i., and lymph nodes & spleen were removed and processed. One million cells (1x10^6) from lymph nodes and spleen were introduced intravenously to naive WT mice (n=6/group) that were then infected with HSV-2 (2000 PFU/vagina). Recipients of spleen cells from naive mice served as controls. Mice were exsanguinated on day 7 p.i. and vaginal tissue, spinal cord, and brain stem were removed, processed and assayed for HSV-2 content by standard plaque assay. The viral titer is expressed as mean log PFU+/- SEM. *, p < 0.05 comparing three different HSV-2-infected groups as determined by ANOVA & Tukey’s post hoc T-test.

CD11b+ sub-mucosal dendritic cells present viral antigens to T cells in the draining lymph nodes as early as 48 h following infection with HSV-2 [121]. Antigen-specific T cells were found to produce IFN-γ in an HSV-2-specific manner which was detectable in the draining lymph nodes prior to T cells from the spleen. We interpret the results to suggest the initial adaptive immune response takes place in the draining lymph nodes. Furthermore, it led us to speculate the magnitude of protection may differ comparing lymph node versus spleen cells after HSV-2 insult. To address this hypothesis, HSV-2-activated lymph node cells (I/ILN) or spleen cells were administered to naive mice intravenously and mice were then challenged with HSV-2. The recipients of the I/ILN cells were found to harbor significantly less infectious virus in the brain stem by day 7 p.i. compared to naive controls (Fig. 3). Likewise, viral titers were also reduced in the spinal cord of I/ILN treated mice but it did not reach significance (p=0.05). By comparison, the virus titer recovered from recipients of activated spleen cells were found to be modestly but insignificantly lower than naive control (Fig. 3). Taken together, effector cells from lymph node seem to be more protective compared to spleen cells following HSV-2 infection. While the results are not conclusive, they do suggest effector cells from the draining lymph nodes can antagonize HSV-2 replication and spread into the CNS following vaginal challenge.

PERSPECTIVE

Chemokines are a group of soluble peptides critical in the orchestration of the immune response to genital HSV-2 infection. Our studies implicate CXCR3 ligands, CXCL9 and CXCL10, as the first line of defense evident by their rapid and tissue-specific expression early following HSV-2 infection. CXCL9 and CXCL10 have specific roles in recruiting NK cells and virus-specific T cells into the primary site of infection and CNS as well as facilitate the generation of effector T cells in the organized lymphoid tissue. The relevance of these ligands has also been demonstrated in CXCR3 deficient mice. However, preliminary data suggest CXCR3 expression is required for appropriate activation/education of effector cells rather than recruitment [unpublished data]. Though there is redundancy within the system, individual expression of chemokine or chemokine receptors seems to be required to maximize the host immune response especially as it relates to effector cell mobilization or function. By resolving the temporal and tissue-specific

Table 1. Chemokines and Chemokine Receptors Critical to Genital HSV-2 Infection

<table>
<thead>
<tr>
<th>Chemokines/Receptors</th>
<th>Target Cells</th>
<th>Site of Expression</th>
<th>Techniques</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCL1</td>
<td>Neutrophils</td>
<td>VG, BS, SC</td>
<td>suspension array, ELISA</td>
<td>[30, 31]</td>
</tr>
<tr>
<td>CXCL9</td>
<td>NK/activated T cells</td>
<td>VG, BS, SC</td>
<td>suspension array, ELISA</td>
<td>[30]</td>
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<tr>
<td>CXCL10</td>
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<td>VG, BS, SC</td>
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<td>[30]</td>
</tr>
<tr>
<td>CCL2</td>
<td>Macrophage/monocytes</td>
<td>VG, BS, SC, Brain sections</td>
<td>In situ hybridization, RT-PCR</td>
<td>[30, 47]</td>
</tr>
<tr>
<td>CCL3</td>
<td>Macrophage/neutrophils</td>
<td>VG, BS, SC, Spleen, Liver, Brain</td>
<td>suspension array, ELISA, RT-PCR</td>
<td>[30, 114]</td>
</tr>
<tr>
<td>CCL5</td>
<td>NK, T cells</td>
<td>VG, BS, SC, Liver, spleen, peritonium</td>
<td>suspension array, ELISA, RT-PCR, ELISA, RT-PCR</td>
<td>[30, 114, 104]</td>
</tr>
<tr>
<td>CXCR3</td>
<td>T cells (Th1)</td>
<td>I/ILN, VG, BS, SC</td>
<td>Flow cytometry</td>
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<tr>
<td>CCR5</td>
<td>NK cells</td>
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<td>Flow cytometry, RT-PCR</td>
<td>[31, 114]</td>
</tr>
</tbody>
</table>

HSV-2, Herpes simplex virus Type 2; VG, vaginal tissue; BS, brain stem; SC, spinal cord; I/ILN, Inguinal/Iliac lymph node; RT-PCR, reverse transcriptase polymerase chain reaction; *, unpublished data.
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expression of chemokines as well as the downstream effector pathways impacted by their expression, a greater understanding in the efficacious and detrimental role of chemokines in the host response to specific disease states will be appreciated. It is anticipated out of this analysis appropriate therapeutic strategies will be developed and applied to fundamental problems inherent within genital herpes infection. The contrast between atypical presentation of genital herpes infection relative to shedding incidence of virus and the local immune response including the production of chemokines within vaginal tissue is beneficial for the genesis of such therapeutic strategies against human HSV-2 infection.

ACKNOWLEDGEMENTS

The authors acknowledge Dr. Josh Farber, Dr. Andrew Luster and Dr. William A. Kuziel for the CXCL9−/−, CXCL10−/− and CCR5−/− mice respectively. All procedures were approved by the University of Oklahoma Health Sciences Center and Dean A. McGee Eye Institute animal care and use committee. This work was supported by NIH grant AI067309.

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