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IL-18, but not IL-12, Regulates NK Cell Activity following Intranasal Herpes Simplex Virus Type 1 Infection

Patrick C. Reading,² Paul G. Whitney, Daniel P. Barr, Magdalena Wojtasiak, Justine D. Minter, Jason Waithman, and Andrew G. Brooks

Infection of the respiratory tract with HSV type 1 (HSV-1) can have severe clinical complications, yet little is known of the immune mechanisms that control the replication and spread of HSV-1 in this site. The present study investigated the protective role of IL-12 and IL-18 in host defense against intranasal HSV-1 infection. Both IL-12 and IL-18 were detected in lung fluids following intranasal infection of C57BL/6 (B6) mice. IL-12-deficient (B6.IL-12−/−) mice were more susceptible to HSV-1 infection than wild-type B6 mice as evidenced by exacerbated weight loss and enhanced virus growth in the lung. IL-12-deficient (B6.IL-12−/−) mice behaved similarly to B6 controls. Enhanced susceptibility of B6.IL-18−/− mice to HSV-1 infection correlated with a profound impairment in the ability of NK cells recovered from the lungs to produce IFN-γ or to mediate cytotoxicity activity ex vivo. The weak cytotoxic capacity of NK cells from the lungs of B6.IL-18−/− mice correlated with reduced expression of the cytolytic effector molecule granzyme B. Moreover, depletion of NK cells from B6 or B6.IL-12−/− mice led to enhanced viral growth in lungs by day 3 postinfection; however, this treatment had no effect on viral titers in lungs of B6.IL-18−/− mice. Together these studies demonstrate that IL-18, but not IL-12, plays a key role in the rapid activation of NK cells and therefore in control of early HSV-1 replication in the lung. The Journal of Immunology, 2007, 179: 3214–3221.

Herpes simplex virus type 1 (HSV-1) is a large dsDNA virus that infects epidermal or epithelial cells before establishment of latent infection in sensory neurons. Reactivation of the virus can cause disease in immunocompetent and immunodeficient individuals, resulting in a variety of illnesses, including disseminated infection, encephalitis, and pneumonia. HSV-1 infection elicits a multifaceted antiviral response to control the virus, requiring elements of both the innate and adaptive immune systems.

NK cells restrict early virus spread by direct lysis of virus-infected cells and through secretion of antiviral cytokines such as IFN-γ and TNF-α. Severe herpesvirus infections have been reported in human patients with NK cell deficiencies (1, 2), and NK cell depletion was associated with exacerbated virus replication and disease in some (3–7), but not all (8–10), murine models of HSV-1 infection. Because activated T cells can express NK cell markers (11), caution must be extended in the interpretation of NK depletion studies using anti-asialo-GM1 (AAGM) and anti-NK1.1 Abs due to their potential to affect T cell responses.

Both CD4 and CD8 T cells have been implicated in limiting the severity of HSV-1 infections. Clear roles for CD4 and CD8 T cells have been described in the resolution of HSV-1 in murine models of infection (12–15), although the mechanisms by which each population can mediate protection have not been fully elucidated. CD4 T cells are classically thought of as “helper” cells, inducing CD8 T cell (CTL) responses through licensing of dendritic cells and class-switching of B cells through the secretion of cytokines. Activated CTLs produce antiviral and proinflammatory cytokines and are potent killers of virus-infected cells.

NK and T cell functions are subject to regulation by a number of cytokines, including IL-12 and IL-18. Both IL-12 and IL-18 induce production of IFN-γ and other cytokines by NK and T cells, and stimulate NK cell activation and T cell proliferation (16–18). Furthermore, IL-12 and IL-18 have a synergistic effect on IFN-γ production (18–20). While IL-12 and IL-18 share some biological activities, there is no significant homology is noted between the two cytokines at the protein level. IL-12 is produced by activated macrophages and dendritic cells as a covalently linked heterodimer (p70) composed of two chains, p40 and p35 (reviewed in Ref. 21). IL-12p40 is often secreted in excess over the p70 heterodimer and can form p80 homodimers that antagonize IL-12 activity (22). IL-18 is produced largely by activated macrophages, dendritic cells, and epithelial cells and is synthesized as a precursor protein (pro-IL-18, 24 kDa), which requires cleavage by IL-1β-converting enzyme to generate the bioactive 18-kDa protein (23).

We hypothesized that if IL-12 and/or IL-18 modulate NK and T cell function, the absence of either might alter disease pathogenesis following HSV-1 infection. We have used a murine intranasal model to examine the responses of mice deficient in either IL-12p40 (B6.IL-12−/−) or IL-18 (B6.IL-18−/−) to HSV-1 infection. Few studies have examined the pathogenesis of HSV-1 in the respiratory tract, despite reports that HSV-1 infection of neonates and immunocompromised patients is associated with a range of pathologic conditions, including pneumonia and meningoencephalitis (24–26). The results reported in this study indicate that IL-18-deficient mice are more susceptible to HSV-1 infection of the lung and that IL-18, but not IL-12, plays an important role in early...
NK cell activation after HSV-1 infection. Of interest, the absence of either IL-12 or IL-18 had no appreciable effects on adaptive responses in the lung. Together, these studies highlight the importance of the innate response, and in particular NK cells, in limiting acute HSV-1 infection of the lung.

Materials and Methods
Mice, viruses, and peptide
C57BL/6 (B6) mice, and mice with a deletion of the gene encoding the p40 subunit of IL-12 (B6.IL-12−/−) (27) or IL-18 (B6.IL-18−/−) (28) were housed in specific pathogen-free conditions in the animal facility at the Department of Microbiology and Immunology, The University of Melbourne (Parkville, Victoria, Australia). Adult male mice (6- to 10-wk-old) were used in all experiments. The KOS strain of HSV-1 was propagated and titrated using Vero cells. The glycoprotein B (gB) peptide with residues 498–505 (gB498−505) with the sequence SSIEFARL was obtained from Auspep. Tetramers containing this immunodominant gB peptide (H-2Kb-gB498−505 tetramer) were prepared as described elsewhere (29).

Infection and treatments of mice
Mice were anesthetized and infected via intranasal route with 106 PFU of HSV-1 (unless otherwise stated) in 50 µl of PBS. Each day, mice were weighed individually and monitored for signs of illness. All research complied with the University of Melbourne Animal Experimentation Ethics guidelines and policies. To determine virus titers in organs, mice were euthanized, and lung, brain, liver, spleen, and trigeminal ganglia (TG) were removed, homogenized, and clarified by centrifugation. The samples were assayed for infectious virus by plaque assay on Vero cells.

Recovery of immune cells for flow cytometry and cytotoxic assays
Bronchoalveolar lavage fluid (BALF) and lung cells were obtained from mock-infected and HSV-1-infected mice at various times postinfection (p.i.). For collection of BALF cells, mice were sacrificed, and the lungs were flushed three times with a 1-ml volume of PBS through a blunted 23-gauge needle inserted into the trachea. The three lavage samples were pooled and the cells treated with Tris-NH4Cl (0.14 M NH4Cl in 17 mM Tris (pH 7.2)) to lyse erythrocytes, washed twice, and resuspended in RPMI 1640 medium supplemented with 10% FBS. Single-cell suspensions of lung cells were prepared by mincing lung tissue before incubation with 2 mg/ml collagenase A (Roche Diagnostics) in serum-free RPMI 1640 at 37°C for 30 min. Lungs were then sieved through wire mesh followed by hypotonic lysis of erythrocytes.

Flow cytometric analysis of cell surface and intracellular Ags
Single-cell suspensions of BALF or lung cells were incubated on ice for 20 min with supernatant from hybridoma 2.4.G2 to block Fc receptors and then stained with the indicated Abs. All Abs were purchased from BD Biosciences. After staining, the cells were washed and propidium iodide (PI) was added before flow cytometric analysis on a FACSCalibur flow cytometer (BD Biosciences). To detect intracellular IFN-γ from HSV-1-specific CD8 T cells, lung cell suspensions were stimulated with gB498−505 peptide in the presence of 10 µg/ml brefeldin A (Sigma-Aldrich) for 6 h at 37°C. In some assays lung cell suspensions were stimulated with an equivalent concentration of irrelevant peptide (SIINFEKL, OVA257−264) under similar conditions. To detect intracellular IFN-γ from NK cells, BALB cells were stained with anti-mouse IFN-γ as described (7). To detect intracellular granzyme B, BALF cells were stained with CD3ε-FITC and NK1.1-PE, fixed and permeabilized before staining with allophycocyanin-conjugated anti-human granzyme B (Caltag Laboratories). Cells were analyzed on a FACSCalibur flow cytometer collecting data on at least 10,000 lymphocytes.

ELISA for IFN-γ, IL-12, IL-18 in BALF
Levels of IFN-γ in BALF were determined by sandwich ELISA from BD Biosciences according to the manufacturer’s instructions. Levels of IL-12 and IL-18 were determined using a BD OptEIA ELISA kit for IL-12 (p70) and a mouse IL-18 ELISA kit from MBL. BALF samples were clarified at 1800 rpm for 10 min before the concentration of each cytokine was determined to a standard curve.

Cytotoxic assays
NK cell cytotoxicity and HSV-1-specific CTL activity in lung cell suspensions was assayed using standard 51Cr release assays. Briefly, NK cell cytotoxicity was tested using YAC-1 target cells, RMA-S cells, or RMA-S cells transfected with Rael1β (RMA-S-Rael1β) (30). EL4 cells were used as targets for HSV-1-specific CTL lysis with or without addition of 1 µM gB498−505 or OVA257−264 Peptide. The percentage of specific 51Cr release was calculated as the percentage of specific lysis = (experimental release − spontaneous release)/(total detergent release − spontaneous release) × 100. The spontaneous release values were always <15% of total lysis.

Statistical analysis
Results from 3 to 10 mice in each group were expressed as mean ± SD unless otherwise stated. Differences in viral titer and cell numbers between two groups were assessed for significance by Student’s t test (two-tailed distribution, two sample equal variance). Values for p < 0.05 were considered significant.

Results
Increased mortality of B6.IL-18−/− mice following intranasal HSV-1 infection
We initially compared the susceptibility of B6, B6.IL-12−/− and B6.IL-18−/− mice to intranasal infection with increasing doses of HSV-1, ranging from 106 up to 5×106 PFU per mouse. Although neither B6 nor B6.IL-12−/− mice succumbed to infection at any dose tested, B6.IL-18−/− mice were significantly more susceptible to the infection with 70% survival (7/10) and 30% survival (3/10) recorded at doses of 106 PFU and 5×106 PFU, respectively (Fig. 1). All deaths were recorded by day 10, and mice displayed symptoms of pneumonia (huddling behavior, rapid breathing) before death. Surviving mice recovered rapidly and did not display respiratory or neurological abnormalities at any time up to day 30. Together, these findings are consistent with an important role for endogenous IL-18, but not IL-12, in host defense against HSV-1-induced pneumonia.

FIGURE 1. B6.IL-18−/− mice are more susceptible to intranasal HSV-1 infection than B6 or B6.IL-12−/− mice. Male B6, B6.IL-12−/−, and B6.IL-18−/− mice were infected via the intranasal route with increasing doses of HSV-1 strain KOS (106, 5×106, 105, 5×105 PFU per mouse). Mice were observed daily and assessed for signs of illness over a 30-day period. Results are shown for inoculum doses of 106 and 5×105 PFU and are expressed as the percentage of survival from groups of 10 mice each. Mice with pronounced signs of pneumonia (huddling, labored breathing, inability to take food/water) were considered moribund and were euthanised. Mice inoculated with doses of <105 PFU did not display pronounced signs of pneumonia and all mice survived the infection to day 30 (data not shown). Data are representative of two independent experiments.
Exacerbation of disease in B6.IL-18−/− mice following intranasal infection with HSV-1

To explore the pathogenesis of HSV-1 in the respiratory tract in more detail, we compared weight loss and signs of disease in mice infected with a nonlethal dose of HSV-1 (Fig. 2A). B6 and B6.IL-12−/− mice showed modest weight loss over the first 5–7 days of infection but recovered rapidly thereafter. B6.IL-18−/− mice lost considerably more weight and took longer to regain normal body weight. At this dose, no visible signs of pneumonia were observed in any infected animals.

Viral replication was examined in the lungs of B6, B6.IL-12−/−, and B6.IL-18−/− mice at various times after intranasal infection with 10⁶ PFU of HSV-1 (Fig. 2B). Low viral titers were recorded 4 h p.i., which had increased 100- to 1000-fold by day 1, consistent with acute viral replication within the lung. As reported previously for B6 mice (7), maximum titers were observed 1–3 days after infection, with clearance achieved 5–7 days after infection.

At day 1 there was a tendency to recover higher virus titers from the lungs of B6.IL-18−/− animals, and this tendency was significant compared with B6 controls in two independent experiments but not in a third (p = 0.038, p = 0.023, and p = 0.058 using Student’s t test). By day 3, however, elevated lung titers in B6.IL-18−/− mice were highly significant when compared with B6 controls (p < 0.01 in three independent experiments, Student’s t test).
were recovered from B6.IL-18−/− mice compared with B6 mice at days 3 and 7 p.i. (\(p < 0.05\), by Student’s \(t\) test). Differential counts confirmed our previous findings (7) that macrophages and lymphocytes were the predominant cell types recruited to the airways during HSV-1 infection; neutrophils comprised <10% of BALF cells from B6, B6.IL-12−/−, and B6.IL-18−/− at all time points tested (data not shown). Flow cytometry showed an early influx of NK cells (NK1.1+CD3−, Fig. 4B) into the BALF followed by the subsequent recruitment of T cells (CD3e+ NK1.1+) (Fig. 4C). Compared with B6 controls, no deficit was observed in the ability of B6.IL-12−/− or B6.IL-18−/−-infected mice to recruit NK cells or T cells to the lung (Fig. 4, B and C). Rather, NK cell and T cell numbers were higher in BALF from B6.IL-18−/− mice at days 3 and 7, respectively, in independent experiments. CD8+ T cells predominated over CD4+ T cells at days 7 and 10 with CD4+ to CD8+ ratios of up to 1:5 observed at day 7 p.i. (Fig. 4D). The absence of endogenous IL-12 or IL-18 had no significant effect on CD4+ to CD8+ ratios.

**No differences in CD8+ T cell responses in the lung after intranasal HSV-1 infection of B6, B6.IL-12−/− and B6.IL-18−/− mice**

Both IL-12 and IL-18 have been reported to play roles in augmenting T cell responses (16–18). As CD8+ T cells are the major cell type recruited to the airways following intranasal infection with HSV-1 (Fig. 4C), we assessed CD8+ T cell activation in B6, B6.IL-12−/−, or B6.IL-18−/− mice and determined 1) the proportion of CD8+ T cells that recognized the immunodominant epitope corresponding to gB498−505 using H-2Kb-gB498−505 tetramers (Fig. 5, A and B), 2) the proportion of CD8+ T cells that produced IFN-\(\gamma\) following in vitro stimulation with peptide gB498−505 (Fig. 5C), and 3) the ability of CD8+ T cells to kill target cells pulsed with the gB498−505 peptide in vitro (Fig. 5D). In all assays, gB498−505-specific CD8+ T cells were first detected in the lung at day 5 p.i., and no significant differences were noted in the responsiveness of cells from B6, B6.IL-12−/−, or B6.IL-18−/− mice. Together, these data indicate that loss of either IL-12 or IL-18 did not significantly impair recruitment and activation of T cells in the lung following intranasal HSV-1 infection.

**Impaired NK cell function in the lungs of B6.IL-18−/− mice infected with HSV-1**

IL-12 and IL-18 also play key roles in innate host defense by enhancing NK cell function, including cytotoxicity and the production of IFN-\(\gamma\). Original studies by Takeda et al. (28) described defective NK cell activity in B6.IL-18−/− mice; however, subsequent studies have indicated that IL-18 is not always required for NK cell activation during viral infections (31, 32). Thus, it was
important to determine whether NK cell function was compromised in B6.IL-18−/−-infected mice following intranasal infection with HSV-1. We first compared lung cell suspensions for their ability to lyse NK-sensitive YAC-1 targets (Fig. 6A). HSV-1-infected B6 and B6.IL-12−/− mice displayed similar profiles of NK cell cytotoxicity and, which was higher than profiles observed in HSV-1-infected B6.IL-18−/− mice on days 1 and 3 p.i., consistent with a defect in NK cell effector function in the absence of IL-18. The number of NK cells present in the lungs of mice 3 days p.i. was determined by flow cytometry and used to calculate NK cell (NK1.1+/CD3−) cells were recovered from infected B6.IL-18−/− mice (data not shown). We did, however, observe a marked decrease in expression of the cytolytic effector molecule granzyme B by BALF NK cells from HSV-1-infected B6.IL-18−/− mice (Fig. 6D), consistent with the poor cytotoxic activity observed ex vivo. Real-time PCR analysis of perforin expression by NK cells (purified by cell sorting from the BALF of HSV-1-infected B6, B6.IL−12−/− and B6.IL-18−/− mice) showed no differences in two independent experiments (data not shown).

We compared BALF NK cells for their ability to produce intracellular IFN-γ directly ex vivo following intranasal HSV-1 infection (Fig. 7A). IFN-γ+ NK cells were detected by day 1, peaked at day 3 and declined at days 5 and 7 (Fig. 7B). Although the total number of BALF NK cells was higher in B6.IL-18−/− mice (Fig. 4B), both proportion and overall number staining for IFN-γ was greatly reduced. The mean fluorescence intensity of IFN-γ+ NK cells was also low in B6.IL-18−/− mice compared with both B6 and B6.IL-12−/− mice (Fig. 7C).

ELISA was used to determine levels of IFN-γ protein in cell-free BALF at days 1, 3, 5, and 7 after HSV-1 infection. Low levels of IFN-γ were detected at days 1 and 3 after infection; however, a striking peak was noted at day 5 and a subsequent decline by day 7 (Fig. 7D). Consistent with a role for NK cells as major producers of early IFN-γ, BALF IFN-γ from B6.IL-18−/− mice was below detection at days 1 and 3 p.i. Of interest, IFN-γ levels were similar in days 5 and 7 BALF from all infected mice, suggesting that other cell types, presumably T cells, produce this cytokine to similar levels in all three mouse strains. Consistent with this notion, depletion of NK cells via treatment of B6 mice with AAGM antiserum profoundly reduced BALF IFN-γ levels at days 1 and 3, but had no effect on peak levels at day 5 p.i. (data not shown).

Enhanced replication of HSV-1 is observed in lungs from B6 and B6.IL-12−/− mice, but not from B6.IL-18−/− mice, following depletion of NK cells

To determine the role of NK cells during HSV-1 infection of the respiratory tract we depleted NK cells from B6, B6.IL-12−/− and B6.IL-18−/− mice using antisera-specific for asialo-GM1 (AAGM) before intranasal infection with 106 PFU of HSV-1. Use of AAGM to deplete NK cells can be complicated as activated T cells express “NK cell” markers such as asialo-GM1 and NK1.1 (11). We therefore examined the effects of AAGM treatment at day 3 p.i. as 1) T cell influx into the lung is minimal at day 3 p.i. as assessed by flow cytometry (Figs. 4 and 5) and by in vivo CTL analysis (7), and 2) treatment of mice with anti-CD4 and anti-CD8 Abs has no effect on HSV-1 titers in the lung at this time, despite >90–95% reductions in resident lung T cells (data not shown).

NK cell (NK1.1+, CD3+) depletion of >90% in BALF was confirmed at day 3 p.i. using flow cytometry, with no significant depletion of neutrophils (GR1high, CD11b+), T cells (CD3+, NK1.1), or macrophages (confirmed by cytoxin analysis and differential staining) at this time. Confirming our previous findings (7), NK cell depletion led to a marked exacerbation of viral titers in the lungs of B6 mice at day 3 p.i. (Fig. 8). Viral titers were also enhanced following NK cell depletion of B6.IL-12−/− mice. Of
interest, AAGM treatment had only modest effects upon viral titers in the lungs of infected B6.IL-18<sup>−/−</sup> mice, and viral titers were not significantly different to control B6.IL-18<sup>−/−</sup> mice (treated with normal rabbit sera) in two independent experiments (p > 0.05, Student’s t test). Together, these data confirm the original findings of Takeda et al. (28) that NK cell function is markedly reduced in IL-18-deficient mice. Collectively, our findings are consistent with a major role for IL-18, but not IL-12, in coordinating rapid NK cell activation in the lung thereby playing a critical role in early control of acute HSV-1 infection.

IL-12 and IL-18 can profoundly influence T cell immunity, acting to enhance proliferation, differentiation and development of T cell effector functions. In addition to promoting the development of Ag-specific T cell immunity, IL-12 and IL-18 can also promote non-Ag-specific IFN-γ production by CD8<sup>+</sup> T cells by microbial products (33), early CD8<sup>+</sup> T cell responses in murine CMV-infected mice before maximal T cell expansion (34) and turnover of CD8<sup>+</sup> memory T cells (35). T cells, in particular CD8<sup>+</sup> T cells, were the predominant inflammatory cells recovered from the lung following HSV-1 infection, yet no appreciable defect was observed in the recruitment of CD4<sup>+</sup> or CD8<sup>+</sup> T cells (Fig. 4D), nor in the function of virus-specific CD8<sup>+</sup> T cells (Fig. 5). Furthermore, at times longer than 4 wk p.i., no differences were noted in 1) the effector memory CD8<sup>+</sup> T cell population, 2) the quantity and isotype profile of HSV-1 specific Abs in sera or BALF, from B6, B6.IL-12<sup>−/−</sup>, or B6.IL-18<sup>−/−</sup> mice (data not shown). Our previous findings describing the clearance of HSV-1 from the lungs of B6.RAG1<sup>−/−</sup> mice or mice depleted of CD8<sup>+</sup> and/or CD4<sup>+</sup> cells (7) suggest that innate mechanisms are sufficient to control and clear HSV-1 at the primary site of infection. The expansion and differentiation of T cells may represent a “safeguard” to ensure effective viral clearance and to prevent/limit the reactivation of latent virus in the nerves. Clearly, the absence of either IL-12 or IL-18 does not lead to any gross defects in the development, magnitude or quality of HSV-1-specific immunity in the intranasal model.

Discussion

NK cells and T cells are components of innate and adaptive responses, respectively, and are recruited to the airways following intranasal HSV-1 infection. The present study shows that a deficiency in IL-18, but not IL-12p40, has profound effects upon the activation status of NK cells in the respiratory tract during HSV-1 infection. In contrast, no functional differences were detected in T cell responses between B6, B6.IL-12<sup>−/−</sup>, or B6.IL-18<sup>−/−</sup> mice infected with HSV-1. IL-18 deficiency was associated with exacerbated disease and increased HSV-1 replication in the lung. Depletion of NK cells in B6 and B6.IL-12<sup>−/−</sup> mice led to increased viral replication in the lung, but had only negligible effects on viral growth in B6.IL-18<sup>−/−</sup> mice. Collectively, our findings are consistent with a major role for IL-18, but not IL-12, in coordinating rapid NK cell activation in the lung thereby playing a critical role in early control of acute HSV-1 infection.
Major differences were, however, noted in the quality of NK cell responses in the lungs of IL-18-deficient mice after HSV-1 infection. NK cells were the predominant cell type recovered from BALF early (<5 days) after infection, consistent with an important role in control of early viral replication. Although impairment of NK cell recruitment and cytotoxicity has been reported in lungs from IL-12-deficient mice infected with respiratory syncytial virus (36), we found no such differences during respiratory infection with HSV-1 (Figs. 4, 6, and 7). Increased numbers of pulmonary NK cells were, however, consistently observed in B6.IL-18−/− mice at day 3 p.i. (Fig. 4), but both cytotoxic function (Fig. 6) and IFN-γ production (Fig. 7) were severely compromised. Levels of the cytotoxic effector molecule granzyme B were reduced in NK cells from B6.IL-18−/− mice (Fig. 6D), offering a possible explanation as to the weak cytotoxic activity of these cells ex vivo. Down-regulation of granzyme B has been associated with the poor cytotoxicity of NK cells observed in murine models of hyperthermia and psychological stress (37, 38). The importance of NK cells in limiting HSV-1 replication in the respiratory tract has been previously reported (7). The current report extends these findings to define a critical role for IL-18 in regulating the activation of NK cells and hence the early control of HSV-1 replication in the lung.

Both IL-12 and IL-18 are capable of augmenting NK cell activation in a number of infectious models. Studies with murine CMV infections have demonstrated that local cytokine requirements for IFN-γ production by NK cells can vary from site to site (for example, IL-18 is required to augment IFN-γ responses in the spleen but not the liver) and that cytokines controlling NK cell IFN-γ production are not necessarily the same as those required for induction of cytotoxic activity (where neither IL-12 nor IL-18 was required for NK cell cytotoxic activity in liver or spleen) (31). In the intranasal model, IL-12 plays the major role in regulating both cytotoxicity and IFN-γ production during HSV-1 infection, whereas IL-12 is largely dispensable. Given that IL-12/p40 mice are also deficient in IL-23 (formed by p40 and p19 heterodimers), an additional cytokine capable of modulating NK and T cell function, it is also clear that IL-23 does not play a major protective role in the respiratory tract following HSV-1 infection. Of interest, IL-12 has been implicated in resistance (39) and corneal scarring (40) following ocular infection of mice with HSV-1. Similarly treating recombinant IL-12 was also shown to protect thermally injured mice from HSV-1 infection (41). HSV-1 infection has been reported to induce up-regulation of IL-23 mRNA in TG (42) and prominent IL-12 responses in the eye (43) following corneal infection. A number of reports confirm IL-12 production in the respiratory tract following infection with viruses such as murine gammaherpesvirus 68 and influenza virus (44, 45). In this study we report that despite early induction of IL-12 in respiratory fluids recovered from HSV-1-infected mice (Fig. 3), deficiency in this cytokine had no obvious effect on the course of HSV-1-induced disease following intranasal infection. Together, these studies indicate that the contribution of IL-12 (and/or IL-23) to innate resistance during HSV-1 infection can be very different in distinct anatomical sites.

IL-18 is a multipotent cytokine, initially identified through its ability to enhance IFN-γ responses from NK cells and T cells (46). Recent evidence suggests IL-18 has a much broader range of biological activities such as stimulating the production of other inflammatory cytokines and chemokines (28, 47, 48), enhancing expression of Fas-ligand and adhesion molecules (49, 50) and modulation of neutrophil responses (51, 52) through IFN-γ-independent pathways. Neutrophil recruitment to the airways of B6.IL-18−/− mice was enhanced at days 1 and 3 p.i. (data not shown), however they still represented a very low proportion (<10%) of BALF cells and are likely recruited as a result of the increased viral burden present in the lungs of B6.IL-18−/− mice at these times. As neutrophils are the predominant cell-type infiltrating lesions early after ocular or skin infections with HSV-1 (53, 54), the effects of IL-18 deficiency on neutrophil responses might be better explored in these models.

Fujioka et al. (55) have demonstrated that IL-18 treatment promoted innate immunity to protect mice from i.p. challenge with HSV-1; this mechanism did not appear to require NK cells or NO, leading the authors to speculate on a role for IFN-γ produced before the HSV-1 infection in promoting IL-18-mediated protection. Clearly, in the intranasal model IL-18 is required for efficient NK cell activation (Figs. 6 and 7) and NK cells play a critical role in the early control of HSV-1 replication in the lung (Fig. 8). Local IFN-γ production in the lung appears to be a feature of NK cells early after HSV-1 infection, as IFN-γ levels were below detection in AAGM-treated mice (which we confirmed had been depleted of BALF NK cells but not BALF T cells or macrophages). Together, these studies demonstrate that IL-18 may modulate the innate response to HSV-1 in different ways depending on the site of infection. Our findings highlight a dominant role for IL-18 in coordinating NK cell responses, and therefore early host defense, to HSV-1 infection of the lung.

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References


