Published in final edited form as:

J Reprod Immunol. 2010 January ; 84(1): 8. doi:10.1016/j.jri.2009.09.007.

Herpes simplex virus (HSV)-specific T cells activated in the absence of IFN-gamma express alternative effector functions but are not protective against genital HSV-2 infection

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Abstract

Interferon gamma (IFN γ) is important for immune resistance to herpes simplex virus (HSV) infection. To examine the influence of IFN γ on the development of HSV-specific immune responses and test for IFN γ -independent adaptive immune mechanisms of protection, IFN γ -deficient mice (IFN $\gamma^{-/-}$) were immunized with thymidine kinase-deficient HSV-2 (HSV-2 333tk⁻). HSV-specific cellular and humoral responses were elicited in immunized IFN $\gamma^{-/-}$ mice resulting in increased resistance relative to non-immune C57BL/6J (B6) mice following challenge with fully virulent HSV-2. CD8⁺ T cells from IFN $\gamma^{-/-}$ mice displayed cytotoxic activity and secreted TNF α . HSV-specific CD4⁺ T cells from immunized IFN $\gamma^{-/-}$ mice secreted IL-4, TNF α , and IL-17, but unlike T cells from HSV-immune B6 mice, could not clear virus from genital tissue following adoptive transfer. HSV-immune IFN $\gamma^{-/-}$ mice produced predominantly IgG₁ HSV-specific antibodies while immune B6 mice produced predominantly IgG_{2c} antibodies. Transfer of equivalent amounts of HSV-specific antibodies from either strain to naïve mice imparted equivalent early resistance against infection of the genital epithelia. However, protection against neurological symptoms mediated by immune B6 antibodies was superior late in infection. Taken together, these results demonstrate that the limited resistance of HSV-immune IFN $\gamma^{-/-}$ mice to HSV-2 infection resulted from the action of HSV-specific Ab rather than IFN γ -independent effector functions of T cells. Further, protection against neurological manifestations of HSV-2 infection was superior in mice receiving Ab from immune B6 mice suggesting that Ab-mediated protective mechanisms involving IFN γ -induced IgG subclasses were more effective once virus had spread to neural tissues.

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Keywords

HSV-2; sensory ganglia; antibody; female genital tract; IFN γ ; IL-17

1. Introduction

Herpes simplex virus type 2 (HSV-2) is an important sexually transmitted pathogen. Severe disease may occur in infected immune-compromised individuals or newborns and HSV-induced genital ulcerations may increase the risk of acquiring HIV (Wald & Link, 2002; Corey et al., 2004). HSV-2 spreads from the genital epithelium to sensory neurons and following brief viral replication, life-long latent infection is established. During reactivation, virus is transported to and shed from the epithelia near the original infection site in the presence or absence of disease symptoms (Wald et al. 2000).

Antibody clearly plays a role in resistance to HSV infection and spread of virus within the nervous system in animal models (Dix et al., 1981; Kohl et al., 1990). Re-infection of immune animals despite high HSV-specific Ab titers has been reported (Kuklin et al., 1998; Milligan et al., 1998), although sterilizing immunity has also been observed (Parr & Parr, 1998). Mechanisms of Ab-mediated protection are not fully understood.

HSV-specific T cells clear infectious virus from epithelial sites of recurrent infection and resolve lesions (Posavad et al., 1997; Koelle et al., 1998). Consistent with this, HSV-specific T cells reside in epithelial/sensory neuron junctions and skin near previous lesions (Zhu et al., 2007). In animal models, CD4⁺ and CD8⁺ T cells secreting primarily type 1 cytokines and exhibiting cytolytic function have been detected at the genital site of HSV-2 infection (Milligan & Bernstein, 1995; Milligan et al., 2004). HSV-specific T cell mechanisms of clearance are not completely understood, although evidence suggests requirements for virus-specific cytolytic activity (Dobbs et al., 2005) and involvement of numerous cytokines including IFN γ , TNF α , type I IFNs, and IL-15 (Ito & O-Malley, 1987; Rossol-Voth et al., 1991; Smith et al., 1994; Bouley et al., 1996; Milligan & Bernstein, 1997; Tsunobuchi et al., 2000; Ashkar & Rosenthal, 2003; Pierce et al., 2005). IFN γ is important in protection against HSV infection. Studies involving IFN γ -deficient mice or *in vivo* neutralization of IFN γ demonstrated significant alteration of outcome including delayed virus clearance from the genital epithelium (Smith et al., 1994; Bouley et al., 1995; Milligan & Bernstein, 1997; Parr & Parr, 1999; Milligan et al., 1998; Harandi et al., 2001a). Rapid resolution of HSV-2 from the female genital tract requires IFN γ stimulation of parenchymal rather than hematopoietic cells (Bird et al., 2007; Ijima et al., 2008).

We previously detected delayed virus clearance in the absence of IFN γ or its receptor (Bird et al., 2007) suggesting alternative, IFN γ -independent clearance mechanisms. T cell-mediated IFN γ -independent protective mechanisms have also been reported during cutaneous HSV-1 infections (Yu et al., 1996). In the present study, IFN γ -deficient mice were immunized with HSV-2 333tk⁻ to examine and evaluate the elicited IFN γ -independent adaptive immune mechanisms in resistance against genital HSV-2 infection.

2. Materials and Methods

2.1. Virus

HSV-2 333tk⁻ (McDermott et al., 1984) was obtained from Mark McDermott (McMaster University, Ontario, Canada). HSV-2 strain 186 was utilized as the challenge virus as described previously (Milligan et al., 2004).

2.2. Virus inoculation and quantification

Mice received 2.0 mg medroxyprogesterone acetate (SICOR Pharmaceuticals, Inc., Irvine, CA) s.c. six days prior to intravaginal inoculation to prepare the genital epithelium for infection (Linehan et al., 2004, Kaushic et al., 2003). Mice were inoculated as described previously (Chu et al., 2008). Mice were not pre-swabbed in Ab passive-transfer experiments to prevent perturbation of Ab-containing vaginal secretions.

To quantify HSV-2 from the vaginal tract, swabs were obtained and titered as described previously (Chu et al., 2008). Infectious virus from homogenized lumbosacral ganglia and adjacent spinal cord was quantified as described previously (Johnson et al., 2008).

2.3. Mice

C57BL/6J (B6), B6.129S7-Ifng^{tm1Ts}/J (IFN γ ^{-/-}), and T-cell receptor (TCR)-transgenic C57BL/6-Tg (Tcr α Tcr β) 425Cbn/J (OT-II, chicken ovalbumin peptide OVA₃₂₃₋₃₃₉-specific CD4⁺ T cells) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were housed in the AAALAC-approved facility at the University of Texas Medical Branch, Galveston, TX. Experiments were approved by the Institutional Animal Care and Use Committee with oversight by staff veterinarians and followed NIH guidelines on the care and use of laboratory animals.

2.4. Quantification of HSV-specific IgG

HSV-specific IgG was quantified as described previously (Dudley et al., 2000). OD₄₉₀ was determined on a Thermomax microplate reader (Molecular Devices, Sunnyvale, CA) and compared to standard (purified mouse IgG) values over the linear portion of the standard curve. Ab concentrations were calculated using Softmax software (Molecular Devices). IgG subclass endpoint titers were obtained by plating serial dilutions of serum followed by addition of HRP-conjugated IgG subclass-specific antibodies to detect specific mouse IgG subclasses. Endpoint dilution was defined as the reciprocal of the final dilution resulting in an OD₄₉₀ greater than 0.1 and greater than twice the values from diluent only controls.

Virus neutralization assays were performed by a previously described plaque reduction method, utilizing rabbit complement (Accurate Chemical and Scientific, Westbury, NY; Milligan et al., 1998).

2.5. Quantification of HSV-specific T lymphocytes

Cytokine-secreting T lymphocytes were quantified by ELISPOT by a modification of the method described previously (Milligan & Bernstein, 1995). CD4⁺ or CD8⁺ populations were isolated using magnetic isolation kits (Miltenyi Biotec, Inc., Auburn, CA) and incubated 40 h with mitomycin C-treated (Sigma-Aldrich) B6 splenocytes pulsed with UV-irradiated HSV-2 (CD4⁺ cells) or gB₄₉₈₋₅₀₅ (CD8⁺ cells) on nitrocellulose plates coated with capture Ab for IL-4, IL-17, TNF α , or IFN γ (BD Pharmingen, San Diego, CA). Plates were developed as described previously (Milligan & Bernstein, 1995). Spot-forming cells (SFC) were quantified using an ImmunoSpot reader and analyzed with ImmunoSpot software (Version 4.0, Cellular Technology Ltd, Cleveland, OH).

2.6. Passive transfer of HSV-specific IgG

B6 or IFN γ ^{-/-} mice were inoculated intravaginally with HSV-2 333tk⁻ (HSV-immune) twice within four weeks. Immune sera were collected at three weeks after the second inoculation, precipitated with ammonium sulfate and dialyzed extensively against PBS as described previously (Chu et al., 2008). Mice received 23.0 μ g of HSV-specific Ab i.p. yielding a serum concentration of approximately 8.0 μ g/ml (within the range of HSV-specific IgG

concentrations normally detected in B6 mice after intravaginal HSV-2 333tk⁻ inoculation). Mice receiving immune IgG, or undiluted normal mouse serum as a control, were challenged three days later with 10⁴ PFU HSV-2 186.

2.7. Adoptive transfer of CD4⁺ or CD8⁺ T cells from HSV-immune B6 or IFN γ ^{-/-} mice

HSV-immune B6 and IFN γ ^{-/-} mice were challenged with 5 × 10⁵ PFU HSV-2 186. Four days later, lymphocytes from spleen and iliac lymph nodes were cultured at 2 × 10⁸ cells per 175 cm² flask for three days with UV-inactivated HSV-2 (CD4⁺ T cells) and 100 U/ml recombinant IL-2. Control cultures of OT-II splenocytes were established similarly using 2 × 10⁷ mitomycin C-treated syngeneic spleen cells pulsed with 100 μ M OVA₃₂₃₋₃₃₉ peptide. CD4⁺ T cells were purified from day 4 cultures using isolation kits (Miltenyi Biotec, Inc.). Irradiated recipient mice (650 cGy) were reconstituted with 2.25 × 10⁶ purified, activated CD4⁺ T cells and challenged with 10⁴ PFU HSV-2 333tk⁻.

2.8. Measurement of in vivo Cytotoxic T-Lymphocyte (CTL) activity

CTL activity was quantified by a modification of the method used previously (Dobbs et al., 2005). HSV-immune B6 or IFN γ ^{-/-} mice were challenged with 10⁶ PFU HSV-2 186. B6 splenocyte target cells were split into fractions: one pulsed with gB₄₉₈₋₅₀₅ peptide and labeled with 2.5 μ M carboxyfluorescein succinimidyl ester (CFSE, Molecular Probes, Eugene, OR), and the second incubated with media only and labeled with 0.25 μ M CFSE. On the indicated days post-challenge, 10⁷ cells from each fraction were injected i.v. After 4 hours, single-cell suspensions of spleens were fixed with 1% formaldehyde and analyzed by flow cytometry. Percent specific lysis = (1 - ratio of naïve cell recipient / ratio of activated CD8⁺ T cell recipient) × 100, where ratio = % CFSE_{low} / % CFSE_{high}.

2.9. Statistics

Survival curves were compared by Logrank test. HSV-specific T cell quantification and HSV-2 titers were analyzed by Student's *t* test when two groups were compared, or ANOVA with Bonferroni correction for multiple groups. Analyses were performed using GraphPad Prism Version 3.0 for Windows (GraphPad Software, Inc., San Diego, CA). *P* values less than 0.05 were considered to indicate statistical significance.

3. Results

3.1. Immunization and HSV-2 genital challenge of B6 and IFN γ ^{-/-} mice

B6 and IFN γ ^{-/-} mice were inoculated intravaginally with HSV-2 333tk⁻. B6 mice were found to clear genital HSV-2 by day 6 post-inoculation (Fig. 1A). Clearance in IFN γ ^{-/-} mice was delayed 2 days and titers were significantly higher on days 4 through 6 post-inoculation compared to B6 mice. Resolution of HSV-2 333tk⁻ vaginal infection allowed immunization of IFN γ ^{-/-} mice and testing for IFN γ -independent cell- or Ab-mediated mechanisms of protection against HSV-2 challenge. HSV-immune B6 and IFN γ ^{-/-} mice were challenged with fully virulent HSV-2 186. All (10/10) naïve B6 mice died by day 8 post-challenge whereas 9/10 HSV-immune B6 mice survived (Fig. 1B). HSV-immune IFN γ ^{-/-} mice survived through day 8, but approximately half succumbed by day 20 (*P* < 0.05 compared to immune B6). As reported previously (Milligan et al., 2004), high HSV-2 titers were detected in naïve B6 mice whereas HSV-immune B6 mice rapidly cleared HSV-2 by day 6 post-challenge (Fig. 1C). Titers in HSV-immune IFN γ ^{-/-} mice were initially high (*P* < 0.001 compared to immune B6), falling rapidly through day 6 before stabilizing through day 20. In a separate experiment of identical design, infectious virus in sensory ganglia (Fig. 1D) and spinal cords (Fig. 1E) of naïve mice reached high levels on day 5 post-challenge (*P* < 0.001 compared to immune mice) and all naïve mice died prior to day 7 sampling. Titers were below detection levels in sensory

ganglia and spinal cords in 11/14 HSV-immune B6 mice on day 5 and all immune B6 mice (14/14) on day 7 post-challenge. HSV-2 titers in HSV-immune IFN γ ^{-/-} mice were significantly lower than naïve mice ($P < 0.001$). Although titers in neural tissues were below detection levels for several HSV-immune IFN γ ^{-/-} mice on days 5 and 7 post-challenge, infectious virus was detected in either sensory ganglia or spinal cords in 9/14 animals.

3.2. Cell-mediated immune responses in HSV-immune B6 and IFN γ ^{-/-} mice

CD8⁺ T cells from HSV-immune B6 mice predominantly secreted IFN γ , although a smaller IL-4-secreting response was detected in spleen (Fig. 2A) and iliac lymph nodes (Fig. 2B). Predictably, CD8⁺ T cells from HSV-immune IFN γ ^{-/-} mice produced IL-4, but not IFN γ . Both strains of immune mice displayed a cytolytic response upon challenge (Fig. 2C). HSV-specific lysis on days 4 and 6 post-challenge in immune IFN γ ^{-/-} mice was significantly higher than B6 mice ($P < 0.005$, $P < 0.001$, respectively), likely reflecting continued presence of viral antigen in these mice or higher CD8⁺ T cell numbers resulting from a dysregulated contraction phase in the absence of IFN γ (Badovinac et al., 2001). Adoptive transfer of activated CD8⁺ T cells from HSV-immune B6, but not IFN γ ^{-/-} mice cleared virus from the genital tract (not shown).

3.3. Inability of HSV-specific CD4⁺ effector T cells from immune IFN γ ^{-/-} mice to clear HSV-2 333tk⁻ from the genital tract

CD4⁺ T cells from iliac lymph nodes of HSV-immune B6 mice predominantly produced IFN γ , with lower magnitude IL-4, IL-17, and TNF α responses (Fig. 3A). Numbers of IL-4-secreting CD4⁺ T cells from HSV-immune IFN γ ^{-/-} mice were significantly higher than B6 mice ($P < 0.05$, day 2; $P < 0.001$, day 5, 7), whereas numbers of IL-17- and TNF α -secreting cells in draining lymph nodes were comparable in immune IFN γ ^{-/-} and B6 mice on days 5 and 7 post-challenge. Comparable numbers were obtained from analysis of genital tract-infiltrating T cells (not shown).

CD4⁺ T cells are the predominant T cell subset for clearing HSV from the genital epithelium of immune mice (Milligan et al., 1998; Kuklin et al., 1998; Harandi et al., 2001b). Transfer of activated HSV-immune B6 CD4⁺ T cells to irradiated recipients resulted in clearance of HSV-2 333 tk⁻ by day 10 post-challenge (Fig. 3B, C) whereas control mice receiving activated OT-II cells failed to clear virus through day 12 post-challenge. Titers remained high in HSV-immune IFN γ ^{-/-} CD4⁺ T cell-recipients and were comparable to OT-II-recipients through day 12 post-challenge. Ultimately, 9/10 HSV-immune IFN γ ^{-/-} T cell-recipients failed to clear virus.

3.4. Resistance to HSV-2 challenge provided by HSV-specific Ab from B6 or IFN γ ^{-/-} mice

The mean HSV-specific serum-IgG titer from immune B6 mice (12.2 $\pm$ 2.0 μ g/ml, range: 3.1 to 36.7 μ g/ml; $n = 23$) was not significantly different from immune IFN γ ^{-/-} mice (16.3 $\pm$ 2.2 μ g/ml, range: 2.3 to 41.6 μ g/ml; $n = 25$). The mean neutralizing serum Ab titer was approximately 1.5-fold higher in immune IFN γ ^{-/-} mice (1:216.3 ($\pm$ 85.7) for immune B6 and 1:319.5 ($\pm$ 97.3) for immune IFN γ ^{-/-} mice). To compare efficacy, 23.0 μ g of HSV-specific IgG isolated from these two strains of immune mice was passively transferred to naïve B6 recipients prior to challenge. IgG Ab from immune B6 mice was predominantly IgG_{2c} whereas HSV-immune IFN γ ^{-/-} mice produced predominantly IgG₁ (Fig. 4A), mirroring the subclass distribution of HSV-specific IgG serum Ab from immune serum donors (not shown). HSV-specific Ab from either strain did not prevent infection. However, initial vaginal HSV-2 titers were reduced on days 1 and 2 post-challenge, prior to the development of the HSV-specific T cell response, compared to non-immune serum-recipients (Fig. 4B). Approximately 95% of non-immune serum-recipients died by day 11 post-challenge (Fig. 4C). Protection was nearly identical through day 12 in immune B6 and IFN γ ^{-/-} Ab-recipients. Resistance waned

in immune IFN $\gamma^{-/-}$ Ab-recipients by day 13 and was significantly lower compared to immune B6 Ab-recipients ($P = 0.039$).

4. Discussion

In murine models, IFN γ is important for resistance to primary HSV-1 and HSV-2 infections (Smith et al., 1994; Bouley et al., 1995; Milligan & Bernstein, 1997; Harandi et al., 2001a). In HSV-immune mice, IFN γ appears critical for resistance despite HSV-specific Ab and CTL responses (Milligan et al., 1998; Parr & Parr, 1999; Milligan et al., 2004). IFN γ impacts infectious virus titer in genital tracts of HSV-immune mice within 24 hours of intravaginal HSV-2 re-challenge (Milligan et al., 1998; Parr & Parr, 1999). Rapid virus clearance by CD8 $^{+}$ or CD4 $^{+}$ T lymphocytes requires IFN γ interaction with parenchymal rather than hematopoietic cells (Bird et al., 2007; Ijima et al., 2008). IFN γ produced by HSV-immune T cells has been suggested to synergize with type I IFNs to inhibit HSV replication (Mikloska & Cunningham, 2001; Sainz & Halford, 2002). In the present study, clearance of an attenuated HSV-2 virus by IFN $\gamma^{-/-}$ mice and development of adaptive immune responses allowed testing for IFN γ -independent mechanisms of immune protection. Using a similar approach, Yu et al. (1996), determined IFN $\gamma^{-/-}$ mice could be fully immunized by HSV-1 inoculation and T lymphocytes from immune mice protected recipients from developing HSV-1 cutaneous lesions in a zosteriform challenge model. In the current studies, immunization rendered IFN $\gamma^{-/-}$ mice partially resistant to intravaginal HSV-2 challenge. Transferred HSV-immune CD4 $^{+}$ or CD8 $^{+}$ T cells from IFN $\gamma^{-/-}$ mice failed to clear genital HSV-2 333tk $^{-}$ while transferred immune serum conferred resistance. Discrepancies between our results and Yu et al. may reflect use of HSV-1 versus HSV-2 or different murine genetic backgrounds.

Naïve CD4 $^{+}$ T cells differentiate into distinct effector cell subsets based on cytokines present during activation. Antigenic stimulation in the absence of IFN γ results in development of T cell populations secreting type 2 cytokines. In addition to IL-4-secreting T cells detected previously in HSV-immune IFN $\gamma^{-/-}$ mice (Bouley et al., 1995; Yu et al., 1996), we detected numerous CD4 $^{+}$ T cells secreting TNF α and IL-17 in secondary lymphoid and vaginal tissues of both strains of immune mice. Although TNF α plays a role in HSV clearance *in vitro* and *in vivo* (Ito & O-Malley, 1987; Rossol-Voth et al., 1991) and IL-17 has been shown to elicit a granulocyte response which may function to limit viral spread (Tumpey et al., 1996; Milligan et al., 2001), adoptive transfer of HSV-specific T cells from immune IFN $\gamma^{-/-}$ mice secreting these cytokines did not result in virus clearance. Thus, we detected no evidence that the T cell response of immune IFN $\gamma^{-/-}$ mice conferred resistance to HSV-2 challenge despite secretion of potentially antiviral cytokines.

Comparable HSV-specific serum IgG levels were detected in HSV-2 333tk $^{-}$ -immunized B6 and IFN $\gamma^{-/-}$ mice. IgG subclass expression reflected the presence or absence of IFN γ during B cell priming. Passive transfer of IgG $_1$ -rich HSV-specific Ab from IFN $\gamma^{-/-}$ mice was as protective early in infection as IgG $_{2c}$ -rich Ab from immune B6 mice, manifested as resistance to initial infection with lower day 1 and 2 vaginal titers and greater protection against mortality through day 12 post-challenge relative to non-immune IgG-recipients. Given comparable neutralization activity of Ab preparations, early protection was likely conferred by HSV neutralization. Lack of protection by HSV-specific IgG from IFN $\gamma^{-/-}$ mice after day 13 suggests the Ab was less effective in preventing virus spread and replication within the nervous system, perhaps reflecting diminished ability of IgG $_1$ to activate complement or mediate Ab-dependent cell-mediated cytotoxicity. Since Ab from immune B6 mice (primarily IgG $_{2c}$) provided superior protection during this time, Ab-mediated protection at neural sites may require biologic functions associated with this IFN γ -influenced IgG subclass. IgG $_1$ Ab binds less well to Fc γ receptors compared to IgG $_{2b}$ or IgG $_{2c}$. Superior protection afforded by

IgG_{2c}-rich HSV-specific IgG is consistent with previous studies demonstrating a role for the Fcγ receptor in Ab-mediated protection against genital HSV-2 infection (Chu et al., 2008).

In conclusion, HSV-immune IFNγ^{-/-} mice were more resistant to HSV-2 challenge than non-immune mice. Partial resistance was not T cell-mediated, but could be transferred by HSV-specific Ab from immune mice. Our results suggest HSV-specific Ab of any IgG subclass may provide initial resistance to HSV-2, though prolonged resistance in neural tissues may require subclasses expressed as a result of IFNγ presence. Different Ab-mediated mechanisms may be involved in protection of genital and neural tissues against HSV-2 infection.

Acknowledgments

We thank Nigel Bourne and Jane Strasser for critical reading of the manuscript. This work was supported by NIH grants AI42815 and AI05444. AJJ was supported by a Vale-Asche Predoctoral Fellowship, MHN by a Sealy Center for Vaccine Development Predoctoral Fellowship, and MDB by a McLaughlin Predoctoral Fellowship.

Abbreviations

CTL	cytotoxic T-lymphocyte
HSV	herpes simplex virus
SFC	spot-forming cell
TCR	T cell receptor
tk ⁻	thymidine kinase-deficient
intravaginal	intravaginal

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Fig. 1.

HSV-2 challenge of HSV-immune B6 and IFN γ ^{-/-} mice. A) Clearance of HSV-2 333tk⁻ from the genital epithelium non-immune of B6 or IFN γ ^{-/-} mice. Mice were inoculated intravaginally with 2×10^5 PFU HSV-2 333tk⁻ ($n = 9$; * $P < 0.05$; ** $P < 0.0001$; ANOVA). B) Survival of HSV-immune B6, -immune IFN γ ^{-/-}, and naïve B6 mice after intravaginal challenge with 10^5 PFU HSV-2 186 ($n = 10$; * $P < 0.0001$ compared to naïve B6 mice; ** $P < 0.05$ compared to immune B6; Logrank test). C) Failure of HSV-immune IFN γ ^{-/-} mice to clear HSV-2 from the vagina. HSV-immune B6, -immune IFN γ ^{-/-} mice, and naïve B6 mice were challenged with HSV-2 186. Vaginal titers were obtained on the indicated days ($n = 9$; * $P < 0.001$; ** $P < 0.0001$ compared to immune B6 mice; ANOVA). HSV-2 titers in lumbosacral ganglia (D) and adjacent spinal cords (E) of HSV-immune B6, -immune IFN γ ^{-/-}, and naïve control mice after challenge with 10^4 PFU HSV-2 186. Mice were sacrificed on days 5 or 7 after challenge and infectious HSV-2 was quantified from the sensory ganglia and spinal cords. Day 5 lumbosacral ganglia and spinal cord titers from naïve B6 mice were significantly greater than HSV-immune B6 and -immune IFN γ ^{-/-} mice ($P < 0.001$; ANOVA).

Fig. 2.

Cytokine secretion by HSV-specific CD8⁺ T cells from HSV-immune B6 and IFN $\gamma^{-/-}$ mice. HSV-immune B6, -immune IFN $\gamma^{-/-}$, and naïve B6 mice were challenged intravaginal with 10⁴ PFU HSV-2 186. Six days later, CD8⁺ T cells isolated from spleens and iliac lymph nodes were stimulated with syngeneic spleen cells pulsed with HSV gB₄₉₈₋₅₀₅ peptide, and IFN γ -secreting cells and IL-4-secreting cells from the spleen (A) or iliac lymph nodes (B) were quantified by ELISPOT (* $P < 0.001$; ANOVA). (C) An *in vivo* CTL assay was performed on the indicated days after challenge of HSV-immune B6 and IFN $\gamma^{-/-}$ mice (* $P < 0.005$; ** $P < 0.0001$; Student's *t* test). Results are representative of 2 experiments performed.

Fig. 3.

Cytokine secretion by HSV-specific CD4⁺ T cells from HSV-immune B6 and $\text{IFN}\gamma^{-/-}$ mice (A). CD4⁺ T cells were isolated and pooled from iliac lymph nodes and vaginal tracts of HSV-immune B6, -immune $\text{IFN}\gamma^{-/-}$ and naïve B6 mice on the indicated day after challenge with 10⁴ PFU HSV-2 186. Results are expressed as the total number of cells obtained from the tissues from 4 mice and are representative of 3 experiments performed (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ for comparisons between immune B6 and immune $\text{IFN}\gamma^{-/-}$ mice; Student's t test). Adoptive transfer of CD4⁺ T cells from HSV-immune B6, but not HSV-immune $\text{IFN}\gamma^{-/-}$ mice, results in clearance of HSV-2 333tk⁻ from the genital epithelium (B). Lymphocytes from HSV-immune B6, -immune $\text{IFN}\gamma^{-/-}$, and naïve OT-II mice were activated

in culture for 4 days with specific antigen. CD4⁺ T cells were isolated and transferred to irradiated B6 recipients as described in Methods. Mice were immediately challenged with 5×10^3 PFU HSV-2 333tk⁻ and virus was quantified on the indicated days. (B) Virus titers following HSV-2 challenge (* $P < 0.001$ compared to HSV-immune B6 mice; ANOVA). (C) Percent of mice shedding virus. Results are pooled from 2 experiments of identical design.

Fig. 4.

Differential protection by passive transfer of HSV-specific antibodies from HSV-immune B6 and IFN $\gamma^{-/-}$ mice. B6 mice received 23.0 μ g HSV-specific IgG from immune B6 or IFN $\gamma^{-/-}$ mice or non-immune mouse serum and were challenged 3 days later with 10^5 PFU HSV-2 186. Mice were swabbed for virus on days 1 and 2 post-challenge and were observed 16 days for disease symptoms. A) HSV-specific IgG subclasses in IgG-enriched Ab preparations from HSV-immune B6, -immune IFN $\gamma^{-/-}$, or naïve mice. B) HSV-2 titers in the genital tracts of HSV-immune B6 IgG-, -immune IFN $\gamma^{-/-}$ IgG-, or non-immune serum recipients (* $P < 0.01$ compared to non-immune serum-recipients; ANOVA). Results are representative of 2 experiments performed. C) Survival of mice following transfer of HSV-immune IgG preparations or non-immune serum (* $P < 0.0001$ compared to non-immune serum recipients; ** $P = 0.039$ compared to immune IFN $\gamma^{-/-}$ serum recipients; Logrank test). Results are pooled from 2 experiments of identical design.