Early Resolution of Herpes Simplex Virus Type 2 Infection of the Murine Genital Tract Involves Stimulation of Genital Parenchymal Cells by Gamma Interferon\(^\text{\dagger}\)

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Received 10 July 2006/Accepted 13 October 2006

Early clearance of a thymidine kinase-deficient strain of herpes simplex virus type 2 from the female genital tract required T-cell-produced gamma interferon (IFN-\(\gamma\)). Transfer of activated CD8\(^+\) T cells to irradiated C57BL/6 mice resulted in rapid virus clearance, but clearance was greatly delayed in recipients deficient in the IFN-\(\gamma\) receptor (IFN-\(\gamma\)R). Early virus clearance was demonstrated in radiation chimeras in which IFN-\(\gamma\)R expression was limited to parenchymal cells, but resolution was significantly delayed in chimeras deficient in IFN-\(\gamma\)R expression and chimeras expressing IFN-\(\gamma\)R only on hematopoietic cells. Together, these results suggest that early IFN-\(\gamma\)-mediated protection was manifested mainly by stimulation of genital parenchymal cells.

Resolution of herpes simplex virus (HSV) lesions from epithelial sites of infection is achieved in healthy individuals by cellular immune mechanisms, but this process may be impaired in immunocompromised individuals. Understanding the cellular and molecular events involved in lesion resolution may be important for the development of therapies to decrease the severity of HSV lesions in these individuals. However, the exact mechanisms involved in clearance of HSV-2 from the genital epithelium are not fully understood. CD8\(^+\) T cells have been identified as important for clearance of genital herpetic lesions, a process that involves both gamma interferon (IFN-\(\gamma\)) secretion and cytolytic mechanisms (6, 7, 12, 13). IFN-\(\gamma\) is a major mediator of HSV-2 clearance (5, 17, 18, 20, 22, 24), presumably due to the activation of multiple immune cell types and/or initiation of numerous antiviral pathways in somatic cells. However, the cell types responding to IFN-\(\gamma\) in the female genital tract and the antiviral mechanisms relevant to the resolution of HSV-2 lesions are not understood.

IFN-\(\gamma\) in the genital secretions of fully immunocompetent mice is derived primarily from NK cells and antigen-specific T lymphocytes, with NK-cell-produced IFN-\(\gamma\) peaking at day 2 after intravaginal (ivag) inoculation and T-cell-produced IFN-\(\gamma\) present after day 3 (2, 17). To test if non-T-cell sources of IFN-\(\gamma\) were sufficient for HSV-2 clearance, Rag1-deficient mice (Rag1\(^{-/-}\)) genetically deficient in adaptive immune cells but possessing an intact innate immune system, including macrophages, dendritic cells, and NK cells (19), were utilized as recipients of activated wild-type OT-1 or IFN-\(\gamma\)-deficient OT-1 (OT-1 IFN-\(\gamma\)\(^{-/-}\)) T cells. Mice were treated with medroxyprogesterone in all experiments to induce susceptibility to genital HSV-2 inoculation (15), most likely reflecting hormonal induction of the HSV entry receptor, nectin-1, on vaginal epithelial cells (14). Rag1\(^{-/-}\) mice were injected intravenously (i.v.) with 3 \(\times\) 10\(^5\) activated OT-I or OT-I IFN-\(\gamma\)\(^{-/-}\) T cells and then challenged ivag with 5 \(\times\) 10\(^3\) PFU of an ovalbumin-expressing virus, HSV-2 tk\(^{-}\) OVA (7). HSV-2 tk\(^{-}\) strains replicate similarly to wild-type HSV-2 in genital epithelial cells but do not replicate well in neurons (15), therefore development of encephalitis and mortality does not occur following ivag inoculation, as is common following inoculation with wild-type HSV-2 (15, 18). The use of a tk\(^{-}\) HSV-2 strain in the present study therefore allowed a focused examination of the T-cell-mediated mechanisms of virus clearance from the genital tract. To confirm the presence of NK cells in the genital epithelia of recipient mice, the vaginas and cervixes were dissected from groups of uninfected or HSV-2 tk\(^{-}\) OVA-infected Rag1\(^{-/-}\) mice three days after virus inoculation and mechanically dissociated. The leukocyte fraction was isolated over Histopaque and stained with fluorescein isothiocyanate-anti NK1.1 or isotype control antibodies. A small population of NK1.1\(^{+}\) cells was detected in the genital tracts of uninfected Rag1\(^{-/-}\) mice (Fig. 1A); however, this population increased following ivag HSV-2 tk\(^{-}\) OVA inoculation (Fig. 1B).

Rag1\(^{-/-}\) mice receiving activated wild-type OT-I cells cleared virus by day 6 postchallenge (Fig. 2A). However, activated OT-I IFN-\(\gamma\)\(^{-/-}\) T-cell recipients were unable to clear HSV-2 tk\(^{-}\) OVA from the genital tract, and 75% (n = 8) of these mice continued to shed virus through day 21 postinoculation (Fig. 2B). These data, together with previous results demonstrating that less IFN-\(\gamma\) is produced by NK cells than with antigen-specific T cells (17), suggest that insufficient NK-cell-produced IFN-\(\gamma\) was available to achieve virus clearance and that T-cell-produced IFN-\(\gamma\) was required for resolution of the genital infection. These results do not preclude the possibility that other proinflammatory cytokines were also involved in HSV-2 clearance such as tumor necrosis factor alpha or interleukin-15 (8, 11, 21).
Cantin et al. showed that mice genetically deficient in the IFN-γ receptor (IFN-γR−/−) were significantly more susceptible to ocular challenge with virulent HSV-1 than were wild-type controls (5). Therefore, as a further test for the importance of IFN-γ in virus clearance, IFN-γR−/− mice (10) and C57BL/6 (B6) mice were sublethally irradiated (650 rads), repopulated with activated OT-I T cells, and inoculated ivag with HSV-2 tk−/− OVA. The majority of B6 mice receiving activated OT-I T cells cleared virus by day 8, and all these recipients cleared by day 10 (Fig. 3A). Virus titers in IFN-γR−/− recipient mice were significantly greater than in B6 OT-I recipients through day 21, although virus titers began dropping by day 18. Eighty-eight percent (n = 8) of the IFN-γR−/− mice were still shedding high titers of virus on day 16, but only 38% were on day 21 (Fig. 3B). Together these results demonstrate a requirement for recipient expression of IFN-γR to achieve rapid virus clearance but suggest an IFN-γR-independent antiviral mechanism may have been acting much later in infection.

We previously showed that OT-I T cells infiltrated the vaginal tracts of HSV-2 tk−/− OVA-infected, but not uninfected, mice (7). In agreement with our previous results, CD8+ T cells were detected in the vaginas of OT-I recipient mice on day 7 after ivag inoculation with HSV-2 tk−/− OVA (Fig. 4A). Further, T cells obtained from the vaginas of identically treated mice produced IFN-γ upon culture with mitomycin C-treated syngeneic spleen cells pulsed with the immunogenic OVA peptide, SIINFEKL (Fig. 4B). Thus, IFN-γ-producing OT-I T cells were present at the site of genital infection at a time concurrent with virus clearance.

The IFN-γR is expressed on nearly all nucleated cells (1, 3), and studies have shown that vaginal epithelial cells expressed class II major histocompatibility complex proteins upon exposure to T-cell-produced IFN-γ (20), thereby demonstrating the ability of genital tissue to respond to IFN-γ. Large numbers of neutrophils and monocytes/macrophages have been shown to infiltrate the vaginal epithelium following genital HSV-2 inoculation, and these cells play an as-yet-undefined role in rapid virus clearance (16). To determine if rapid resolution of HSV-2 infection of the genital epithelium correlated with IFN-γ activation of these infiltrating innate cells or of genital parenchymal cells, we constructed IFN-γR−/− mice. B6 or IFN-γR−/− mice were lethally irradiated (900 rads) to fully deplete immune cells and then repopulated i.v. with 1.2 × 10^7 T-cell-depleted bone marrow and spleen cells from B6 or IFN-γR−/− donors. T-cell depletion was confirmed by flow cytometric analysis of donor cell preparations (G. N. Milligan, unpublished results). The resulting chimeras expressed the IFN-γR on all cells (R− R−), on the parenchymal cells only (R−/− R+), on hematopoietic immune cells only (R+ R−/−), or they lacked the re-
receptor on both cell types (R−/− R+/−). The mice were rested for one week, injected i.v. with 3 × 10^6 activated OT-I CD8+ T cells, and challenged i.vag with 5 × 10^3 PFU HSV-2 tk− OVA virus. As shown in Fig. 4C, R+ R+ and R+/− R+ mice cleared virus by day 8 compared to R− R−/− and R−/− R−/− chimeras, which were unable to clear virus until day 14. Mice receiving T-cell-depleted hematopoietic cells only were unable to clear virus and were shedding significantly higher titers of virus on day 14 (P < 0.05; analysis of variance [ANOVA]). These data suggest rapid clearance of HSV-2 is correlated with IFN-γR expression on genital parenchymal cells. These results are consistent with a recent report that clearance of lymphocytic choriomeningitis virus was dependent on parenchymal cell expression of the IFN-γR (9).

The delayed clearance in R−/− R−/− chimeras (Fig. 4C) and IFN-γR−/− recipients (Fig. 3A) suggests the presence of a less-efficient, IFN-γR-independent antiviral mechanism, distinct from the IFN-γR-dependent mechanism responsible for early resolution of infection. This late-acting mechanism apparently did not require IFN-γR expression on either transferred hematopoietic cells or on the genital parenchymal cells and may have involved enhanced production of cytokines such as IFN-α/β, tumor necrosis factor alpha, or interleukin-15 which are thought to be involved in controlling HSV infections (4, 8, 11, 21). Because the transfer of accessory cells alone did not mediate clearance (Fig. 4), the process also involved the activity of T lymphocytes. The difference in clearance kinetics between IFN-γR−/− recipient mice (Fig. 3) and IFN-γR−/− R−/− chimeras (Fig. 4C) most likely reflects differences in experimental design. The IFN-γR−/− recipients (Fig. 3) were irradiated and reconstituted only with activated OT-I T cells immediately before virus inoculation, whereas radiation chimeras received T-cell-depleted accessory cells from bone marrow and spleen one week before T-cell reconstitution and virus
inoculation. It is possible that the irradiation and cell reconsti-
tution regimen used to construct chimeras may have resulted in
expansion of critical accessory cell populations involved in
important interactions with T cells or responsible for produc-
tion of higher amounts of antiviral cytokines or other effector
molecules relative to the IFN-γR−/− recipients described in
Fig. 3.

Although alternative IFN-γ-independent mechanisms may
ultimately result in a delayed resolution of the genital tract
infection, early resolution of the infection required interaction
of IFN-γ with the genital parenchymal cells and not the
recruited hematopoietic immune cells present in the HSV-in-
fected vagina. IFN-γ may therefore be necessary for promoting an
antiviral state in the genital epithelial cells, possibly by
initiating antiviral gene cascades or through increasing the
expression of molecules necessary for enhancing antigen pro-
cessing and presentation which in turn may promote recogni-
tion and cytolysis of HSV-infected cells.

We thank Nigel Bourne and Mark Estes for critical reading of the
manuscript and Mark Griffin of the University of Texas Medical Branch
Flow Cytometry Core Facility for assistance with flow cytometry.

This work was supported by research grants AI42815 and AI054444
from the National Institutes of Health. M. D. Bird was supported by a
Flow Cytometry Core Facility for assistance with flow cytometry.

The authors have no conflicting financial interests.

REFERENCES

and NKT cells play a critical role in innate protection against genital herpes
a paradigm for cytokine receptor signaling. Annu. Rev. Immunol. 15:563–
591.
interferon (IFN-γ) receptor null-mutant mice are more susceptible to
herpes simplex virus type 1 infection than IFN-γ ligand null-mutant mice.
Merigan. 1985. Evolution of recurrent herpes lesions. An immuno-
 Clearance of herpes simplex virus type 2 by CD8+ T cells requires gamma
interferon and either perforin- or Fas-mediated cytolytic mechanisms. J. Vi-
rol. 79:14546–14554.
dependent contribution of interleukin-15 to innate protection against mu-
cosal viral infection. J. Virol. 79:4470–4478.
Impaired virus control and severe CD8+ T-cell-mediated immunopathology
in chimeric mice deficient in gamma interferon receptor expression on both
10. Huang, S., W. Hendriks, A. Althage, S. Hemmi, H. Bluethmann, R. Kamijo,
recovery of HSV-specific T lymphocyte clones from human recurrent HSV-2
Investig. 101:1500–1508.
14. Linehan, M. M., S. Richman, C. Krummenacher, R. J. Eisenberg, G. H.
simplex virus type 1 (HSV-1) and HSV-2 from the vaginal mucosa. J. Vi-
rol. 78:2520–2536.
Bienenstock. 1984. Immunity in the female genital tract after intravaginal
vaccination of mice with an attenuated strain of herpes simplex virus type 2.
16. Milligan, G. N. 1999. Neutrophil aid in protection of the vaginal mucosa of
immune mice against challenge with herpes simplex virus type 2. J. Virol.
73:6380–6386.
resolution of herpes simplex virus type 2 infection in the murine genital tract.
Virology 229:259–268.
required for protection of the vaginal mucosa and sensory ganglia of im-
cune mice against reinfection with herpes simplex virus type 2. J. Immunol.
160:6093–6100.
19. Mombaerts, P., J. Iacomini, R. S. Johnson, K. Herrup, S. Tonegawa, and
V. E. Papaioannou. 1994. Direct role of RAG-1 in antibody production and
immunity in the female genital tract after intravaginal vaccination of mice with
vivo protective effect of tumor necrosis factor alpha against experimental
Control of acute cutaneous herpes simplex virus infection: T cell-mediated
viral clearance is dependent upon interferon-gamma (IFN-gamma). Virology
23. Reference deleted.