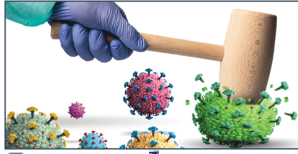
COVID-19 Research Tools
Defeat the SARS-CoV-2 Variants
InvivoGen

The Journal of Immunology

RESEARCH ARTICLE | APRIL 15 2007

Oligoadenylate Synthetase/Protein Kinase R Pathways and αβ TCR+ T Cells Are Required for Adenovirus Vector: IFN-γ Inhibition of Herpes Simplex Virus-1 in Cornea[1] FREE

Bobbie Ann Austin; ... et. al

J Immunol (2007) 178 (8): 5166–5172.
https://doi.org/10.4049/jimmunol.178.8.5166

Downloaded from http://journals.aai.org/jimmunol/article-pdf/178/8/5166/1241991/zim00807005166.pdf by guest on 27 January 2023

Oligoadenylate Synthetase/Protein Kinase R Pathways and $\alpha\beta$ TCR^+ T Cells Are Required for Adenovirus Vector: IFN-γ Inhibition of Herpes Simplex Virus-1 in Cornea[1]

Bobbie Ann Austin,[2]* William P. Halford,‡ Bryan R. G. Williams,§ and Daniel J. J. Carr[3]*†

An adenoviral (Ad) vector containing the murine IFN-γ transgene (Ad:IFN-γ) was evaluated for its capacity to inhibit HSV-1. To measure effectiveness, viral titers were analyzed in cornea and trigeminal ganglia (TG) during acute ocular HSV-1 infection. Ad:IFN-γ potently suppressed HSV-1 replication in a dose-dependent fashion, requiring IFN-γ receptor. Moreover, Ad:IFN-γ was effective when delivered −72 and −24 h before infection as well as 24 h postinfection. Associated with antiviral opposition, TG from Ad:IFN-γ-transduced mice harbored fewer T cells. Also related to T cell involvement, Ad:IFN-γ was effective but attenuated in TG from $\alpha\beta$ TCR-deficient mice. In corneas, $\alpha\beta$ TCR^+ T cells were obligatory for protection against viral multiplication. Type I IFN involvement amid antiviral efficacy of Ad:IFN-γ was further investigated because types I and II IFN pathways have synergistic anti-HSV-1 activity. Ad:IFN-γ inhibited viral reproduction in corneas and TG from $\alpha\beta$ IFNR-deficient ($CD118^{-/-}$) mice, although viral titers were 2- to 3-fold higher in cornea and TG compared with wild-type mice. The absence of IFN-stimulated antiviral proteins, 2′-5′ oligoadenylate synthetase/RNase L, and dsRNA-dependent protein kinase R completely eliminated the antiviral effectiveness of Ad:IFN-γ. Collectively, the results demonstrate the following: 1) nonexistence of type I IFN receptor does not abolish defense of Ad:IFN-γ against HSV-1; 2) antiviral pathways oligoadenylate synthetase-RNase L and protein kinase R are mandatory; and 3) $\alpha\beta$ TCR^+ T cells are compulsory for Ad:IFN-γ effectiveness against HSV-1 in cornea but not in TG. *The Journal of Immunology,* **2007, 178: 5166–5172.**

Interferon γ is a potent endogenous antiviral cytokine, made by leukocytes including T, NK, and NKT cells, which operate through a single IFN-γ receptor (1, 2). Inhibition of neurotropic viruses by IFN-γ involves activation of dsRNA-dependent protein kinase R (PKR)[4] (3–5), oligoadenylate synthetases (OAS) (6, 7), Mx (8–10), and NO synthetase (11–13) antiviral pathways (14). In contrast to these findings, adenoviral (Ad) vector delivery of the IFN-γ transgene to explant trigeminal ganglion (TG) cultures inhibits acute HSV-1 replication independently of recognized strategies for other neurotropic viruses (15).

As a follow-up to prior in vitro studies, an objective of the current report was to examine how murine IFN-γ transgene delivered by an Ad vector (Ad:IFN-γ) inhibits acute ocular HSV-1 infection in vivo. Adenovirus is a useful vector for delivering IFN transgenes to the cornea and TG (16–18). To examine the mechanisms used by IFN-γ to inhibit HSV-1, we studied parameters including participation of type I IFN-inducible antiviral pathways, OAS with downstream effector RNase L (RL), PKR, the need for type I and type II IFNR expression, and the requirement for $\alpha\beta$ TCR^+ T cells.

A prediction for type I IFN involvement follows: in mice lacking type I IFN antiviral pathways, infectious HSV-1 will reach higher levels in cornea and TG when compared with wild-type (WT) mice. We reasoned that because the IFN $\alpha\beta$ system alone controls herpes infection for the first several days of infection without T, NK, or B cells (19), then viral burden will likely be greater in the absence of this protective mechanism.

Type I and type II IFN anti-HSV activities have been linked. Specifically, type I and type II IFNs have synergistic anti-HSV-1 activity (20–22). In response to some viral infections, both IFN pathways are required and considered nonredundant (23). To examine the relationship of type I and type II IFN antiviral pathways during HSV-1 infection, the following hypothesis was tested: if type I IFN pathways are not required for Ad:IFN-γ effectiveness against acute HSV-1 infection, then transduction of IFN $\alpha\beta$ receptor-deficient ($CD118^{-/-}$) mouse corneas with Ad:IFN-γ will provide protection against HSV-1 replication.

Downstream effector molecules of IFN-inducible antiviral OAS and PKR pathways have proven antiviral activity. Yet, cells lacking OAS and PKR can still mount an antiviral response (24), and effectiveness of Ad:IFN-γ in dissociated TG cultures is not lost in the absence of OAS and PKR (15). To resolve the necessity for OAS and PKR in vivo, we tested the following hypothesis: if effectiveness of Ad:IFN-γ is independent of OAS and PKR, then the ability of Ad:IFN-γ transduction to inhibit viral replication will be lost in RL and PKR double knockout mice ($RL/PKR^{-/-}$ mice).

*Department of Ophthalmology and †Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104; ‡Montana State University, Bozeman, MT 59718; and §Monash Institute of Medical Research, Monash Medical Center, Clayton, Victoria, Australia

Received for publication November 30, 2006. Accepted for publication January 15, 2007.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by a Research to Prevent Blindness Stein Research Professorship (to D.J.J.C.) and by Grant AI053108 (to D.J.J.C.) and Core Grant EY12190 from the National Institutes of Health.

[2] Current address: Laboratory of Immunology, National Institutes of Health, National Eye Institute, 10 Center Drive, Building 10, Room 10N112, Bethesda, MD 20982-1857.

[3] Address correspondence and reprint requests to Dr. Daniel J. J. Carr, Department of Microbiology and Immunology, Room 415, Dean A. McGee Eye Institute, University of Oklahoma Health Sciences Center, 608 Stanton L. Young Boulevard, Oklahoma City, OK 73104. E-mail address: dan-carr@ouhsc.edu

[4] Abbreviations used in this paper: PKR, protein kinase R; OAS, oligoadenylate synthetase; Ad, adenoviral; TG, trigeminal ganglia; RL, RNase L; WT, wild type; p.i., postinfection; TU, transducing unit; DTH, delayed-type hypersensitivity.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/178/8/5166/1241991/zim00807005166.pdf by guest on 27 January 2023

In addition to effects on IFN-stimulated gene expression, the antiviral outcome of Ad:IFN-γ transduction is anticipated to involve T cells. Specifically, T cells are found in TG during acute HSV-1 infection (25), and T cell-deficient mice are highly susceptible to HSV-1 (26). T cells accumulate around neurons that are infected with HSV-1, and IFN-γ is present in the vicinity amid T cell infiltrates (27, 28). Therefore, the question arises: will protective antiviral activity of Ad:IFN-γ be lost in the absence of $\alpha\beta$ TCR$^+$ T cells? We predict the dearth of $\alpha\beta$ TCR$^+$ T cells to impact antiviral activity of Ad:IFN-γ because 1) T cells are a source of antiviral cytokines including TNF-α, which has supplementary anti-HSV-1 properties (29), and 2) virus surveillance by T cells involves direct cytolytic action by effector T cells. Therefore, we proposed the following: if T cells are required for antiviral effectiveness of Ad:IFN-γ, then antiviral activity following Ad: IFN-γ transduction will be attenuated in mice lacking $\alpha\beta$ TCR$^+$ T cells compared with WT mice.

Materials and Methods

Mice

Animal treatment was consistent with the National Institutes of Health Guidelines on the Care and Use of Laboratory Animals. All procedures were approved by the University of Oklahoma Health Sciences Center and Dean A. McGee Eye Institute Institutional Animal Care and Use Committee. WT (C57BL/6), CD119/IFN-γ receptor-deficient (CD119$^{-/-}$), and $\alpha\beta$ TCR knockout (TCR$^{-/-}$) mice were obtained from The Jackson Laboratory. CD118$^{-/-}$ mice (30) and RL/PKR$^{-/-}$ mice on a WT background (24, 31) are maintained at Dean A. McGee Eye Institute (Oklahoma City, OK). Mice at age 6–8 wks were anesthetized by i.p. injection with xylazine (2 mg/ml; 6.6 mg/kg) and ketamine (30 mg/ml; 100 mg/kg).

Cells

All cell culture reagents were obtained from Invitrogen Life Technologies. Vero (African green monkey kidney) cells were obtained from the American Type Culture Collection (ATCC) and cultured in RPMI 1640, supplemented with 10% FBS, 2% antibiotic/antimycotic, and 0.2% gentamicin. Vero cells were plated in 96-well flat-bottom plates (50,000 cells/well), and incubated at 37°C in an atmosphere of 5% CO_2 and 95% humidity.

HSV-1 infection

Corneas were scarified 40 times with a 25 5/8-gauge needle. Next, tear films were blotted before topical inoculation with 10,000 PFU HSV-1 (McKrae strain), prepared as described previously (32).

Ad vector transduction

We used two replication defective Ad vectors (ΔE1-ΔE3), constructed as previously described (33, 34). Ad vectors were propagated in E1-complementing E293 cells (ATCC) in DMEM, supplemented with 5% FBS, 2% antibiotic/antimycotic, and 0.2% gentamicin. Cells were maintained at 37°C, with 5% CO_2 and 95% humidity. Mouse corneas were scarified as before, and tear films were blotted preceding topical application of 1×10^5–1×10^7 transducing units (TU) control adenovirus vector without IFN transgene (Ad:Null) or an adenovirus expressing the murine IFN-γ transgene (Ad:IFN-γ).

Measurement of IFN-γ levels

At 48 h posttransduction, corneas and TG were dissected. Each tissue sample was homogenized with a tissue miser (Fisher Scientific) in 500 μl of T-PER tissue protein extraction reagent (Pierce), supplemented with a protease inhibitor mixture (Calbiochem). Tissue homogenates were clarified by centrifugation at 10,000 $\times$ *g* for 1–2 min. Levels of IFN-γ were determined by ELISA according to the manufacturer's instructions (Quantikine immunoassay; R&D Systems).

Determination of HSV-1 titer

HSV-1 viral titers were examined at time points postinfection (p.i.). Specifically at days 3–7 p.i., mice were euthanized, and corneas and TG were dissected under sterile conditions. Tissues were frozen in 500 μl of RPMI 1640 medium, thawed, and homogenized using a tissue miser (Fisher Scientific). Tissue homogenates were then centrifuged at 10,000 $\times$ *g* for 1–2 min. Clarified supernatants were serially diluted and incubated on Vero cell monolayers in 96-well microtiter plates for 60 min. Supernatants were subsequently discarded and replaced with a 100-μl overlay of RPMI 1640 containing 10% FBS, antibiotic/antimycotic, and 0.5% methylcellulose. Cultures were incubated at 37°C in 5% CO_2 and 95% humidity for an additional 31 h. Amount of infectious virus has been reported as mean log PFU per cornea or per TG.

Flow cytometry

Mice were anesthetized and perfused with 20 ml PBS (pH 7.4). TG were collected and homogenized in complete DMEM, using a Dounce-type homogenizer. Homogenates were subsequently forced through a cell strainer (BD Biosciences). Corneas were collected and digested with 1 mg/ml collagenase type I (Sigma-Aldrich) for 90 min at 37°C. Single-cell suspensions from cornea or TG were pelleted by centrifugation. Cells were incubated on ice for 15 min with 4 μl of anti-mouse CD16/32 (BD Pharmingen) in 46 μl of PBS-1% BSA. After the incubation, cells were centrifuged (300 $\times$ *g*, 5 min) and resuspended in PBS-1% BSA containing 5% normal rat serum (Jackson ImmunoResearch Laboratories) for an additional 15 min on ice. Cells were then triple-stained in the dark at 4°C for 30 min in 50 μl of PBS-1% BSA with BD Pharmingen Abs: 2 μl of FITC-conjugated anti-mouse CD3, 2 μl of PE-Cy5-conjugated anti-CD45 (clone 30-F11), and 2 μl of PE-labeled anti-mouse NK-1.1. Cells were then washed three times with PBS-1% BSA, pelleted each time by centrifugation (300 $\times$ *g*, 5 min), and resuspended in PBS containing 1% paraformaldehyde. After overnight fixation at 4°C, cells were pelleted and resuspended in PBS-1% BSA. Before analysis, CountBright absolute counting beads (Invitrogen Life Technologies) were added (28,000/sample). Cell suspensions were gated on CD45^{high}-expressing cells, and the percentage of T cells (CD3$^+$NK1.1$^-$) and NK cells (CD3$^-$NK1.1$^+$) were determined at this gate setting. A second gate was used to count the number of beads that passed through during the sampling time. Samples were analyzed for 550 s; the absolute number of leukocytes (CD45^{high}) in TG and cornea were determined by calculating: (the number of input beads)/(the number of beads counted per sample) $\times$ (the number of CD45^{high} events). The absolute number of T and NK cells was then calculated: (number of CD45^{high} cells) $\times$ (the percentage of each lymphocyte population per sample). Isotypic control Abs were used to establish background fluorescence levels.

Statistical analysis

Statistical analysis was performed using the GBSTAT program (Dynamic Microsystems). Specifically, Student's *t* test (two samples per separate variances) was used to determine significant differences between the Ad: Null and Ad:IFN-γ transduced groups.

Results

Ad:IFN-γ transduction before infection results in a dose-dependent reduction in infectious virus

Previously, 1×10^6 TU Ad:IFN-β was shown to be effective at enhancing the survival of HSV-1-infected mice (35). As a starting point to examine Ad:IFN-γ effectiveness, we also chose to use 1×10^6 TU Ad vector, as well as 1 log more and 1 log less than the effective dose seen in prior studies using Ad:IFN-β (35). With 1×10^5 TU Ad:IFN-γ, no IFN-γ was detected in cornea at 48 h posttransduction. By comparison, transduction of corneas with 1×10^6 or 1×10^7 TU Ad:IFN-γ resulted in detection of IFN-γ in 50% of corneas, ranging from 24 to 36 pg/cornea. We were unable to detect IFN-γ in Ad:Null-transduced corneas with the same dose.

Because IFN-γ levels were successfully measured in the cornea following in situ transduction, WT mouse corneas were next transduced with 1×10^5–1×10^7 TU Ad:IFN-γ and assessed for antiviral activity following inoculation of corneas with HSV-1. No differences in viral titers were found in mouse corneas or TG from animals transduced with 1×10^5 TU Ad:Null vs Ad:IFN-γ (Fig. 1). With 1×10^6 TU Ad vector, infectious virus levels were less in corneas and TG from Ad:IFN-γ-transduced mice, but the difference did not achieve significance (Fig. 1). With 1×10^7 TU, corneas and TG from mice transduced with Ad:IFN-γ possessed significantly less virus, compared with corneas and TG from Ad: Null-transduced mice (Fig. 1).

Downloaded from http://journals.aai.org/jimmunol/article-pdf/178/8/5166/1241991/zim00807005166.pdf by guest on 27 January 2023

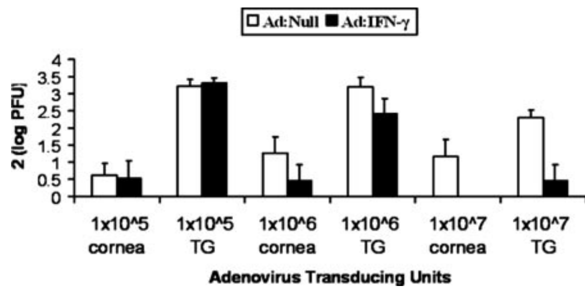

FIGURE 1. Dose-dependent effectiveness of Ad:IFN-γ transduction. WT mouse corneas were transduced with 1×10^5–1×10^7 TU Ad:Null or Ad:IFN-γ 24 h before infection with 10,000 PFU HSV-1. At day 7 p.i., the amount of infectious virus was analyzed in each cornea and TG by plaque assay. Data represent collective mean log PFU/tissue (cornea and TG tissue) from a representative experiment ($n = 4$ samples/group), which was repeated three times.

In the absence of IFN-γ receptor, antiviral action of Ad:IFN-γ transduction is lost

The IFN-γ receptor is composed of a ligand binding subunit, referred to as IFN-γ R1, IFN-γ Rα, or CDw119, and a second receptor subunit, referred to as IFN-γ R2, IFN-γ Rβ, or AF-1 (1). To verify the specificity of Ad:IFN-γ, mice deficient in IFN-γR1 ($CD119^{-/-}$) were transduced with 1×10^7 TU Ad:Null or Ad:IFN-γ 24 h before infection with HSV-1. In the first experiment, we attempted to analyze infectious virus levels at day 7 p.i., but $CD119^{-/-}$ mice died at day 6 p.i. In two subsequent experiments, infectious virus levels were analyzed in corneas and TG at day 5 p.i. Reduction in infectious virus was not found in corneas or TG from $CD119^{-/-}$ mice, transduced with Ad:IFN-γ vs Ad:Null (data not shown), suggesting that the Ad:IFN-γ-elicited antiviral effect is mediated through CD119.

The antiviral effect of Ad:IFN-γ over time

Mouse corneas were transduced with Ad:Null or Ad:IFN-γ 24 h before infection, and infectious virus levels were monitored at days 3, 5, and 7 p.i. in the cornea and TG. At day 3 p.i., there was no difference in viral titer in corneas from mice transduced with Ad:Null vs Ad:IFN-γ (Fig. 2). In cornea at days 5 and 7 p.i. and in TG at days 3–7 p.i., infectious virus levels were reduced 1.5–2.5 mean log PFU in tissues from Ad:IFN-γ-transduced mice, compared with Ad:Null control (Fig. 2). Notably, no virus was detected at day 5 p.i. in 10 of 10 corneas from mice transduced with 1×10^7 TU Ad:IFN-γ (Fig. 2).

Next, the prophylactic vs therapeutic application of Ad:IFN-γ was determined. Because, on occasion, Ad:Null-transduced WT mice succumbed to infection before day-7 p.i., tissue was harvested day 5 p.i. in all transduced groups of mice. Transduction of mouse corneas with Ad:IFN-γ 24 h prior (Fig. 2 and see Figs. 4–6) and 72 h before infection (Fig. 3) resulted in significant reduction of viral titers in TG and cornea, compared with virus levels

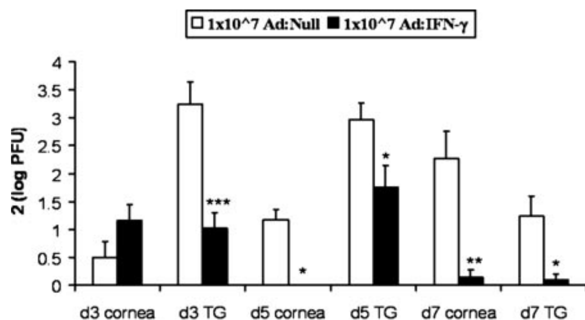

FIGURE 2. Time course analysis of Ad:IFN-γ efficacy. Following scarification, WT mouse corneas were transduced with Ad:Null or Ad:IFN-γ using 1×10^7 TU. Corneas were infected with 10,000 PFU HSV-1 24 h later. Viral titers were determined in corneas and TG by plaque assay at days 3, 5, and 7 p.i. Data represent collective mean log PFU/tissue (both cornea and TG tissue) from two to three separate experiments ($n = 9$–15 samples/time point and treatment). *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.001$.

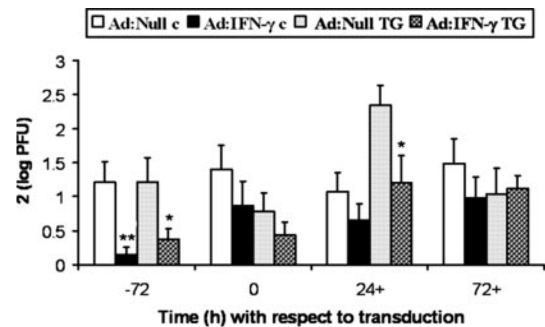

FIGURE 3. Kinetics of Ad:IFN-γ protection. Mouse corneas were scarified and transduced with 1×10^7 TU Ad:Null or Ad:IFN-γ 72 h prior (−72), at the same time (0), 24 h after (24+), or 72 h after (72+) infection with 10,000 PFU HSV-1. Infectious HSV-1 levels in the cornea (c) and TG were analyzed by plaque assays at day 5 p.i. Data represent collective mean log PFU/tissue (both cornea and TG tissues) from two separate experiments ($n = 10$–12 samples/time point and treatment). *, $p < 0.05$; **, $p < 0.005$.

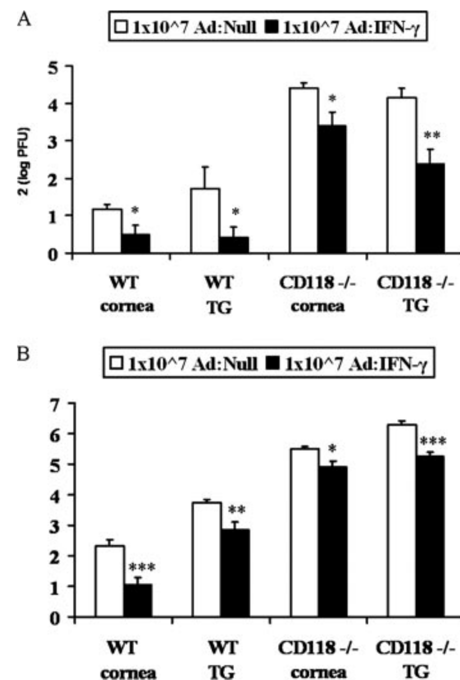

FIGURE 4. Efficacy of Ad:IFN-γ in $CD118^{-/-}$ compared with WT mice. WT and $CD118^{-/-}$ mouse corneas were scarified and transduced with 1×10^7 TU Ad:Null or Ad:IFN-γ 24 h before infection with 10,000 PFU HSV-1. Infectious virus levels were determined by plaque assay. *A*, Infectious virus levels at day 3 p.i. are reported as cumulative mean log PFU/tissue ± SEM (of both cornea and TG tissues) from two separate experiments ($n = 8$–10 samples/group). *B*, Infectious virus levels at day 5 p.i. are reported as cumulative mean log PFU/tissue (both cornea and TG tissues) ± SEM from three separate experiments ($n = 4$–12 samples/group). *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.001$.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/178/8/5166/1241991/zim00807005166.pdf by guest on 27 January 2023

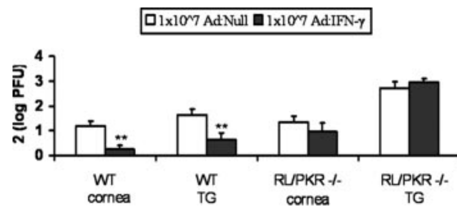

FIGURE 5. Ineffectiveness of Ad:IFN-γ transduction in RL/PKR$^{-/-}$ mice. WT and RL/PKR-deficient (RL/PKR$^{-/-}$) mouse corneas were transduced with 1×10^7 TU Ad:Null or Ad:IFN-γ 24 h before infection with 10,000 PFU HSV-1. At day 5 p.i., levels of infectious virus were determined by plaque assay for each cornea and TG. Data represent collective mean log PFU/tissue (for each cornea and TG) from two to three separate experiments ($n = 12$–16 samples/group). **, $p < 0.01$.

in these tissues from Ad:Null-transduced mice. When mice were transduced 24–72 h after infection, the antiviral effect was no longer observed in the cornea (Fig. 3). However, transduction of corneas 24 h, but not 72 h, after infection suppressed viral replication in TG (Fig. 3). Ironically, transduction of Ad:IFN-γ at the time of infection did not impact HSV-1 replication in cornea or TG (Fig. 3).

The antiviral effect of Ad:IFN-γ is not lost in the absence of CD118 (αβ IFNR)

To examine potential type I IFN involvement, the effectiveness of Ad:IFN-γ in mice devoid of one subunit of the type I IFNR (23, 30) was examined. In the absence of CD118, Ad:IFN-γ was capable of suppressing HSV-1 replication in both the cornea and TG at day 3 p.i. (Fig. 4*A*) or day 5 p.i. (Fig. 4*B*). It should be noted that 4 of 6 Ad:Null-transduced CD118$^{-/-}$ mice succumbed to infection at day 4 p.i., whereas none of the Ad:IFN-γ-transduced mice died by day 5 p.i. This observation reinforces the protective effect of Ad:IFN-γ even in the absence of a functional type I IFNR.

The antiviral effect of Ad:IFN-γ is lost in mice devoid of functional OAS and PKR pathways

PKR and OAS are inducible following exposure to IFN-γ, and both have proven antiviral activity (14). To determine whether these pathways are involved in the Ad:IFN-γ-inducible antiviral effect in vivo, viral titers were compared in WT and RL/PKR-deficient mice, transduced with Ad:Null vs Ad:IFN-γ before infection with HSV-1. Consistent with previous results, Ad:IFN-γ transduced WT mice were found to possess significantly less HSV-1 in cornea and TG (Fig. 5). In contrast, the effect was lost in RL/PKR-deficient mice (Fig. 5).

Ad:IFN-γ partially protects against HSV-1 infection in TG from mice lacking αβ TCR

Because activated CD4 and CD8 T cells are a rich source of IFN-γ, and both T cells and IFN-γ are found in the TG during acute ocular

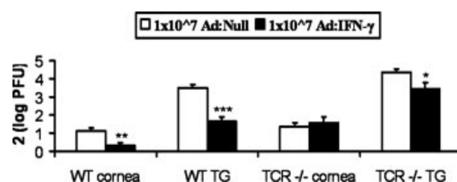

FIGURE 6. Ad:IFN-γ protection in TCR$^{-/-}$ mice. WT and TCR-deficient (TCR$^{-/-}$) mouse corneas were scarified before transduction with 1×10^7 TU Ad:Null or Ad:IFN-γ. At 24 h later, corneas were infected with 10,000 PFU HSV-1. Infectious virus levels were analyzed by plaque assay in cornea and TG at day 5 p.i. Data represent mean log PFU/tissue (for each cornea and TG) ± SEM from three to four experiments ($n = 13$–19 samples/group). *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.001$.

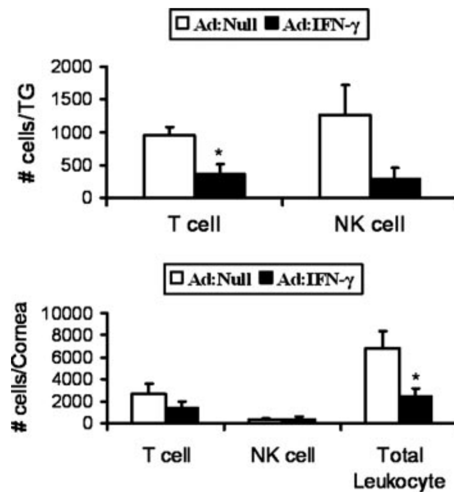

FIGURE 7. Recruitment of leukocytes to the cornea of Ad:Null-transduced and Ad:IFN-γ-transduced mice in response to HSV-1. WT mouse corneas were scarified and transduced with 1×10^7 TU Ad:Null or Ad:IFN-γ. Corneas were infected with 10,000 PFU HSV-1 24 h posttransduction. Mice were euthanized at day 5 p.i., and single-cell suspensions were prepared from the TG (*A*) and cornea (*B*) and labeled for the detection of the number (#) of T cells (NK1.1$^-$CD3$^+$), NK cells (NK1.1$^+$CD3$^-$), and total leukocytes (CD45^{high}) subsequently analyzed by flow cytometry. The results are shown as mean ± SEM from two to three separate experiments ($n = 3$–6 samples/group). *, $p < 0.05$.

HSV-1 infection (25, 27, 28), we questioned whether Ad:IFN-γ could substitute for T cells in protecting mice against HSV-1 infection. Therefore, the antiviral effect of Ad:IFN-γ was evaluated in αβ TCR$^{-/-}$ mice. The results show in mice lacking αβ TCR$^+$ T cells, the antiviral activity of Ad:IFN-γ was lost in the cornea and attenuated in the TG, compared with WT mice (Fig. 6).

T cell and NK cell recruitment to the TG of HSV-1-infected mice is diminished following Ad:IFN-γ transduction

Because Ad:IFN-γ antagonized HSV-1 replication, we reasoned that the reduced antigenic stimulus would equate to fewer leukocytes infiltrating the tissue. Therefore, leukocyte trafficking into TG and cornea was measured in mice transduced with Ad:IFN-γ. Within the TG, there were significantly fewer T cells in the Ad:IFN-γ-transduced mice compared with TG from Ad:Null-transduced mice (Fig. 7, *top*). Although not significant, a similar trend was found assessing NK cells with a reduction residing in the TG from Ad:IFN-γ-transduced mice compared with the Ad:Null-transduced animals (Fig. 7, *top*). The total leukocyte (CD45^{high}) population infiltrating the TG of Ad:IFN-γ and Ad:Null was not significantly different (data not shown). In contrast, the total leukocyte infiltration in the cornea of Ad:Null-transduced mice was significantly elevated in comparison to the cornea from Ad:IFN-γ-transduced animals (Fig. 7, *bottom*). Neither NK cell nor T cell populations residing in the cornea of mice transduced with Ad:Null or Ad:IFN-γ were found to be different (Fig. 7, *bottom*).

Discussion

HSV-1 infection of cornea triggers a potent inflammatory response that serves to eliminate virus. However, repeated inflammatory events lead to permanent corneal scarring and vision loss (36). Corneal replacement is not a viable solution because individuals with past incidence of herpetic keratitis are likely to have chronic recurrence (37, 38). Development of better therapeutic strategies to prevent vision loss from herpetic keratitis requires an understanding of protective endogenous antiviral systems. Type I IFN and

Downloaded from http://journals.aai.org/jimmunol/article-pdf/178/8/5166/1241991/zim00807005166.pdf by guest on 27 January 2023

downstream effector protection against HSV-1 morbidity and mortality is well documented (16–18, 23, 24, 35, 39–45). Type II IFNs are equally important for clearing virus from cornea and TG (46, 47), but knowledge of the mechanism used by IFN-γ to control HSV-1 is deficient. Previously, type I and type II IFN have been shown synergist control of HSV-1 (20–23, 48). Therefore, we sought to determine whether type I IFNs and downstream effectors, OAS and PKR, are required for IFN-γ inhibition of HSV-1. Because CD3$^+$ T and NK cells contribute to viral clearance (11, 14, 19, 25–27, 41, 46, 49–65), we further examined involvement of $\alpha\beta$ TCR and NK and CD3$^+$ T cells. A replication defective Ad vector to deliver the IFN-γ transgene was chosen for the current study instead of treatment with rIFN-γ because a continuous source of IFN-γ would be available. In addition, we reasoned this approach to be an improved model because host cells profoundly affect posttranslational processing, the three-dimensional structure of the glycoprotein, and its biological function (66–69).

In this study, transduction of mouse corneas with Ad:IFN-γ was effective at reducing HSV-1 replication before or within 24 h after infection. However, transduction at the time of infection did not significantly impact viral replication. This result suggests that either IFN-γ protein was made at lower levels during infection, or the protein made was less functional. One explanation is that the virus caused a shut down of host cell protein synthesis, so IFN-γ was made at lower levels (70). Alternatively, alterations in cornea cell biology during infection caused IFN-γ to be less functional either by altering posttranslational modifications or by a secondary effect on the conformation of the protein. Although there are many possible explanations for viral interference with protein function, the activity of IFN-γ is known to be dependent on interactions with heparan sulfate. Specifically, binding to heparan sulfate influences both the activity and availability of IFN-γ (71). HSV-1 also uses heparan sulfate as its primary receptor (72), so availability of heparan sulfate for IFN-γ may be reduced at the time of infection.

In the present study, we found the levels of infectious virus were elevated in corneas and TG from type I IFNR-deficient mice compared with WT mice. Yet, in the absence of CD118, the antiviral efficacy of the Ad:IFN-γ was not lost. It was anticipated the effectiveness of Ad:IFN-γ would not require antiviral pathways including OAS and PKR pathways implicated in the inhibition of HSV-1 (16, 17, 39, 42, 43). In contrast to previous observations in vitro (15), effectiveness of IFN-γ transgene was lost in vivo in the absence of intact OAS or PKR signaling. Local levels of OAS and PKR transcripts were only modestly elevated (2-fold by real-time RT-PCR) following Ad:IFN-γ transduction (data not shown). Cumulatively, the results suggest the effect of IFN-γ on OAS and PKR is likely systemic. One possible interpretation is that the IFN-γ transgenes reach distal locations and exert antiviral activity, mediated by OAS and PKR pathways, via organized immune tissue (18).

Along with activation of endogenous host antiviral pathways, such as OAS and PKR, HSV-1 infection of cornea triggers a potent inflammatory response. TLRs are activated, leading to production of CXC chemokines (73), also induced by IFN-γ, which act as chemoattractants for T and NK cells (72). Although IFN-γ indirectly activates chemokines that function as chemoattractants for leukocytes, it could be argued that with IFN-γ treatment, infectious virus levels and viral Ag levels are lower, and therefore, there will be less immune cell infiltration. In mice lacking $\alpha\beta$ TCR$^+$ T cells, the efficacy of Ad:IFN-γ against HSV-1 replication was completely lost in cornea and partially lost in TG. We interpret these results to suggest that in TG the presence of IFN-γ can partially override the requirement for T cells. In the TG, fewer T cells were found in mice transduced with Ad:IFN-γ. But in cornea, there was no difference in T cell infiltrates in the mice transduced with Ad:Null or Ad:IFN-γ. Whether T cells trafficking to corneas of Ad:IFN-γ- vs Ad:Null-transduced mice was functionally different was not analyzed. Although the total number of T cells was not significantly different, the phenotype of CD4$^+$ T cells could be different in Ad:IFN-γ-transduced mice. We reason this difference may be the case from previous reports examining the immunosuppressive environment of the anterior ocular chamber. Under homeostatic conditions, the anterior chamber of the eye has a specific deficiency of Ag-specific delayed-type hypersensitivity (DTH)-mediating T cells, mediated by Th1 cells, and a shift toward a Th2 phenotype of CD4$^+$ T cells (74). This situation would therefore be different from the normal immune environment in the TG, potentially explaining some of the differences observed in the effect of Ad:IFN-γ in cornea vs TG. Another factor that could contribute to differences in T cell phenotypes includes products of Th1 and Th2 cells cross-regulate each other (74–76). Specifically, IFN-γ, a cytokine of Th1 cells, inhibits Th2 proliferation (75). It is difficult to separate out the players in ocular immune suppression in our experimental model because most Ags introduced into the anterior chamber prime/induce suppression of DTH (such as albumin, which would be present in the medium used to prepare our adenovirus and HSV-1 stocks), but herpes simplex introduction into the anterior chamber of C57BL/6 mice induces positive DTH (77), and local expression of IFN-γ also abolishes intraocular immune privilege (78).

In conclusion, IFN-γ transgene delivery with an Ad vector potently reduced levels of infectious HSV-1 in cornea and TG during primary infection. The effectiveness of transgene delivery was dose-dependent and required IFN-γ receptor, intact antiviral pathways (OAS and PKR) and the presence of $\alpha\beta$ TCR$^+$ T cells. A reduction in the total leukocyte infiltrate into the cornea of Ad: IFN-γ-transduced mice following HSV-1 was also discovered. Excluding the T and NK cell population, the predominant infiltrating population of leukocytes includes polymorphonuclear neutrophils and macrophages known to contribute in the inflammatory response through the production of soluble mediators including TNF-α and matrix metalloproteins that often lead to undesirable collateral damage (79). Therefore, the contribution of the Ad: IFN-γ transduction of corneal tissue may not only reside in a reduction in virus replication but also a reduction in the trafficking of polymorphonuclear neutrophils and macrophages that would significantly contribute to impairment of the visual axis.

Acknowledgments

We acknowledge the technical assistance provided by Gabrielle Nguyen, Manoj Thapa, and Todd Wuest.

Disclosures

The authors have no financial conflict of interest.

References

1. Bach, E. A., M. Aguet, and R. D. Schreiber. 1997. The IFNγ receptor: a paradigm for cytokine receptor signaling. *Annu. Rev. Immunol.* 15: 563–591.
2. Farrar, M. A., and R. D. Schreiber. 1993. The molecular cell biology of interferon-γ and its receptor. *Annu. Rev. Immunol.* 11: 571–611.
3. Iordanov, M. S., J. M. Paranjape, A. Zhou, J. Wong, B. R. Williams, E. F. Meurs, R. H. Silverman, and B. E. Magun. 2000. Activation of p38 mitogen-activated protein kinase and c-Jun NH_2-terminal kinase by double-stranded RNA and encephalomyocarditis virus: involvement of RNase L, protein kinase R, and alternative pathways. *Mol. Cell Biol.* 20: 617–627.
4. Lee, S. B., R. Bablanian, and M. Esteban. 1996. Regulated expression of the interferon-induced protein kinase p68 (PKR) by vaccinia virus recombinants inhibits the replication of vesicular stomatitis virus but not that of poliovirus. *J. Interferon Cytokine Res.* 16: 1073–1078.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/178/8/5166/1241991/zim00807005166.pdf by guest on 27 January 2023

5. Khabar, K. S., M. Dhalla, Y. Siddiqui, A. Zhou, M. N. Al-Ahdal, S. D. Der, R. H. Silverman, and B. R. Williams. 2000. Effect of deficiency of the double-stranded RNA-dependent protein kinase, PKR, on antiviral resistance in the presence or absence of ribonuclease L: HSV-1 replication is particularly sensitive to deficiency of the major IFN-mediated enzymes. *J. Interferon Cytokine Res.* 20: 653–659.
6. Theerasurakarn, S., and S. Ubol. 1998. Apoptosis induction in brain during the fixed strain of rabies virus infection correlates with onset and severity of illness. *J. Neurovirol.* 4: 407–414.
7. Díaz-Guerra, M., C. Rivas, and M. Esteban. 1997. Inducible expression of the 2–5A synthetase/RNase L system results in inhibition of vaccinia virus replication. *Virology* 227: 220–228.
8. Patterson, C. E., D. M. Lawrence, L. A. Echols, and G. F. Rall. 2002. Immune-mediated protection from measles virus-induced central nervous system disease is noncytolytic and γ interferon dependent. *J. Virol.* 76: 4497–4506.
9. Pavlovic, J., H. A. Arzet, H. P. Hefti, M. Frese, D. Rost, B. Ernst, E. Kolb, P. Staeheli, and O. Haller. 1995. Enhanced virus resistance of transgenic mice expressing the human MxA protein. *J. Virol.* 69: 4506–4510.
10. Staeheli, P., and J. Pavlovic. 1991. Inhibition of vesicular stomatitis virus mRNA synthesis by human MxA protein. *J. Virol.* 65: 4498–4501.
11. Komatsu, T., Z. Bi, and C. S. Reiss. 1996. Interferon-γ induced type I nitric oxide synthase activity inhibits viral replication in neurons. *J. Neuroimmunol.* 68: 101–108.
12. Lowenstein, C. J., S. L. Hill, A. Lafond-Walker, J. Wu, G. Allen, M. Landavere, N. R. Rose, and A. Herskowitz. 1996. Nitric oxide inhibits viral replication in murine myocarditis. *J. Clin. Invest.* 97: 1837–1843.
13. Detels, R., J. A. Brody, J. McNew, and A. H. Edgar. 1973. Further epidemiological studies of subacute sclerosing panencephalitis. *Lancet* 2: 11–14.
14. Chesler, D. A., and C. S. Reiss. 2002. The role of IFN-γ in immune responses to viral infections of the central nervous system. *Cytokine Growth Factor Rev.* 13: 441–454.
15. Austin, B. A., W. Halford, R. H. Silverman, B. R. Williams, and D. J. Carr. 2006. OAS and PKR are not required for the antiviral effect of Ad:IFN-γ against acute HSV-1 in primary trigeminal ganglia cultures. *J. Interferon Cytokine Res.* 26: 220–225.
16. Al-Khatib, K., B. R. Williams, R. H. Silverman, W. Halford, and D. J. Carr. 2004. Distinctive roles for 2′,5′-oligoadenylate synthetases and double-stranded RNA-dependent protein kinase R in the in vivo antiviral effect of an adenoviral vector expressing murine IFN-β. *J. Immunol.* 172: 5638–5647.
17. Al-khatib, K., B. R. Williams, R. H. Silverman, W. Halford, and D. J. Carr. 2003. The murine double-stranded RNA-dependent protein kinase PKR and the murine 2′,5′-oligoadenylate synthetase-dependent RNase L are required for IFN-β-mediated resistance against herpes simplex virus type 1 in primary trigeminal ganglion culture. *Virology* 313: 126–135.
18. Noisakran, S., and D. J. Carr. 2001. Topical application of the cornea post-infection with plasmid DNA encoding interferon-α1 but not recombinant interferon-αA reduces herpes simplex virus type 1-induced mortality in mice. *J. Neuroimmunol.* 121: 49–58.
19. Vollstedt, S., S. Arnold, C. Schwerdel, M. Franchini, G. Alber, J. P. Di Santo, M. Ackermann, and M. Suter. 2004. Interplay between $\alpha\beta$ and γ interferons with B, T, and natural killer cells in the defense against herpes simplex virus type 1. *J. Virol.* 78: 3846–3850.
20. Pierce, A. T., J. DeSalvo, T. P. Foster, A. Kosinski, S. K. Weller, and W. P. Halford. 2005. β Interferon and γ interferon synergize to block viral DNA and virion synthesis in herpes simplex virus-infected cells. *J. Gen. Virol.* 86: 2421–2432.
21. Halford, W. P., K. J. Halford, and A. T. Pierce. 2005. Mathematical analysis demonstrates that interferons-β and -γ interact in a multiplicative manner to disrupt herpes simplex virus replication. *J. Theor. Biol.* 234: 439–454.
22. Neumann-Haefelin, D., R. Sundmacher, H. Frey, and W. Merk. 1985. Recombinant HuIFN-γ prevents herpes simplex keratitis in African green monkeys: demonstration of synergism with recombinant HuIFN-α2. *Med. Microbiol. Immunol.* 174: 81–86.
23. Muller, U., U. Steinhoff, L. F. Reis, S. Hemmi, J. Pavlovic, R. M. Zinkernagel, and M. Aguet. 1994. Functional role of type I and type II interferons in antiviral defense. *Science* 264: 1918–1921.
24. Zhou, A., J. Paranjape, T. L. Brown, H. Nie, S. Naik, B. Dong, A. Chang, B. Trapp, R. Fairchild, C. Colmenares, and R. H. Silverman. 1997. Interferon action and apoptosis are defective in mice devoid of 2′,5′-oligoadenylate-dependent RNase L. *EMBO J.* 16: 6355–6363.
25. Shimeld, C., J. L. Whiteland, S. M. Nicholls, E. Grinfeld, D. L. Easty, H. Gao, and T. J. Hill. 1995. Immune cell infiltration and persistence in the mouse trigeminal ganglion after infection of the cornea with herpes simplex virus type 1. *J. Neuroimmunol.* 61: 7–16.
26. Manickan, E., and B. T. Rouse. 1995. Roles of different T-cell subsets in control of herpes simplex virus infection determined by using T-cell-deficient mouse-models. *J. Virol.* 69: 8178–8179.
27. Cantin, E. M., D. R. Hinton, J. Chen, and H. Openshaw. 1995. γ Interferon expression during acute and latent nervous system infection by herpes simplex virus type 1. *J. Virol.* 69: 4898–4905.
28. Shimeld, C., J. L. Whiteland, N. A. Williams, D. L. Easty, and T. J. Hill. 1997. Cytokine production in the nervous system of mice during acute and latent infection with herpes simplex virus type 1. *J. Gen. Virol.* 78: 3317–3325.
29. Feduchi, E., M. A. Alonso, and L. Carrasco. 1989. Human γ interferon and tumor necrosis factor exert a synergistic blockade on the replication of herpes simplex virus. *J. Virol.* 63: 1354–1359.
30. Garvey, T. L., K. D. Dyer, J. A. Ellis, C. A. Bonville, B. Foster, C. Prussin, A. J. Easton, J. B. Domachowske, and H. F. Rosenberg. 2005. Inflammatory responses to pneumovirus infection in IFN-$\alpha\beta$ R gene-deleted mice. *J. Immunol.* 175: 4735–4744.
31. Yang, Y. L., L. F. Reis, J. Pavlovic, A. Aguzzi, R. Schafer, A. Kumar, B. R. Williams, M. Aguet, and C. Weissmann. 1995. Deficient signaling in mice devoid of double-stranded RNA-dependent protein kinase. *EMBO J.* 14: 6095–6106.
32. Halford, W. P., B. M. Gebhardt, and D. J. Carr. 1996. Mechanisms of herpes simplex virus type 1 reactivation. *J. Virol.* 70: 5051–5060.
33. Kolls, J., K. Peppel, M. Silva, and B. Beutler. 1994. Prolonged and effective blockade of tumor necrosis factor activity through adenovirus-mediated gene transfer. *Proc. Natl. Acad. Sci. USA* 91: 215–219.
34. Lei, D., J. R. Lancaster, Jr., M. S. Joshi, S. Nelson, D. Stoltz, G. J. Bagby, G. Odom, J. E. Shellito, and J. K. Kolls. 1997. Activation of alveolar macrophages and lung host defenses using transfer of the interferon-γ gene. *Am. J. Physiol.* 272: L852–L859.
35. Al-Khatib, K., B. R. Williams, R. H. Silverman, W. Halford, and D. J. Carr. 2005. Dichotomy between survival and lytic gene expression in RNase L- and PKR-deficient mice transduced with an adenoviral vector expressing murine IFN-β following ocular HSV-1 infection. *Exp. Eye Res.* 80: 167–173.
36. Whitcher, J. P., M. Srinivasan, and M. P. Upadhyay. 2001. Corneal blindness: a global perspective. *Bull. World Health Organ.* 79: 214–221.
37. Mitchell, W. J., P. Gressens, J. R. Martin, and R. DeSanto. 1994. Herpes simplex virus type 1 DNA persistence, progressive disease and transgenic immediate early gene promoter activity in chronic corneal infections in mice. *J. Gen. Virol.* 75: 1201–1210.
38. Nesburn, A. B. 1975. Recurrence in ocular herpes simplex infection. *Int. Ophthalmol. Clin.* 15: 101–110.
39. Carr, D. J., K. Al-khatib, C. M. James, and R. Silverman. 2003. Interferon-β suppresses herpes simplex virus type 1 replication in trigeminal ganglion cells through an RNase L-dependent pathway. *J. Neuroimmunol.* 141: 40–46.
40. Samuel, C. E. 2001. Antiviral actions of interferons. *Clin. Microbiol. Rev.* 14: 778–809.
41. Sen, G. C. 2001. Viruses and interferons. *Annu. Rev. Microbiol.* 55: 255–281.
42. Al-Khatib, K., B. R. Williams, R. H. Silverman, W. P. Halford, and D. J. Carr. 2002. Absence of PKR attenuates the anti-HSV-1 activity of an adenoviral vector expressing murine IFN-β. *J. Interferon Cytokine Res.* 22: 861–871.
43. Austin, B. A., C. James, R. H. Silverman, and D. J. Carr. 2005. Critical role for the oligoadenylate synthetase/RNase L pathway in response to IFN-β during acute ocular herpes simplex virus type 1 infection. *J. Immunol.* 175: 1100–1106.
44. Carr, D. J., L. A. Veress, S. Noisakran, and I. L. Campbell. 1998. Astrocyte-targeted expression of IFN-α1 protects mice from acute ocular herpes simplex virus type 1 infection. *J. Immunol.* 161: 4859–4865.
45. Kimura, T., Y. Murata, T. Yamaguchi, and K. Okumura. 1989. Immunopathological analysis of T and non-T cell involvement in corneal inflammation caused by HSV type-1 infection. *Nippon Ganka Gakkai Zasshi* 93: 124–131.
46. Bouley, D. M., S. Kanangat, W. Wire, and B. T. Rouse. 1995. Characterization of herpes simplex virus type-1 infection and herpetic stromal keratitis development in IFN-γ knockout mice. *J. Immunol.* 155: 3964–3971.
47. He, J., H. Ichimura, T. Iida, M. Minami, K. Kobayashi, M. Kita, C. Sotozono, Y. I. Tagawa, Y. Iwakura, and J. Imanishi. 1999. Kinetics of cytokine production in the cornea and trigeminal ganglion of C57BL/6 mice after corneal HSV-1 infection. *J. Interferon Cytokine Res.* 19: 609–615.
48. Sainz, B., Jr., and W. P. Halford. 2002. $\alpha\beta$ Interferon and γ interferon synergize to inhibit the replication of herpes simplex virus type 1. *J. Virol.* 76: 11541–11550.
49. Heiligenhaus, A., D. Bauer, M. Zheng, S. Mrzyk, and K. P. Steuhl. 1999. $CD4^+$ T-cell type 1 and type 2 cytokines in the HSV-1 infected cornea. *Graefes Arch. Clin. Exp. Ophthalmol.* 237: 399–406.
50. Geiger, K. D., T. C. Nash, S. Sawyer, T. Krahl, G. Patstone, J. C. Reed, S. Krajewski, D. Dalton, M. J. Buchmeier, and N. Sarvetnick. 1997. Interferon-γ protects against herpes simplex virus type 1-mediated neuronal death. *Virology* 238: 189–197.
51. Banerjee, K., P. S. Biswas, U. Kumaraguru, S. P. Schoenberger, and B. T. Rouse. 2004. Protective and pathological roles of virus-specific and bystander $CD8^+$ T cells in herpetic stromal keratitis. *J. Immunol.* 173: 7575–7583.
52. Banerjee, K., P. S. Biswas, and B. T. Rouse. 2005. Elucidating the protective and pathologic T cell species in the virus-induced corneal immunoinflammatory condition herpetic stromal keratitis. *J. Leukocyte Biol.* 77: 24–32.
53. Banerjee, K., S. Deshpande, M. Zheng, U. Kumaraguru, S. P. Schoenberger, and B. T. Rouse. 2002. Herpetic stromal keratitis in the absence of viral antigen recognition. *Cell. Immunol.* 219: 108–118.
54. Hendricks, R. L., T. M. Tumpey, and A. Finnegan. 1992. IFN-γ and IL-2 are protective in the skin but pathologic in the corneas of HSV-1-infected mice. *J. Immunol.* 149: 3023–3028.
55. Brandt, C. R., and C. A. Salkowski. 1992. Activation of NK cells in mice following corneal infection with herpes simplex virus type-1. *Invest. Ophthalmol. Vis. Sci.* 33: 113–120.
56. Cantin, E., B. Tanamachi, and H. Openshaw. 1999. Role for γ interferon in control of herpes simplex virus type 1 reactivation. *J. Virol.* 73: 3418–3423.
57. Carr, D. J. 1998. Increased levels of IFN-γ in the trigeminal ganglion correlate with protection against HSV-1-induced encephalitis following subcutaneous administration with androstenediol. *J. Neuroimmunol.* 89: 160–167.
58. Costa-Pereira, A. P., T. M. Williams, B. Strobl, D. Watling, J. Briscoe, and I. M. Kerr. 2002. The antiviral response to γ interferon. *J. Virol.* 76: 9060–9068.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/178/8/5166/1241991/zim00807005166.pdf by guest on 27 January 2023

59. Decman, V., P. R. Kinchington, S. A. Harvey, and R. L. Hendricks. 2005. γ Interferon can block herpes simplex virus type 1 reactivation from latency, even in the presence of late gene expression. *J. Virol.* 79: 10339–10347.
60. Glenn, J. A., J. B. Sonceau, H. J. Wynder, and W. E. Thomas. 1993. Histochemical evidence for microglia-like macrophages in the rat trigeminal ganglion. *J. Anat.* 183: 475–481.
61. Liu, T., K. M. Khanna, X. Chen, D. J. Fink, and R. L. Hendricks. 2000. $CD8^+$ T cells can block herpes simplex virus type 1 (HSV-1) reactivation from latency in sensory neurons. *J. Exp. Med.* 191: 1459–1466.
62. Liu, T., Q. Tang, and R. L. Hendricks. 1996. Inflammatory infiltration of the trigeminal ganglion after herpes simplex virus type 1 corneal infection. *J. Virol.* 70: 264–271.
63. Xia, L. K., J. S. Zhang, X. L. Chen, and A. Y. Wang. 2005. Cytokine expression in murine cornea tissue during recurrent herpetic stromal keratitis. *Zhonghua Yan Ke Za Zhi* 41: 403–408.
64. Seo, S. K., B. M. Gebhardt, H. Y. Lim, S. W. Kang, S. Higaki, E. D. Varnell, J. M. Hill, H. E. Kaufman, and B. S. Kwon. 2001. Murine keratocytes function as antigen-presenting cells. *Eur. J. Immunol.* 31: 3318–3328.
65. Hendricks, R. L. 1997. An immunologist's view of herpes simplex keratitis: Thygeson Lecture 1996, presented at the Ocular Microbiology and Immunology Group meeting, October 26, 1996. *Cornea* 16: 503–506.
66. James, D. C., R. B. Freedman, M. Hoare, O. W. Ogonah, B. C. Rooney, O. A. Larionov, V. N. Dobrovolsky, O. V. Lagutin, and N. Jenkins. 1995. *N*-glycosylation of recombinant human interferon-γ produced in different animal expression systems. *Biotechnology* 13: 592–596.
67. James, D. C., M. H. Goldman, M. Hoare, N. Jenkins, R. W. Oliver, B. N. Green, and R. B. Freedman. 1996. Posttranslational processing of recombinant human interferon-γ in animal expression systems. *Protein Sci.* 5: 331–340.
68. Hooker, A. D., N. H. Green, A. J. Baines, A. T. Bull, N. Jenkins, P. G. Strange, and D. C. James. 1999. Constraints on the transport and glycosylation of recombinant IFN-γ in Chinese hamster ovary and insect cells. *Biotechnol. Bioeng.* 63: 559–572.
69. Cencic, A., F. LeFevre, S. Koren, and C. La Bonnardiere. 1999. Tetracycline-controlled expression of glycosylated porcine interferon-γ in mammalian cells. *Anim. Biotechnol.* 10: 63–79.
70. Laurent, A. M., J. J. Madjar, and A. Greco. 1998. Translational control of viral and host protein synthesis during the course of herpes simplex virus type 1 infection: evidence that initiation of translation is the limiting step. *J. Gen. Virol.* 79: 2765–2775.
71. Lortat-Jacob, H., P. Esterre, and J. A. Grimaud. 1994. Interferon-γ, an anti-fibrogenic cytokine which binds to heparan sulfate. *Pathol. Res. Pract.* 190: 920–922.
72. WuDunn, D., and P. G. Spear. 1989. Initial interaction of herpes simplex virus with cells is binding to heparan sulfate. *J. Virol.* 63: 52–58.
73. Wuest, T., B. A. Austin, S. Uematsu, M. Thapa, S. Akira, and D. J. Carr. 2006. Intact TRL 9 and type I interferon signaling pathways are required to augment HSV-1 induced corneal CXCL9 and CXCL10. *J. Neuroimmunol.* 179: 46–52.
74. Li, X. Y., L. T. D'Orazio, and J. Y. Niederkorn. 1996. Role of Th1 and Th2 cells in anterior chamber-associated immune deviation. *Immunology* 89: 34–40.
75. Garside, P., and A. M. Mowat. 1995. Polarization of Th-cell responses: a phylogenetic consequence of nonspecific immune defence. *Immunol. Today* 16: 220–223.
76. Mosmann, T. R., and K. W. Moore. 1991. The role of IL-10 in crossregulation of TH1 and TH2 responses. *Immunol. Today* 12: A49–A53.
77. Kielty, D., S. W. Cousins, and S. S. Atherton. 1987. HSV-1 retinitis and delayed hypersensitivity in DBA/2 and C57BL/6 mice. *Invest. Ophthalmol. Vis. Sci.* 28: 1994–1999.
78. Geiger, K., and N. Sarvetnick. 1994. Local production of IFN-γ abrogates the intraocular immune privilege in transgenic mice and prevents the induction of ACAID. *J. Immunol.* 153: 5239–5246.
79. Thomas, J., S. Gangappa, S. Kanangat, and B. T. Rouse. 1997. On the essential involvement of neutrophils in the immunopathological disease: herpetic stromal keratitis. *J. Immunol.* 158: 1383–1391.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/178/8/5166/1241991/zim00807005166.pdf by guest on 27 January 2023