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Short communication

Protective effects of interferon-gamma in intraocular herpes simplex type 1 infection do not depend on major histocompatibility complex class I or class II expression

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Intraocular infection with herpes simplex virus type I strain F (HSV-1) induces bilateral retinitis, the expression of both MHC class I and II molecules and activation of CD4 and CD8 cells. To investigate the role of MHC upregulation in IFN-γ mediated antiviral effects in intraocular infection with HSV-1, we infected MHC deficient mice and mice with an additional ectopic site of IFN-γ production in their retina (rhoγ) intravitreally with HSV-1 into one eye. Protective effects of IFN-γ in intraocular HSV-1 infection were notable as sparing of the contralateral non-inoculated eye from retinitis, and were not dependent on MHC class I and class II expression, thus limiting the importance of MHC expression for the outcome of viral infection *in vivo*.

Keywords: HSV-1; intraocular infection; MHC class I; MHC class II; intracerebral infection, IFN-γ

The development of pathology in viral infection of the CNS is a summary of viral toxicity and host immune response, involving cytotoxic T cells, presentation of viral antigens on MHC molecules, and cytokine effects, which are not yet fully understood. This situation is complicated by the fact that the CNS is regarded as an immunoprivileged site, a property that is shared by the brain (Sloan *et al*, 1991) and the eyes (Streilein *et al*, 1992), rendering the CNS vulnerable to viral infection, with the eyes as a possible efficient route of entrance to the brain.

We have previously demonstrated that the cytokine IFN-γ provided protection for transgenic mice infected with HSV-1 using a model of intravitreal infection in mice that expressed IFN-γ ectopically in the photoreceptors of the retina (Geiger *et al*, 1994b). This protection consisted of sparing of the second non-inoculated eye, and was associated with upregulation of MHC class I and class II expression, and with increased numbers of CD4 and CD8 cells in the eyes of protected animals (Geiger *et al*, 1994b). Yet, effects of IFN-γ in this system did not appear to be mediated by a suppression of viral replication. We became interested in determining whether the upregulation of MHC class I or II molecules within the eye was responsible for the observed protection from intraocular viral infection. To address this question, we performed infection with HSV-1 (Atherton and Streilein, 1987; Pepose and Whittum-Hudson, 1987; Streilein *et al*, 1987). We compared the outcome of intravitreal inoculation of HSV-1 strain F (Roizman *et al*, 1972), on wild-type, MHC class I-deficient mice, MHC class II deficient mice and on mice which had ectopic expression of IFN-γ in the photoreceptors of the retina (rhoγ) (Geiger *et al*, 1994a) in addition to their MHC deficiency and SCID mice lacking mature T and B cells. In mice of all groups (Table 1) infection with HSV-1 F spread from the uvea and retina of the inoculated eye into the brain via the optic nerve and the trigeminal nerve, similar to earlier observations (Vann and Atherton, 1991). SCID mice lacking mature B and T cells developed lethargy, rough coat, ataxia and paralysis, and died from encephalitis by d7–9. The mice of all other groups survived infection with only minor symptoms such as inflammation of the eyelids and rough coat. Encephalitis was not observed. PBS injected controls remained free from morphological changes and viral antigen. In infected mice, destruction of the ipsilateral retina, cellular infiltration and positive

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Table 1 Outcome of intravitreal infection with HSV-1 strain F

Mice[a]					*Pathology*			*Statistics*	
SCID	*Retinal IFN-γ (rhoγ)*	*MHC class I*	*MHC class II*	*Group size*	*Death (number of animals)*	*Localization of viral antigen (number of animals[b])*	*Sparing second eye (number of animals)*	*Significance retinal sparing[c]*	*c2 (P)*
+	-	+	+	10	10	Both eyes, brain	2	-	–
-	-	+	+	9	0	Both eyes, brain	0	-	–
-	+	+	+	11	0	Right eye, brain	11	+	< 0.001
-	-	-	+	9	0	Both eyes, brain, 6	3	-	0.1 > 0.05
-	+	-	+	11	0	Right eyes, brain, 8	3	+	< 0.05
-	-	+	-	9	0	Right eye, brain	9	+_	< 0.001
-	+	+	-	10	0	Right eye, brain	10	+	< 0.001

[a] Mice, distribution of the used phenotypes
[b] Numbers of animals were given if condition did not apply to all animals of the group
[c] Significance using χ^2 test in comparison to normal infected mice

staining for viral antigen appeared almost simultaneously in the injected eyes of all mice by d4–5 (Figure 1A–G) and in the brains by d5–6. Although there was no significant difference in the amount of pathology or the time course of disease between the studied groups of surviving animals, the involvement of the second non-inoculated eye represented a distinguishing factor. As demonstrated previously (Geiger *et al*, 1994b), rhoγ mice enjoyed protection of the contralateral, non-treated eye, while normal control mice of the same background had bilateral retinitis. All C57BL/6 mice and 80% of the SCID mice developed bilateral retinitis (Table 1). Of the MHC class I deficient mice, 67% developed bilateral retinitis, as established by morphology and immunostaining for viral antigen. However, when MHC class I deficiency was combined with retinal expression of IFN-γ, the percentage of mice with bilateral retinitis dropped to 27% (Table 1). MHC class II deficient mice of the same background also enjoyed protection of the untreated eye, independent of additional expression of the rhoγ transgene. Among the groups tested, tissue destruction appeared slightly more extensive in the eyes of MHC class I deficient animals without additional expression of IFn-γ, (not shown) as compared to the other groups (Table 1). Statistical evaluation of the outcome of infection, using comparison to normal control mice by χ2 test, found the results highly significant for MHC class II deficient mice but only a tendency towards improvement in MHC class I deficient mice. When MHC class I deficiency was combined with the rhoγ transgene, improvement became statistically significant in comparison to normal mice.

Inflammatory cells in the eyes and brain of these mice consisted of natural killer cells, macrophages, and lymphocytes containing subsets of CD4 and CD8 cells, as determined by immunostaining with the antibodies to NK, Mac-1, F4/80, LY2 and L3T4. The overall amount of infiltrating cells was lower in SCID mice and normal control mice as compared to all other groups. However, the distribution of the different cell types tested varied between the groups. As expected, SCID mice had neither CD4 nor CD8 cells. MHC class I deficient mice had no CD8 cells, whereas MHC class II deficient mice had no CD4 cells in either the eyes or brains. However, in control sections from spleens of MHC class II deficient animals, atypically located CD4 cells were observed within B cell areas as described previously (Cosgrove *et al*, 1991; Markovitz *et al*, 1993). MHC class I deficient mice also had single CD8 cells in their spleens (Zijlstra *et al*, 1989). In class II deficient mice higher numbers of CD8 cells were found in the eyes and brains. This compensatory increase in CD8 cells is consistent with previous reports (Cosgrove *et al*, 1991). MHC class I deficient mice and normal control mice appeared to have slightly more NK cells than the other groups.

Our results confirm that the more virulent HSV-1 F has characteristics distinguishing this strain from the more commonly used KOS strain (Whittum-Hudson and Pepose, 1987), by inducing bilateral retinitis in control mice; similar to infection patterns described for a glycoprotein C deficient HSV-1 strain (Liu *et al*, 1993). Furthermore C57BL/6 mice were sensitive to infection. Destruction of the retina in both eyes appeared to be independent of T cell function, since it was observed in SCID mice as well. SCID mice had less cellular infiltration of their eyes than did normal controls, relating the observed retinal destruction to lytic capacity of the virus we used, rather than to host immune mechanisms.

Protective effects of IFN-γ expression were not mediated by upregulation of MHC class I or class II, since MHC deficient rhoγ mice enjoyed protection similar to their MHC expressing litter mates. The better outcome of intraocular infection in MHC class II deficient mice as compared to MHC class I deficient animals could indicate an actually harmful effect of infiltrating CD4 cells as suggested earlier (Alters *et al*, 1990; Goldman and Abramowicz,

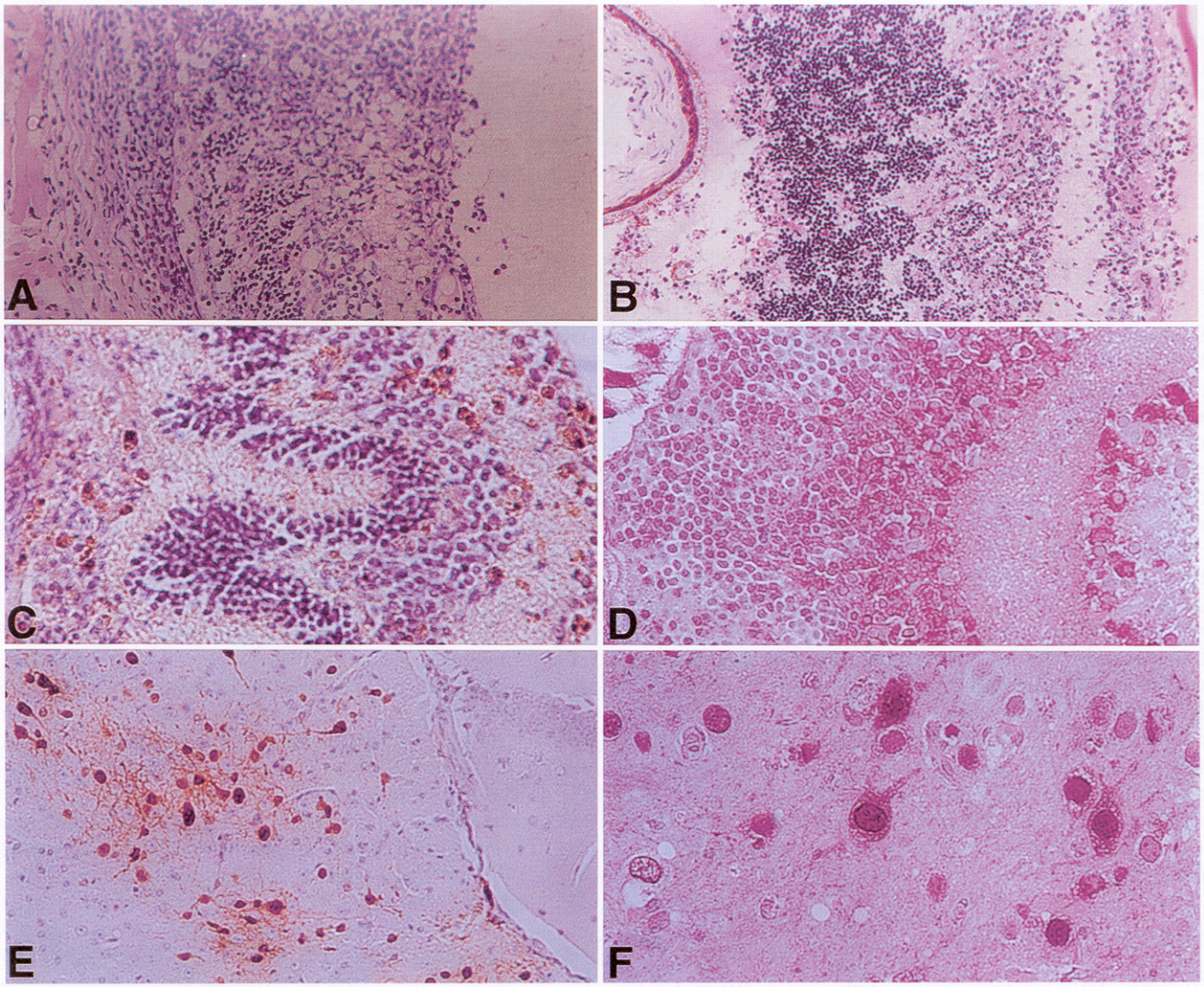

Figure 1 Pathology of the eyes after injection of HSV-1. (A) Detail of the retina in a C57Bl/6 mouse at d6 after intravitreal inoculation with cellular infiltration, consisting mainly of small lymphocytes and macrophages in all layers of the retina and the choroid. Destruction of the retinal architecture with loss of the photoreceptors and destruction of the ganglion cells, paraffin-embedded tissue, PAS magnification orig. × 20. (B) IAß null mouse at d7 afger intravitreal inoculation with similar retinal destruction as the mouse in A, apart from better preservation of choroid and retinal pigment epithelium, paraffin-embedded tissue, PAS, magnification orig. × 40. (C) Retina of a rhoγ mouse at d6 after intravitreal inoculation with brown staining for viral antigen present in the choroid and all layers of the retina, paraffin-embedded tissue, H&E, immunostaining with DAB as a chromogen, magnification orig. × 40. (D) Retina of a SCID mouse at d6 after intravitreal inoculation with purple staining for viral antigen. Note the destruction of the ganglion cell layer and the folding of the photoreceptor layer with beginning cellular loss, paraffin-embedded tissue, H&E immunostaining with VIP as a chromogen, magnification orig. × 40. (E) Brain of rhoγ mouse at d6 after intravitreal inoculation. Detail of an area adjacent to the lateral geniculate with brown staining for viral antigen, paraffin-embedded tissue, H&E, immunostaining with DAB as a chromogen, magnification orig. × 20. (F) Similar area in the brain of a SCID mouse at d6 after intravitreal inoculation with dark staining for viral antigen, paraffin-embedded tissue, H&E, immunostaining with VIP as a chromogen, magnification orig. × 40.

1994; Hutchings *et al*, 1993), or a protective effect of CD8 cells (Hendricks and Tumpey, 1990; Niemialtowski *et al*, 1992). However, experiments using intraocular infection of CD4-or CD8-depleted mice with HSV-1 found no differences in the outcome of the disease, nor protective effects in CD4 depleted mice (Igietseme *et al*, 1991, Azumi and Atherton, 1994; Johnson *et al*, 1992; Streilein *et al*, 1987) which rather implies that the effect is mediated directly by loss of MHC expression. Interestingly, depletion of CD8 or CD4 cells did not prevent the development of contralateral retinitis (Azumi and Atherton, 1994) which has been associated with the immunosuppressive properties of the intraocular microenviroment (Atherton and Streilein, 1987; Streilein *et al*, 1987; Whittum-Hudson and Pepose, 1987). Rhoγ mice and animals deficient in MHC class I are known to lack intraocular immune privi-

lege, possibly accounting for part of our results, whereas this information is not yet available concerning the role of MHC class II expression. However, other factors, such as availability of preferred target cells for the virus, may have a role as well. Apart from MHC (Neely *et al*, 1985) restricted killing, macrophage activation by IFN-γ, which may render these cells more resistant to HSV-infection (Lucchiari and Pereira, 1990) could have a role in the observed protection.

The roles of MHC expression, CD4 and CD8 cells in intraocular HSV infections have been subject to several recent studies (Hendricks and Tumpey, 1990; 1991; Jayaraman *et al*, 1993; Johnson *et al*, 1992; Niemialtowski and Rouse, 1992; Vasilakos and Michael, 1993; Yasukawa *et al*, 1991). We have demonstrated earlier that the cytokine IFN-γ does not block the replication of HSV-1 in a substantial way, yet the cytokine affords protection in intraocular infection with HSV-1 (Geiger *et al*, 1994b) correlated with the upregulation of MHC class I and class II within the eye. The cytokine IFN-γ is also capable of abrogating the intraocular immune privilege (Geiger and Sarvetnick, 1994). In the present study we have demonstrated that these protective effects of intraocular IFN-γ expression are not dependent on the availability of MHC molecules in the eye. This study has narrowed the possible mechanisms of IFN-γ mediated protection from intraocular HSV-infection. Apart from possible direct effects on macrophages, IFN-γ may exert antiviral activity by influencing characteristics of the virus itself, thus limiting viral spread, or by ensuring survival of infected cells.

Methods

We used CB17/SCID mice, C57BL/6 mice, C57 BL/6-derived β_2m null mice, which do not express a functional MHC class I molecule (Zijstra *et al*, 1989) (kindly provided by R Jaenisch), and IAβ null mice, which are deficient in MHC class II expression (Cosgrove *et al*, 1991; Grusby *et al*, 1991) (kindly provided by L Glimcher). These mice were crossed once with BALB/c derived rhoγ mice with ectopic expression of IFn-γ in the retina (Geiger *et al*, 1994a) and then backcrossed to mice of the C57BL/6 background for at least three generations to provide a consistent genetic background. Age-matched groups of 5- to 8-week-old mice (4th generation) with MHC class I or class II deficiency, and mice in which these traits were combined with the rhoγ transgene, were infected with HSV-1. All experiments were performed at least twice, unless stated otherwise. PBS-injected mice served as controls. All procedures adhered to the NIH guidelines.

MHC deficient alleles were screened by PCR of tail DNA. In the case of MHC class II deficient mice, the primer sequences CTCTGCAGATCCCCAGCCTCCTC and DCTGTTCCAGTACTCGGCGTCTG were used with 35 cycles of 30 seconds (s) denaturation time at 94°C, annealing for 45 s at 66°C and polymerisation for 1 min at 72°C. For typing of MHC class I deficient mice, the primers were CTTCAGCAAGGACTGGTCTTTC and GCAGGCGTATGTATCAGTCTC, run at 35 cycles with 30 s denaturation at 94°, 45 s annealing at 60°C and 45 s polymerization at 72°C. Primers were chosen to flank the inserted neomycin resistance coding regions (knock out areas) which then could be identified by long amplified fragments, whereas the wild type genes were characterized by short fragments. MHC deficiency was additionally tested by immunocytochemistry with antibodies against MHC class I and II and with antibodies against CD4 (L3T4) and CD8 (LY2) cells as described below. The presence of the SCID mutation was established by morphology of the spleen and by a two antibody sandwich ELISA for IgG using an indirect immunoperoxidase system.

For intravitreal injection, 1 µl (2×10^5 pfu) HSV-1 strain F (Roizman *et al*, 1972) was injected into the vitreous of each animal's right eye using a 10 µl Hamilton syringe with replaceable needles (33 gauge). Animals were monitored daily for signs of disease such as rough coat, local inflammation, lethargy, ataxia, paralysis and seizures. The mice were sacrificed at different time points between day (d) 4 and 12 after inoculation of the virus or when displaying symptoms of severe encephalitis including paralysis and seizures.

We examined the consequences of viral infection by histology and immunohistochemistry (Geiger *et al*, 1994b) on both eyes and the brains of test mice. Sections of tissues either fixed in formalin and embedded in paraffin or snap frozen in OCT compound were stained with hematoxylin/eosin (H&E) or periodic acid-Schiff (PAS) and studied for virus induced pathology, including disruption of morphology and inflammatory changes. For immunocytochemistry the indirect avidin-biotin-peroxidase complex method (Vector Laboratories) was used. Primary antibodies were applied at a concentration of 5 µg ml^{-1}, and consisted of polyclonal anti-HSV-1 (Dako, Serotec), monoclonal anti-H-2, LY-2, L3T4, NK (Pharmigen, San Diego, CA), monoclonal F4/80 (Serotec), and monoclonal Mac-1, IA (Boehringer Mannheim, Indianapolis, IN). DAB (0.05%) with 0.04% nickel sulfate and 0.02% hydrogen peroxide, or the Vector VIP kit served as chromogens. The sections were counterstained in hematoxylin or in methyl green before dehydration and mounting.

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References

Alters SE, Sakai K, Steinman L, Oi VT (1990). Mechanisms of anti-CD4-mediated depletion and immunotherapy. A study using a set of chimeric anti-CD4 antibodies. *J Immunol* **144**: 4587–4592.

Atherton SS, Streilein JW (1987). Virus-specific DTH prevents contralateral retinitis following intracameral inoculation of HSV-2. *Curr Eye Res* **6**: 133–139.

Azumi A, Atherton SW (1994). Sparing of the ipsilateral retina after anterior chamber inoculation of HSV-1: Requirement for either CD4+ or CD8+ T cells. *Invest Ophthalmol Vis Sci* **35**: 3251–3259.

Cosgrove D, Gray D, Dierich A, Kaufman J, Lemeur M, Benoist C, Mathis D (1991). Mice lacking MHC class II molecules. *Cell* **66**: 1051–1066.

Geiger K, Howes E, Gallina M, Huang XJ, Travis GH Sarvetnick N (1994a). Transgenic mice expressing IFN-γ in the retina develop inflammation and photoreceptor loss. *Invest Ophthalmol Vis Sci* **35**: 2667–2681.

Geiger K, Howes EL, Sarvetnick N (1994b). Ectopic expression of IFN-γ in the eye protects transgenic mice from intraocular HSV-1 infections. *J Virol* **69**: 5556–5567.

Geiger K, Sarvetnick N (1994). Local prodiction of IFN-γ abrogates the intraocular immune privilege in transgenic mice and prevents the induction of ACAID. *J Immunol* **153**: 5239–5246.

Goldman M, Abramowicz D (1994). Allogenic reaction, a model of autoimmunity. *Rev Prat* **44**: 53–56.

Grusby MJ, Johnson RS, Papaionnou VE, Glimcher LH (1991). Depletion of CD4+ T cells in major histocompatibility complex class II-deficient mice. *Science* **253**: 1417–1420.

Hara Y, Okamoto S, Rouse B, Streilein JW (1993). Evidence that peritoneal exudate cells cultured with eye-derived fluids are the proximate antigen presenting cells in immune deviation of the ocular type. *J Immunol* **151**: 5162–5171.

Hendricks RL, Tumpey TM (1990). Contribution of virus and immune factors to herpes simplex virus type I-induced corneal pathology. *Invest Opthalmol Vis Sci* **31**: 1929–1939.

Hendricks Rl, Tumpey TM (1991). Concurrent regeneration of T Lymphocytes and susceptibility to HSV-1 corneal stromal disease. *Curr Eye Res* **10** (suppl): 47–53.

Hutchings P, Parish N, O'Reilly L, Dawe K, Roitt IM, Cooke A (1993). The regulation of autoimmunity through CD4+ T cells. *Autoimmunity* **15** (suppl): 21–23.

Igietseme JU, Streilein W, Miranda F, Feinerman SJA, Atherton SS (1991). Mechanisms of protection against herpes simplex virus type 1-induced retinal necrosis by in vitro-activated T lymohocytes. *J Virol* **65**: 763–768.

Jayaraman S, Heiligenhaus A, Rodriguez A, Soukiasian S, Dorf ME, Foster CS (1993). Exacerbation of murine herpes simples virus-mediated stromal keratitis by Th2 type T cells. *J Immunol* **151**: 5777–5789.

Johnson RM, Lancki DW, Sperling AI, Dick RF, Spear PG, Fitch FW, Bluestone JA (1992). A murine CD4-, CD8- T cell receptor-γδ T lymphocyte clone specific for herpes simplex virus glycoprotein I. *J Immunol* **148**: 983–988.

Liu Y, Sakai Y, Minagawa H, Toh Y, Ishibashi T, Inomata H, Mori R (1993). Iduction of bilateral retinal necrosis in mice by unilateral intracameral inoculation of a glycoprotein-c deficient clinical isolate of herpes simplex virus type 1. *Arch Virol* **129**: 105–118.

Lucchiari MA, Pereira CA (1990). A major role of macrophage activation by interferon-gamma during mouse hepatitis virus type 3 infection. II Age-dependent resistance. *Immunobiology* **181**: 31–39.

Markowitz JS, Auchincloss HJ, Grusby MJ, Glimcher LH (1993). Class II- positive hematopoietic cells cannot mediate positive selection of CD4+ T lymphocyytes in class II-defiecient mice. *Proceedings of the National Academy of Science USA* **90**: 2779–2783.

Neeley SP, Cross AJ, Crow TJ, Johnson JA, Taylor GR (1985). Herpes simplex virus encephalitis. Neuroanatomical and neurochemical selectivity. *J Neurol Sci* **71**: 325–337.

Niemialtowski MG, Rouse BT (1992). Phenotypic and functional studies on ocular T cels during herpetic infections of the eye. *J Immunol* **148**: 1864–1870.

Pepose JS, Whittum-Hudson JA (1987). An immunogenetic analysis of resistance to herpes simplex retinitis in inbred strains of mice. *Invest Ophthalmol Vis Sci* **28**: 1549–1522.

Roizman B, Spear PG, Kieff ED (1972). Herpes simplex viruses I and II: a biochemical definition. *Perspect Virol* **8**: 129–169.

Sloan DJ, Wood MJ, Charlton HM (1991). The immune response to intracerebral neural grafts. *Trends Neurosci* **14**: 341–346.

Streilein JW, Atherton SS, Vann V (1987). A critical role for ACAID in the distinctive pattern of retinitis that follows anterior chamber inoculation of HSV 1. *Curr Eye Res* **6**: 127–131.

Streilein JW, Wilbanks GA, Cousins SW (1992). Immunoregulatory mechanisms in the eye. *J Neuroimmunol* **39**: 185–200.

Vann VR, Atherton SS (1991), Neural spread of herpes simplex virus after anterior chamber inoculation. *Invest Ophthalmol Vis Sci* **32**: 2462–2472.

Vasilakos JP, Michael JG (1993). Herpes simplex virus class I-restricted peptide induces cytotoxic T lymphocytes in vivo independent of CD4+ cells. *J Immunol* **150**: 2346–2355.

Whittum-Hudson JA, Pepose JS (1987). Immunologic modulation of virus-induced pathology in a murine model of acute herpetic retinal necrosis. *Invest Ophthalmol Vis Sci*, **28**: 1541–1548.

Yasukawa M, Inatsuki A, Horiuchi T, Kobayshi Y (1991). Functional heterogeneity among herpes simplex virus-specific human CD4+ T cells. *J Immunol*, **146**: 1341–1347.

Zijlstra M, Li E, Saijadi F, Subramani S, Jaenisch R (1989). Germ-line transmission of a disrupted $ß_2$-microglobulin gene produced by homologous recombination. *Nature* **342**: 435–439.