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Transgenic expression of interleukin-6 in the central nervous system confers protection against acute herpes simplex virus type-1 infection

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IL-6 is a pro-inflammatory cytokine that has previously been associated with herpes simplex virus type 1 reactivation. To further investigate this relationship during acute infection, ocular HSV-1 infection was studied in transgenic mice homozygous or heterozygous expression of IL-6 by astrocytes in the central nervous system. The virus load in both the eye and trigeminal ganglia was significantly reduced at day 6 but not day 3 post infection in the homozygous IL-6 transgenic mice compared to the wild type and heterozygous littermates. IL-6 protein and mRNA levels in the eye coincided with the level of transgene expression in mice acutely infected with virus (i.e., day 3 post infection). Likewise, IL-6 transcript levels in the TG mirrored the expression of the transgene in the mice throughout the course of the infection into latency. The HSV-1 α lytic phase gene ICP27 was rapidly down-regulated by day 6 post infection in the TG of homozygous IL-6 transgenic mice compared to the wild type and heterozygous littermates. The resistance to acute HSV-1 infection in the homozygous IL-6 transgenic mice correlated with a significant elevation in IFN-α/β in the eye compared to the wild type or heterozygous IL-6 transgenic animals. Heterozygous and homozygous IL-6 transgenic mice latently infected with HSV-1 showed elevated anti-HSV-1 antibody titers compared to the latently infected wild type controls. Collectively, the results suggest dose-dependent IL-6 antagonism of acute HSV-1 infection *in vivo*.

Keywords: Neuroimmunology; HSV-1; IL-6; transgenic mice

Introduction

Ocular HSV-1 infection rapidly induces or up-regulates the expression of cytokines and chemokines in the eye and trigeminal ganglion (TG) of mice (Bouley *et al*, 1995; Cantin *et al*, 1995; Halford *et al*, 1996; Su *et al*, 1996). Included within the molecules expressed are the pro-inflammatory cytokines TNF-α, IL-1, IL-6, and IL-12 (Staats and Lausch, 1993; Kanangat *et al*, 1996a; Liu *et al*, 1996). The production of the pro-inflammatory cytokines directs the initial immune response by promoting the expression of adhesion molecules, stimulating the production of chemokines which recruit leukocyte populations including neutrophils and T lymphocytes, and inducing the acute-phase response. Relative to HSV-1, IL-12 has been found to protect mice from HSV-1-mediated death (Carr *et al*, 1997) probably through the induction of IFN-γ (Ozmen *et al*, 1995). Alternatively, the rapid expression of the pro-inflammatory cytokines may, in fact, facilitate the pathogenesis associated with HSV-1 not only by the inflammatory nature of these molecules but also by augmenting the replication of the virus. Specifically, HSV-1 reactivation and replication is enhanced in the presence of TNF-α (Walev *et al*, 1995), and the virus utilizes a TNF/nerve growth factor receptor protein member to enter into cells (Montgomery *et al*, 1996). Likewise, in a dose-dependent fashion anti-IL-6 antibody but not control antibody has been found to significantly reduce the frequency of reactivation of HSV-1 following ultraviolet light exposure in latent mice (Kriesel *et al*, 1997a). Recently, hyperthermic stress of latently infected mice which induces HSV-1

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reactivation has been found to cause a transient rise in TG IL-6 mRNA and protein but not other cytokines including IL-1 and TNF-α (Noisakran *et al*, 1998). Blocking the stress-mediated rise in TG IL-6 mRNA antagonized HSV-1 reactivation. HSV-1 has also been found to induce the selective up-regulation of IL-6 in the HSV-1 permissive murine epithelial-like cell line EMT-6 (Kanangat *et al*, 1996b). However, the relationship between IL-6 and HSV-1 has not been firmly identified. One group suggests that the causal relationship between IL-6 and genes that prevent apoptotic death of cells including MyD116 and growth arrest and DNA damage gene 34 may protect neurons from undergoing apoptosis and provide an environment conducive to HSV-1 (Kanangat *et al*, 1996b). Consistent with this idea is the finding that up-regulated expression of IL-6 accelerates nerve regeneration following trauma (Hirota *et al*, 1996). While IL-6 may directly or indirectly facilitate HSV-1 replication (Kriesel *et al*, 1997b), IL-6 may act in concert with other cytokines in blocking HSV-1 replication during an acute infection. Specifically, the addition of neutralizing IL-6 antibody to IL-6 generating, astrocyte-enriched cultures has been found to augment (twofold) HSV-1 replication in these cultures (Baker, Carr and Gebhardt, unpublished observation).

The present study was undertaken to further elucidate the relationship between IL-6 and HSV-1 infection using a transgenic mouse (termed GFAP-IL6) model in which expression of IL-6 is targeted in astrocytes under the transcriptional control of the glial fibrillary acidic protein (GFAP) promoter (Campbell *et al*, 1993). The constitutive expression of the transgene within the central nervous system (CNS) causes progressive neuropathological changes. Homozygous GFAP-IL6 mice (G169) develop more moderate neurologic disease manifested by neurodegeneration, astrocytosis, angiogenesis, learning impairment, and induction of acute-phase proteins while the GFAP-IL6 heterozygous mice have similar but less neuropathology over time (Campbell *et al*, 1993; Heyser *et al*, 1997). However, whether the expression of IL-6 in the CNS would modify the immune response to acute HSV-1 infection or viral replication and routing in the peripheral nervous system was unknown. Consequently, these animals would be useful in serving as a model to begin to address the nature of the relationship between IL-6 and HSV-1 replication and pathogenesis following ocular inoculation.

Results

GFAP-IL6 mice have a reduced viral load in the TG 6 days post infection

The premise of this study was based on the hypothesis that IL-6 facilitates HSV-1 replication either through promoting gene transcription through the activation of transcription binding proteins (e.g., NF-IL-6 or STATs) or neural differentiation. Transgenic mice expressing IL-6 constitutively in the CNS were evaluated for HSV-1 infection following ocular inoculation. All mice inoculated with HSV-1 had detectable virus in the eye 3 days post infection (p.i.). Likewise, all mice screened ($n = 6-9$/group) showed similar clearance rates with the complete absence of virus in the eye by day 9 p.i. While the viral loads in the TG and eyes were not significantly different comparing all groups of mice 3 days p.i., heterozygous (IL-6 tg/+) and homozygous (IL-6 tg/tg) transgenic mice showed significantly reduced viral loads in the TG 6 days p.i. compared to the wild type (IL-6+/+) controls (Figure 1). In addition, there was a significant reduction in the amount of infectious virus in the eye of the IL-6 tg/tg mice compared to the IL-6 tg/+ and wild type mice (Figure 1). Whereas 100% (12/12) of the wild type and IL-6 tg/+ mice had detectable virus in the eye and TG 6 days p.i., only 25% (2/8) of the TG samples and 50% (4/8) of the eye samples from the IL-6 tg/tg mice possessed detectable infectious virus. Furthermore, 17% of

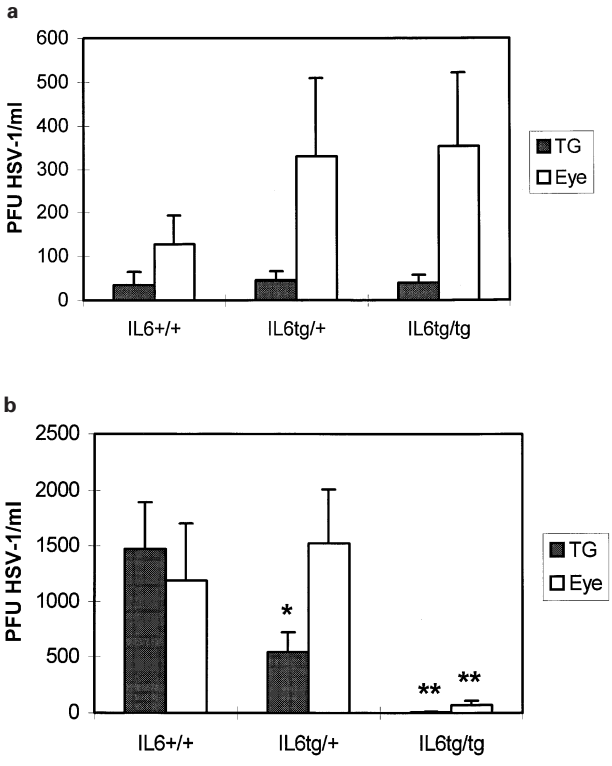

Figure 1 Homozygous (GFAP-IL6 tg/tg) mice antagonize HSV-1 replication in the eye and trigeminal ganglion. IL-6 homozygous transgenic (IL-6 tg/tg), IL-6 heterozygous transgenic (IL-6 tg/+) and wild type (IL-6+/+) mice (n=8–12/group) were infected with HSV-1 (210 p.f.u./eye) and sacrificed 3 (**a**) or 6 (**b**) days p.i. The eyes and TG were recovered, homogenized, and surveyed for infectious virus by plaque assay using CV-1 indicator cells. Bars represent s.e.m. $^{**}P<0.01$, $^{*}P<0.05$.

the wild type mice had detectable virus in the cerebellum compared with 0% of the HSV-1-infected GFAP-IL6 mice measured 6 days p.i.

Expression of HSV-1 ICP27 day 6 post infection is down-regulated in the TG of homozygous GFAP-IL6 mice

The α regulatory protein ICP27 which is associated with the lytic phase of HSV-1 infection peaks early during viral replication. In addition, infiltrating lymphocytes and cytokines and chemokines have been characterized in acute HSV-1 infection as well (Halford *et al*, 1996). Therefore, the expression of ICP27 mRNA was used as a marker to assess viral replication in the transgenic and wild type mice during the acute infection. In addition, the expression of cytokine, chemokine, and CD8 transcript levels were assessed to determine if the constitutive expression of IL-6 in the transgenic mice influenced the immune response in the eye and TG to HSV-1 infection. There were no differences in the expression of ICP27 mRNA in the eye of transgenic or wild type mice 3 days p.i (Table 1). Likewise, there were no differences in the expression of chemokines (including cytokine response gene [CRG]-2, monocyte chemoattractant protein [MCP]-1, macrophage inhibitory protein [MIP]-1β) or CD8 mRNA in the eye of transgenic or non-transgenic mice with the exception of RANTES and IL-6 (Table 1). IL-6 transcript expression was highest in the IL-6 tg/tg mice followed by the IL-6 tg/+ mice with the wild type mice expressing very little mRNA as determined by RT–PCR. Conversely, RANTES mRNA expression was lower in the eye of the IL-6 tg/tg mice compared to the IL-6 tg/+ or wild type mice. Similar to the eye, IL-6 mRNA expression was significantly elevated in the TG obtained from transgenic mice 3 days p.i. (Table 1). Quantitative PCR measuring IL-6 transcript levels in the eye and TG confirmed the RT-PCR results (Figure 2). Likewise, IL-6 protein levels measured in the eye 3 days p.i. showed a significant increase in the homozygotic IL-6 transgenic mice consistent with the

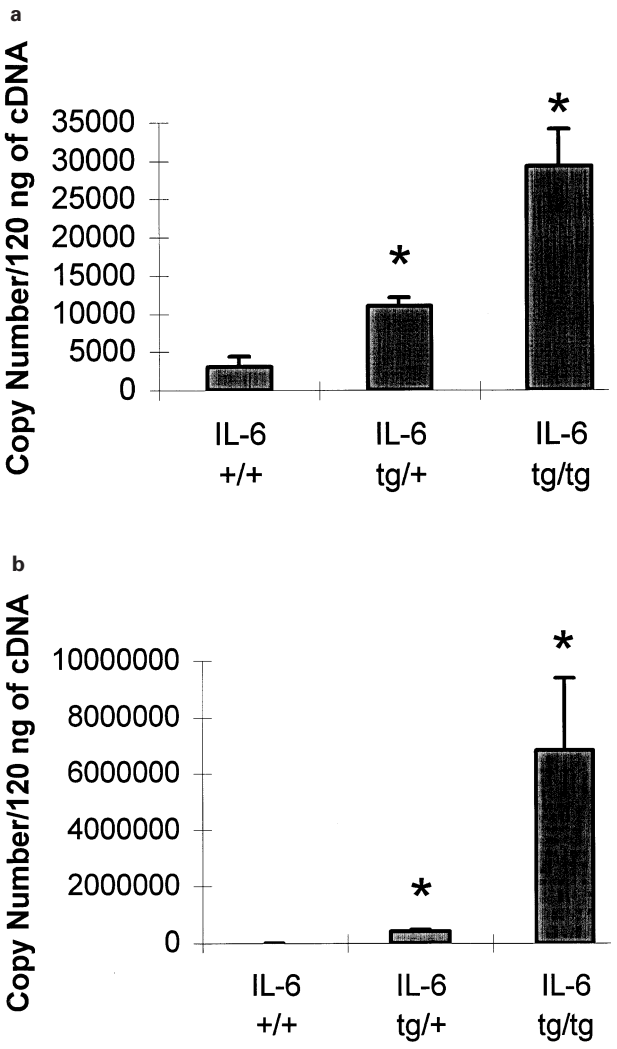

Figure 2 IL-6 mRNA levels in the trigeminal ganglion are elevated in the homozygous IL-6 transgenic mice. The eyes (**a**) and trigeminal ganglia (**b**) of HSV-1-infected mice were collected and homogenized 3 days post infection. RNA was extracted from the homogenized tissue and quantitated for IL-6 mRNA using a q-PCR protocol. Bars represent mean $\pm$ s.e.m. $n = 3$/group. $^*P < 05$ comparing the transgenic to wild type mice.

Table 1 IL-6 homozygous transgenic mice express elevated levels of ICP27 mRNA in the trigeminal ganglion 3 days post infection with HSV-1†

	Eye			*Trigeminal ganglion*		
Transcript	*IL-6+/+*	*IL-6 tg/+*	*IL-6 tg/tg*	*IL-6 +/+*	*IL-6 tg/+*	*IL-6 tg/tg*
ICP27	0.19 $\pm$ 0.06	0.28 $\pm$ 0.07	0.36 $\pm$ 0.16	0.06 $\pm$ 0.06	0.03 $\pm$ 0.02	0.33 $\pm$ 0.13*
IL6	0.19 $\pm$ 0.13	0.67 $\pm$ 0.11*	1.19 $\pm$ 0.07**	0.05 $\pm$ 0.04	1.32 $\pm$ 0.08**	1.48 $\pm$ 0.25**
RANTES	0.70 $\pm$ 0.08	0.80 $\pm$ 0.15	0.20 $\pm$ 0.03*	0.33 $\pm$ 0.09	0.39 $\pm$ 0.19	0.15 $\pm$ 0.09

†This table summarizes the results of 2–4 experiments/group of mice ($n = 3$ mice/group). IL-6 homozygous transgenic (IL-6 tg/tg), IL-6 heterozygous transgenic (IL-6 tg/+), and wild type (IL-6+/+) mice were sacrificed 3 days following ocular infection with 210 p.f.u./eye HSV-1 (McKrae strain). The eyes and TG were collected and RNA processed and analyzed by RT–PCR. Numbers represent the RT–PCR product yield expressed in terms of the integrated volume of pixels associated with each ethidium bromide-stained band. The results are summarized and expressed as a ratio of the target gene of interest and the housekeeping gene, GADPH. Numbers are displayed as the mean $\pm$ s.e.m. $^*P < 0.05$, $^{**}P < 0.01$ comparing the transgenic to wild type groups for the designated transcript as determined by ANOVA and Tukey's *post-hoc t*-test.

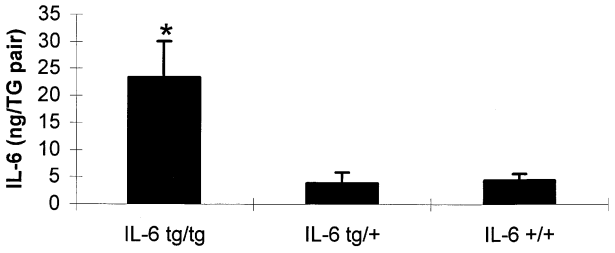

Figure 3 IL-6 homozygotic mice express high levels of IL-6 in the eye following HSV-1 infection. IL-6 homozygotic (IL-6 tg/tg), heterozygotic (IL-6 tg/+), and wild type (IL-6 +/+) mice were infected with 210 p.f.u./eye HSV-1 (McKrae strain) and sacrificed 3 days p.i. The eyes were removed and homogenized in RPMI-1640. Clarified homogenates were assayed for IL-6 by ELISA. Bars represent the mean/TG pair ± s.e.m. (n=4/group). *$P<0.05$ comparing the IL-6 tg/tg mice to the other groups of animals.

mRNA levels (Figure 3). No other transcripts measured were found to be modified between the transgenic and wild type animals 3 days p.i. in the TG with the exception of ICP27. HSV-1 ICP27 mRNA levels were significantly elevated in the IL-6 tg/tg mice compared to the heterozygous IL-6 tg/+ or wild type animals (Table 1). By comparison, the HSV-1 late (γ) gene transcript viral protein (VP) 16 was not readily detectable in the TG of any group of mice assessed but was detected in the eyes of 7/7 wild type, 5/5 heterozygous IL-6 transgenic, and 2/4 homozygous IL-6 transgenic mice 3 days p.i.

Consistent with IL-6 expression in the TG 3 days p.i., IL-6 mRNA levels were elevated in the TG of HSV-1 infected transgenic mice in comparison to HSV-1 infected wild type controls 6 days p.i. (Table 2). However, TG ICP27 mRNA levels were significantly reduced in the IL-6 tg/tg animals completely opposite as to what was found 3 days p.i. In addition, VP16 which was present in the TG of wild type mice (4/5 mice) 6 days p.i. was not detectable in the IL-6 tg/tg animals (0/5 mice). Likewise, there was no detectable IFN-γ mRNA in the TG of IL-6 tg/tg mice 6 days p.i. No other transcripts (RANTES, CD8, and IL-10) measured within the TG were found to be different between the three groups of HSV-1 infected transgenic and wild type mice.

The expression of viral and immune transcripts were measured in the TG of latent HSV-1 infected transgenic and wild type mice as well. All mice surveyed during latency expressed LAT (Table 3). Similar to the early time points, IL-6 expression coincided with the constitutive expression of the IL-6 transgene comparing the homozygous and heterozygous GFAP-IL6 mice to the wild type controls. However, no other transcripts (IFN-α, CD3, CD8, and RANTES) measured were found to be significantly different comparing the groups of latently infected animals to one another.

Table 2 IL-6 homozygous (IL-6 tg/tg) transgenic mice antagonize HSV-1 replication in the trigeminal ganglion 6 days post infection as evidenced by reduced ICP27 expression†

Transcript	*IL-6+/+*	*IL-6 tg/+*	*IL-6 tg/tg*
ICP27	0.62 ± 0.21	0.81 ± 0.15	0.02 ± 0.01*
IFN-γ	0.22 ± 0.13	0.04 ± 0.02	0*
IL-6	0.3 ± 0.07	0.93 ± 0.09*	1.54 ± 0.11**

†IL-6 homozygous transgenic (IL-6 tg/tg), IL-6 heterozygous transgenic (IL-6 tg/+), and wild type (IL-6 +/+) mice were sacrificed 6 days following ocular infection with 210 p.f.u./eye HSV-1 (McKrae strain). The TG were collected and RNA processed and analyzed by RT–PCR. Numbers represent the RT–PCR product yield expressed in terms of the integrated volume of pixels associated with each ethidium bromide-stained band. The results are summarized and expressed as a ratio of the target gene of interest and the housekeeping gene, GAPDH. This table is a summary of 2–3 experiments (n=7–9 mice/group). *$P<0.05$, **$P<0.01$ comparing the transgenic to wild type mice.

Table 3 Homozygous IL-6 transgenics (IL-6 tg/tg) express elevated IL-6 mRNA levels in the trigeminal ganglion of HSV-1 latent mice†

Transcript	*IL-6 +/+*	*IL-6 tg/+*	*IL-6 tg/tg*
LAT	0.25 ± 0.06	0.24 ± 0.04	0.12 ± 0.05
IL-6	0.14 ± 0.05	0.31 ± 0.12	0.58 ± 0.09*

†IL-6 homozygous transgenic (IL-6 tg/tg), IL-6 heterozygous transgenic (IL-6 tg/+), and wild type (IL-6 +/+) mice were sacrificed 30 days following ocular infection with 210 p.f.u./eye HSV-1 (McKrae strain). The TG were collected and RNA processed and analyzed by RT–PCR. Numbers represent the RT–PCR product yield expressed in terms of the integrated volume of pixels associated with each ethidium bromide-stained band. The results are summarized and expressed as a ratio of the target gene of interest and the housekeeping gene, GADPH. This table is a summary of 2–4 experiments (n=5–12 mice/group). *$P<0.05$ comparing the IL-6 tg/tg mice to the wild type animals.

Uninfected IL-6 transgenic and wild type mice were assessed for the expression of transcripts in the TG as well. Transcripts were either not detected or were rarely detected with the exception of IL-6, RANTES, and CD8 (Table 4). IL-6 mRNA expression coincided with transgene levels whereas RANTES and CD8 mRNA was not significantly elevated in the IL-6 transgenic mice (Table 4).

Homozygous GFAP-IL6 transgenic mice produce elevated levels of IFN-α/β in the eye following ocular HSV-1 infection

Type 1 IFNs (i.e., IFN-α and IFN-β) have previously been shown to play a central role in controlling HSV-1 replication during the course of the acute infection (Hendricks *et al*, 1991; Halford *et al*, 1997). Since there was a significant reduction in the viral load of the IL-6 tg/tg mice compared to the IL-6 tg/+ and wild type animals, changes in the type

Table 4 Uninfected IL-6 transgenic mice express elevated levels of IL-6 mRNA in the trigeminal ganglia†

Transcript	*IL-6 +/+*	*IL-6 tg/+*	*IL-6 tg/tg*
IL-6	0.02 ± 0.01	0.12 ± 0.05	0.27 ± 0.04
RANTES	0.05 ± 0.03	0.14 ± 0.07	0.14 ± 0.05
CD8	0.10 ± 0.02	0.27 ± 0.13	0.20 ± 0.14

†IL-6 homozygous transgenic (IL-6 tg/tg), IL-6 heterozygous transgenic (IL-6 tg/+), and wild type (IL-6 +/+) mice were sacrificed and the trigeminal ganglia were separately collected, and the RNA was processed and analyzed by RT–PCR for the levels of IL-6, RANTES, and CD8. Numbers represent the RT–PCR product yield expressed in terms of the integrated volume of pixels associated with each ethidium bromide-stained band. The results are summarized and expressed as a ratio of the target gene and the housekeeping gene, GAPDH. The numbers represent the mean ± s.e.m., $n = 3$/group.

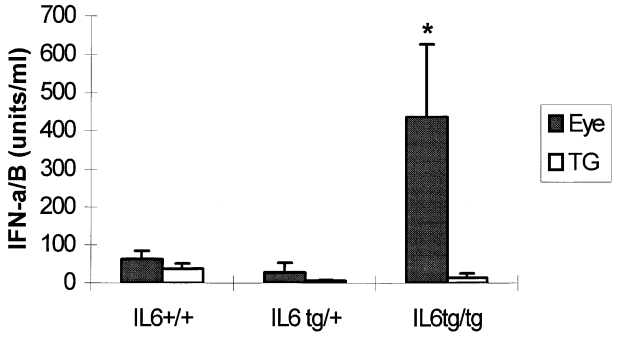

Figure 4 Homozygous GFAP-IL6 transgenic mice produce elevated levels of IFN-α/β in the eye during acute ocular HSV-1 infection. IL-6 transgenic and wild type mice (n=4/group) were infected with HSV-1 (210 p.f.u./eye, McKrae strain) and sacrificed 6 days p.i. The eyes and TG were removed, processed, and the IFN-α/β levels were determined by bioassay. $^*P < 0.05$ comparing the IL-6 tg/tg mice to the other two groups.

1 IFN levels may contribute to the resistance to HSV-1 replication seen in the GFAP-IL6 tg/tg mice. Therefore, the levels of IFN-α/β were measured in the eye and TG of the IL-6 transgenic and wild type mice during the acute stage of the ocular infection. While there were no differences in the level of IFN-α/β in the TG 3 days p.i., IL-6 homozygotic transgenic mice had considerably higher levels of IFN-α/β in the eye in comparison to the other two groups which is consistent with the reduction in the viral load in the eye and TG 6 days p.i. (Figure 4).

Discussion

IL-6 is a pleiotropic cytokine whose expression is induced in the CNS following infection with various viruses (Lieberman *et al*, 1989; Lewandowski *et al*, 1994; Bencsik *et al*, 1996). In fact, enriched astrocyte cultures produce microgram quantities of IL-6 in response to HSV-1 infection (Baker, Carr, and Gebhardt, unpublished observation). In the present study, IL-6 homozygotic transgenic mice were more resistant to acute HSV-1 infection compared to the IL-6 heterozygotic or wild type mice based on the recovery of infectious virus from the eyes and TG 6 days p.i. The elevated IL-6 mRNA and protein in the IL-6 tg/tg mouse eye may have contributed to the viral resistance. Specifically, IL-6 is known to enhance parameters that control viral infection including antibody synthesis (Hirano *et al*, 1986) and the development of cytotoxic T cells (Renauld *et al*, 1989). Recently, IL-6 has been found to enhance the expression of cytolytic molecules (including granzyme B and perforin) associated with cytotoxic effector cells that monitor viral infection (Greene *et al*, 1997) as well as act as a cytokine adjuvant enhancing antiviral immunity (Larsen *et al*, 1998).

Consequently, the level of viral resistance observed in the IL-6 transgenic mice may be due, in part, to promoting effector cell development or cytolytic activity. Additional resistance to HSV-1 infection would include the increased production of IFN-α/β observed in the eyes of the IL-6 tg/tg mice during the early acute infection. It is unclear why a similar finding was not observed in the TG. However, the lack of an elevation in IFN-α/β in the TG of IL-6 tg/tg mice may reflect a difference in cell constituency comparing the TG and eye rather than transgene expression *per se*. Collectively, the IL-6 tg/tg mice may have been in a pre-activated state facilitating a rapid response to the viral insult compared to the IL-6 tg/+ and wild type mice. However, a comparison of other cytokine and chemokine profiles in the eye and TG of transgenic and wild type animals by RT–PCR prior to or after infection with HSV-1 showed no significant differences in the level of expression except for IL-6 which was significantly elevated in the IL-6 tg/tg mice and less so in the IL-6 tg/+ animals. It is possible that high concentrations of IL-6 may modify the expression of cellular factors that facilitate HSV-1 replication or alternatively, may modify neuroendocrine processes that may be involved in HSV-1 replication including corticosteroids (Lyson and McCann, 1991) as was previously noted in these GFAP-IL6 mice (Raber *et al*, 1997). However, the protective effect elicited by IL-6 is a two-edged sword. Specifically, uncontrolled production of IL-6 within the CNS resulting in a localized inflammatory response is thought to significantly contribute to neurological disease (Gijbels *et al*, 1990; Benveniste, 1992) consistent with the CNS pathological state of the GFAP-IL6 mice (Campbell *et al*, 1997).

The transgenic mice used in this study (G167) exhibit a dose-dependent neuropathology independent of viral infection (Campbell *et al*, 1993).

In fact, the production of IL-6 constitutively within the astrocyte population would predictably induce astrogliosis and astrocyte proliferation resulting in scar formation and activation of microglia (Benveniste, 1997). Such an episode would elicit the production of cytokines by the microglia which might interfere (antagonize) with HSV-1 infection. However, we found in uninfected IL-6 transgenic or wild type mice measuring a variety of cytokines and chemokines (IL-6, IL-10, IFN-α, IFN-γ, CRG-2 MCP-1, MIP-1β, and RANTES) and immune cells (CD3 and CD8) by RT–PCR that only CD8, IL-6, and RANTES expression was observed in the TG with only an elevation in IL-6 transcript levels in the transgenic mice compared to the wild type animals. Therefore, the modest neuropathology associated with the transgene expression of the homozygous GFAP-IL-6 mice in the CNS did not elicit cytokine or chemokine expression in the peripheral nervous system (i.e., TG) that would explain the resistance to the acute HSV-1 infection with the exception of IL-6. This point is also supported by the data showing equivalent levels of virus in both the TG and eye of mice (transgenic and wild type) measured 3 days p.i. IL-6 has recently been shown to antagonize HSV-1 replication in astrocyte-enriched cultures (Baker, Carr, and Gebhardt, unpublished observation). Therefore, the hindrance of HSV-1 replication in the eye and TG 6 days p.i. may either be the result of interference via IL-6 or alternatively, the indirect action of IL-6 on anti-viral mediators including type I IFNs.

Even though HSV-1 replication was significantly restricted in the IL-6 tg/tg mice compared to the IL-6 tg/+ and wild type mice based on the viral load and expression of ICP27 and VP 16 mRNA in the TG 6 days p.i., all of the homozygous IL-6 transgenic animals evaluated were found to be latently infected as defined by the detection of LAT expression in the TG 30 days p.i. Likewise, all IL-6 tg/tg mice seroconverted with anti-HSV-1 antibody titers similar to the levels found in the IL-6 tg/+ transgenic animals (data not shown). Although the LAT levels were reduced in the IL-6 tg/tg mice compared to the IL-6 tg/+ or wild type animals, the difference was not significant suggesting that the establishment of latency is most probably committed within the first 3–6 days p.i. This idea is supported by the data showing the recovery of infectious HSV-1 was significantly reduced in the TG of IL-6 tg/tg mice compared to wild type 6 days p.i. but similar levels were found 3 days p.i.

The expression of IL-6 mRNA was elevated in the eye and TG of the IL-6 transgenic mice p.i. coinciding with the transgene level. Muller cells in the eye (Humphrey *et al*, 1997) and Schwann cells in the TG (Riol *et al*, 1997) can express GFAP following injury suggesting that it is possible that the transgene can be expressed in these tissues. In fact, it has recently been shown that HSV-1 significantly elevates the expression of GFAP in the TG during an acute ocular infection of mice with HSV-1 (Carr *et al*, 1998).

Attention in the present study was primarily focused on HSV-1 replication and the immune response to the viral insult during the acute infection. However, the recent studies suggesting a relationship between IL-6 and HSV-1 reactivation (Kriesel *et al*, 1997a; Noisakran *et al*, 1998) suggest that the primary influence of IL-6 on HSV-1 may not be during the acute infection but rather, on the reactivation from latency. Specifically, there appears to be differences in the temporal expression of viral genes in the process of HSV-1 replication in comparison to reactivation (Tal-Singer *et al*, 1997). Consequently, IL-6 may modify the expression of the lytic immediate early (α) viral genes in the TG as shown day 3 p.i. as well as those genes more closely associated with reactivation. The frequency of induced reactivation following the establishment of latency in these transgenic mice will be a focus of future research.

Materials and methods

Mice and virus infection

Homozygous or heterozygous GFAP-IL6 mice or wild type littermates (male and female, 8–12 weeks old) of the low expressor G167 line were used. A description of the construction of the IL-6 fusion gene and production of the GFAP-IL6 mice using a C57BL/6 X SJL hybrid strain has been described in detail (Campbell *et al*, 1993). Briefly, the fusion gene construct used was composed of an entire modified GFAP gene into which was inserted in the first exon, the cDNA fragment corresponding to the coding region of IL-6, together with an upstream SV-40 intron sequence and a downstream SV-40 polyadenylation signal. Astrocyte expression of the transgene was confirmed at both the RNA and protein levels.

Mice were anaesthetized by i.p. administration of 0.1 ml PBS containing xylazine (6.6 mg/kg) and ketamine (100 mg/kg). Following corneal scarification, tear film was blotted from the eyes and the mice were inoculated with 210 plaque forming units (p.f.u.)/eye of HSV-1 (McKrae strain) in a volume of 3 μl. Under such conditions, we have found the LD_{50} for this strain ranges from 100–210 p.f.u./eye in outbred mice. Infection was verified by swabbing the eyes 2–3 days post infection (p.i.), placing the swabs in CV-1 monolayer cultures, and observing the cells for cytopathic effects (CPE). Additional eye swabbings were performed at 6, 9, and 12 days p.i. in mice destined to establish a latent infection to assess the clearance rate of the virus in the tear film. Animals were sacrificed at the indicated time p.i.

Virus and cells
CV-1 African monkey kidney cells (American Type Culture Collection [ATCC], Manassas, VA, USA) were cultured in RPMI-1640 (Mediatech, Washington, DC, USA) containing 5% FCS (Life Technologies, Gaithersburg, MD, USA) and an antibiotic/antimycotic solution (Sigma Chemical Co., St. Louis, MO, USA). Cells were incubated at 37°C, 5% CO_2, 95% humidity. HSV-1 (McKrae strain) was grown up and harvested as described (Halford *et al*, 1996).

Measurement of tissue HSV-1 titers
Eyes, TG, and cerebella were removed 3 or 6 days p.i. and homogenized in 0.8 ml RMPI-1640 containing 5% FCS in 2.0 ml microcentrifuge tubes. Homogenates were clarified by centrifugation for 1 min at 13 000 × g. HSV-1 titer in clarified supernatants was determined by plaque assay using CV-1 cells and reported as p.f.u./ml.

Reverse transcription–polymerase chain reaction
RT–PCR was carried out as described (Halford *et al*, 1996). Briefly, eye and TG RNA were extracted in Ultraspec™ RNA isolation reagent (Biotecx Inc., Houston, TX, USA). First strand cDNA was synthesized using AMV reverse transcriptase (Promega Corp. Madison, WI, USA). PCR was performed in a thermal cycler (Ericomp Delta cycler I, Ericomp, San Diego, CA, USA) with 30–35 cycles of 94°C (1′ 15″)→57–65°C (1–1′15″)→ 72°C (30–45″). PCR primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), infected cell polypeptide 27 (ICP27), IFN-γ, latency associated transcript (LAT), IL-10 and RANTES were as previously described (Halford *et al*, 1996). IFN-α (consensus sequence for IFN-α1, 2, and 7) and CD8 primer sequences were obtained from Clontech Laboratories, Inc. (Palo Alto, CA, USA). Primers for CD3 were 5′-ATGGAGCAGAGGAAGGGTCTG-3′ (sense) and 5′-TCACTTCTTCCTCAGTTGGTT-3′ (antisense). Primers for IL-6 were 5′-TTCCATCCAGTTGCCTTCTTGG-3′ (sense) and 5′-CTTCATGTACTCCAGGTAG-3′ (antisense). Primers for JE/monocyte chemoattractant protein-1 (MCP-1) and macrophage inhibitory protein (MIP)-1β, and the settings for the amplification of the specific products were as described (Bouley *et al*, 1995). Primers for cytokine response gene (CRG)-2 were 5′-CAGCACCATGAACCCAAGTGC-3′ (sense) and 5′-GCTGGTCACCTTTCAGAAGACC-3′ (antisense). Primers for VP16 were 5′-FFACTCGTATTCCAGCTTCAC-3′ (sense) and 5′-CGTCCTCGCCGTCTAAGTG-3′ (antisense). Following electrophoresis of the amplified product, ethidium bromide-stained PCR products were visualized with a Bio-Rad 1000 gel documentation system (Bio-Rad, Hercules, CA, USA). Densitometric analysis of gel images was performed using Molecular analysis software™ 3.3 (Bio-Rad, Hercules, CA, USA). Quantitative PCR was performed to measure the copy number of IL-6/input RNA as determined using a commercially available kit (Biosource International, Camarillo, CA, USA). Essentially, this competitive PCR is a quantitative adaptation using a known copy number of an exogenous synthesized DNA (provided in the kit) which serves as the internal calibration standard and is mixed with sample cDNA. The standard amplicon can be distinguished from the sample based on a unique capture binding site engineered in the amplified product. Following the PCR, the amplicon is denatured and captured using engineered IL-6 specific capture oligonucleotides. The captured amplicons are then detected and quantified by addition of an enzyme-strepavidin conjugate followed by substrate. The signal generated is proportional to the amount of amplicon present. Based on the readout, it is possible to determine the amount of input IL-6 cDNA from each sample.

Measurement of tissue interferon (IFN)-α/β
The eyes and TG obtained from IL-6 transgenic and wild type mice were homogenized in RPMI-1640. Clarified supernatant was assayed for IFN bioactivity as previously described (Halford *et al*, 1998). The authenticity of the IFN type was determined in assays using neutralizing antibody (neutralizing capacity=510 units) to IFN-α/β (Lee Biomolecular Research Lab. Inc., San Diego, CA, USA) or normal rabbit serum.

Statistics
One-way ANOVA and Tukey's *post hoc t*-test were used to determine significant ($P<0.05$) differences between the indicated groups using the GBSTAT™ program (Dynamic Microsystems, Silver Springs, MD, USA).

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