Effect of Fas and Fas Ligand Deficiency in Resistance of C57BL/6 Mice to HSV-1 Keratitis and Chorioretinitis

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PURPOSE. To investigate the effect of Fas and Fas ligand (FasL) deficiency on the development of herpes stromal keratitis and on the von Szily model of herpes retinitis in C57BL/6 mice, which are ordinarily resistant to development of both of these herpetic diseases.

METHODS. Anterior chamber inoculation of the right eye of each mouse with various titters of HSV-1 (KOS strain) was performed. Both eyes of each mouse were enucleated on postinoculation day 15 and processed for histopathologic examination. HSV-1 was inoculated into one cornea of other mice, and the severity of stromal keratitis was scored.

RESULTS. Contralateral destructive chorioretinitis developed in susceptible Balb/cByj mice (19/23); ipsilateral chorioretinitis did not occur (0/23). Stromal keratitis developed in susceptible C.AL-20 mice (15/16). None of the C57BL/6 (0/10 for keratitis or 0/20 for retinitis) developed inflammation. Neither did B6.SMN.C3H.gld (Fas deficient; 0/12 or 0/28) or B6.MRL1pr (Fas deficient; 0/11 or 0/34) mice (keratitis or contralateral chorioretinitis). Minimal scattering of inflammatory cells in the contralateral retina but no destructive chorioretinitis was observed in two C57BL/6, three B6.SMN.C3H.gld, and five B6.MRL1pr mice. Few inflammatory cells were also found in the ipsilateral vitreous and vitreoretinal interface (but not destructive chorioretinitis) of all C57BL/6, two gld, and three lpr mice.

CONCLUSIONS. Immune dysregulation secondary to deficiency in Fas or FasL system does not influence the resistance of the C57BL/6 mice to develop herpes simplex keratitis or destructive herpes simplex chorioretinitis. (Invest Ophthalmol Vis Sci. 2001;42:2505–2509)

Unocular anterior chamber inoculation of herpes simplex virus type 1 (HSV-1) into Balb/cByj mice results in ipsilateral inflammation of the anterior segment with relative choroidal sparing and contralateral herpes simplex retinitis (HSR).1 This was first described by von Szily in 1924 in rabbits,2 was adapted to mice by Whittum and colleagues in 1998.1,3 Recently, Fas and FasL have been implicated in curtailing HSV-induced inflammatory response to HSV in von Szily retinitis. Thus, Balb/cByj mice (Igh-1a) are susceptible, whereas their Igh-1 congenic relatives C.B-17 (Igh-1b) and C57BL/6 mice (Igh-1b) are resistant to contralateral retinitis.17 The Igh-1-associated gene products control both humoral and cellular aspects of the immune response,18 and we and others have demonstrated that T cells,18–21 B cells,22 and natural killer cells and macrophages23 play a role in the pathogenesis of or protection from contralateral HSR and ipsilateral retinal sparing.16,20,23 The kinetics of the immune response, virus specific delayed-type hypersensitivity reactions and virus neutralizing antibodies, has been shown to differ in the resistant and susceptible mice.21 Resistance to a second HSV-1–induced ocular disease, herpes simplex keratitis (HSK), is also controlled by the Igh1 locus.24 Inbred mouse strains that carry the Ighb24–26 alleles (e.g., C.AL-20 [Ighb]) are susceptible to necrotizing stromal keratitis induced by infection with HSV-1, whereas congenic strains that carry the Ighb allele (C.B-17) are highly resistant.25 The resistance to HSK in mice is associated with allotypic variation in immunoglobulin genes,26 possibly because circulating immunoglobulin-derived peptides can cross-tolerize T cells specific for corneal tissue autoantigens.27

Fas (CD95) is a 36-kDa cell surface protein that induces apoptosis in appropriate target cells. Fas is expressed on a variety of cell types, including hemopoietic, epithelial, and other cells.28 The ligand for Fas (FasL) has a more restricted pattern of expression. Expression of FasL is induced on mature CD4+ and CD8+ T lymphocytes after their activation, but it is not expressed on any other hemopoietic cells.29,30 FasL is also constitutively expressed in two immunologically privileged tissues: the eye and the testis.31 Recently, Fas and FasL have been implicated in regulating the normal immune responses by influencing various immune effector cell functions. Fas-FasL interactions have been shown to limit the magnitude of helper T-cell–dependent B-cell activation.28 The constitutive expression of FasL in ocular tissue has been reported to play an important role in curtailing HSV-induced inflammatory response.32 However, these studies did not establish a role of Fas–FasL-dependent apoptosis in corneal and retinal destruction after HSV-1 infection of resistant mouse strains.

In this study, we ask if Fas–FasL interactions are responsible for prevention of ocular inflammatory damage after corneal or anterior chamber inoculation of HSV in HSK- and HSR-resistant mice. Therefore, we studied the effects of immune dysregula-
tion secondary to Fas or FasL deficiency on lpr (Fas deficient) and in gld (FasL deficient) mice, on the development of von Szily HSR and HSK in resistant C57BL/6 mice.

**METHODS**

**Virus**

HSV-1 (strain KOS) was cultured in VERO cells derived from African Green Monkey kidneys (CCL; American Type Culture Collection, Manassas, VA). Plaque assays were performed in the same cell line. Viral and cell cultures were grown according to previously described methods.10,24

**Animals**

Male and female HSV-1–susceptible Balb/cByJ (Igh-1a), C.AL-20 (Igh-1b), HSV-1–resistant C57BL/6 (Igh-1b), B6.SMN.C3H.gld (Fas-ligand deficient), and B6.MRL.lpr (Fas deficient) mice, aged 7 to 9 weeks, were obtained from the Jackson Laboratories (Bar Harbor, ME). Mice were housed in microisolators mounted in ventilated animal racks (VR-1; obtained from the Jackson Laboratories). Mice were divided into two groups. The first group consisted of 18 Balb/cByJ, 15 C57BL/6, 13 B6.SMN.C3H.gld, and 19 B6.MRL.lpr mice; each strain experiment was conducted in a masked manner.

**Corneal Inoculation in Various Strains of Mice**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Cumulative</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.AL-20</td>
<td>9/9 (100)</td>
<td>6/7 (85)</td>
<td>15/16 (94)*</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>0/5 (0)</td>
<td>0/5 (0)</td>
<td>0/10 (0)</td>
</tr>
<tr>
<td>B6.SMN.C3H.gld</td>
<td>0/5 (0)</td>
<td>0/7 (0)</td>
<td>0/12 (0)</td>
</tr>
<tr>
<td>B6.MRL.lpr</td>
<td>0/5 (0)</td>
<td>0/6 (0)</td>
<td>0/11 (0)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.

* Fifteen C.AL-20 mice developed grade 4+ ipsilateral keratitis; one developed only grade 2+ keratitis. None of the mice in the other three strains developed any degree of keratitis.

**RESULTS**

**Herpes Simplex Keratitis**

Ninety-four percent (15/16) of the susceptible C.AL-20 mice developed grade 4+ ipsilateral keratitis; one mouse (1/16) developed grade 2+ keratitis. None of the C57BL/6/6 (0/10), B6.SMN.C3H.gld (0/11), or B6.MRL.lpr (0/12) mice developed any degree of keratitis (Table 1).

**Herpes Simplex Retinitis**

Contralateral chorioretinitis developed in 83% (19/23) of HSR-susceptible Balb/cByj mice: 16 of 18 mice inoculated with 4.0 × 10⁵ PFU of HSV-1 and 3 of 5 mice inoculated with 6.2 × 10⁴ PFU of HSV-1 (Fig. 1). Ipsilateral chorioretinitis was defined as the presence of focal or diffuse chorioretinal inflammation or destruction; contralateral chorioretinitis was defined as the presence of chorioretinal necrosis, vitreous cellular infiltration, or retinal edema. All experiments were replicated, and all evaluations were conducted in a masked manner.
DISCUSSION

There is currently no clear explanation for the exact mechanism of the pathogenesis of contralateral retinitis and relative sparing of ipsilateral retina after uniocular anterior chamber HSV inoculation in the von Szily mouse model of HSR. Evidence suggests that the presence of live virus in the posterior segment of the contralateral eye is necessary, although not sufficient for the development of HSR. Tracing studies have demonstrated in euthymic mice that the virus spreads from the injected eye to the contralateral optic nerve and retina via synaptically related neurons but does not spread to the ipsilateral optic nerve and the retina.

HSK resulting from HSV-1 (KOS) infection of C.AL-20 mice represents a virally induced autoimmune reaction against corneal tissues initiated by T cells. HSK can be mediated by T-cell clones specific for corneal self-antigens, which also recognizes an allotype-bearing peptide derived from IgG2a, and exposure of HSK-susceptible mice to a soluble form of this peptide (tolerization to it) confers resistance to HSK. More recently, UL6, a coat protein of HSV-1 (KOS), has been shown to be recognized by these autoreactive T cells, which target corneal antigens, suggesting that this model of autoimmune disease may be exacerbated by viral mimicry. No such virion-associated protein has been identified in the HSR model.

Several studies have implicated immunologic processes in the pathogenesis of ipsilateral retinal sparing and the contralateral HSR after uniocular anterior chamber HSV-1 inoculation. The contributions by various participants of the immune system, such as T cells, B cells, and natural killer cells and macrophages, have been reported. The role of T cells in this interesting model has been elucidated by studies involving T-cell depletion in euthymic BALB/c mice using anti-CD4...
(helper/inducer) and CD8 (cytotoxic/suppressor) antibodies and adoptive transfer of selective T-cell subsets in athymic BALB/c and SCID mice. In athymic BALB/c (nude) mice, bilateral necrotizing retinitis develops after unilateral anterior chamber injection with HSV. Adoptive transfer studies have revealed that CD4+ cells can mediate destruction of the contralateral retina, whereas CD8 cells have been implicated in preventing contralateral retinitis.

Because depletion of B cells in resistant CB-17 mice results in bilateral chorioretinitis, B cells may indirectly contribute to inhibition of contralateral retinitis. We have also shown that the contralateral retina is protected in susceptible mice after intravitreal injection of anti-CD11, an antibody against immune effector cells, macrophages, and natural killer cells. Together, these studies suggest a role for immune-mediated protection of the ipsilateral retina and for immune-mediated destruction of the contralateral retina.

Recently, the Fas–FasL system has been demonstrated to limit the magnitude of helper T-cell-dependent B-cell activation. FasL is expressed constitutively on ocular tissue and has been suggested to play an important role in curtailing the virus-induced inflammatory response. We therefore studied the effect of immune dysregulation in Fas- and FasL-deficient mice on the development of retinitis in resistant C57BL/6 mice.

Griffith and colleagues reported the results of their studies in resistant CB-17 mice. They showed that the contralateral retina is protected in susceptible mice after intravitreal injection of anti-CD11, an antibody against immune effector cells, macrophages, and natural killer cells. Together, these studies suggest a role for immune-mediated protection of the ipsilateral retina and for immune-mediated destruction of the contralateral retina.

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Recent studies have suggested that defects in the Fas or FasL system may contribute to the development of retinitis. FasL-deficient congenic mice showed no evidence of retinal architectural destruction. The results of our studies indicate that defects in the Fas or FasL system may contribute to the development of retinitis. FasL-deficient congenic mice showed no evidence of retinal architectural destruction.

**Table 2. Development of Ipsilateral and Contralateral Retinitis after HSV-1 Anterior Chamber Inoculation in Various Strains of Mice**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ipsilateral Eye</th>
<th>Contralateral Eye</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1 (4.0 × 10^5 PFU of HSV-1)</td>
<td>Group 2 (6.2 × 10^6 PFU of HSV-1)</td>
</tr>
<tr>
<td>B6/MRL.lpr</td>
<td>0/19</td>
<td>0/15</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>0/13</td>
<td>0/15</td>
</tr>
<tr>
<td>C57BL/6.lpr</td>
<td>0/13</td>
<td>1/15</td>
</tr>
<tr>
<td>C57BL/6.lpr</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>C57BL/6.lpr</td>
<td>16/18</td>
<td>3/5</td>
</tr>
<tr>
<td>C57BL/6.lpr</td>
<td>0/15</td>
<td>0/5</td>
</tr>
<tr>
<td>C57BL/6.lpr</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>C57BL/6.lpr</td>
<td>0/19</td>
<td>0/15</td>
</tr>
</tbody>
</table>

Values are number of mice, with percentage in parentheses.

PFU, plaque-forming units.
evidence to show the role of Fas and Fasl). A very small percentage of our gld and lpr mice showed scattered vitreous inflammatory cells, which may be similar to the findings that Griffith et al. reported. However, ipsilateral or contralateral destructive retinitis was not seen. Thus, we cannot conclude that Fas and/or Fasl system influences the resistance (to the full extent of keratitis or retinitis) of C57BL/6 to von Shizy HSR or to the development of HSK, as suggested by Griffith and colleagues. It has been shown that in the von Shizy model, herpes virus is transmitted from the central nervous system to the retina of the contralateral eye by retrograde axonal transport through the optic nerve along the endocrine–optic pathway between the retina and the suprachiasmatic nucleus of the hypothalamus.5,6 The Fas or Fasl system may play a partial role in the resistance of certain murine strains (e.g., C57BL/6) to keratitis or retinitis. However, there may be other more dominant factors along the optic nerve transmission route, which maintain most of the HSV-1 resistance, even in the absence of the Fas or Fasl system. Although resistance to HSK and herpes retinitis in mice may, to some degree, involve activation-inhibited cell death, the presence or absence of Fas and FasL does not play a significant role in genetic resistance to HSK and necrotizing retinitis.

References