Recombinant Varicella-Zoster Virus Glycoproteins E and I: Immunologic Responses and Clearance of Virus in a Guinea Pig Model of Chronic Uveitis

Hiroshi Kimura,* Yun Wang, Lesley Pesnicak, Jeffrey I. Cohen, John J. Hooks, Stephen E. Straus, and Richard K. Williams

Guinea pigs immunized with recombinant varicella-zoster virus (VZV) glycoproteins E (gE) and I (gI) developed antigen-specific antibodies in the sera, vitreous, and conjunctival washes. Sera from immunized animals neutralized both cell-free and cell-associated VZV, and peripheral blood lymphocytes proliferated in vitro in response to recombinant gE and gI and to antigens from VZV-infected cells. Immunized guinea pigs were inoculated intravitreally with VZV, which induces chronic uveitis. VZV DNA was more rapidly cleared and infectious VZV was isolated less frequently from the retinas of animals immunized with gE and gI compared with controls receiving vehicle alone. Nonetheless, cellular infiltrates in the vitreous, retina, and choroid were prevalent at 21 days after VZV inoculation in both the adjuvant-alone– and gE-gI–immunized animals. Immunization with VZV gE and gI induced potent humoral and cellular responses that accelerated the clearance of VZV DNA and may neutralize virus within the eye.

Specific immunity to varicella-zoster virus (VZV) develops during the primary infection, varicella, and confers long-term protection from further exogenous VZV reinfection. Protection may be lost in situations in which immunity is suppressed or deficient or in which immunity has waned, especially in the elderly (reviewed in [1]). Ophthalmic involvement is rare in varicella [2] but occurs frequently in herpes zoster and is potentially sight-threatening. The ocular manifestations of herpes zoster include acute and chronic forms of keratitis, uveitis, and retinitis [3, 4]. VZV is also associated with acute retinal necrosis, which can cause retinal detachment in both immunocompetent and immunocompromised individuals in the absence of symptoms of zoster [5, 6].

Immune mechanisms that protect the eye against VZV infection consist of both humoral and cellular components. Some components of the immune response to VZV may also contribute to immunopathologic injury to the eye. Although both protective and pathologic immune mechanisms have been described for ocular infection with herpes simplex virus [7–13], these mechanisms have not been elucidated for VZV infections of the eye.

No animal species appears to be fully susceptible to VZV and, therefore, the full range of VZV disease has not been seen in a model system. Recently, we described a guinea pig model in which VZV inoculated into the vitreous evoked a chronic uveitis with virus detected in the iris, ciliary body, and retinal pigment epithelium [14]. We have used this model system to determine the effect of immunization with recombinant gE and gI on VZV-induced eye disease and clearance of infectious VZV and viral DNA from the eye and to characterize the immune responses to these viral glycoproteins.

VZV gE and gI are glycoproteins of the virion envelope that form a non–covalently linked complex [15–17]. The biochemical analysis of these envelope components has been aided by their production as recombinant proteins [16–18]. Recently, we purified gE, gI, and the gE-gI complex from the culture supernatants of insect cells infected with recombinant baculoviruses into which the VZV gE and gI genes had been inserted [17].

VZV gE and gI were chosen for study because they are critical targets of host responses to VZV. Both humoral and cellular immune responses to gE and gI are elicited following varicella infections in humans [1]. Monoclonal antibodies to both gE and gI neutralize VZV in vitro in a complement-dependent manner [18, 19]. Compared with immunocompetent animals [20], animals immunized with either VZV gE or gI were protected from subsequent challenge with VZV, as evidenced by a reduced induction of antibody to VZV open-reading frame 62 (ORF 62) protein, which was used as a marker of viral replication.

In this study, we immunized guinea pigs with the recombinant gE-gI complex or with a mixture of these recombinant glycoproteins and documented the specific humoral and cellular immune responses to these glycoproteins. Immunized and control animals were then challenged by intravitreal inoculation with VZV. The effect of immunization on VZV clearance from the eye was examined by virus isolation and quantitative DNA polymerase chain reaction (PCR). Finally, the severity of uveitis was assayed by measuring the extent of inflammatory cell infiltration of the eye.
Materials and Methods

Recombinant proteins. Recombinant VZV gE and gI and the gE-gI complex were expressed in insect cells and purified in soluble forms by metal affinity chromatography, as previously described [17]. Each purified protein was dialyzed against PBS and stored at −70°C until use.

Immunization of guinea pigs. Female Hartley guinea pigs (5 weeks old) were immunized with either a mixture of purified recombinant gE and gI (25 µg each) or the recombinant gE-gI complex (50 µg). Purified protein solutions (0.25 mL) were injected intramuscularly with 0.25 mL of complete Freund’s adjuvant at the first injection followed with incomplete Freund’s adjuvant at the second and third injections at 2-week intervals. Control animals received 0.25 mL of PBS and an equal amount of adjuvant.

ELISA. Purified recombinant VZV glycoproteins (125 ng/well) were coated onto 96-well plates (Immulon; Dynatech Laboratories, Chantilly, VA) overnight. After blocking with 5% dry milk in PBS, serially diluted pre- or postimmune sera were added to the plate in duplicate and incubated for 1 h. The bound antibodies were detected by horseradish peroxidase–labeled anti–guinea pig IgG antibody (Sigma, St. Louis) and tetramethylbenzidine substrate kit (Kirkegaard & Perry Laboratories, Gaithersburg, MD), and optical densities were measured at 450 nm. The serum titers were determined as the highest dilution in which an optical density value exceeded 0.2.

For the detection of antibodies in conjunctival washes, a cotton-tipped swab was rolled over the upper and lower conjunctival cul-de-sac and retained in the nasal fornix for 10 s. Conjunctival fluid was eluted from the swabs in 0.5 mL of PBS, and serial dilutions were then tested in the gE and gI ELISAs.

Neutralization assays. Neutralizing antibody titers were determined by plaque-reduction assay on MRC-5 cells. Cell-free VZV (clinical grade Oka strain varicella vaccine, a gift from Merck, West Point, PA) was incubated for 1 h at 37°C with serially diluted sera in the presence of 10% guinea pig complement (Life Technologies Gibco BRL, Gaithersburg, MD) and inoculated on MRC-5 cells. After 7 days, cells were stained with crystal violet and plaques were counted. Neutralizing titers were defined as the minimal dilution in which the plaque number was reduced to 50% of the mean value of the control.

For neutralization of cell-associated virus, VZV-infected guinea pig embryo fibroblasts were incubated for 1 h at 37°C with serially diluted postimmune serum in the presence of the complement and were inoculated onto MRC-5 cells. After 5 days, cells were stained with crystal violet and plaques were counted.

Lymphocyte proliferation assay. Blood was obtained from guinea pigs by cardiac puncture, and peripheral blood mononuclear cells (PBMC) were separated with Ficoll-Paque (Pharmacia Biotechnology, Piscataway, NJ). PBMC were cultured at 2 × 10^5 cells/well (0.2 mL total volume) in RPMI 1640 with 10% guinea pig serum containing 30 mM HEPES and 30 µM 2-mercaptoethanol. The cells were stimulated with 10 µg of purified gE or gI or with VZV antigens [20] consisting of sonicated VZV-infected guinea pig fibroblasts. In each assay, PBS and sonicated uninfected guinea pig fibroblasts were used as negative controls. After 5 days, cells were pulsed with 1 µCi/well [3H]thymidine (Amersham, Arlington Heights, IL) for 16 h and harvested. The uptake of [3H]thymidine in each well was measured by scintillation counting. The stimulation index was calculated as the ratio of mean counts per minute (cpm) in antigen-stimulated wells to the mean cpm in control wells.

Ocular VZV infections and sample preparations. Recombinant VZV strain Oka expressing β-galactosidase, termed ROka-LacZ [14], was passaged 10 times in guinea pig embryo fibroblasts. Guinea pigs were inoculated bilaterally by the intravitreal route with guinea pig fibroblasts containing 200 pfu of VZV in 10 µL. At various times after infection, animals were sacrificed and eyes were harvested for virus isolation, PCR analysis, and histology. For virus isolation and PCR, each eye was cut in half sagittally, frozen, and stored at −70°C. To isolate retinas, the eyes were thawed, and the lens and vitreous were removed. The retinal preparations, consisting mainly of the neural retina and retinal pigment epithelial layer and ciliary bodies, were isolated for further study.

For virus isolation, retinas were inoculated onto MRC-5 cells. After 7 days, plaques were counted by light microscopy. VZV plaques were confirmed by X-gal staining for the β-galactosidase enzyme expressed by the challenge virus.

DNA for PCR assays was extracted from the retinas (Puregene DNA isolation kit; Gentra Systems, Minneapolis), and the concentration of the DNA was determined by spectrophotometry.

Vitreal humor was obtained for the detection of antibodies. To avoid contamination with serum, frozen vitreous bodies were removed from eyes before thawing. Isolated vitreous humor was then used in an ELISA.

For determination of cellular infiltration, whole eyes were fixed in 10% formalin and stained with hematoxylin-eosin. The number of inflammatory cells in the vitreous was determined from stained sections.

Quantitative DNA PCR. VZV DNA was quantitated by two methods, using a phosphorescent imager and by “real-time” fluorogenic assay.

Phosphorescent imager. PCR was done using primers specific for the sequence encoding VZV glycoprotein B (gB), as previously described [21]. Briefly, 1 µg of extracted DNA in a total volume of 100 mL was amplified for 35 cycles at 95°C for 1 min, 60°C for 30 s, and 72°C for 1 min, with a final 7-min extension interval. Each experiment contained at least 2 negative controls containing DNA from mock-infected eyes. Amplified products were separated on a 2% agarose gel, transferred onto a nylon membrane, and hybridized with a 32P-labeled oligonucleotide probe located internal to the amplified DNA segment. The membrane was washed and exposed to X-OMAT film (Eastman Kodak Company, Rochester, NY) for 16 h. The same membrane was used for the quantitation.

To estimate the copy number of VZV, the radioactivity of the probe hybridized to the amplified product was quantitated by laser imaging of phosphorescent storage plates (PhosphorImager; Molecular Dynamics, Sunnyvale, CA). A plasmid containing the complete gB gene in pUC19 (pUC19-VZgB; gift of Liyanye Perera, NIH, Bethesda, MD) was used as a positive control. A standard curve was constructed based on the amount of product obtained from serially diluted PUC19-VZgB (figure 1A). Each sample of the standard curve also contained 1 µg of genomic DNA derived from uninfected guinea pig eyes.

Although controls containing >10^7 copies of DNA were beyond the linear range, this method was quite sensitive and could detect two copies of plasmid DNA per sample. The amount of product obtained after amplification of each specimen was compared with the standard curve to determine the number of copies of VZV DNA.
Figure 1. Standard curves for quantitation of VZV DNA. A, plasmid containing VZV glycoprotein B (gB) was serially diluted, amplified by polymerase chain reaction (PCR) in presence of guinea pig genomic DNA, and quantitated by Southern blotting followed by phosphorescent imaging. Signal intensity of amplified product was plotted as function of input VZV gB plasmid copies. B, standard curve using fluorogenic 5′-nuclease assay. Serially diluted VZV gB plasmid was amplified in presence of guinea pig genomic DNA and analyzed in real time, and threshold cycle (C_T) values were plotted as function of copy number.

Real-time quantitative PCR of VZV DNA by fluorogenic 5′-3′ nuclease assay. Primers for this PCR system were also selected from the gene encoding gB. The upstream and downstream primer sequences were 5′-GATGGTGCATACAGAGAACATTCC-3′ and 5′-CCGGTTAAATGAGGCGTGACTAA-3′, respectively. Amplification with these primers yielded a 139-bp product. A fluorogenic probe (5′-ATTACTGGAACCTGCAGCGGA-3′), the sequence of which was located between the above primers, was synthesized by PE Applied Biosystems (Foster City, CA). The probe was derivatized with a fluorescent reporter dye (6-carboxyfluorescein, FAM) at its 5′ end and a quencher dye (6-carboxytetramethyl-rhodamine, TAMRA) at the 3′ end. PCR reactions were performed using the TaqMan PCR kit (PE Applied Biosystems). The 50-μL PCR mixture contained 10 mM Tris (pH 8.3), 50 mM KCl, 10 mM EDTA, 3.5 mM MgCl_2, 200 μM dUTP, dATP, dCTP, dGTP, 0.3 μM each primer and the fluorogenic probe, 0.025 U/μL AmpliTaq Gold, 0.01 U/μL AmpErase UNG, and 0.5 μg template DNA. Following a reaction of AmpErase UNG for 2 min at 50°C and activation of AmpliTaq Gold for 10 min at 95°C, 50 cycles of 15 s at 95°C, and 1 min at 60°C were carried out using a PCR cycler with an interconnected fluorescence detector (model 7700; PE Applied Biosystems).

Real-time fluorescent measurements were taken, and a threshold cycle (C_T) value for each sample was calculated by determining the point at which the fluorescence exceeded a threshold limit (10 times the SD of the baseline). A standard curve was constructed by the C_T values obtained from serially diluted PUC19-VZVgB (figure 1B). Each sample of the standard curve also contained 1 μg/100 μL PCR reaction of genomic DNA derived from uninfected guinea pig eyes. Using the fluorogenic PCR method for quantitation of the VZV gB gene, a linear range was established, beginning at a minimum of 20 copies and extending through 10^6 copies of gB plasmid.

Statistical analysis. The software package JMP (SAS Institute, Cary, NC) was used for data analysis. Comparison of means was done using the Student’s t test or the Wilcoxon rank sum test.

Results

Recombinant gE and gI induce high levels of glycoprotein-specific antibodies. Guinea pigs were immunized three times with either a mixture of purified recombinant gE and gI or the purified recombinant gE-gI complex. Control animals received adjuvant alone. One week after the last immunization, sera were obtained from each animal and tested using an ELISA. High titers of antibodies against gE and gI (>1:32,000) were detected in the sera of all animals immunized with a mixture of gE and gI or with the gE-gI complex but not in the sera of control animals or in preimmune sera (figure 2A).

Conjunctival washes and vitreous humor from immunized guinea pigs were tested for antibodies to the VZV glycoproteins by ELISA. Antibodies against both gE and gI were detected in the conjunctival washes of both immunized groups but not controls (figure 2B). Antibodies were also detected in the vitreous humor from animals immunized with the gE-gI mixture.

Sera obtained at 1, 5, and 9 weeks after the final immunization were used for the detection and quantitation of neutralizing antibody. VZV was neutralized using cell-free VZV in the presence of complement. Neutralizing antibody titers peaked...
Figure 2. Glycoprotein-specific antibodies in serum (A), conjunctival washes, and vitreous humor (B) induced by immunization with recombinant VZV glycoproteins E (gE) and I (gI). Purified recombinant proteins were coated onto 96-well plates, and anti–gE and anti–gI antibodies in serially diluted samples were measured by ELISA. C. Neutralization of VZV by serum from immunized guinea pigs in presence of complement on MRC-5 cells. Guinea pigs immunized with a mixture of gE and gI ●, gE-gI complex ▲, or adjuvant only ○.
Table 1. Lymphocyte proliferation induced by purified recombinant glycoproteins (gE and gI) and antigens from VZV-infected cells.

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>gE</th>
<th>gI</th>
<th>VZV antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td>gE-gI + adjuvant (n = 6)</td>
<td>20.0 ± 21.0*</td>
<td>20.1 ± 21.0*</td>
<td>4.1 ± 5.0*</td>
</tr>
<tr>
<td>Adjuvant alone (n = 4)</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>0.8 ± 0.2</td>
</tr>
</tbody>
</table>

NOTE. Data are results of lymphocyte proliferation assays expressed as stimulation indexes (mean ± SD).
* Statistically significant at $P < .015$, Wilcoxon rank sum test.

Induction of lymphocyte proliferation responses to gE and gI. Animals immunized with the mixture of gE and gI were tested for cell-mediated immunity (CMI) to VZV and compared with control animals that received adjuvant alone.

PBMC were obtained from animals 3 weeks after the final immunization and were tested for antigen-specific lymphocyte proliferation. Lymphocytes from gE-gI–immunized animals proliferated in response to purified recombinant gE and gI and also to antigens obtained from VZV-infected cells (table 1).

Persistence of infectious VZV in the eye. To determine whether immunization with gE and gI can enhance the clearance of VZV in vivo, both immunized and control (adjuvant-alone) guinea pigs were challenged with VZV. Animals (28/group) were inoculated intravitreally with 200 pfu of cell-associated VZV 4 weeks after the final immunization. At 4 h after inoculation, 4 animals from each group were sacrificed, and their eyes were harvested to test for infectious VZV.

Figure 3. Neutralization of cell-associated virus. VZV-infected guinea pig embryo fibroblasts were incubated for 1 h with serially diluted postimmune serum in presence of complement prior to inoculation onto MRC-5 cells. Sera from animals in each immunized group and 2 animals in each control group were tested. Mean nos. of plaques in each group are indicated with SD (error bars). Differences were statistically significant at $*P = .02$ and $**P = .002$ by Student's t test.
In contrast to these results, there was less VZV DNA 3 days after infection in the immunized group than in the control animals. Quantitative PCR assay by Southern blot showed that the copy numbers of VZV DNA in the retinas were 73% lower in the gE-gI-immunized group than in the control group (figure 4, table 3). These differences were statistically significant at $P < .05$ when compared using the Wilcoxon rank sum test. Because the sensitivity of the fluorogenic PCR in our setting was lower than that of the PCR–Southern blot system (figure 1), we could not detect VZV DNA in most retinas at day 3 by the fluorogenic PCR system (table 3). Quantitation by Southern blot was repeated using retinal DNA obtained from the opposite eye of each animal, and the results were similar: The mean copy numbers of the immunized group were again approximately one-fourth that of the control group. Taken together, these results suggest that immunization with recombinant gE-gI led to faster clearance of both infectious VZV and VZV DNA from the guinea pig retinas.

**Uveitis is not prevented by vaccination.** Because we had shown that VZV inoculated into the posterior chamber of the guinea pig eye causes chronic uveitis [14], we wanted to determine if immunization with the recombinant gE and gI could prevent or otherwise alter the inflammatory response. At day 21 after challenge, 20 animals in each group were sacrificed, and the mean numbers of inflammatory cells in the retina and vitreous were counted in a masked fashion. Uninfected animals had no detectable infiltrating cells regardless of the immunogen used. In contrast, infected animals had large increases in infiltrating cells. VZV-infected guinea pigs that had been given adjuvant alone or gE-gI in adjuvant had significant increases in infiltrating cells compared with infected animals given no adjuvant ($P = .03$ and .08, respectively, Student’s $t$ test; table 4). Infected animals administered gE-gI in adjuvant showed a slightly greater but nonsignificant number of infiltrating cells than the infected recipients of adjuvant alone.

**Discussion**

The guinea pig intravitreal model of VZV infection was developed with the goal of using it to study host defense factors that control VZV spread, both within and beyond the eye, as well as factors that contribute to VZV disease pathogenesis in the eye. To further explore this model, we infected guinea pigs that had previously been vaccinated with recombinant VZV glycoproteins. VZV gE, the most abundant VZV envelope glycoprotein, has been proposed as a subunit vaccine [22, 23].

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### Table 2. Immune responses, virus recovery, and VZV DNA load in guinea pigs immunized with glycoproteins E (gE) and I (gI) at 4 h after infection.

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Immunogen</th>
<th>CMI</th>
<th>Anti-gE</th>
<th>Anti-gI</th>
<th>Virus isolation</th>
<th>VZV DNA (copies/μg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>gE-gI</td>
<td>14.2</td>
<td>4096</td>
<td>4096</td>
<td>–</td>
<td>131</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>1.4</td>
<td>256</td>
<td>256</td>
<td>+</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>3.4</td>
<td>1024</td>
<td>1024</td>
<td>–</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>2.7</td>
<td>1024</td>
<td>1024</td>
<td>–</td>
<td>671</td>
</tr>
<tr>
<td>11</td>
<td>Adjuvant alone</td>
<td>0.6</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>+</td>
<td>8192</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>0.8</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>+</td>
<td>192</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>1.0</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>+</td>
<td>49</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>0.9</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>–</td>
<td>671</td>
</tr>
</tbody>
</table>

*NOTE.* CMI (cell-mediated immunity) was determined by lymphocyte proliferation assay and shown as stimulation index. PCR, polymerase chain reaction.

* Reciprocal antibody titer.

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Figure 4. Detection of VZV DNA in guinea pig eyes after challenge. Eyes were tested in inoculated animals at 4 h or 3 days after VZV inoculation. VZV DNA was amplified by polymerase chain reaction coupled with Southern blotting and film autoradiography.
Table 3. Immune responses, virus recovery, and VZV DNA load in guinea pigs immunized with glycoproteins E (gE) and I (gI) 3 days after infection.

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Immunogen</th>
<th>Neutralizing antibody*</th>
<th>Virus isolation</th>
<th>VZV DNA (copies/μg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Southern blot</td>
</tr>
<tr>
<td>7</td>
<td>gE-gI</td>
<td>512</td>
<td>+</td>
<td>9</td>
</tr>
<tr>
<td>8</td>
<td>gE-gI</td>
<td>512</td>
<td>–</td>
<td>13</td>
</tr>
<tr>
<td>9</td>
<td>2058</td>
<td>–</td>
<td>2058</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>2058</td>
<td>–</td>
<td>2058</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>Adjuvant alone</td>
<td>&lt;8</td>
<td>+</td>
<td>20</td>
</tr>
<tr>
<td>16</td>
<td>&lt;8</td>
<td>–</td>
<td>8</td>
<td>41</td>
</tr>
<tr>
<td>17</td>
<td>&lt;8</td>
<td>+</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>18</td>
<td>&lt;8</td>
<td>+</td>
<td>8</td>
<td>14</td>
</tr>
</tbody>
</table>

NOTE. PCR, polymerase chain reaction.

* Reciprocal serum titer.

† Statistically significant at $P < .05$ by Wilcoxon rank sum test, mean values.

VZV gI forms a heterodimer with gE [17] and is necessary for correct biosynthesis and subcellular localization of gE [16, 24, 25]. Therefore, we vaccinated animals with gE and gI, quantitated humoral and cellular immune responses to this vaccine, and assayed their impact on subsequent intravitreal challenge with VZV.

Our results show that immunization with recombinant VZV gE and gI produced high titers of serum antibodies directed to these VZV envelope components. These serum antibodies neutralized in vitro both the cell-associated and cell-free forms of VZV. Whereas humoral responses to VZV do not protect against established varicella [26], antibodies are important in modifying the early stages of VZV infection [27, 28]. In the current study, antibodies against both VZV gE and gI were found in the vitreous and tear fluid of immunized animals. Thus, in the immunized guinea pig, the specific humoral response to gE and gI is positioned to limit the spread of VZV from the initial site of infection. Since the immunizations with recombinant VZV glycoproteins were given intramuscularly, the presence of these antibodies in the vitreous before challenge with virus is likely to have resulted from leakage into the eye from the systemic circulation.

Cellular immunity to VZV is essential to the clearance of infected cells in varicella and zoster [1]. Immunodeficient patients are at greater risk for cutaneous and visceral spread of VZV, which results in significant morbidity following either an initial or a recurrent VZV infection. In this respect, immunization of guinea pigs with recombinant gE and gI elicited substantial levels of VZV-specific cellular immunity as assayed by proliferative responses to the recombinant proteins and to infected cell sonicates.

The effect of specific immunity on clearance of infectious VZV from the guinea pig eye was apparent in the present experiments, even at the earliest time points. We found that VZV could not be isolated from most gE-gI–immunized animals within 4 h of inoculation, suggesting that antibodies or cytotoxic cells in the eyes of immunized animals quickly suppressed the initial infection of VZV. VZV DNA levels measured 4 h after inoculation, however, were not yet reduced in immunized animals compared with adjuvant-alone control animals. Therefore, while the infectivity may have been neutralized quickly in the eye, the VZV DNA had not yet been cleared. At 3 days, VZV DNA was significantly reduced and infectious virus was recovered less often in the gE-gI–vaccinated guinea pigs than in the control animals. The levels of VZV DNA observed in the control animals on day 3, however, were also lower than those observed in this group at 4 h after inoculation. Thus, VZV DNA was also being cleared, albeit more slowly, in the absence of immunization. This indicates that intravitreal inoculation with VZV in these experiments did not result in extensive replication of the virus, even in the adjuvant-alone immunized guinea pigs.

Inactivation of VZV within the eye might have been expected to lessen the extent of uveitis in the immunized animals since previous studies showed that live VZV produced significantly more cellular infiltration in the guinea pig eye than did inactivated VZV [14]. In the current experiments, immunization of guinea pigs with a VZV subunit vaccine appeared to accelerate clearance of virus but did not lessen the development of uveitis. Systemic treatment of guinea pigs with adjuvant

Table 4. Ocular inflammation in immunized guinea pigs 21 days after challenge with VZV.

<table>
<thead>
<tr>
<th>Group</th>
<th>Immunogen</th>
<th>n</th>
<th>Inflammatory cells/section (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VZV-challenged</td>
<td>Adjuvant alone</td>
<td>20</td>
<td>139.8 ± 47.4</td>
</tr>
<tr>
<td></td>
<td>gE-gI</td>
<td>20</td>
<td>163.4 ± 93.0</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>4</td>
<td>73.5 ± 7.18</td>
</tr>
<tr>
<td></td>
<td>gE-gI</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Adjuvant alone</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Unchallenged</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
alone predisposed them to more severe uveitis in response to VZV infection. Under these conditions, any protection afforded by the gE-gI immunizations may have been masked by strong immunologic responses elicited by the adjuvant. Other adjuvant formulations may enhance protective effects of the gE-gI vaccine without resulting in excessive inflammatory cell responses. Alternatively, routes of immunization other than intramuscular may effectively shift the balance of the immune response to one which triggers less uveitis.

Because VZV infects retinal pigment epithelial cells [14], which can express MHC class I and class II antigens, release proinflammatory cytokines, and present antigen to T cells [29–31], the cellular infiltration of the retina observed here may involve cytokines and CMI responses induced by infection of retinal pigment epithelial layer cells. The cytokine responses to VZV within the eye are not known, but human peripheral blood lymphocytes stimulated with VZV antigen produce primarily interferon-γ, and to a lesser extent, interleukin-4 [32]. Further study of the immune responses to VZV within the eye and the induction of uveitis is needed. These studies may provide important insights into ocular disease caused by VZV in humans.

References