Nonneutralizing Antibody Against the Glycoprotein K of Herpes Simplex Virus Type-1 Exacerbates Herpes Simplex Virus Type-1-Induced Corneal Scarring in Various Virus–Mouse Strain Combinations

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Purpose. To determine whether the exacerbation of herpes simplex virus type-1 (HSV-1) induced corneal scarring that the authors reported previously in HSV-1 glycoprotein K (gK) vaccinated BALB/c mice challenged with HSV-1 strain McKrae was a general phenomenon independent of virus and mouse strains. To determine the gK-induced immune response leading to exacerbation of HSV-1-induced corneal scarring.

Methods. BALB/c or C57BL/6 mice were vaccinated with gK, ocularly challenged with HSV-1 strain KOS or McKrae, and the relative amount of corneal scarring determined 28 days after challenge. The T cells, total serum, or purified immunoglobulin G (IgG) isolated from gK-vaccinated mice was transferred individually to naive mice, and the affects on corneal scarring after HSV-1 challenge were determined.

Results. The KOS challenge of gK-vaccinated BALB/c mice resulted in significant corneal scarring \( (P = 0.0003) \), despite the fact that KOS normally produces no corneal scarring. McKrae challenge of gK-vaccinated C57BL/6 mice resulted in significant corneal scarring \( (P < 0.0001) \), despite the fact that C57BL/6 mice are normally refractory to HSV-1-induced corneal scarring. Passive transfer of total anti-gK mouse sera or purified anti-gK mouse IgG, but not adoptive transfer of total anti-gK T-cells to naive mice, resulted in exacerbation of corneal scarring after HSV-1 challenge \( (P < 0.0001) \). Mice defective for T-cell-dependent antibody production were not susceptible to exacerbation of HSV-1-induced corneal scarring by gK vaccination \( (P < 0.0001) \).

Conclusions. The ability of gK vaccination to exacerbate HSV-1-induced corneal scarring was not mouse strain or HSV-1 strain specific. The gK-induced exacerbation of corneal scarring was related to anti-gK IgG. How anti-gK IgG exacerbated HSV-1 induced corneal scarring remains to be determined. Invest Ophthalmol Vis Sci. 1997;38:1213-1221.

After primary herpes simplex virus (HSV) infection, the virus establishes a latent infection in sensory neurons with the complete absence of infectious virus.1-5 The majority of primary HSV infections are either asymptomatic or so mildly symptomatic that they are almost completely unrecognized.6-9 By way of unknown mechanisms, HSV reactivates sporadically and travels back to the initial site of infection, where it can cause recurrent clinical disease. Symptomatic eye disease as a result of recurrent ocular infection is the leading cause of infectious blindness in the United States.8-11 Blindness is the result of corneal scarring.12 Although both viral antigens and the immune response of the host appear to contribute to corneal disease,13,14 neither the specific immune response nor the specific HSV-1 protein against which the harmful immune response is directed is known. Most of our knowledge about the immune responses involved in herpes simplex virus type-1 (HSV-1)-induced corneal scarring is...
based on studies using ocular challenge of naive mice. In naive mice, most T-lymphocyte depletion experiments suggest that CD4+ T cells are involved in induction of eye disease and that CD8+ T cells act in a protective or possibly regulatory way. In contrast, other reports concluded that CD8+ T cells were involved in eye disease and CD4+ T cells were protective.

Corneal scarring tends to be more common and more severe during recurrent infections rather than primary infections, presumably because of the preexisting immune response. Unfortunately, HSV-1 spontaneous reactivation is rare in mice, and it is not feasible to study corneal scarring because of spontaneous recurrent infections in this system. Although induced reactivation is possible in the mouse eye model, the induction rate is well below 100%. In addition, multiple rounds of induced reactivation would be necessary to simulate the repeated recurrences that lead to corneal scarring in humans. In this report, we studied corneal scarring after challenge of vaccinated mice in an effort to simulate immune responses that might occur after repeated recurrent infections.

Most HSV-1-induced immune responses are directed against the viral glycoproteins. Recently, we expressed individually all 11 of the known HSV-1 glycoproteins (gB, gC, gD, gE, gG, gH, gI, gJ, gK, gL, and gM) in baculovirus and then tested their vaccine efficacy against that of primary ocular HSV-1 challenge in mice. Five of the glycoproteins (gB, gC, gD, gE, and gI) provided strong-to-moderate protection against ocular disease, whereas five of the glycoproteins (gG, gH, gJ, gL, and gM) had little or no impact on ocular disease. In sharp contrast, vaccination of mice with gK exacerbated corneal scarring severely after ocular HSV-1 challenge. These preliminary studies were done using the McKrae strain of HSV-1 in BALB/c mice. This HSV-1 strain–mice strain combination normally produces a significant amount of corneal scarring.

In naive mice, the severity of HSV-1-induced corneal scarring is dependent on both the mouse and the virus strain. The studies in this report were directed at determining whether exacerbation of corneal scarring by gK vaccination was specific for the virus strain. Plaque-purified viral stocks of HSV-1 strains McKrae normally causes corneal scarring in BALB/c mice. HSV-1 strain KOS normally does not cause corneal scarring. Plaque-purified viral stocks were grown as described previously. General virus manipulations, such as plaque assays and yield determinations, were done using standard virologic techniques.

MATERIALS AND METHODS

Viruses

HSV-1 strains McKrae normally causes corneal scarring in BALB/c mice. HSV-1 strain KOS normally does not cause corneal scarring. Plaque-purified viral stocks were grown as described previously. General virus manipulations, such as plaque assays and yield determinations, were done using standard virologic techniques.

Mice

Inbred 6- to 8-week-old female BALB/c and C57BL/6 mice were obtained from The Jackson Laboratory. For experiments with major histocompatibility (MHC) class II-deficient mice, Aβ/o/o mice and the C57BL/6 parental mice were both purchased from Taconic Farm (Germantown, NY). The Aβ/o/o mice are congenic for the null allele of the Aβ gene, have reduced levels of CD4+ T cells, and produce little or no T-cell-dependent antibody. Animals were handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Vaccination

Mice were vaccinated either one time or three times (at 3-week intervals) with total cells extract from Sf9 cells infected with baculovirus expressing gK as we described previously. Each inoculation consisted of extract from 1 × 10⁶ cells, which contained 5 to 7 μg of gK based on intensity of the expressed gK band on stained gels. Mock vaccine consisted of extract from the same number of cells infected with wild-type baculovirus. Each mouse was inoculated both intraperitoneally (antigen + buffer) and subcutaneously (antigen + Freund's complete adjuvant for inoculation 1; antigen + Freund's incomplete adjuvant for inoculations 2 and 3).

Ocular Challenge

A total of 2 × 10⁸ plaque forming units of McKrae was placed in each eye without corneal scarification and...
the lid rubbed gently for 30 seconds. Mice were challenged 3 weeks after the final vaccination or 4 hours after passive or adoptive transfer.

Monitoring Corneal Scarring
The severity of corneal scarring was scored on a 0-to-4 scale in a masked fashion using 1% fluorescein to delineate epithelial ulceration as we described previously.30

Passive Transfer
Vaccinated or mock-vaccinated donor mice were killed 3 weeks after the third vaccination and bled from the vena cava. The sera from each donor group were combined and the equivalent of one mouse’s worth of serum or IgG (i.e., the average amount of serum or IgG recovered from one donor mouse) then was injected into each naive BALB/c recipient mouse 4 hours before ocular challenge.

Purification of Immunoglobulin G
The serum IgG fraction was isolated 3 weeks after the final vaccination from sera of donor mice in each group using an immobilized antimouse IgG antibody purification kit (Calbiochem, San Diego, CA).

Adoptive Transfer
Vaccinated or mock-vaccinated donor mice were killed 3 weeks after the third vaccination. Spleens were removed and single-cell suspensions prepared as described.50 For each immunization group, the total spleen cells were pooled and total T cells were separated using T-cell enrichment columns (MTCC 2000; R & D Systems, Minneapolis, MN). Each recipient mouse received an amount of T cells equivalent to that from one donor mouse.

Statistical Analysis
Data were analyzed by the Student’s t test using Instat (Graph Pad, San Diego, CA), a personal computer program. Results were considered statistically significant if P < 0.05.
RESULTS

Exacerbation of HSV-1 McKrae-Induced Corneal Scarring in BALB/c Mice Vaccinated With Baculovirus-Expressed Glycoprotein K

BALB/c mice were vaccinated once with baculovirus-gK, mock vaccine (wild-type baculovirus), or baculovirus-D (protective vaccine) and challenged ocularly with McKrae as described in the Materials and Methods section. In the baculovirus-gK- and mock-vaccinated mice, keratitis in the form of geographic ulcerations first appeared on day 3 and proceeded to corneal ulceration and finally corneal scarring by day 28 in the surviving mice (6 of 20 mice in the gK-vaccinated group and 8 of 20 mice in the mock-vaccinated group survived the viral infection. All deaths occurred within the first 14 days). The baculovirus-gK-vaccinated mice appeared to have more severe eye disease than did the mock-vaccinated mice throughout the observation period. All of the eyes in the gK-vaccinated mice showed severe corneal scarring on day 28, with an average corneal scarring score of >3 on a 0-to-4 scale (Fig. 1A). In contrast, the mock-vaccinated eyes had an average corneal scarring score of approximately 1. This difference was statistically significant (P = 0.005, Student’s t-test). As we reported previously, gD-vaccinated mice were protected against HSV-1-induced corneal scarring (Fig. 1A, gD, 0 of 20 eyes). These results are consistent with those of our previous report in which severely exacerbated HSV-1 McKrae-induced corneal scarring developed in BALB/c mice vaccinated three times with baculovirus-gK (rather than once as shown here).25 Mock ocular challenge of baculovirus-gK-vaccinated BALB/c mice produced no corneal scarring (not shown).

Herpes Simplex Virus Type-1 KOS Ocular Challenge of Glycoprotein K-Vaccinated Mice

To determine whether baculovirus-gK vaccination could produce corneal scarring with an HSV-1 strain that normally causes no corneal scarring, mice immunized as above were challenged with KOS (Fig. 1B). KOS normally does not cause corneal scarring, even at challenge doses 1000-fold higher than used here (2 × 10^8 plaque forming units/eye; Ghiasi et al, unpublished data, 1996). Consistent with this result was that none of the mock-vaccinated eyes showed any corneal scarring. In contrast, corneal scarring developed in 8 of 10 eyes from mice vaccinated with gK, with an average severity score of approximately 0.5 on day 28 after challenge (Fig. 1B). A reading of 0.5 indicates that approximately 7/8 or 12% of the surface of the cornea is involved in scarring. It is, therefore, easy to distinguish between no scarring in the mock-vaccinated eyes and the average scarring score of 0.5 in the gK-vaccinated eyes (Fig. 1B; P = 0.0003 compared with that of mock-vaccinated mice).

Ocular Challenge of Glycoprotein K-Vaccinated C57BL/6 Mice

To determine whether baculovirus-gK vaccination could induce HSV-1 scarring in a mouse strain that normally is resistant to HSV-1-induced corneal scarring, C57BL/6 mice were immunized with gK and challenged ocularly with McKrae as above. As expected, corneal scarring did not develop in any of the eyes from mock- (0 of 20) or gD- (0 of 20) vaccinated C57BL/6 mice (Fig. 1C). In addition, no corneal scarring was detected in mock-vaccinated C57BL/6 mice, even at a 10-fold higher challenge dose (2 × 10^6 plaque forming units/eye; 0 of 20 eyes; data not shown). In contrast, significant HSV-1-induced corneal scarring developed in 8 of 10 C57BL/6 mice vaccinated with gK in at least 1 eye (10 of 20 eyes). The mean severity score in the baculovirus-gK-vaccinated group was >1 (Fig. 1C), which was significantly more than in the mock- or gD-vaccinated group (P < 0.0001, Student’s t-test).

Adoptive Transfer

To determine whether the exacerbation of corneal scarring associated with gK vaccination could be transferred by T cells, an adoptive transfer experiment was done. Donor mice were vaccinated and total T cells were purified and injected into 10 naive BALB/c recipient mice per vaccine, as described in the Materials and Methods section. Recipient mice that received T cells from KOS- (live avirulent virus) vaccinated mice had no detectable corneal scarring (Fig. 2). This positive control result confirmed the effectiveness of the adoptive transfer procedure. In contrast, significant corneal scarring developed in both the gK vaccine and mock vaccine recipient mice, indicating that neither transfer provided significant protection. In addition, the mean HSV-1-induced corneal scarring scores in these two groups were similar (Fig. 2; P = 0.5). Thus, transfer of T cells from gK-vaccinated mice did not appear to exacerbate HSV-1-induced corneal scarring.

Passive Transfer

We have found previously that sera from gK-vaccinated mice did not induce neutralizing antibody titers, but it induced enzyme-linked immunosorbent assay titers and also immunoprecipitated gK from extracts of HSV-1-infected rabbit skin cell cultures (Ghiasi et al, unpublished data, 1996). To determine whether the exacerbation of HSV-1-induced corneal scarring associated with gK vaccination could be transferred by serum, serum was collected from the donor mice used above, injected into each of 10 naive BALB/c recipient mice per group, and the recipient mice...
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were challenged ocularly with McKrae 4 hours later. No corneal scarring after ocular challenge developed in mice receiving serum from KOS-vaccinated mice (Fig. 3A). This positive control result confirmed the effectiveness of the passive transfer procedure. Mice receiving mock-vaccinated serum had an average corneal scarring score of approximately 1.5 (Fig. 3A). In contrast, mice receiving anti-gK serum had a score of >3.5 (P = 0.002 compared with that of mock). These corneal scarring scores in the recipient mice were similar to those of the gK- and mock-vaccinated mice in Figure 1.

Passive Transfer of Anti-Glycoprotein K Immunoglobulin G

To determine whether the exacerbation of HSV-1-induced corneal disease transferable by anti-gK serum was caused by IgG, as described in the Materials and Methods section, the following was performed. IgG purified from mice that were vaccinated as described above was injected into naive recipient BALB/c mice.

The effectiveness of the IgG passive transfer procedure was confirmed by the lack of any corneal scarring in mice receiving purified IgG from the positive control KOS-vaccinated donor mice (Fig. 3B). The average corneal scarring severity score was >3 in mice.
FIGURE 4. Corneal scarring in $A_n^{o/o}$ mice. $A_n^{o/o}$ and C57BL/6 parental mice were vaccinated three times with glycoprotein K (gK) or mock vaccine and then challenged with McKrae. The mean corneal scarring scores on day 28 after challenge are shown. gK-C57BL/6 = 22 eyes; mock-C57BL/6 = 20 eyes; gK-$A_n^{o/o}$ = 10 eyes; mock-$A_n^{o/o}$ = 10 eyes.

receiving anti-gK IgG. In contrast, mice receiving IgG from mock-vaccinated donors had an average corneal scarring score of only approximately 1 (Fig. 3B; $P = 0.0001$). Again, the HSV-1-induced corneal scarring scores in the IgG recipient mice were similar to those seen in the corresponding vaccinated mice in Figure 1. Because we found previously that no detectable HSV-1 neutralizing antibody developed in mice vaccinated with baculovirus-gK, the above results support the notion that nonneutralizing anti-gK IgG can exacerbate HSV-1-induced corneal scarring.

**Decreased Corneal Scarring in $A_n^{o/o}$ Mice**

If anti-gK antibody is involved in exacerbation of HSV-1 corneal scarring, then baculovirus-gK vaccination of mice deficient in antibody production should not exacerbate HSV-1-induced corneal scarring. To test this, $A_n^{o/o}$ mice, which produce little or no T-cell-dependent antibody, and their parental C57BL/6 control mice were vaccinated three times with baculovirus-gK or mock (wild-type baculovirus) vaccine and challenged with McKrae. Similar to the results shown in Figure 1C, gK-vaccinated C57BL/6 mice had an average corneal scarring severity score of just >1 (Fig. 4). In contrast, no HSV-1-induced corneal scarring was detected in any of the $A_n^{o/o}$ gK-vaccinated mice ($P < 0.0001$ compared with that of C57BL/6 gK-vaccinated mice) or $A_n^{o/o}$ mock-vaccinated mice. The lack of HSV-1-induced corneal scarring in the gK-vaccinated $A_n^{o/o}$ mice mirrored that seen previously in the mock-vaccinated parental C57BL/6 mice ($P > 0.05$) Thus, gK vaccination of $A_n^{o/o}$ mice did not exacerbate subsequent HSV-1-induced corneal scarring. Because $A_n^{o/o}$ mice are MHC class II deficient, this result suggests that an MHC class II function, such as T-cell-dependent antibody, is necessary for exacerbation of HSV-1-induced corneal scarring by prior gK vaccination. This is consistent with the notion that gK antibody is involved in exacerbation of HSV-1-induced corneal scarring in baculovirus-gK-vaccinated mice.

**DISCUSSION**

Most previous reports investigating the immune responses involved in HSV-1-induced corneal scarring have been limited to the investigation of primary HSV-1 ocular infection of naive animals. These studies, therefore, did not address the question of potentially harmful, preexisting HSV-1-induced immune responses or potentially harmful secondary immune responses, both of which might occur during recurrent infection. Secondary immune responses, after exposure of a previously primed animal to the same antigen, usually differ from primary immune responses after exposure of an animal to a specific antigen for the first time. Thus, the immune response during recurrent HSV-1 infection is likely to differ significantly from the immune response to primary HSV-1 infection. One or more of these differences may be instrumental in clinical corneal scarring induced by recurrent HSV-1 infection. Unfortunately, it is difficult to study recurrent HSV-1-induced corneal scarring in the mouse, because of the scarcity of spontaneous reactivation in this model. To simulate more closely the secondary immune response seen during recurrent infection, in this report, we examined HSV-1-induced corneal scarring in mice that were vaccinated. Ocular challenge of these mice stimulates a secondary immune response and therefore is likely to simulate more closely the situation that occurs during spontaneous reactivation.

**Herpes Simplex Virus Type-1-Induced Corneal Scarring**

Although the specific immune responses and the specific viral antigens involved remain ill defined, HSV-1-induced corneal scarring has long been thought to be the result of host immune interaction with virus antigen. This is based partially on two observations. First, the worst clinical manifestations of herpetic stromal disease and scarring develop in patients with exuberant immune responses, whereas patients who are immunosuppressed show only minimal stromal disease. Second, HSV-1-induced corneal scarring is much more prevalent during recurrent infection,
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which occurs in the face of an already primed immune response, than during primary ocular infection.33,34

Immune Response to Glycoprotein K-Exacerbated Corneal Scarring

The results presented here extend our previous report by showing that a single gK vaccination can exacerbate corneal scarring with a virus–mouse strain combination (McKrae–BALB/c) that normally produces corneal scarring. More important, we also show the following:

1. Prior gK vaccination led to HSV-1-induced corneal scarring by a virus (KOS) that normally does not produce corneal scarring.
2. Prior gK vaccination led to HSV-1-induced corneal scarring in a mouse strain (C57BL/6) that is normally not susceptible to HSV-1-induced corneal scarring.
3. Passive transfer of total serum or purified IgG, but not adoptive transfer of T cells, from gK-vaccinated mice to naive mice, resulted in exacerbation of HSV-1-induced corneal scarring after ocular challenge.
4. gK vaccination of antibody-deficient A6/6 mice did not exacerbate HSV-1-induced corneal scarring.

Because baculovirus-gK does not induce HSV-1-neutralizing antibody,25 these results support the notion that preexisting, nonneutralizing anti-gK IgG can result in exacerbation of HSV-1-induced corneal scarring after subsequent ocular challenge.

Anti-Glycoprotein K Immunoglobulin G Probably Exacerbates Corneal Scarring Through an Indirect Mechanism

Anti-gK IgG might interact directly with HSV-1-infected cells to produce corneal scarring. However, because there is a great deal of evidence that corneal scarring is the result of a cell-mediated immune response rather than a humoral immune response,16 we think that a direct effect of anti-gK IgG on exacerbation of corneal scarring is unlikely. It seems more likely that the anti-gK IgG acts in a more indirect manner to exacerbate corneal scarring.

Although gK appears to be a minor component of infected cells and virus, it is possible that nonneutralizing anti-gK IgG might bind to the virus or to infected cells, either by direct interaction with gK or by way of cross-reactivity to other viral or cellular proteins, and by stearic hindrance interfere with a normal protective immune response to HSV-1. Alternatively, the nonneutralizing anti-gK IgG might interfere with a normal protective immune response by binding to immune cells or immune factors, thereby blocking their activity. In either case, such interference with an important aspect of the HSV-1-protective immune response would be expected to result in an extended and/or more vigorous HSV-1 infection. We would expect this to result in increased corneal scarring by allowing the as-yet undefined immune response that normally leads to HSV-1-induced corneal scarring more opportunity to produce corneal scarring. In the above scenario, the gK antibody is not the direct cause of the exacerbated corneal scarring. Rather, the exacerbated HSV-1-induced corneal scarring is because of the same “harmful” immune response that produces HSV-1-induced corneal scarring in naive HSV-1 challenged mice. gK antibody’s role in the exacerbation would be to extend the duration of the corneal infection by partially shielding the virus from the protective immune response. Consistent with this notion, we found previously that baculovirus-gK vaccination of mice results in HSV-1 challenge virus detection in the eye for an extended period.25

Several viruses, including HSV-1, make proteins that allow the virus to partially elude the host immune response. The VCP vaccinia protein blocks the classical complement pathway by binding to the C4b complement component.15 Another vaccinia protein interferes with interleukin-1β.36,37 Adenovirus makes a protein that binds to MHC-1 antigens, thus blocking lysis by cytotoxic T cells.38 Epstein-Barr virus makes a protein that blocks gamma interferon synthesis, protecting the virus against immune clearance.39 The HSV ICP47 protein reduces lysis of infected cells by CD8+ T cells by blocking the association of HSV peptides with MHC-1 proteins.40-41 HSV gC and its homologs in several other herpes viruses binds C3b, thus protecting against antibody-dependent and antibody-independent lysis by complement.42-44 gC also protects against antibody-independent complement neutralization.45 The HSV gE and gI glycoproteins form a complex that binds the Fc region of IgG and helps protect the virus from antibody-dependent cellular cytotoxicity and antibody-mediated complement enhanced neutralization.46-49 Thus, there are numerous examples of viral proteins that help the virus survive by directly interfering with the normal protective immune response. We hypothesize that in an analogous fashion, gK also helps the virus survive by interfering with the protective immune response. However, gK’s activity is indirect. It induces an immune response that interferes with the normal protective immune response. When this occurs in the eye, corneal scarring is exacerbated.

Regardless of the mode of action by which gK vaccination exacerbates HSV-1-induced corneal scarring, the results reported here strongly suggest that gK should be excluded from subunit candidate HSV vaccines.
Relevance and Importance of the Glycoprotein K Immune Response

We do not know whether the immune response to gK described here plays a role in natural HSV-1 infection. There certainly is precedent for a virus eliciting an immune response that aids in protecting the virus from other aspects of the immune response, as we have speculated here for gK. It also is possible that during natural infection, the response to gK differs from that seen with a subunit gK vaccine, because of differences in presentation of gK to immune surveillance. In this regard, it has been suggested that gK differs from all the other HSV-1 glycoproteins because it is embedded in the membrane and is not a transmembrane glycoprotein. It is likely that a membrane-embedded glycoprotein would not elicit a strong immune response and that a gK subunit vaccine might therefore result in a unique HSV-1-directed immune response.

Regardless of the clinical relevance of the anti-gK IgG response, the model presented in this report may be a useful tool in determining and characterizing the critical, currently undefined, protective immune responses against HSV-1. If, as we expect, anti-gK IgG exacerbates corneal scarring by interfering with the normally protective immune response, it may be possible to approach the question of which immune responses are most important for protection against HSV-1 infection by determining the immune responses with which anti-gK IgG interferes.

Key Words

eye disease, glycoproteins, HSV-1, mice, ocular, vaccine

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References