Herpes Simplex Virus-Specific Antibodies Present in Tears During Herpes Keratitis (42961)

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Abstract. **We examined the specificity and levels of antibodies present in rabbit tears after induced infection of the rabbit cornea. Two strains of herpes simplex virus-1 (HSV) with different patterns of ocular disease were used: RE which produces stromal disease, and F which produces epithelial disease. We found that (i) IgG, IgA, and IgM antibodies were produced, (ii) the number of specific HSV antigens recognized by these antibodies was no significantly different, and (iii) postinfection (PI) timing and concentration of antibodies varied according to the disease pattern of the virus strain. The animals infected with strain F produced high levels of IgG antibodies early PI which remained constant, while IgA and IgM antibodies also increased early PI but declined after Day 16 PI. Animals infected with strain RE showed low levels of IgA and IgM antibodies which remained low. IgG antibodies increased early PI but declined at Day 16 PI. These differences in times of appearance and in amounts of antibodies in tears may be related to the clinical course of the disease. It has been shown that stromal disease has an immunopathologic basis. Inflammation, cellular infiltration of lymphocytes, and plasma cells are seen in the stroma of RE-infected animals, but these are not present in the stroma of F-infected animals. Infectious virus was not isolated from corneal explants taken from animals during the quiescent stage of the disease. The difference in pathogenicity cannot be explained in terms of specificity of tear antibodies. Even though the disease patterns were different, the number and types of HSV polypeptides recognized by both sets of tears was similar. Consequently, we believe that the immunopathology seen in the stromal disease may be due to the anatomical site of HSV antigens, rather than to differences in specificity of tear antibodies.** [P.S.E.B.M. 1989, Vol 192]

Both the humoral and the cellular immune systems are stimulated by a herpes simplex virus (HSV) infection. Antibodies belonging to the IgM, IgG, and IgA immunoglobulin classes are subsequently produced, but their roles in the immune defense against disease are not clearly understood. Production of HSV specific IgA antibodies in patients with localized HSV infections (e.g., herpes keratitis) has been described (1). Pedersen *et al.*[2] have suggested that these IgA antibodies play a role in the neutralization of infectious virus (2). HSV-specific secretory IgA antibodies have been identified in tears collected from patients with herpes keratitis, indicating that these antibodies are locally produced (3). Similar results have been obtained from studies of the rabbit cornea (4).

In the New Zealand White rabbit, induced primary epithelial disease was self-limiting, peaking at about Day 5 post infection (PI) and subsiding thereafter (5–9). During the first week of disease, pathologic changes were due primarily to viral growth in the epithelial layer of the cornea. At about the time of disappearance of epithelial disease (second week), stromal disciform edema was seen which persisted for at least 2 weeks. A necrotizing keratitis and corneal vascularization continued to develop in some animals. Antivirals given during the first week of primary stromal keratitis (6) prevented stromal involvement. In contrast, a late initiation of antiviral therapy did not prevent subsequent stromal

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involvement. Seemingly, the early penetration and proliferation of the virus was necessary for the development of disease.

The immune reaction consisted of plasma cells and lymphocytes seen at the limbus. The central cornea showed subepithelial foci of necrosis and infiltration of a mixed population of lymphocytes and macrophages. These findings demonstrated that the disease has an immunopathologic basis and indicated the presence of an HSV antigenic moiety in the cornea. The nature and source of these antigens are subject to controversy. Viral antigens have been demonstrated by immunofluorescence techniques (10, 11) and, in some instances, virus particles have been seen by electron microscopy (12), but the isolation of infectious virus particles has been difficult (13, 14). Recently, it has been implied that HSV can remain latent in non-neural cells (15) and that the virus may reside within the keratocytes of the corneal stroma (16–18).

Because antibodies in tears are the first line of immunologic defense in a superficial epithelial disease, we chose to compare the immune response, as measured by antibodies in tears, in animals infected with a stromal or epithelial disease-producing virus. Our study utilized two HSV strains with reproducible and well-defined disease patterns: the HSV-1(F) strain (an epithelial disease-producing virus) and the HSV-1(RE) strain (a stromal disease-producing virus). This study examined (i) immunoglubulin production, (ii) antibody production, and (iii) specificity of these antibodies. It also addressed the question of whether HSV could be isolated from the stromal layer of the cornea in the absence of overt disease.

Materials and Methods

Cells and Viruses. Vero and Hep-2 cells (Whittaker Bioproducts, Inc., Walkersville, MD) were grown with minimal essential medium supplemented with 10% calf serum, glutamine, sodium bicarbonate, and antibiotics. Two of the HSV-1 strains were studied: F, a prototype for epithelial disease, and RE, which is a stromal disease-producing virus (19–21).

Ocular Disease. New Zealand White rabbits (2–3 kg body wt) were topically infected with HSV-1 on a scarified cornea with 10^5 plaque-forming units/per eye. the severity of epithelial disease was graded from 1 to 4 as described previously (22). Stromal disease was monitored from the third week PI until the end of the experiment, usually 60–90 days. At appropriate intervals, some animals were sacrificed and the corneas removed for histology and tissue culture.

We obtained two kinds of corneal specimens: those with stromal involvement but no apparent changes in the epithelium, and others in which stromal edema was accompanied by changes in the epithelium.

All animals used in this study received care and treatment in accordance with the ARVO Resolution on the Use of Animals in Research.

Tear Collection. For collection of tears, a Schirmer test strip was placed on the rabbit conjunctiva and the equivalent of 10 μl of tears was collected per eye. The strips from 10 rabbits were pooled by day of collection and eluted in 50 ml of phosphate-buffered saline (PBS) diluted 1/10 overnight at 4°C. The samples were then concentrated to a final volume of 5 ml. These concentrated tears were used for the immunoblotting assays.

A second set of tears was similarly collected every other day; however, each Schirmer paper was eluted overnight with 0.5 ml of PBS and no concentration was made. These tears were used for the determination of antibodies belonging to the various classes of immunoglobulins and the immune precipitation assays.

Preparation of Radioactively Labeled Viral Antigen. Cells grown in 150-cm^2 flasks were infected with HSV-1(F) at a multiplicity of 10 plaque-forming units/cell. After 1 hour of adsorption at 37°C, the inoculum was aspirated and maintenance medium supplemented with 1/10 the normal concentration of methionine and 20 μCi of [^{35}S]methionine (1026 μCi/mmol; New England Nuclear, Dreieich, Germany)/ml of medium were added. At the end of the labeling period, cells labeled from 2 to 4, 6 to 8, and 10 to 12 hr PI were pooled and the soluble-infected cell proteins were extracted in 1% Triton X-100-containing buffer as described previously (23).

Immunoprecipitation by Antibodies of the IgG Class Present in Tears. The radioactively labeled antigen was preadsorbed with formalin-fixed staphylococcus (Cowan strain I) prepared as described by Kessler (24). A 10% suspension of staphylococcus was used in an amount corresponding to twice the volume of the antigen. The adsorption continued for 1 hr at 4°C, and the staphylococcus was pelleted by centrifugation for 1 min in an Eppendorf centrifuge. Aliquots (25 μl) of the adsorbed antigen were incubated for 1 hr at 4°C with a constant volume of tears (10 μl) and the immune complexes bound to the staphylococcus were isolated by centrifugation. The pellet was washed four times in buffer containing 0.05 *M* Tris-HCl at pH 7.4, with 0.1 *M* NaCl. The precipitated proteins were solubilized in a disruption mixture (25).

Immunoprecipitation by Antibodies of the IgA/IgM Class Present in Tears. Using the HSV-1 antigen described above, antibodies of the IgG class were removed from the tears in the manner described previously. The supernatant thus contained only antibodies of the IgA/IgM and of the IgG subclass 3. Indirect immunoprecipitation was accomplished as follows: 25 μl of antigen was mixed with 10 μl of adsorbed tears and incubated for 1 hr at 4°C; 20 μl of a 10% suspension of fixed staphylococcus coated with swine IgG directed against rabbit immunoglobulins (Dako, Copenhagen, Denmark) were added and incubated for 1 hr at 4°C.

Immunocomplexes bound to the staphylococcus were isolated by centrifugation, and the proteins were solubilized in disruption mixture (25) after extensive washing.

As a control for possible IgG remaining after the absorption of tears, direct immunoprecipitation was done where antigen was incubated with adsorbed tears and fixed uncoated staphylococcus.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. The radioactively labeled proteins isolated by immunoprecipitation were electrophoretically separated in 9.25% acrylamide gels. The stained, dried gels were autoradiographed by exposure to Kodak XPR-1 film for 1 to 2 weeks, and the proteins were numbered according to the nomenclature of Morse *et al.* (25).

Quantitative Analysis of Immunoglobulins Present in Tears. *IgG.* Microtiter plates (Nunc 269620) were coated with infected cell extract as described for the antigen preparation. Fifty microliters of infected cell or mock extract diluted 1/100 were added to each well, and the plates were incubated for 4 hr at room temperature. The plates were emptied and 200 μl of 3% bovine serum albumin in PBS was added for 30 min. The plates were then washed and 50 μl of tears diluted 2-fold were added to both HSV and mock-coated wells. Serial dilutions (1/2) were made in order to titrate the amount of immunoglobulin present in each sample. The plates were incubated at 37°C for 1 hr, and 50 μl of an appropriate dilution of ^{125}I-protein A (80 μCi/μg; New England Nuclear) were added to each well after extensive washing. Binding was done for 1 hr at 37°C and was followed by extensive washing. Specifically bound protein A was quantitated after solubilization of each sample in 100 μl of 10% sodium dodecyl sulfate (SDS), followed by transfer to test tubes for counting in a gamma counter. All washes were done in PBS with 0.05% Tween-20.

The amount of HSV-specific IgG in the lacrimal fluid was calculated as counts per minute of protein A bound to HSV-coated wells, less the counts per minute of protein A bound to the corresponding mock-coated wells. All data in the tear sample are presented as cpm/μg total protein as measured by the Lowry standard method (26). In this study, the range averaged 3000–4000 cpm/μg of protein.

IgA/IgM. Analysis was basically as described for the quantitation of IgG, but the tears were adsorbed on formalin-fixed staphylococcus to remove IgG before the procedure. Serial dilutions were made onto both HSV and mock-coated plates, which were thereafter processed as described above. Determinations of IgA/IgM and IgG antibodies were done in duplicate.

Immunoblotting. Analysis of HSV specific antibodies of the IgG, IgM, and IgA immunoglobulin class was done by immunoblotting (27). Infected cell polypeptides separated by polyacrylamide gel electrophoresis were transferred to nitrocellulose paper. Protein bands were resolved by using 3,3′-diaminobenzidine/hydrogen peroxide as the substrate. Determination of antibodies of the IgG or IgM class were incubated for 2 hr with peroxidase-labeled goat anti-rabbit IgG or peroxidase-labeled goat anti-rabbit IgM.

Determination of antibodies of the IgA class was done by incubating the blocked strips with IgG fraction goat anti-rabbit IgA for 2 hr at room temperature, followed by peroxidase-conjugated rabbit anti-goat IgG for 2 hr. (This was necessary as the peroxidase-labeled goat anti-rabbit IgA was not available.

Histology. Specimens for light microscopy were fixed in 10% buffered formalin, paraffin embedded, and stained with hematoxylin-eosin/periodic acid-Schiff in the routine fashion. Sections from the same paraffin block were used for the immunoperodoxidase staining of HSV-1 antigens. After the paraffin is removed, the sections were then acetone fixed for 10 min.

The sections were incubated with a normal goat serum (1/50) for 20 min and exposed to anti-HSV-1 serum for 30 min. Following washes in PBS, the sections were incubated with biotinylated goat anti-rabbit IgG antibody (1:200; Vector, Burlingame, CA), and a final layer of avidin-biotin-peroxidase complex reagent was then applied for 45 min. The immunoperoxidase reaction was developed with 3-amino-9-ethylcarbazole (Sigma, St. Louis, MO) in dimethyl formamide and 0.01% hydrogen peroxide in sodium acetate until a positive result was obtained (red reaction product). All slides were countersigned with hematoxylin and mounted with Gelvatol (Monsanto, Springfield, MA). Control slides (negative for HSV-1 antigen) were also included in each assay.

Results

HSV-Specific Antibodies Present in Tears of Infected Animals. By use of radioimmunoassay, sequential samples of tears collected from rabbits infected with the F and RE strains of HSV were analyzed. The amount of HSV-specific IgA/IgM present in tears of rabbits infected with the F strain increased from Day 5 until Day 16 PI, after which it decreased (Fig. 1). The results obtained from the RE-infected rabbits were different in that no increase was found during the first week PI. A slight increase was observed until Day 15, and it reached a plateau thereafter.

The same sequentially collected tears were also analyzed for the amount of HSV-specific IgG present. The tears from F-infected animals showed a rapid increase in IgG from Day 4 through Day 21 PI. RE-infected rabbits showed an even more pronounced increase in the specific IgG from Day 6 through Day 15 PI, whereafter the amount of IgG antibodies decreased (Fig. 2). All determinations were done in duplicates.

Immunoprecipitation of HSV-1 Proteins by Antibodies Present in Tears of Rabbits Infected with

HSV-1(F). The [^{35}S]methionine-labeled HSV-1 proteins, which were immunoprecipitated with antibodies present in lacrimal fluid, were electrophoretically separated on SDS-polyacrylamide gels (Fig. 3). This method allows the identification of antibodies which can precipitate soluble, nondenatured proteins. These infected cell proteins were precipitated with both IgG and IgA/IgM portions of the tears. Slots 2, 4, 6, and 8 show proteins precipitated with IgA/IgM antibodies. As seen from the autoradiographic image of the separated proteins, the amount of protein precipitated with IgG antibodies increased from 5 days to 3 weeks PI, but no new bands occurred. The IgG immunoglobulins of unabsorbed tears precipitated most of the soluble HSV-1 proteins; these are shown in Slots 3, 5, 7, and 9. The proteins with M_r of 103,000 (ICP15), 68,000 (ICP24), 54,500 (ICP29), and 42,500 (ICP36) were particularly evident.

The antibodies of the IgA and the IgM immunoglobulin classes could not be analyzed separately, as the reagents were not commercially available. The intensity of the protein bands obtained by precipitation with the combined IgA/IgM immunoglobulins remained constant in the sequential samples analyzed. The IgA/IgM immunoglobulins specifically precipitated four polypeptides with M_r of 4,000, 51,000, 68,000 (ICP24), and 82,000 (Fig. 3, Slot 9, and Table I).

As a control, the precipitating capacity of the adsorbed tears was tested in a direct immunoprecipitation test. A small number of proteins appeared as faint bands in the autoradiogram (Fig. 3, Slot 10).

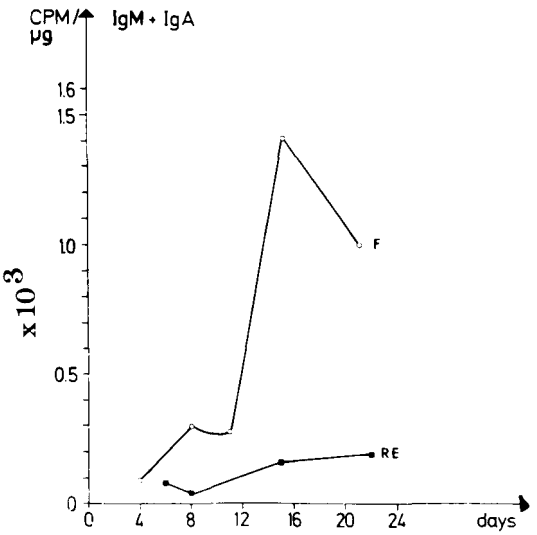

Figure 1. Quantitative analysis of the HSV-specific immunoglobulins of the IgA and the IgM class. Tear samples collected sequentially from rabbits infected with the HSV-1(F) and HSV-1(RE) strains were absorbed on formalin fixed staphylococcus to remove immunoglobulins of the IgG class. The amount of HSV-specific IgA and IgM immunoglobulins per microgram of total protein is shown in the graph: O, HSV-1(F); ■, HSV-1(RE).

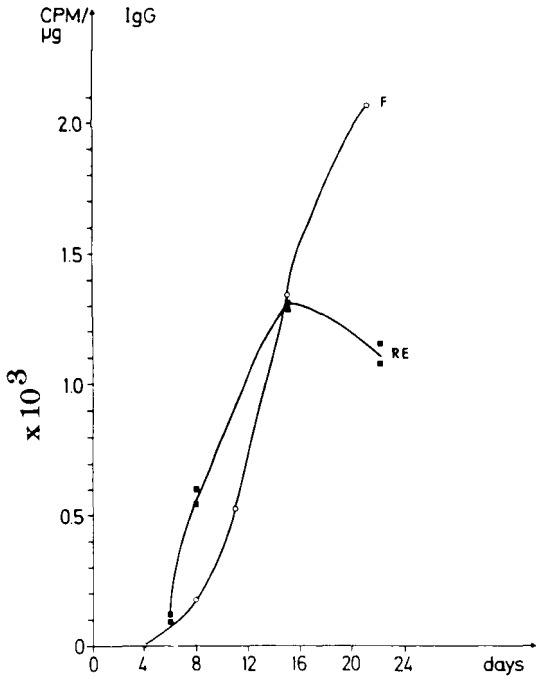

Figure 2. Quantitative analysis of the HSV-specific immunoglobulins of the IgG class. Tear samples from the rabbits described in Figure 1 were analyzed unabsorbed for the amount of HSV-specific IgG. The quantity per microgram of total protein is shown in the graph; O, HSV-1(F); ■, HSV-1(RE).

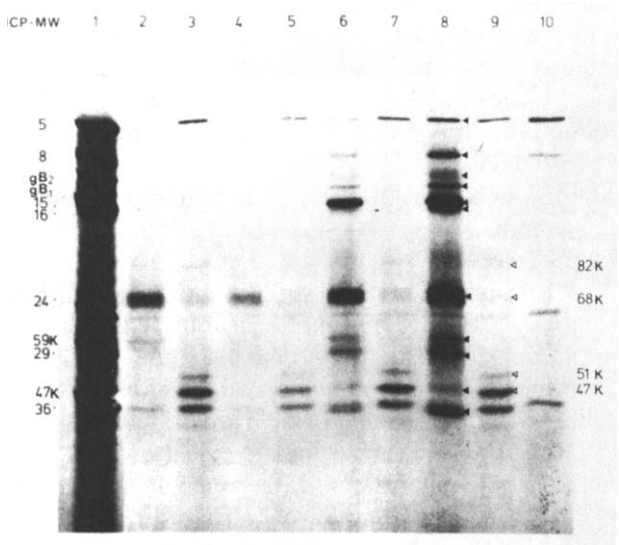

Figure 3. The autoradiographic image of the HSV-1-infected cell polypeptides precipitated with tear samples collected sequentially from HSV-1)F) infected rabbits. The proteins precipitated with HSV-specific antibodies of the IgG class are marked with filled triangles (Slot 8); the ones precipitated specifically with antibodies of the IgA/IgM class are marked with open triangles (Slot 9). Slot 1, [^{35}S] methionine-labeled HSV-1(F) cell extract; Slots 2 and 3, tear sample from 4 days PI; Slots 4 and 5, tears 8 days PI; Slots 6 and 7; tears 15 days PI; Slots 8 and 9, tears 21 days PI. The precipitation profiles obtained with the IgG antibodies are shown in Slots 2, 4, 6, and 8. The profiles obtained with the IgA/IgM antibodies are shown in Slots 3, 5, 7, and 9. Slot 10, the control precipitation with absorbed tears from 21 days PI.

Table I. HSV-1(F) Proteins Identified with Antibodies Present in Lacrimal Fluid Collected from Rabbits Infected with the F and RE Strains

ICP	M_r ($\times 10^{-3}$)	F		RE	
		IgA/IgM	IgG	IgA/IgM	IgG
135	153	+	+	+	+
6	146	+	+	+	+
8	128	+	+	+	+
gB_2	117		+		+
gB_1	114		+		+
15	103		+		+
16	101		+		+
17	92				
	82,000	+			
20	77				
21	72,500				
23	71				
24	68	+	+	+	+
25	64				
	59,000		+		+
29	54,500		+		+
	51	+		+	+
	47,000	+	+		+
36	42,500	+	+	+	+
	39,000				
	38,000				

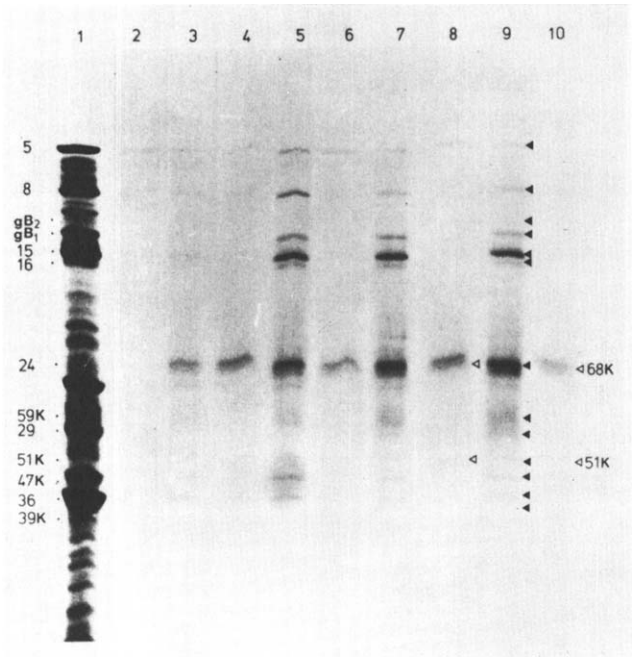

Figure 4. The autoradiographic image of the HSV-1-infected cell polypeptides precipitated with tear samples collected sequentially from HSV-1(RE)-infected rabbits. Slot 1, [^{35}S]methionine-labeled HSV-1(F) cell extract. Slot 2, control precipitation with absorbed tears collected 22 days PI, Slots 3 and 4, tears from 6 days PI; slots 5 and 6, tears from 8 days PI; Slots 7 and 8, tears from 15 days PI; Slots 9 and 10, tears from 22 days PI. The precipitation profiles obtained with the IgG antibodies are shown in slots 3, 5, 7, and 9. The profiles obtained with the IgA/IgM antibodies are shown in slots 4, 6, 8, and 10. The triangle marks are as described in Figure 3.

Immunoprecipitation of HSV-1 Proteins by Antibodies Present in Tears of Rabbits Infected with HSV-1(RE). The HSV-1 proteins precipitated with the sequential lacrimal fluid samples from RE-infected rabbits and separated on SDS-polyacrylamide gels are shown in Figure 4. HSV-specific IgA/IgM antibodies were present in the lacrimal fluid as early as 48 hr PI. HSV proteins reactive specifically with the IgA/IgM antibodies had M_r of 51,000 and 68,000 (ICP24). The data in Figure 4 describe the specimens collected from Day 6 until Day 22 PI. Profiles of the HSV-1 proteins precipitated with the IgG antibodies were faint early in the infection, but an increasing intensity and number of proteins were identified in samples collected later in the infection. The profile of the proteins identified was similar to that obtained with tears collected from F-infected rabbits. The molecular weights are listed in Table I.

Examination of the immunoprecipitation profile of both the RE and F strains of HSV-1 reveals a high degree of comparability. The number of HSV polypeptides resolved by antibodies of the IgG class was similar for both viruses. However, a small difference in two of the HSV polypeptides resolved by the IgA and IgM antibodies was seen. For this reason, we decided to further elucidate this difference by analyzing the IgA antibodies from both strains through the immunoblotting technique.

Immunoblotting. The specificity of the antibodies to HSV present in the tears of infected animals was analyzed by immunoblotting, a procedure in which the antibodies react with polypeptides of denatured and solubilized HSV proteins.

Antibodies of the IgA and IgG class were detected in the tears of both RE- and F-infected animals. Figure 5 shows the antibodies of the IgG class present in tears of RE-infected and F-infected animals. A complete enhanced scan of the blot revealed a total of 21 polypeptides for the RE strain and 19 for the F strain. A variation on the intensity of the bands was seen in the RE strain polypeptide profile.

Antibodies of the IgA class were the most abundant, were present very early in infection (48–72 hr), and remained at a constant level. A total of 22 polypeptides was resolved by these antibodies. In the tears collected from the F-infected animals, antibodies of the IgA class were the most abundant. While their relative concentration did not seem to vary with time, the IgG antibodies initially increased and then decreased by the third week (Fig. 6). The fluctuations in the levels of IgG antibodies could be attributed to transudation from the circulating humoral antibodies.

Ocular Disease. Epithelial disease was seen in both the RE- and F-infected animals during the first week PI. At Days 5 and 7 PI, the animals infected with the F strain showed maximum disease severity. This subsided

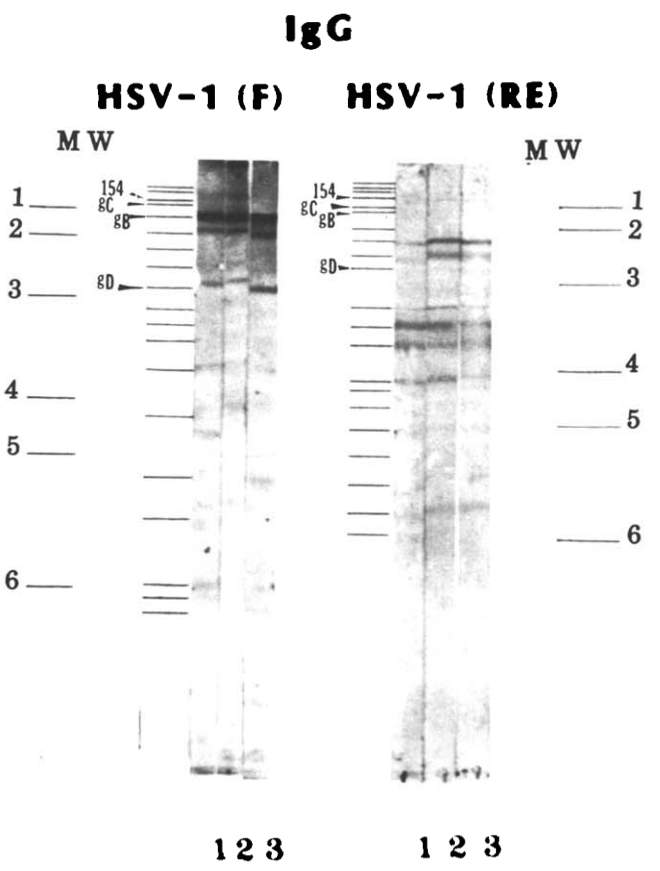

Figure 5. Western blot of HSV-infected cell polypeptides detected by IgG antibodies in tears of rabbits infected with HSV-1(RE) and HSV-1(F). One, 2, and 3 refers to Weeks 1, 2, and 3 PI of sequentially collected tears. A line drawing of resolved polypeptide bands is included at the left side of each blot. The molecular weights (MW) are marked at the left and right margins. Markers 1 through 6 correspond to 130,000, 75,000, 50,000, 39,000, and 17,000, respectively.

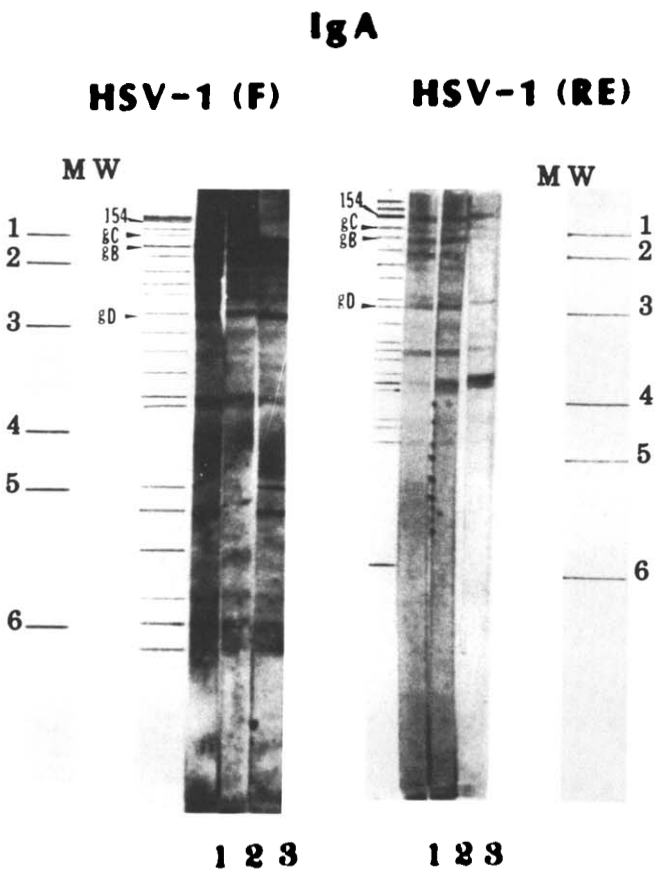

Figure 6. Western blot of HSV-infected cell polypeptides detected by IgA antibodies in tears of rabbits infected with HSV-1(RE) and HSV-1(F). One, 2, and 3 refers to Weeks 1, 2, and 3 PI of sequentially collected tears. A line drawing of resolved polypeptide bands is included at the left side of each blot. The molecular weights (MW) are marked at the left and right margins. Markers 1 through 6 correspond to 130,000, 75,000, 50,000, 39,000, 27,000, and 17,000, respectively.

thereafter, and the corneas became clear by the end of the second week. In contrast, the animals infected with the RE strain showed stromal edema by the second week, and some of the eyes developed necrotizing keratitis after 30–45 days PI. Two types of corneal samples were obtained from the RE-infected animals. Six corneas were obtained during the quiescent phase of the disease, that is to say from animals with some stromal edema present but no overt epithelial disease. Six corneas were grown as explant cultures. Although the cultures were maintained for 6 to 8 weeks with sampling of the supernatant fluid for infectious virus recovery three times per week, we failed to recover any virus in these cultures. Two other corneas were obtained from eyes in which the edema had progressed to a more severe condition and epithelial changes had begun. In these corneal cultures, infectious virus was isolated after 1 week.

Histopathologic examination of the RE-infected corneas showed intraepithelial edema and small areas of bullous keratopathy. In addition, focal chronic interstitial keratitis with proliferation of keratocytes and chronic inflammatory infiltrate (primarily lymphocytes, plasma cells, and a few macrophages) were present within the anterior one-third of the stroma. Numerous small blood vessels were also identified, and although most of the blood vessels were located in the anterior one-third of the stroma, some were observed in deeper layers. The Descemet's membrane and endothelial cell layer failed to show any abnormalities (Fig. 7). No significant histopathologic abnormalities were detected in the F-infected corneas (Fig. 8).

Discussion

The role of immunity, both humoral and cell mediated, in controlling or modifying the course of an infectious disease is well established. In the case of herpes simplex ocular infections, overt disease takes place in the presence of both circulating and locally produced antibodies.

In this study, we compared the specificity and levels of antibodies in the tears of animals infected with two viruses with strikingly different patterns of corneal disease. The HSV-1(RE) strain produces stromal disease,

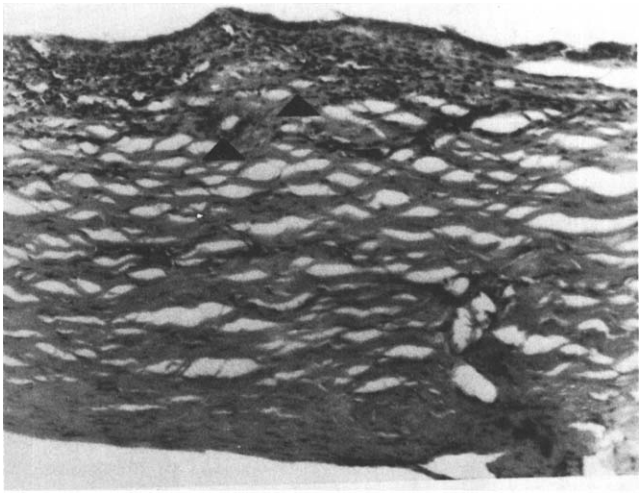

Figure 7. A histopathologic section from an HSV-1(RE)-infected cornea depicting chronic interstitial keratitis and vascularization, primarily involving the anterior one-third of the stroma. Arrows indicate blood vessels (hematoxylin-eosin; original magnification ×100).

and the HSV-1(F) strain produces epithelial disease. We hoped to determine whether the initial antibody response during the primary disease had any correlation with the ensuing disease pattern.

Different amounts of specific IgA/IgM and IgG antibodies were present in tear samples collected sequentially up to 21 days PI from rabbits infected with the F and RE strains. Infection with the HSV-1(F) strain induced the largest amounts of antibodies which reached the maximum value after 14 days PI. The RE strain induced very low amounts of IgA/IgM antibodies. In contrast, both the F and RE strains induced high levels of specific IgG antibodies, although the amount of RE-induced antibodies leveled off after 14 days.

The observation that higher amounts of IgA/IgM antibodies are produced after infection with the F strain, as compared with the levels obtained after infection with the RE strain, implies that these antibodies might have an influence on the duration of the disease. The disease caused by the F strain is self-limiting and confined to the epithelial layer of the cornea; the amount of virus reaches its maximum on Days 5 to 7 PI. In contrast, the RE strain-induced disease is of longer duration, and virus could be cultured up to Day 14 PI (8). The stromal involvement begins thereafter, usually in the presence of high levels of specific IgG.

The specificity of the tear antibodies was measured by immune precipitation, which recognizes undenatured soluble HSV proteins, and by immunoblotting, which recognizes epitopes on denatured proteins. In both cases, with minor variations, the numbers of polypeptides detected were similar (Table I).

The role of tear antibodies in controlling the disease is not well defined. In the initial phase (first 2 weeks), there is a marked difference between the level of IgA/IgM antibodies in the tears of both groups of animals. These antibodies have access to extracellular infectious virus present in the superficial epithelial lesions, but not to cell-associated virus. After primary infection, the IgG class of antibodies was present in rather similar amounts in the tears of animals infected with both F and RE strains, although their disease patterns are very different. Since it is known that virus or virus antigens are present in the stroma of RE-infected animals (10, 28), we can then theorize that the difference in disease manifestations is due to the invasive nature of the RE strain, and that the immunopathology is not due to an inherent difference in levels of specificity of antibodies in tears between these two viruses, but is due to the anatomical site where the viral antigens reside.

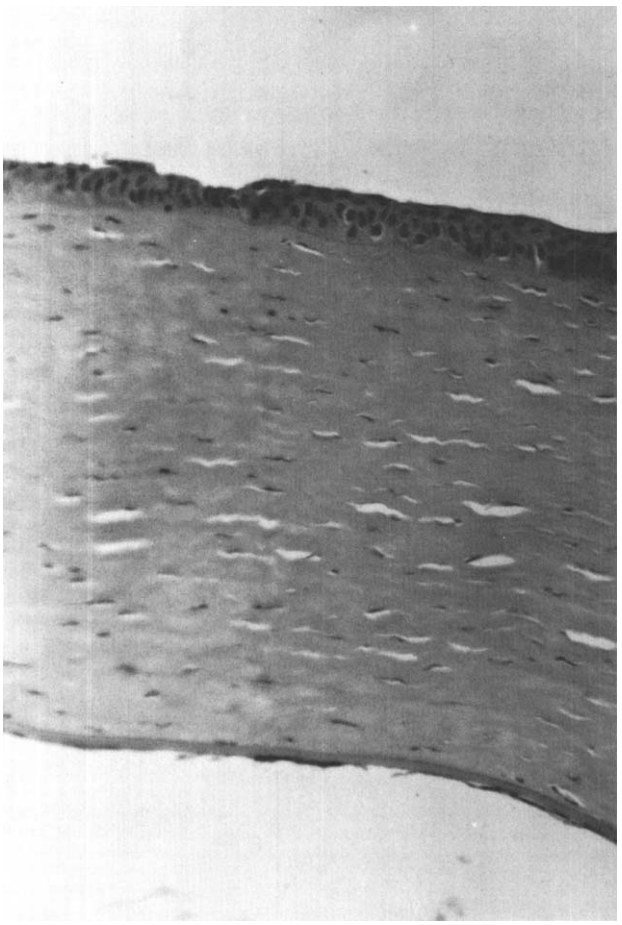

Figure 8. A histopathologic section from an HSV-1(F)-infected cornea at 45 days PI (hematoxylin-eosin; original magnification ×100).

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