Immunopathogenesis of corneal inflammation in herpes simplex virus stromal keratitis: role of the polymorphonuclear leukocyte

Roberta H. Meyers-Elliott and Patricia A. Chitjian

The present studies suggest that polymorphonuclear leukocytes (PMNs) play an essential role in the development of corneal infiltrates in stromal herpes simplex virus (HSV) keratitis. Corneal infiltration was seen rarely in herpes-infected animals treated with anti-PMN serum or with chemotherapy to reduce the numbers of circulating PMNs. By contrast, at least two thirds of the control animals with intact PMNs and infected with herpes virus developed stromal infiltrates. Host complement was localized with HSV antigen and rabbit gamma globulin along with inflammatory cells in the corneas of animals with stromal infiltrates. In the absence of PMN infiltrates, neither complement nor a significant amount of gamma globulin was localized in the corneal stroma. In the PMN-depleted animals, only viral antigen was detected in the stromal keratocytes.

Key words: immunopathogenesis, herpetic stromal keratitis, tissue injury, allergic inflammation, antigen-antibody interaction, complement, polymorphonuclear leukocyte, rabbit cornea

In the course of studies on the pathogenesis of corneal inflammation produced by infection of rabbit corneas with herpes simplex virus (HSV), viral antigen, host antibody, and complement were localized along with inflammatory cells in the corneal stroma during the stage of stromal keratitis. Localized antigen-antibody complexes have been documented to induce increased vascular permeability, leukocytic infiltration, and tissue necrosis in experimental models of immune tissue disease in which bovine serum albumin (BSA)\(^2\) and murine viral injections were used.\(^4\) Tissue damage results from the interactions of these complexes with host humoral, cellular, and tissue factors. The polymorphonuclear leukocyte (PMN) plays an important role in immunologic inflammation processes\(^5\) and is essential for the development of vasculitis and necrosis in Arthus' lesions and in removing antigen-antibody complexes from damaged vessels.\(^6\) The serum complement system is another host factor that is involved in immunologically induced tissue damage.\(^7\) Since the inflammatory cells in the cornea during herpetic stromal keratitis are predominantly PMNs, the role of the PMN in the pathogenesis of corneal inflammation was investigated. This article describes the marked inhibition of
Table I. Experimental protocol for evaluating leukopenia on herpetic stromal keratitis

<table>
<thead>
<tr>
<th>Experimental group (no. of animals)</th>
<th>Experimental day and treatment</th>
<th>Day 1, HSV OD&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Days 3-6, leukocyte-depletion regimen</th>
<th>Day 6, BSA OS&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Days 7-14, leukocyte-depletion regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (20)</td>
<td>+</td>
<td>None</td>
<td>+</td>
<td>None</td>
<td>Systemic anti-PMN globulin&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>II (12)</td>
<td>+</td>
<td>Systemic anti-PMN globulin&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+</td>
<td>Systemic anti-PMN globulin</td>
<td>Subconjunctival anti-PMN globulin&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>III (6)</td>
<td>+</td>
<td>Subconjunctival anti-PMN globulin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+</td>
<td>Subconjunctival anti-PMN globulin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+</td>
</tr>
<tr>
<td>IV (6)</td>
<td>+</td>
<td>Systemic sheep globulin&lt;sup&gt;e&lt;/sup&gt;</td>
<td>+</td>
<td>Systemic sheep globulin&lt;sup&gt;e&lt;/sup&gt;</td>
<td>+</td>
</tr>
<tr>
<td>V (20)</td>
<td>+</td>
<td>Nitrogen mustard&lt;sup&gt;d&lt;/sup&gt;</td>
<td>+</td>
<td>Nitrogen mustard&lt;sup&gt;d&lt;/sup&gt;</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup>HSV administered by topical instillation onto the right cornea. See methods.

<sup>b</sup>BSA intracorneal challenge injection in the left cornea. See methods.

<sup>c</sup>Animals received the first dose of sheep-anti-rabbit PMN globulin systemically. See methods.

<sup>d</sup>Animals received 1 ml of anti-PMN globulin subconjunctivally. See methods.

<sup>e</sup>Animals received normal sheep globulin systemically. See methods.

<sup>f</sup>Animals were given nitrogen mustard, mechlorethamine HCl, intravenously. See methods.

Table II. Clinical course of herpes virus keratitis

<table>
<thead>
<tr>
<th>Group (treatment)</th>
<th>Epithelial disease</th>
<th>Stromal disease</th>
<th>Vascularization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. involved</td>
<td>Clinical severity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No. involved</td>
</tr>
<tr>
<td></td>
<td>(eyes/total)</td>
<td></td>
<td>(eyes/total)</td>
</tr>
<tr>
<td>I (Untreated control)</td>
<td>20/20</td>
<td>4+</td>
<td>12/20</td>
</tr>
<tr>
<td>II (Anti-PMN globulin)</td>
<td>12/12</td>
<td>3+</td>
<td>6/6</td>
</tr>
<tr>
<td>III (Anti-PMN globulin)</td>
<td>6/6</td>
<td>4+</td>
<td>6/6</td>
</tr>
<tr>
<td>IV (Normal globulin)</td>
<td>6/6</td>
<td>4+</td>
<td>6/6</td>
</tr>
<tr>
<td>V (Nitrogen mustard)</td>
<td>20/20</td>
<td>3+</td>
<td>4/20</td>
</tr>
</tbody>
</table>

<sup>a</sup>Score for clinical severity of epithelial disease: 0 = none; 1+ = lesion of any type (punctate, dendritic, or geographic) affecting one quarter or less of total cornea surface; 2+ = lesions affecting more than one quarter but less than one half of cornea; 3+ = lesions affecting one half or more but less than three quarters of cornea; 4+ = lesions affecting three-quarters or more of cornea.

<sup>b</sup>Score for clinical severity of stromal disease (edema and opacity): 0 = none; 1+ = slight edema or surface irregularity with or without slight infiltrate; 2+ = hazy with edema; 3+ = translucent gross clouding with edema and infiltration; pupillary border seen indistinctly; 4+ = opaque cornea with edema and infiltration.

<sup>c</sup>Score for clinical severity of vascularization of cornea: 0 = none; 1+ = limbus vessels enlarged but not extending into cornea; 2+ = vessels extending to localized lesions only; 3+ = vessels extending into cornea along periphery; 4+ = vessels extending throughout entire cornea.

corneal inflammation after PMN depletion of rabbits with HSV keratitis.

Material and methods

**Animals.** Separately caged 1.5 to 2 kg New Zealand albino male rabbits were used throughout this study.

**Antigen and immunization.** Thrice-crystallized BSA (Armour Pharmaceutical Co., Kankakee, Ill.) was injected in complete Freund adjuvant according to the procedure of Cochrane et al. as an immunologic control for PMN depletion in the ocular Arthus reaction to subsequent BSA intracorneal challenge.

**Skin testing.** Following immunization, all animals were tested for a positive Arthus skin reaction to an intradermal injection of 100 μg of BSA nitrogen in a total volume of 0.2 ml. Representative biopsies of the reaction site were made at 12, 24, and 48 hr after challenge and fixed in 10% formalin for hematoxylin and eosin sections. The degree of inflammation in the Arthus site was scored macroscopically according to Cochrane et al. Rabbits with a positive Arthus skin reaction to BSA were selected for this study.

**Corneal inoculation.** For challenge, 0.03 ml of BSA at a concentration of 150 mg/ml was injected into the center of the left cornea, which had been anesthetized locally with 0.05% proparacaine hydrochloride (Ophthaine, E. R. Squibb, Princeton, N.J.)

**Virus.** Type 1 HSV, W strain (initially obtained...
Fig. 1. A, HSV keratitis at day 5 after infection. Note central stromal edema and corneal vessels. B, Day 5 after infection, after HSV corneal infection of PMN-depleted rabbit given systemic anti-PMN globulin. Stromal edema is negligible.

Fig. 2. A, Day 7 after infection. Disciform edema and stromal opacity are apparent. Conjunctivitis is present. B, Day 7 after infection of PMN-depleted rabbit. Stromal edema is present, but stromal infiltrate is absent.

Viral infection. After inducing topical anesthetia with 0.05% proparacaine hydrochloride, the corneas of the right eyes of the experimental BSA-sensitized rabbits were traumatized by rubbing a sterile cotton applicator over the epithelium 10 times in two directions. Two drops of the virus (3 x 10⁶ pfu in 0.1 ml) were instilled on the abraded cornea of the right eye, and the lids held closed for 30 sec. At intervals of 1 to 2 days after infection, the eyes of the experimental animals were examined by means of gross inspection with fluorescein staining and by biomicroscopy. At each examination the degree of epithelial involvement and the degree of stromal involvement (edema, opacity) were recorded.

PMN suppression regimen. The animals used in this study were all systemically sensitized to BSA and divided into five groups. Group I served as a control and received no leukogenic depletion regimen. Group II received specific anti-PMN globulin systemically to deplete circulating PMNs. Group III received anti-PMN globulin subconjunctivally for local periorcular PMN depletion. Group IV received normal sheep globulin systemically as a control for the heterologous sera. Group V received nitrogen mustard systemically to deplete circulating PMNs.

Anti-PMN globulin. Antisera to rabbit PMNs were prepared in a sheep by the method of Cohn...
Fig. 3. A, Day 11 after infection. Corneal stromal opacification with neovascularization. B, Day 11 after infection of PMN-depleted rabbit. Mild stromal edema with peripheral vascularization.

Fig. 4. A, Day 27 after infection. Corneal inflammation has cleared. B, Day 27 after infection. Chronic stromal necrotizing keratitis with vascularization and scarring. C, Day 27 after infection of PMN-depleted rabbit. Clear cornea.

and Hirsch. The final suspension of PMN was washed, adjusted to 20,000/mm³ in saline, and incorporated into incomplete Freund adjuvant for immunization. A 5 ml volume was injected subcutaneously at monthly intervals for 6 months, and periodic bleedings were made after 3 months.

To determine the presence of antibodies in the sheep antisera to the rabbit PMNs, agglutination tests were performed with PMN suspensions as prepared for immunization of the sheep as test cells.

To remove cross-reacting antibodies, the sheep
antisera were absorbed repeatedly with rabbit plasma, red blood cells, and lymph node cells. Agglutinating titers to the PMN species dropped only slightly; whereas titers to the other cells used for absorption were reduced markedly or abolished. The absorbed pooled antisera were fractioned in 40% ammonium sulfate; after dialysis against buffered saline, they were passed through a 0.45 μ Millipore filter (Millipore Corp., Bedford, Mass.) and stored at −40° C. Just before experimental use the globulin solution was ultracentrifuged in a Spinco model L centrifuge (Beckman Instruments, Palo Alto, Calif.) using a 50.1 head at 78,000 × g for 2 hr; the sediment was discarded. Protein concentration determined by the biuret method was in the range of 4 gm/dl.

Pooled normal sheep sera were fractionated and centrifuged in the same manner.

Experimental protocol. The experiment to determine the effects of PMN depletion on experimental herpetic stromal keratitis is outlined in Table I. All animals were systemically sensitized to BSA. On day 1, all animals received a corneal infection with HSV in the right eye. On days 3 through 6, all animals in groups II, III, and V received the PMN-depletion regimens with group I serving as untreated control and group IV serving as a serum control. On day 6, all animals received an intracorneal challenge of BSA in the left eye. On days 7 through 14, groups II, III, and V received a second series of PMN-depletion regimens.

Anti-PMN globulin was used in the experimental rabbits in a dose sufficient to depress the circulating PMNs below 1000/mm³ during the course of the acute HSV corneal infection. Group II animals received the first dose, 6 ml intravenously and 6 ml intraperitoneally, within 72 hr of infection. Subsequent doses were administered intravenously on alternate days. By the time of sacrifice a total of 40 to 60 ml of anti-PMN globulin had been administered systemically. Group III animals received 1 ml of anti-PMN globulin subconjunctivally, with the first dose administered within 72 hr after infection and subsequent doses on alternate days. Group IV animals received normal sheep globulin systemically following the same schedule as group II animals. Group V animals were given nitrogen mustard, mechlorethamine HCl (HN2) (Merck, Sharp, & Dohme, Piscataway, N.J.), intravenously (1.75 mg/kg) in dosages of 0.2 to 1 mg/kg every 3 to 4 days.

During the treatment period frequent total leukocyte and differential blood counts were obtained. Hematologic parameters were followed on alternate days in order to monitor the circulating blood PMNs. Skin tests with 10 and 100 μg of BSA in a total volume of 0.1 ml were performed on representative animals during the treatment regimen to serve as indicators of the degree of PMN suppression.

Histology. Representative animals from each group were sacrificed beginning at day 3 and continuing through day 40. Histologic studies were made on both the HSV and BSA enucleated eyes by examination of formalin-fixed paraffin sections stained with hematoxylin and eosin (H & E).

Immunofluorescence studies. Representative HSV eyes were obtained from rabbits on experimental days 3 through 40. The presence of HSV antigens in the corneal stroma of infected rabbits was studied by applying fluorescein isothiocyanate (FITC)-labeled human immune globulin with anti-HSV neutralizing antibody activity to the corneal sections; likewise, the presence of host antiviral antibody (host IgG) and complement was determined, respectively, by the use of goat anti-rabbit IgG labeled with FITC and guinea pig anti-rabbit complement labeled with FITC. 11

Guinea pig anti-rabbit complement for immunofluorescence studies was prepared according to the procedure of Mardinay and Müller-Eberhard. 12 Goat antiserum to rabbit immune globulin was obtained from Meloy Laboratories (Springfield, Va.) and human immune globulin with antibody activity to HSV was obtained from Lederle Laboratories (Pearl River, N.Y.). Details

Fig. 5A. Day 7 after infection. Cornea from HSV-infected rabbit given anti-PMN globulin subconjunctivally. Severe conjunctivitis and corneal edema.
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Fig. 5B. Histologic section of cornea obtained at 7 days after infection. Plasma cells and lymphocytes are seen at the limbus. (H & E; X400.)

of preparation of the monospecific antisera used and their conjugation to FITC have been described. The fluorescent antibody technique, as described by Coons and Kaplan, was employed with minor modifications. Enucleated eyes were fixed in 10% buffered formalin for 4 hr, followed by the 30% sucrose for 24 to 48 hr according to the procedure of Eidelman and Davis. Next, the corneas were removed, embedded in 2% gelatin, and quick-frozen in a Dry Ice-alcohol bath. The HSV-infected corneas were sectioned in a cryostat, and serial sections stained with the following fluorescein-conjugated antisera: anti-HSV, anti-rabbit Ig, and anti-rabbit complement. Controls included staining of uninfected corneas and staining of infected corneas with fluorescein-labeled anti-BSA antibody. In a prior study not reported here, we employed our fluoresceinated anti-HSV antibody to stain the corneas of rabbits with a BSA immune corneal ring and found no nonspecific staining with our reagent. Blocking studies were performed by the application of unlabeled specific antibody prior to the addition of fluoresceinated anti-HSV antibody.

Antibody. To determine humoral immunologic responses, anti-BSA antibody titers were measured by hemagglutination 2 weeks after the last immunizing injection and at the time of sacrifice. Anti-HSV antibody titers were determined by microradioimmunoassay at the time of sacrifice.

Results

Effect of PMN depletion on the immunologic response. The Arthus skin reactivity to BSA challenge was tested in all groups at 14 to 16 days after treatment. Positive reactions occurred only in the control group (group I), the group receiving normal sheep globulin (group IV), and the group receiving anti-PMN globulin subconjunctivally (group III) (Table II). Skin test sites of treated animals remained negative if the PMN levels were maintained below 1000/mm³.

Antibody levels were determined on the sera when the animals were sacrificed. Anti-BSA antibody titers, as determined by hemagglutination, ranged from 1:128 to 1:2048 and were found to be comparable in both
leukopenic and untreated sensitized controls. The anti-HSV antibody titers were lower but still of a significant titer to neutralize HSV in the leukopenic animals as compared with untreated infected controls (group I). Anti-HSV antibody, as determined by microneutralization, ranged in titers from 1:64 to 1:512 in the nonleukopenic animals (groups I and IV). The leukopenic animals (groups II, III, and IV) had titers of 1:16 to 1:128.

Effect of PMN depletion on the clinical course of HSV keratitis. Scoring of epithelial and stromal disease in HSV-infected leukopenic rabbits is shown in Table II. All eyes had a slight to moderate conjunctivitis. In nonleukopenic control rabbits (group I) punctate epithelial erosion with small dendrites appeared within 48 hr after topical corneal infection with HSV and later progressed to form a dendrite or geographic ulcer. Extension into the central stroma was noted by day 5 (Fig. 1, A) and reached a peak of severity by days 7 to 9 (Fig. 2, A). Epithelial keratitis was clearing by this time. The stromal inflammatory response reached maximum intensity within 11 to 15 days after infection (Fig. 3, A). Iritis appeared between days 4 and 7. The disease resolved within 3 to 4 weeks in most animals (Fig. 4, A). However, some animals had severe corneal opacification with permanent corneal scarring (Fig. 4, B). As compared with the nonleukopenic controls, the PMN-depleted rabbits exhibited less corneal inflammation (Figs. 1, B, 2, B, 3, B, and 4, C; Table II). The area of cornea involved in epithelial keratitis was not significantly smaller (Table II), but the striking difference was the severity of stromal keratitis. Both edema and stromal opacity were reduced (Figs. 3, B, and 4, C; Table II). However, vascularization was still observed in the PMN-depleted animals. The treatment control group receiving sheep globulin intravenously was similar to the untreated control group.

Some of the animals that received subcon-
Table III. Hematologic parameters at time of sacrifice

<table>
<thead>
<tr>
<th>Group (treatment)</th>
<th>Total white blood cells</th>
<th>PMNs</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
<th>Eosinophils</th>
<th>Basophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Untreated control)</td>
<td>10,922/mm³ * (8,300-13,750)*</td>
<td>48.3%</td>
<td>36%</td>
<td>15.6%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>II (Anti-PMN globulin)</td>
<td>1,675/mm³ * (650-2,300)</td>
<td>2%</td>
<td>66%</td>
<td>32.0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>III (Anti-PMN globulin subconj.)</td>
<td>7,008/mm³ * (14,400-10,800)</td>
<td>41.3%</td>
<td>46.6%</td>
<td>13.2%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>IV (Normal globulin)</td>
<td>962/mm³ * (700-1,200)</td>
<td>14.5%</td>
<td>63.0%</td>
<td>22.0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

*Range.

Junctival injections of anti-PMN serum had severe corneal inflammation (Table II and Fig. 5A). This could be due to an increase in vascular permeability of the eye caused by local antibody formation to the sheep antirabbit globulin that was injected in large volumes at multiple times. Histologically, large numbers of plasma cells were noted at the corneal limbus and in the corneas of these animals (Fig. 5B). In the untreated animals, widespread infiltration of PMNs was observed in the infected cornea during stromal keratitis (Figs. 6A and 6B). However, only occasional PMNs were observed in the corneal stroma of the HSV-infected leukopenic animals (Fig. 6C).
Fig. 6C. Corneal stroma from a PMN-depleted rabbit 11 days after topical infection with HSV. Only occasional PMNs are seen in the stroma. (H & E; X250.)

Table IV. Immunologic response to BSA

<table>
<thead>
<tr>
<th>Group (treatment)</th>
<th>No. of animals</th>
<th>Dermal Arthus reaction</th>
<th>Immunologic response</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Untreated control)</td>
<td>20</td>
<td>20/20</td>
<td>18/20</td>
</tr>
<tr>
<td>II (Anti-PMN globulin)</td>
<td>12</td>
<td>0/12</td>
<td>0/12</td>
</tr>
<tr>
<td>III (Anti-PMN globulin subconj.)</td>
<td>6</td>
<td>3/6</td>
<td>3/6</td>
</tr>
<tr>
<td>IV (Normal globulin)</td>
<td>6</td>
<td>6/6</td>
<td>6/6</td>
</tr>
<tr>
<td>V (Nitrogen mustard)</td>
<td>20</td>
<td>0/20</td>
<td>0/20</td>
</tr>
</tbody>
</table>

Effect of PMN depletion on hematologic parameters. Total white cell counts of the circulating peripheral blood lymphocytes were followed on alternate days to maintain circulating blood PMNs below 1000/mm³. Hematologic parameters at the time of sacrifice are shown in Table III. Anti-PMN globulin administered systemically (group II) was the most effective method of keeping the numbers of total PMNs below 1000/mm³. Nitrogen mustard systemically (group V) produced PMN depletion but not as effectively. A relative lymphocytosis occurred in the presence of a leukopenia in these treated animals (groups II and V). Normal globulin (group IV) produced a mild leukopenia and lymphocytosis. Anti-PMN globulin administered subconjunctivally (group IV) was not effective in eliciting leukopenia.

Effect of PMN depletion on corneal ring

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Fig. 7. A, Corneal immune ring appears 24 to 48 hr after intracorneal challenge of BSA-sensitized rabbits. B, No immune ring develops in PMN-depleted rabbits following corneal challenge with BSA.

Fig. 8A. Immunofluorescent localization of HSV antigen along with the inflammatory infiltrate in the corneal stroma of an HSV-infected eye in an animal with normal PMNs. (×200.)

*formation.* A peripheral opaque ring or arc of antigen-antibody precipitation, the Wessely ring, developed in the cornea within 12 to 24 hr after intracorneal challenge with BSA in all sensitized rabbits of group I that did not receive the PMN-depletion regimen as well as in rabbits in group IV that received normal sheep globulin systemically (Fig. 7, A; Table IV). At 48 hr a massive infiltration of PMNs was noted. Clearing of the cornea occurred after about the fourth day. No immune rings were noted in the rabbits that received PMN-depletion regimens with anti-PMN serum (groups II and III) or with nitrogen mustard (group V) (Fig. 7, B, and Table IV). Occasionally a mild transitory corneal infiltrate was noted in the leukopenic animals, but only in animals with total circulating PMN counts exceeding 1200/mm³. The leukopenic animals did not show the striking
Fig. 8B. Immunofluorescent localization of complement along with the inflammatory infiltrate in the stroma of an HSV-infected eye in an animal with normal PMN. (X300.) Similar localization of anti-HSV antibody was noted on an adjacent section not shown here.

conjunctival hyperemia, focal hemorrhage, and edema which occurred in the untreated animals.

Immunohistologic studies on the effects of PMN depletion on corneal inflammation in HSV-infected eyes. Fluorescent antibody studies of HSV-infected corneas revealed the presence of HSV antigens within polymorphs at day 9 after infection (Fig. 8A). Coating the corneal sections with nonfluorescent anti-HSV and then with fluorescent anti-HSV greatly reduced the intensity of the staining. Fluorescent anti-IgG and fluorescent anti-C3 showed similar localization of positive staining within the polymorphs on sequential sections from the corneas that displayed staining for HSV antigens (Fig. 8B). Only occasional PMNs were noted in the corneal stroma of the PMN-depleted animals although there were some inflammatory cells at the corneal limbus. Immunofluorescence staining of these corneas was positive for the presence of viral antigens in the infected keratocytes (Fig. 8C), whereas these corneas did not show fluorescence for antibody and stained only weakly for complement (Fig. 8D).

Discussion
Our study suggests an essential role played by the PMNs in development of ocular inflammation and subsequent tissue injury in herpetic stromal keratitis. We have shown
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Fig. 8C. Immunofluorescent localization of HSV antigens in the central corneal stroma of an HSV-infected eye of animal treated with anti-PMN globulin. (×200.)

that depletion of circulating PMNs resulted in nearly complete abrogation of structural injury in the corneal stroma.

Our study centered on the requirement for PMNs in the inflammatory response in herpes stromal keratitis. The effects of PMN depletion on viral clearance from the cornea were not followed. PMNs not only are essential in the development of the inflammatory necrotizing reactions, as has been observed previously, but also are instrumental in removing antigen from the reaction site, allowing repair of the inflammatory site. In addition, mononuclear cells, lymphocytes, and macrophages in particular, in the absence of inflammatory reactions, have been reported to be important in antigenic catabolism. Since an infectious virus can be isolated from HSV-infected rabbit corneas through days 5 to 7 after infection for most strains of HSV-1, and since it cannot be recovered during stromal keratitis, it would seem that PMN depletion would not affect viral catabolism in eyes with stromal keratitis but certainly could affect antigen processing. The log2 decrease in anti-HSV antibody titer but no decrease in anti-BSA antibody titer in animals receiving PMN-depletion regimens may be explained by antigen presentation to the PMNs and macrophages, since HSV was employed as a particulate antigen and BSA as a soluble antigen.

The mechanism by which neutrophils enter the site where antigen and antibody have localized has been clarified in part. Complement components have been shown to chemotactically attract PMNs to antigen-antibody complexes both in vivo and in vitro. We utilized the Boyden chamber to quantitate neutrophil chemotaxis in vitro in corneal samples from HSV-infected rabbits. We found that lysates of neutrophils obtained only from the stroma of rabbits with HSV stromal keratitis were chemotactic for other neutrophils. We suggested that these lysates, in addition to the chemotactic HSV antigen-antibody complexes, might provide the basis for PMN migration into the corneal stroma in HSV keratitis. We also have shown that depletion of one of the complement components required for neutrophil chemotaxis, i.e., complement component C3, is
Fig. 8D. Corneal stroma of an HSV-infected eye in an animal treated with anti-PMN globulin. Section is stained with fluorescein-labeled anti-rabbit complement. Weak staining was found.

...just as effective as direct depletion of circulating neutrophils in preventing the development of stromal lesions in HSV keratitis. Our preliminary studies showed that rabbits with HSV corneal infection that received treatment with purified cobra venom factor beginning day 3 after infection to deplete C3 had greatly reduced corneal stromal inflammation as compared with untreated animals. Further studies on the effects of complement depletion in the presence of intact PMN function are needed to elucidate the role of complement in the pathogenesis of tissue damage in stromal keratitis.

As a control for neutrophil depletion in the corneal immune ring, we used the Wessely ring model. Intracorneal challenge with BSA into the avascular cornea of BSA-sensitized rabbits is followed by the development of a white ring of opacification in the cornea. Immunohistologically the ring is composed of antigen, antibody, and complement, along with inflammatory cells. Our studies reported here have shown that an immune ring is not formed after intracorneal challenge of BSA in rabbits made neutropenic by anti-PMN globulin or nitrogen mustard. This confirms the earlier report by Germuth et al. who used nitrogen mustard to inhibit the Wessely ring. We have produced similar Wessely rings in the rabbit cornea by the intrastromal challenge injection...
tion of HSV antigens in animals that were systemically sensitized previously. This experimentally produced ring represents a local accumulation of inflammatory PMNs, HSV antigen, host antiviral antibody, and complement. Clinically when ulceration is not present, stromal herpetic may be present as a partial or complete ring infiltrate identical to the Wessely ring phenomenon produced experimentally in corneas of animals and that found in herpetic stromal keratitis in man.

Our results demonstrate that the PMN plays a role as effector of tissue injury and inflammation in deep stromal keratitis associated with the presence of HSV antigens, antiviral antibody, and complement. That the PMNs are essential for this type of tissue injury and inflammation can be demonstrated in our experimental model. When the experimental animals are rendered neutropenic, the stromal infiltrate diminishes. The rabbit eye experimental model of infection may permit a dissection of the inflammatory events from induced immunity in the cornea by the selective manipulation of these components with specific immunomodulators.

REFERENCES

24. Meyers RL and Pettit TH: Chemotaxis of polymor-


Erratum

In Fig. 4, A, of "Retinal vessel photocoagulation: a quantitative comparison of argon and krypton laser effects" by Michael Wieder, Oleg Pomerantzeff, and Julianne Schneider (Invest Ophthalmol Vis Sci 20:418, 1981), a shaded area indicating retinal burns with extensive damage around the vessel and at the level of photoreceptors was poorly reproduced. The corrected illustration (below) is available in reprints.