Ocular Inoculation of Monkeys With Simian Varicella Virus: Clinical and Histopathologic Observations

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Purpose. To explore the possibility that inoculation of the eyes of African green monkeys with simian varicella virus (SVV) induces the symptoms of herpes zoster ophthalmicus (HZO), as seen in humans, and to develop a realistic and reproducible animal model of herpes zoster ophthalmicus for experimental studies.

Methods. In the first experiment, the right eyes of three African green monkeys were inoculated by intrastromal and subconjunctival injections with a suspension of SVV-infected Vero cells. In the second experiment, three additional monkeys were pretreated with intramuscular injections of methylprednisolone (41 mg/kg) for 7 days before ocular inoculation with SVV and for 3 weeks at 14 mg/kg after virus inoculation. The eyes were examined by slit-lamp biomicroscopy. Histologic, immunohistochemical, and electron microscopic studies were performed.

Results. In the first experiment, all three animals developed high titers of anti-SVV antibodies (IgG). Diffuse stromal opacity, with keratic precipitates, stromal edema, and mild vascularization of the cornea, appeared 12 to 14 days after inoculation. The onset of ocular disease was correlated with the rise in serum antibody levels. There was no clinical evidence of a systemic viral infection resulting from the corneal inoculations in these monkeys. In the second experiment, all three animals treated with methylprednisolone developed severe ocular pathology within 1 week of inoculation. The clinical appearance of the diseased eyes strongly indicated that local viral infection had occurred. Dendritiform keratitis, corneal erosion, and stromal necrosis with vascularization of the cornea was seen in all the eyes. The disease resolved within 4 to 5 weeks of inoculation, leaving opaque, vascularized corneas. Histologic studies showed that inflammatory cells and viral antigens were widespread throughout the diseased corneas. A high titer of anti-SVV antibody (IgG) was detected in the immunosuppressed monkeys, but no evidence of systemic viral infection was observed.

Conclusions. The authors propose that inoculation of the eyes of methylprednisolone-treated African green monkeys with simian varicella virus provides an appropriate animal model for studies of the virology and immunopathology of ocular varicella virus infection. Invest Ophthalmol Vis Sci. 1995;36:41-51.

Varicella–zoster virus (VZV) causes an acute, vesicular exanthem in children called varicella, or chickenpox, because the vesicles resemble a chick pea (from the French, pois chicke).1,2 During the period of active replication, the virus invades the sensory nerves, innervating the tissues, and establishes latent infections in the dorsal root and trigeminal ganglia.3–6 Later in life, reactivation of the latent virus in the trigeminal ganglion induces a multiplicity of ocular complications referred to as herpes zoster ophthalmicus (HZO). A particularly severe infection may be seen in patients with malignancy, in those on immunosuppressive drugs, and in patients with acquired immunodeficiency syndrome (AIDS).7–9 The most frequent ocular complications of VZV reactivation include dendritiform keratopathy, stromal keratitis, anterior uveitis, and postherpetic neuralgia.2,10,11 VZV reactivation has also been implicated in the pathogenesis of acute retinal necrosis syndrome.12,13 Patients

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with severe ophthalmic zoster may beg to have the affected eye enucleated, or may contemplate suicide because of the unrelenting pain.14

Varicella-zoster virus can be recovered from dendritic lesions of the cornea during the early stages of the disease, but attempts to demonstrate infectious virus in the eye at later stages have been unsuccessful.15 Histopathologic studies of chronically scarred eyes enucleated from patients with zoster ophthalmicus reveal numerous lymphocytic inflammatory cells,16 suggesting that cell-mediated immunopathology may play an important role in the induction of ocular disease. It has been suggested that anti-VZV antibodies may also contribute to the pathogenesis of ophthalmic zoster.17 For these reasons, there is considerable interest in developing a reproducible animal model of zoster ophthalmicus for experimental studies to define the roles of VZV infection and host immune responses to viral antigens in periorcular disease, keratoouveitis, and postherpetic neuralgia.17 Therefore, a pilot study was performed to determine the feasibility of developing a primate model of herpes zoster ophthalmicus in the African green monkey, using simian varicella virus (SVV) to inoculate the eyes.

MATERIALS AND METHODS

Monkeys
Young adult African green monkeys, *Cercopithecus aethiops*, were obtained from Charles River Primate Center (Port Washington, NY). All experimental treatments and handling of the animals were carried out under veterinary supervision in full compliance with Public Health Service policy and Institutional Animal Care and Use Committee guidelines. The experiments conform to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Simian Varicella Virus
Simian varicella virus was obtained from Dr. Kenneth Soike, Delta Regional Primate Research Center (Covington, LA). The virus grows readily in African green monkey kidney cells (Vero, ATCC CCL 81) with the appearance of typical cytopathic effects characterized by grape-like clusters of round refractile cells. Virus preparations to be used for ocular inoculations were harvested by scraping the flask with a rubber policeman, then washing the cells three times in Hank's balanced salt solution before inoculation of the eyes. The simian varicella virus is highly cell associated, and virus preparations are stored as trypsin-dispersed suspensions of infected cells in 10% dimethyl sulfoxide in a liquid nitrogen freezer. The infectivity of the virus preparations to be used for ocular inoculations was determined by titration of serial dilutions on Vero cells under a methylcellulose overlay in 25-cm² flasks. The number of infectious centers or plaques was counted by phase-contrast microscopy at low magnification. A plaque-forming unit (pfu) is assumed to represent one virus-infected cell, not a single virion. The titer of the virus preparations used for ocular inoculations was adjusted to 5 × 10⁶ pfu/ml.

Preparation of Soluble Simian Varicella Virus Antigen
A cell-free preparation of soluble SVV antigens was prepared by precipitation from the supernatant medium of infected cultures with polyethylene glycol 6000, as described by Forghani et al.18 A precipitate was also obtained from uninfected Vero cells for use as a control.

Immunosuppression of Monkeys
Methylprednisolone sodium succinate for injection (A-Methapred; Abbott Laboratories, Chicago, IL) was administered by intramuscular injection (41 mg/kg) daily for 7 days before ocular inoculation with simian varicella virus and for 2 days after inoculation. On day 10, the dose was reduced to 14 mg/kg, and it was continued at this level until day 22 after inoculation.

Ocular Inoculations
Before ocular inoculations with virus, the animals were anesthetized by intramuscular injection with a mixture of ketamine and xylazine (20 mg/kg ketamine, 5 mg/kg xylazine), followed by topical application of 0.5% proparacaine HCl to the corneas. The virus suspension was injected into the ocular tissues with a 28-gauge needle using an insulin syringe. Injections were made subconjunctivally, intrastromally, into the anterior chamber, or intradermally into the periocular tissues (eyelids) to initiate SVV infection of the eyes.

Virus Assays
At weekly intervals after ocular inoculation with SVV, the monkeys were bled for detection of viremia. The lymphocytes in 3 ml of heparinized blood were separated on a Ficoll-Hypaque gradient (Litton Bionetics, Charleston, SC), washed in Dulbecco's medium containing 10% heat-inactivated newborn calf serum, then cocultured with Vero cells in 25-cm² flasks. The tear film was also assayed for virus shedding. A sterile cotton-tipped applicator was used to swab the inferior cul-de-sac and nasal fornix. The swab was eluted with 1.0 ml of medium, which was then used to inoculate a 25-cm² flask of Vero cells. Homogenates of the trigeminal ganglia were also cocultured with Vero cells. The flasks were examined by phase-contrast microscopy (Olympus [New Hyde Park, NY] inverted research microscope model IMT) for detection of viral antibodies may also contribute to the pathogenesis of ophthalmic zoster.17 For these reasons, there is considerable interest in developing a reproducible animal model of herpes zoster ophthalmicus in the African green monkey, using simian varicella virus (SVV) to inoculate the eyes.
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FIGURE 1. Eyes of monkey 2 inoculated intrastromally and subconjunctivally with (A) Vero cells (left eye) or (B) SVV-infected Vero cells (right eye). Moderate corneal opacity developed in the virus-inoculated right eye on day 12.

cytopathic effects. Negative cultures were blind-passaged to fresh Vero cells after 7 days of incubation.

Biomicroscopy and Photography

Biomicroscopic observations and photography of the eyes and periocular tissues of virus-infected monkeys were made at frequent intervals (2 to 3 times per week) to evaluate the severity of the disease and to record the observed symptoms.

Antibody Titers

Titers of anti-SVV antibody (IgG) in the blood of virus-inoculated monkeys were determined by enzyme immunoassay. The reciprocal of the serum dilution at which the optical density extrapolated to the background reading was taken as the antibody titer.

Histology and Immunohistochemistry

Ocular tissues were fixed overnight in a solution of 4% formaldehyde, 1% glutaraldehyde in phosphate-buffered saline for histology and immunohistochemistry, embedded in paraffin, and sectioned at 10 microns. Alternate slides were stained with hematoxylin and eosin or used for SVV antigen detection by immunoperoxidase histochemistry. Corneas from eyes fixed in 2% formaldehyde and 2% glutaraldehyde were postfixed in 1% osmium tetroxide in phosphate-buffered saline, then embedded in Spurr’s low-viscosity medium (Polysciences, Warrington, PA) and sectioned for electron microscopy. Thin sections (80 nm) stained with uranyl acetate and lead citrate were examined and photographed with a Phillips 301 (Phillips Electronic, Mount Vernon, NY) or Zeiss EM 109 (Carl Zeiss, Thornwood, NY) transmission electron microscope.

RESULTS AND OBSERVATIONS

Experiment 1: Ocular Inoculation of Normal, Untreated Monkeys

The purpose of this experiment was to determine if normal, immunocompetent monkeys were susceptible to SVV infection by the ocular route, and, if so, would they develop symptoms of herpes zoster ophthalmicus. Three young adult African green monkeys (numbers 1, 2, and 3) were each inoculated in the right eye with a suspension of simian varicella virus-infected Vero cells by intrastromal and subconjunctival routes simultaneously. The intrastromal inoculations (10 µl) produced a “bleb” containing 5 x 10^4 virus-infected cells. Approximately 20 µl of cell suspension (10^5 virus-infected cells) was injected subconjunctivally into the same eye. The left eyes were injected similarly with uninfected Vero cells. The animals were examined two to three times each week by slit-lamp biomicroscopy for evidence of ocular disease. There was no evidence

FIGURE 2. Seroconversion of African green monkeys after ocular inoculation with SVV. The rise in serum titer of anti-SVV antibody (IgG) is correlated with the onset of corneal opacity shown in Figure 1. Antigen challenge in the cornea and on the forearms of monkey 2 on day 43 after inoculation boosted the antibody titer of this animal.
of viral infection or ocular disease in either of the
monkeys until day 12 after inoculation, when a moderate
corneal opacity was observed in the virus-inocu-
LATED eyes of two of the animals (Fig. 1). On day 16
after inoculation, the third monkey also developed
moderate corneal opacity. Slit-lamp examination of
the eyes (day 22 after inoculation) revealed disciform
areas of stromal opacity or haze, with thickening of
the cornea and a flocculent infiltrate in the deep stroma.
Blood vessels were seen at all levels in the cornea,
extending from the limbus toward the stromal infil-
trates. Keratitic precipitates were seen in one eye.
There was no evidence of conjunctivitis or scleritis
in either of the eyes, in spite of the subconjunctival
inoculation with SVV-infected cells. The pupils were
normal and symmetric. Aside from the keratitic precipi-
itates, there was no evidence of obvious anterior
chamber reaction. Contralateral (control) corneas in-
oculated with Vero cells were clear, except for scattered
subepithelial infiltrates of unknown origin in
one eye.
On day 43 after inoculation, the corneas were no-
ticeably clearer and less inflamed than on day 22.
Areas of haze were more discrete and localized. Blood
vessels were smaller and fewer in number. However,
areas of interstitial keratitis were evident in the deep
stroma. These symptoms of corneal disease resolved
slowly with time, except for a few "ghost" vessels and
minimal haze near the limbus. There were no clinical
signs of systemic viral infection in these animals at any
time after ocular inoculation with SVV. Blood and tear
film cultures were consistently negative for virus, and
erithema or vesicles did not develop in the animals.
Body temperatures remained normal.

**Antibody Titers**

Antibody to SVV was undetectable by enzyme immu-
noassay on day 5 after inoculation, but high titers were
found on days 14 and 16. As shown in Figure 2, anti-
body titers of 30,000 were found at 5 months after
inoculation and remained at this level for at least 9
months after inoculation. The onset of corneal disease
(stromal opacity and neovascularization), which first
appeared on day 12, correlated with the rise in serum
antibody levels (Fig. 2). On the basis of these observa-
tions, together with the absence of other symptoms or
evidence of virus infection, we suggest that the corneal
pathology observed in these normal, immunocompe-
ten monkeys resulted from an immune (antibody-
mediated) response to the presence of a foreign anti-
gen in the cornea, and not viral infection.

**Antigen Challenge**

If the corneal opacity observed in the SVV-infected
monkeys (Fig. 1) was due to an antibody-mediated
reaction with viral antigens, then one would expect

![Figure 3](image-url)

**FIGURE 3.** Right eye of monkey 3 after intrastromal challenge
with SV at 9 months after the initial ocular inoculation. Before
challenge, the eye was essentially clear. A dense stromal ulcer
with diffuse corneal opacity and vascularization is seen in the
temporal cornea at 24 hours after challenge.

![Figure 4A](image-url)

**Figure 4A.** On the eighth day after inoculation, the right eyelid
was red and swollen, and conjunctival chemosis was evident.
The control eye (left, injected with Vero cells, remained clear.

![Figure 4B](image-url)

**Figure 4B.** A dense, white opacity was seen in the central
cornea of the right eye. Vascularization extended 360° around
the circumference of the cornea; blood vessels were
seen at all levels throughout the stroma.

![Figure 5](image-url)

**Figure 5.** Right eye of monkey 4 after intrastromal challenge
with SV at 9 months after the initial ocular inoculation.
<table>
<thead>
<tr>
<th>Monkey Description</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 8</th>
<th>Day 16</th>
<th>Day 20</th>
<th>Day 24</th>
<th>Day 36</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 4: Anterior chamber and intradermal eyelid inoculations</td>
<td>Thickened, hazy cornea. Whole cornea involved; no focal lesions. Iris swollen and pressed against endothelium at limbus.</td>
<td>Opaque cornea. Watery discharge. Small vesicle on eyelid.</td>
<td>Opaque cornea; conjunctival chemosis. Eyelid red and swollen (Fig. 4A). Monkey rubbing head and pulling right ear.</td>
<td>Opaque cornea with 1 x 1.75-mm epithelial defect that stains intensely with fluorescein.</td>
<td>Opaque cornea; short (1 mm) blood vessels appear at the limbus. Epithelium and stroma unchanged.</td>
<td>Opaque cornea with milky, white center (Fig. 4B). Blood vessels extend through stroma from limbus to central opacity.</td>
<td>Opaque, vascularized cornea with large central epithelial defect.</td>
</tr>
<tr>
<td>No. 5: Intrastromal and intradermal eyelid inoculations</td>
<td>Mild stromal haze with reticular opacity at inoculation site. Mildly injected conjunctiva.</td>
<td>No change in appearance of cornea. Small, red vesicle on eyelid.</td>
<td>Dense, flocculent, full-thickness stromal opacity. Posterior bowing of Descemet’s membrane. Injected, swollen eyelid; diffusely injected conjunctiva.</td>
<td>Opaque cornea with large epithelial defect that stains intensely with fluorescein (Fig. 5B). Severely inflamed right eyelid with vesicle at inoculation site.</td>
<td>No change in cornea. Blood vessels seen at limbus in temporal cornea.</td>
<td>Dense vascularized stromal opacity and epithelial defect in temporal cornea. Scab on eyelid vesicle</td>
<td>Dense, vascularized stromal scar centrally, with large epithelial defect in temporal cornea (Fig. 5C). Healed vesicle on eyelid.</td>
</tr>
<tr>
<td>No. 6: Intrastromal and intradermal eyelid inoculations</td>
<td>Clear cornea, except for reticular stromal opacity at inoculation site.</td>
<td>No change in appearance of cornea. No evidence of viral infection. Eyelid clear.</td>
<td>Mild stromal opacity at inoculation site.</td>
<td>Epithelial defect and dense stromal opacity that stains with fluorescein in temporal cornea. Folds in Descemet’s membrane.</td>
<td>No change. Same as Day 16. Eyelid clear with no evidence of infection.</td>
<td>Dense stromal opacity and epithelial defect temporally. Mild iritis with injection of iris vessels.</td>
<td>Cornea similar to No. 5, but less severe.</td>
</tr>
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</table>
to observe a rapid reappearance of corneal opacity following challenge with SVV antigens by intrastromal injection. To test this hypothesis, a cell-free preparation of soluble SVV antigens (see Methods) was used. On day 43 after inoculation, when the eyes were essentially clear, Monkey No. 2 was given intrastromal and intradermal injections in the right cornea and forearm, respectively, with the antigenic material. A similar preparation obtained from uninfected Vero cells was used to inoculate the left eye and forearm. At 48 hours post-challenge, a dense white opacity surrounded by an area of moderate stromal haze was observed in the right eye. A mild reaction with minimal haze was seen in the left (control) eye. An inflammatory response was not observed in the forearms, however. The corneal opacity slowly resolved with time. As shown in Figure 2, an increase in serum anti-SVV antibody titer of monkey 2 was elicited by the antigen challenge.

At 9 months after inoculation, when the eyes were essentially clear except for a few ghost vessels near the limbus, two of the monkeys (monkeys 1 and 3) were challenged with an intrastromal inoculation of SVV-infected Vero cells. As expected, this resulted in severe stromal opacity and vascularization of the cornea, which appeared within 24 hours after challenge (Fig. 3). A heat-inactivated virus preparation also elicited an immune response in the cornea of monkey 2. Therefore, viral replication was not required for this reaction. The left eyes, injected with Vero cells, remained clear except for mild stromal haze. These symptoms slowly resolved with time but were still evident by slit-lamp examination at 3 weeks after challenge.

**Experiment 2: Effect of Immunosuppression on the Induction and Severity of SVV-Induced Ocular Disease**

It is generally recognized that immunosuppression exacerbates herpes virus infections of the eye. Therefore, three other African green monkeys (monkeys 4, 5, and 6) were immunosuppressed by intramuscular
FIGURE 6. Histologic sections of the right eye of monkey 4 (anterior chamber inoculation with SW). (A) Severe degeneration and keratinization of the epithelium was seen. (B) A massive infiltrate of mononuclear inflammatory cells was seen in the corneal stroma. (C) Note the marked folding of Descemet's membrane. Numerous macrophages were found in the anterior chamber but were not seen in the stroma of this eye.

injections of methylprednisolone (A-Methapred, Abbott) to determine the effect of this treatment on the severity of SVV-induced ocular disease in the monkey.

**Clinical Observations, Monkey 4**

Monkey 4 was inoculated with $5 \times 10^4$ pfu (10 µl) of SVV into the anterior chamber of the right eye and also intradermally into the right eyelid. The left eye was inoculated similarly with uninfected Vero cells. At 2 days after inoculation, a thickened, diffusely hazy cornea without focal lesions was seen in the right eye. The iris was pressed against the endothelium near the limbus. On day 4, the cornea was opaque, a small reddish bump was visible on the eyelid at the inoculation site, and a watery discharge was seen. On day 8, the animal appeared to be experiencing pain in the right eye, occasionally rubbing her head and pulling her right ear. Buprenex (Reckitt and Colman Pharmaceuticals, Richmond, VA) was administered for pain. The right eyelid was red and swollen, and conjunctival chemosis was evident (Fig. 4). The cornea was completely opaque on day 16, and there was a 1 mm × 1.75 mm epithelial defect that stained with fluorescein in the central cornea. On day 20, short blood vessels were seen entering the cornea from the limbus between the 3 o'clock meridian and the 10 o'clock meridian in the inferior cornea. A dense, white opacity was seen in the central cornea. On day 24, the peripheral corneal vascularization extended for 360° around the circumference of the cornea. Blood vessels were seen at all levels throughout the stroma. On day 36, the entire cornea was white and opaque. The left (control) eye remained quiet and clear. These clinical observations are summarized in Table 1.

Monkey 4 was killed on day 37 after inoculation. The virus-inoculated right eye was phthisical, indicated by its small size and shrunken appearance. The left (control) eye was normal.

**Clinical Observations, Monkeys 5 and 6**

Monkey 5 was inoculated with $5 \times 10^4$ pfu of SW by both the intrastromal and intradermal (eyelid) routes. Monkey 6 was inoculated similarly, except that only $1 \times 10^4$ pfu was injected into the cornea. Both animals developed severe stromal keratitis. However, the onset of disease was delayed in monkey 6, which received only one fifth the dose of virus intrastromally as monkey 5. On day 4 after inoculation, there was no evidence of infection or ocular disease in either animal except for a small red vesicle on the eyelid of monkey 5 at the site of inoculation. On day 8 after inoculation, monkey 5 had a severely inflamed right eyelid, with a prominent vesicle at the inoculation site. A dense, flocculent, full-thickness stromal opacity with posterior bowing of Descemet's membrane was present in the temporal cornea. The pupils were equal in size, and there was no obvious anterior chamber reaction. By comparison, monkey 6 had a small subcutaneous hemorrhage of the eyelid and a mild stromal opacity at the inoculation site in the central cornea. On day 16, monkey 5 had a severely inflamed right eyelid with a vesicular lesion at the inoculation site. A large epithelial lesion that stained intensely with fluorescein.
was seen in the temporal cornea. A thickened stroma with folds in Descemet's membrane was evident. The general appearance of this eye suggested that an active viral infection was present in the cornea (Fig. 5). Dilated vessels were seen on the anterior iris surface, but no other evidence of anterior chamber reaction was noted. On day 24 after inoculation, a large epithelial defect was present in the temporal cornea of monkey 5, with a disorganized mass of tissue centrally. The eyelid vesicle had healed and formed a scab. Monkey 6 had a dendritiform lesion in the temporal cornea that stained intensely with fluorescein. A shallow depression of eroded epithelium was seen near the temporal limbus. On day 36 after inoculation, the appearance of the virus-inoculated eyes of monkeys 5 and 6 were similar, except that stromal keratitis was more severe in monkey 5, which had received the larger inoculum. A mass of necrotic tissue was seen near the center of the cornea, with shallow depressions of eroded epithelium in the temporal corneas of both animals. Fascicles of blood vessels extended from the limbus at all levels throughout the cornea. There was little anterior chamber reaction visible in the eyes of these monkeys. Control (left) eyes inoculated with Vero cells remained clear throughout the study. These clinical observations are summarized in Table 1. Monkeys 5 and 6 were killed on day 37 after inoculation. The eyes were fixed in 2% formaldehyde, 2% glutaraldehyde for light and electron microscopic studies.

**Figure 7.** (A) Histologic section of the right cornea of monkey 5 (intrastromal inoculation) showing numerous lymphocytes and pigmented macrophages in the stroma. (B) Histologic section of the cornea from a control eye injected with normal Vero cells. There was no evidence of an inflammatory reaction in this eye.

**Figure 8.** (A) A heavy deposit of SVV antigens was found by immunoperoxidase histochemistry in the cornea of monkey 4. (B) No evidence of viral antigens or inflammation was found in the control (left) eye.

**Antibody Titers**

Anti-SVV antibody titers of Monkeys 4, 5, and 6 were 100,000 on days 20, 29, and 36 after inoculation. These titers are slightly higher than those observed in the immunocompetent monkeys (30,000), as shown in Figure 2. Thus, methylprednisolone did not abrogate the antibody response to SVV infection.

**Histologic and Immunohistochemical Observations**

The epithelium of the virus-inoculated corneas was severely degenerated and keratinized, with invaginations extending deep into the stroma. In the phthisical eye that received the anterior chamber inocula-
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FIGURE 9. Electron micrographs from the right, SVV-inoculated, cornea of monkey 5. (A) Polymorphonuclear leukocytes (PMN) and stromal keratocyte. (B, C) Lymphocytes (L) in close contact with degenerating cells. (D) Virus particle (100 nm diameter) and cell debris in the stroma.

Discussion of Data

Inoculation (monkey 4), Descemet's membrane was folded in an accordion-like pattern. Numerous large pigmented macrophages were seen in the anterior chamber. A massive infiltrate of mononuclear cells, but no macrophages, was seen in the stroma of this eye (Fig. 6). The corneas inoculated by intrastromal injection (monkeys 5 and 6) were heavily infiltrated with inflammatory cells, including numerous large pigmented macrophages in the stroma (Fig. 7). The control eyes, inoculated with Vero cells only, were clear with no evidence of an inflammatory reaction.

A heavy deposit of varicella virus-specific antigen was found in the right cornea of monkey 4 (Fig. 8). Because this eye was inoculated by the anterior chamber route, rather than intrastromally, the presence of SVV antigens in the corneal stroma indicates that viral replication and dissemination must have occurred in this eye. Viral antigens were not detected in the control (left) eye, and no evidence of ocular disease was observed in either cornea or retina.

Virus Assays

Attempts to recover SVV from the blood, tear film, or trigeminal ganglia of the immunosuppressed monkeys were consistently negative.

Electron Microscopy

Lymphocytes, polymorphonuclear leukocytes, macrophages, and an occasional eosinophil were found in the right virus-inoculated cornea of monkey 5 (Fig. 9). The stromal keratocytes were pleomorphic and contained dense lamellae bodies and lipid-filled vesicles. Mononuclear inflammatory cells (lymphocytes) were found in close proximity to degenerating keratocytes and disrupted collagen fibrils. There was no evidence of a bacterial infection in the ocular tissues. However, occasional virus particles were found in the corneal stroma (Fig. 9).

Discussion

Studies of the pathogenesis and therapy of herpes zoster ophthalmicus have been hindered by the lack of
well-defined animal models. Varicella-zoster virus (VZV) infection of the guinea pig cornea was recently described by Pavan-Langston and Dunkel. Although this model could be useful for studies of antiviral chemotherapy, there is no evidence for involvement of the immune system in ocular disease in the guinea pig.

An alternative approach to the study of ophthalmic zoster involving the use of simian varicella virus infection of the monkey eye as an experimental model has been proposed. Simian varicella virus (SVV) causes a varicella-like disease in monkeys characterized by a vesicular skin rash resembling chickenpox.

Also, the simian virus cross-reacts with antibodies against human VZV and shares at least 70% DNA homology with the human virus. SVV DNA was recently identified in trigeminal, cervical, and thoracic ganglia obtained from an African green monkey infected 5 months earlier. Reactivation of latent SVV in monkeys resulting in disseminated varicella infection has also been reported. In addition, the immune cells of the monkey express membrane markers that cross-react with antibodies to the corresponding human antigens. These similarities between the human and simian varicella viruses, and the diseases they produce, suggest that a close phylogenetic relationship exists between them and that information obtained by studying the infection of monkeys with the simian virus can readily be extrapolated to humans.

The experimental observations described in this article indicate that systemic administration of methylprednisolone, followed by ocular inoculation of African green monkeys with simian varicella virus, induces symptoms characteristic of herpes zoster ophthalmicus seen in humans. The absence of systemic or neurologic symptoms of viral infection and failure to isolate VZV from the blood or trigeminal ganglia indicate that the viral infection remained localized in the ocular tissues. Humoral and cell-mediated immunologic processes after an initial viral infection appear to have been responsible for the ocular disease.

Clinical observations of SVV-infected monkeys, carried out in conjunction with extensive virologic, histopathologic, and serologic studies of the same animals, are needed to determine the feasibility of using African green monkeys as a model system for therapeudic studies of VZV ocular infection in humans.

Key Words
simian varicella virus, African green monkey, methylprednisolone, primates, histopathology

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References
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