Experimental *Pseudomonas* keratitis in the rabbit: bacteriologic, clinical, and microscopic observations

Diane L. Van Horn, Starkey D. Davis, Robert A. Hyndiuk, and Harlan J. Pederson

Uniformly severe corneal infections were produced in rabbits by intracorneal injection of a few viable *Pseudomonas aeruginosa*. The bacteria multiplied rapidly, and within 24 hr, about 10 million organisms were present. The numbers remained stable thereafter. Polymorphonuclear leukocytes (PMNs) began to infiltrate peripheral stroma 24 hr after inoculation. By 32 hr, ring-shaped dense accumulations of PMNs were apparent in the anterior stroma with moderate stromal edema. By 48 hr, the anterior one third of central stroma was severely involved with abscess formation and loss of epithelium, and PMNs had invaded full corneal thickness. The area of liquefactive necrosis eventually involved the entire cornea from limbus to limbus, and collagen staining was lost. Transmission electron microscopy revealed the accumulation of small electron-dense particles in association with collagen fibrils and degranulating PMNs.

Key words: bacterial keratitis, cornea, histopathology, *Pseudomonas aeruginosa*, *Pseudomonas* keratitis, rabbit, ultrastructure

A severe corneal infection which progresses rapidly, is difficult to treat, and frequently results in extensive scarring or perforation of the cornea is produced by *Pseudomonas aeruginosa*.1-4 The pathogenesis of experimental *Pseudomonas* keratitis has been studied by light and electron microscopy in rabbit4 and guinea pig6 with conflicting results. Gray and Kreger5 reported that the combination of acute inflammation and liquefaction necrosis seen in *Pseudomonas* keratitis in rabbit 24 hr after injection was due to extensive corneal edema and loss of proteoglycan ground substance, resulting in dispersal of undamaged collagen fibrils, weakening of the cornea, and subsequent descemetocele formation and corneal perforation. In contrast, we6 recently described sequential changes in *Pseudomonas* keratitis in guinea pig and showed that acute inflammation led to progressive liquefactive necrosis and eventual perforation due to apparent collagen breakdown in the vicinity of high concentrations of degranulating PMNs.

The purpose of the present study was to correlate sequential clinical alterations with light and electron microscopic alterations occurring in rabbit cornneas infected in the same manner and with the same strain of *Pseudomonas* as we used in guinea pigs.6

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Table I. Multiplication of *Pseudomonas* strain 107 in rabbit cornea*

<table>
<thead>
<tr>
<th>Time</th>
<th>Corneal colony counts</th>
</tr>
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<tbody>
<tr>
<td>1 day</td>
<td>6.94 ± 0.38 (7)</td>
</tr>
<tr>
<td>2 days</td>
<td>6.99 ± 0.31 (8)</td>
</tr>
<tr>
<td>3 days</td>
<td>5.73 ± 1.01 (7)</td>
</tr>
<tr>
<td>4 days</td>
<td>5.67 ± 0.89 (6)</td>
</tr>
<tr>
<td>7 days</td>
<td>5.71 ± 0.57 (2)</td>
</tr>
</tbody>
</table>

Values are mean ± S.D.; number of samples in parentheses.

* A total of 16 rabbits were infected intracorneally in two trials with about 36 colony-forming units. Numbers of viable bacteria are expressed as common logarithms (base 10).

Materials and methods

New Zealand white rabbits, weighing 2 to 3 kg, were fed rabbit feed and tap water ad lib. After topical and general anesthesia, 48 rabbits were infected intracorneally with 20 μl of a dilute broth of viable *P. aeruginosa* strain 107 adjusted to contain about 10 viable organisms.

Eight infected eyes were examined grossly and biomicroscopically before and at frequent intervals for 4 days after infection by an ophthalmologist (R. A. H.). In control studies, two corneas were inoculated intracorneally with heat-killed bacteria. There was no stromal edema or inflammatory infiltrate, and the control corneas remained clear throughout the study (except for linear, opaque inoculation tracts, which disappeared by 4 days).

For quantitation of the bacteria, infected animals were sacrificed at intervals, and numbers of viable bacteria in the cornea were determined.\(^8\)\(^7\) Two trials with a total of 16 animals were done. Results in the two trials did not differ by analysis of variance, and the data were pooled.

For light and electron microscopic studies, animals were sacrificed at 8 hr intervals from 8 to 96 hr after intracorneal infection with viable *Pseudomonas*. Two animals were sacrificed at each time period, for a total of 24 animals. The corneas were removed, and one of each pair was fixed in...
## Table II

<table>
<thead>
<tr>
<th>Time after infection (hr)</th>
<th>Clinical observations</th>
<th>Histologic observations</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Epithelium</td>
</tr>
<tr>
<td>16</td>
<td>Corneas clear except for linear opacity of inoculation tract</td>
<td>Intact</td>
</tr>
<tr>
<td>24</td>
<td>Stromal edema in all 8 eyes; 4 eyes containing dense anterior stromal infiltrates; 1 containing diffuse infiltrate from limbus to limbus; flare and cells in anterior chamber</td>
<td>No large areas of epithelial involvement; small focal ulcerations of peripheral epithelium; inflammatory cells on or near basement membrane and on anterior surface of intact epithelium</td>
</tr>
<tr>
<td>32</td>
<td>Not made</td>
<td>Epithelium completely missing over corneal abscesses but intact over areas with less stromal involvement</td>
</tr>
<tr>
<td>48</td>
<td>6 of 8 eyes with ring-shaped areas of infiltration and edema; infiltrate whitish yellow and dense in anterior stroma</td>
<td>Epithelium completely stripped off of underlying abscesses that extend almost from limbus to limbus; intact epithelium still found on small and less edematous areas of peripheral cornea</td>
</tr>
<tr>
<td>56-96</td>
<td>Dense necrotic infiltrates from limbus to limbus in all eyes; epithelium and anterior stromal ulcerated, resulting in early ectasia, by 4 days corneas soft and severely ectatic; anterior chamber details obscured</td>
<td>No epithelium present on any of the specimens from these time periods</td>
</tr>
</tbody>
</table>

buffered formalin for light microscopy, and the other in buffered glutaraldehyde for electron microscopy. Paraffin-embedded sections were prepared and stained with hematoxylin and eosin and Masson’s trichrome collagen stain. For electron microscopy, the corneas were fixed and processed as previously described for guinea pig corneas and flat-embedded in a low-viscosity epoxy resin. Ultra-thin sections were stained with uranyl acetate and lead citrate.
Results

Quantitative bacteriology. Numbers of viable bacteria in rabbit corneas after infection are shown in Table I. Organisms multiplied rapidly in the first 24 hr, and numbers then remained relatively stable.

Clinical and microscopic observations. The clinical and light and electron microscopic alterations at various times after infection of the corneal stroma with P. aeruginosa are described in Table II and Figs. 1 to 7.

Discussion

Intracorneal injection of about 10 viable P. aeruginosa strain 107 into rabbit corneas was uniformly followed by rapid multiplication of bacteria in the first 24 hr. The numbers of Pseudomonas organisms then remained stable for several days and were similar to those observed in guinea pigs infected with the same strain.6, 7

Early ring-shaped abscess formations were made up of PMNs and limited to the anterior one third of the stroma. The abscesses were associated with loss of the overlying epithelium. Liquefactive necrosis and gradual enlargement of the infiltration to include central cornea eventually resulted in destruction of the entire cornea. The increase in corneal thickness as well as damage to the corneal stroma, as observed by both light and electron microscopy, were closely associated with the infiltration and degranulation of the PMNs and lagged considerably behind the early rapid increase in the numbers of Pseudomonas.
Ultrastructural evidence of collagen breakdown (tactoid formation and accumulation of amorphous electron-dense material), such as we described in the guinea pig, was not seen in rabbit corneas. Instead, accumulation of variable-sized electron-dense particles was seen within the abscesses in association with collagen fibrils and degranulating PMNs. With increasing time after inoculation, the electron-dense granules were found over an increasingly larger area of the stroma. The particles may be breakdown products of collagen and/or proteoglycans. Similar accumulations of electron-dense particles have also been described in unpublished electron micrographs by Gray and Kreger (personal communication).

Apparent loss of collagen staining was seen in histologic sections, but this could have been due to the presence of corneal edema. We were unable to determine the roles of collagen and/or proteoglycan alterations in the observed liquefactive necrosis in rabbit. Gray and Kreger reported that a loss of proteoglycan-ground substance caused a dispersal of undamaged collagen fibrils in experimental Pseudomonas keratitis in rabbits; however, edema may also cause proteoglycan dilution, and their data are therefore difficult to interpret.

By electron microscopy, undigested bacteria were found in completely degranulated PMNs as long as 88 hr after inoculation. This sequestration may be an explanation for the persistence of Pseudomonas in the cornea despite chemotherapy. Relapse of Pseudo-
Fig. 4. Area of confrontation between the bacteria and PMNs in central anterior stroma of 32 hr specimen. Most collagen fibrils appear to be intact. Some bacteria (arrow) are engulfed by PMNs, but four others remain free in the stroma. (×9900.)
Fig. 5. Aggregation of degenerating and necrotic PMNs found 40 hr after inoculation. Accumulation of small electron-dense particles of various sizes was apparent in such abscesses. (×7300.)
**Fig. 6.** Higher magnification of indistinct collagen fibrils and electron-dense particles in same specimen as Fig. 5. Bacteria found free in the stroma appear to possess a glycocalyx not seen at earlier times. (x39,000.)

*Pseudomonas* keratitis has been reported in humans after apparently adequate chemotherapy.9–11 Electron microscopy of bacteria found free in the stroma of rabbit corneas revealed that they were not surrounded by a clear or electron-lucent area as was the case with bacteria found in guinea pig corneas. However, as the keratitis in rabbit progressed, bacteria were encompassed with a glycocalyx not seen at earlier times and never seen at any time during the course of the disease in guinea pigs. No significant proteolytic alterations were observed in association with bacteria alone.

The results of this study indicate that the damage to the corneal stroma is most likely mediated by proteolytic enzymes released by the PMNs, as is the case in many inflammatory conditions of the cornea.12 Epithelial and stromal cell collagenases may also be contributory.

**REFERENCES**


Fig. 7. High magnification of collagen fibrils and electron-dense granules in specimen 72 hr after infection. (x29,000.)


