Experimental staphylococcic keratitis

Michael J. Hogan, Victor Diaz-Bonnet, Masao Okumoto, and Samuel J. Kimura

The human clinical manifestations of staphylococcic infections of the eyelids, conjunctiva, and cornea are discussed. The corneal lesions occurring during the course of staphylococcic infections of the lid develop after the disease has been present in the eyelid for several years, but are not always correlated with the degree of activity. The peripheral infiltrations and ulcerations either are due to hypersensitivity or are a result of some product produced by Staphylococcus aureus. Repeated instillations of staphylococcic antigen and toxin onto the corneas of nonimmunized rabbits failed to produce the superficial epithelial keratitis which is so common in human beings with chronic staphylococcic infections of the lid. Injection of a small challenging dose of staphylococcic antigen into the center of the corneas of rabbits, which had been immunized over a period of time with subcutaneous staphylococcic antigen or toxoid, produced corneal rings resembling those in antigen-antibody reactions of the cornea. The incidence of rings was twice as common in those animals immunized with killed and disrupted staphylococci as in those immunized with toxoid. The ring infiltrations which were produced appeared within 24 to 48 hours, were complete or incomplete, lasted 3 to 5 days, and gradually disappeared without scarring. A second ring developed in a few animals. Intracorneal injection of similar doses of toxoid into another series of similarly immunized animals failed to produce ring infiltrations.

The staphylococcus is one of the most important eye pathogens. Lesions produced by this organism on the lid, conjunctiva, and cornea have been studied for many years.

Cutaneous staphylococcic infections affecting the lids, such as the pyoderma which produce folliculitis, furuncles, and cellulitis often are associated with secondary conjunctivitis and keratitis. Involvement of the lids in other cutaneous diseases, such as acne rosacea, is frequently associated with secondary staphylococcic infections. Most often, however, the lid margins are affected primarily by a staphylococcic infection which produces blepharitis. Seborrheic blepharitis is another common disease of the eyelid which often becomes secondarily infected with pathogenic staphylococci. The infection in blepharitis may extend to the conjunctival sac to produce a conjunctivitis which is either acute or chronic. Alterations of the conjunctival inflammation may or may not occur with exacerbations and remissions of the inflammation of the lid. Since staphylococci are not epithelial parasites, it has been postulated that they produce keratoconjunctivitis through the action of potent exotoxins. Allen showed experimentally that Staphylococcus aureus toxin, when...
dropped into the eye, can result in superficial punctate erosions of the corneal epithelium.

Except for fine diffuse punctate superficial epithelial erosions which stain with fluorescein, the human cornea rarely is affected during all these staphylococccic infections. Occasionally, during the course of some infections of the lid however, marginal corneal infiltrations and ulcerations occur. Most often cultures from the ulcers show no growth of organisms. It has been postulated that these peripheral corneal lesions are due to the effects of staphylococccic toxin, either as a direct toxic effect, or to hypersensitivity. Woods and Burky demonstrated that hypersensitivity to staphylococccic toxin may produce conjunctivitis. Very little, however, has been done to explain the pathogenesis of the corneal lesions in human beings. Germuth and coworkers showed that an opaque ring could be produced in the peripheral cornea of the rabbit with bovine serum globulin and albumin. This demonstrates that immunologic phenomena can be produced in the corneal stroma of experimental animals. The Wessely phenomenon in experimental animals also provides evidence that hypersensitivity plays a part in the development of experimental infiltrations of the peripheral cornea.

This study is one of a series designed to develop an experimental model in rabbits to gain further information on the pathogenesis of staphylococccic keratitis.

**Clinical manifestations**

It is known that corneal lesions are uncommon in children. It also is known that in cases of staphylococccic blepharitis the lid inflammation may be mild at the time the keratitis develops.

The epithelial keratitis most often produces mild symptoms, such as smarting or burning. It is characterized by punctate staining, which involves the lower half of the cornea, but occasionally it is more diffuse, even involving the upper limbal area. The staining varies in intensity from time to time, especially in the chronic varieties of conjunctivitis. It tends to be more pronounced on arising in the morning, when the symptoms also are worse. During the day the erosions heal and symptoms diminish. Scrapings of the lid margin usually show an abundance of gram-positive cocci, and cultures verify the presence of pathogenic *Staphylococcus aureus*. Conjunctival scrapings show epithelial cells, lymphocytes, polymorphonuclear leukocytes, and, at times, staphylococci.

Peripheral marginal infiltrations and ulcerations also may appear during the quiet or active phase of the blepharitis. Thygeson found *Staphylococcus aureus* on the lids in 133 of 156 catarrhal ulcers associated with chronic catarrhal conjunctivitis, and indicated the importance of conjunctivitis in the production of the corneal lesions. Single or multiple gray, rounded, or crescentic lesions may develop in the peripheral corneal stroma, separated from the limbus by a narrow rim of normal corneal tissue (Fig. 1). Any portion of the peripheral cornea may be affected, and at times the multiple lesions extend to form partial ring infiltrates. Only occasionally a complete ring infiltration occurs around the corneal periphery. The infiltration is 1 to 2 mm. in breadth, and rarely extends deeper than the midstroma. Ulceration is common (catarrhal ulcer). Recurrences are common over a period of years. Vascularization occurs from the limbus. Cultures from the ulcers are negative. Cytologic studies show acute inflammatory cells and necrotic corneal debris. There is a rapid response of the lesions to corticosteroid therapy. This suggests either that the lesions are due to some type of antigen-antibody reaction or that they are toxic and are benefited by the anti-inflammatory corticosteroid effect.

Similar corneal ulcerations have been reported rarely with *Moraxella lacunata* and by *Hemophilus aegyptius*, but in these conditions the organism can be recovered from the conjunctiva.

Thygeson reported 14 cases of ring in-
filtration and ulceration of the cornea in his series. The flora of the conjunctiva and lid margin was normal in 12 cases, and in 2 a coagulase-positive staphylococcus was found. It is known that similar lesions can also occur during the course of bacillary dysentery, periarteritis, and ulcerative colitis.

Materials

Antigen. A strain of mannitol- and coagulase-positive, hemolytic *Staphylococcus aureus*, isolated from the lid of a patient with an external hordeolum was used. The organism was inoculated on nutrient agar, incubated for 24 hours at 37° C., and harvested by scraping the colonies and suspending the organisms in saline solution. The resulting suspension showed a plate count of 10^10 live organisms per milliliter. A portion of this antigen was heated for 2 hours at 60° C. for immunization of some rabbits. It was found that heating the suspension for 2 hours at 60° C. failed to kill all the organisms. Therefore, the freshly prepared suspension was placed in a sonic oscillator* for 2 hours at 0° to -3° C. The frequency was 10 kilocycles per second at 250 watts. This failed to kill all organisms. Therefore, the suspension was centrifuged at 1,000 r.p.m. for 10 minutes. The supernatant fluid was then passed through a millipore filter (H.A. 0.47 micron pore size). The filtrate was cultured and found to be sterile. It was placed in a sterile container and refrigerated at 4° C.

Toxoid. A commercial preparation of toxoid* containing 10,000 units per milliliter was used.

Toxin. Crude *Staphylococcus* toxin† was used (Lot No. 42925-199), and was preserved with thimerosal 0.01 per cent.

Rabbits. Black Dutch rabbits of medium size were purchased from various animal suppliers. The eyes were examined with a slit lamp prior to carrying out the experimental work. All had normal corneas.

Methods

Immunization. Immunization with *Staphylococcus aureus* was carried out as follows: A partially heat-killed suspension was mixed with an equal volume of Freund's complete adjuvant. One milliliter of this mixture was given subcutaneously each week for a total of 6 weeks. Agglutinin titers were determined at the end of 6 weeks, and were positive in all animals at a titer of 1:32 or more.

Immunization of another series of rabbits was carried out by giving 1.0 ml. of toxoid and Freund's adjuvant subcutaneously each week for 6 weeks. The presence of precipitin antibody to toxin was demonstrated by use of the agar diffusion technique. The results showed strongly positive bands with all sera.

Challenge. The immunized and normal control rabbits were challenged either by local instillation

*Raytheon Model DF 101.
†Lederle Laboratories, Pearl River, N. Y.
‡Difco Laboratories, Detroit, Mich.
or intracorneal injection of the antigen and its corresponding control fluid.

Local instillation. One drop of sonically disrupted and filtered staphylococcus antigen was applied to the right cornea of 3 of the staphylococcus-immunized rabbits from a 1 ml. Luer tuberculin syringe every 5 minutes for 10 times. The left eyes of these rabbits were treated similarly with sterile normal saline. Three nonimmunized rabbits were given the same antigen in the same way to the right eye and sterile normal saline solution was instilled into the left eye. Three of the toxoid-immunized rabbits were challenged in the right eye by instillation of the crude staphylococcal toxin. As a control, a solution of 0.01 per cent thimerosal (the preservative for the toxin) was instilled in the left eyes. At the same time 3 nonimmunized rabbits received drops of toxin in the right eye and thimerosal in the left eye in the same manner.

Intracorneal injection. (1) Thirty-one eyes suitable for challenge were selected from the group of rabbits immunized with the staphylococcal antigen. A 1 ml. tuberculin syringe with a 30-gauge needle was used to make an injection of 0.01 to 0.02 ml. in the center of the corneal stroma. Twenty-four corneas were challenged with the staphylococcal antigen, 4 with undiluted crude toxin, and 3 with normal saline.

(2) Similarly, 68 rabbit eyes immunized with toxoid were challenged by intracorneal injection as follows: 23 corneas with staphylococcal antigen, 12 corneas with commercial staphylococcal toxoid, 21 corneas with varying dilutions of crude toxin, 6 corneas with 0.01 per cent thimerosal, and 6 corneas with sterile normal saline.

(3) Twenty normal nonimmunized rabbit eyes were also injected with each of the challenge fluids; 4 eyes were used for each fluid. All animals were subjected to careful prechallenge examination by ordinary illumination and with a slit lamp. Subsequent examinations were carried out immediately after injection and daily thereafter.

Results

Local instillation. The conjunctival instillation of staphylococcal antigen in both immunized and nonimmunized rabbits produced a very mild conjunctivitis for several hours after the drops. Corneal punctate staining failed to occur in any of the animals. The instillation of crude staphylococcal toxin in toxoid-immune and normal rabbits failed to produce any corneal changes.

Intracorneal instillation (Table I).

Staphylococcus-immunized rabbits. Thirteen of the 31 eyes challenged by intracorneal injection developed a ringlike corneal opacity which was thought to be an antigen-antibody reaction (Fig. 2). The 13 eyes which developed a ring were all in the group challenged with staphylococcal

<table>
<thead>
<tr>
<th>Challenged with</th>
<th>Toxin</th>
<th>Toxoid</th>
<th>Staph. filtrate</th>
<th>Saline</th>
<th>Control fluid</th>
<th>Ring</th>
<th>Uveitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus-immunized (31 eyes)</td>
<td>4</td>
<td>24</td>
<td>0</td>
<td>13</td>
<td>23</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>&quot;toxoid-immunized (68 eyes)</td>
<td>21*</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>21</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Normal (20 eyes)</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4 (with keratitis)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Of the 21 eyes 3 received undiluted toxin; 3, a 1:1 solution; 8, a 1:4 solution; and 7 a 1:8 solution.
antigen. All 24 which received this antigen had a coincident marked uveitis with fibrin deposits, aqueous flare, and cells. The saline-injected corneas cleared within a few hours after the injection and remained clear. The 4 eyes challenged with crude toxin developed marked uveitis, keratitis, and corneal necrosis at the site of injection.

Toxoid-immunized rabbits. Six of the 68 eyes challenged intracorneally developed a ring peripheral to the injection site. The 6 eyes were in the group of rabbits challenged with staphylococcal antigen. All rabbits, however, developed a marked uveitis, except for those challenged with normal saline and 0.01 per cent thimerosal.

Normal rabbits. None of the 20 eyes used developed a ringlike reaction. Five out of 20 eyes developed a uveitis. Four eyes injected with toxin had corneal necrosis and a marked uveitis. One eye challenged with staphylococcal antigen developed a very mild uveitis.

Discussion
The failure of the nonimmunized rabbits to develop epithelial keratitis after repeated instillations of staphylococcal antigen as well as toxin is puzzling. Allen² was able to produce conjunctivitis and an epithelial keratitis by the repeated local instillation of toxin into the conjunctival sac of various animals and human beings. We are repeating this experiment in animals with toxins from known strains of pathogenic staphylococci. It is possible our results differ from those of Allen² because of the method of preparation of the antigen and the toxin.

At the present stage it is impossible to correlate the observations in this series of experimental animals with the type of lesion seen in human eyes. A number of differences are noted, principally in the development of the rings, their location, and method of fading. In the human eye the peripheral cornea is believed to be the site of the antigen-antibody reaction. Except for circumferential spread, lesions in human beings do not migrate once they are fully developed, whereas in the rabbit the corneal ring varies in position. Most often a single ring forms, and, as successive rings are formed, they seem to migrate...
toward the central cornea before fading completely.

The rings observed in the corneas were either complete or incomplete, appeared within 24 to 48 hours, were visible for 3 to 8 days, and varied in breadth and density. Corneal scarring did not result at their sites of appearance. Some scarring nearly always occurred at the site of injection.

It is apparent that only the intracorneally injected staphylococcal antigen produced corneal rings in both the staphylococcus as well as the toxoid-immunized animals. Toxoid is a crude antigen containing modified toxin as well as other products of staphylococcal cell bodies. It is demonstrated by this study that immunization can be carried out with toxoid. The failure of intracorneally injected toxoid to produce rings in immunized animals may be due to the small amount of antigen injected. As evidence of the greater effectiveness of the staphylococcal bacterial antigen, corneal rings developed about twice as frequently in the staphylococcus-immunized group as in the toxoid-immunized group.

The keratitis in the affected animals was accompanied by edema, haziness, and thickening of the stroma. It lasted for several weeks, then healed. The uveitis produced by toxin injected intracorneally was interesting in that it ran a violent course, at times with an associated hypopyon. Toxoid given to nonimmunized animals produced no uveitis, whereas in immunized animals it produced uveal inflammation.

Microscopic examination of the anterior portion of the eyes of the affected rabbits showed the cornea to be markedly edematous and thickened. There was a diffuse infiltration of polymorphonuclear leukocytes. These cells formed heavy aggregations at the site of ring formation. The anterior chamber contained a considerable number of acute and chronic inflammatory cells and the iris also showed considerable acute inflammation.

REFERENCES