preclude the contact between these enzymes and membrane phospholipids with a consequent reduction in the availability of arachidonic acid for PG synthesis and release. This hypothesis is supported by the evidence that only pharmacological doses of corticosteroids which stabilized lysosomes also inhibited PGE release.

In addition, corticosteroids may reduce the availability of arachidonic acid by several alternative mechanisms, such as (1) inhibition of the release of cell membrane phospholipase, (2) inhibition of phospholipase activity, and (3) interference with the transport of the newly formed arachidonic acid to the microsomes.

Since CS and aspirin-like drugs act on separate loci of PG biosynthesis, it is suggested that combined treatment with both types of drugs, in submaximal doses, may prove beneficial in ocular inflammation.

We are grateful to Dr. F. Kohen for supplying us with anti-PGE serum.

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Pathogenesis of corneal damage from Pseudomonas exotoxin A. BARBARA H. IGLEWSKI, ROBERT P. BURNS, AND ILENE K. GIPSON.

Pseudomonas aeruginosa exotoxin A was injected into rabbit corneas. Death of epithelial, endothelial, and stromal cells resulted, and necrosis of the cornea followed. Control eyes with exotoxin neutralized by specific antitoxin showed minimal damage. A dose-response pattern was evident. Antitoxin neutralization of pseudomonas exotoxin A in corneal ulcers may have possible therapeutic implications.

Pseudomonas aeruginosa, an opportunistic pathogen, is a common cause of severe corneal infections which progress rapidly and are very destructive. While endotoxin-induced damage from bacterial cell walls is the usual mechanism of gram-negative bacterial injury, a number of studies have implicated extracellular pseudomonal proteases as the substances responsible for corneal damage. Recently Liu produced from Pseudomonas aeruginosa a heat-labile protein exotoxin (exotoxin A) which is lethal for many experimental animals and cultured mammalian cells. Exotoxin A has the same enzymatic activity as diphtheria toxin fragment A. Both toxins catalyze the transfer of the adenosine diphosphate ribose (ADP-R) portion of nicotinamide adenine dinucleotide (NAD) to mammalian elongation factor 2 (EF-2), thereby inhibiting cellular protein synthesis. This preliminary report de-
scribes our studies on the effect of pseudomonas exotoxin A on rabbit eyes.

Methods. *Pseudomonas aeruginosa* (PA103) was used for the production of exotoxin A. Exotoxin A was produced and purified as previously described, with the following modification. The (NH₄)₂SO₄ precipitated toxin was chromatographed on columns of Sephadex G200, then DEAE-cellulose. The purified toxin had a mouse median lethal dose (LD₅₀) of 1 µg per 22 grams of Swiss Webster mice when injected intraperitoneally and contained approximately 0.01 µg of endotoxin per gram of protein. The toxin did not contain detectable protease as assayed by liquefaction of gelatin, or destruction of the enzyme activity of the toxin at 52°C. Pony antiserum to exotoxin A was kindly provided by Liu.

Rabbits, albino or Dutch belted, 2 to 3 kilograms in weight, were anesthetized by intramuscular ketamine supplemented by topical proparacaine. The eyes were exposed with a lid speculum and intracorneal injections were made with a 30 gauge needle from a 1 c.c. tuberculin syringe. Exotoxin A was administered in 2.5, 1.0, and 0.1 µg amounts, diluted to 0.1 ml. volume in one eye, and the same weight and volume of exotoxin, neutralized by specific antitoxin, was injected into the other eye. The needle was placed just into the corneal stroma, approximately 0.01 ml. injected, then the needle advanced into the resultant bleb while the rest of the 0.1 ml. dose was injected, resulting in a milky bleb that covered approximately two thirds of the cornea. Some of the injected material leaked out through the injection site, but generally at least two thirds to three fourths remained intrastromally. Within a few hours the edema of the injection cleared. Eyes were examined biomicroscopically daily, then photographed and enucleated for pathologic study at 1, 3, and 10 day intervals. Comparisons described are between high dose (2.5 µg of toxin) and low dose (0.1 µg of toxin) and controls.

Results. One day after toxin injection, by biomicroscopy the entire cornea was cloudy and so grossly thickened the iris was barely visible. There was sometimes a purulent discharge on the eyelids, and an abscess at the injection site, and the needle track could be seen easily. The reaction may have been more intense in albino than the Dutch belted rabbits but this is difficult to ascertain with the number of experiments performed to date, since exact quantitation in small numbers is difficult with either biomicroscopic or histopathologic preparation. Control eyes showed only the trauma of injection at the needle track and slight stromal opacity. Rabbit corneas injected with low doses of exotoxin A were much less opaque than those given the high dose, and there was less conjunctival hyperemia and discharge.

Histopathologic examination of the whole cornea in formalin-fixed, hematoxylin and eosin-stained eyes of high-dose-treated rabbits showed complete absence of epithelium and endothelium and almost total absence of keratocytes on the first day (Fig. 1). At the corneal periphery a few cells remained. Sometimes an abscess appeared at the injection site, composed of rabbit polymorphonuclear leukocytes (PMN) or pseudoeosinophils, but PMN's did not always migrate into the cornea the first day. The low-dose exotoxin A eyes also

![Fig. 1. Central cornea of high-dose toxin-injected rabbits at 24 hours with loss of epithelium and endothelium (A) and only minimal remnants of keratocytes (C). Antitoxin-neutralized exotoxin corneas show normal cellular pattern (B). (A and B x30; C x102.)](image-url)
Fig. 2. Three days after high-dose exotoxin injection, epithelium and endothelium absent and stroma infiltrated with leukocytes. (×102.) Dead keratocyte being phagocytized by PMN, with collagen bundles remaining intact. (×12,000.)

showed almost total absence of corneal cells in the center but at the periphery endothelial, epithelial, and stromal cells persisted. Acid mucopolysaccharide (AMP) staining, by a colloidal iron technique, showed very marked increase in blue staining, principally around the area where keratocyte nuclei had been.

Electron microscopic examination of plastic-embedded corneas showed de-epithelialized anterior cornea with intact subepithelial basement membrane and collagen bundles. At the periphery of the ulcer a few epithelial cells remained, but these had notable lipid globules and vacuoles in the cytoplasm and disruption of the nuclear chromatin. Endothelium also was absent centrally, though a few abnormal endothelial cells remained in the peripheral cornea.

By 3 days the corneas were biomicroscopically much more cloudy, with more purulent exudate, although the control eyes showed only minimal corneal haze, minimal anterior chamber protein, and moderate hyperemia of the iris. Low doses of toxin caused much less opacity at 3 days than the high doses.

At 3 days, histopathologic examination of eyes receiving high doses showed absence of endothelium and epithelium and PMN infiltration into the stroma, with some ulceration (Fig. 2). Acid mucopolysaccharide staining was less marked on the third day than on the first. Central loss of endothelium was verified by electron microscopy but some peripheral cells showed morphologic changes suggesting regeneration. Keratocytes appeared vacuolized and some keratocytes were undergoing phagocytosis by PMN's. The collagen remained intact (Fig. 2). Eyes receiving low doses showed some intact cells through most of the cornea.

By 10 days the high-dose exotoxin-injected corneas were completely opaque, and often severely ulcerated, with a hypopyon. Postinjection cultures never showed Pseudomonas aeruginosa, only saprophytes and nonpathogens. The control corneas showed minimal corneal haze and moderate iris hyperemia. The eyes injected by low doses of toxin had minimal opacity by 10 days. In the most severely involved eyes receiving high doses all corneal cells were absent except for an infiltration of PMN's. Epithelial regeneration seemed to occur in rabbits receiving low doses. Antitoxin-treated control eyes had intense cellular infiltration of the cornea, trabecular meshwork (which may possibly contribute to increased intraocular pressure), and limbus.

The ulcerative process continued in the most severely damaged eyes until 14 days, when some eyes perforated. Animals treated with low doses had minimal corneal opacity at 14 days, and the control eyes were almost clear except for moderate iritis.

Discussion. It is apparent that pseudomonas exotoxin A is a potent factor in pathogenesis of corneal ulceration. This exotoxin kills epithelial and endothelial cells, and presumably stromal cells as well, in 1 day. Loss of endothelium allows the cornea to become edematous and cloudy; loss of epithelium and keratocytes promote later ulceration and perforation. Morphologically, these events occur before collagen destruction and corneal stromal ulceration.
It is of interest that AMP stains are more positive in toxin-treated corneas than in controls. Since the colloidal iron stain demonstrates nuclear fragments as well as proteoglycans, disintegrating nuclei may give a false impression of enhanced proteoglycan content.

The possibility of neutralizing exotoxin A by horse-serum antitoxin as a therapeutic approach remains to be studied.

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REFERENCES


Oral vaccination and multivalent vaccine against Pseudomonas aeruginosa keratitis. JOHN R. GERKE AND JIM S. NELSON.

Active immunization against Pseudomonas aeruginosa keratitis and systemic disease in mice was studied. In the first series of experiments, monovalent vaccine, administered orally or intraperitoneally, protected against subsequent corneal and intraperitoneal challenge with the homologous strain of P. aeruginosa; however, oral administration of vaccine elicited less protection than intraperitoneal administration. After both routes, protection was observed at 11 and 32 days post-vaccination, but it was greater at 11 days. In the second series of experiments, multivalent vaccine administered intraperitoneally protected against corneal challenge with 56 to 78 percent of 18 strains.

Pseudomonas aeruginosa keratitis following trauma to the cornea may result in blindness. In addition, pseudomonal septicemia and pneumonia often lead to death. Improvements in antimicrobial therapy have reduced these threats; however, additional means are needed.

The potential of active and passive immunization against P. aeruginosa keratitis was first shown in 1927.1 Active and passive immunotherapy based on a multivalent vaccine have shown promise in combating disease caused by a diversity of antigenic types of P. aeruginosa.2 In some types of disease, the parenteral vaccine causes severe reactions.2 Oral vaccine offers reduced risk of vaccine reactions.

This study was designed to determine the answer, in part, to two questions. Does immunization with a multivalent vaccine protect against experimental keratitis caused by diverse strains of P. aeruginosa? Does oral immunization protect against Pseudomonas keratitis?

Materials and methods.

P. aeruginosa cultures. The strains (Table III) were selected for their ability to damage mouse corneas and to represent the seven Fisher, Devlin, and Gnanask immotypes. George Cole of Parke, Davis and Co. (Detroit, Mich.) kindly provided the immunotyping information. Four of the strains have been reported on previously: strains 119 and 120 were identified3 as PA7 and PA103, and strains 186 and 187 are respectively the type 6 and type 7 components of the heptavalent vaccine Pseudogen (Parke, Davis and Co.). All strains were maintained in 10 percent glycerol-