Clinicohistopathologic Correlations in Xerophthalmic Ulceration and Necrosis

Alfred Sommer, MD; W. Richard Green, MD; Kenneth R. Kenyon, MD

Corneal tissue from five eyes of three children with active xerophthalmic keratopathy and stromal loss was studied histopathologically. Stromal dissolution was strikingly focal, sometimes occurring beneath an intact epithelium and often, though not always, accompanied by extensive inflammatory reaction and bacteria.

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At least a quarter of a million children with xerophthalmia have corneal involvement develop every year. A majority suffer potentially blinding stromal loss: either sharp-margined ulceration, or localized or total corneal dissolution. The pathogenesis of these changes remains obscure. Clinical reports suggest bacterial infection, while animal experiments suggest that release of proteolytic enzymes by invading leukocytes or regenerating epithelium play an important role. This report presents histopathologic material from three well-documented cases in human subjects.

METHODS

One hundred sixty-two consecutive patients with vitamin A deficiency-related corneal xerophthalmia were studied, treated, and followed up at the Cirendo Eye Hospital, Bandung, Indonesia, between June 1977 and September 1978. Corneal tissue was obtained from a small proportion, either at enucleation or necropsy (always within two hours of death). All corneal material was immediately immersed in Karnovsky’s 1% formaldehyde-glutaraldehyde fixative, and transported to the Eye Pathology Laboratory of the Wilmer Institute for processing and examination by light, phase contrast, and transmission electron microscopy.

Serum vitamin A levels were determined by the method of Neeld and Pearson, holo-retinal-binding protein (holo-RBP) by that of Glover et al., and transferrin by the technique of Mancini et al. Vitamin A determinations were all carried out within two weeks, and holo-RBP within eight months of collection. Anthropometric status was compared with Western standards.

RESULTS

Histopathologic material was available from five eyes of three patients with active stromal loss. One cornea contained small, sharply circum-
Fig 3.—Case 1, left eye. Top left, Survey light microscopy of peripheral area of lesion illustrates sharp delineation of saucer-shaped ulcerated zone with intact keratinized epithelium (hematoxylin-eosin, X45). Top right, Phase contrast microscopy of corresponding area shows desquamating keratinized epithelium (asterisk) overlying stroma that is heavily infiltrated by inflammatory cells (paraphenylenediamine, X650). Bottom, Transmission electron microscopy of this area confirms presence of densely staining keratinized epithelium (E) plus several polymorphonuclear leukocytes in various stages of degranulation, phagocytosis, and degeneration. Stromal collagen (asterisks) shows great fibrillar disorganization and degradation (X13,000).
Fig 1.—Case 1, left eye. Top left, Survey light microscopy shows keratinized corneal epithelium overlying sharply delineated saucer-shaped area of stromal disintegration. Descemet's membrane is disrupted centrally (hematoxylin-eosin [HE], X30). Top right, Higher magnification of area circled (in top left) shows edematous and keratinized epithelium covering disorganized stroma that contains some fibroblasts and inflammatory cells (HE, X310). Center left, Light microscopy at margin of lesion demonstrates sharp delineation (between arrows) with normal-appearing stroma to right, but with decreased numbers of keratocytes in marginal zone and stromal dissolution to left (HE, X100). Center right, Phase contrast microscopy of ulcerated area (above) and normal stroma (below) shows former to contain numerous clusters of inflammatory and fibroblastic cells (paraphenylenediamine, X650). Bottom, Transmission electron microscopy of ulcerated area (compare with center right) depicts several polymorphonuclear neutrophils and macrophages clustered between disorganized stromal lamellae (X7,500).

Fig 2.—Case 1, left eye. Top left, Light microscopy of lateral posterior aspect of affected area emphasizes sharp delineation between normal stroma (right) and affected area (left), with latter zone having loosely fibrillar lamellae and numerous spindle-shaped fibroblasts (arrowheads) but relatively few inflammatory cells (hematoxylin-eosin, X320). Top right, Higher-magnification transmission electron microscopy resolves normal diameter and macroperiodicity of stromal collagen fibrils whose lamellar organization has been disrupted (X45,000). Bottom, Transmission electron microscopy resolves attenuated fibroblastic cell centrally with rough-surfaced endoplasmic reticulum, bordered by leukocytes (X11,000).
Fig 1. Case 1 right eye. Top left: Light microscopy shows intact, keratinized epithelium covering fibrous remnants of stroma (bracketed) that is apposed by inflammatory and fibroblastic cells (bracketed as well). The epithelium abuts posterior corneal surface (PAS, ×5/5). Top right, Phase contrast microscopy reveals infiltrated, thickened epithelial layer overlying approximately 25-μm-thick remnant of stroma (5). Fragment of ruptured Descemet’s membrane (DM) is also apparent (paraphenylene-diamine, ×1000). Bottom, Transmission electron microscopy of this stroma demonstrates active fibroblast (F), polymorphonuclear neutrophil (PMN), and macrophage (M) within degraded stroma (×10,000). Inset (bottom, upper right) resolves lamellar remnants of collagen and probable fibrin (×50,000).
scribed ulcers. The other four appeared totally necrotic in two; necrosis occurred just prior to necrosis in the other two, the process had been present longer.

REPORT OF CASES

Case 1.—This patient was a marasmic weight for height, <30% of standard 1-year-old boy. His serum albumin level was 2.2 g/dl; transferrin level was 10 mg/dl; vitamin A level was 10 μg/dl; and holo-RBP level was 2 μg/dl, all extremely low values. He had a history of night blindness.

The left cornea contained two sharply punched-out, adjacent ulcers (Color Figure, top left). Each was three-fourths deep, 1 to 2 mm in diameter, and surrounded by a thin rim of infiltrate. The anterior chamber was deep and intact. The right cornea appeared to be a total descemetocele, except for its peripheral 1 mm, which was hazy but otherwise normal.

He received 200,000 IU of vitamin A orally on admission and again the following day, as well as frequent topical doses of antibioties and systemic doses of penicillin G potassium and streptomycin sulfate. By the day following admission, the ulcers in the left cornea were already filling in. By the second day, the ulcerated area appeared more infiltrated. The appearance of the right cornea was unchanged. The child died the morning of the next day and biopsy specimens were taken of both corneas.

Histopathologic examination of sections of the left cornea disclosed a central area where there was a loss of most of the corneal stroma and disruption of Descemet's membrane (Fig 1). A loose fibrillary material covered by edematous and keratinized epithelium bridged this central defect in the posterior aspect of the cornea. The central area exhibited an abrupt loss of Bowman's layer and stroma in a saucer-shaped configuration. At the margin of the lesion, there was an abrupt transition between normal intact collagen, but with decreased numbers of keratocytes, and the central zone of collagen necrosis and dissolution (Fig 1). Between the central area of stromal loss and peripheral normal stroma, the stroma appeared loose, fibrillar, and contained numerous clusters of acute inflammatory cells and fibroblasts, both superficially (Fig 1) and deep (Fig 2).

Sections at the periphery of the lesion
Fig 6.—Case 2, left eye. Top left, Light microscopy illustrates stromal necrosis (arrow) and moderately intense polymorphonuclear neutrophil infiltration (hematoxylin-eosin, X100). Top right, Phase contrast microscopy of ulcerated area exhibits inflammatory cell debris and innumerable filamentous and spherical microorganisms (paraphenylenediamine, X700). Center, Transmission electron microscopy of this area shows necrotic inflammatory cellular debris at left and several densely staining microorganisms at right (X10,600). Bottom, At higher magnification, encapsulated filaments of approximately 0.5 μm in diameter are evident interspersed among cellular and extracellular debris (X 19,000).
Fig. 7—Case 3: left eye. Top left. Light microscopy of corneal biopsy shows fibrinopurulent exudate (hematoxylin eosin, \( \times 25 \)). Top right. Phase contrast microscopy resolves clusters of bacterial forms (circled) within polymorphonuclear neutrophil-infiltrated stroma (paraphenylene diamine, \( \times 850 \)). Bottom. Transmission electron microscopy of this area shows numerous diplococci within degenerating inflammatory cell. Other polymorphonuclear neutrophils are adjacent. Stroma remains as degradated filamentary aggregates. Remains of stromal collagen appear as dispersed filamentous aggregates (\( \times 9,000 \)).
again showed a distinct delineation between the normal and affected area (Fig 3). Hypercellularity due to an increased number of polymorphonuclear neutrophils was noted superficially. Bacteria were not present in any of the sections.

The right cornea showed total dissolution of corneal stroma and consisted of keratinized epithelium, a fibrous material with fibroblasts, macrophages and neutrophils, and iris adherent to the posterior corneal surface (Fig 4). No bacteria were seen.

Case 2.—During the ten days preceding admission, an 8-year-old girl had a rise in body temperature, a hemorrhagic rash of her face and extremities, and "white spots" in her peripheral vision (Fig 5). Phacoanaphylactic pseudophakia developed in both eyes on the second hospital day (Fig 6). Collagenase release was noted in the right cornea (Fig 7).

Histopathologic examination of the right cornea showed extensive necrosis (Fig 8) and foci of Gram-negative rods. The peripheral stroma was densely infiltrated by polymorphonuclear neutrophils. Centrally, necrotic stroma was relatively devoid of inflammatory cells.

The left cornea showed a central ulceration, with near total loss of stroma. The superficial margin of the ulcer showed prominent necrosis. An intense infiltration of polymorphonuclear leukocytes was present throughout the involved stroma (Fig 6). Thinned, keratinized epithelium lined the corneal peripheral to the ulcer. Actinomyces-like organisms were present in the superficial portion of the ulcer near the margin (Fig 6).

Case 3.—This patient was a 1-year-old girl who was admitted with a recurrent anterior uveitis and cataract (Fig 5). She had a rise in temperature, herpes zoster, and a white blood cell count of 30,000. The conjunctiva was injected, and on day 2, a central ulcer was noted in the left cornea.

Fig 6.—Case 3, left eye. Left, Enucleated globe exhibits only peripheral cornea (at arrowheads) remaining. Iris is prolapsed centrally and covered by fibrinopurulent exudate. Retina, choroid, and ciliary body are greatly detached. Extensive abscess is present in collapsed and partially prolapsed vitreous (asterisks) (hematoxylin-eosin, X5). Right, Higher-magnification light microscopy shows numerous Gram-positive cocci within vitreous abscesses (Brown and Hopps, X1,100).

COMMENT

Our data confirm, histologically, unique aspects of corneal dissolution in human xerophthalmia. One of the most striking characteristics of xerophthalmic ulceration is the sharp focal nature. In each of the five cases studied histologically, the transition between normal and abnormal stroma was remarkably abrupt. Clinical reports suggest that xerophthalmic dissolution may proceed between an intact epithelium. Wason reported similar changes in the vitamin A-deficient rat. Histopathologic changes in one of our subjects support these clinical observations. Areas of stromal dissolution of both the ulcerated left cornea and necrotic right cornea of the first case were covered by epithelium. Since healing had already begun before the biopsy specimens were obtained, it is possible that in areas where the epithelium is thinned, it had been absent when necrosis originally occurred. In other areas, however, it is thickly keratinized. In at least one area of the ulcerated left cornea, destruction was more severe posteriorly than anteriorly, with rupture of Descemet's membrane after the biopsy specimens were obtained, it is possible that in areas where the epithelium is thinned, it had been absent when necrosis originally occurred. In other areas, however, it is thickly keratinized. In at least one area of the ulcerated left cornea, destruction was more severe posteriorly than anteriorly, with rupture of Descemet's membrane after the biopsy specimens were obtained. 

Pirie has suggested that collagenases released by fibroblasts (Fig 5) would not explain this pattern of stromal loss, suggesting that interruption of the epithelial layer may not be essential to initiation of corneal melting in xerophthalmia. Pirc has suggested that collagenases released by fibroblasts (Fig 5) would not explain this pattern of stromal loss, suggesting that interruption of the epithelial layer may not be essential to initiation of corneal melting in xerophthalmia. Pirc has suggested that collagenases released by fibroblasts (Fig 5) would not explain this pattern of stromal loss, suggesting that interruption of the epithelial layer may not be essential to initiation of corneal melting in xerophthalmia.
Fig 9 — Case 3, left eye. Top left, Light microscopy of enucleation specimen shows intact peripheral cornea (asterisk) that is covered by epithelium. Fibrinopurulent exudate (between arrowheads) replaces central cornea and is lined posteriorly by iris (hematoxylin-eosin, ×100). Top right, Phase contrast microscopy of peripheral cornea confirms keratinized epithelium and inflammatory cells within disorganized stroma (paraphenylenediamine, ×750). Bottom, Transmission electron microscopy discloses active polymorphonuclear neutrophil containing numerous phagocytosed diplococci. Diplococci are also evident extracellularly (×13,000).
produced more collagenase, and were more likely to ulcerate than those from pair-fed control animals. Although several clinical observers have reported corneal infiltration early in xerophthalmic keratopathy, they may have confused stromal edema with true inflammatory infiltration. Other clinical reports suggested that corneal infiltration is a relatively late change. Histopathologic studies of corneal necrosis in vitamin A-deficient animals revealed intense inflammatory infiltration, but ocular and periocular inflammation, early and prominent manifestations of xerophthalmia in rats, are often minimal or absent in the human condition. Smith et al. noted a lack of inflammatory cells in their relatively fresh corneal samples. In our four eyes demonstrating recent, active necrosis, acute inflammatory cells were prominent, particularly at the periphery of the lesions. Two of these eyes, however, contained areas of necrosis relatively devoid of inflammatory infiltrate.

Valenten and Tann found a direct correlation between the severity of corneal involvement and the frequency with which pathogenic organisms were recovered. Necrotic corneas of vitamin A-deficient rats often contain large numbers of bacteria. However, in several clinical studies, positive cultures were not substantially more frequent from ulcerated than nonulcerated eyes, and healing was unrelated to the use of antibiotics. Of the two early cases of necrosis in the present series, one (case 1) received systemic doses of antibiotics. Both eyes of this patient and one eye of the other patient (case 2) received topical doses of antibiotics. However, the course of treatment was short and unlikely to account for the absence of organisms from both eyes of the former patient. No doubt secondary, potentially contributory infections do occur, accounting for the panophthalmitis in the most long-standing case (case 3).

The roles of inflammatory cells and bacterial infection in the keratoma-like ulceration of the vitamin A-deficient rat cornea following corneal abrasion have been recently emphasized. Studies of human noninfected corneal ulcers have demonstrated the correlation between collagenolytic enzymes and polymorphonuclear neutrophils in tears. The ability of a glued-on contact lens or tissue adhesive alone to prevent stromal ulceration in the chemically or thermally burned rabbit cornea is related to the exclusion of acute inflammatory cells. The presence of bacteria and inflammatory infiltrate in our patients suggests that similar mechanisms may be responsible for at least some aspects of corneal ulceration and necrosis in human xerophthalmia. It is still uncertain, however, whether infection and inflammation initiate the necrosis or merely contribute to it. Loss of keratocytes from the marginal zone of otherwise normal-looking stroma and necrosis deep to an intact epithelium in the left eye of our first patient, absence of bacteria from both eyes of the same patient, and the relative lack of inflammatory cells in some areas of necrosis in our first two patients suggest that as yet undefined biochemical abnormalities consequent to impaired vitamin A metabolism may play an important role.

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