Collagen content and types in trachomatous conjunctivitis

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LABORATORY STUDY

Abstract

Purpose **To study alterations in conjunctival collagen in the conjunctiva of patients with active trachoma.**

Methods **We studied conjunctival biopsy specimens obtained from nine subjects with active trachoma and from four control subjects. We used immunohistochemical techniques and a panel of monoclonal and polyclonal antibodies directed against types I, III, IV and V collagen.**

Results **In normal conjunctiva, the staining for types I and III collagen was localised to the substantia propria. Type IV collagen was located in the epithelial and capillary endothelial basement membranes. The staining for type V collagen was absent. In trachoma biopsy specimens, staining for types I and III collagen showed collagen fibrils among epithelial cells, patchy increase in staining intensity in the upper stroma, and thicker and irregularly arranged collagen fibrils in the substantia propria. Staining for type IV collagen showed irregularly thickened epithelial basement membrane. Staining for type V collagen showed patchy staining in the upper substantia propria; it was also noted in the cytoplasm of fibroblasts, in the walls of blood vessels in the substantia propria, and in the walls of accessory lacrimal glands.**

Conclusions **Our data indicate new type V collagen formation, and increased types I, III and IV collagen content, in the conjunctiva from patients with active trachoma.**

Key words Chlamydia, Collagen, Conjunctiva, Trachoma

Trachoma, a chronic follicular conjunctivitis, remains a major worldwide infectious cause of blindness, and a major public health problem, particularly in developing countries. It is estimated to affect 500 million people, 7 million of whom are blind.[1] It is caused by serovars A, B, Ba and C of *Chlamydia trachomatis*.[2] The blinding complications of trachoma are associated with progressive conjunctival and subconjunctival fibrosis that may lead to dry eye syndrome, entropion, trichiasis and corneal blindness. The possible factors involved in chronic progressive conjunctival cicatrisation in trachoma that lead to blindness remain unclear. However, previous immunohistochemical studies demonstrated that the tissue damage might result from immunological mechanisms.[3–5]

The metabolic alterations of extracellular matrix components in trachoma may be the result of cytokines released by resident conjunctival cells and by numerous inflammatory cell types infiltrating the tissue.[6] The banded collagen fibrils are the most abundant and widely distributed matrix components. Increased collagen content is a feature of many inflammatory and fibroproliferative diseases.[7–17]

We analysed conjunctival biopsy specimens from patients with active trachoma in order to determine some of the mechanisms that control conjunctival scarring. Using immunohistochemical methods we examined the nature and distribution of types I, III, IV and V collagen in conjunctival biopsy specimens from patients with active trachoma.

Patients and methods

Study subjects and specimen collection

School children in a village in the Eastern province of Saudi Arabia were examined using a binocular magnifying loupe (×4 magnification) and a portable light source. The diagnosis of active trachoma and the grading system of the intensity of the disease were performed using the recommended World Health Organization criteria.[1] A total of nine children with active trachoma between 7 and 16 years of age were included in the study (mean age 9.4 years). All patients were asymptomatic and had mild to moderate active trachoma. A 2 × 2 mm upper palpebral conjunctival biopsy specimen was obtained from each patient. None of the patients was on topical or systemic therapy. The study was approved by the Research Center, College of Medicine, King Saud University and the patients admitted to the study gave their informed consent.

In addition, four upper palpebral conjunctival biopsy specimens were obtained from patients undergoing strabismus surgery

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without obvious inflammation and served as controls. The control patients were in a similar age group.

Immunohistochemical staining

The conjunctival biopsy specimens were immediately snap-frozen in Tissue-Tek optimum cutting temperature (OCT) compound (Miles Laboratories, IN) and maintained at −80 °C until use. For immunohistochemistry, 5 μm serially cut cryostat sections were dried overnight at room temperature, fixed in absolute acetone for 10 min and stained with a three-step avidin/biotin peroxidase-labelled complex procedure. Rehydrated slides were incubated for 30 min with the monoclonal and polyclonal antibodies listed in Table 1, which were diluted to an optimum concentration. The secondary and tertiary antibodies consisted of biotin-conjugated rabbit anti-mouse immunoglobulin and the avidin/biotin peroxidase-labelled complex, respectively, which were both purchased from Dakopatts (Copenhagen, Denmark). All incubations were carried out for 30 min at room temperature, then washed in three changes of phosphate-buffered saline at pH 7.2 for 15 min. The reaction product was developed by addition of diaminobenzidine and H_2O_2, resulting in brown immunoreactive sites. The slides were faintly counterstained with Harris haematoxylin. Finally, the sections were rinsed with distilled water and coverslipped with glycerol. Controls, which were invariably negative, consisted of omission of primary or secondary antibody and use of chromogen alone.

Results

In normal conjunctival tissue specimens, the substantia propria showed diffuse, fine fibrillar staining with antibodies to types I and III collagen. Staining was more intense in a band-shaped area located just underneath the epithelium and in the perivascular areas. The epithelial and capillary endothelial basement membranes reacted with type IV collagen antibody. No staining was observed with antibody to type V collagen (Fig. 1).

Trachoma specimens showed abnormal deposition of fibrils of types I and III collagen among epithelial cells. Patchy increase in staining intensity with antibodies to types I and III collagen was noted in the substantia propria just underneath the epithelium. Thicker and irregularly arranged collagen type I and III fibrils were noted between inflammatory cells in the stromal lymphoid follicles, suggesting an increased production of collagen types I and III. Antibody to type IV collagen showed irregularly thickened epithelial basement membrane. Patchy anti-type V collagen staining was observed in the upper part of the substantia propria. Type V collagen staining was also observed in the cytoplasm of fibroblasts, in the walls of blood vessels in the substantia propria, and in the walls of accessory lacrimal glands (Fig. 2).

Discussion

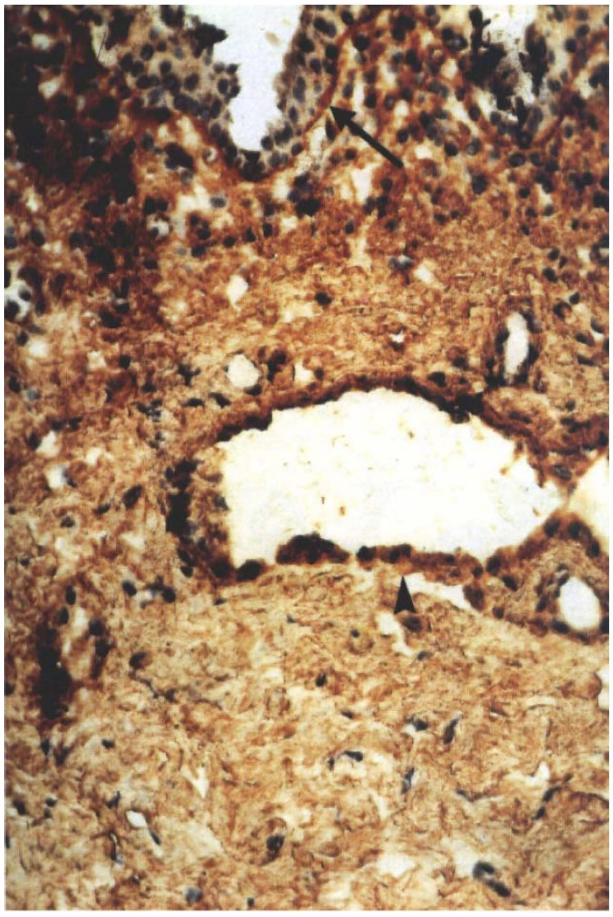
Biochemical alterations in the connective tissue matrix are a common feature of many diseases, and account in major part for their functional impairment. Such alterations are especially important in acute and chronic inflammatory diseases, where they may take the form of degradation of matrix components or their excessive accumulation leading to fibrosis. Histochemically, we observed that the conjunctival stroma from patients with active trachoma contained type V collagen. On the other hand, type V collagen was not detected in the normal conjunctiva. This is consistent with the observations of others who reported accumulation of type V collagen in conditions associated with tissue remodelling and new collagen synthesis. Increased type V collagen content has been detected in other diseased tissues such as cardiac hypertrophy,[8] neoplasia,[9,10] Crohn's disease,[11] atherosclerotic lesions,[12,13] pseudointima of vascular grafts[7] and chronically inflamed gingival tissue,[16] suggesting that it may play an important role in the pathology of these diseases.

Type V collagen was first identified in human placenta[18] and skin extracts.[19] It has since been identified in many tissues, including cornea.[7,20,21] Some studies showed a preferential pericellular localisation of collagen V.[7,22] Due to its pericellular localisation type V collagen may interact with cell surfaces and modulate cellular activities such as adhesion, differentiation, migration or synthesis. Several cell lines including fibroblasts were found to adhere and spread on native and denatured type V collagen.[23] Cell-surface-associated heparan sulphate appears to mediate the attachment of cells to type V collagen.[24] In addition, type V collagen has been postulated to play a role in regulating the diameter of heterotypic fibrils composed of type V collagen molecules co-assembled along those of type I collagen. Increased expression of type V relative to type I collagen

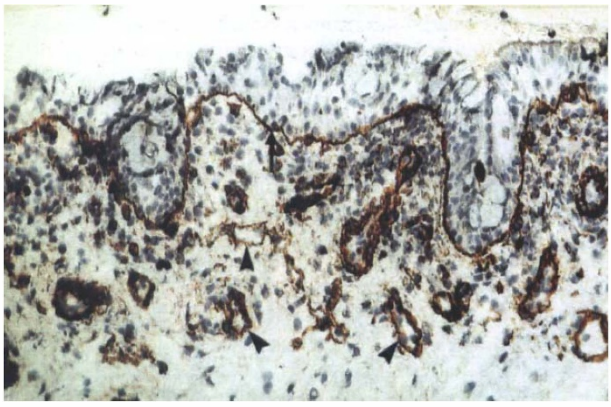
Table 1. *Monoclonal and polyclonal antibodies used in this study*

Antibody	Specificity	Working dilution	Source[a]
Anti-type I collagen (pc)	Type I collagen	1:10	Campro
Anti-type III collagen (pc)	Type III collagen	1:10	Chemicon
Anti-type IV collagen	Type IV collagen	1:20	Euro-diagnostics
Anti-type V collagen (pc)	Type V collagen	1:20	Chemicon

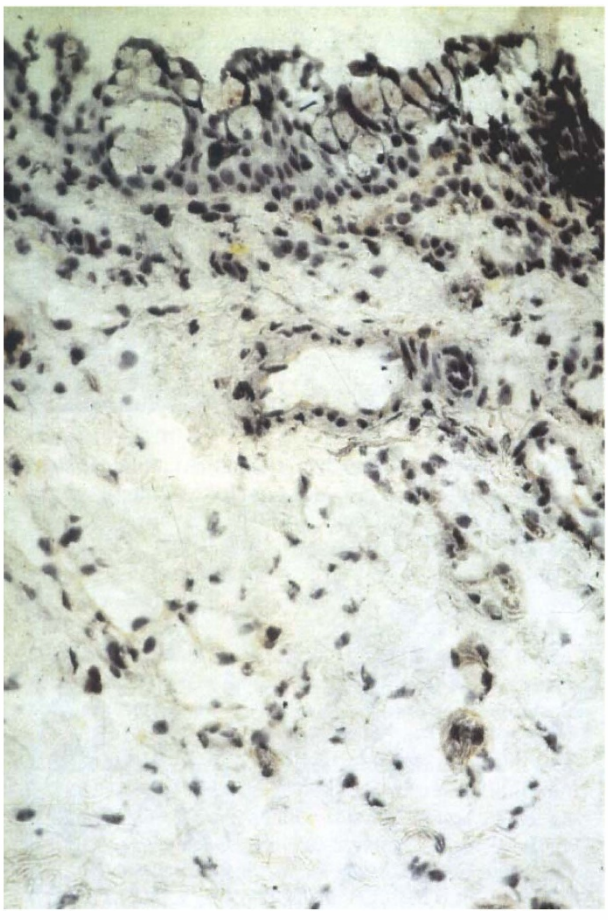
pc, polyclonal antibodies.
[a]Location of manufacturers: Campro, Veenendaal, Netherlands; Chemicon, Temecula, CA, USA; Euro-diagnostics, Malmö, Sweden.



(a)



(b)



(c)

Fig. 1. *Frozen section immunohistochemical staining of normal conjunctiva. (a) Stromal diffuse, fine, fibrillar staining with the antibody to type I collagen, with more intense staining in the subepithelial stroma (arrow) and in the perivascular areas (arrowhead). Note that normal epithelium does not contain type I collagen. (b) Epithelial (arrow) and capillary endothelial (arrowheads) basement membranes are stained with the antibody to type IV collagen. (c) No staining with antibody to type V collagen (three-step avidin/biotin peroxidase-labelled complex procedure;* ×300).

decreases the diameter of these heterofibrils.[25] It is thought that the high content of type V collagen within the corneal collagen fibrils is one factor responsible for the small, uniform fibrillar diameter characteristic of this tissue.[20,25] Animals that produce structurally abnormal type V collagen exhibit skin and eye abnormalities caused by disorganised type I collagen fibrils.[26] These data indicate that type V collagen is a key determinant in the assembly of tissue-specific matrices.

New type V collagen formation observed in conjunctival tissues from patients with active trachoma is most probably produced by conjunctival fibroblasts. *In vitro* studies have shown that corneal fibroblasts and epithelial cells can synthesise type V collagen.[20,27] A number of factors have been shown to induce type V collagen expression, including transforming growth factor β1,[28,29] epidermal growth factor,[30] hepatic fibrogenic factor[31] and platelet derived growth factor.[29]

Type I collagen is the most abundant collagen in the body; its function is to give tissue tensile strength. Type III collagen is associated with tissues and organs that require a motile structural scaffolding such as uterus, arteries, skin, intestines and lung.[32] Type IV collagen is found only within basement membranes.[32] In the present study, the localisation of types I, III and IV collagen in normal conjunctiva was consistent with that reported by Dake *et al*.[33] We observed that the conjunctival tissue

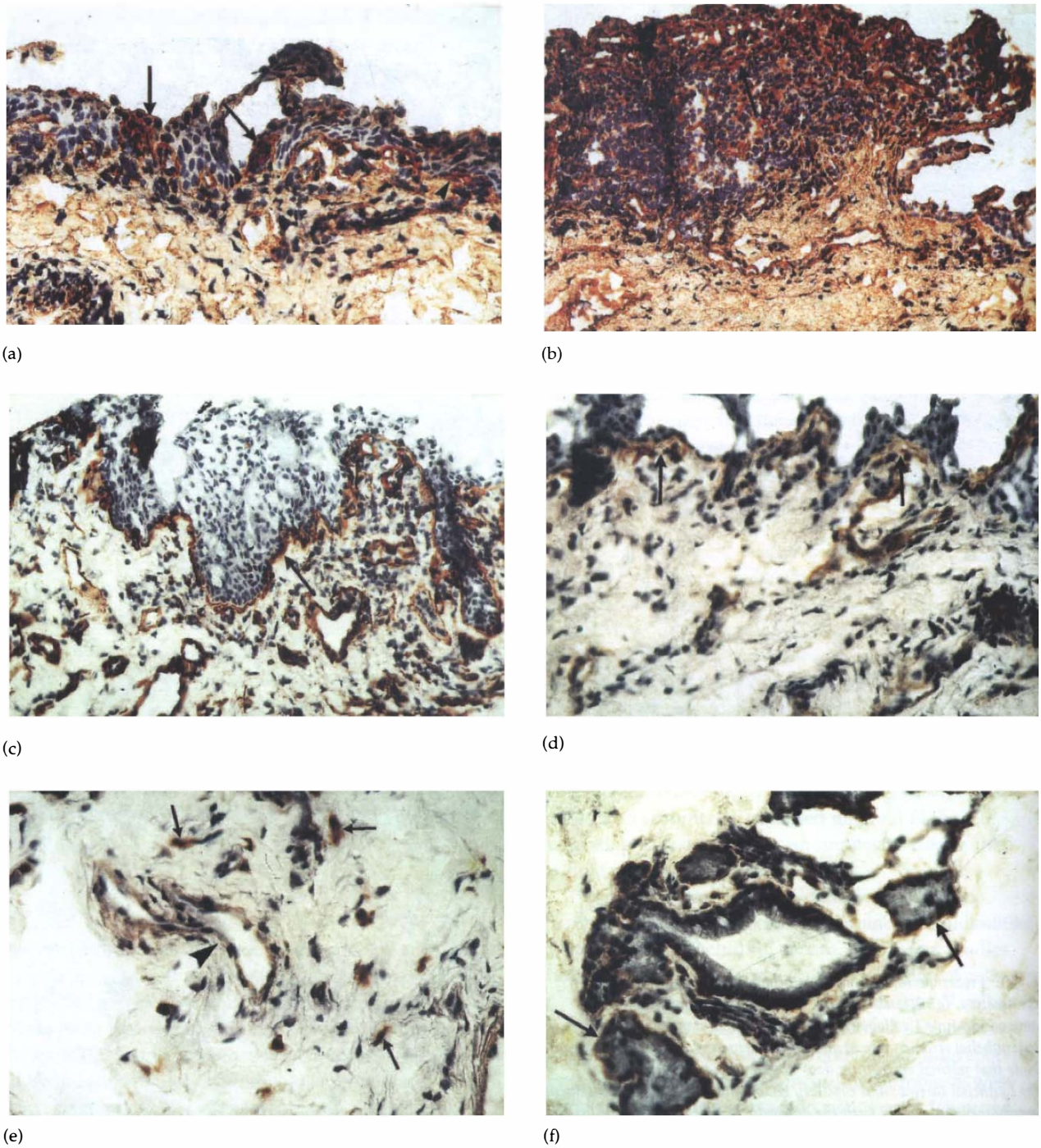


Fig. 2. *Frozen section immunohistochemical staining of trachomatous conjunctiva. (a) Staining among epithelial cells (arrows) indicating abnormal deposition, and patchy more intense staining in the upper stroma (arrowhead) with the antibody to type I collagen. (b) Newly deposited thick, irregularly arranged collagen fibrils between inflammatory cells in the stromal lymphoid follicle (arrow) with the antibody to type III collagen. (c) Irregularly thickened epithelial basement membrane with the antibody to type IV collagen (arrow). (d) Patchy anti-type V collagen staining in upper stroma (arrows). (e) Staining with the antibody to type V collagen in the cytoplasm of fibroblasts (arrows) and in the walls of blood vessels (arrowhead). (f) Staining with the antibody to type V collagen in the walls of accessory lacrimal glands (arrows) (three-step avidin/biotin peroxidase-labelled complex procedure; ×300).*

from patients with active trachoma displayed increased amounts of types I, III and IV collagen. Increased deposition of types I, III and IV collagen was also noted in atherosclerotic arterial wall.[12,13] In addition, increased amounts of type III collagen was noted in the giant papillae of vernal keratoconjunctivitis patients,[14] in corneal wound healing[15] and in the conjunctiva of ocular cicatricial pemphigoid.[17]

In conclusion, new type V collagen formation and increased deposition of types I, III and IV collagen occurs in the conjunctiva from patients with active trachoma. These findings suggest that alteration of collagen metabolism has occurred in trachomatous conjunctiva.

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