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Mast Cell Hyperplasia in Atopic Keratoconjunctivitis
An immunohistochemical study

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Summary

Immunohistochemical staining of conjunctival biopsies from four normal subjects and five patients with atopic keratoconjunctivitis (AKC) was performed, using a monoclonal antibody to human mast cell tryptase. A massive hyperplasia of mast cells in the patients with AKC was demonstrated. The mean mast cell density in the normal patients was 55.6/mm^2 and in the patients with AKC, 172/mm^2. The appearance of large numbers of mast cells in the conjunctival epithelium of AKC patients was also demonstrated. The possible pathophysiological significance of these findings is discussed.

Atopic keratoconjunctivitis (AKC) is a severe, chronic external ocular inflammation invariably associated with atopic dermatitis. First described by Hogan in 1953,[1] the disease remains poorly understood despite its undoubted association with sight threatening complications such as atopic and herpetic keratitis,[2,3,4] keratoconus,[5] and atopic cataracts.[6,7]

The immunopathology of atopic dermatitis probably represents a mixture of type I hypersensitivity reactions and cell mediated immunity. Thus, patients have raised serum IgE titres,[8] multiple positive reactions to skin prick testing with common allergens,[9,10] personal and family histories of hay fever, asthma and other atopic diseases,[10] T cell infiltrates in the skin lesions,[11] and deficiencies in certain T cell subsets.[12]

Previous work in this department using a specific monoclonal antibody to mast cell tryptase, AA1,[13] has demonstrated a mild mast cell hyperplasia in the upper tarsal conjunctiva of patients with seasonal allergic conjunctivitis due to hay fever, together with evidence of intraepithelial migration of mast cells.[14] We now present the results of a study on the mast cell populations of the conjunctiva in AKC and discuss the pathophysiological implications of these findings.

Patients and methods

Patients

Four normal subjects (age 22 to 39 years, mean 30.75 years) and five patients with AKC (age 16 to 44 years, mean 28.2 years) gave informed consent to the taking of a punch biopsy of upper tarsal conjunctiva as previously described.[14] AKC was diagnosed on

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The Southampton & South West Hants Ethical Committee gave its approval of this study.

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the basis of a history of atopic dermatitis associated with a chronic recurrent conjunctivitis, in which typical signs of papillary hypertrophy, mucous discharge, punctate epithelial keratitis and corneal vascularisation were observed by slit lamp biomicroscopy. Four of the five AKC patients had multiple, strongly positive skin prick reactions to common allergens including grass pollen, house dust, house dust mite and animal dander; this information was not available for the fifth patient. One of the five AKC patients (number 2) had keratoconus and one (number 4) had undergone bilateral glaucoma drainage surgery some months previously for glaucoma possibly related to long term topical steroid use.

Laboratory techniques

Biopsies were bisected. One half was snap frozen in liquid nitrogen. This specimen was used for staining of basement membrane collagen. The other was fixed in 10% neutral buffered formalin for 24 h, transferred to 70% ethanol, processed on a 5 h schedule through to paraffin wax and then orientated correctly before embedding. 4 μm sections were cut. Sections were dewaxed with two washes in xylene then hydrated by serial passage through graded alcohols prior to staining. For each specimen, the following stains and immunohistochemical labelling were performed:

(1) Haematoxylin and Eosin.
(2) Toluidine blue: Sections were immersed in 0.5% toluidine blue in 0.5% HCl, pH 1, for 30 minutes. They were dehydrated by rapid passage through graded alcohols then washed twice in xylene and mounted in DPX under glass coverslips.
(3) AA1 anti-tryptase antibody: A three stage immunoperoxidase technique was employed as previously described.[14] Briefly, this involves first blocking both non-specific avidin binding activity and endogenous peroxidase, then application of the mouse monoclonal antibody. A second stage rabbit anti-mouse antibody complexed with biotin was then applied. The sites at which the biotin was bound were detected using complexes of avidin, biotin and horseradish peroxidase. The addition of the peroxidase substrate DAB (3, 3′ diaminobenzidine tetrahydrochloride) revealed the binding sites with a brown reaction product. Control sections were prepared in which the monoclonal AA1 antibody was omitted but all other stages were carried out as above.

Frozen tissue sections 7 μm thick were cut on a cryostat at −20°C and were stained for the presence of collagen IV (basement membrane collagen) using a proprietary mouse monoclonal antibody (PHM 12, Serotec, Oxford, UK) and an otherwise identical technique. Controls omitting the monoclonal antibody and sections stained with H&E were also performed on frozen material.

Analysis of tissue sections

All analyses were performed by a single observer who was not aware of the identity of each section.

H and E sections were examined by light microscopy for the presence of an inflammatory cell infiltrate, and specifically for the presence of eosinophils. The degree of inflammatory cell infiltrate was graded as nil (0), mild (+) or marked (++). The degree of epithelial convolution was assessed on a similar scale.

Toluidine blue and AA1 sections were examined on a Leitz Dialux microscope with video monitoring. Using an Apple IIe PC with a Vids II Area Fractions software package (Analytical Measuring Systems Ltd, Pampisford, Cambridge, UK) the outlines of sectioned epithelium and stroma were carefully drawn and their cross-sectional areas calculated. Mast cells in epithelium and stroma were then counted visually on either an Olympus BH microscope or a Leitz Orthomat photomicroscope. Between two and six tissue sections from each block were counted in this way for each of the two mast cell stains, the number of sections examined depending solely upon the number of sections that were successfully stained by each method. The mean density of mast cells per unit cross-sectional area for epithelium and stroma could then be calculated for each stain.

Results

H and E stained sections

Four out of five AKC patients showed epi-

thelial convolution which was marked in one patient (Fig. 1). None of the normal patients showed any epithelial convolution. All five AKC patients showed an inflammatory cell infiltrate rated mild in three and marked in two. One normal patient showed a mild infiltrate (Table I). Eosinophils were seen in H and E sections in small numbers in all AKC patients, but not in every section (12 of 30 sections examined). They were not observed in normal controls.

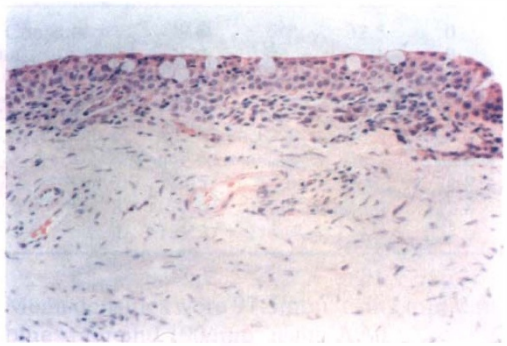

Fig. 1a. *Normal upper tarsal conjunctiva. H&E.*

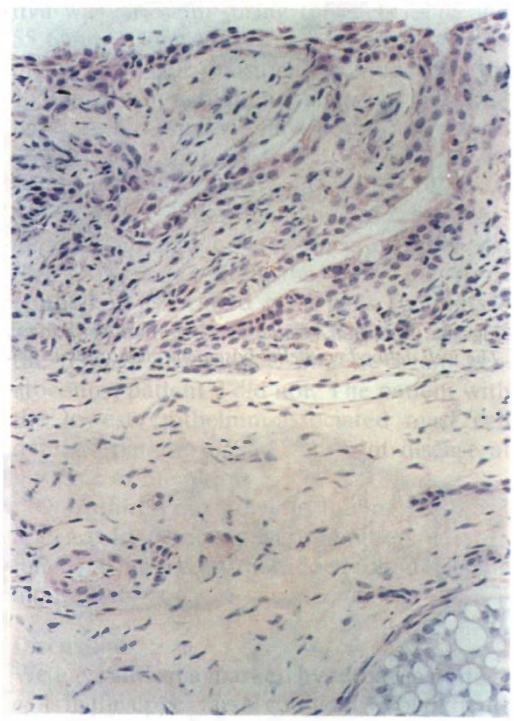

Fig. 1b. *Upper tarsal conjunctiva, AKC. H&E. Note the pronounced epithelial convolution and inflammatory cell infiltrate.*

Mast cell stains

The distribution of mast cells in the biopsies showed marked differences between the normal and AKC specimens. In normals (Fig. 2a), there was a subepithelial population of moderate numbers of mast cells, many of which lay close to capillaries. These cells were never observed in the conjunctival epithelium but were nevertheless closely related to it. Small groups of mast cells were observed in the deeper parts of the tarsal conjunctiva, often clustered around Meibomian acini or blood vessels.

In the biopsies from AKC patients (Fig. 2b), much higher densities of mast cells were observed in the area defined by the deep limits of the convolutions of the conjunctival epithelium. This area enclosed small fibrovascular stalks forming the cores of the conjunctival papillae which were not separable from the epithelium for purposes of area measurement. The disposition of the conjunctival epithelium was confirmed using frozen tissue sections stained for basement membrane collagen (collagen IV, Fig. 3). The papillary cores contained high densities of mast cells and smaller numbers of mast cells were also visible within the epithelium itself. The mast cell hyperplasia was particularly marked in two of the five specimens (numbers 5 and 9, Table II), which also showed the highest density of intraepithelial mast cells. The term 'epithelium-associated' was used to describe the superficial population of mast cells in papillae and epithelium.

Table I *Histological characteristics of upper tarsal conjunctiva in H&E sections*

	Age/Sex	Degree of epithelial convolution	Degree of inflammatory cell infiltrate
Controls	1. 22/M	0	0
	2. 31/M	0	0
	3. 31/F	0	0
	4. 39/M	0	+
AKC	5. 16/M	+	++
	6. 19/M	−	+
	7. 22/F	+	+
	8. 40/M	++	+
	9. 44/M	+	++

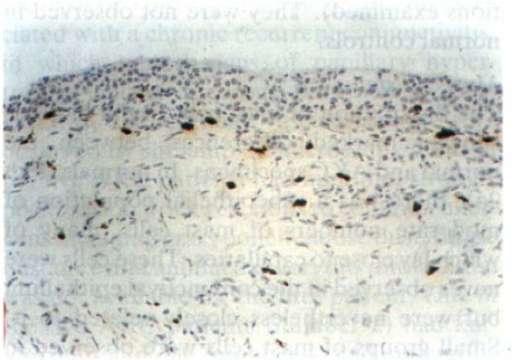

Fig. 2a. *Normal. AA1/immunoperoxidase. Note the small numbers of mast cells in sub-epithelial location.*

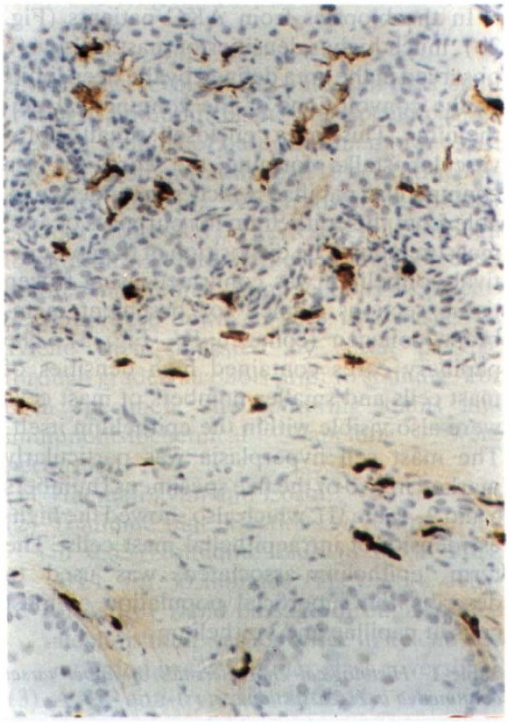

Fig. 2b. *AKC. AA1/immunoperoxidase. Note the presence of mast cells in the conjunctival papillae and also in the epithelium.*

The deeper parts of the tarsal conjunctiva also appeared to contain increased numbers of mast cells, although their distribution was similar to that found in normals.

The densities of mast cells detected in the individual biopsies are presented in Table II. In the conjunctival stroma, the mean mast cell density in the normal group was 33.8/mm^2 using toluidine blue and 58.1/mm^2 using AA1. In the AKC group, mean values were substantially higher: 160/mm^2 using toluidine blue and 158/mm^2 using AA1. In the epithelium, no mast cells were observed in any of the 38 sections of normal tissue studied. Since the epithelium was flat, papillary cores were absent. In AKC, large numbers of epithelium-associated mast cells were observed, in the epithelium and in the cores of the papillae.

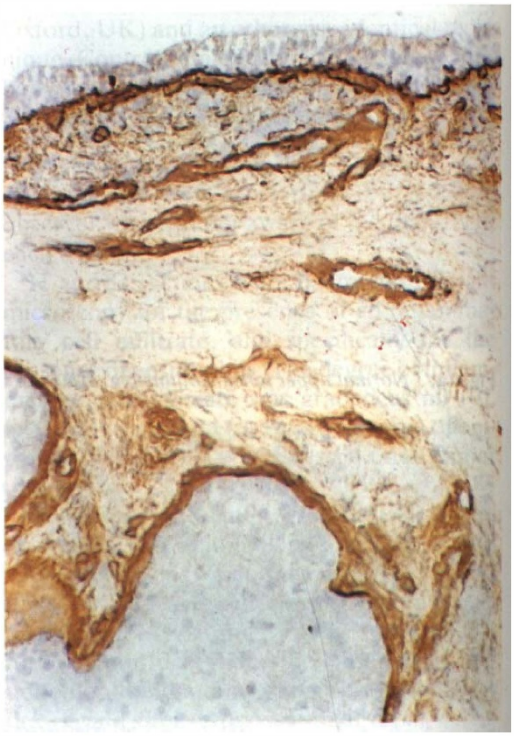

Fig. 3a. *Normal. Collagen IV/immunoperoxidase. The well defined epithelial basement membrane is flat.*

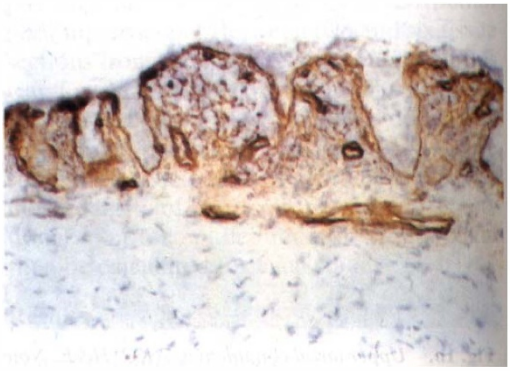

Fig. 3b. *AKC. Collagen IV/immunoperoxidase. Note the convoluted epithelial basement membrane.*

Table II *Distribution of mast cells in the upper tarsal conjunctiva of controls and AKC patients. (Figures in the table are numbers of mast cells observed per mm² of section examined)*

		Labelling method for mast cell detection			
		Toluidine blue		AA1 monoclonal antibody	
		Stroma	Epithelium	Stroma	Epithelium
Controls	1.	39.5	0	32.5	0
	2.	26.0	0	41.1	0
	3.	45.8	0	113.0	0
	4.	23.7	0	45.7	0
AKC	5.	63.4	82.1	60.3	387.2
	6.	449.5	40.0	328.3	25.0
	7.	78.5	55.6	120.6	74.8
	8.	77.0	92.9	146.3	112.5
	9.	131.2	216.9	134.4	352.6

Mean densities were 97.5/mm² using toluidine blue stain and 190/mm² using AA1.

Taking the specimens as a whole, the mean mast cell density detected in normal conjunctiva was 32.3/mm² using toluidine blue and 55.6/mm² using AA1. For AKC patients, the mean mast cell density in conjunctiva was 162/mm² using toluidine blue and 172/mm² using AA1. Thus we have found an overall increase in the mast cell population of between three and five fold in AKC patients.

The mast cell density appeared to be unrelated to patient age but it was interesting that the two patients yielding the highest density of epithelium-associated mast cells (patients 5 and 9) both had severe and active disease at the time of the biopsy. Patient 5 had keratoconus; patient 9 did not. The patient with the lowest epithelium-associated mast cell density (patient 6) had quiescent disease at the time of the biopsy.

We confirm our previous finding that AA1 appears to detect greater numbers of conjunctival mast cells than does toluidine blue alone.[14]

Discussion

We have shown a marked hyperplasia of mast cells in the upper tarsal conjunctiva of patients with atopic keratoconjunctivitis (AKC). We have also demonstrated a marked change in the distribution of mast cells in this tissue in AKC, with the appearance of large numbers of mast cells in the conjunctival epithelium and its immediate vicinity. The development of papillary hypertrophy with resultant folding of the conjunctival epithelium is associated with infiltration of the papillary cores with high densities of mast cells and with the presence of mast cells in the epithelium itself. It seems reasonable to consider the mast cells in this superficial location as a single group for counting purposes. Superficial mast cells are also present in normal tarsal conjunctiva but there is no clear anatomical feature which would allow them to be counted as a separate group.

The magnitude of this mast cell hyperplasia appears to be much greater than that which we have described previously for seasonal pollen-related conjunctivitis,[14] although we accept the need for statistical confirmation of our finding with larger numbers of patients.

The role of the mast cell is generally conceived as the primary effector cell in immediate hypersensitivity (type I) reactions. Antigen specific immunoglobulin IgE bound to the mast cell membrane is cross-linked by binding with the appropriate antigen, leading to the liberation of pre-formed and newly synthesised mediators including histamine, neutral proteases, prostaglandins, sulphidopeptide leukotrienes, and platelet activation factor.[15] Thus, an inflammatory cascade is set in motion leading to the clinical manifestations of immediate hypersensitivity, the most severe form of which is fatal anaphylactic shock.

However, mast cells may have other biological effects upon tissues indicating that they may have other roles to play in disease. Some of these may be particularly relevant to AKC. For example, factors contained in mast cell granules have been shown to cause proliferation of human microvascular endothelial cells.[16] In the tarsal conjunctiva of AKC patients, the fibrovascular cores of the papillae frequently show marked capillary proliferation. Perhaps the infiltration with mast cells helps to bring about the papillary hypertrophy. Heparin is also capable of stimulating fibroblast proliferation.[17] Conjunctival scarring is frequently clinically observed in patients with chronic AKC, resulting in dry-

ness and surface wetting disorders. Tryptase, a neutral protease enzyme released by degranulating mast cells, is known to activate collagenases.[18] Tryptase activity is detectable in increased amounts in the tears of allergic subjects after topical allergen challenge.[19] Keratoconus, in which there is progressive thinning and ectasia of the corneal stroma, whose main structural component is collagen, is known to be associated with atopy in general, and AKC in particular.[5,20] It is thus tempting to speculate that conjunctival mast cell hyperplasia with release of proteolytic enzymes has some role in the pathogenesis of this condition. Comparisons are therefore needed of the conjunctival mast cell populations in atopic and non-atopic keratoconus patients, as well as in atopes with and without keratoconus.

In conclusion we have shown a clear increase in conjunctival mast cell numbers in AKC that is likely to be relevant to the pathogenesis of this disorder. The recent demonstration of relative clonal selection of a subset of T cells designated the TH_2 subtype, with the capacity to generate IL-4, IL-5 and GM-CSF but not IFN-8 indicates an important role of these cytokines in disease pathogenesis.[21] With the capacity of IL-4 to serve as a mast cell growth factor and promote isotype switching of B cells from IgM to IgE, a mechanism is provided for the allergic response. Indeed Tepper *et al.*[22] have recently shown that constitutive expression of the IL-4 gene in mice leads to a form of conjunctivitis almost identical with AKC. These observations have provided new avenues for research which will hopefully explain the pathogenesis of this distressing condition.

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Key words: Atopic keratoconjunctivitis. Mast cell. Monoclonal antibody. Tryptase.

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