

Original article

Reduced structural proteins in the conjunctival epithelium in allergic eye disease

Aims: Allergic eye disease affects up to 20% of the population with varying severity. The conjunctival epithelium plays a key role in allergic eye disease. The purpose of this study was to determine whether the conjunctival epithelium is abnormal in allergic eye disease.

Methods: Conjunctival biopsy samples were taken from patients with seasonal allergic conjunctivitis (SAC) 'in' and 'out of season' and nonatopic control subjects. Specimens were fixed in glycol methacrylate, 2 µm serial sections cut and Image-J used to assess the sites and areas of immuno-staining.

Results: E-cadherin, CD44, keratins K5/6, K8, K13, K14, K18 and pan-keratin immuno-staining were all significantly lower in patients 'out of season' compared with normal controls. No structural differences in the epithelium were observed between the two groups. The epithelium of patients 'in season' was thicker and immuno-staining of the above markers similar to controls.

Conclusions: The expression of a wide spectrum of epithelial cell adhesion proteins and cytoskeletal elements is downregulated in the conjunctiva of SAC patients 'out of season' compared with normal controls. We suggest that this could have an important impact on the ability of the epithelium to protect itself against allergen penetration, potentially influencing the development and course of allergic eye disease and offering a novel area for therapeutic control.

**J. L. Hughes, P. M. Lackie,
S. J. Wilson, M. K. Church,
J. I. McGill**

Allergy and Inflammation Research, Division of Infection, Inflammation and Repair, School of Medicine, University of Southampton, Southampton General Hospital, Southampton, UK

Key words: CD44; cell adhesion; E-cadherin; epithelium; keratin; seasonal allergic conjunctivitis.

Prof. Martin K. Church
Division of Infection Inflammation and Repair
School of Medicine
Mail Point 825 South Block
Southampton General Hospital
Southampton SO16 6YD
UK

Accepted for publication 16 June 2006

Little is known about whether there are either macroscopic or microscopic differences in the conjunctival epithelial structure in allergic eye disease compared with normal. This is important as such differences could affect the pathogenesis of allergic conjunctivitis. Allergic conjunctivitis, which affects up to 20% of the UK population, comprises a family of conditions increasing in severity from seasonal allergic conjunctivitis (SAC) and perennial allergic conjunctivitis to vernal kerato-conjunctivitis and atopic kerato-conjunctivitis. The milder and the commonest forms, seasonal and perennial allergic conjunctivitis, have symptoms of itch, tearing, mucus discharge and redness, which, while irritating, are not sight-threatening. In contrast, vernal kerato-conjunctivitis and atopic kerato-conjunctivitis have severe perennial symptoms and signs, whose treatment is often inadequate and result in much ocular morbidity and sight loss (1). While all of these conditions have a clear allergic basis, our understanding of how the allergic cascade actually starts is poor.

To address this, we have studied SAC patients, whose seasonal symptoms correlate with environmental increases in grass pollens or other aero-allergens, indicating that their disease is allergen-driven. Out of season there are no symptoms and clinically the conjunctiva looks normal (2). This seasonal nature makes it an ideal condition to

compare the epithelial biopsies from individuals with SAC not currently exposed ('out of season') and 'normal' nonatopic individuals with those currently exposed to allergen ('in season').

One of the major unknown factors in allergy is why all individuals who are atopic, i.e. have raised immunoglobulin E (IgE) levels and positive skin test reactions to one or more common allergens, do not express overt allergic disease. Even if they do so, this may be seen at one site and not another, such as lung disease but not eye disease. In this context, the UK prevalence of atopy is 40% (3, 4) and yet the prevalence of rhino-conjunctivitis and atopic eczema is only ~20%. A possible critical factor is the epithelial integrity of mucosal surfaces, which would influence the ability of allergen to penetrate these surfaces and activate the underlying mast cells, which store manufacture and release the factors responsible for initiating the allergic cascade. It is known that in the mild forms of allergic eye disease, such as SAC, there is an increase in sub-epithelial mast cell numbers and a phenotypic shift from subtype MC_{TC} to subtype MC_T, and in chronic cases, such as atopic kerato-conjunctivitis, there is also an accompanying increase in sub-epithelial neutrophils and eosinophils cells (1, 2, 5, 6), with migration of mast cells into the epithelial layers (7).

Epithelial integrity depends on epithelial cell adhesion, mediated by junctional and nonjunctional cell adhesion molecules, and the cell cytoskeleton. The structural adhesion molecules are essential for the dynamic adhesion and interaction of epithelial cells, and changes in their expression or function may result in cell malfunction. Epithelial cell–cell adhesion is dependent on structural adhesion proteins, such as E-cadherin and CD44, and the cell cytoskeleton on the keratins. It has been shown recently that in inflammatory bowel disease epithelial tight junctions are weakened and paracellular permeability increased (8). Also, epithelial permeability is increased in both asthma (9) and in bronchial epithelial cultures after allergen challenge (J. L. Hughes unpublished observations). This suggests that a reduced or abnormal cell–cell adhesion may weaken epithelial barrier function thus allowing easier allergen penetration.

Many of the major allergens contain both cysteine and serine proteases capable of causing tissue damage. The major allergen of *Dermatophagoides pteronyssinus*, *Der p 1*, is able to increase epithelial permeability (10, 11) by proteolytic cleavage of occludin, therefore disrupting tight junctions, cleaving intercellular adhesion molecules (11–15) and causing structural damage to the epithelium (14, 16). A normal conjunctival epithelium would act as a protective barrier to these allergens making it difficult for them to penetrate. A question arises concerning the epithelium in allergic eye disease, whether it is abnormal thus allowing easier allergen penetration.

In order to investigate possible abnormalities in the conjunctival epithelium, we have examined sections of conjunctival biopsies from patients with allergic conjunctivitis (SAC) 'out of season' and compared them with biopsies taken from SAC patients 'in season' and non-atopic controls. All sections were immunostained with monoclonal antibodies to cell adhesion molecules and cytoskeletal elements to study the degree and distribution of their expression.

Methods

Biopsy samples

Forty-six volunteers (23 nonatopic controls, mean age: 54 years, range: 20–82 years; nine SAC patients 'out of season', mean age: 35.9 years, range: 19–70 years; and 14 SAC patients 'in season', mean age: 49.1 years, range: 23–70 years) were enrolled into the study. There was no statistical difference between the ages of the groups. Control patients, with no history of allergic disease, were recruited from patients, age matched, who were undergoing cataract surgery and who had no history of chronic eye disease. Ethical approval was granted by the Southampton and South West Joint Research Ethics Committee and the study conducted in concordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants after explanation of the nature and possible consequences of the study.

All individuals were skin tested with mixed grass, *Der p 1*, cat and dog dander, tree pollen, and aspergillus allergens to determine their

atopic status. Symptoms of allergic conjunctivitis were scored on the Abelson scale (17) on a 0–4 basis for symptoms of tearing and itch and signs for hyperaemia. A minimum score overall of eight of 12 together with a positive allergen test was taken to be indicative of 'in season' SAC. To be included in the 'out of season' SAC group, patients needed to have had a positive skin test, be symptomless at the time of the biopsy, but have symptoms of SAC in the appropriate season when the aero-pollen count was high. They were tested after the preceding seasons symptom's had subsided. There was no difference between the mean symptom score of the 'in season' group and the 'out of season' group when the latter had symptoms in the appropriate season. To be included in the SAC 'in season' group, patients had to have positive skin test, a history of SAC and to be currently displaying signs and symptoms as a result of a high pollen count.

Bulbar conjunctival specimens were taken with scissors from beneath the upper fornix under local anaesthesia (topical benoxinate 0.4% and subconjunctival lignocaine 2%) as previously described (1, 2, 5, 6, 18, 19). The specimens were fixed in acetone containing the enzyme inhibitors iodoacetamide (20 mM), and phenyl methyl sulphonyl fluoride (2 mM) and stored overnight at –20°C before processing into glycolmethacrylate (20) (JB4 kit, Park Science Ltd, Northants, UK), using the method previously described (2, 5, 6, 18, 19). Sections of 2 µm thickness were cut using a Leica supercut microtome and mounted on poly-L-lysine coated glass slides, and were air-dried for about an hour and immunostained within 1 week of cutting (2, 5, 6, 18, 19). Sections were immuno-stained using a three step avidin–biotin peroxidase method visualized using 3,3'-diaminobenzidine tetrahydrochloride (DAB) as the chromogen and counterstained with Meyer's haematoxylin. (20) Endogenous peroxidase was inhibited by 0.1% sodium azide. Antibodies for immunohistochemistry were titrated to determine the optimal concentration. Positive controls (antibody AA1), which also acted as a isotypic control ruling out nonspecific background staining, and negative controls (omission of primary antibody) were included in each staining run and conditions for staining were standardized. Secondary antibodies and other immunological reagents were obtained from DakoCytomation (Ely, UK).

Imaging and image analysis

As previously described (1, 2, 5, 6, 19), a minimum of three sections from different parts of each biopsy were examined to ensure that they were representative of the whole biopsy. The sections were assessed blindly by at least three observers independently and the mean of the results recorded. Fields were chosen where the epithelium was sectioned perpendicular to the plane of the basement membrane. Slides were viewed using a Leica DM-RBE upright microscope (Leica Milton Keynes, UK) with a 10× objective and images captured using a JVC KY-55 3 chip CCD video camera and saved as Tagged Image Format Files (TIFF) using the Acquis image program (Synoptics, Cambridge UK). The public domain Image-J program (21) was used to quantify epithelial morphology and the extent and localization of immuno-staining in the epithelial layer. A stage micrometer (Graticules Ltd, Tonbridge, UK) was used as a scale calibration standard. Epithelial damage was assessed by looking for any physical damage or irregular epithelium.

Epithelial cross-sectional height was measured perpendicular to the basement membrane, at 10 equally spaced points along the epithelium. For each biopsy section, 4–10 images were taken of different, nonoverlapping microscope fields. Once all the images had been measured, the mean height for each image was calculated and these were used to calculate the mean height and the standard error of the mean for each biopsy.

Cell cross-sectional area was measured by using Image-J in representative areas of epithelium. A straight line parallel to the basement membrane was overlaid on the image and used as the base line for a tetrahedral box incorporating all the epithelial cross-section. To determine the expression of each adhesion molecule or cytoskeletal molecule, primary staining was carried out with monoclonal antibodies to CD44, E-Cadherin, and the keratins K5/6, K8, K12, K13, K14, K18, and a pan-keratin (Table 1).

The percentage of the epithelium that had positive staining was quantified using Image-J. An area of intact epithelium was selected from the epithelial layer and a pixel intensity threshold was changed to only include the pixels in immunopositive areas. In the thresholded image, the total number of positive pixels and total pixel count in the selected area were used to calculate the percentage immuno-staining area. Such a method overcomes any changes in the total area of immunostaining resulting from differences in epithelial thickness, and is expressed as a percentage of immuno-staining per unit area.

To calculate the distribution of staining throughout the epithelium, two lines were drawn, one along the basement membrane and the other corresponding to the apical limit of the epithelium. Lines were then constructed running from the basement membrane intersecting the epithelial layer up to the apical membrane at 10 pixel intervals. The pixel threshold was adjusted to highlight positive pixels, and the distribution of pixels along the line which exceeded the threshold was recorded running basal to apical. The length of each epithelial dissecting line was then normalized to 100 and the values from the lines combined and expressed as a percentage of immunoreactive area (thresholded normalized pixels/total normalized pixels $\times 100$) at each of 100 levels through the epithelium allowing an overall staining distribution pattern in the epithelium to be assessed. In order to eliminate the possibility that any variation in staining was because of differences in the uptake of the immunostain, the immuno-staining was carried out in batches, each containing sections from a mixture of patient groups. The results were expressed as the percentage of the epithelium showing staining. Measurement of the staining intensity of individual areas or cells was not sufficiently accurate. To confirm that the results were not because of physical differences between the conditions of the tissue blocks, the numbers of mast cells staining with the anti-tryptase antibody, AA1, were assessed. Mast cell numbers were expressed as the number staining, and not the intensity of staining.

Table 1. Antibodies used in this study (all are mouse monoclonal antibodies)

Antigen	Clone/name	Dilution	Source
CD44	25-32	1 + 4 of hybridoma supernatant	ECACC (Salisbury, UK)
E-Cadherin	HECD-1	1 + 200	Zymed (CambridgeBioscience, Cambridge, UK)
Keratin 5/6	D5/16 B4	1 + 300	Dako, Ely, UK
Keratin 7	OV-TL 12/30	1 + 800	Dako, Ely, UK
Keratin 8	35 β H11	1 + 4500	Dako, Ely, UK
Keratin 13	KS-1A3	1 + 1600	Sigma (Gillingham, UK)
Keratin 14	CKB1	1 + 2000	Sigma (Gillingham, UK)
Keratin 18	CY-90	1 + 1800	Sigma (Gillingham, UK)
Pan Keratins	C 2562 [C-11, PCK-26,CY-90, KS-1A3,M20, A53-B/A2]	1 + 8000	Sigma (Gillingham, UK)
Mast cell tryptase	AA1	1 + 1000	A Walls, Southampton

All data are expressed as mean $\pm$ SEM and statistical analysis was performed using Student's *t*-test for unpaired data. A probability value of $P < 0.05$ was taken as statistically significant.

Results

The average symptoms score for the normal and 'out of season' group was zero, when the pollen count was zero. For the 'in season' SAC group, the score was 6.75 (range: 5–8), with a mean pollen count of 116.7 grains/m³. Examination of 2 μ m thick sections from biopsies from all groups showed that the stratified epithelium was continuous over the conjunctival surface and, with the exception of the extremities of the biopsies, which were damaged as a result of surgical removal; no areas of gross histological damage were seen. The thickness of the epithelium in SAC 'out of season' ($46.1 \pm 7.0 \mu\text{m}$) was not significantly different from that of the normal conjunctiva, ($48.1 \pm 3.3 \mu\text{m}$, $P = 0.803$). Also, the mean cell area in the epithelium of both groups was similar, being $135.4 \pm 6.2 \mu\text{m}^2$ and $133.5 \pm 17.3 \mu\text{m}^2$ for normal and SAC 'out of season' respectively ($P = 0.921$). However, the presence of an active allergic reaction in SAC in season was associated with a significant thickening of the epithelium to $72.6 \pm 5.9 \mu\text{m}$ ($P = 0.004$ versus normal) caused primarily by an increased mean cell volume reflected by the increase in cross sectional area to $169.6 \pm 14.0 \mu\text{m}^2$ ($P = 0.05$ vs normal) rather than hyperplasia.

Immunocytochemical analysis of sections from the conjunctival biopsies showed that the area of immuno-staining for E-cadherin of $1.9 \pm 0.8\%$ of the epithelial area in samples from 'out of season' SAC was significantly ($P < 0.0001$) lower than the $30.9 \pm 2.4\%$ observed in normal conjunctiva (Figs 1 and 2). The distribution of E-cadherin immuno-staining within the normal epithelium showed it to be most concentrated in the mid zone. It was from this zone that the majority of staining was lost in 'out of season' SAC (Fig. 3). The area of immuno-staining for CD44 in 'out of season' SAC of $1.7 \pm 0.6\%$ was significantly lower ($P < 0.0001$) than the $29.3 \pm 4.1\%$ in normal conjunctival samples (Fig. 1). The distribution of CD44 in normal and SAC 'out of season' epithelium was similar to that of E-cadherin (Fig. 3).

Similarly, the expression of keratins was also significantly lower (Fig. 3). The mean reduction in epithelial staining in the 'out of season' group compared with normal controls for the keratins was: K5/6, 45% ($P = 0.004$); K7, 52% ($P = 0.003$); K8, 59% ($P = 0.017$); K13, 85% ($P = 0.0001$); and pan-keratin 41% ($P = 0.001$). Although the staining of K14 and K18 was reduced by 93% and 95% respectively in the 'out of season' group, the weak and variable staining precluded statistical significance from control (Fig. 4).

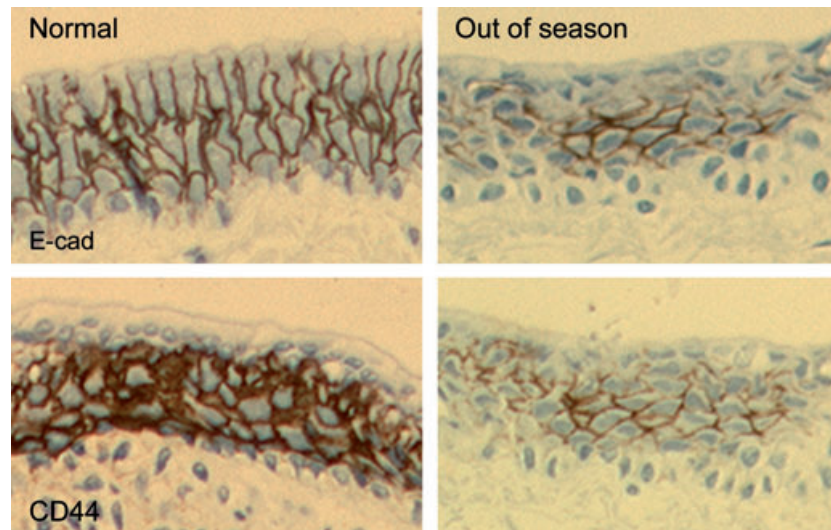


Figure 1. Micrographs of sections of conjunctival epithelium demonstrating that the epithelium in SAC 'out of season' is intact with no visible damage but has reduced expression of E-cadherin and CD-44 compared with normal.

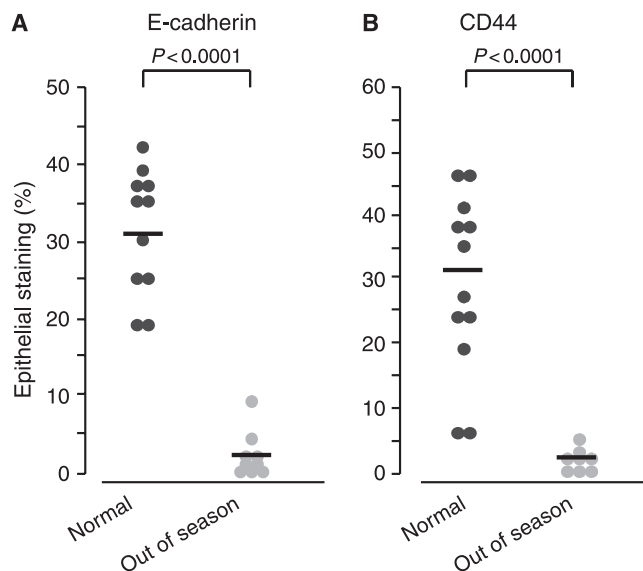


Figure 2. The expression of E-cadherin and CD44 immunoreactivity in the conjunctival epithelium of SAC patients 'out of season' and normal controls. Bars indicate mean values.

To assess whether the cells of the conjunctival epithelium of SAC patients could be stimulated to express the above molecules, biopsies were also taken 'in season'. Unfortunately, ethical considerations prevented us from taking a second biopsy from persons examined 'out of season', so different individuals were used. During active SAC, when there was clinically overt conjunctival inflammation, the areas of immuno-staining of E-cadherin ($29.9 \pm 2.5\%$) and CD44 ($25.8 \pm 2.2\%$) were significantly higher (both $P < 0.0001$) than in samples of 'out

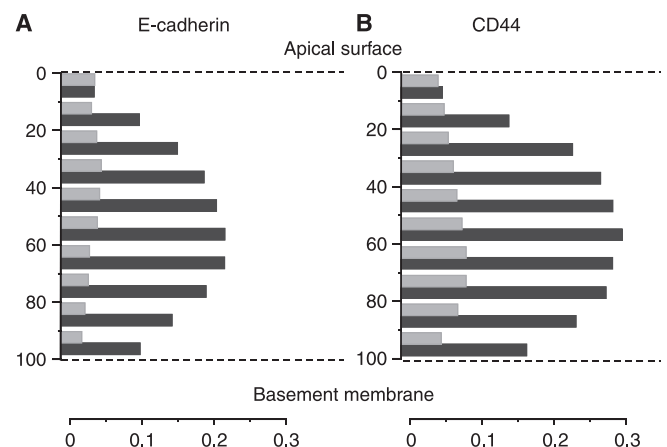


Figure 3. The distribution of E-cadherin and CD44 immunoreactivity, expressed as % area stained, throughout the conjunctival epithelium of SAC patients 'out of season' (green bars) and normal controls (blue bars). The bars represent sequential 10% sections of the epithelium from the apical surface at the top to the basement membrane at the bottom.

of season' SAC. These values were not statistically different from those of normal controls. The degrees of 'in season' keratin staining with statistical significance from 'out of season' were: K5/6, $38.6 \pm 4.5\%$ ($P = 0.037$); K7, $34.2 \pm 4.3\%$ ($P = 0.035$); K8, $20.7 \pm 2.3\%$ (N.S.); K13, $42.4 \pm 5.3\%$ ($P < 0.001$); K14, $5.8 \pm 2.0\%$ ($P = 0.043$); K18, $2.6 \pm 1.1\%$ (N.S.); Pan keratin, $46.5 \pm 4.5\%$ ($P = 0.042$). As with E cadherin and CD 44, in the SAC patients 'in season', the areas of staining of all the keratins were not statistically significantly different from those in the normal control group.

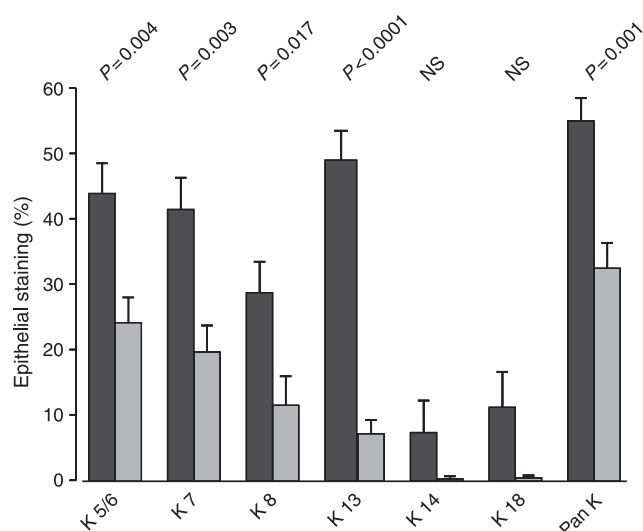


Figure 4. The expression of cytokeratin immunoreactivity in the conjunctival epithelium of SAC patients 'out of season' (green bars) and normal controls (blue bars). Bars indicate mean values $\pm$ SEM for keratins 5/6, 7, 8, 13, 14, 18 and a pan-cytokeratin antibody.

Mast cells staining, with the anti-tryptase antibody, AA1, were assessed, as a positive control (see Methods). These numbers did not differ significantly between tissues from any of the three groups, being 17.2 ± 1.2 , 27.62 ± 2.4 and $23.9 \pm 2.1/\text{mm}^2$ in normal, 'out of season' SAC and 'in season' SAC respectively.

Discussion

The results of this study show that although the conjunctival epithelium appeared microscopically normal as we have reported previously (2, 5, 6, 18), the expression of epithelial cell adhesion and cytoskeletal proteins is reduced in 'out of season' SAC compared with normal controls.

E-cadherin and CD44 were selected as examples of cell adhesion molecules because of their known involvement in epithelial structure and repair. Normal expression and functional activity of E-cadherin are critical for the maintenance of tight junctions and normal epithelial barrier function (22), whilst CD44 is a widely distributed multi-functional trans-membrane protein involved in epithelial repair (23). Its affinity for hyaluronic acid facilitates its role in cell-cell and cell-matrix adhesion (24). The reduced levels of E-cadherin and CD44 found in 'out of season' SAC patients would suggest that the conjunctiva in these cases may be structurally weaker than normal, therefore being more susceptible to allergen damage and penetration. There are parallels in other diseases where there are alterations in adhesion molecule expression, such as in inflammatory bowel (8), asthma

(D. E. Davies personal communication) and pulmonary fibrosis (25), but in no other condition has it been shown that the levels are abnormal in the resting state. In the guinea pig lung after allergen challenge, E-cadherin is expressed in the lateral sides of the epithelial cells and a soluble form is released into the lung lumen at the same time as an increase in epithelial permeability (26). A potential allergen would be able to enter the epithelium more easily under such conditions (22). Low calcium concentrations and the presence of a blocking antibody to E-cadherin lead to diffusion of E-cadherin, actin filaments and the tight junction protein ZO-1 (27) and cause functional and morphological disruption of the tight junction barrier (28).

It has been shown that after allergen challenge in the mouse lung, the E-cadherin extracellular domain is cleaved destabilizing the tight and adherens junctions, and cell-cell contact so causing an alteration in the epithelial surface permeability (26). Similarly, the allergen *Der p 1* cleaves tight junctions in tissue culture (14, 16). If E-cadherin levels were reduced in the out of season atopic patient it would suggest that epithelial integrity may be reduced, making the conjunctiva more permeable, so allowing greater allergen access. After any such inflammation following allergen challenge, there is a neutrophil influx (5,19). Neutrophils contain elastase, which cleaves endothelial cadherin to release its extra-cellular domain (16). If the structural protein levels were initially abnormally low, any such challenge would result in greater damage and so greater allergen penetration and a more pronounced allergic cascade.

It was interesting to find the changes that occur in the conjunctival epithelial intermediate filament protein patterns, as little is known about such changes in disease (29). The keratin pairs expressed in epithelial cells are adapted to the structural and functional requirements of the epithelium and the role of the particular cell type within that. For example keratin-1 (K1), K2, K3, K6, K9 and K17 are found in stratified epithelium, K14 and K9 in the basal cells of nonkeratinizing stratified epithelium of mucosal surfaces, while K4 and K13 are markers of differentiating supra-basal cells.(29). The conjunctival epithelium contains K4, K5, K13, K14, K15, and K19, with little expression of K6, K10, K12, K16 or K17 (25, 30,31). Expression of K8, K18 and K19 in conjunctival goblet cells is the same as seen for these cells in other mucosal epithelia and different from the surrounding epithelium (32).

But in diseased states, an abnormal expression of these keratins has been described. We have shown that keratin-14, is reduced in 'out of season' SAC, though the weak staining prevented statistical analysis. It is a basal epithelial cell marker (33), is present in corneal pterygium where abnormal conjunctival cell growth causes structural changes in the epithelium (34,35). Knockout studies with K14 have shown that loss of expression is associated with rupture of stratified epithelial tissue, which could be

of relevance as intermediate filaments give strong mechanical support to the epithelium, thus retaining cell shape even under repeated episodes of stress (36). Thus the loss of K14 could be associated with an increased risk of cell-cell rupture. Keratin-18 has not previously been found either in the rat conjunctiva (37), or in the human conjunctiva (38), while we found K18 immunoreactivity in all epithelial layers. This difference may reflect the impression cytology used by Krenzer and Freddo (38) which only reliably captures superficial cells, whereas our sections are of the full-epithelial thickness. K18 has not been found in paraffin sections (37), while we used resin embedded sections.

SAC 'out of season' patients have no symptoms and clinically the conjunctiva appears normal, when the pollen count is zero. At this time conjunctival ICAM-1 expression is normal, and only rises after the allergen challenge (1,2), which contrasts with the suggestion that in asymptomatic rhinitis patients sensitive to the house dust allergen, there is persistent inflammation and ICAM-1 expression in both the nasal mucosa and conjunctiva (39). In these patients there was a continuous exposure to the allergen whilst in our patients there was only exposure during the pollen season, which would explain the finding of low CD44 and E-cadherin counts in our 'out of season' patients. Reduction in the immunoreactivity of cell adhesion molecules and cytoskeletal proteins in 'out of season' conjunctiva could be due to general reduction in antibody accessibility or epitope masking in the quiescent state. Both seem unlikely because of the number of molecules whose immunostaining was reduced and that the pancytokeratin stain was also reduced. Also, AA1 immunostaining for mast cells was similar in both controls and patients. In other studies using some of the same tissue blocks, we have shown that the expression of the adhesion proteins E-selectin and ICAM-1, were similar in normal and 'out of season' tissues (18).

We have shown that the conjunctiva is abnormal in atopic subjects with quiescent SAC with alterations in expression of cell adhesion molecules and cytoskeletal proteins, but no change in cell density or numbers. A question which remains to be answered is whether these

changes are because of SAC or a result of previous active disease. Which ever it is, the defect is more likely to reside in a cytokine or growth factor, which regulates the synthesis or maturation of a spectrum of structural elements within the epithelium than in the individual elements. We suggest this for two reasons. First, at least seven adhesion molecules and cytoskeletal proteins are affected to similar degrees. Second, in SAC 'in season' all these molecules are at levels not significantly different from levels in normal controls indicating that their synthesis may be increased under appropriate conditions.

The functional consequences of the epithelial abnormalities are yet to be explored. The observation that <50% of atopic individuals show clinical expression of allergic conjunctivitis strongly suggests that something more than the ability to produce allergen specific IgE determines an individual's susceptibility to disease. We suggest that abnormalities in the structure of the conjunctival epithelium may contribute to the pathogenesis of seasonal allergic conjunctivitis. It is known that epithelial permeability is increased after allergen challenge (14) and epithelial tight junctions are altered in inflammatory bowel disease (8) and asthma (9). That the loss of epithelial barrier function may lead to disease has been shown in pemphigus vulgaris, an autoimmune disorder directed against desmosomes (40). Also, genetic variants in the gene encoding filaggrin, a key protein that facilitates terminal differentiation of the epidermis and formation of the skin barrier, are associated with atopic dermatitis and the subsequent development of asthma (41).

Acknowledgments

Biopsy samples were collected with the help of Mr David Anderson FRCOphth., Siti Yussof, Carley Bowman-Burns, Zaid Al-Najjar and Poonam Ahluwalia. JLH is in receipt of a studentship part sponsored by the School of Medicine research strategy committee and the AAIR charity. Part of this work was presented in abstract form at the International Symposium on Frontiers in Ocular Immunology, Inflammation and Transplantation, London, 2002.

References

- McGill JI, Holgate ST, Church MK, Anderson DF, Bacon A. Allergic eye disease mechanisms. *Br J Ophthalmol* 1998;**82**:1203–1214.
- Bacon AS, Ahluwalia P, Irani AM, Schwartz LB, Holgate ST, Church MK et al. Tear and conjunctival changes during the allergen-induced early and late phase responses. *J Allergy Clin Immunol* 2000;**106**:948–954.
- Cullinan P, Harris JM, Newman Taylor AJ, Jones M, Taylor P, Dave JR et al. Can early infection explain the sibling effect in adult atopy? *Eur Respir J* 2003;**22**:956–961.
- Arshad SH, Bateman B, Matthews SM. Primary prevention of asthma and atopy during childhood by allergen avoidance in infancy: a randomised controlled study. *Thorax* 2003;**58**:489–493.
- Anderson DF, MacLeod JD, Baddeley SM, Bacon AS, McGill JI, Holgate ST et al. Seasonal allergic conjunctivitis is accompanied by increased mast cell numbers in the absence of leucocyte infiltration. *Clin Exp Allergy* 1997;**27**:1060–1066.
- MacLeod JD, Anderson DF, Baddeley SM, Holgate ST, McGill JI, Roche WR. Immunolocalization of cytokines to mast cells in normal and allergic conjunctiva. *Clin Exp Allergy* 1997;**27**:1328–1334.

7. Allansmith MR, Ross RN. Ocular allergy. *Clin Allergy* 1988;**18**:1–13.
8. Prasad S, Mingrino R, Kaukinen K, Hayes KL, Powell RM, MacDonald TT et al. Inflammatory processes have differential effects on claudins 2, 3 and 4 in colonic epithelial cells. *Lab Invest* 2005;**85**:1139–1162.
9. Puddicombe SM, Steel M, Powell RM, Thornton DJ, Swallow DM, Holgate ST et al. Asthma specific up-regulation of the gel-forming mucin MUC5AC during epithelial differentiation in the presence of interleukin (IL)-13. *Am J Respir Crit Care Med* 2004;**169**:A536.
10. Herbert CA, Holgate ST, Robinson C, Thompson PJ, Stewart GA. Effect of mite allergen on permeability of bronchial mucosa. *Lancet* 1990;**336**:1132.
11. Herbert CA, King CM, Ring PC, Holgate ST, Stewart GA, Thompson PJ et al. Augmentation of permeability in the bronchial epithelium by the house dust mite allergen Der p1. *Am J Respir Cell Mol Biol* 1995;**12**:369–378.
12. Petersen A, Grobe K, Schramm G, Vieths S, Altmann F, Schlaak M et al. Implications of the grass group I allergens on the sensitization and provocation process. *Int Arch Allergy Immunol* 1999;**118**:411–413.
13. Stewart GA, Lake FR, Thompson PJ. Faecally derived hydrolytic enzymes from *Dermatophagoides pteronyssinus*: physicochemical characterisation of potential allergens. *Int Arch Allergy Appl Immunol* 1991;**95**:248–256.
14. Wan H, Winton HL, Soeller C, Tovey ER, Gruenert DC, Thompson PJ et al. Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. *J Clin Invest* 1999;**104**:123–133.
15. Widmer F, Hayes PJ, Whittaker RG, Kumar RK. Substrate preference profiles of proteases released by allergenic pollens. *Clin Exp Allergy* 2000;**30**:571–576.
16. Robinson C, Baker SF, Garrod DR. Peptidase allergens, occludin and claudins. Do their interactions facilitate the development of hypersensitivity reactions at mucosal surfaces? *Clin Exp Allergy* 2001;**31**:186–192.
17. Abelson MB, Chambers WA, Smith LM. Conjunctival allergen challenge. A clinical approach to studying allergic conjunctivitis. *Arch Ophthalmol* 1990;**108**:84–88.
18. Ahluwalia P, Anderson DF, Wilson SJ, McGill JI, Church MK. Nedocromil sodium and levocabastine reduce the symptoms of conjunctival allergen challenge by different mechanisms. *J Allergy Clin Immunol* 2001;**108**:449–454.
19. Bacon AS, McGill JI, Anderson DF, Baddeley S, Lightman SL, Holgate ST. Adhesion molecules and relationship to leukocyte levels in allergic eye disease. *Invest Ophthalmol Vis Sci* 1998;**39**:322–330.
20. Britten KM, Howarth PH, Roche WR. Immunohistochemistry on resin sections: a comparison of resin embedding techniques for small mucosal biopsies. *Biotech Histochem* 1993;**68**:271–280.
21. Abramoff MD, Magelhaes PJ, Ram SJ. Image processing with image J. *Biophotonics Int* 2004;**11**:36–42.
22. Alattia JR, Tong KI, Takeichi M, Ikura M. Cadherins. *Methods Mol Biol* 2002;**172**:199–210.
23. Leir SH, Baker JE, Holgate ST, Lackie PM. Increased CD44 expression in human bronchial epithelial repair after damage or plating at low cell densities. *Am J Physiol Lung Cell Mol Physiol* 2000;**278**:L1129–L1137.
24. Miyake K, Underhill CB, Lesley J, Kincade PW. Hyaluronate can function as a cell adhesion molecule and CD44 participates in hyaluronate recognition. *J Exp Med* 1990;**172**:69–75.
25. Ryder MI, Weinreb RN. Cytokeratin patterns in corneal, limbal, and conjunctival epithelium. An immunofluorescence study with PKK-1, 8.12, 8.60, and 4.62 anticytokeratin antibodies. *Invest Ophthalmol Vis Sci* 1990;**31**:2230–2234.
26. Goto Y, Uchida Y, Nomura A, Sakamoto T, Ishii Y, Morishima Y et al. Dislocation of E-cadherin in the airway epithelium during an antigen-induced asthmatic response. *Am J Respir Cell Mol Biol* 2000;**23**:712–718.
27. Gumbiner B, Stevenson B, Grimaldi A. The role of the cell adhesion molecule uvomorulin in the formation and maintenance of the epithelial junctional complex. *J Cell Biol* 1988;**107**:1575–1587.
28. Meza I, Ibarra G, Sabanero M, Martinez-Palomo A, Cerejido M. Occluding junctions and cytoskeletal components in a cultured transporting epithelium. *J Cell Biol* 1980;**87**:746–754.
29. Pitz S, Moll R. Intermediate-filament expression in ocular tissue. *Prog Retin Eye Res* 2002;**21**:241–262.
30. Kasper M, Moll R, Stosiek P, Karsten U. Patterns of cytokeratin and vimentin expression in the human eye. *Histochemistry* 1988;**89**:369–377.
31. Kurpakus MA, Stock EL, Jones JC. Expression of the 55-kD/64-kD corneal keratins in ocular surface epithelium. *Invest Ophthalmol Vis Sci* 1990;**31**:448–456.
32. Dushku N, Reid TW. Immunohistochemical evidence that human pterygia originate from an invasion of vimentin-expressing altered limbal epithelial basal cells. *Curr Eye Res* 1994;**13**:473–481.
33. Porter RM, Lane EB. Phenotypes, genotypes and their contribution to understanding keratin function. *Trends Genet* 2003;**19**:278–285.
34. Komine M, Okinaga M, Takeda F, Nashiro K, Kikuchi K, Murakami T et al. Patterns of basal cell keratin 14 expression in Bowen's disease: a possible marker for tumour progression. *Br J Dermatol* 2001;**145**:223–228.
35. Yu FX, Gipson IK, Guo Y. Differential gene expression in healing rat corneal epithelium. *Invest Ophthalmol Vis Sci* 1995;**36**:1997–2007.
36. Galou M, Gao J, Humbert J, Mericskay M, Li Z, Paulin D et al. The importance of intermediate filaments in the adaptation of tissues to mechanical stress: evidence from gene knockout studies. *Biol Cell* 1997;**89**:85–97.
37. Kasper M. Heterogeneity in the immunolocalization of cytokeratin specific monoclonal antibodies in the rat eye: evaluation of unusual epithelial tissue entities. *Histochemistry* 1991;**95**:613–620.
38. Krenzer KL, Freddo TF. Cytokeratin expression in normal human bulbar conjunctiva obtained by impression cytology. *Invest Ophthalmol Vis Sci* 1997;**38**:142–152.
39. Ciprandi G, Buscaglia S, Pesce GP, Bagnasco M, Canonica GW. ICAM-1/CD54 expression on conjunctival epithelium during pollen season. *Allergy* 1995;**50**:184–187.
40. Calkins CC, Setzer SV, Jennings JM, Summers S, Tsunoda K, Amagai M et al. Desmoglein endocytosis and desmosome disassembly are coordinated responses to pemphigus autoantibodies. *J Biol Chem* 2006;**281**:7623–7634.
41. Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006;**38**:441–446.