

## SCIENTIFIC REPORT

# Inflammatory cells in brush cytology samples correlate with the severity of corneal lesions in atopic keratoconjunctivitis

Y Takano, K Fukagawa, M Dogru, N Asano-Kato, K Tsubota, H Fujishima

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**Background:** Inflammatory cells infiltrating to the tarsal conjunctiva are thought to be involved in the pathogenesis of corneal lesions in severe allergic conjunctival diseases. The relation between such cells and the severity of corneal lesions was studied.

**Methods:** Six patients with atopic keratoconjunctivitis (AKC) were enrolled in this study. Tarsal brush cytology findings and the severity of corneal damage at that point were recorded and analysed for correlation.

**Results:** Four out of six patients exhibited correlation between eosinophils and corneal damage. Three out of six patients exhibited correlation between neutrophils and corneal damage. Two out of six patients exhibited correlation between both eosinophils and neutrophils and corneal damage. Analysis of all data from all patients taken together revealed that both eosinophils and neutrophils in brush cytology samples significantly correlated with corneal damage.

**Conclusions:** Inflammatory cells in brush cytology samples correlated with corneal damage. Evaluation of the relative percentages of inflammatory cells in brush cytology samples is a useful method of assessing disease activity in allergic conjunctival disease.

Atopic keratoconjunctivitis (AKC) is a severe chronic allergic conjunctival disease and is often associated with corneal complications such as erosion and ulcers. Histologically, these diseases are characterised by the infiltration of lymphocytes, neutrophils, mast cells, eosinophils, and basophils to the conjunctiva. We have previously reported that the percentages of eosinophils and neutrophils in tarsal conjunctival brush cytology samples in patients with vernal keratoconjunctivitis (VKC) were significantly higher in subjects with severe corneal lesions such as ulcers.<sup>1</sup> Inflammatory cells infiltrating from the tarsal conjunctiva are therefore thought to have an important role in the pathogenesis of corneal lesions.

In this study we examined the relation between the percentage of each inflammatory cell in tarsal conjunctival brush cytology samples and the severity of corneal damage at that point in individual patients over a period of time.

## METHODS

Six patients with AKC (range 5–20 years, all male) with severe corneal lesions including ulcers were enrolled in this study. All patients were diagnosed as atopic dermatitis by dermatologists and diagnoses of AKC were made from slit lamp examinations of the ocular surface findings. After informed consent was obtained, brush cytology was performed on the upper tarsal conjunctiva as previously described.<sup>2</sup>

After topical anaesthesia (0.4% oxybuprocaine hydrochloride), mucous discharge was carefully removed and the tarsal conjunctiva was scraped several times with Cytobrush-S (Medscand, Malmö, Sweden). The collected cells were suspended in Hanks solution and were then fixed onto glass slides by cytospin (28 g for 5 minutes, Sakura Seiki Co Ltd, Tokyo, Japan). The slides were stained by May-Giemsa and the numbers of various types of cells were counted up to a total of 500 under microscopic observation and the percentages of each cell type was recorded. All subjects were assessed by brush cytology at more than five time points.

The severity of corneal lesions was assessed by fluorescein staining. The cornea was divided into three sections horizontally and each section was scored from 0 to 3 and the total score was recorded.<sup>3</sup> Eyes with ulcers or erosions were scored as 10. The relation between the percentage of inflammatory cells and corneal epithelium score was plotted and statistically analysed for correlation. *p* Values of less than 0.05 were considered to be statistically significant.

## RESULTS

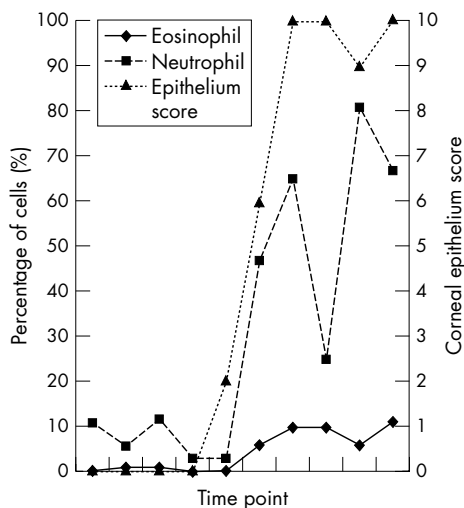
Four out of six patients exhibited correlation between eosinophils and the severity of corneal damage. Three out of six patients exhibited correlation between neutrophils and the severity of corneal damage. Two out of six patients exhibited correlation between both eosinophils and neutrophils and the severity of corneal damage. Figure 1 shows the percentage of inflammatory cells and the severity of corneal lesions in a representative case. Eosinophils exhibited a tendency to parallel the severity of corneal lesions and decreased to almost zero when corneal lesions were not present. Neutrophils also tended to be increased in acute exacerbations; however, neutrophils were also present in many cases in decreased numbers even when there was little corneal damage.

Statistical analysis of all data from all cases obtained in this study taken together also revealed that eosinophils and neutrophils in brush cytology samples significantly correlated with corneal damage ( $r = 0.41$ ,  $p < 0.001$  for both cell types).

## DISCUSSION

Eosinophils are known to be important effector cells in the pathogenesis of corneal lesions in severe conjunctival allergies. Eosinophils and cytotoxic eosinophil granule proteins such as eosinophil cationic protein (ECP)<sup>4,5</sup> and major basic protein (MBP)<sup>6</sup> are present in the tears of patients with VKC. In a previous study, we detected large numbers of eosinophils and high concentrations of eotaxin, a potent chemoattractant for eosinophils, in tear samples of patients with severe corneal lesions.<sup>7</sup> ECP and MBP have

**Abbreviations:** AKC, atopic keratoconjunctivitis; ECP, eosinophil cationic protein; MBP, major basic protein; VKC, vernal keratoconjunctivitis



**Figure 1** A case that exhibited positive correlation between the percentage of eosinophils and corneal damage. The percentage of eosinophils almost parallels corneal damage.

been shown to affect corneal epithelial cell viability and morphology *in vitro*.<sup>8</sup>

In contrast, the role of neutrophils in the pathogenesis of ocular allergies is less clearly defined. Neutrophils contain proteolytic enzymes such as collagenase, elastase, and myeloperoxidase that may cause primary tissue damage or may perpetuate the injurious process. We have previously reported that the percentage of neutrophils in brush cytology samples from patients with VKC were significantly higher in patients with corneal lesions.<sup>1</sup>

Brush cytology is a useful method of collecting cells including inflammatory cells from the conjunctival epithelium.<sup>2</sup> In order to gain insights into the relative importance of each type of cell in the pathogenesis of such lesions, we investigated the relation between the number of each type of cell and the severity of the corneal lesions.

In our study we found that eosinophils correlated with corneal damage in four out of six patients compared to three out of six patients in the case of neutrophils. In some cases (representative case shown in fig 1), the changes in the number of eosinophils paralleled corneal damage and decreased to almost zero when corneal lesions were not

present. Neutrophils also tended to be increased in acute exacerbations; however, neutrophils were also present in many cases in decreased numbers even when there was little corneal damage. This difference in the relation of each type of cell to corneal damage may be because neutrophils not only cause primary tissue damage through their granular constituents but also have a critical role in the non-specific reactions of scavenging inflammatory stimuli and damaged connective tissue.

To conclude, inflammatory cells, in particular eosinophils, exhibited a tendency to parallel corneal damage. Such cells may play an important part in the pathogenesis of corneal lesions in severe ocular allergies. Evaluation of the relative percentages of inflammatory cells in brush cytology samples is a useful method of assessing disease activity in allergic conjunctival disease.

#### Authors' affiliations

Y Takano, K Fukagawa, M Dogru, N Asano-Kato, K Tsubota, H Fujishima, Department of Ophthalmology, Keio University, Tokyo, Japan

Correspondence to: Yoji Takano, Department of Ophthalmology, Kawasaki Municipal Hospital, 12-1, Shinkawa-dori, Kawasaki-ku, Kawasaki-shi, Kanagawa, Japan 210-0013; RXN12332@nifty.ne.jp

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#### REFERENCES

- Miyoshi T, Fukagawa K, Shimmura S, *et al*. Interleukin-8 concentrations in conjunctival epithelium brush cytology samples correlate with neutrophil, eosinophil infiltration, and corneal damage. *Cornea* 2001;**20**:743-7.
- Tsubota K, Takamura E, Hasegawa T, *et al*. Detection by brush cytology of mast cells and eosinophils in allergic and vernal conjunctivitis. *Cornea* 1991;**10**:525-31.
- Shimmura S, Ono M, Shinozaki K, *et al*. Sodium hyaluronate eyedrops in the treatment of dry eyes. *Br J Ophthalmol* 1995;**79**:1007-11.
- Leonardi A, Borghesan F, Faggian D, *et al*. Eosinophil cationic protein in tears of normal subjects and patients affected by vernal keratoconjunctivitis. *Allergy* 1995;**50**:610-13.
- Montan PG, van Hage-Hamsten M. Eosinophil cationic protein in tears in allergic conjunctivitis. *Br J Ophthalmol* 1996;**80**:556-60.
- Udell JJ, Gleich GJ, Allansmith MR, *et al*. Eosinophil granule major basic protein and Charcot-Leyden crystal protein in human tears. *Am J Ophthalmol* 1981;**92**:824-8.
- Fukagawa K, Nakajima T, Tsubota K, *et al*. Presence of eotaxin in tears of patients with atopic keratoconjunctivitis with severe corneal damage. *J Allergy Clin Immunol* 1999;**103**:1220-1.
- Trocme SD, Hallberg CK, Gill KS, *et al*. Effects of eosinophil granule proteins on human corneal epithelial cell viability and morphology. *Invest Ophthalmol Vis Sci* 1997;**38**:593-9.