

Cytological changes in tears during the secondary conjunctival response induced by nasal allergy

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Accepted 3 March 2012

ABSTRACT

Purpose Allergic conjunctivitis (AC) can be caused by an allergic reaction localised exclusively in the conjunctivae and initiated by direct exposure of conjunctivae to an allergen (primary AC form) or it can be induced secondarily by an allergic reaction occurring primarily in the nose (secondary AC form).

Objectives To investigate the cellular profiles in tears accompanying the particular types of secondary conjunctival response (SCR).

Methods In 53 seasonal AC or perennial AC patients developing 53 positive SCRs (17 immediate, 21 late, 15, delayed) and 31 negative responses to the nasal provocation test with allergen (NPT), the NPTs were repeated and supplemented with, cytological examination of the tears.

Results The immediate SCRs ($p < 0.01$), appearing 10–120 min after the NPT, were associated with increased eosinophil and mast cell counts. The late SCRs ($p < 0.01$), occurring 5–12 h, were accompanied by increased eosinophil, neutrophil, basophil and epithelial cell counts. The delayed SCRs ($p < 0.05$), appearing 24–48 h, were associated with increased lymphocyte, neutrophil, monocyte, epithelial and goblet cell counts.

Conclusions The secondary immediate, late and delayed conjunctival responses, induced by nasal allergy, were associated with different cellular profiles in the tears. The cells, except mast, epithelial and goblet cells, displaying no intracellular changes, had probably migrated from conjunctival capillaries, affected by factors released during the initial nasal allergic reaction, without participating in the allergy processes. In AC patients demonstrating insufficient therapeutic compliance, the role of nasal allergy should be examined.

INTRODUCTION

Disorders of the conjunctiva in which an allergic component plays a key role include three groups: (1) allergic conjunctivitis (AC) affecting only conjunctiva, such as seasonal AC or perennial AC; (2) allergic keratoconjunctivitis, affecting conjunctiva and cornea, such as vernal keratoconjunctivitis and atopic keratoconjunctivitis; and (3) giant papillary conjunctivitis.^{1–5} Two forms of AC can be distinguished; an AC form, where the allergic reaction takes place primarily in the conjunctiva due to the direct exposure of conjunctiva to an external (inhalant) allergen (so-called primary AC form), and an AC form, which is secondarily induced by the allergic reaction occurring primarily in the nasal mucosa due to the exposure of nasal mucosa to an allergen (so-called secondary AC form).^{6–8}

Various hypersensitivity mechanisms, such as immediate (immunoglobulin E (IgE)-mediated,

type I), late (type III) or delayed type (cell-mediated, type IV) can participate in the primary and in the secondary form of AC.^{1–16} The involvement of different immunological mechanism results, then, in the development of different types of conjunctival response (CR), an immediate CR (ICR), a late CR (LCR) or a delayed CR (DYCR).^{1–3 6–11 13–16}

There is a dearth of information concerning the role of an allergic reaction occurring initially in the nasal mucosa in the induction of the secondary conjunctival response (SCR).⁸ Moreover, no data are available to illustrate the cytological changes in the tears accompanying the particular types of SCR.^{6–8}

The purpose of this study being a continuation of our earlier work^{7 8} was: (1) to investigate the appearance and changes in the counts of particular cell lineages in tears during the SCR types; (2) to evaluate the significance of the individual cell types and the changes in their counts in tears for the possible mechanism(s) underlying the SCRs.

PATIENTS AND METHODS

Patients

Fifty-three of the 87 patients suffering from AC for more than 4 years, responding insufficiently to the ophthalmologic treatment, referred to our Department of Allergology & Immunology (Institute of Medical Sciences 'De Klokkenberg', Breda, The Netherlands) for more extensive diagnostic analysis, have developed SCR to the nasal provocation tests (NPT) with allergen, performed as a part of the routine diagnostic procedure. They volunteered to participate in this study. These patients, 24 men and 29 women, 18–41 (27 ± 5 ; mean $\pm$ SD) years of age, seasonal AC (SAC; $n = 24$) or perennial AC (PAC; $n = 29$), showed conjunctivitis symptoms and positive skin tests to various inhalant allergens. They all had normal intraocular pressure and did not suffer from other disease. The conjunctival provocation tests (CPT) with various inhalant allergens ($n = 27$) performed previously in 16 of these patients were negative. Patients were treated with topical and systemic H1-receptor antagonists, ocular glucocorticosteroids, topical vasoconstrictors, decongestants and non-steroid anti-inflammatory drugs without substantial improvement in their conjunctival complaints.

Patients underwent a routine diagnostic procedure consisting of a detailed disease history recording, general examination, laboratory tests, bacteriological screening of the tears and nasal secretions (NS), skin tests with inhalant allergens, x-ray of the paranasal sinuses, nasoscopy, cytological examination of the NS and tears, ophthalmoscopy, slit-lamp evaluation and vital staining with fluorescein.

The diagnostic procedure revealed a positive nasal allergy history (n=46; table 1S), positive skin (intradermal) tests with various inhalant allergens (n=53; table 1S), oedematous nasal mucosa, increased eosinophil and neutrophil counts in the NS (n=51), conjunctival hyperaemia, hypersecretions (n=53), blood eosinophilia (n=7) and positive specific IgE in the serum (n=11). In these 53 patients, 84 NPT with inhalant allergens, with respect to the suspect disease history and/or positive skin tests (table 2S) and 53 phosphate-buffered saline (PBS) control tests were performed by rhinomanometry^{6–8 17 18} combined with recording of the ocular signs and symptoms.^{6–8} The patients were investigated in a period without acute ocular and nasal complaints, outside the allergen-relevant season.

The 53 positive NPTs and 53 PBS controls (table 1S; figure 1C, figure 2C, figure 3C) were repeated 1–2 weeks later and supplemented with tear collection for the cytological examination. Long-acting H1-receptor antagonists and topical glucocorticosteroids were withdrawn 6 weeks, whereas other treatments 48 h before each of the NPTs.

Ethical approval

The study protocol was approved by the Ethical Committee IBR-MCK (Institute of Medical Sciences 'De Klokkenberg', Breda, The Netherlands) and oral informed consent was obtained from all study participants.

Allergens, skin tests, nasal challenges with allergens and control challenges with PBS are described in the online supplementary files.

Conjunctival response

The conjunctival and subjective symptoms were registered before and during all NPTs with allergens and PBS controls at the same time-points as the NPG (nasopharynx-nostril pressure gradients = ΔP in cm H₂O) values.^{6–8} The conjunctiva was assessed by ophthalmoscopy including a slit lamp. The conjunctival signs (hyperaemia/injection, chemosis, hyperlacrimation, and palpebral oedema) and the subjective symptoms (itching/burning, blurred vision and photophobia) were evaluated by means of the Pelikan's scoring system (table 3S).^{7 8} The evaluation criteria were as follows: 0 = absent, 1 = mild (present to a slight degree), 2 = moderate, 3 = pronounced (moderately severe), 4 = severe (table 3S). Differences in total sign score of ≥ 4 points (3 ± 1 , mean $\pm$ SE), recorded at least at three consecutive time-intervals, were found to be statistically significant ($p < 0.05$).⁷

Collection and processing of tears

The tears were collected from the fornix of each eye separately by means of micropipette before, and repeatedly after the NPTs by the following schedule: (1) Secondary ICR (SICR): before and 0–10, 20–30, 60, 90, 120, 180 and 240 min after the challenge (the tear samples collected from 0 to 10 and from 20 to 30 min were pooled with respect to the small amounts); (2) Secondary LCR (SLCR): before and then 30, 60 and 90 min and every hour up to the 12th h and at 24th and 28th h after the challenge; (3) Secondary DYCR (SDYCR): before and 30, 60 and 120 min and every second hour during the time-periods between the 24th–36th and 48th–56th h after the NPTs.

The tear specimens (10–20 μ l; $14 \pm 5 = \text{mean} \pm \text{SD}$) were spread out on the slide surface by means of a glass probe. The air-dried specimens were fixed by polyethylene glycol and stained by modified May-Grünwald-Giemsa method.^{6 17 18}

Specimens were dehydrated by methyl alcohol, mounted in Canada balsam and scanned microscopically by a double-blind

manner.^{6 17–19} The particular cell types were distinguished according to the cytological criteria described in detail in our previous papers.^{6 17–19} The absolute numbers of particular cell types were counted per microscopic field at magnification $\times 250$, and the mean values were, calculated from 20 fields per each eye separately. The mean values from both the eyes were finally calculated and expressed in numbers of cells per 1 microscopic field ($\times 250$ magnification). Doubtful cells were re-examined under oil immersion (magnification $\times 1200$).

Control group

In 12 patients with allergic rhinitis without ocular disease history and with normal ophthalmological findings who volunteered to participate as controls, the 12 repeated NPTs were supplemented with conjunctival feature registration and cytological examination of the tears.

Statistical analysis

The mean NPG and the mean total conjunctival scores of the same response type were compared with corresponding PBS values at each of the time-points and analysed by the Mann–Whitney U test. The changes in the cell counts during the NPTs and PBS controls were analysed by the Wilcoxon matched-pair signed rank test, comparing the post-challenge with the mean pre-challenge values at each of the time-points. The cellular changes were evaluated separately from each eye and then their means from both the eyes were calculated. A p value < 0.05 was considered to be statistically significant.²⁰

Additional statistical analysis is presented in the online supplementary files.

RESULTS

Nasal responses (NRs) are presented in the online supplementary files.

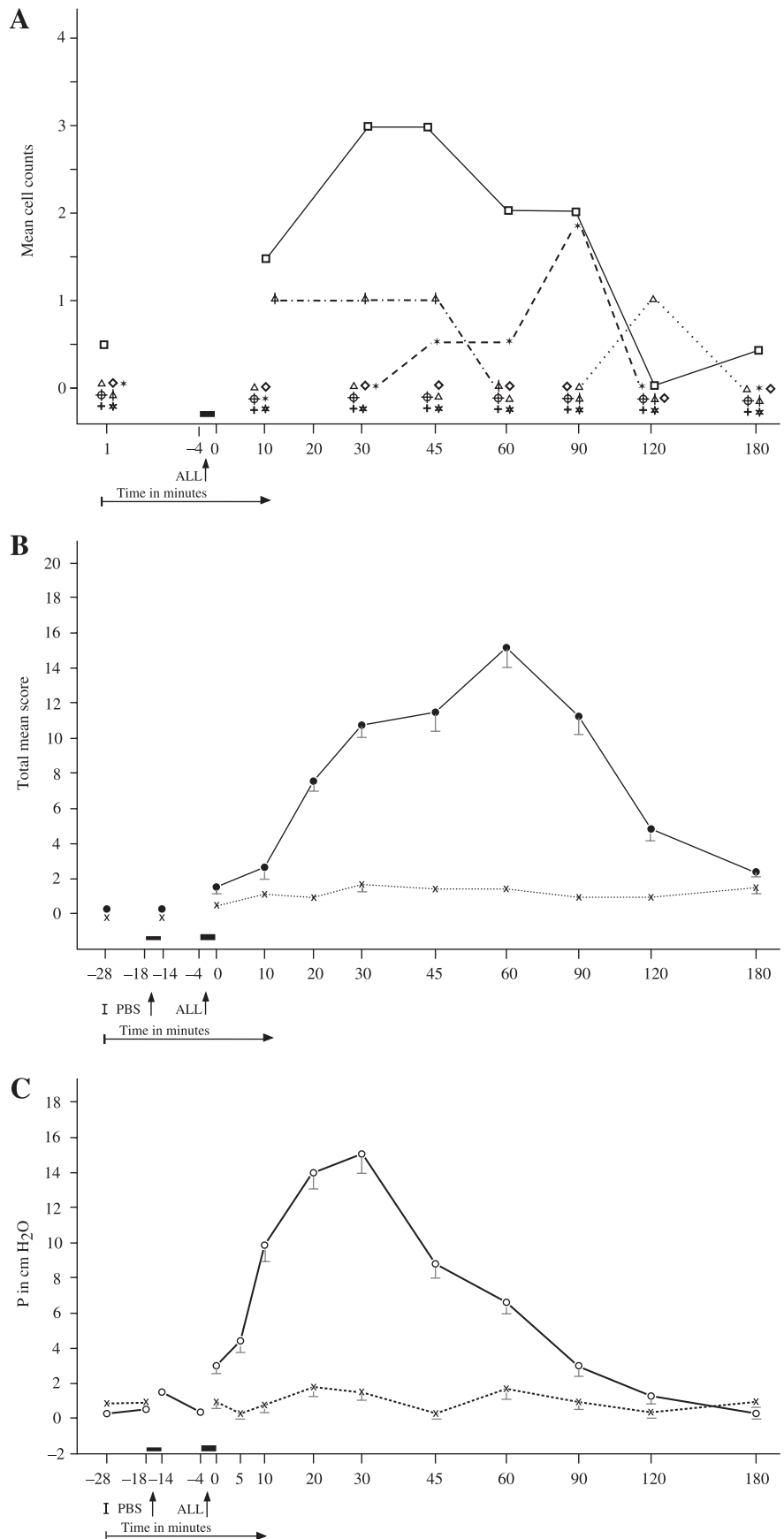
Secondary conjunctival responses (SCRs)

The 53 repeated NRs were associated with following positive SCRs (4 or more total score points at least at three consecutive time-intervals)⁷: (1) 17 immediate response (SICR; figure 1B): onset within 10 min, maximum between 45 and 60 and resolving within 120 min ($p < 0.01$); (2) 21 late response (SLCR; figure 2B): onset between 5 and 6, maximum between 7 and 10 and resolving within 12 h ($p < 0.001$); (3) 15 delayed response (SDYCR; figure 3B): onset between 24 and 28, maximum between 30 and 36 and resolving within 56 h after the NPT ($p < 0.05$). No corneal signs were observed during the SCR of any type. No significant conjunctival changes were recorded during the 31 negative nasal responses (NNRs; $p > 0.05$) or the 53 PBS controls ($p > 0.2$). No differences in the conjunctival features were observed between the right and left eye ($p > 0.1$). Agreement of SCRs with disease history and skin tests is presented in table 2S.

Cytological changes in the tears during the SCRs

The SCRs were accompanied by relatively low cell counts in tears as compared with the primary types of AC.^{9–11 16 21–25} The SICRs were associated with increased counts ($p < 0.05$) of eosinophils and mast cells in tears between 30 and 60 min after the NPT (figure 1A, table 1). The mast cells displayed disappearance of most intracellular granules, intracellular vacuolisation, cellular membrane wrinkling, diminished stain intake and some of them were disrupted. The eosinophils were intact. The SLCRs were accompanied by increased counts ($p < 0.05$) of eosinophils between 3 and 7 h, neutrophils between 5 and

Figure 1 The secondary immediate conjunctival responses (SICRs; n=18) accompanying the isolated immediate nasal responses (n=17). (A) The mean score of particular cell counts during the SICR: $\square$ = eosinophils; $\triangle$ = neutrophils; $\oplus$ = basophils; $\blacktriangle$ = mast cells; $\diamond$ = lymphocytes; $+$ = monocytes; $\star$ = goblet cells; $\star$ = conjunctival epithelial cells. (B) The mean total score of conjunctival signs and symptoms during the SICR $\bullet$ and phosphate buffered saline (PBS) $\times$. (C) The mean rhinomanometric values (NPG) recorded during the isolated immediate nasal responses $\circ$ and PBS $\times$. ALL, Allergen challenge; Bars, mean $\pm$ SEM; I, Initial (baseline) values.

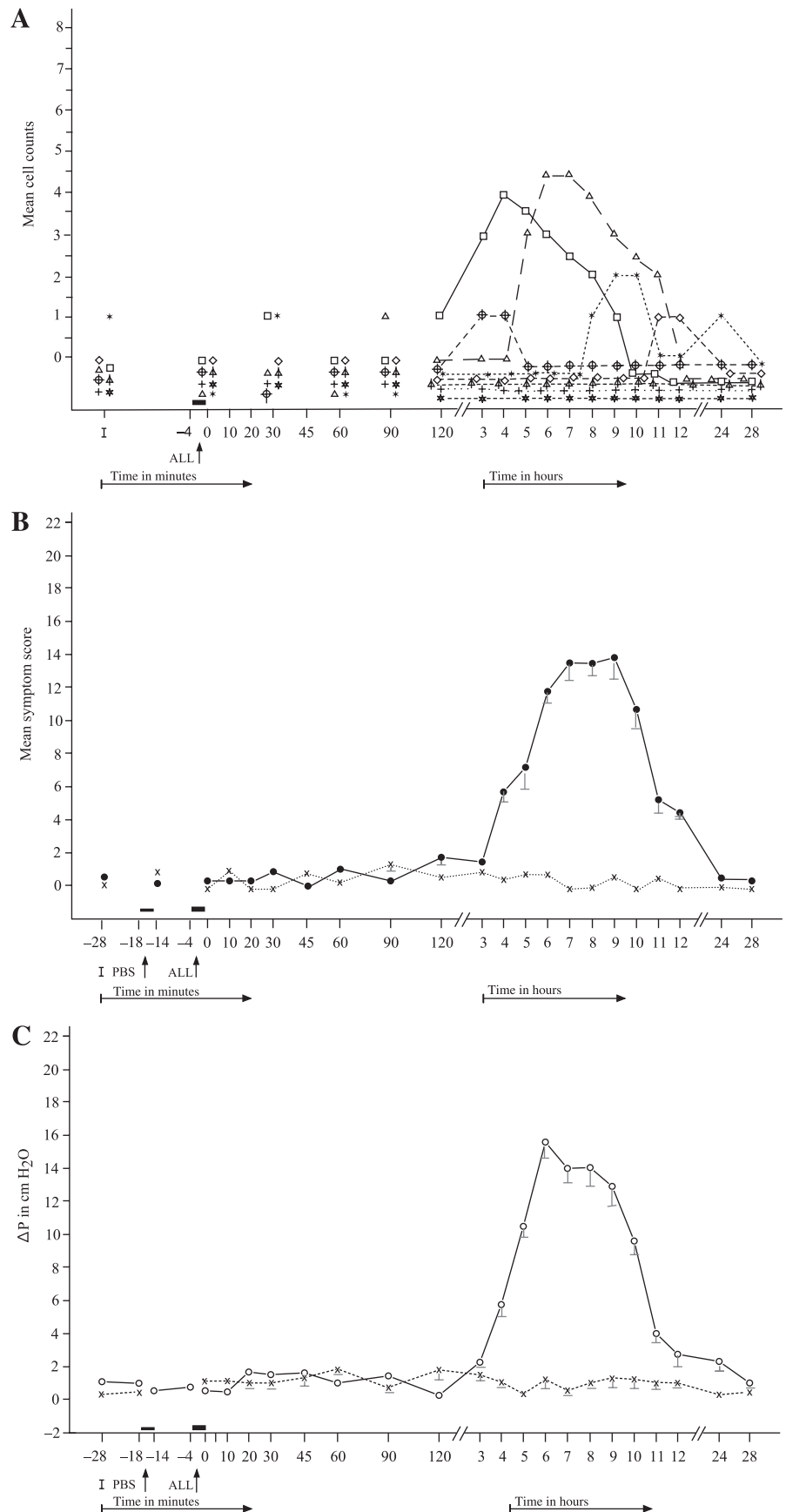


10 h, basophils at 3 h and epithelial cells between 9 and 10 h after the NPT (figure 2A, table 2). The cells were intact. The epithelial cells exhibited cellular deformation, cellular membrane

wrinkling and diminished cytoplasmic homogeneity. The SDYCRs were associated with increased counts ($p<0.05$) of neutrophils between 12 and 32 h, lymphocytes between 24

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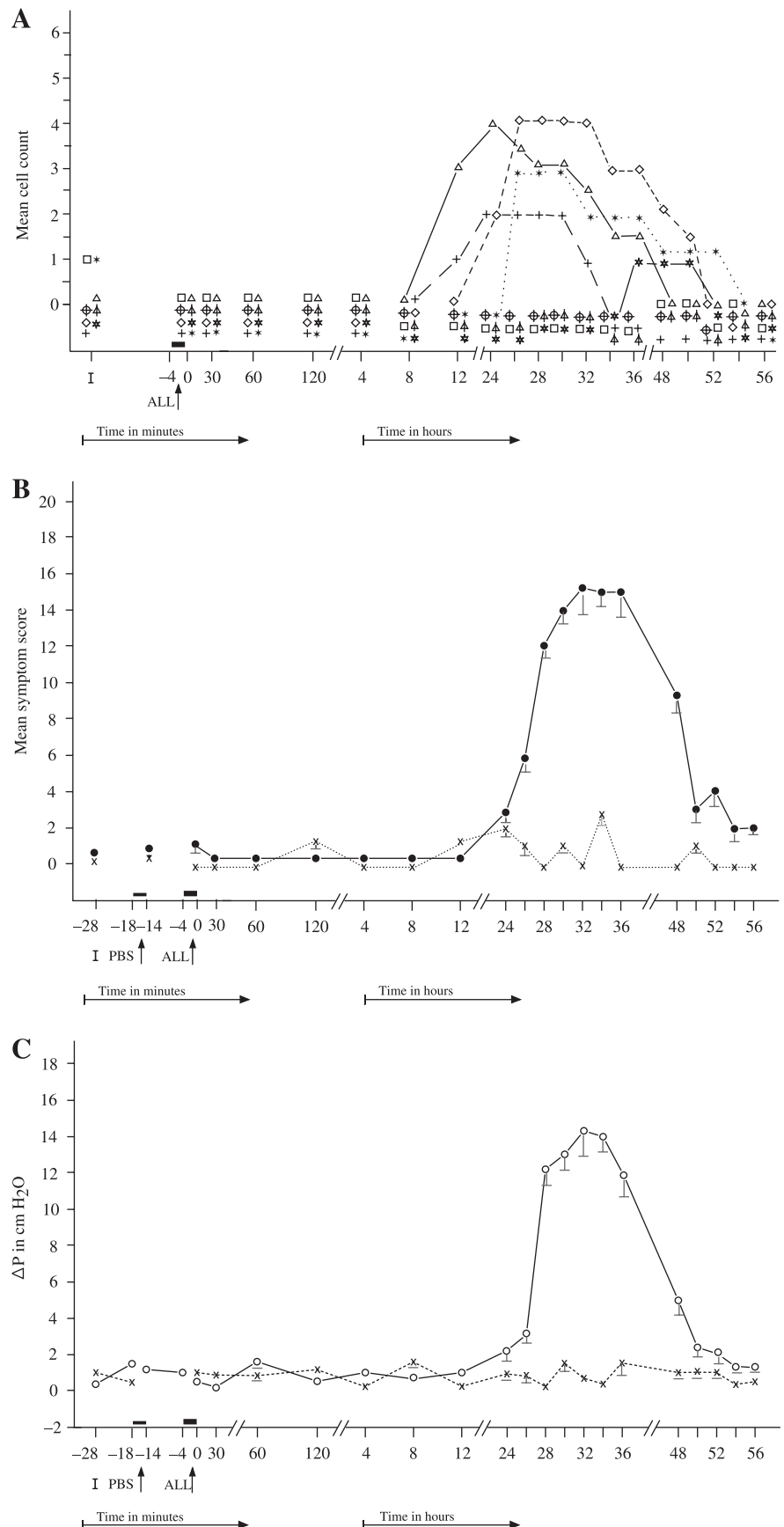
Figure 2 The secondary late conjunctival responses (SLCRs; n=20) accompanying the isolated late nasal responses (ILNR; n=21). (A). The mean score of particular cell counts during the SLCR: $\square$ = eosinophils; $\triangle$ = neutrophils; $\oplus$ = basophils; $\blacktriangle$ = mast cells; $\diamond$ = lymphocytes; $+$ = monocytes; $\star$ = goblet cells; $\ast$ = conjunctival epithelial cells. (B) The mean total score of conjunctival signs and symptoms during the SLCR $\bullet$ and PBS $\times$. (C) The mean rhinomanometric values (NPG) recorded during ILNR $\circ$ and PBS $\times$. ALL, Allergen challenge; Bars, mean $\pm$ SEM; I, Initial (baseline) values; PBS, Phosphate buffered saline.



and 48 h, monocytes between 12 and 32 h, epithelial cells between 26 and 36 h and goblet cells between 36 and 50 h after the NPT (figure 3A, table 3). These cells were intact. The

epithelial cells were deformed, displayed cellular membrane wrinkling, diminished stain intake, and the goblet cells were empty and wrinkled. During the 53 PBS controls and 31

Figure 3 The secondary delayed conjunctival responses (SDYCRs; n=15) accompanying the isolated delayed nasal responses (IDYNR; n=15). (A) The mean score of particular cell counts during the SDYCR: □ = eosinophils; △ = neutrophils; ⊕ = basophils; ▴ = mast cells; ◇ = lymphocytes; + = monocytes; ☆ = goblet cells; ★ = conjunctival epithelial cells. (B) The mean total score of conjunctival signs and symptoms during the SDYCR ● and PBS x. (C) The mean rhinomanometric values (NPG) recorded during IDYNR ○ and PBS x. ALL, Allergen challenge; Bars, mean±SEM; I, Initial (baseline) values, PBS, Phosphate buffered saline.



NNRs, only sporadic epithelial cells were observed. No significantly different results were found between both the eyes ($p>0.1$).

Control subjects

In 12 control subjects developing four immediate ($p<0.01$), six late ($p<0.02$) and two delayed ($p<0.05$) NRs to repeated NPTs,

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Table 1 Mean numbers of particular cell types in tears during the positive secondary immediate conjunctival response (SICR) and phosphate buffered saline (PBS) control challenge (n=17)

		Before the challenge	After the challenge (min)							
			0–10	20–30	45	60	90	120	180	240
Eosinophils										
SICR	0.5	2†	3*	3*	2†	2†	0	0.5	0	
PBS	0	0	0	0	0	0	0.5	0	0.5	
Neutrophils										
SICR	0	0	0	0	0	0	1	0	0	
PBS	0	0	0	0	0	0	0	0	0	
Mast cells										
SICR	0	1*	1*	1*	0	0	0	0	0	
PBS	0	0	0	0	0	0	0	0	0	
Basophils										
SICR	0	0	0	0	0	0	0	0	0	
PBS	0	0	0	0	0	0	0	0	0	
Lymphocytes										
SICR	0	0	0	0	0	0	0	0	0	
PBS	0	0	0	0	0	0	0	0	0	
Monocytes										
SICR	0	0	0	0	0	0	0	0	0	
PBS	0	0	0	0	0	0	0	0	0	
Goblet cells										
SICR	0	0	0	0	0	0	0	0	0	
PBS	0	0	0	0	0	0	0	0	0	
Epithelial cells										
SICR	0	0	0	0.5	0.5	2.0*	0	0	0	
PBS	0	0	0	0	0	0	0	0	0	

The statistically significant magnitude of changes in the cells in tears (mean $\pm$ SD), were as follows: eosinophils 2 (2.10 $\pm$ 0.14); neutrophils 2 (2.25 $\pm$ 0.21); basophils 0.3 (0.28 $\pm$ 0.04); mast cells 0.5 (0.35 $\pm$ 0.11); lymphocytes 1 (1.23 $\pm$ 0.16); monocytes 0.4 (0.41 $\pm$ 0.19); epithelial cells 2 (2.17 $\pm$ 0.41); goblet cells 0.2 (0.20 $\pm$ 0.04).^{6–8}

Significance with respect to the baseline (before the challenge).

*=p<0.05; †=p $\leq$ 0.05.

Table 2 Mean numbers of particular cell types in tears during the positive secondary late conjunctival response (SLCR) and phosphate buffered saline (PBS) control challenge (n=21)

		Before the challenge	After the challenge (h)															
			½	1	1½	2	3	4	5	6	7	8	9	10	11	12	24	28
Eosinophils																		
SLCR	0	1	0	0	1	3*	4*	3.5*	3.0*	2.5*	2†	1	0	0	0	0	0	0
PBS	0	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0.5	0
Neutrophils																		
SLCR	0	0	0	1	0	0	0	3*	4.5*	4.5*	4.0*	3*	2.5*	0	0	0	0	0
PBS	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
Mast cells																		
SLCR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PBS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Basophils																		
SLCR	0	0	0	0	0	1*	0	0	0	0	0	0	0	0	0	0	0	0
PBS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lymphocytes																		
SLCR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1†	1†	0	0
PBS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Monocytes																		
SLCR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PBS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Goblet cells																		
SLCR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PBS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Epithelial cells																		
SLCR	1	0	0	0	0	0	0	0	0	0	1	2*	2*	0	0	1	0	0
PBS	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

The statistically significant magnitude of changes in the cells in tears (mean $\pm$ SD), were as follows: eosinophils 2 (2.10 $\pm$ 0.14); neutrophils 2 (2.25 $\pm$ 0.21); basophils 0.3 (0.28 $\pm$ 0.04); mast cells 0.5 (0.35 $\pm$ 0.11); lymphocytes 1 (1.23 $\pm$ 0.16); monocytes 0.4 (0.41 $\pm$ 0.19); epithelial cells 2 (2.17 $\pm$ 0.41); goblet cells 0.2 (0.20 $\pm$ 0.04).^{6–8}

Significance with respect to the baseline (before the challenge).

*=p<0.05; †=p $\leq$ 0.05.

Table 3 Mean numbers of particular cell types in tears during the positive secondary delayed conjunctival response (SDYCR) and phosphate buffered saline (PBS) control challenge (n=15)

	Before the challenge	After the challenge (h)																	
		½	1	2	4	8	12	24	26	28	30	32	34	36	48	50	52	54	56
Eosinophils																			
SDYCR	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PBS	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Neutrophils																			
SDYCR	0	0	0	0	0	0	3*	4*	3.5*	3*	3*	2.5*	1	0	0	0	0	0	0
PBS	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0
Mast cells																			
SDYCR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PBS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Basophils																			
SDYCR	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PBS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lymphocytes																			
SDYCR	0	0	0	0	0	0	0	2*	4*	4*	4*	4*	3*	3*	2*	1.5†	0	0	0
PBS	0	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0
Monocytes																			
SDYCR	0	0	0	0	0	0	1*	2*	2*	2*	2*	2*	0	0	0	0	0	0	0
PBS	0	0	0	0	0	0	0	0	0	0	0	0.2	0	0	0	0	0	0	0
Goblet cells																			
SDYCR	0	0	0	0	0	0	0	0	0	0	0	0	0	1*	1*	1*	0	0	0
PBS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Epithelial cells																			
SDYCR	1	0	0	0	0	0	0	0	3*	3*	3*	2*	2*	2*	1	1	1	0	0
PBS	1	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0

The statistically significant magnitude of changes in the cells in tears (mean±SD), were as follows: eosinophils 2 (2.10±0.14); neutrophils 2 (2.25±0.21); basophils 0.3 (0.28±0.04); mast cells 0.5 (0.35±0.11); lymphocytes 1 (1.23±0.16); monocytes 0.4 (0.41±0.19); epithelial cells 2 (2.17±0.41); goblet cells 0.2 (0.20±0.04).^{6–8}

Significance with respect to the baseline (before the challenge).

*=p<0.05; †=p≤0.05.

no conjunctival signs were recorded ($p>0.1$). In the tears, only sporadic intact neutrophils, eosinophils or epithelial cells (<1 cell per 5 microscopic fields) were recorded.

DISCUSSION

The objective signs and subjective symptoms of AC can be evaluated by various scoring (grading) systems, differing with respect to the parameters registered and the numbers of the grading points.^{1–4 24–28} We have applied our (Pelikan's) scoring system including, additionally, conjunctival chemosis, palpebral oedema, burning, blurred vision and photophobia (table 3S).^{7 8} In our system, the differences between the post-challenge and the pre-challenge mean values of the signs and symptoms were considered as basic parameters for the assessment of the CR.

The relationship between the conjunctivae and lacrimal ways and the nasal mucosa is realised both on the anatomical and on the functional levels,^{1 3 4 6–14 26 29} including (1) connection of the conjunctiva with the nasal cavity through the naso-lacrimal duct, facilitating the tear drainage into the nasal cavity and allowing also a retrograde penetration of various factors and cells from the nasal cavity into the conjunctivae, and (2) connection of both the organs through the blood vessels, lymphatic and the neurogenic network. These connection modalities share some common properties and show some links between both the organs. An allergic reaction occurring in the nasal mucosa can affect the conjunctiva in various ways upon involving of different mechanisms: (1) factors released in the nasal mucosa can reach the conjunctiva via a retrograde penetration through the naso-lacrimal duct;^{4 7–15} or they can be transported by the bloodstream through the local blood vessels^{4 6–13 29}; (2) this

reaction can stimulate the nasal neurogenic network releasing neuropeptides reaching the conjunctiva along and/or through the particular nerves^{7 8}; (3) various cells participating in the nasal allergic reaction can migrate through the blood and/or the lymphatic stream into the ocular tissue, or they can be expelled into NS and migrate through the lacrimal system into the conjunctivae;^{6–8} (4) the factors released during the nasal allergic reaction can stimulate the nose-associated lymphatic tissue (NALT), communicating with tear duct-associated lymphatic tissue (TALT), lacrimal drainage-associated lymphoid tissue (LDALT), conjunctival-associated lymphatic tissue (CALT) and eye-associated lymphatic tissue (EALT).^{6–8 29} In this way, the transmission of certain intercellular and cellular-tissue receptor signals and cellular traffic of various cells can be realised.^{4–13 23–26}

An additional mechanism involved in the cellular traffic among the particular organ-associated lymphatic tissues, is the defective 'homing mechanism' of the B- and T-lymphocytes, controlled by a number of homing factors.^{6–8 29} The allergic reactions in the conjunctival tissue, similar to the nasal mucosa, are dynamic processes in which a variety of cell types are involved in various steps of this process.^{1–16 21–26} These are also exfoliative processes leading to release of various cells into the NS or tears.^{1–13 15–19} These fluids execute the conditioning function of particular organs, and the drainage function of the cells being eliminated by the mucosal tissues after finishing their active participation in the allergic reaction.^{1 3 6 9–11 13 16–19 21 25 26} However, to distinguish exactly the portion of tears resulting from a genuine immunological mechanism from that portion being caused by reflex mechanisms, is a very difficult if not impossible task. Nevertheless, we are convinced that the tear specimens, collected by the very careful and non-irritating

technique used in this study, have been produced by the genuine immunological mechanism(s) initiated in the nasal mucosa and inducing secondarily the conjunctival response.

The cytological examination of tears is an easy and valuable technique for evaluation of changes in the counts and condition of particular cell types appearing in tears during the allergic reaction.^{9 11 13 16 21–25} However, the results generated by this method are limited only to tears and they do not indicate the cellular changes in the mucosal membrane.^{9 24 25}

The cytological examination of the conjunctiva can be performed by means of various techniques, such as brush, impression and scraping technique and biopsy technique.^{4 5 9 11 13 16 22–25 29}

The major disadvantages of these techniques, except the brush method, is the use of anaesthetics and certain traumatising effects of the conjunctival tissue and blood capillary neurogenic receptors causing undesirable reflexes. These techniques cannot be performed repeatedly on the same conjunctival location owing to traumatising effects.

By contrast, the cytological examination of the tears, collected by aspiration with a micropipette, is an easy non-traumatising method which does not require anaesthesia and which can be repeated almost endlessly.^{9 10 13 15 16 22–25} The tear specimens can be processed and stained by various methods.^{5 13 16 22 23 25} We have used May-Grünwald-Giemsa staining as a basic method.^{6 17–19}

Studies concerning the cytological examination of tears in AC patients are not numerous.^{9 16 22} The cell appearance has usually been investigated by means of a single-tear cytogram, revealing increased numbers of eosinophils, mast cells, epithelial cells and sometimes neutrophils. The cytological data generated by brush, impression or scraping methods and conjunctival biopsy, demonstrated some variations in the presence of eosinophils, neutrophils, mast cells and, sporadically, lymphocytes in the conjunctival tissue.^{9 16 22 25}

There is a dearth of data documenting dynamic course of the count changes of individual cell types in tears both during the primary CR types to the CPT with allergen^{6 13 16 23–25 27} and cytological changes in the tears during the SCRs due to the NPTs.^{6–8}

Our findings of low counts of almost intact cells in tears during the particular types of the SCR, would suggest the following conclusions: (1) the SICR, SLICR and SDYCR are associated with different cellular profiles in the tears; (2) the low counts of intact cells in tears during the SCRs, in contrast to the higher cell counts in tears during the primary CR to CPTs with allergen,^{9 13–15 24} and the high counts of the same cell lineages in the NS during the nasal allergic reaction,^{6 17–19} would exclude their participation in allergic reactions localised in the conjunctivae. These cells had probably migrated directly from the conjunctival capillaries affected by factors released during the initial allergic reaction in the nose and penetrating into the conjunctival tissue. By contrast, the mast, epithelial and goblet cells, appearing in the tears during the SCRs, displaying manifold cellular and intracellular changes were discharged from the conjunctival epithelium affected by factors released during the nasal allergic reaction and penetrating, subsequently, into the conjunctival tissue.^{6 9 16 17 25}

In AC patients, demonstrating insufficient therapeutic compliance to the usual ophthalmological treatment and low cell counts in the tears, the involvement of nasal allergy should be examined by means of the nasal allergen challenge(s) combined with monitoring of the conjunctival features and with respect to the outcomes, the therapeutic measures should be, if necessary, adjusted.

Contributors ZP is the sole contributor to this article and also takes full responsibility for the content of the paper and data presented.

Competing interests None.

Patient consent Obtained.

Ethics approval Ethics approval was provided by the ethical committee of the Institute of Medical Science "De Klokenberg", Breda, The Netherlands. The study has been carried out according to the Declaration of Helsinki and the study protocol was approved by the local ethics committee and informed consent was obtained from all study participants.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1. **Barney NP**, Graziano FM, Cook EB, *et al.* Allergic and immunologic diseases of the eye. In: Adkinson NF, Bochner BS, Busse WW, *et al.*, eds. *Middleton's Allergy, Principles & Practice*. 7th edn. Philadelphia: Mosby-Elsevier Inc, 2009:1117–37.
2. **Bielory L**, Friedlaender MH. Allergic conjunctivitis. *Immunol Allergy Clin N Am* 2008;**28**:43–57.
3. **Hingorani M**, Lightman S. Ocular allergy. In: Kay AB, ed. *Allergy and Allergic Diseases*. Oxford: Blackwell Sci, 1997:1645–70.
4. **McGill JI**, Holgate ST, Church MK, *et al.* Allergic eye disease mechanisms. *Br J Ophthalmol* 1998;**82**:1203–14.
5. **Dart JK**, Buckley RJ, Monnickendan M, *et al.* Perennial allergic conjunctivitis: definition, clinical characteristics and prevalence. A comparison with seasonal allergic conjunctivitis. *Trans Ophthalmol Sci UK* 1986;**105**:513–20.
6. **Pelikan Z**. *The Late Nasal Response*. Thesis. Amsterdam: The Free University of Amsterdam, 1996.
7. **Pelikan Z**. Seasonal and perennial allergic conjunctivitis: the possible role of nasal allergy. *Clin Exp Ophthalmol* 2009;**37**:448–57.
8. **Pelikan Z**. Allergic conjunctivitis and nasal allergy. *Curr Allergy Asthma Rep* 2010;**10**:295–302.
9. **Bacon AS**, Ahluwalia P, Irani AM, *et al.* Tear and conjunctival changes during the allergen-induced early- and late-phase responses. *J Allergy Clin Immunol* 2000;**106**:948–54.
10. **Anderson DF**. The conjunctival late-phase reaction and allergen provocation in the eye. *Clin Exp Allergy* 1996;**26**:1105–7.
11. **Calder VL**. Cellular mechanisms of chronic cell-mediated allergic conjunctivitis. *Clin Exp Allergy* 2002;**32**:814–17.
12. **Metz DP**, Hingorani M, Calder VL, *et al.* T-cell cytokines in chronic allergic eye disease. *J Allergy Clin Immunol* 1997;**100**:817–24.
13. **Leonardi A**, De Dominics C, Motterle L. Immunopathogenesis of ocular allergy: a schematic approach to different clinical entities. *Curr Opin Allergy Clin Immunol* 2007;**7**:429–35.
14. **Choi SH**, Bielory L. Late-phase reaction in ocular allergy. *Curr Opin Allergy Clin Immunol* 2008;**8**:438–44.
15. **Leonardi A**, Fregona IA, Plebani M, *et al.* Th1- and Th2-type cytokines in chronic ocular allergy. *Graefes Arch Clin Exp Ophthalmol* 2006;**244**:1240–5.
16. **Bonini S**, Bonini S, Berruto A, *et al.* Conjunctival provocation test as a model for the study of allergy and inflammation in humans. *Int Arch Allergy Appl Immunol* 1989;**88**:144–8.
17. **Pelikan Z**, Pelikan-Filipek M. Cytologic changes in the nasal secretions during the late nasal response. *J Allergy Clin Immunol* 1989;**83**:1068–79.
18. **Pelikan Z**, Pelikan-Filipek M. Cytologic changes in the nasal secretions during the immediate nasal response. *J Allergy Clin Immunol* 1988;**82**:1103–12.
19. **Pelikan Z**, Pelikan-Filipek M. Intracellular changes of eosinophils (EO), neutrophils (NE) and basophils (BS) in nasal secretions (NS) during the early (ENR) and late (LNR) nasal response. *Allergy Clin Immunol News* 1994;(Suppl 2):336.
20. **Tabachnick BG**, Fidell LS. *Using Multivariate Statistics*. New York: Harper Collins College Publishers, 1996.
21. **Trocme SD**, Leiferman KM, George T, *et al.* Neutrophil and eosinophil participation in atopic and vernal kerato-conjunctivitis. *Curr Eye Res* 2003;**26**:319–25.
22. **Kari O**. Atopic conjunctivitis, a cytologic examination. *Acta Ophthalmol (Copenh)* 1988;**66**:381–6.
23. **Bonini S**, Bonini S, Vecchione A, *et al.* Inflammatory changes in conjunctival scrapings after allergen provocation in humans. *J Allergy Clin Immunol* 1988;**82**:462–9.
24. **Helintö M**, Renkonen R, Tervo T, *et al.* Direct in vivo monitoring of acute allergic reactions in human conjunctiva. *J Immunol* 2004;**172**:3235–42.
25. **Leonardi A**. In-vivo diagnostic measurements of ocular inflammation. *Curr Opin Allergy Clin Immunol* 2005;**5**:464–72.
26. **Bielory L**. Allergic and immunologic disorders of the eye. I. Immunology of the eye; II. Ocular allergy. *J Allergy Clin Immunol* 2000;**106**:805–16; 1019–32.
27. **Abelson MB**, Chambers WA, Smith LM. Conjunctival allergen challenge. A clinical approach to studying allergic conjunctivitis. *Arch Ophthalmol* 1990;**108**:84–8.
28. **Abelson MB**, George MA, Schaefer K, *et al.* Evaluation of the new ophthalmic antihistamine, 0.05% levocabastine, in the clinical allergen challenge model of allergic conjunctivitis. *J Allergy Clin Immunol* 1994;**94**:458–64.
29. **Dua HS**, Gomes JA, Donoso LA, *et al.* The ocular surface as part of the mucosal immune system: conjunctival mucosa-specific lymphocytes in ocular surface pathology. *Eye* 1995;**9**:261–7.



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Br J Ophthalmol 2012 96: 941-948

doi: 10.1136/bjophthalmol-2011-301382

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