Tear and conjunctival changes during the allergen-induced early- and late-phase responses

Annette S. Bacon, FRCS,[a]* Poonam Ahluwalia, PhD,[b] Anne-Marie Irani, MD,[c] Lawrence B. Schwartz, MD, PhD,[c] Stephen T. Holgate, MD, DSc,[d] Martin K. Church, PhD, DSc,[b] and James I. McGill, DPhil, FRCS[a] *Southampton, United Kingdom, and Richmond, Va*

Background: Allergic eye disease is common, but little is known about the underlying disease mechanisms. Conjunctival allergen challenge causes symptoms similar to those of seasonal allergic conjunctivitis and is a useful model to study.
Objective: We have used allergen challenge to investigate the course of the ocular response, tear inflammatory mediators, tissue adhesion protein expression, and cellular infiltration.
Methods: Eighteen atopic patients and 4 nonatopic control subjects were challenged with extracted mixed grass or *Dermatophagoides pteronyssinus* in one eye and control vehicle in the other. The clinical response was recorded, and tears were collected over a 6-hour period. Conjunctival biopsy specimens were taken from the challenged eye at 6 or 24 hours.
Results: An early-phase response (maximal at 20 minutes) showed a significant increase in tear histamine and tryptase levels, reducing to control levels again by 40 minutes. At 6 hours, a late-phase response occurred with increased symptoms, a second peak of tear histamine and eosinophil cationic protein but not tryptase, upregulation of the adhesion molecules E-selectin and intercellular adhesion molecule, and a cellular infiltrate of mast cells, neutrophils, eosinophils, macrophages, and basophils, with T cells increased only in bulbar biopsy specimens.
Conclusions: The early peaks of tear histamine plus tryptase indicate that the mast cell is responsible for the early-phase response, but basophils may be involved in the late-phase response. Both tear and biopsy findings underline the significance of the late-phase response as the transition between a type I response and clinical disease. (J Allergy Clin Immunol 2000;106:948-54.)

Key words: *Allergy, conjunctivitis, histamine, tryptase, eosinophil cationic protein, mast cell, basophil, eosinophil, T cell, E-selectin, intercellular adhesion molecule 1, vascular cellular adhesion molecule 1*

The slow development of ocular and nasal allergic symptoms after exposure to plant products was originally noted by Blackley[1] in 1873. Since then, seasonal allergic conjunctivitis (SAC [hay fever]) and perennial allergic conjunctivitis have become universally recognized as self-limiting non–sight-threatening diseases that cause significant morbidity. The seasonal incidence of SAC is linked closely with cycles of release of airborne plant-derived allergens, and specific IgE against grass and tree pollens has been found in the tears of patients with this condition.[2] The IgE-dependent degranulation of mast cells by allergen gives rise to a typical type I allergic response, with the conjunctiva becoming edematous and red and the sufferer complaining of itching and watery eyes. In perennial allergic conjunctivitis, which does not follow a seasonal pattern, specific IgE against epitopes of the house dust mite has been detected in tears,[3] confirming that this is also an example of a type I allergic response.

Allergen challenge has been used by workers in many fields of allergology as a tool to investigate the mechanisms of clinical disease.[4] Type I or immediate hypersensitivity is usually considered to be a short-lived event not necessarily leading to prolonged inflammation.

However, evidence from allergen challenges of subjects with perennial allergic rhinitis,[5] atopic dermatitis,[6] and asthma[7] has indicated that, in addition to an early-phase response (EPR), there may be a late-phase response (LPR) beginning 4 to 6 hours after allergen challenge. It has been proposed[8,9] that the late response explains the transition of the early acute inflammatory response into days or weeks of disease. The unpredictable (although repeatable within an individual) nature of the LPR in a given organ, for example in the nose versus the skin,[9] may be a clue to the pattern of affliction of an individual by allergic disease. One reason that the LPR is taken as one of the most relevant models of clinical disease is the change in reactivity that occurs as a part of the reaction. For example, exposure to allergen has been reported to induce an increase of up to 100-fold in the sensitivity to nasal rechallenge 11 hours later in some individuals.[9,10] This increased reactivity may still be seen 24 hours later and correlates with markedly increased basophil and eosinophil infiltration into the

From [a]Southampton Eye Unit and [b]Allergy and Inflammation Sciences, Southampton General Hospital, Southampton; [c]the Departments of Pediatrics and Internal Medicine, Virginia Commonwealth University, Richmond, Va; and [d]Respiratory Cell and Molecular Biology Research Division, Southampton General Hospital, Southampton.

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*Currently affiliated with Royal Berkshire Hospital, Reading, United Kingdom.

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Reprint requests: James McGill, DPhil, FRCS, Southampton Eye Unit, Southampton University Hospital, Tremona Rd, Southampton, SO16 6YD, United Kingdom.

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Abbreviations used
ECP: Eosinophil cationic protein
EPR: Early-phase response
ICAM-1: Intercellular adhesion molecule 1
LPR: Late-phase response
SAC: Seasonal allergic conjunctivitis
VCAM-1: Vascular cellular adhesion molecule 1

nasal mucosa. The phenomenon of increased reactivity is similar to that of priming in allergic rhinitis, which was recognized in 1969.[11] A nonspecific increase in the reactivity may also occur in, for example, the nasal[10] or conjunctival[12] response to histamine. Thus LPRs appear to contribute substantially to the symptoms of allergic conjunctivitis, as well as to those of asthma, allergic rhinitis, urticaria, and atopic dermatitis.

Conjunctival allergen challenge has been undertaken since 1932.[4] Several different mediators have been shown to be released into the tears immediately after allergen challenge on the conjunctiva, including histamine, leukotrienes,[13] kinins, PGD2, and tosyl arginyl methylester esterase activity.[14] However, there has been little information about events during the LPR, although there is recent evidence to suggest that this response to allergen challenge in SAC is dose dependent.[15]

In this study we have used local allergen challenge in patients with SAC to investigate the secretion of mediators into the tears in both the EPR and LPR and to assess the expression of adhesion proteins and the infiltration of inflammatory cells in the conjunctiva during the LPR.

METHODS

Patients and control subjects

The study was carried out during January and February in the Northern Hemisphere (Southampton, United Kingdom) outside of the hay fever season, so as to avoid patients with ongoing disease and those on drug therapy. Patients were asked to confirm that they had been off drug treatment for at least a month. Inclusion criteria included a positive history of atopic eye disease (SAC), a positive allergen skin test response, and an age of 18 to 70 years. Exclusion criteria were active allergic eye disease, current topical or systemic therapy, and pregnancy. Eighteen atopic patients, with no symptoms at the time of entry to the trial and with histories compatible with SAC, were recruited and underwent conjunctival allergen challenge (mean age, 38.7 years). Four nonatopic subjects were also selected to serve as control subjects (mean age, 45.5 years). Because ethically we could only perform a biopsy on one eye of a subject, a further 8 out-of-season patients with SAC (mean age, 32.4 years) underwent conjunctival biopsy to serve as control subjects. Results were presented as a change from these control subjects. Basal cell counts were made from 4 normal control subjects and 7 out-of-season patients with SAC, 3 patients undergoing biopsy at 6 hours after challenge, and 4 patients undergoing biopsy at 24 hours after challenge. Tear samples were obtained from each eye, one eye being challenged with allergen and one with vehicle (control), and the results were expressed as a change from these control levels. The study was approved by the Southampton and South West Hampshire ethics committees, and all patients and control subjects gave informed consent. The tenets of the Declaration of Helsinki were followed.

Skin prick testing

Patients and control subjects were tested by means of forearm skin prick with a routine panel of allergen extracts, including grass, tree and nettle pollens, *Dermatophagoides pteronyssinus*, cat and dog dander, feathers, *Aspergillus fumigatus*, and *Candida albicans* (Soluprick, ALK Ltd). Responses to a 0.1% histamine solution and vehicle were included as positive and negative controls. The diameter of each wheal was measured with a ruler 10 minutes later and recorded, with a positive result being a wheal greater than 5 mm in diameter.

Allergen challenge

Mixed grass pollen extract was used for challenge in all but 3 atopic individuals, in whom *D pteronyssinus* was used instead. Challenge was elicited with a 30-μL drop of undiluted allergen instilled in the lachrymal sac area of the lower fomix of the challenged eye. To serve as a control, a similar volume of vehicle was instilled into the contralateral eye.

Tear collection

Tears were collected from both challenged and unchallenged eyes before challenge and 20 minutes, 40 minutes, and 6 hours after challenge by using cellulose sponges placed in the inferior fomix for periods of 1 minute.[14,16]

Biopsy

Tarsoconjunctival wedges were collected from the middle third of the upper lid after achievement of local anesthesia (topical benoxinate 0.4% and subconjunctival lignocaine 2%) by using a 3-mm trephine. Bulbar conjunctival specimens were also taken with scissors from beneath the upper fomix. The specimens were placed in acetone containing the enzyme inhibitors iodoacetamide (20 mmol/L) and phenylmethylsulfonyl fluoride and stored overnight at –20°C before processing.

Specimens were processed into glycolmethacrylate[17] (JB4 kit, Park Science Ltd) and immunostained, and mast cells, neutrophils, eosinophils, T cells, and macrophages were counted, as previously described.[18,19] Adhesion molecule expression was measured by recording the percentage of the total stromal vessel endothelial length of a blood vessel, assessed with Ulex lectin, staining for that particular mAb.[20] The following antibodies were used: mast cell tryptase (AA1)[21]; neutrophil elastase; eosinophil cationic protein (ECP; EG2); pan-T cell ($CD3^+$); T-cell subset ($CD4^+$); T-cell subset ($CD8^+$); macrophages (CD68; all from Dakopatts); intercellular adhesion molecule 1 (ICAM-1), E-selectin, vascular cellular adhesion molecule 1 (VCAM-1), all from British Biotechnology; and Ulex lectin (Sigma). For basophil counting, 2D7, a murine monoclonal anti-basophil antibody was used.[22] Biotinylated mouse anti-mouse F(ab)2 (Dakopatts) was used as the secondary antibody. Cell counting was carried out as previously described.[18,19,23]

Tear mediator assays

Histamine was measured by using a specific RIA with a detection limit of 0.2 nmol/L (Immunotech International). Tryptase levels were measured by using an RIA method (Pharmacia) with a detection limit of 0.5 μg/L. ECP was measured by using a double-antibody RIA with a detection limit of 2 μg/L (Pharmacia).

Scoring of clinical signs and symptoms

Signs and symptoms were scored for each eye by a single observer (A.S.B.) at 4 time points: before challenge and 20 minutes, 40 minutes, and 6 hours after challenge. Each sign or symptom was graded on a 0 to 4 scale by using the scales and scoring system devised by Abelson et al.[24] The maximum symptom score for any individual was 16 (Table I).

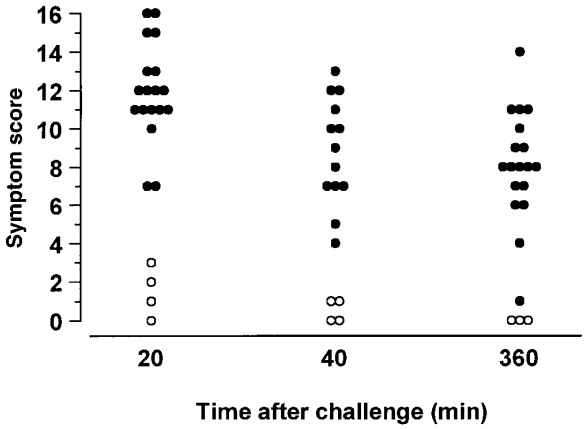

FIG 1. Time scale of patient score in 18 challenged atopic subjects and 4 control subjects (redness, itching, edema, and tearing). Note that at 40 minutes, only 13 of 18 patients were scored. *Filled circles,* Challenged atopic patient; *open circles,* challenged control subjects.

TABLE I. Basophil numbers in bulbar biopsy specimens taken from nonatopic eyes (control eyes) or out-of-season atopic eyes at either 6 or 24 hours after allergen challenge

No. of basophils/mm²		
Control	**6 h**	**24 h**
7.0	84.7	5.9
14.2	49.6	6.3
8.5	22.6	3.7
8.7		14.8

Bulbar biopsy sections were stained for human basophils by using antibody 2D7.[21]

The following scoring systems were used:

- Redness: 1, mild dilatation of conjunctival vessels; 2, moderate and/or patchy areas of hyperemia; 3, evenly distributed marked hyperemia; 4, extreme redness with dilatation of episcleral vessels.
- Edema: 1, a simple "glassy" (increased light reflex) look to the conjunctiva; 2, mild or patchy areas of fluid separating the conjunctival and deeper layers; 3, more marked and uniform areas of fluid separating the conjunctival and deeper layers; 4, gross swelling of the whole conjunctiva.
- Itching: Patients were asked to grade the sensation of itching on a scale of 0 to 4, with 1 representing mild irritation and 4 very intense and unpleasant itching.
- Tearing: 1, slightly watery eyes; 2, need to wipe the eyes at least once in the previous 10 minutes; 3, overt watering that required frequent wiping; 4, tears that poured onto the cheek.

RESULTS

Clinical course of signs and symptoms

The signs and symptoms score for each patient were combined (Fig 1). In 16 of 18 allergen-challenged atopic individuals, an EPR with edema, severe itching, and tearing appeared within 5 to 10 minutes and was maximal at 15 to 20 minutes. Redness was less pronounced at this stage, possibly because it was masked by edema. The median symptom score of the symptoms at 20 minutes was 12 (range, 7-16). Two individuals had symptom scores of only 7. However, both of these subjects reported that their symptoms were similar to those present when they had hay fever. The EPR decreased within 30 minutes in half of the patients, whereas in the rest of the patients, the symptoms continued for longer. At 40 minutes, the itching was reported to be less intense, but the signs were not much altered from the EPR stage, with the median symptom score at this time being 9 (range, 4-13).

The LPR began from 2 to 6 hours after allergen challenge. Redness, tearing, and a feeling of discomfort dominated the symptoms, which reached a maximum at 6 hours when the median score in 16 of 18 subjects was 8 (range, 4-14). Two subjects did not produce a significant clinical LPR, scoring 4 of 16 and 1 of 16, respectively. These indi-

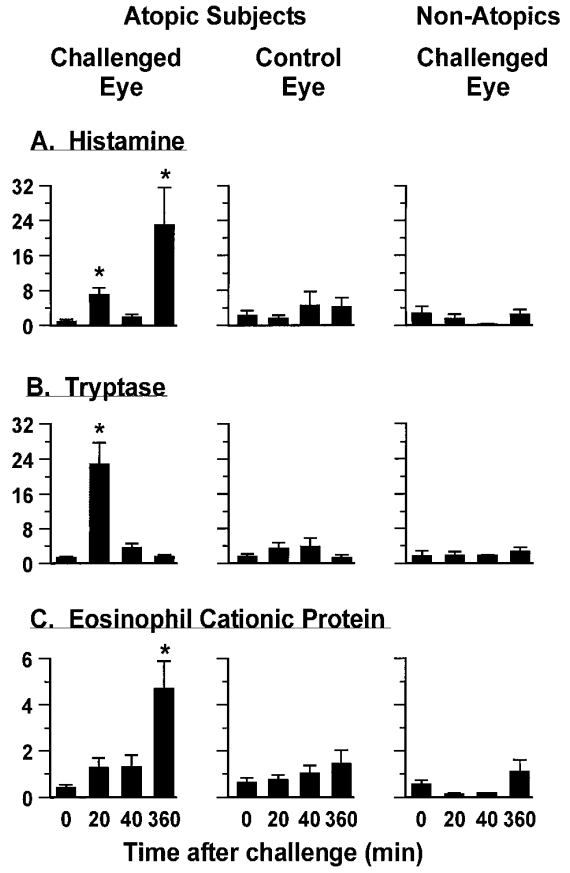

FIG 2. The generation of histamine, tryptase, and ECP into atopic subjects' tears after conjunctival allergen challenge. Tears were collected by cellulose sponges placed in the inferior fornix for periods of 1 minute at the times stated above. Two controls were used: vehicle instillation into the contralateral eye of the subjects receiving allergen challenge and allergen challenge of nonatopic subjects. Each result is the mean ± SEM for group sizes, as defined in the "Results" section. *Asterisks* indicate significant ($P < .05$) differences from baseline results in the same subjects.

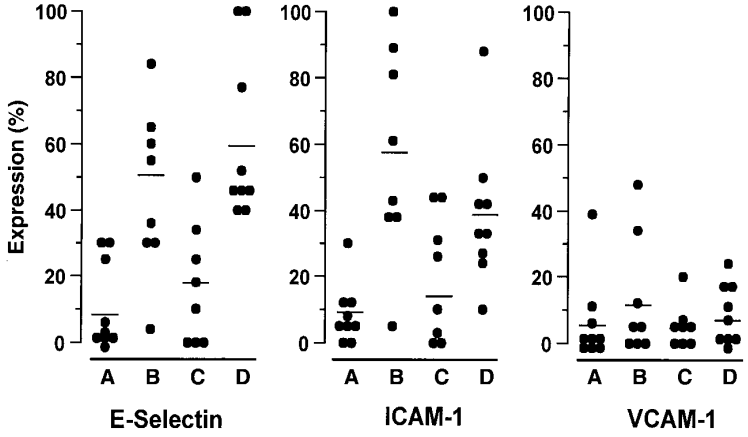

FIG 3. The expression of the adhesion proteins E-selectin, ICAM-1, and VCAM-1 in bulbar *(B)* and tarsal *(D)* biopsy specimens taken from atopic subjects 6 hours after conjunctival allergen challenge. Control biopsy specimens from bulbar *(A)* and tarsal *(C)* conjunctiva were from patients with SAC taken out of season. Results are the length of blood vessel endothelium staining with mAbs to adhesion proteins expressed as a percentage of the total blood vessel endothelial length assessed with Ulex lectin. The means of each group of 8 subjects are indicated by *horizontal lines*. Expression of E-selectin and ICAM-1, but not that of VCAM-1, was significantly greater in both bulbar (E-selectin, $P < .01$; ICAM-1, $P < .01$) and tarsal biopsy specimens (E-selectin, $P < .001$; ICAM-1, $P < .01$) from allergen-challenged eyes compared with control eyes.

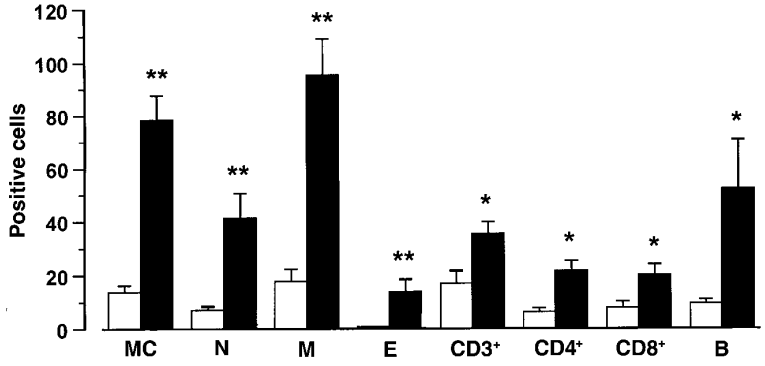

FIG 4. Individual cellular levels in the substantia propria of bulbar conjunctival biopsy specimens 6 hours after allergen challenge in 9 atopic and 22 normal subjects. The means of the counts are expressed as the number of cells per square millimeter. *White columns,* Control eyes; *black columns,* challenged eyes (**$P < .001$, *$P < .05$ to .003). Antibodies used were AA1 tryptase[21]; neutrophil elastase; ECP (EG2); pan-T cell (CD3); T-cell subset (CD4); T-cell subset (CD8); and macrophages (CD68; all Dakopatts). *MC*, Mast cells; *N*, neutrophils; *M*, macrophages; *E*, eosinophils; *B*, basophils.

viduals, who were not the same two who had low EPR symptom scores, were excluded at this stage. Two subjects were examined the following day, and all the others were telephoned to enquire about the duration of their symptoms. These had lasted 8 to 12 hours, and no individual had any residual symptoms or signs the following day.

Tear assay results

The results of the assays are shown in Fig 2. Before challenge, the amounts of mediators recovered from the eyes of all subjects were not significantly different when expressed as absolute amounts (mean ± SEM) corrected for total tear volumes.

At 20 minutes, there was a significant ($P < .01$) increase in the amount of histamine and tryptase in tears collected from the challenged eyes of 14 atopic patients compared with the control eyes of the same subjects. There was a small and variable increase in ECP in the challenged eyes of the atopic subjects, but this was not statistically significant.

At 40 minutes, histamine and tryptase levels in the tears returned to near to control levels. The levels of ECP, however, remained slightly and variably elevated, but again, the difference between the challenged and control eyes was not statistically significant.

Six hours after challenge, there was a second significant ($P < .05$) peak of histamine in the tears of the challenged eyes of 15 atopic subjects. There was no parallel rise in tryptase levels at this time. There was, however, a statistically significant ($P < .05$) increase in the amount of ECP in the tears of the challenged eyes of the atopic subjects.

At no time was there a statistically significant rise in tear levels of histamine, tryptase, or ECP in the control patients (Fig 2).

Expression of adhesion molecules

To assess the expression of adhesion proteins, the length of blood vessel endothelium staining with mAbs to E-selectin, ICAM-1, and VCAM-1 was expressed as a percentage of the total blood vessel endothelial length assessed with Ulex lectin.[18,20] Measurements made in biopsy specimens from 8 atopic subjects 6 hours after allergen challenge were compared with similar measurements made in a group of 8 subjects with SAC out of season. The results (Fig 3) show an upregulation of both E-selectin and ICAM-1 but not VCAM-1. The expression of E-selectin was increased 5.7-fold from 8.0% ± 4.3% to 45.9% ± 8.9% ($P < .01$) in bulbar biopsy specimens and 3.6-fold from 17.1% ± 6.5% to 60.8% ± 8.3% ($P < .001$) in tarsal biopsy specimens. Similarly, the expression of ICAM-1 increased 6.3-fold from 9.0% ± 3.4% to 56.9% ± 11.1% ($P < .01$) in bulbar biopsy specimens and 2.7-fold from 14.6% ± 6.0% to 38.8% ± 7.3% ($P < .01$) in tarsal biopsy specimens. There was no significant difference between tarsal and bulbar adhesion molecule expression.

Inflammatory cell infiltration

Six hours after allergen challenge in the atopic group, there was increased cellular infiltrate in both the epithelium and substantia propria. The epithelium demonstrated significantly increased numbers of mast cells, neutrophils, eosinophils, and macrophages but not $CD4^+$ or $CD8^+$ cells. In the superficial substantia propria there was a significant increase in mast cells, eosinophils, and macrophages and a smaller but significant increase in bulbar, but not tarsal, $CD3^+/CD4^+$ and $CD8^+$ T cells (Fig 4, *A* and *B*). No infiltrating cells were seen in the control groups. Comparing tarsal and bulbar cell counts, there was a significant increase in bulbar macrophage and $CD3^+$, $CD4^+$, and $CD8^+$ cell counts compared with those found in tarsal biopsy specimens.

After the completion of this study, when the majority of the biopsy specimens had been used, 2D7, a murine monoclonal anti-basophil antibody[22] was made available to address the possibility of basophil infiltration during the LPR. Only bulbar conjunctiva was available, and the results of basal cell counts indicate the presence of significantly increased numbers of basophils at 6 hours compared with control eyes ($P < .05$), returning to normal in 24 hours. All were in the substantia propria (Table I).

DISCUSSION

These results confirm that the conjunctival LPR is a repeatable clinical phenomenon, is associated with characteristic tear and tissue changes, and is therefore useful for evaluating new therapeutic agents.

Histologic studies to date have indicated that the mast cells are the predominant cell types involved in seasonal and perennial allergic conjunctivitis,[19,23-26] many of which are degranulated, and also eosinophils and basophils. Mast cells numbers are also increased in vernal and giant papillary conjunctivitis.[27] It has therefore been commonly assumed that a type I allergic response forms the basis of the pathogenesis of these diseases. There are no published data describing the cellular infiltrate found in conjunctival biopsy specimens taken during the LPR, although eosinophils and lymphocytes have been found in surface scrapings.[28]

The finding that after allergen challenge there is increased expression of E-selectin and ICAM-1 is of interest and would explain the recruitment of cells into the conjunctiva. Previous work has shown that 6 hours after conjunctival challenge, there is an increase in E-selectin[18] and ICAM-1[18,29] expression correlating with lymphocyte and granulocyte levels.[18] This would explain our finding of increased infiltration of these cell types found in the conjunctiva. VCAM-1 induction is slower and has been shown to peak at 24 hours after conjunctival allergen challenge.[18] Increased E-selectin and ICAM-1 have been found at 6 hours, and increased VCAM-1 has been found at 24 hours, after endobronchial and skin challenge with allergen.[30-32] This explains why in this study no increased levels of VCAM-1 were observed 6 hours after allergen challenge.

Increased numbers of mast cells, neutrophils, eosinophils, and macrophages were detected in the epithelium 6 hours after allergen challenge, although it is realized that this time point may not be optimal for all parameters assessed.[18,31] $CD3^+$, $CD4^+$, and $CD8^+$ T cells

were only significantly increased in the bulbar layers, possibly because the bulbar surface is more accessible to topical challenge. The greatest increase was in the mast cell, macrophage, neutrophil, and eosinophil numbers, possibly because the EPR and LPR are mast cell and not T-cell driven, which is confirmed by the finding of increased mast cell levels in SAC[19,23] and the demonstration here of increased tryptase and histamine tear levels in the EPR. There was also an increase in basophil numbers at 6 hours. The IgE dependency of both the EPR and the LPR[6,33] implicates the mast cell, basophil, or both (which possess the FcεRI) in these phases. Mast cells are able to generate many of the mediators capable of eliciting an LPR.[34]

The most striking piece of evidence in support of the clinical LPR is the presence of 2 separate peaks of histamine in tears at 20 minutes and 6 hours after challenge, which we and other authors have found after ocular[24,35] and skin challenge.[36] It has been shown that careful repeated tear collection does not affect conjunctival permeability,[37] so that conjunctival trauma would not have been responsible for the findings reported here. In other tissues an LPR has been demonstrated, such as in the nose, where basophils predominate.[38] Nasal allergen challenge leads to the prompt release of histamine, leukotriene C4, PGD2, and tosyl arginyl methylester esterase esterase.[39,40] Conversely, in the LPR histamine release was not accompanied by PGD_2,[41] strongly suggesting that basophils, and not mast cells, are responsible for this response in the nose. This concept is supported by the findings reported here of increased numbers of basophils in the conjunctiva at 6 hours during the LPR. Basophils have been detected during the LPR in the skin also.[42]

The LPR represents, with its associated increase in inflammatory cells and adhesion molecules, the transition between a transient type 1 response and clinical disease, with a marked increase in tissue reactivity to challenge, contributing to the symptoms seen in asthma, atopic dermatitis, and allergic eye disease. It offers not only a model for the study of allergic eye disease but the means of studying the effect of therapeutic agents and a possible site for therapeutic intervention.

REFERENCES

1. Blackley CH. Experimental researches on the causes and cure of Catarrhus Aestivus (Hayfever or hay asthma). London: Tindall and Cox Ltd; 1873. p. 77.
2. Allansmith MR, O'Connor GR. Immunoglobulins: structure, function and relation to the eye. Surv Ophthalmol 1970;14:367-402.
3. Dart JKG, Buckley RJ, Monnickendan M, Prasad J. Perennial allergic conjunctivitis: definition, clinical characteristics and prevalence. Trans Ophthalmol Soc UK 1986;105:513-20.
4. Peshkin NM. A dry pollen ophthalmic test in pollen asthma and hayfever patients negative to cutaneous tests. J Allergy 1932;3:20-9.
5. Pelikan Z. Late and delayed responses of the nasal mucosa to allergen challenge. Ann Allergy 1978;41:37-47.
6. Solley GO, Gleich GJ, Jordan RE, Schroeter AL. The late phase of the immediate wheal and flare skin reaction: its dependence upon IgE antibodies. J Clin Invest 1976;58:408-20.
7. Pepys J, Hutchcroft BJ. Bronchial provocation tests in etiologic diagnosis and analysis of asthma. Am Rev Respir Dis 1975;112:829-59.
8. Gleich GJ. The late phase of the immunoglobulin E-mediated reaction: a link between anaphylaxis and common allergic disease? J Allergy Clin Immunol 1982;70:160-9.
9. Iliopoulos O, Proud D, Adkinson NF, et al. Relationship be between the early, late and rechallenge reaction to nasal challenge with antigen: observations on the role of inflammatory mediators and cells. J Allergy Clin Immunol 1990;86:851-61.
10. Majchel AM, Proud D, Freidhoff L, et al. The nasal response to histamine challenge: effect of the pollen season and immunotherapy. J Allergy Clin Immunol 1992;90:85-91.
11. Connell JT. Quantitative intranasal pollen challenge. III. The priming effect in allergic rhinitis. J Allergy 1969;43:33-6.
12. Ciprandi G, Buscaglia S, Pesce GP, Bagnasco M, Canonica GW. Ocular challenge and hyperresponsiveness to histamine in patients with allergic conjunctivitis. J Allergy Clin Immunol 1993;91:1227-30.
13. Bisgaard H, Ford-Hutchinson AW, Charleson S, Taudorf E. Production of leukotrienes in human skin and conjunctival mucosa after specific allergen challenge. Allergy 1985;40:417-23.
14. Proud D, Sweet J, Stein P, et al. Inflammatory mediator release on conjunctival provocation of allergic subjects with allergen. J Allergy Clin Immunol 1990;85:896-905.
15. Bonini St, Bonini Se, Bucci MG, et al. Allergen dose response and late symptoms in a human model of ocular allergy. J Allergy Clin Immunol 1990;86:869-76.
16. Seal DV, McGill JI, Mackie IA, Liakos GM, Jacobs P, Goulding NJ. Bacteriology and tear protein profiles of the dry eye. Br J Ophthalmol 1986;70:122-5.
17. Britten K, Howarth P, Roche W. Immunohistochemistry of small mucosal biopsies: a comparison of different resin-embedding techniques. Biotech Histochem 1993;68:271-80.
18. Bacon AS, Baddeley S, Metz D, Lightman SL, McGill JI, Holgate ST. Upregulation of adhesion molecules in allergic eye disease and relationship to leucocyte infiltration. Invest Ophthalmol Vis Sci 1998;39:322-30.
19. Baddeley SM, Bacon AS, McGill JI, Holgate ST. Mast cell distribution and neutral phase expression in acute and chronic allergic conjunctivitis. Clin Exp Allergy 1995;25:41-50.
20. Montefort S, Feather IH, Wilson SJ, et al. The expression of leukocyte-endothelial adhesion molecules is increased in perennial allergic rhinitis. Am J Respir Cell Mol Biol 1992;7:393-8.
21. Walls AF, Bennett AR, McBride HM, Glennie MJ, Holgate ST, Church MK. Production and characterisation of monoclonal antibodies specific for human mast cell tryptase. Clin Exp Allergy 1990;20:581-9.
22. Kepley CL, Craig SS, Schwartz LB. Identification and partial characterization of a unique marker for human basophils. J Immunol 1995;154:6548-55.
23. Morgan ST, Williams JW, Walls AF, Church MK, Holgate ST, McGill JI. Mast cell numbers and staining characteristics in the normal and allergic human conjunctiva. J Allergy Clin Immunol 1991;87:111-6.
24. Abelson MB, Chambers WA, Smith LM. Conjunctival allergen challenge. A clinical approach to studying allergic conjunctivitis. Arch Ophthalmol 1990;108:84-8.
25. Allansmith MR, Ross RN. Ocular allergy and mast cell stabilisers. Surv Ophthalmol 1986;30:229-44.
26. Anderson DF, Macleod JDA, Baddeley SM, et al. Seasonal allergic conjunctivitis is accompanied by increased mast cell numbers in the absence of leukocyte infiltration. Clin Exp Allergy 1997;27:1060-6.
27. Irani AA, Butrus SI, Tabbara KF, Schwartz LB. Human conjunctival mast cells: Distribution of MCT and MCTC in vernal conjunctivitis and giant papillary conjunctivitis. J Allergy Clin Immunol 1990;86:34-40.
28. Bonini SE, Bonini ST, Bucci MG, Balsano F. Allergic mechanisms of the eye. Allergologie 1989;12s:158-62.
29. Ciprandi G, Buscaglia S, Pesce G, Villagio B, Bagnasco M, Canonica GW. Allergic subjects express intercellular adhesion molecule-1 (ICAM-1 or CD54) on epithelial cells of conj unctiva after allergen challenge. J Allergy Clin Immunol 1993;91:783-92.
30. Montefort S, Gratziou C, Goulding D, Polosa R, Haskard DO, Howarth P, et al. Bronchial biopsy evidence for leukocyte infiltration and upregulation of leukocyte-endothelial cell adhesion molecules 6 hours after local allergen challenge of sensitized asthmatic airways. J Clin Invest 1994;93:1411-21.
31. Bentley AM, Durham SR, Robinson DS, Cromwell O, Kay AB, Wardlaw AJ. Expression of the endothelial and leucocyte adhesion molecules ICAM-1, E-selectin and VCAM-1 in the bronchial mucosa in steady state asthma and allergen induced asthma. J Allergy Clin Immunol 1993;92:857-68.

32. Leung DY, Pober JS, Cotran RS. Expression of endothelial-leukocyte adhesion molecules in elicited late phase allergic reactions. J Clin Invest 1991;87:1805-9.
33. Dolovich J, Hargreave FE, Chalmers R, et al. Late cutaneous allergic responses in isolated IgE-dependent reactions. J Allergy Clin Immunol 1973;52:38-46.
34. Costa JJ, Galli SJ, Church MK. Mast cell cytokines in allergic inflammation. In: Holgate ST, Busse WW, eds. Inflammatory mechanisms in asthma. New York: Marcel Dekker; 1998. p. 111-28.
35. Helleboid L, Khatami M, Wei Z-G, Rockey JH. Histamine and prostacyclin. Primary and secondary release in allergic conjunctivitis. Invest Ophthalmol Vis Sci 1991;32:2281-9.
36. Charlesworth EN, Hood AF, Kagey-Sobotka A. The cutaneous late phase response to allergen: mediator release and inflammatory cell infiltration. J Allergy Clin Immunol 1989;73:905-12.
37. Kari O, Solo O, Halmepuro L, Suvilehto K. Tear histamine during allergic conjunctivitis. Graefes Arch Clin Exp Ophthalmol 1985;223:60-2.
38. Bascom R, Wachs M, Naclerio RM, et al. Basophil influx occurs after nasal antigen challenge: effects of topical corticosteroid pretreatment. J Allergy Clin Immunol 1988;81:580-9.
39. Naclerio RM, Proud D, Togias AG, et al. Inflammatory mediators in late allergen-induced rhinitis. N Engl J Med 1985;313:65-70.
40. Naclerio RM, Meier HL, Kagey-Sobotka A, et al. Mediator release after nasal airway challenge with allergen. Am Rev Respir Dis 1983;128:597-602.
41. Pipkorn U, Proud D, Lichtenstein LM ,Kagey-Sobotka A, Norman PS, Naclerio RM. Inhibition of mediator release in allergic rhinitis by pretreatment with topical glucocorticosteroids. N Engl J Med 1987;316:1506-10.
42. Irani AA, Huang C, Xia HZ, et al. Immunohistochemical detection of human basophils in late-phase skin reactions. J Allergy Clin Immunol 1998;101:354-62.