

proposed that the defective skin barrier in AE promotes sensitization after cutaneous contact with allergens.⁶

During the first year of life, children with early/recurrent AE were more likely to develop allergic sensitization (to food and/or aeroallergen) compared with the healthy/transient and late-onset AE phenotypes (Table I). Following the cohort longitudinally, children with early/recurrent AE continued to have significantly higher rates of allergen-specific IgE at age 2, 5, and 6 years (Fig 1, C and see this article's Fig E2, A and B in the Online Repository at www.jacionline.org), whereas total serum IgE levels did not significantly vary between the defined AE groups until age 6 years. At age 6, children with none/transient AE had a higher total IgE (see this article's Fig E2, C, in the Online Repository at www.jacionline.org).

Interestingly, dog in the home at the time of birth was less common in the early/recurrent group (10%) compared with the healthy/transient (43%) and late-onset (50%) groups (Table I). Exclusive breast-feeding for the first 6 months of life was associated with late onset disease versus early/recurrent disease ($P = .05$; Table I). Other factors, including daycare attendance, older siblings, and environmental tobacco smoke exposure, were not different. Early/recurrent AE was more closely associated with atopic biomarkers and other atopic diseases throughout early childhood (see this article's Fig E3 in the Online Repository at www.jacionline.org).

The increasing prevalence of AE has led to the search for environmental factors that modify the risk or natural history of AE. In our study, lack of exposure to dogs, but not cats, was associated with early/recurrent AE. This finding is consistent with other reports that exposure to dogs, particularly near the time of birth, has been associated with distinct changes in immune development in early childhood, and lower rates of wheezing by age 3 years.⁷ In contrast, breast-feeding was associated with late-onset atopic eczema. It is possible that exclusive breast-feeding delays the onset of AE in children who might otherwise have developed it in infancy. Similarly, our data suggest that breast-feeding may have differential effects depending on the specific phenotype of AE. Published studies have had varied results with respect to effects of breast-feeding on AE, and findings may differ depending on the atopic history of the parents or the failure to consider different phenotypes of AE.^{5,8,9}

In conclusion, children in this high-risk cohort can be grouped into 1 of 3 distinct AE phenotypes: healthy/transient, late-onset, and early/recurrent AE. Our data suggest that frequent significant respiratory viral illnesses, early onset of allergic sensitization, and wheezing are associated with early/recurrent AE, whereas exposure to dogs may have beneficial effects. Further studies to define the underlying mechanisms linking AE to respiratory illnesses and other potentially modifiable factors are warranted to identify new therapeutic targets and strategies for prevention.

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Supported by NIAID/NIH R01 HL080072-01, NHLBI/NIH P01 HL70831-01, and NIAID/NIH T32 AI007635 (T32).

Disclosure of potential conflict of interest: R. F. Lemanske, Jr, is on the speakers' bureau for Merck, AstraZeneca, Doernbecher Children's Hospital, Washington University, Medicus Group, Park Nicolet Institute, ACAA, LA Allergy Society, Michigan Allergy/Asthma Society, Medical College of Wisconsin, Fund for Medical Research and Education (Detroit), Children's Hospital of Minnesota, Toronto Allergy Society, AAAAI, Beaumont Hospital (Detroit), University of Illinois, Canadian Society of Allergy and Clinical Immunology, and New York Presbyterian; has consultant arrangements with AstraZeneca, Map Pharmaceuticals, Gray Consulting, Smith Research, Inc, Merck Childhood Asthma Network, Novartis, Quintiles/Innovax, RC Horowitz & Co, Inc, International Meetings and Science, and Scienomics; is an author of Up-to-Date; and is a textbook editor for Elsevier, Inc. Christine M. Seroogy receives grant support from the NIH, the AAAAI Research Trust, and Midwest Athletes Against Childhood Cancer. The rest of the authors have declared that they have no conflict of interest.

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doi:10.1016/j.jaci.2009.11.026

Reciprocal interaction of the conjunctiva and cornea in ocular allergy

To the Editor:

Severe ocular allergic diseases such as vernal and atopic keratoconjunctivitis involve not only conjunctival allergic inflammation but also various corneal disorders. Corneal complications such as persistent corneal erosion can threaten vision and are often resistant to therapy, thus remaining a challenge in the treatment of ocular allergy.¹

The cornea and conjunctiva are physically apposed, separated by only a thin film of tear fluid. However, these tissues have distinct characteristics. The conjunctiva is rich in immune cells and vasculature and possesses only weak barrier properties, with allergic inflammation thus readily evoked. In contrast, primary allergic reactions do not occur in the cornea, given that this tissue

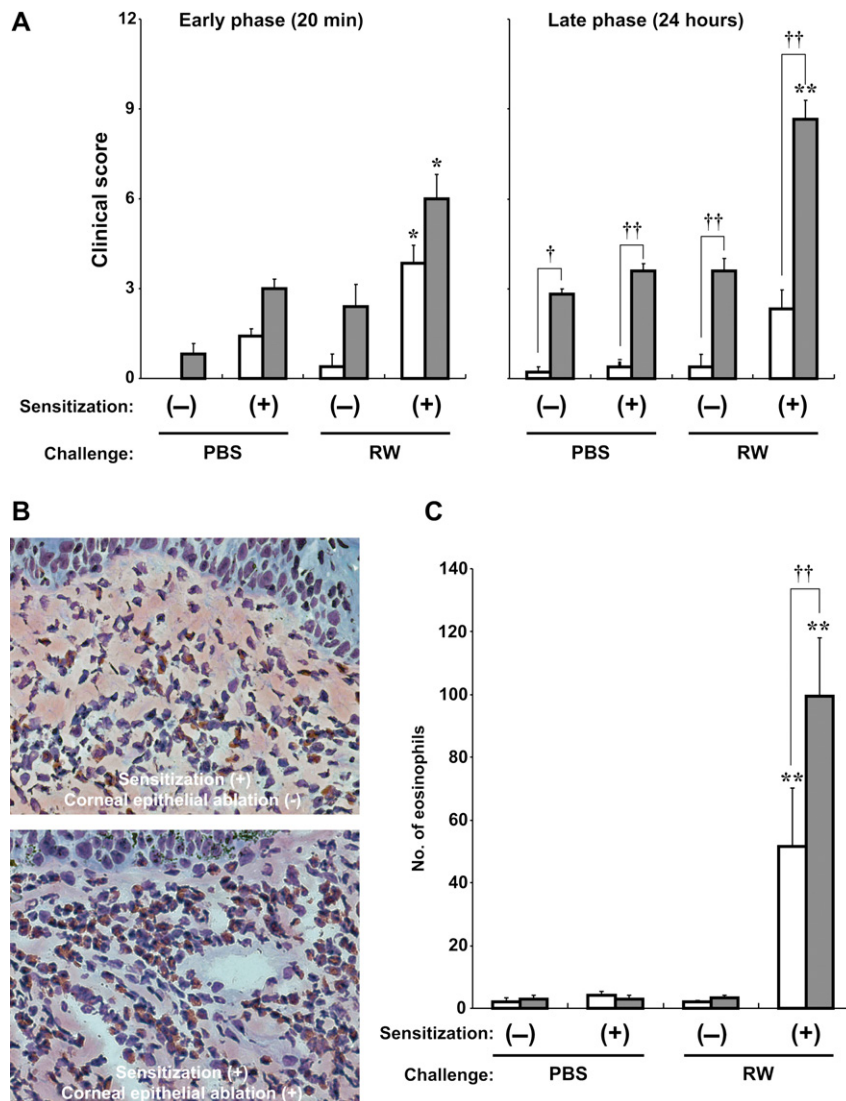


FIG 1. Effects of corneal epithelial ablation and antigen challenge on symptoms and eosinophil infiltration into the conjunctiva. Clinical score (**A**) and the number of conjunctival eosinophils (**C**) after antigen challenge with (closed bars) or without (open bars) corneal epithelial ablation in sensitized or nonsensitized rats. **B**, Sections of the conjunctiva from sensitized rats at 24 hours after antigen challenge with or without corneal epithelial ablation were stained with Congo red. * $P < .05$, ** $P < .01$ versus the corresponding value for nonsensitized rats; † $P < .05$, †† $P < .01$ (Tukey-Kramer test).

does not normally contain immune cells or vasculature and that it possesses strong barrier properties conferred by the corneal epithelium. These 2 tissues are thought to influence each other through the release of bioactive substances such as chemical mediators or cytokines into tear fluid. However, direct evidence of interaction between the cornea and conjunctiva during ocular allergic inflammation *in vivo* has remained elusive. We have now investigated whether such interaction occurs in a rat model of ocular allergy.

Male Brown Norway rats 8 to 12 weeks old (CLEA Japan, Tokyo, Japan) were sensitized by injection with 100 μ g short ragweed pollen (RW; ICN Biomedicals, Aurora, Ohio) in aluminum hydroxide (Sigma-Aldrich, St Louis, Mo) or were injected with aluminum hydroxide only (control) into the left foot pad. Three weeks later, the rats were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg) and the application of 0.4% oxybuprocaine hydrochloride to each eye, the entire corneal

epithelium of the right eye was removed with a razor blade, and the animals were challenged by topical instillation of 10 μ L PBS containing 2 mg RW or PBS alone into both eyes.² Symptoms including conjunctival chemosis and redness, lid swelling, and mucus secretion were scored on a scale of 0 to 3 (maximal total score of 12) in a blind manner at 20 minutes and 24 hours after challenge, as previously described.³ At 24 hours after antigen challenge, the rats were killed and the eyes, including each conjunctiva, were harvested and immediately frozen in OCT compound. Vertical 4- μ m-thick sections were cut and stained with Congo red. Infiltrating eosinophils in the lamina propria mucosa of the conjunctiva were counted by blind observers. All animal procedures conformed to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research, and the study protocol was approved by the Yamaguchi University Committee on Animal

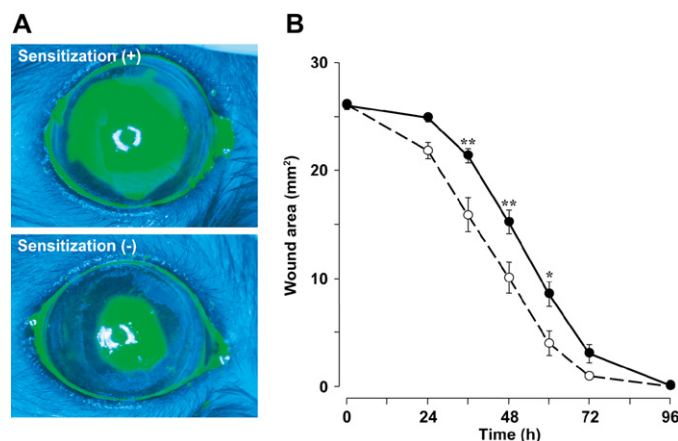


FIG 2. Effect of allergic inflammation in the conjunctiva on corneal epithelial wound healing. Fluorescein staining of corneal epithelial defects at 48 hours (**A**) and time course of wound healing (**B**) after removal of the corneal epithelium and antigen challenge in non-sensitized (open circles) or sensitized (closed circles) rats. * $P < .05$, ** $P < .01$ versus the corresponding value for non-sensitized rats (Tukey-Kramer test).

Welfare. All quantitative data are means $\pm$ SEMs from 5 eyes, statistical analysis was performed with the Tukey-Kramer multiple comparison test and Spearman rank correlation coefficient, and a P value $< .05$ was considered statistically significant.

We first examined the effects of corneal epithelial ablation on ocular symptoms. At 20 minutes after RW challenge (early phase), mild symptoms were apparent only in sensitized rats (Fig 1, A). Corneal epithelial ablation induced only mild symptoms such as conjunctival redness and edema in non-sensitized rats, and it did not augment the symptoms induced by antigen challenge in sensitized rats. At 24 hours after RW challenge (late phase), corneal epithelial ablation in non-sensitized rats or antigen challenge without corneal ablation in sensitized rats induced only mild clinical symptoms; however, antigen challenge in the presence of corneal epithelial ablation induced a large increase in clinical score in sensitized rats.

Antigen challenge induced significant eosinophil infiltration into the conjunctiva only in sensitized rats, indicative of antigen-specific inflammation (Fig 1, B and C). Corneal ablation did not induce eosinophil infiltration in non-sensitized rats. However, it significantly augmented the eosinophil infiltration induced by antigen challenge in sensitized rats. Spearman rank correlation coefficient (r_s) revealed a positive correlation between the number of eosinophils and clinical score in both early ($r_s = 0.54$; $P < .01$) and late ($r_s = 0.42$; $P < .01$) phases.

To examine the mechanism of the observed conjunctival eosinophilia, we measured the amounts of mRNAs for the chemokines CC chemokine ligands (CCL)5 and CCL11 as well as for intercellular adhesion molecule 1 in the conjunctiva of sensitized rats. CCL5 rather than CCL11 was previously implicated as responsible for eosinophil infiltration in a rat model of allergic conjunctivitis.² Consistent with this previous finding, the amount of CCL5 mRNA (but not that of CCL11 mRNA) tended to be increased by antigen challenge (see this article's Fig E1 in the Online Repository at www.jacionline.org). Moreover, corneal epithelial ablation followed by antigen challenge induced a significant increase in the amount of CCL5 mRNA. The amount of the mRNA for intercellular adhesion molecule 1, an adhesion molecule for eosinophils, was also upregulated by the combination of epithelial ablation and antigen challenge.

Finally, we investigated the effect of allergic inflammation in the conjunctiva on corneal epithelial wound healing. The area of

the epithelial wound stained with 1% fluorescein was measured on photographs with a computer-assisted image analyzer (Fig 2, A). The wound area of rats challenged with PBS did not differ between non-sensitized and sensitized animals at times up to 96 hours (data not shown). For RW-challenged rats, however, the wound area of sensitized animals was significantly larger than that of non-sensitized animals at 36 to 60 hours after the challenge (Fig 2, B). Corneal epithelial wound healing was thus significantly delayed by conjunctival allergic inflammation.

Our results have shown that the conjunctiva and cornea reciprocally interact with each other in a rat model of ocular allergy. Loss of barrier function of the corneal epithelium was thus found to exacerbate conjunctival inflammation, whereas conjunctival inflammation suppressed corneal epithelial wound healing, suggestive of the operation of a vicious cycle.

The pathogenesis of corneal lesions in vernal and atopic keratoconjunctivitis remains unclear, although eosinophils are implicated in this process on the basis that eosinophilic granule proteins have been detected in corneal ulcers and that these proteins adversely affect corneal epithelial cell viability and wound healing *in vitro*.⁴ We have shown here that eosinophil infiltration in the conjunctiva was associated with a delay in corneal epithelial wound healing *in vivo*.

We previously showed that exposure of stromal fibroblasts (but not epithelial cells) of the cornea to T_H2 cytokines results in upregulation of both chemokines and adhesion molecules⁵⁻⁷ that together promote the activation and infiltration of eosinophils and T_H2 lymphocytes.^{8,9} Loss of the barrier function of the corneal epithelium and consequent exposure of corneal fibroblasts to allergic cytokines in tear fluid are thus likely to exacerbate ocular allergic inflammation.

Restoration of the barrier function of the ocular surface is therefore a potential goal for the treatment of ocular allergic reactions. Indeed, surgical removal of corneal plaque and restoration of the corneal epithelium suppress conjunctival inflammation in some individuals.¹ Further studies into the local factors specific to ocular inflammation, such as the structural cells of the cornea and conjunctiva, might provide a basis for the development of novel treatments for ocular allergic diseases.

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Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

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doi:10.1016/j.jaci.2009.10.041

Nasal inflammation in vernal keratoconjunctivitis

To the Editor:

Vernal keratoconjunctivitis (VKC) is a bilateral ocular disease characterized by chronic inflammation of the whole conjunctiva with variable corneal involvement.¹ Conjunctival "cobblestone papillae" are a characteristic of the disease; the cornea can develop punctate keratitis, erosions, shield ulcers, plaques, and neovascularization.^{1,2} VKC typically appears before the age of 10 years and tends to improve or remit in adulthood.³ The term *vernal* is largely accepted for historical reasons because VKC sometimes displays spring or autumn exacerbations that are followed by periods of remission. Nonetheless, the term is not completely appropriate, because many patients with VKC have exacerbations during winter or develop a perennial form of the disease. VKC might occur in people with a family history of atopy or with allergic sensitization, although the role allergy in the pathophysiology of VKC is unclear. Allergic rhinitis is commonly associated with conjunctivitis, and the term *rhinoconjunctivitis*⁴ is frequently used. We therefore studied the nasal characteristics of patients with VKC, to determine if the nasal mucosa is involved as in classic allergic rhinoconjunctivitis.

Consecutive outpatients, at their first diagnosis of VKC, were given an ear, nose, and throat examination before they were treated. The parents of pediatric patients signed an informed consent form, and the protocol was approved by the local ethical committee. The diagnosis of VKC was always made by an ophthalmologist. The presence of at least typical papillae or corneal involvement was required.⁵ The subjective severity was graded by patients >12 years (or by their parents) by scoring for each eye, from 0 (absent) to 3 (severe), for the following

features: ocular itching, foreign body sensation, photophobia, and watering. An objective score of values from 0 to 3 was assigned by the ophthalmologist to rate the following: hyperemia, tarsal/limbal papillae, neovascularization, and cornea ulceration. The family history of atopy was determined by asking parents about the presence of rhinitis and/or asthma and/or atopic dermatitis. The history of rhinitis symptoms was assessed according to the International Study on Allergy and Asthma in Childhood (ISAAC) questionnaire. Patients/parents recorded the instantaneous rhinitis symptoms, assigning scores of 0 to 3 for nasal itching, obstruction, rhinorrhea, and sneezing. All patients underwent skin prick tests and CAP-RAST, and levels of total IgE were assayed. Skin tests were performed with commercial extracts (Lofarma SpA, Milan, Italy) of house dust mites, grass, *Parietaria*, olive, cypress, birch, hazelnut, ragweed, and cat and dog dander. Nasal and conjunctival scrapings were collected for cytology analysis by using a RhinoProbe device (Cardinal Health, Dublin, Ohio) according to a validated methodology.^{6,7} Nasal smears were obtained from the middle portion of the inferior turbinate. Conjunctival scrapings were obtained from the middle portion of the inferior palpebra. At the time of evaluation, patients had not yet been treated for allergic symptoms (eg, with antihistamines, steroids, or mast-cell stabilizers). The smear samples were fixed, air dried, stained with May-Grünwald-Giemsa (Carlo Erba, Milan, Italy), and examined by light microscopy (at 1000-fold magnification using a Nikon E600; Nikon, Milan, Italy). Total numbers of cells, eosinophils, and mast cells were counted in 10 fields of each sample.⁷ Each specimen was examined by 2 blind investigators, and mean values were obtained. Correlations between parameters were calculated using the Pearson test.

Thirty-eight patients (30 boys; age range, 3-18 years) were studied; 13 subjects (34%) had atopy (1 or both parents had allergic hypersensitivity). Two subjects had asthma, and 8 had atopic dermatitis. Four patients (11%) tested positive to at least 1 allergen (cypress, grass, or olive), confirmed by the CAP-RAST, and 18 subjects had total IgE levels greater than 200 kU/L. A mild septal deviation was observed in 7 patients and a tonsil hypertrophy in 12. A clinical history of rhinitis was present in 15% of subjects. The rhinitis scores were 0 of 12 in 21 subjects and 4 of 12 or less in the remaining 17 patients. The conjunctival objective score assigned by the ophthalmologist was <6 in 6 patients and >10 in 12.

The mean total cell count in nasal samples was 23.8 ± 19 , including 18.4 ± 15 eosinophils (range, 0-60) and 4.9 ± 2 mast cells (range, 3-22). Nasal scrape samples were negative in 9 (23.6%) patients; samples from 10 patients (26.3%) contained only eosinophils, 5 (13.2%) had only mast cells, and 14 (36.9%) had a mixed inflammatory cell population (eosinophils and mast cells). Conjunctival scrape samples were abnormal in all subjects. The mean total cell count was 103.6 ± 44 (range, 22-165), with 54.8 ± 19 eosinophils (range, 22-118) and 48.1 ± 25 mast cells (range, 7-125). One subject had only eosinophils, 5 had only mast cells, and 32 (84.2%) subjects had a mixed cell population. The conjunctival total cell count correlated with the subjective ($r = 0.45$; $P = .04$) and objective ($r = 0.44$; $P = .04$) conjunctival scores (Fig 1). No correlation was found between nasal cell count and nasal score or between the nasal and conjunctival cell counts ($r = 0.02$; $P = .8$; Fig 1). Similar observations were made when eosinophils and mast cells were analyzed separately. A correlation between cell counts and skin sensitizations could not be determined because of the small number of patients with a positive

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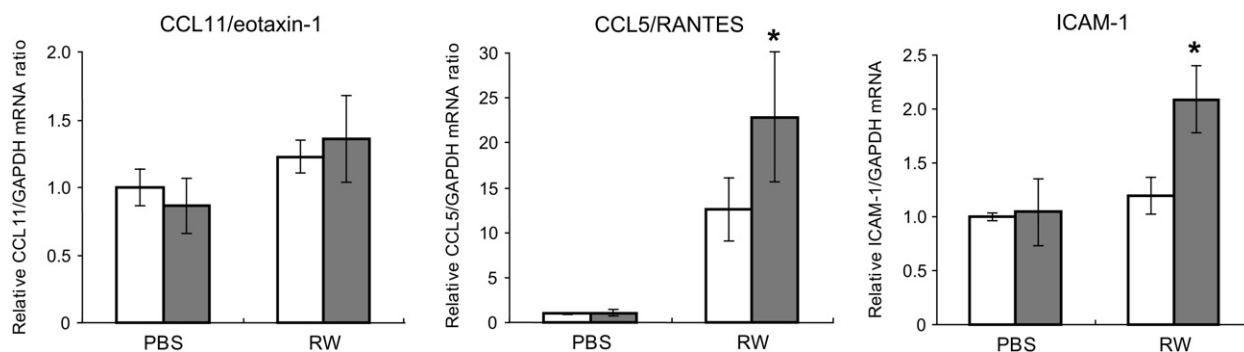


FIG E1. Effects of corneal epithelial ablation and antigen challenge on the abundance of chemokine and adhesion molecule mRNAs in the conjunctiva. Sensitized rats were challenged with PBS or RW in the presence (*closed bars*) or absence (*open bars*) of corneal epithelial ablation. The abundance of CCL11, CCL5, and intercellular adhesion molecule 1 (*ICAM-1*) mRNAs in the conjunctiva at 18 hours after challenge was determined by reverse transcriptase polymerase chain reaction (RT-PCR) analysis as described previously.^{E1} In brief, total RNA was extracted from conjunctival tissue with the use of an RNeasy Mini Kit (Qiagen, Tokyo, Japan) and was subjected to RT, and the resulting cDNA was then subjected to real-time PCR analysis with specific primers (Qiagen) and with the use of a LightCycler instrument (Roche Molecular Biochemicals, Mannheim, Germany). Transcripts of the constitutively expressed gene for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served to normalize the amount of target mRNA in each sample. Data are means $\pm$ SEMs from 5 eyes. * $P < .05$ versus the corresponding value for PBS-challenged rats (Turkey-Kramer multiple comparisons test).