

A new murine model of allergic conjunctivitis and effectiveness of nedocromil sodium

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Background: Allergic conjunctivitis is the most common atopic disease affecting the eye. To study the pathophysiology and effectiveness of antiallergic drugs, it is necessary to develop animal models that closely mimic human allergic conjunctivitis.

Objective: The study was performed to develop an experimental murine model of ocular allergic conjunctivitis to an airborne allergen.

Methods: SWR/J mice were divided into the following groups: group 1, untreated, experimental; group 2, phosphate-buffered saline-treated; group 3, nedocromil sodium-treated; and group 4, unmanipulated controls. Groups 1, 2, and 3 were exposed to ragweed by topical contact with the nasal and conjunctival mucosae. Allergic conjunctivitis was evaluated by scoring of clinical signs, serum IgE levels, and histologic findings.

Results: Mice exposed to ragweed had clinicopathologic signs of allergic conjunctivitis and specific anti-ragweed IgE. Allergic conjunctivitis was modulated by nedocromil sodium. Treated mice had fewer clinical signs of allergy, lower levels of ragweed-specific IgE, reduction of conjunctival eosinophil infiltration, decrease in the number of intact and degranulating mast cells, and reduction of cytokine release.

Conclusion: This is the first report of a murine model of allergic conjunctivitis to an airborne allergen that can be used to study the disease pathophysiology and its response to treatment. (*J Allergy Clin Immunol* 1996;97:1129-40.)

Key words: Cytokines, hay fever conjunctivitis, histopathology, IgE, nedocromil sodium, ragweed

Allergic conjunctivitis, an atopic phenomenon that affects the ocular surface,¹ is one of the most common entities encountered in general ophthalmic practice.² Patients with allergic conjunctivitis have symptoms of itching and burning in response to a variety of ubiquitous airborne allergens, which are ordinarily innocuous to the normal population.^{2,3} Clinical signs consist of chemosis, tearing, conjunctival hyperemia, and lid edema.²⁻⁴ Current

Abbreviations used

LM:	Light microscopy
PBS:	Phosphate-buffered saline
TEM:	Transmission electron microscopy

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medical therapy is symptomatic and consists of vasoconstriction, mast-cell stabilization, antihistaminic agents, and topical corticosteroids.⁵ To assess the activity and therapeutic value of new drugs and to study the pathophysiology of allergic conjunctivitis, animal models that closely resemble human allergic conjunctivitis must be developed. The experimental models previously described were created in rats⁶⁻⁸ and guinea pigs^{9,10} and involved intraperitoneal immunization with a non-airborne allergen mixed with adjuvant and subsequent conjunctival challenge with a solution of the same antigen. Mice have not been used in the development of a model of allergic conjunctivitis, despite their immunologic similarity to human beings and the extensive array of reagents available

TABLE I. Scoring of clinical signs

Clinical signs	None (0+)	Minimal (1+)	Mild (2+)	Moderate (3+)	Severe (4+)
Conjunctival redness	Absent	Minimal redness	Easily detectable redness	Obvious redness	Diffuse redness
Conjunctival edema	Absent	Minimal edema	Focal areas of edema	Obvious chemosis	Severe chemosis
Lid redness	Absent	Minimal redness	Easily detectable redness	Obvious redness	Severe redness
Lid edema	Absent	Minimal edema	Easily detectable edema	Obvious lid edema	Severe lid edema
Mucus secretion	Absent	Filaments of mucus present	Patchy mucus	Obvious mucus secretion	Severe mucus secretion

Adapted from Ballas et al. *Surv Ophthalmol* 1993;38:141-8 and Merayo-Llodes, Calonge, and Foster. *Curr Eye Res* (in press).

for study and manipulation of their immune parameters. We hypothesized that mice can develop allergic conjunctivitis after exposure to an airborne allergen through the nasal and conjunctival mucosae and that this reaction can be modulated by antiallergy drugs.

METHODS

Animals

Sixty-four 8- to 10-week-old female SWR/J mice (Jackson Laboratories, Bar Harbor, Maine) were housed in microisolators within a VR-1 laminar flow isolation unit (Lab Products Inc., BioMedic Corporation, Rochelle Park, N.J.), given mouse chow and water ad libitum, and cared for in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.¹¹

The animals were divided into four groups of 16 each: group 1, untreated, experimental or positive controls; group 2, phosphate-buffered saline (PBS)-treated; group 3, nedocromil sodium-treated; and group 4, unmanipulated controls.

Immunization

Immediately after its arrival, the short ragweed pollen powder, *Ambrosia artemisiaefolia*, (International Biologicals, Piedmont, Okla.) was divided into aliquots of 0.5 gm under a laminar flow hood and stored at -20°C . With a fresh aliquot each day, 1.25 mg of ragweed pollen powder was administered to each mouse in groups 1, 2, and 3 on days 1 to 5 under a laminar flow hood with an Eppendorf micropipette (USA/Scientific Plastics, Ocala, Fla.) calibrated to 10 μl . On day 8, the mice were challenged with the same amount of ragweed pollen powder delivered to the conjunctival sac. Group 4 animals did not undergo immunization or challenge. The details of the sensitization procedure are as follows: the tip of the micropipette was put into the tube containing ragweed, and the powder was aspirated. The tip of the micropipette was then placed proximal to the conjunc-

tival sac and the nostrils of the mouse, and the ragweed was released by pressing the superior part of the Eppendorf micropipette. This procedure releases from the micropipette 1.25 mg of ragweed powder in each application. Before beginning the animals studies, we conducted a series of experiments in which we pipetted ragweed pollen powder 100 times with the procedure described above; the amount of ragweed delivered averaged 1.25 mg (variation from 1.10 mg to 1.40 mg over 5 series of weighing). The investigator, according to the Massachusetts Eye & Ear Infirmary Animal Care Committee recommendations, was protected by gloves, a dust mask, and goggles.

Treatment

Groups 1 (untreated, experimental) and 4 (normal) did not undergo treatment. Group 2 animals received 2 μl of PBS, pH 7.2, alone. Group 3 mice received 2 μl of 2% nedocromil sodium in PBS, pH 7.2, in one eye and PBS alone in the other eye in blinded fashion. Each solution was administered with an Eppendorf micropipette twice daily (8:00 AM and 12:00 PM) on days 1 to 5 and day 8. Antigen exposure was performed 3 hours after the second administration of the drug.

Evaluation

Five clinical signs were judged in room light under an operating microscope (Zeiss, Munich, Germany): conjunctival edema and redness, lid swelling and redness, and mucus secretion. The evaluation was performed in blinded fashion at 20 minutes after allergen challenge. The clinical signs were scored on a scale of 0 to 4+ (0+, absent; 1+, minimal; 2+, mild; 3+, moderate; and 4+, severe) (Table I), modified from previous reports on allergic conjunctivitis¹² and used previously by us in a guinea pig model of allergic conjunctivitis.¹³

Histologic findings

Light microscopy (LM). The orbits were exenterated after the animals were killed with a 50 mg/ml pentobarbital overdose (Nembutal; Abbott Laboratories, Chi-

TABLE II. Scoring of clinical signs by group

	Group			
	Experimental (n = 8)	PBS (n = 14)	Nedocromil (n = 10)	Control (n = 10)
Conjunctival redness	2.250 ± 0.707*†	2.214 ± 0.426*‡	1.400 ± 0.516*	0 ± 0
Conjunctival edema	2 ± 0*‡	2.143 ± 0.363*‡	1.400 ± 0.516*	0 ± 0
Mucus secretion	0.375 ± 0.518	0.286 ± 0.469	0.300 ± 0.483	0 ± 0
Lid redness	0.250 ± 0.463	0.214 ± 0.426	0.100 ± 0.316	0 ± 0
Lid edema	0.250 ± 0.463	0.357 ± 0.497	0.300 ± 0.483	0 ± 0

**p* ≤ 0.0001 versus control.†*p* ≤ 0.001 versus nedocromil.‡*p* ≤ 0.0001 versus nedocromil.

cago, Ill.), and specimens were processed for LM as previously described.¹⁴ Briefly, by fixation in Karnovsky's solution (1% paraformaldehyde, 1.25% glutaraldehyde, 0.13% sucrose, and 25 mmol/L sodium phosphate in 150 mmol/L sodium cacodylate buffer) for 24 hours, dehydrated through a graded series of ethanol, and embedded in glycol methacrylate (Historesin; Reichert-Jung, Heidelberg, Germany) with an LKB ultraprocessor (LKB-producer AB, Bromma, Sweden). Two and a half-micron sections were stained with hematoxylin-eosin, periodic acid-Schiff, and alkaline Giemsa stains. An Olympus B-H microscope (Olympus Optical Co. Ltd., Tokyo, Japan) and a micrometer ocular grid were used to evaluate the slides (magnification, ×400). Cells in the conjunctival epithelium and the immediate subepithelial region were counted with a 0.25 × 0.025 mm grid; stromal cells were counted with a 0.25 × 0.25 mm grid. Three fields were counted for each region.

Immunohistology. Representative mouse eyes were snap-frozen in liquid nitrogen and embedded in Tissue Tek OCT compound (Ames Company Division, Miles Laboratory, Elkhart, Ind.). Four-micron sections were cut in a Cryocut 1800 cryostat microtome (Reicher-Jung, Nussloch, Germany), mounted on coated slides (Accurate Chemical and Scientific Corporation, Westbury, N.Y.), and stored at -70°C. Before immunolabeling, sections were fixed in acetone for 10 minutes. Nonspecific binding of immunoglobulins was blocked with 5% donkey serum (Jackson ImmunoResearch, West Grove, Pa.) in 1% PBS-bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.). The sections were incubated overnight at 4°C with a 1:10 dilution of the primary goat antibodies in 1% PBS-bovine serum albumin with 1% Triton X-100 (Mallinckrodt Inc., Paris, Ky.), directed against the following mouse cytokines: IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, and tumor necrosis factor-α (R & D Systems, Minneapolis, Minn.). The samples were then washed and incubated for 30 minutes with a 1:500 dilution in 1% PBS-bovine serum albumin of the biotinylated secondary antibody, donkey anti-goat IgG (Jackson ImmunoResearch). After an additional 30-minute incubation with alkaline phosphatase-conjugated streptavidin (Jackson ImmunoResearch), the sub-

strate, Fast Red Naphtol AS-MX (Sigma), was added for approximately 4 to 5 minutes. The slides were then fixed in 10% formalin for 10 minutes and counterstained with aqueous hematoxylin (Biomedica Corp., Foster City, Calif.) for 1 minute; cover slips were applied with Airvol-203 (Air Products and Chemicals Inc., Allentown, Pa.). The specificity of the immunolabeling was tested by replacing the primary antiserum with normal goat serum (Jackson ImmunoResearch) as a negative control. All slides were evaluated by two observers in blinded fashion. Positive staining of the corneal epithelium, the conjunctival epithelium and stroma, and the perivascularities were scored on a scale of 0 to 3+. Positive cells were counted separately in three high-power fields (magnification, ×400).

Transmission electron microscopy (TEM). Representative eyes from each group were processed for TEM. Specimens were immediately fixed in 0.2 mol/L cacodylate buffer overnight at 4°C, and postfixed in 1% osmium tetroxide in 0.2% cacodylate buffer for 2 hours at the same temperature. After fixation, tissues were dehydrated with a graded series of ethanol and two changes in propylene oxide, followed by embedding in epoxy resin. Thin sections were made on an LKB ultramicrotome and stained with uranyl acetate and Sato's lead stain and examined with a Phillips CM 10 electron microscope (Phillips Industrial and Electro-Acoustic Systems, Eindhoven, The Netherlands) at the Eye Pathology Laboratory of the Massachusetts Eye & Ear Infirmary.

IgE determination

Mice were bled from the orbital sinus, and blood was collected. After 10 minutes of centrifugation at 333 g, serum was removed and stored at -70°C. Determinations of total and specific anti-ragweed IgE were performed by an ELISA according to previous reports¹⁵ and the recommendations of Pharmingen Laboratories (Pharmingen, San Diego, Calif.).

For total IgE determinations, 96-well, flexible microtiter plates (Costar Laboratories, Cambridge, Mass.) were coated with 100 μl of 2 μg/ml rat anti-mouse IgE capture reagent, clone R35-72 (Pharmingen), overnight

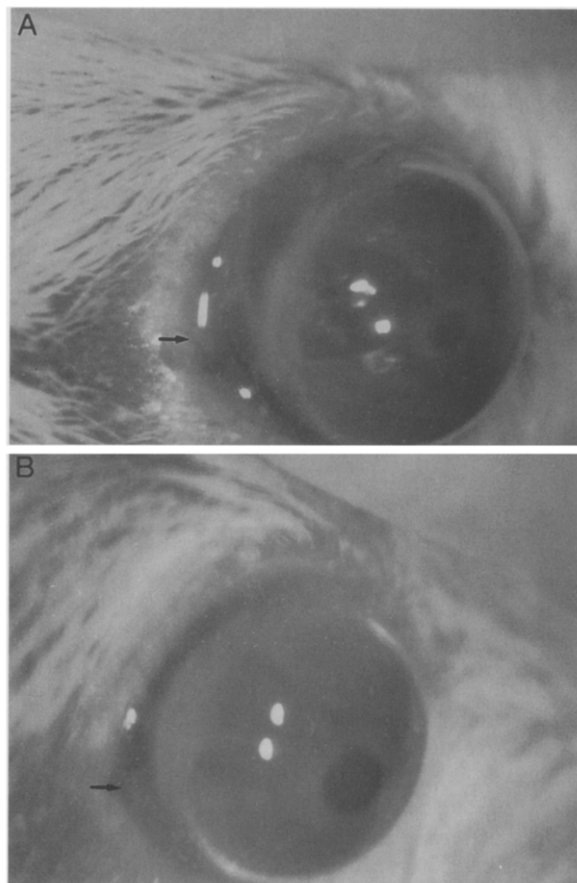


FIG. 1. **A**, Mouse eye 20 minutes after conjunctival challenge with ragweed pollen. Notice presence of conjunctival edema and redness (arrow). **B**, Mouse eye treated topically with nedocromil sodium. Notice reduction of conjunctival edema and redness (arrow).

at 4° C, followed by the addition of either serum diluted 1:10 in PBS or serial dilutions of IgE standard (Pharmingen). Samples were analyzed by the addition of 100 μ l of 1 μ g/ml biotinylated rat anti-mouse IgE, clone R35-118 (Pharmingen), followed by peroxidase-conjugated streptavidin (Jackson ImmunoResearch). After the addition of o-phenylenediamine dihydrochloride substrate (Sigma), the absorbance at 492nm was measured by a Titertek Multiscan Plus spectrophotometer (Lab-systems, Helsinki, Finland). The total IgE concentration in the samples was calculated by linear regression between the standard IgE and the sample values of absorbance.

For specific anti-ragweed IgE determinations, 96-well Linbro/Titertek EIA microtitration plates (Biomedicals Inc., Horsham, Pa.) were coated with 100 μ l of 1 mg/ml short ragweed pollen in coating buffer. Serum samples were added to the wells according to the biotin-avidin technique described previously. The absorbance measurement of each group was used directly for comparisons among the groups.

Data analysis

Differences among groups for each parameter were analyzed by analysis of variance, followed by the Student-Newman-Keuls test with Superanova Software (Abacus Concepts Inc., Berkeley, Calif.) for Macintosh computers (Apple Computer, Inc., Cupertino, Calif.). A *p* value of 0.05 or less was considered statistically significant.

RESULTS

Clinical signs

All mice exposed to ragweed (groups 1, 2, and 3) had signs of allergic conjunctivitis after the conjunctival challenge (Table II). Significant differences in conjunctival redness and edema were found in the groups of mice exposed to ragweed when pre- and postchallenge parameters were compared. The untreated, experimental (Fig. 1, *A*) and the PBS-treated mice had more conjunctival redness and edema than did the nedocromil sodium-treated (Fig. 1, *B*) and untreated control animals (*p* < 0.001). The variation between investigators in evaluating clinical signs was $\pm$ 0.5.

Histologic findings

LM. Table III shows the results of the histologic studies. The mice in group 1 (untreated, experimental) and group 2 (PBS-treated) had eosinophils in the epithelium; these cells were not found in the nedocromil sodium-treated mice or in controls. The stroma closest to the epithelium had a great infiltration of eosinophils (Fig. 2, *A*) in the untreated experimental and PBS-treated groups, in striking contrast to the nedocromil sodium group (Fig. 2, *B*).

LM did not reveal mast cells in the epithelium in any group. However, in the subepithelium, the group 3 (nedocromil sodium-treated) eyes showed significantly less mast-cell infiltration than did the group 2 (PBS-treated) eyes. In the rest of the substantia propria, significant differences were found between the group 1 (untreated, experimental) and group 2 (PBS-treated) mice compared with the group 3 (nedocromil sodium-treated) mice. Significantly more degranulating mast cells were counted in the group 1 (untreated, experimental) animals (Fig. 3, *A*) compared with the group 4 (normal control) and the group 3 (nedocromil sodium-treated) animals (Fig. 3, *B*).

Immunohistopathology. Table III shows the results of immunohistochemical staining for conjunctival cytokine expression. The group 1 (untreated, experimental) eyes showed more positive staining (*p* < 0.05) for IL-1 α , IL-1 β , IL-3, IL-4, IL-5, and

TABLE III. Results of light histology

	Group			
	Experimental (<i>n</i> = 8)	PBS (<i>n</i> = 5)	Nedocromil (<i>n</i> = 10)	Control (<i>n</i> = 10)
Eosinophils in epithelium (cells/mm ²)	6.66 ± 18.856	10.6 ± 23.7	0 ± 0	0 ± 0
Eosinophils in subepithelium (cells/mm ²)	2426.66 ± 553.53*†	2026 ± 905.74*†	171.9 ± 163.06	42.63 ± 42.15
Mast cells (cells/mm ²)	191.11 ± 54.32‡§	258.73 ± 96.82*	106.2 ± 28.05	95.4 ± 76.23
Degranulating mast cells (cells/mm ²)	160 ± 120.88§¶	85.4 ± 60.9*	48 ± 68.65	15.9 ± 50.28

Values are expressed as means ± SD.

**p* ≤ 0.0001 versus control.

†*p* ≤ 0.0001 versus nedocromil.

‡*p* ≤ 0.001 versus control.

§*p* ≤ 0.05 versus nedocromil.

||*p* ≤ 0.01 versus nedocromil.

¶*p* ≤ 0.05 versus control.

IL-6, especially in the epithelium (Fig. 4, *A*). This upregulation of cytokine expression by allergen exposure was dramatically modulated by nedocromil sodium therapy (Fig. 4, *B*). IL-1α and IL-3 showed statistically significant differences between group 2 (PBS-treated) and group 3 (nedocromil sodium-treated) animals (Table IV). There was positive staining of cells in the conjunctival stroma for IL-1α and IL-6 in the experimental and PBS-treated groups. No significant differences in cytokine expression were found between the nedocromil sodium-treated mice and the negative controls.

TEM. More eosinophils and degranulating mast cells were observed in the experimental and PBS-treated eyes than in the nedocromil sodium-treated eyes, and more degranulated goblet cells were also observed in the eyes not treated with nedocromil sodium. Macrophages that contained crystalline proteins from eosinophils were found in the conjunctiva of mice exposed to ragweed and treated with nedocromil sodium (Fig. 5).

IgE levels

No statistically significant differences were found among the groups in total IgE levels (data not shown); but the untreated, experimental mice and PBS-treated mice had high levels of specific anti-ragweed IgE. Interestingly, animals treated with nedocromil sodium showed lower levels of spe-

cific IgE than the untreated, experimental animals and PBS-treated animals, similar to results reported in previous in vitro experiments¹⁶ (Table V).

DISCUSSION

The model described in this study mimics human allergic conjunctivitis more closely than previously described models for the following reasons: the antigen used is the most common airborne allergen responsible for causing allergic rhinoconjunctivitis in the United States¹⁷; the route (nasal and conjunctival mucosae) is the natural means of allergen exposure; no adjuvants were used; the animals had high levels of anti-ragweed IgE and clinical and histologic signs of allergic conjunctivitis; and the conjunctivitis was modulated by a well-known antiallergy drug, nedocromil sodium. This new murine model is reliable, economical, rapid, easy to perform, and quantifiable.

We selected female mice on the basis of the sex predilection for females in the spontaneous allergic disease developed in dogs¹⁸ and on the sex dimorphism of ocular mucosal immunity with the protective role of male hormones.¹⁹ We selected the SWR/J strain on the basis of our preliminary work with four high reagenic responder mouse strains: C3H/J, SWR/J, C57BL/6J, A/HeJ,²⁰⁻²² and BALB/c. In these studies, SWR/J mice showed the greatest clinical and histologic conjunctival allergic reactions.²³

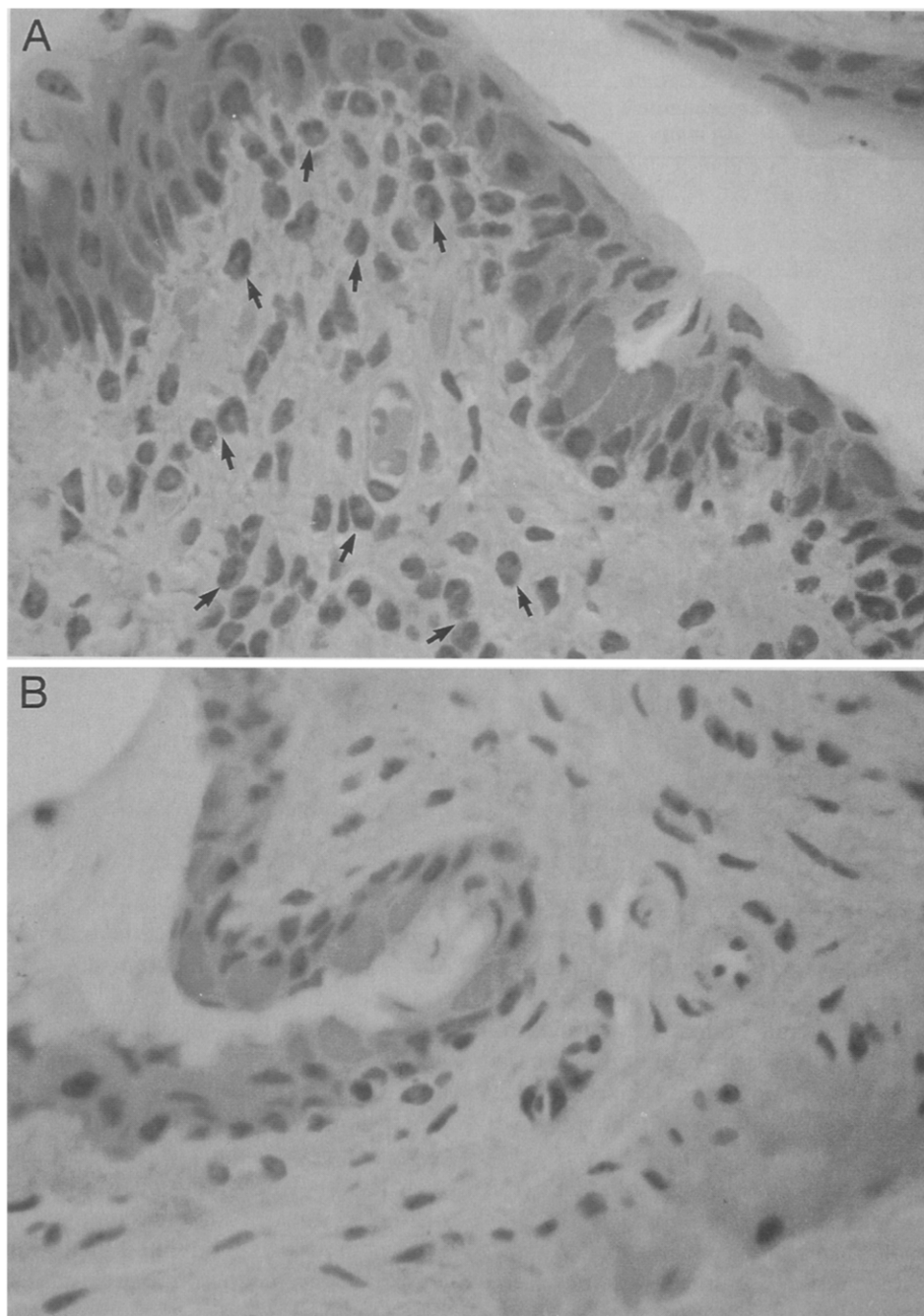


FIG. 2. Eosinophil infiltration in conjunctival substantia propria closest to epithelium (*arrows*). PBS-treated group. Mouse conjunctiva (hematoxylin-eosin, $\times 160$). **B.** Reduction of eosinophil infiltration by nedocromil sodium. Nedocromil-treated group. Mouse conjunctiva (hematoxylin-eosin, $\times 160$).

To expose the animals to the allergen we used powder, which was delivered with micropipettes. Micropipettes are designed to be used with liquids, and therefore powder delivery was less accurate. However, by using the method described, we obtained an average value of 1.25 mg with each

application. Preliminary experiments (data not shown) also confirmed that when the ragweed pollen was diluted to different concentrations in a liquid vehicle, the animals did not exhibit the degree of conjunctival allergic reaction that developed with the powder form; other authors failed to

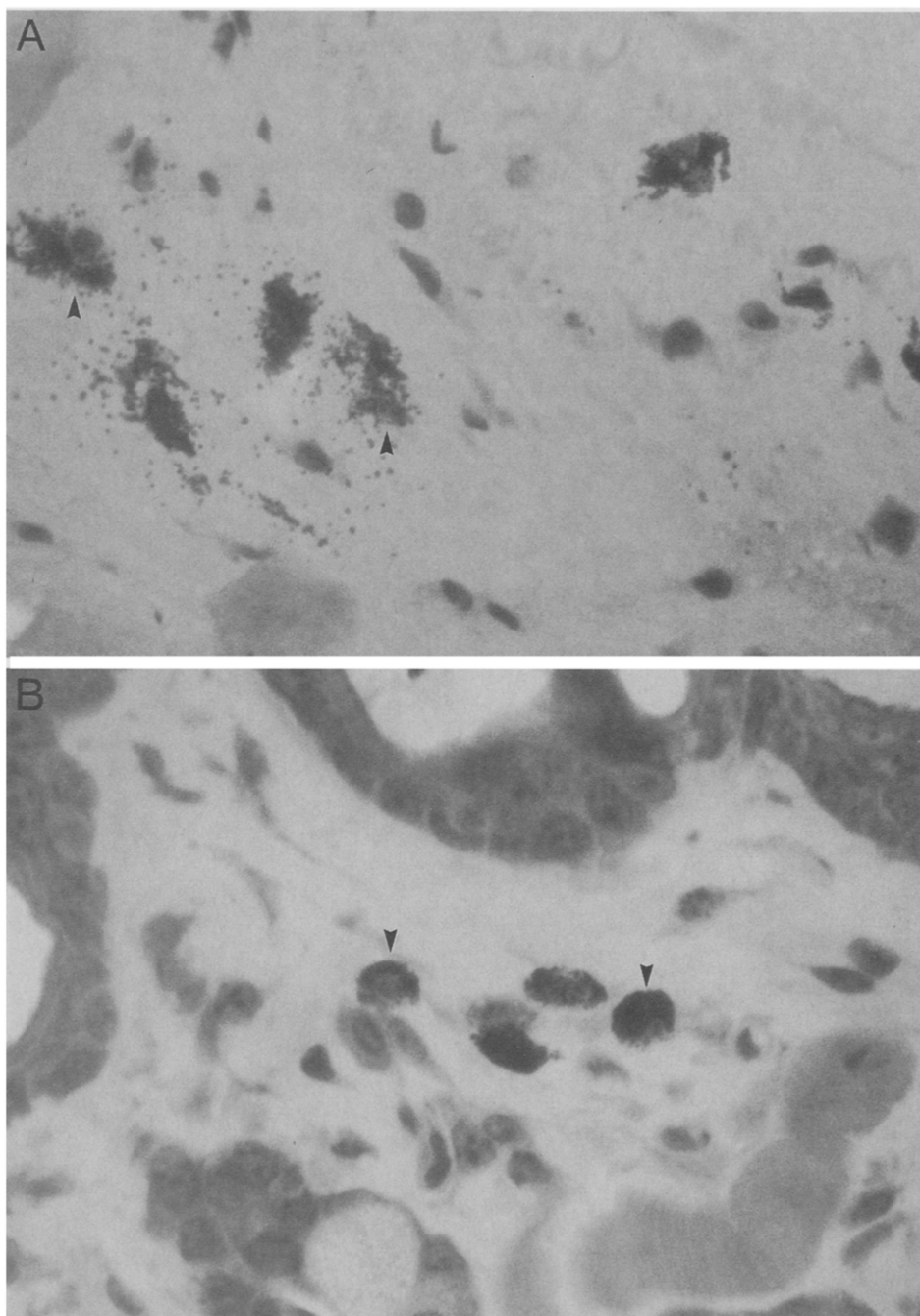


FIG. 3. **A**, Degranulating mast cells in PBS-treated group (*arrows*). Mouse conjunctiva (Giemsa, $\times 256$). **B**, Intact mast cells in nedocromil-treated group (*arrows*). Mouse conjunctiva (Giemsa, $\times 256$).

induce an allergic reaction in mice by using antigen in solution.²⁴ On the basis of these experiences, it is unlikely that the antigen administered in powder form could act as a local irritant; and the clinical, histologic, and serologic allergic alterations are associated with an allergic response elicited by the interaction of the antigen with the mucosa.

Our mice were exposed to ragweed pollen for 5 days and then challenged 3 days later. This short immunization period was chosen on the basis of a previous report,²⁰ which showed antigen-specific IgE in mice 7 to 10 days after the first antigen exposure.

Our mice had signs of conjunctival edema and

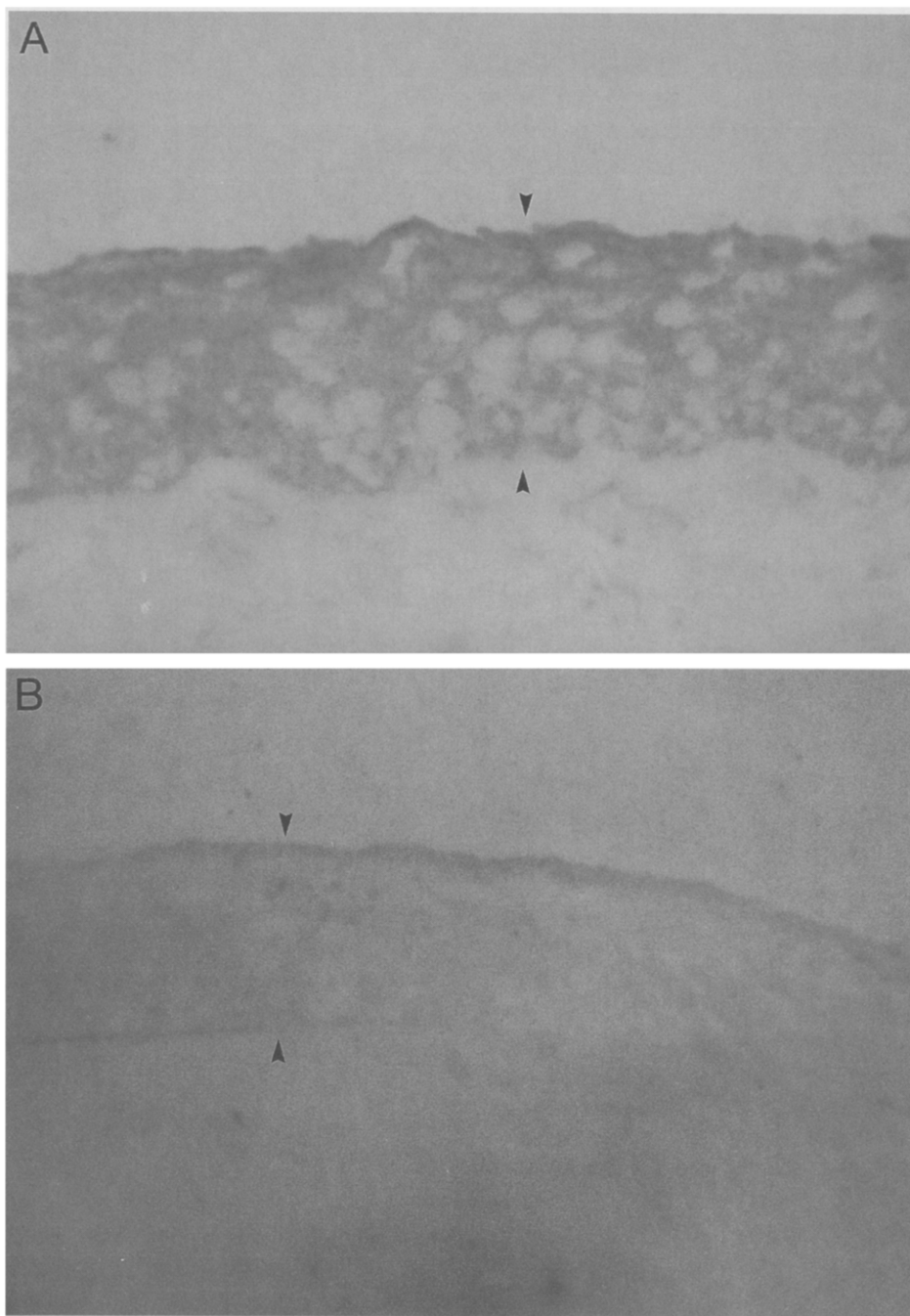


FIG. 4. **A**, Positive staining for IL-1 α in corneal epithelium (*arrows*). PBS-treated group. Mouse cornea (alkaline phosphatase immunochemical staining, $\times 160$). **B**, Reduction of IL-1 α expression in corneal epithelium in an allergic mouse treated with nedocromil sodium (*arrows*). Nedocromil-treated group. Mouse cornea (alkaline phosphatase immunochemical staining, $\times 128$).

redness. The group treated with nedocromil sodium had fewer clinical signs than the other groups exposed to ragweed. These findings are in accordance with human seasonal allergic conjunctivitis in which mild conjunctival chemosis and erythema are usually present.^{2,3} In atopic dogs with ragweed

sensitivity, clinical signs of self-inflicted trauma associated with scratching were reported previously.¹⁸ No clinical signs of scratching were found in the lids or perinasal area. The animals developed a washing maneuver in the nose and the mouth, possibly related to itching, that never in-

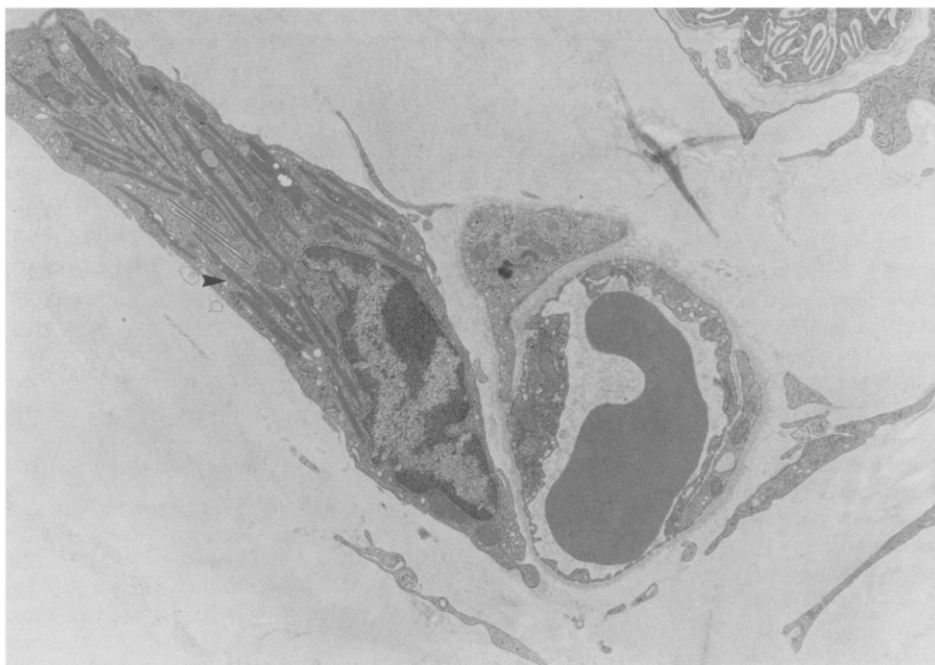


FIG. 5. Macrophages with crystalline proteins from eosinophils (arrow). Mouse conjunctiva. Nedocromil-treated group (TEM, $\times 16,000$).

volved the eye. In addition, more tearing was found in the groups exposed to ragweed.

The LM studies showed that the untreated, experimental mice had an enormous eosinophil infiltration (mean, 2426.66 cells/mm²) in the stroma close to the epithelium, and some eosinophils were present in the epithelium. The infiltration of eosinophils into the conjunctiva is the hallmark of the conjunctival histologic findings in patients with allergic conjunctivitis,^{25, 26} and eosinophils are the predominant cell in nasal mucosal biopsy specimens of patients with allergic rhinitis.²⁷ This infiltration was dramatically reduced to 171.9 cells/mm² ($p < 0.05$) in the eyes treated with nedocromil sodium.

The differences in the number of mast cells were not as striking, possibly because the cells that were totally degranulated were not visible by LM^{28, 29}; but more mast cells were observed in the groups exposed to ragweed and not treated with nedocromil sodium. Results of human studies³⁰ showed more mast cells in the subepithelium of patients with allergic conjunctivitis than in healthy volunteers. Allansmith et al.^{28, 31} reported more degranulating mast cells in the conjunctiva of allergic rats and human beings, and we found more degranulating mast cells in the untreated, experimental group compared with the nedocromil sodium-treated group. No significant differences

were found by LM in the number of goblet cells among the groups. A similar observation was reported in rats, suggesting that mucus secretion may not be attributable to an anaphylactic mechanism alone.³²

The activation of mast cells and eosinophils releases the classical mediators (vasoactive amines and eosinophil proteins) that are responsible for the clinical features of allergy.^{33, 34} However, T_{H2} CD4⁺ T cells,³⁵ eosinophils, and mast cells also release proinflammatory cytokines that play a central role in the pathophysiology of the allergic inflammatory response.³⁶⁻³⁸ Further, epithelial cells can be induced to secrete proinflammatory cytokines³⁹ and to express adhesion molecules⁴⁰ under the influence of other cytokines. Our experimental murine model provides the opportunity for cytokine measurement because of the ready availability of reagents for detecting mouse cytokines. We selected the cytokines tested on the basis of previous reports of cytokine involvement in allergic^{38, 41} and inflammatory diseases⁴² and the availability of commercial reagents. Our immunohistochemical studies showed increased proinflammatory (IL-1 α , IL-1 β , IL-2) and specifically allergic (IL-3, IL-4, IL-5, IL-6) cytokines in the corneal and conjunctival epithelium and stroma in the experimental groups, and a great reduction of these molecules in the nedocromil sodium-treated

TABLE IV. Immunohistochemical staining for conjunctival cytokine expression

	Group			
	Experimental (n = 6)	PBS (n = 5)	Nedocromil (n = 5)	Control (n = 5)
IL-1 α	4.83 $\pm$ 1.50*†	4.7 $\pm$ 1.78*‡	1.90 $\pm$ 0.37	1.65 $\pm$ 2.19
IL-1 β	4.0 $\pm$ 0.89*†	2.0 $\pm$ 1.39	1.35 $\pm$ 0.22	1.45 $\pm$ 2.11
IL-2	3.95 $\pm$ 2.87	2.8 $\pm$ 1.89	1.55 $\pm$ 1.06	1.50 $\pm$ 1.4
IL-3	4.16 $\pm$ 0.98*‡	3.6 $\pm$ 2.23*‡	1.80 $\pm$ 0.73	1.10 $\pm$ 0.9
IL-4	3.83 $\pm$ 2.29	3.4 $\pm$ 1.29	1.05 $\pm$ 0.78	1.55 $\pm$ 2.0
IL-5	3.33 $\pm$ 0.93*†	2.5 $\pm$ 1.45	1.20 $\pm$ 0.87	1.10 $\pm$ 1.0
IL-6	3.66 $\pm$ 1.53*‡	3.7 $\pm$ 1.95*‡	1.55 $\pm$ 1.06	0.5 $\pm$ 0.86
IL-10	2.33 $\pm$ 1.99	0.7 $\pm$ 0.54	0.25 $\pm$ 0.35	0.65 $\pm$ 0.85
TNF- α	3.58 $\pm$ 2.45	2.95 $\pm$ 2.15	1.05 $\pm$ 1.03	1.1 $\pm$ 1.38

Positive staining in the corneal epithelium, conjunctival epithelium and stroma, and the perivasculature was scored on a scale from 0 to 3+ for each area. Values are expressed as mean scores $\pm$ SD.

TNF- α , Tumor necrosis factor- α .

* $p \leq 0.001$ versus control.

† $p \leq 0.001$ versus nedocromil.

‡ $p \leq 0.05$ versus nedocromil.

TABLE V. Specific anti-ragweed IgE values

	Group			
	Experimental (n = 16)	PBS (n = 9)	Nedocromil (n = 16)	Control (n = 16)
Specific anti-ragweed IgE	161.72 $\pm$ 82.6*†	177.46 $\pm$ 36.72*†	75.38 $\pm$ 30.25	33.4 $\pm$ 19.1

Values are expressed as means $\pm$ SD (10^{-4} Absorbance).

* $p \leq 0.0001$ versus control.

† $p \leq 0.0001$ versus nedocromil.

eyes. IL-1 α and IL-3 showed statistically significant differences between the PBS- and nedocromil sodium-treated groups. Mast cells have been identified in a study of the nasal mucosa in allergic rhinitis as responsible for 90% of IL-6 secretion and 50% of IL-5 secretion, with eosinophils responsible for the other 50% of IL-5 production.⁴¹ Positive staining of cells in the conjunctival stroma for IL-1 α and IL-6 was observed in the experimental groups with reductions in the number of such cells in the nedocromil sodium-treated eyes.

TEM provided the surprising observation that the nedocromil sodium group had macrophages with phagocytosed crystalline protein structures, similar to Charcot-Leyden crystal protein,⁴³ identified as proteins derived from eosinophils (personal communication, Dr. Ann Dvorak, Pathology Service, Beth Israel Hospital, Boston, Mass.). This finding may be in accordance with the report that addresses the possible effects of nedocromil sodium on eosinophil survival⁴⁴ and subsequent macrophage engulfment of apoptotic cells. Similar

crystal-containing macrophages were found in lung biopsy specimens of IL-4 transgenic mice (personal communication, Dr. Ann Dvorak) that showed an allergy-like inflammatory disease in the lids.^{45, 46}

Specific anti-ragweed IgE was significantly elevated in the experimental and PBS-treated mice, whereas mice treated with nedocromil sodium showed lower levels of specific anti-ragweed IgE. This correlates with in vitro experiments that showed an IgE reduction when nedocromil sodium was added to a cell culture of IgE-producing B cells.^{16, 47}

Allergic conjunctivitis was developed in SWR/J mice after topical exposure to the airborne allergen, ragweed pollen. This conjunctivitis exhibited the clinical (conjunctival injection and chemosis), histopathologic (eosinophil and mast-cell migration into the conjunctiva with mast-cell degranulation), and serologic (specific anti-ragweed IgE) characteristics of human seasonal allergic conjunctivitis. This animal model of seasonal allergic conjunctivitis was modulated by topical application of

the mast-cell stabilizer, nedocromil sodium. The drug decreased the clinical signs, reduced the number of eosinophils and degranulating mast cells, and reduced the release of allergic and proinflammatory cytokines in the conjunctiva. To the best of our knowledge, this is the first report of the development of an experimental murine model of allergic conjunctivitis to an airborne allergen that can be used to study the disease pathophysiology and its treatment response.

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