

Pollen-specific IgG antibodies in the tears of patients with allergic-like conjunctivitis

Mark Ballow, M.D., Louis Mendelson, M.D., Peter Donshik, M.D.,
Anthony Rooklin, M.D., and Pamela Rapacz, B.S.

Chester, Pa., and Farmington, Conn.

Specific-IgG antibodies to rye grass and/or AgE in the tear secretions were demonstrated in studies of patients with the clinical features of allergic conjunctivitis but negative immediate skin reactivity and absent IgE antibodies by RAST to the inhalant pollen allergens. Studies by use of transferrin as a marker for the vascular leakage of plasma proteins into the tear secretions indicated that more than 98% of these pollen-specific IgG antibodies were locally produced by the conjunctival tissues of the external eye. These findings suggest that IgG-mediated immune mechanism(s) are important in the pathogenesis of some patients with allergic-like conjunctivitis. (J ALLERGY CLIN IMMUNOL 73:376-380, 1984.)

Allergic disorders of the eye are common clinical problems. Clinical features include hyperemia and edema of the conjunctiva, intense itching, and tearing. Symptoms are often seasonal, and patients exhibit immediate skin reactivity to various airborne pollens. These characteristics and laboratory data suggest that the inflammatory reaction is mediated by IgE immune mechanisms.¹⁻³

We present in this article a group of patients with the clinical features of allergic conjunctivitis but who have negative immediate skin reactivity and absent specific-IgE antibodies by RAST in serum and tears to the inhalant pollen allergens. Pollen-specific IgG antibodies were demonstrated in the tear secretions. These specific antibodies were demonstrated to be locally produced by the conjunctival tissues. These findings together with the studies of Ballow and Mendelson⁴ and Easty et al.⁵ of patients with vernal conjunctivitis who have pollen-specific tear IgE antibodies indicate that the external eye, i.e., conjunctival tissues, can mount a local immune response. Further-

more, our findings suggest that an IgG-mediated immune mechanism(s) may be responsible for the pathogenesis of tissue inflammation in some patients with symptoms of allergic-like conjunctivitis.

METHODS

Patient populations

Twelve patients with typical symptoms of seasonal allergic-like conjunctivitis were referred for study because of negative intradermal skin testing to the inhalant pollen allergens. Symptoms included tearing, itching, light sensitivity, and scratchiness of the eyes. Papillae (cobblestoning) on the tarsal conjunctiva as seen in patients with vernal conjunctivitis were not present. Nine patients also had nasal symptoms of rhinorrhea and pruritus. Four patients had other atopic disorders (asthma and eczema); most (10 of 12) had a family history of atopic diseases. Maximum symptoms occurred from June through Sept. with three patients having some symptoms throughout the year. The patients (six male patients and six female patients) ranged in age from 12 to 47 yr (median 17 yr). Study populations included subjects without conjunctivitis (control group: eight non-atopic individuals and four patients with allergic rhinitis) and 12 patients with seasonal ARC. All patients with ARC had multiple positive skin reactivities and serum RAST to the inhalant pollen allergens.

Serum was obtained by venipuncture and stored at -20°C . Tears were induced either spontaneously by staring into a bright light or by stimulation with several small crystals of sodium chloride. The tear samples (100 to 300 μl) were collected in glass capillary tubes and stored at -70°C .

Specific-IgE antibodies to the pollen allergens were assayed in serum and tears by RAST (Pharmacia Diagnostics, Piscataway, N.J.), and total IgE was quantitated by PRIST

From the University of Connecticut School of Medicine, Farmington, Conn., and the Crozer Chester Medical Center, Chester, Pa.

Supported by Public Health Service grant EY02036 from the National Eye Institute.

Received for publication May 16, 1983.

Accepted for publication Sept. 22, 1983.

Reprint requests: Mark Ballow, M.D., Department of Pediatrics, University of Connecticut Health Center, Farmington, CT 06032.

Abbreviations used

ARC:	Allergic rhinoconjunctivitis
RAST:	Radioallergosorbent test
PRIST:	Paper radioimmunosorbent test
PBS-T:	Tween 20
ELISA:	Enzyme linked immunosorbent assay
RG:	Rye grass group I antigen
AgE:	Ragweed antigen E

(Pharmacia Diagnostics, Piscataway, N.J.) as previously described.⁴ Transferrin, a serum protein of approximately 70,000 daltons, which is not normally found in tears, was used as a marker for the leakage of serum proteins into the tear secretions. Transferrin was measured by radial immunodiffusion by use of monospecific antisera (Boehringer Mannheim Biochemicals, Indianapolis, Ind.).

Total IgG, IgM, and IgA were quantitated in the tears by ELISA.⁶ The lower limit of immunoglobulin quantitation was 2.4 µg/ml for IgG, 4.7 µg/ml for IgM, and 2.4 µg/ml for IgA.

Specific-IgG antibodies to RG and AgE were measured by a double antibody-ELISA method.⁷ IgG-subclass antibodies were assayed by use of mouse monoclonal antibodies to IgG₁ (Fab portion, clone SG-16, Miles Labs, Slough, England) and IgG₄ (clone 1D7, Bethesda Research Lab., Gaithersburg, Md.). The partially purified pollen antigens were obtained from the National Institute of Allergy and Infectious Disease, Bethesda, Md. Briefly, polystyrene microtiter plates (Flow Labs, McLean, Va.) were coated with antigen in a carbonate-bicarbonate buffer. After removing excess antigen, serial dilutions of serum or tears were added to wells of the microtiter plate, incubated at 37° C for 1 hr, and washed with buffered saline containing 0.05% PBS-T. Polyvalent rabbit anti-human IgG (Cappel Labs, Cochranville, Pa.) or mouse monoclonal anti-IgG₁ or IgG₄ were added to the plates, incubated, and washed with PBS-T. Peroxidase-conjugated goat anti-rabbit IgG (Cappel Labs) or alkaline phosphatase-conjugated goat anti-mouse IgG (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) was added and incubated 1 hr or overnight, respectively. After a final PBS-T wash, the appropriate enzyme substrate was added, and the color reaction was read on a Titertek Multiskan ELISA plate reader (Flow Labs). Antibody specificity to these pollen antigens were demonstrated by absorption and blocking studies. A positive control serum from a hay fever patient undergoing desensitization was run on each plate to correct for daily variation. Saliva from a nonatopic individual was used as a negative control. The absorbance was plotted against the log of the reciprocal serum or tear dilution. Quantitation of specific antibody to RG and AgE was performed by calculation of the area under the serum or tear dilution curve, and the results were expressed in arbitrary ELISA units.^{7, 8}

Assessment of the local production of specific-IgG antibody in the tears was performed as described by Donovan et al.⁹ In a different approach as described by Mathews,¹⁰ an

index of local secretion was calculated. This formula takes into account the variability and dilutional effects of sample collection. An index over 0.2 represents appreciable local production of specific-IgG antibody.

RESULTS

Total IgE levels in the tears and serum are shown in Table I. Patients with ARC had significantly ($p < 0.05$) elevated levels of IgE in both the tears and serum compared to the tears and serum levels of IgE in control individuals and the patients with allergic-like conjunctivitis. Nine of 12 patients with ARC had a positive serum RAST to one or more inhalant pollen allergens; five also had a positive tear RAST. In contrast, none of the patients with allergic-like conjunctivitis had either a positive tear or serum RAST.

Levels of IgG, IgM, and IgA were measured in the tear secretions (Table II). The tear levels of IgG were moderately increased (18.7 µg/ml) in the allergic-like conjunctivitis group compared to the control (13.2 µg/ml), and ARC (12.3 µg/ml) groups. However, this difference was not statistically significant ($p > 0.05$). In the presence of normal levels of tear IgE and the absence of pollen-specific IgE antibodies, the slightly increased levels of IgG in the tear secretions suggested an IgG-mediated immune mechanism.

Table III demonstrates the tear levels of IgG antibodies to RG- and AgE-pollen antigens. Patients with allergic-like conjunctivitis had markedly elevated levels of specific-IgG antibodies to RG (41.6 ELISA units) and AgE (30.5 ELISA units) compared to control subjects ($p < 0.005$) and patients with ARC ($p < 0.01$) (Table III). Ten of 12 and nine of 12 individuals with allergic-like conjunctivitis had levels of specific-IgG antibodies to RG and AgE, respectively, that were greater than 2 standard deviations above the mean IgG levels to these two pollen antigens in the control group. Serum levels of IgG-specific antibodies to RG and AgE did not differ significantly among the various study groups.

In those patients with sufficient sample, IgG₁- and IgG₄-subclass antibodies to RG and AgE were assayed in the tear secretions. Both IgG₁- and IgG₄-specific antibodies were detected in the tears. Four of five patients with allergic-like conjunctivitis had high levels of both IgG₁ and IgG₄ antibodies to RG (Fig. 1) and AgE (Fig. 2). These subclass-IgG antibodies to RG in the tears of patients with allergic-like conjunctivitis patients were significantly elevated ($p < 0.05$) compared to the tear levels in the patients with ARC.

Since the serum contained IgG antibodies to RG and AgE, we evaluated the proportion of IgG antibodies to these two pollen allergens that were locally

TABLE I. Tear and serum total IgE and RAST in patients with conjunctivitis

Patient group	Total IgE*		RAST	
	Serum	Tears	Serum	Tears
Control (12)†	22.8‡ (3.5 to 148)§	1.4 (0 to 2.0)	4/12	0/12
ARC (12)	59 (15.8 to 220)	3.1 (1.4 to 6.7)	9/12	5/12
Allergic-like conjunctivitis (12)	13.9 (3.2 to 61)	1.5 (0 to 2.4)	0/12	0/12

*Units per milliliter.

†Number of patients in group.

‡Geometric mean.

§ ± 1 SD.

||Number positive/total number tested in group.

TABLE II. Tear immunoglobulin levels*

Patient group	P	IgG	IgM	IgA
		(μ g/ml)		
Control (12)		13.2† (4.0 to 43.4)†	0	166 (75.5 to 366)
ARC (12)	NS	12.3 (3.5 to 42.8)	‡	94 (16.9 to 521)
Allergic-like conjunctivitis (12)	NS	18.7 (4.7 to 74.1)	§	126 (53 to 297)

NS = not significantly different from control values.

*Tear immunoglobulins measured by ELISA.

†Geometric mean ± 1 SD.‡Three patients had measurable levels ($>4.7 \mu$ g/ml) of tear IgM.§Only 1 patient had detectable levels ($>4.7 \mu$ g/ml) of tear IgM.

TABLE III. Tear IgG-specific antibodies to rye grass and ragweed pollen antigens

Patient groups	RG	AgE
	(ELISA units)	
Control (12)	2.9* (0 to 10.2)	2.2 (0 to 6.2)
ARC (12)	4.5 (0 to 20.4)	6.4 (2 to 24)
Allergic-like conjunctivitis (12)	41.6 (11.5 to 151)	30.5 (12 to 78)
p values	<0.005 †	<0.01

*Geometric mean ± 1 SD.

†Student's t test comparison between ARC and allergic-like conjunctivitis groups.

produced by the conjunctival tissues. The formulas of Donovan et al.⁹ and Mathews¹⁰ were used with transferrin as a marker for the transudation of plasma proteins into the tear secretions. In 11 patients with allergic-like conjunctivitis for whom we had adequate tear samples for study, the percentage of local production of IgG antibodies to RG and AgE ranged from

87.8% to 99.9% (mean 98.7%). Similarly, the index of local secretion for these pollen-specific IgG antibodies was greater than 0.2 in all 11 patients for RG (range 3.47 to 258) and in 10 of 11 patients for AgE (range 1.6 to 142). In two patients the levels of IgG antibody to RG were higher in the tears than in the serum.

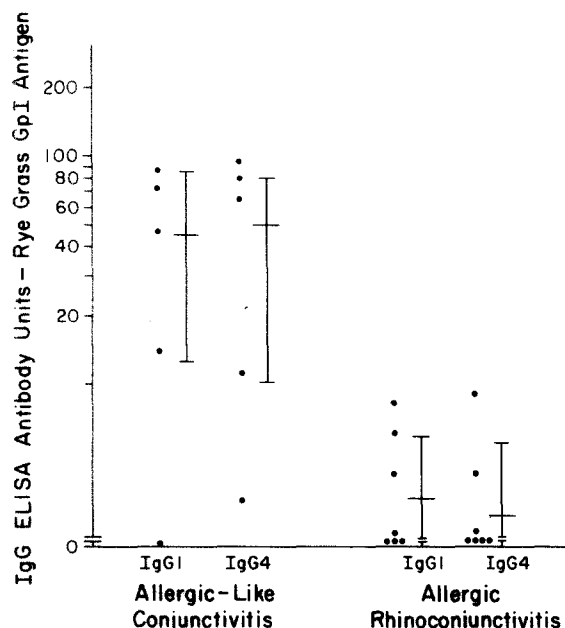


FIG. 1. IgG-subclass antibodies in tear secretions to RG. The mean value (± 1 SD) for each group of data points is shown (horizontal bars). Tear levels of IgG₁ and IgG₄ antibodies to RG were significantly ($p < 0.05$) elevated in the patients with allergic-like conjunctivitis.

DISCUSSION

Although the findings in the group of patients who are described in this article are uncommon, they represent a clinical entity that is an enigma to the physician and a frustration for the patient. The usual diagnostic studies, such as skin testing, serum IgE, and RAST suggested that the pathogenesis of the conjunctivitis was not IgE mediated. Nevertheless, the symptoms and the clinical features were consistent with an immunologic mechanism. The prevalence of this type of hypersensitivity conjunctivitis is difficult to estimate, but, in general, one to two new patients per year are usually observed in a busy allergy practice.

Further evaluation confirmed the initial clinical and laboratory testing of these patients. Tear IgE was normal, and pollen-specific IgE antibodies by RAST of the tear secretions were negative. These findings are in contrast to patients with ARC and vernal conjunctivitis in which the total IgE is elevated and specific-IgE antibodies to inhalant pollen allergens and other allergens can be demonstrated in the tears.^{4, 5} The seasonal component with maximum symptomatology in summer and early fall suggested that other types of immune mechanisms may be playing a role in the pathogenesis of this ocular disease. Our studies support this concept with the demonstration of specific-IgG antibodies in the tear secretions to

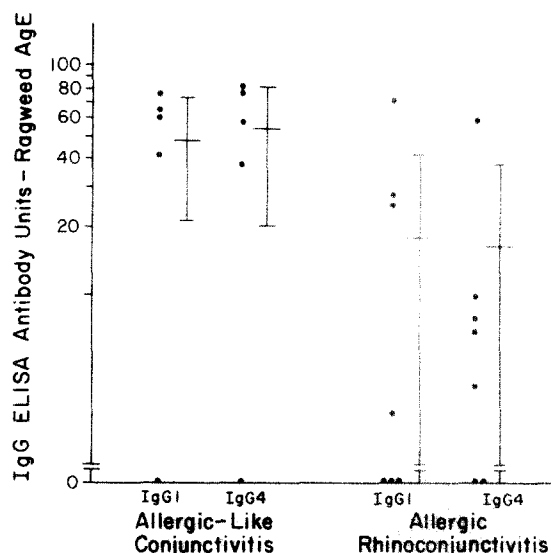


FIG. 2. IgG-subclass antibodies to AgE in tears. The mean value (± 1 SD) for each group of data points is shown (horizontal bars).

RG- and AgE-pollen antigens. Studies in our laboratory of patients with vernal conjunctivitis revealed that IgE antibodies⁴ and more recently IgG antibodies⁷ to pollen allergens in the tear secretions were locally produced by the conjunctival tissues. Since individuals in all study groups had serum-IgG antibodies to RG and/or AgE, transferrin, a plasma protein produced in the liver, was used to examine the proportion of IgG antibodies in the tears that were locally produced versus transudation from the plasma. By use of two approaches, the formulas of Donovan et al.⁹ and Mathews,¹⁰ most (98% to 99%) of the IgG antibodies to RG and/or AgE were demonstrated to be derived by immune responses of the conjunctival tissues of the external eye.

The causal role, if any, of these tear pollen-specific IgG antibodies in the mechanism(s) of inflammation and the resulting conjunctivitis is not entirely clear. Classically in allergic diseases, IgG antibodies are believed to have an important role as blocking antibodies for IgE-mediated immune disorders.¹¹ In our patients, an IgE-mediated immune mechanism does not appear to be present. However, IgG antibodies may be involved in tissue inflammation in an immune complex-type reaction with activation of complement. Several complement proteins have been detected in the tears including C4,¹² C3,¹³ and factor B.¹⁴ Activation of complement by IgG antibodies to RG/AgE could result in the release of vasoactive amines from basophils and mast cells that would then lead to changes in vascular permeability.¹⁵ On the other hand recent reports have suggested that certain

hypersensitivity mechanisms in allergic disorders are associated with the presence of skin sensitizing, heat-stable IgG antibodies¹⁶ that may belong to the IgG₄ subclass.¹⁷ This is of interest since IgG₄ has been suggested to act as a reaginic immunoglobulin and thus mimic IgE-mediated type disorders.¹⁸ Both these possibilities are supported by the findings in our study of IgG₁- and IgG₄-subclass antibodies to RG and/or ragweed AgE in the tears. IgG₁ antibodies have a relatively high binding affinity for C1q. Thus they are capable of activating the classic complement cascade leading to the production of anaphylatoxins, e.g., C3a and C5a, and the release of vasoactive amines from mast cells. The function of IgG₄-subclass antibodies remains controversial. As suggested by several investigators, IgG₄ may have reaginic properties binding to basophils and mediate immediate-type hypersensitivity reactions.¹⁷⁻²⁰ In contrast, van Toorenbergen and Aalberse^{21, 22} could not demonstrate IgG₄-mediated degranulation of basophils. These workers²³ suggested that IgG₄ antibodies may serve as blocking antibodies. Further studies will be necessary in our patients with allergic-like conjunctivitis to elucidate the nature and role of these allergen specific-IgG antibodies in inflammation of the external eye.

We thank Drs. Palm, Wheeler, Englander, Kordansky, Gupta, and other Connecticut Allergists and Ophthalmologists for allowing us to study their patients. We also wish to thank Maureen G. Yates and Lorraine Fox for their secretarial assistance.

REFERENCES

- Theodore FH, Schlossman A: Ocular allergy. Baltimore, 1958, Williams & Wilkins
- Settipane GA, Connell JT, Sherman WB: Reagin in tears. *J ALLERGY* 3:37, 1965
- Patten JT, Allansmith MR: Ocular allergy. in Middleton E, Reed CE, Ellis EF, editors: *Allergy. Principles and practice*. St. Louis, 1978, The CV Mosby Co, p 1116
- Ballow M, Mendelson L: Specific immunoglobulin E antibodies in tear secretions of patients with vernal conjunctivitis. *J ALLERGY CLIN IMMUNOL* 66:112, 1980
- Easty DL, Birkenshaw M, Merrett T, Merrett J, Entwistle C, Amer B: Immunological investigations in vernal eye disease. *Trans Ophthalmol Soc UK* 100:98, 1980
- Ballow M, Mendelson L, Donshik PC, Sparks K: IgG specific antibodies to rye grass and ragweed antigens in the tear secretions of patients with vernal conjunctivitis. *J ALLERGY CLIN IMMUNOL* 69:114, 1982
- Ballow M, Donshik PC, Mendelson L, Rapacz P, Sparks K: IgG specific antibodies to rye grass and ragweed pollen antigens in the tear secretions of patients with vernal conjunctivitis. *Am J Ophthalmol* 95:161, 1983
- Sparks K, Ballow M: The indirect ELISA for quantification of specific antibody: Analysis of antibody dilution curves. *Diag Immunol* 1:269, 1983
- Donovan R, Johansson SGO, Bennich H, Soothill JF: Immunoglobulins in nasal polyp fluid. *Int Arch Allergy Appl Immunol* 37:154, 1970
- Mathews KP: Calculation of secretory antibodies and immunoglobulins. *J ALLERGY CLIN IMMUNOL* 68:46, 1981
- Lichtenstein LM, Holtzman NA, Burnett LS: A quantitative in vitro study of the chromatographic distribution and immunoglobulin characteristics of human blocking antibody. *J Immunol* 10:317, 1968
- Chandler JW, Leder R, Kaufman HE, Caldwell JR Jr: Quantitative determinations of complement components and immunoglobulins in tears and aqueous humor. *Invest Ophthalmol* 13:151, 1974
- Bluestone R, Easty DL, Goldberg LS, Jones BR, Pettit TH: Lacrimal immunoglobulins and complement quantified by counter-immunoelectrophoresis. *Br J Ophthalmol* 59:279, 1975
- Yamamoto GK, Allansmith MR: Complement in tears from normal humans. *Am J Ophthalmol* 17:58, 1979
- Johnson AR, Hugli TE, Muller-Eberhard HJ: Release of histamine from mast cells by the complement peptides C3a and C5a. *Immunology* 28:1067, 1975
- Pepys J, Parish WE, Stenius-Aarniala B, Wide L: Clinical correlations between long term (IgE) and short-term (IgG₄-TS) anaphylactic antibodies in atopic and "non-atopic" subjects with respiratory allergic disease. *Clin Allergy* 9:645, 1979
- Gwynn CM, Ingram J, Almousawi T, Stanworth DR: Bronchial provocation tests in atopic patients with allergen-specific IgG₄ antibodies. *Lancet* 1:254, 1982
- Stanworth DR, Smith AK: Inhibition of reagin-mediated PCA reactions in baboons by the human IgG₄ sub-class. *Clin Allergy* 3:37, 1973
- Nakagawa T, Stadler BM, Heiner DC, Skvaril F, De Weck AL: Flow-cytometric analysis of human basophil degranulation. II. Degranulation induced by anti-IgE, anti IgG₄ and the calcium ionophore A23187. *Clin Allergy* 11:21, 1981
- Gwynn CM, Almosawi T, Stanworth DR: Clinical associations with serum allergen-specific IgG₄ antibodies. *Clin Allergy* 12:459, 1982.
- van Toorenbergen AW, Aalberse RC: IgG₄ and passive sensitization of basophil leukocytes. *Int Arch Allergy Appl Immunol* 65:432, 1981
- Toorenbergen AW, Aalberse RC: IgG₄ and release of histamine from human peripheral blood leukocytes. *Int Arch Allergy Appl Immunol* 67:117, 1982
- Aalberse RC, van der Gaag R, van Leeuwen J: Serologic aspects of IgG₄ antibodies. 1. Prolonged immunization results in an IgG-4 restricted response. *J Immunol* 130:722, 1983