

Reagin in tears

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Skin-sensitizing antibody in low titer was present in the lacrimal secretion of 2 out of 7 atopic individuals whose sera showed a high skin-sensitizing antibody titer. Blocking antibody to ragweed was not found in the lacrimal secretion of one treated patient who had an elevated serum blocking antibody titer. Serum protein components found in tears were albumin and beta-2A globulin.

Skin-sensitizing antibody has been found in nasal secretions,¹ nasal polyps,² and recently in saliva³ of allergic patients. The common eye complaints in hay fever, together with the well-known conjunctival test for pollens, has suggested to us the possibility that skin-sensitizing antibody may be present in lacrimal secretion. The presence of reagin in nasal discharge encouraged this hypothesis.

In the present investigation tears from ragweed hay fever patients and normal individuals were studied for reagin and various protein fractions.

MATERIALS AND METHODS

The tears of 10 individuals were studied. Eight of these were ragweed hay fever patients. Two were normal subjects with negative intracutaneous tests to 0.025 ml. of aqueous ragweed extract, 1,000 protein nitrogen units (PNU) per milliliter.

Lacrimal secretion was stimulated by the use of irritants, such as spirits of ammonia or freshly cut onions. An 18.5 gauge sterile polyethylene catheter attached to a tuberculin syringe was used for aspiration of tears from the con-

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Supported by United States Public Health Training Grant 5T1 AI 201-3 and United States Public Health Research Grant AI-02682-05 from the National Institute of Allergy and Infectious Diseases, Bethesda, Md.

Received for publication July 6, 1964.

Table 1. *Skin-sensitizing antibody*

<i>Patient</i>	<i>Skin-sensitizing antibody titer</i>	
	<i>Serum</i>	<i>Tears</i>
A. C.	500	Positive
I. A.	500	Positive
J. L.	500	Negative*
K. R.	500	Negative
J. M.	500	Negative
B. A.	500	Not done
C. P.	300	Negative
R. S.	200	Negative
M. S.†	0	Not done
E. P.†	0	Not done

*Tested in the 1 to 5 dilution only.

†Nonallergic patients.

junctival sac of the lower eyelid. The tears were immediately cultured and stored at -3°C .

Skin-sensitizing antibody titers of tears and serum of 7 hay fever patients were determined by the Prausnitz-Küstner procedure.⁴ One-tenth milliliter of sterile tears undiluted and diluted 1 to 5 and 1 to 10 were injected into the skin of normal recipients and challenged 24 to 48 hours later with 0.025 ml. of aqueous ragweed extract 1,000 PNU per milliliter. Positive reactions were defined as a wheal and erythema greater than 5 mm. Controls were negative. Skin-sensitizing antibody titer is reported as the reciprocal of the highest dilution giving a positive reaction.

Blocking antibody determination was done by a modification of the Prausnitz-Küstner technique,⁵ using a 1 to 10 dilution of tears from a patient with a serum blocking antibody titer to ragweed of 600 PNU per milliliter.

Precipitins were studied by immunoelectrophoresis using the Scheidegger modification on microscopic slides,⁶ and by double diffusion on agar plates using the method of Ouchterlony.⁷ The following monospecific, goat antihuman sera were obtained from Hyland Laboratories: antiwhole serum (3802u5), anti- $\beta_2\text{A}$ -globulin (G206-7P1063), and anti- $\alpha_2\text{M}$ -globulin (G70ABSP7-1961) anti- $\beta_2\text{M}$ -globulin (GP164), anti- β -lipoprotein (G7-363), anticerculoplasmin (GP1263), and antitransferrin (G197P4-1363). Rabbit antihuman gamma globulin and antihuman albumin were obtained through the courtesy of Dr. A. E. O. Menzel.*

RESULTS

Two of the 7 samples of tears used in the Prausnitz-Küstner technique showed positive reactions on ragweed challenge, one a moderate reaction, and the other a slight but definite reaction. The 1 to 5 and 1 to 10 dilutions were

*Rabbits were immunized with human gamma globulin that had been purified by an ammonium sulfate procedure and found to be, in moving boundary electrophoresis, remarkably homogenous, and exhibiting a mobility of 1.23×10^{-5} cm.² volt⁻¹ sec.⁻¹ in Longworth buffer. The antihuman gamma globulin serum was not absorbed with either beta-2A globulin or beta-2M globulin. Rabbit antihuman albumin was prepared from a highly pure albumin obtained from Mann Research Laboratories, Inc.

negative. The 2 patients with reagin in their lacrimal secretion had a serum skin-sensitizing antibody titer greater than 500, and both had received hyposensitization treatment. Of the 5 patients whose tears gave a negative Prausnitz-Küstner reaction, 3 had serum skin-sensitizing antibody titers of 500 or greater, and 2 had titers of 300 or less (Table I).

Lacrimal secretions studied by immunoelectrophoresis using antiwhole serum revealed 2 precipitin bands. One was in the albumin region, and the other in the beta globulin region. On using anti-beta-2A globulin instead of antiwhole serum, a similar precipitin band was noted in the beta globulin region (Fig. 1).

The presence of beta-2A globulin and albumin was confirmed by Ouchterlony plate analysis. All tears tested in this manner revealed a positive precipitin band when anti-beta-2A globulin was placed in the center well and tears from allergic patients and 1 normal subject were placed in the peripheral wells

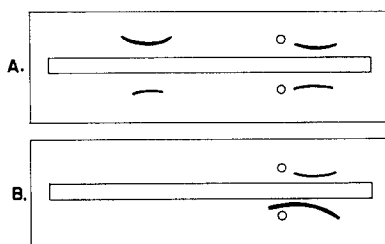


Fig. 1

Diagrams of immunoelectrophoresis of tears: *A*, Trough was filled with antiwhole serum and wells with tears from 2 allergic patients. *B*, Trough filled with anti- β_{2A} globulin. Upper well was filled with tears from an allergic patient, and lower well was filled with the corresponding serum.

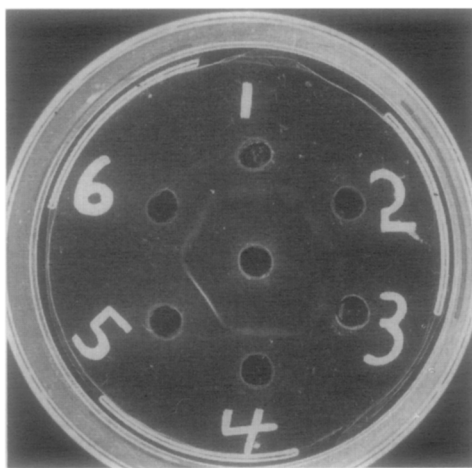


Fig. 2

Ouchterlony plate analysis of lacrimal secretion. Peripheral wells were filled with tears from 5 allergic patients and one normal individual (5). Center well was filled with anti- β_{2A} globulin serum.

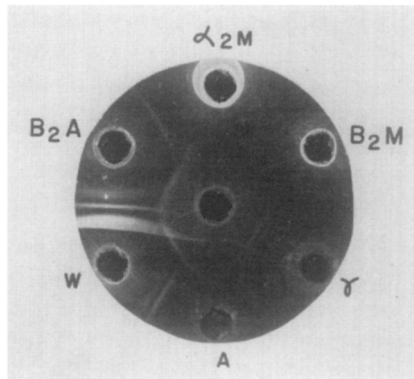


Fig. 3

Center well was filled with tears from an allergic patient. Peripheral wells were filled with the following antihuman serum: antiwhole serum (*W*), anti- β_{2A} -globulin, anti- α_{2M} -globulin, anti- β_{2M} -globulin, antigamma globulin (γ) and antialbumin (*A*). Precipitin bands between antisera are due to human proteins present in specific antisera.

(Fig. 2). The double diffusion gel technique also revealed a weak precipitin band to albumin in 2 out of 3 tears, and a band to gamma globulin in all 3 tears tested (Fig. 3). No reaction was found on using anti-gamma-2M globulin, anti-beta-2M globulin, anti-beta lipoprotein, anticerculoplasmin and antitransferrin.

The protein fractions in the lacrimal secretions that gave a positive Prausnitz-Küstner response did not differ from that of other tears. No significant difference was found between tears of allergic and normal individuals.

Blocking antibody was not found in the tears of one patient whose serum blocking antibody to ragweed was 600 PNU per milliliter.

DISCUSSION

The two samples of tears that demonstrated a positive Prausnitz-Küstner reaction came from patients with serum skin-sensitizing antibody titers of greater than 500. However, lacrimal secretion from normal flow and that obtained from irritation reportedly differ in protein concentration,⁸ a fact that complicates the interpretation of our results.

By paper electrophoresis, Krause⁹ discovered 6 protein fractions in concentrated tears: albumin, gamma-2 globulin, beta globulin, an unknown fraction 4, gamma globulin, and lysozyme. He stated that the albumin in tears moved significantly more slowly than serum albumin, and he felt that there may be a question as to the true identity of this fraction. His mean value for total proteins in lacrimal secretions from 29 normal subjects was 355.8 mg. per cent. Of this, 14.7 per cent was "albumin," 4.2 per cent gamma-2 globulin, and 44.4 per cent lysozyme. Beta globulin was estimated at 19 per cent, fraction 4 at 11 per cent, and gamma globulin at 7 per cent.

By immunoelectrophoresis and/or Ouchterlony procedures, we were definitely able to identify only albumin and beta-2A globulin as the serum proteins present

in lacrimal secretion. The precipitin band with anti-gamma globulin serum in Fig. 3 may have been due to a cross reaction between this antiserum and the beta-2A globulin in tears. Chodirker and Tomasi¹⁰ in a study of globulin in lacrimal secretion by the gel double diffusion technique reported the presence of predominantly beta-2A globulin with little or no gamma-2 globulin or beta-2M globulin.

Beta-2A globulin has been frequently implicated as one of the carriers of reagin.¹¹⁻¹⁴ Ishizaka and his associates³ demonstrated the presence of reagin in saliva, where the predominant protein was found to be beta-2A globulin. In this study beta-2A globulin was found in all tears, but reagin was present in only 2 out of the 7 samples from allergic patients. Therefore, no definite statement can be made between the relationship of beta-2A globulin and reagin.

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