

A comparative study of the allergens of cat urine, serum, saliva, and pelt

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In direct RAST analyses of sera from 43 individuals with a history of cat allergy, 39.5% were positive to cat pelt, 37.5% to cat saliva, and 12% each to cat urine and serum. The cat pelt and saliva extracts contained allergen 1, but cat serum and cat urine collected by bladder puncture had no detectable levels of this allergen. A crossed immunoelectrophoresis/crossed radioimmuno-electrophoresis analysis failed to reveal any allergen in urine or serum that was not also present in the saliva or pelt preparations, although urine had two allergens not present in serum. When serum from a patient who was direct RAST positive to cat pelt, serum, saliva, and urine was tested by crossed radioimmuno-electrophoresis, it was determined that a total of six allergens were detectable in cat pelt, three in cat urine, and six in cat serum. Since cat serum contains no detectable cat allergen 1, it may be concluded that at least seven allergens derived from the cat are capable of binding to IgE antibody in humans. (J ALLERGY CLIN IMMUNOL 76:563-9, 1985.)

Studies have demonstrated that allergen 1 plays a major role in cat allergy. Skin reactivity to cat extracts has been demonstrated to correlate with the cat allergen 1 content of the extracts. This allergen has been identified in cat saliva, cat pelt extracts, cat washes, sebaceous glands of cat skin, and in samples of the air from a room where cats reside.¹⁻⁷ It has also been demonstrated that cat extracts contain other allergens, but the importance of these other allergens has not been investigated. Little data have been reported that would establish the importance of cat serum or urinary allergens in cat allergy, although the latter are abundantly available in cat litter boxes. Because it has been demonstrated that rat and mouse urine are the most important sources of allergens in these species, the investigation of urinary allergens is especially important.

MATERIAL AND METHODS

Patient sera

Three clinics provided sera from patients who claimed to have cat allergy symptoms. All patients were skin test positive to commercial cat extracts in the various clinics.

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Abbreviations used

CIE: Crossed immunoelectrophoresis
CRIE: Crossed radioimmuno-electrophoresis
RID: Radial immunodiffusion test

Serum samples from these patients were collected during a 3-year period and were stored at -20°C . The control sera used in the tests were from four individuals that claimed no symptoms of cat allergy, a 5% solution of human serum albumin and two human serum pools that demonstrated direct RAST activity to pollen extracts.

Allergen sources

Two cat pelt extracts (A and B) were used in the direct RAST and CIE/CRIE studies. Pelt extract A was prepared by extracting three whole dried cat pelts followed by filtration and freeze-drying. Pelt extract B, prepared in a commercial establishment, was an aqueous extract of many whole cat pelts, which was precipitated with acetone. The precipitate was rehydrated and freeze-dried. Cat saliva, serum, and urine samples were obtained from healthy cats who were examined by staff veterinarians of National Institutes of Health before any samples were obtained. Free flowing saliva was collected in a beaker from cats anesthetized with Ketamine HCl (Parke-Davis, Detroit, Mich.). The cat saliva was freeze-dried in order to facilitate filtration after rehydration. The saliva was reconstituted to its original volume with deionized water, filtered through a $0.22\text{ }\mu\text{m}$ membrane, and stored at -20°C . Cat serum was separated from whole blood obtained by venipuncture, and cat urine was collected by bladder puncture of a male cat. The urine

TABLE I. Characteristics of cat allergen preparations

Sample	Protein (mg/ml)	Cat allergen 1 (U/ml)
Pelt A	0.5	1.3
Pelt B	3.8	10.8
Saliva	4.0	1.8
Serum	7.1	ND*
Urine†	3.7	ND

*ND = not detectable, i.e., <1.3 U/ml.

†Obtained by bladder puncture from a male cat; see text for details.

Acceptable variability for both methods is $\pm 25\%$.

was dialyzed through tubing with a 1000 dalton cutoff (Spectrapor No. 6, Spectrum Medical Industries, Inc., Los Angeles, Calif.) against four changes of 300 volumes of normal saline at 4° C over a 48-hour period. After freeze-drying, the cat urine was reconstituted to 25% of its original volume with deionized water and stored at -20° C. Separate collections of urine from male and female cats housed in metabolism cages were used to immunize rabbits for CIE/CRIE studies but not in any direct RAST analyses. These urine preparations were demonstrated to contain cat allergen 1 by RID.

Test methods

All preparations were tested for allergen 1 by a RID procedure when the allergen 1 content is expressed in arbitrary units based on Food and Drug Administration reference preparation.⁶ Total protein was calculated by multiplying Kjeldahl nitrogen by 6.25 or by a modified ninhydrin assay⁸ by use of bovine serum albumin as a standard.

The direct RAST procedure used paper discs (Type 595, Schleicher & Schuell Inc., Keene, N. H.) activated by cyanogen bromide⁹ as the solid support. Each disc was individually sensitized with 100 μ l of a solution containing approximately 10 μ g of protein. When tests were conducted with discs sensitized with as much as 350 μ g of protein, the counts per minute did not increase in either the positive or the control samples. After incubation with allergen at room temperature for 1 hour, the discs were washed with a solution of 0.1 mol/L phosphate buffer, pH 7.5, containing 5% bovine serum albumin and 2% Tween 20, and were incubated in this solution overnight at room temperature to block any unreacted sites. The solution was then aspirated, and 50 μ l of patient serum was added to each disc and allowed to incubate for 3 hours at room temperature. The discs were washed three times with 0.1 mol/L phosphate buffer, pH 7.5, containing 1% Tween 20, and were incubated overnight at room temperature with ¹²⁵I-labeled anti-human IgE (approximately 25,000 cpm containing 4 ng of affinity purified antihuman IgE prepared from the epsilon chain of the PS myeloma [Atlantic Antibodies, Scarborough, Me.]). After washing, the discs were counted in a gamma counter. Duplicate counts per minute values within each test were averaged, and the mean value of all tests on each patient were calculated. Serum samples from the four

TABLE II. Direct RAST activity ratios* of patients reporting allergic symptoms to cats

Patient No.	Urine	Serum	Saliva	Pelt
1	0.76	0.47	0.83	0.41
2	0.72	0.58	1.69	1.82
3	0.73	0.82	2.26	1.72
4	0.75	0.75	4.23	6.50
5	0.55	0.41	0.57	0.91
6	0.64	0.45	0.74	0.75
7	0.46	0.46	0.46	0.41
8	0.42	0.47	0.86	1.49
9	0.41	0.50	0.73	0.60
10	0.41	0.61	2.91	2.37
11	0.45	0.49	0.48	0.68
12	0.64	0.47	0.91	0.45
13	0.57	0.47	0.60	0.53
14	0.58	1.44	0.89	2.05
15	0.48	0.56	0.83	0.98
16	0.48	0.63	0.83	0.53
17	0.84	0.85	1.33	1.96
18	0.52	0.65	0.58	0.40
19	0.51	0.62	2.88	3.63
20	0.54	0.52	0.43	0.61
21	0.49	0.52	0.82	0.66
22	0.67	0.63	0.75	0.84
23	0.47	0.47	0.53	0.39
24	0.59	0.37	1.41	1.30
25	0.44	0.32	0.48	0.87
26	0.57	0.56	0.63	0.60
27	0.56	0.48	1.03	0.61
28	0.56	0.48	0.90	0.83
29	0.54	0.71	0.51	0.37
30	0.47	0.50	0.53	0.63
31	1.44	3.72	1.60	3.11
32	1.38	0.60	2.00	2.91
33	0.45	0.52	0.68	0.56
34	0.84	0.81	2.49	2.64
35	4.09	6.25	10.86	11.46
36	0.52	0.66	0.90	0.95
37	2.26	1.51	4.52	7.84
38	0.48	0.41	1.47	2.63
39	0.78	0.59	1.31	1.83
40	0.56	0.33	0.87	0.64
41	0.49	0.45	0.77	0.78
42	0.41	0.69	0.62	0.59
43	3.75	5.11	5.12	6.74
No. of positives	5	5	16	17
Percent positive	11.8	11.8	37.1	39.5

*See test methods in text.

asymptomatic patients, the two serum pools, and human serum albumin samples were analyzed several times; the mean counts per minute and standard deviation of 40 values was 277 ± 116 . A test serum was considered to be direct RAST positive if its mean counts per minute was >2 SD above the mean counts per minute of the control sera (i.e.,

509 cpm). The value of each test serum was expressed as the ratio of its mean counts per minute to 509 cpm. Therefore, a score ≥ 1 indicated that the individual was considered direct RAST positive to the allergen.

CIE/CRIE

Rabbit antisera to cat pelt and sheep anti-allergen 1 were produced by the investigators and used in procedures similar to those described in earlier articles.^{4,6,10} Rabbit antifemale cat urine and rabbit antimale cat urine was prepared by use of the urine samples collected from cats housed in metabolism cages. New Zealand white rabbits, weighing approximately 2.2 kg were injected in numerous subcutaneous sites with 1 mg of nondialyzable solids from each type of urine emulsified in complete Freund's adjuvant. After 30 days a booster injection of 1 mg solids in incomplete Freund's adjuvant was administered. Serum was obtained from whole blood collected 2 weeks after the booster. Goat antiserum to cat serum was purchased commercially (Cappel Laboratories, West Chester, Pa.). Cat serum, urine, saliva, or pelt was added to intermediate gels for some studies to detect common allergens. Sera from patient No. 4, direct RAST positive to cat pelt and saliva, and patient No. 43, direct RAST positive to cat pelt, saliva, urine, and serum were used for CRIE.

RESULTS

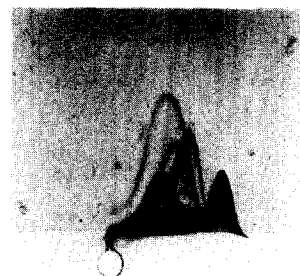
Table I summarizes the analyses of the individual cat allergen preparations and indicates that cat pelt and cat saliva contain allergen 1, whereas male cat urine obtained by bladder puncture and cat serum had no detectable quantity of this allergen. However, allergen 1 was detected in the male urine samples collected from cats housed in metabolism cages, although urine collected from female cats housed in the same manner had no detectable allergen 1.

The ratios calculated from the results of the direct RAST analysis of serum samples from symptomatic patients are presented in Table II. Seventeen of the 43 symptomatic patients were RAST positive to cat pelt, and 16 were RAST positive to cat saliva. Two patients were RAST positive to pelt and not saliva (Nos. 8 and 14), whereas one patient (No. 27) was positive only to saliva. Of the six symptomatic patients that were RAST positive to cat urine or serum, four were positive to both preparations. The two patients that reacted differently to the cat urine and serum (Nos. 14 and 32) were both RAST positive to cat pelt.

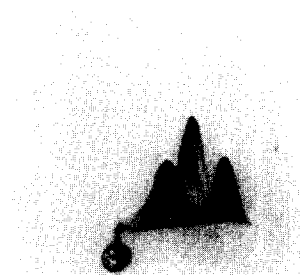
The direct RAST ratios expressed in Table II were subjected to rank correlation analysis; these results are presented in Table III. The highest correlation (0.77) was found between cat saliva and cat pelt. Cat saliva and cat urine demonstrated a fairly high correlation (0.52), whereas the rank correlations of direct RAST activity between other reagents were all found to be less than 0.4.

CIE AND CRIE OF CAT PELT

(A) CIE



(B) CRIE with Serum from Pt 43



(C) CRIE with Serum from Pt 4

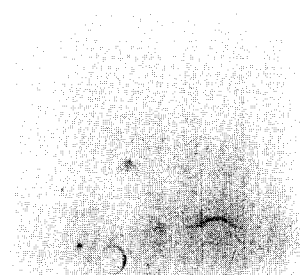


FIG. 1. CIE pattern of cat pelt A with rabbit antipelt and resulting CRIEs with serum from two allergic individuals. A, Allergen 1 and B, albumin are marked in the CIE.

The CIE pattern of cat pelt extract A and rabbit anti-pelt (Fig. 1, A) demonstrates the presence of at least 11 antigens; a CRIE pattern by use of serum from patient No. 43 who was direct RAST positive to all preparations demonstrates that six of these antigens are allergens (Fig. 1, B). The CRIE pattern that was created with the serum from patient No. 4 who was RAST positive only to cat pelt and saliva recognized only two of the antigen peaks as allergens; these allergens were allergen 1 and cat albumin (Fig. 1, C). The albumin peak was very faint.

Fig. 2, No. 1, a series of photographs, illustrates a second CIE pattern of cat pelt A accompanied by the CIE patterns of cat serum (Fig. 2, No. 2, a series) and urine (Fig. 2, No. 3, a series) by use of corresponding antisera in the upper gel and intermediate gels without antisera or antigens. Pelt A has 10 visible antigen peaks in this assay that involved a larger quantity of antigen and less upper gel area caused by the presence of the intermediate gel. This caused one of

ALLERGENS OF CAT PELT, SERUM AND URINE

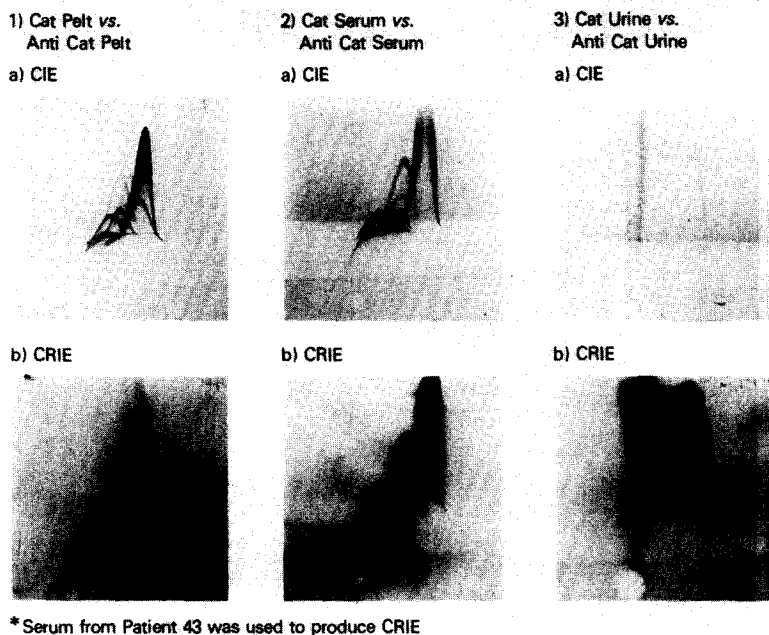


FIG. 2. The CIE of homologous antigen and antibody to cat pelt, serum, and urine are illustrated in the *a* series of photographs. Allergen 1 (No. 1) and albumin (No. 2) are marked in the CIE of cat pelt A (No. 1, *a* series). The CRIE patterns generated by use of serum from patient No. 43 is illustrated in the *b* series.

the fainter peaks to migrate out of the upper gel. Cat serum demonstrates eight peaks, and cat urine has three peaks in the other CIE photographs of Fig. 2. The line from the origin to the top of the gel in the urine CIE may be either the result of the absence of a particular antibody in the rabbit antimale urine serum or an artifact, since it occurs regularly in CIE analyses of cat urine with other antibody preparations.

The second row of photographs in Fig. 2 illustrates the corresponding CRIE patterns created with serum from patient No. 43. These photographs illustrate the presence of at least six allergens in cat pelt (Fig. 2, No. 1, *b* series), six in serum (Fig. 2, No. 2, *b* series), and three in urine (Fig. 2, No. 3, *b* series). Similar studies with serum from patient No. 4 resulted in a faint autoradiographic tracing of the albumin peak in cat serum and no detectable allergens in urine (not shown). Allergen 1, which was not detectable in cat serum and urine by RID procedures, was absent in CIE/CRIE analyses (not shown).

Since four of the five patients who displayed direct RAST activity to cat serum also reacted to cat urine in direct RAST tests, CIE/CRIE was used to determine whether there were common allergens in these two preparations. Fig. 3, A, a CRIE photograph, illustrates the effect of incorporating cat serum in the intermediate gel of the cat urine/anticat urine CRIE

illustrated in Fig. 2, No. 3, *b* series. This CRIE pattern, created with serum from patient No. 43, illustrates that two radiostained allergen peaks do not migrate with the serum front, demonstrating that cat urine contains at least two allergens that are not present in cat serum.

Since it has been previously reported that cat saliva contains all the detectable allergens of cat pelt,⁴ the presence of cat urinary allergens in saliva was explored. Cat saliva was placed in the intermediate gel of the urine/antiurine CIE/CRIE gels; the resulting CRIE pattern with the use of serum from patient No. 43 is illustrated in Fig. 3, B. All peaks normally displayed as a result of cat urine travel with the cat saliva front, demonstrating that cat saliva contains the allergens of urine that are detected in CIE/CRIE.

To examine the specificity of the CRIE patterns, sera of patients Nos. 43 and 4 were heated at 56° C for 1 hour and used in a repeat CRIE with cat pelt. The autoradiograph of the heated sera samples demonstrated no pattern, which is compatible with an antibody reaction of the IgE class. Further evidence of specificity is that serum from patient No. 43 did not react to all cat pelt antigens, demonstrating that all precipitin peaks do not react with allergic human serum or radiolabeled antihuman IgE.

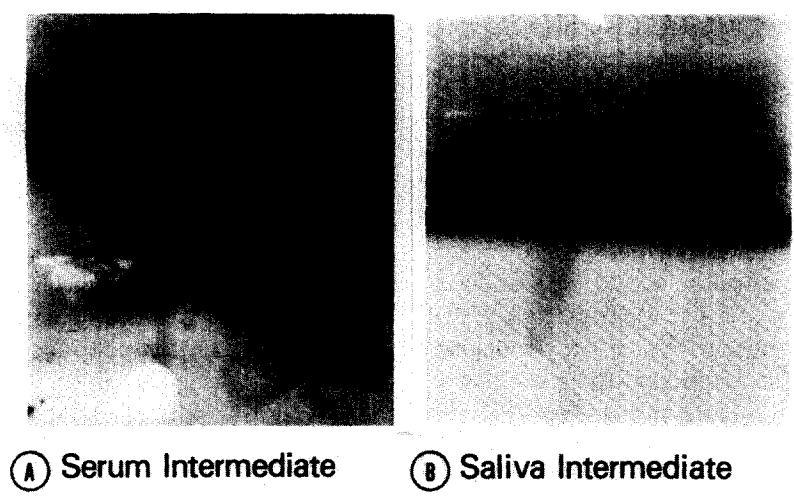


FIG. 3. CRIE patterns of bladder puncture urine and rabbit antimale cat urine antibody with intermediate gels containing cat serum in the *a* series of photographs and cat saliva in the *b* series.

DISCUSSION

It is generally accepted that allergen 1 is the most important cat allergen. Table I clearly indicates that cat pelt and saliva are good sources of this allergen, although the ratio of allergen 1 to protein is somewhat lower for cat saliva than for cat pelt. However, the ratio of allergen 1 to protein in the two pelt extracts is very similar, despite the fact that they were prepared in different establishments by use of different methods. Cat urine, however, is a more complicated product. Three samples of cat urine obtained by bladder puncture and one sample of voided urine from female cats did not contain detectable allergen 1. A sample of voided urine from male cats had detectable allergen 1 by RID and CIE/CRIE procedures. It has not been possible to determine if voided urine adventitiously contains allergen 1, if the allergen is secreted in the process of urination, or if the sex of the animal is a factor. Cat serum also had no detectable allergen 1; therefore, cat serum and bladder puncture urine were used to compare preparations without detectable cat allergen 1 to extracts of pelts and saliva that contained measurable allergen 1.

We have previously demonstrated with CIE and CRIE that, with one possible exception, cat saliva and cat pelt share common antigens and allergens.⁴ Table III indicates that analysis of the direct RAST results of the symptomatic patients produced a high rank correlation value of 0.77. If cat saliva and pelt share all of the same allergens, the rank correlation value would be 1. The failure of two individuals who were direct RAST positive to cat pelt to be RAST positive to cat saliva (No. 14 and No. 19) may have resulted from different ratios of allergen 1 to total protein in cat

TABLE III. Rank correlation values of direct RAST ratios of patients with cat allergy symptoms

	Serum	Saliva	Pelt
Urine	0.34	0.52	0.39
Serum	—	0.39	0.36
Saliva	—	—	0.77

Correlation coefficient $df = 43; p < 0.05 = 0.294$.

saliva and cat pelt or may have been a statistical phenomenon that resulted from the method used to define direct RAST positives. This same situation may have also resulted in the discovery of one saliva positive, pelt negative patient (No. 27). This one direct RAST result does not provide sufficient proof of the existence of a unique cat salivary allergen.

Of the tested individuals claiming to be allergic to cats, 39.5% were direct RAST positive to cat pelt, and 37.1% were direct RAST positive to saliva. Therefore, direct RAST analysis by use of criteria outlined earlier to establish a positive response detects fewer than 50% of the individuals in this study who claimed to have cat allergy. Only 12% of these cat-symptomatic patients were direct RAST positive to cat urine or serum. No patients were both cat serum and urine positive and cat pelt or saliva negative, so that it may be concluded that cat serum and urine contain only allergens that exist in cat saliva and pelt. Unfortunately, because of the length of time that has elapsed since the collection of these serum samples, it is no longer possible to reevaluate the severity of the symptoms in the patients tested or to examine how

individual or multiple direct RAST positives correlate with allergic symptoms experienced by these patients. The fairly high correlation of direct RAST positives to cat urine and saliva (0.52) supports the idea that cat saliva contains the urinary allergens as well as those of cat pelt. The inability of the direct RAST to detect significant serum IgE activity in patients with clinical allergy has been reported previously.¹¹

The CRIE patterns in Fig. 1 that were produced from the CIE pattern of cat pelt and anticat pelt that demonstrates eight antigens demonstrate the dramatically different patterns of allergenicity produced with the sera from two individuals. It should be noted that both individuals have IgE to allergen 1 by direct RAST and CRIE analyses. The effect of the difference in the IgE antibody population of individual patients will require further investigation to determine the relationship of such different antibody populations to severity of allergic symptoms.

The valuable information that may be obtained by use of multiple CIE/CRIE systems is displayed in Fig. 2. This particular patient shows six allergens to cat pelt and six to cat serum; at least one of the allergens in the cat serum CRIE is different, since cat serum does not contain allergen 1. Consequently, this patient has IgE antibody to at least seven allergens of the cat. Some or all of the three allergens demonstrated in cat urine may also be detected in cat pelt or cat serum, but again, this urine did not contain allergen 1.

Fig. 3 illustrates the CRIEs of the cat urine/anti-urine system when intermediate gels that contain either cat serum or saliva are introduced. The migration of one of the cat urinary allergens with the serum front demonstrated the presence of one common allergen that is most probably albumin. However, two of the cat urinary allergens are not found in serum, as evidenced by the fact that they remain in their original CIE position. When cat saliva is incorporated in an intermediate gel in the urine system, all peaks migrate with the cat saliva front, illustrating the presence of all urinary allergens in cat saliva. As a result of the failure of this preparation of rabbit anticat pelt anti-serum to recognize urinary allergens other than albumin, the incorporation of cat urine in an intermediate gel in the pelt CIE/CRIE system provided no additional information as to the presence of urinary allergens in either pelt extract used in this study.

CONCLUSION

The allergenic activity of cat pelt, saliva, urine, and serum and the correlation of direct RAST activity to cat urine and serum was demonstrated in patients reporting symptoms associated with allergy to cats. The

study also illustrates that, with these reagents, it is not possible to detect allergens in cat urine or serum that were not present in either cat pelt or saliva. The fact that only 39.5% of the symptomatic patients tested were direct RAST positive to cat pelt demonstrates need for additional study to determine the relationship between direct RAST, skin test reactivity, and severity of symptoms. It also indicates that, in order to prepare a satisfactory reagent for direct RAST, the extract used for coupling to the solid support should be demonstrated to contain other allergens as well as allergen 1. An extract without allergen 1 will still detect some RAST positive individuals, but the number would be very small.

The two extracts prepared from cat pelt by use of different methods were found to have similar analytic properties. This suggests that cat pelt protein content is not necessarily determined by the presence of adventitious quantities of serum or saliva and confirms earlier studies that the content of cat pelt and saliva allergens is very similar and perhaps identical.⁴

The presence of allergen 1 in voided urine from male cats strongly indicates that litter boxes kept in homes may be an important source of environmental allergen contamination. The failure to detect allergen 1 in voided urine from female cats presents an intriguing issue of practical importance and will require a wide survey of cat urine specimens to confirm the possible relationship of sex to allergen 1 content.

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Molecular forms of tear IgA and distribution of IgA subclasses in human lacrimal glands

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*Tears from subjects without conjunctival inflammation contained 93% polymeric IgA (pIgA) and of secretory IgA standard for tear samples and monomeric IgA standard for serum samples. Tears from subjects without conjunctival inflammation contained 93% polymeric IgA (pIgA) and 7% monomeric IgA; tears from subjects with conjunctival inflammation had less pIgA (79%). Biopsy specimens of 11 lacrimal glands and one accessory lacrimal gland were obtained from 12 additional subjects. Tissues were stained with polyclonal antisera for immunoglobulins, J chain, and secretory component. In addition, tissues were stained for IgA subclasses with monoclonal reagents specific for IgA1 and IgA2. IgA plasma cells predominated over other types of plasma cells, and most were J chain positive. Secretory component, although absent from the interstitium of the gland, was found in most acinar and ductal cells. The average proportion of IgA1 to IgA2 cells was 56% to 44%. We concluded that the lacrimal system of the human resembles other exocrine systems in the production of predominantly pIgA and the nearly equal occurrence of IgA1 and IgA2 plasma cells. (*J ALLERGY CLIN IMMUNOL* 76:569-76, 1985.)*

Tears exhibit properties of the secretory immune system fluids. IgA is the dominant immunoglobulin.¹ SC is present in tears.² A high proportion of the IgA occurs in the IgA2 subclass,³ and in pooled tears IgA is predominantly polymeric.^{3,4} The lacrimal gland has characteristics of the secretory immune tissue⁵: IgA-containing plasma cells are dominant⁵ and SC is localized in gland acini and ducts.^{5,6}

To characterize further the human ocular secretory

Abbreviations used

SC:	Secretory component
pIgA:	Polymeric IgA
RIA:	Radioimmunoassay
mIgA:	Monomeric IgA
S-IgA:	Secretory IgA
FITC:	Fluorescein isothiocyanate
TRITC:	Tetramethylrhodamine isothiocyanate

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immune system, we measured the levels of pIgA and mIgA in *individual* samples of tears from normal subjects and from patients with a variety of ocular disorders and determined the distribution of SC, J chain, and subclasses of IgA in lacrimal glands.

MATERIAL AND METHODS

Tears

Tears from 14 subjects were collected on Weck-Cel sponges (Edward Weck Co., Long Island, N. Y.) as de-