

Immunologic Studies of a Patient with Seminal Plasma Allergy¹

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Immunological studies of a patient with allergic reaction to seminal plasma are described. The reaction is IgE mediated. The antigen eliciting the reaction is present in all the seminal plasmas tested. It is a protein with a molecular weight of 20,000–30,000 daltons. The antigenic activity is not destroyed by enzymes which degrade carbohydrate groups but is destroyed by proteolytic digestion. The antigen is heat stable and probably prostatic in origin. Leukocytes from this patient release histamine on challenge with the seminal plasma antigen. This release is markedly enhanced by the presence of serum.

INTRODUCTION

Allergic reaction to human ejaculate is a rarely reported phenomenon (1–3). This report will present studies on the immunological nature of the reaction, the character of the antibody, and the nature and source of the antigen.

The case history of this 30-yr-old female has been reported in detail previously (2). She was essentially healthy except for ragweed hayfever of 15-yr duration. At the age of 28 she developed an anaphylactic type reaction within minutes after intercourse. The symptoms included generalized hives, sneezing, and periorbital swelling; these episodes persisted thereafter. Immunological studies revealed positive skin tests with seminal plasma at dilutions of 1:10⁷; the antigen was present in all 15 seminal plasmas tested but was absent in normal male serum. Fractionation of seminal plasma showed that the antigen has a molecular size of 20,000–30,000 daltons and was active in eliciting a skin reaction at a concentration of 2.5×10^{-4} μ g/ml. *In vitro* studies demonstrated histamine release from the blood of the patient on challenge with antigen.

Investigations reported here demonstrate that the reaction is due to IgE antibody, that the seminal plasma antigen(s) is destroyed by proteolytic enzymes but not by enzymes that digest carbohydrate groups, and the histamine-release reaction from basophils has a unique requirement for serum.

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MATERIALS AND METHODS

Materials. Rabbit anti-human IgE, Fc specific, was kindly provided by Dr. K. Ishizaka and contained 710 μg AbN per ml. Mixed ragweed allergen was obtained from a commercial source. Human serum albumin was from Behring Diagnostics, Hoechst, Somerville, NJ.

Buffers

A Tris buffer was used in the histamine-release studies. It contained NaCl 120 mM, KCl 5 mM, and Tris buffer (pH 7.4) 25 mM. Tris A was prepared by adding human serum albumin to the buffer at a final concentration of 0.3 mg/ml. The reaction mixtures for histamine release contained $1 \times 10^{-3}M$ Ca^{2+} and $1 \times 10^{-3}M$ Mg^{2+} .

Allergens

Seminal plasma. Ejaculates were obtained from healthy men with functioning vasectomies. The ejaculate supernatants were found to give strong intradermal wheal-and-flare reactions at dilutions of $1:10^4$ and weak reactions at $1:10^7$. A pool of seminal plasma was made, centrifuged, and fractionated by molecular sieving on Sephadex G-200. Repeated fractionation revealed that all the allergenic activity as assayed by histamine release and skin test was recovered from the column. About 90% of the activity was in fraction 4 corresponding to a molecular weight of 20,000–30,000 daltons. Fraction 4 contained approximately 250 μg per ml of protein. This fraction was used in all the studies being reported in this paper.

Prostate extract. Five grams of prostatic tissue were obtained from a surgical specimen at suprapubic prostatectomy (microscopic diagnosis of benign prostatic hypertrophy). It was washed thoroughly, then minced and extracted with Tris-buffered saline. The mixture was centrifuged and the supernatant was sterilized by Millipore filtration.

Methods

Protein determinations were done with a Beckman D.U. Spectrophotometer equipped with a Gilford read-out attachment. Calculations were based on the absorptions at 260 and 280 nm with the use of the equation proposed by Layne (4).

Enzymatic digestion. The fraction 4 of seminal plasma described above was subjected to enzymatic digestion. Duplicate tubes containing approximately 200 μg fraction 4 protein in 2 ml in the appropriate buffer were incubated for 16 hr at 37°C unless otherwise indicated. The amount of enzyme added was from 1–5% by weight in all cases. The details of the enzyme treatments are in Table 1. Control tubes consisted of fraction 4 incubated without enzymes but with the added buffers and compounds.

Histamine release from whole blood. Different concentrations of the antigen fractions to be tested were added in a volume of 0.5 ml to 12×75 -mm plastic tubes in an ice bath. Heparinized blood (heparin 50 unit/ml final concentration) was added to the tubes in a volume of 0.5 ml after a dilution of 20–40%. Control tubes received buffer and blood instead of antigen. In each experiment

TABLE I
CONDITIONS FOR THE ENZYMATIC DIGESTION OF SEMINAL PLASMA ANTIGEN

Tube no.	Enzyme	Buffer	Other additions
1	Neuraminidase (<i>Cl. perfringens</i>)	0.1 M Acetate pH 5.0	None
2	Trypsin	1 M Tris pH 8.0	100 λ 0.1 M Ca ₂ Cl
3	Lysozyme	0.1 M Acetate pH 5.0	None
4	Pronase	0.1 M PO ₄ pH 7.0	None
5	Chymotrypsin	0.1 M Tris pH 8.0	100 λ 0.1 M Ca ₂ Cl
6	β -Glucosidase	0.1 M Acetate pH 5.0	None
7	Papain ^a	0.1 M PO ₄ pH 7.0	100 λ each of 0.01 M EDTA, 0.1 M 2-ME, ^b 0.1 M Cysteine

^a Incubated at 25°C.

^b 2-ME = Mercaptoethanol.

Fraction 4 at a concentration of 200 μ g protein in 2 ml of the appropriate buffer was incubated for 16 hr at 37°C unless otherwise indicated. The amount of enzymes added was 1–5% by weight. At the end of 16-hr incubation a trace precipitate was noted in tubes 3, 4, 6, and 7, the other tubes were clear.

perchloric acid at a final concentration of 3% was added to some tubes to obtain the total histamine content. At the end of 60 min the reaction was stopped by transferring the tubes to an ice bath followed by centrifugation at 2500 rpm (1100g) for 20 min at 4°C. The supernatants were assayed for histamine content.

Histamine release from washed leukocytes. Thirty to thirty-five milliliters of venous blood were drawn into plastic syringes containing EDTA (0.01 M final concentration). After sedimentation the leukocytes were isolated and washed by previously described techniques (5).

Histamine assay. Histamine was assayed by a recently developed automated fluorometric technique (6). The method allows the analysis of small sample volumes (0.4 ml) with great sensitivity and precision.

Calculations. Percentage of histamine release was calculated using the formula: % release = $100(E-B/C-B)$, where C is the histamine content of the tubes with perchloric acid added (completes); B is the histamine in the supernatants lacking antigen (blank), and E is the histamine in the supernatants of the experimental tubes. Blanks were consistently less than 5% of the completes.

RESULTS

Studies of the nature of the antigen. Previous studies have indicated that the antigen to which the patient is reactive is present in seminal plasma. The present investigations showed that the antigen was present in the prostate; skin tests with the prostate extract gave a strong wheal-erythema reaction (12-mm wheal with pseudopodia) at a dilution of 1:10⁴ and a mild reaction at 1:10⁶. Other investigations were conducted with fraction 4 from the Sephadex G-200 fractiona-

tion. Studies revealed that this antigen was essentially heat stable with no loss of activity after boiling for 60 min.

The nature of the antigen was further investigated by studying the effect of various enzymes on its activity. The ability of the enzyme-treated allergen to release histamine was tested utilizing dilution curves of the enzyme-treated fraction 4 (Fig. 1). In these experiments, it is clear that pronase, trypsin, and chymotrypsin markedly reduced the allergenic activity of the fraction, whereas papain was less active. In contrast, there was little change in the activity of the fraction when it was treated with carbohydrate-destroying enzymes. The enzymes tested alone at the same concentrations did not release any histamine. A summary of these results is given in Table 2; neuraminidase, lysozyme, and β -glucosidase have little or no ability to destroy the antigenic activity.

Histamine-release studies. Heparinized whole blood was used for histamine release on numerous occasions. There was 26–88% release with dilutions of whole seminal plasma, or with the isolated fractions. The highest release was 88% which was observed 3 wk after an anaphylactic reaction.

Early in these studies it was noted that, whereas whole blood was active in releasing histamine, there was little release from washed leukocytes (Table 3). The 88% release from the whole blood with the seminal plasma fraction 4 antigen dropped to 12% when the antigen was added to washed cells. The addition of 10% of the patient's plasma to the reaction mixture markedly enhanced the release. Decreased histamine release was also observed when whole seminal plasma was used as antigen in the absence of added serum or plasma. In

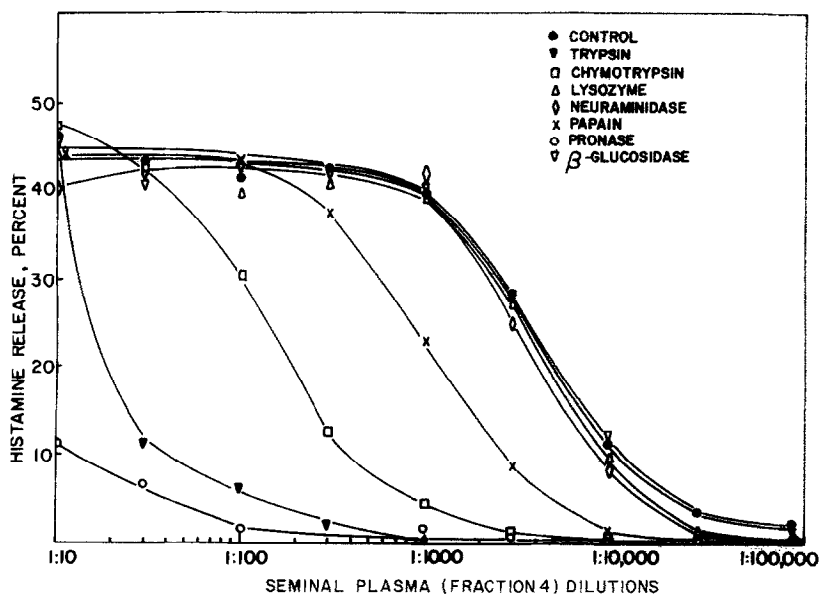


FIG. 1. Histamine release with enzyme-treated seminal plasma antigen. Seminal plasma fraction 4 was treated with the different enzymes as indicated in Table 1. Dilutions of each tube were then tested for histamine release from whole blood. Control consisted of antigen incubated in the absence of enzymes. Enzymes at the same concentrations did not release histamine. Reaction mixture contained 2.1×10^6 leukocytes/ml, total histamine 37 ng/ml.

TABLE 2
THE EFFECT OF DIFFERENT ENZYMES ON THE ALLERGENIC ACTIVITY
OF SEMINAL PLASMA FRACTION 4

Enzyme	Loss of activity ^a
Pronase	100
Trypsin	99.5
Chymotrypsin	96.0
Papain	70
Neuraminidase	16
Lysozyme	9
β -Glucosidase	4

^a Seminal plasma fraction 4 was treated with the different enzymes; control tubes received the same treatment but no enzyme. The activity of all samples was determined by measuring the histamine release from leukocytes induced by increasing dilutions of the treated antigens.

contrast, the effect of serum was much less pronounced on the histamine release induced by ragweed antigen to which the patient was also allergic. Other studies indicated that serum from normal donors had the same enhancing effect on histamine release (7).

TABLE 3
HISTAMINE RELEASE FROM THE LEUKOCYTES OF A PATIENT
WITH SEMINAL PLASMA ALLERGY

Allergen	Concentrations	Percent histamine release		
		From blood	From washed WBC	Washed WBC 10% plasma ^a
1 Seminal plasma G.200 fraction 4	(μ g/ml)			
	0.013	2	0	4
	0.04	14	6	22
	0.13	61	7	38
	0.42	76	12	58
	1.25	88	12	64
2 Mixed ragweed	(PNU/ml) ^b			
	0.07	2	2	9
	0.2	17	9	23
	0.7	51	57	70
	2.0	61	69	80
	7.0	57	56	78
	20.0	40	46	80
Leukocyte concentration/ml		2.3×10^6	0.9×10^6	0.8×10^6
Histamine content ng/ml		28.5	19.4	18.0

Reaction time was 60 min at 37°C.

^a Washed leukocytes were incubated with 10% autologous plasma for 10 min before the addition of antigen.

^b PNU = Protein nitrogen units.

TABLE 4
ABSORPTION OF P-K ACTIVITY WITH ANTI-IgE SERUM^a

Patient's serum concentration	Seminal plasma sites					Ragweed sites				
	Control serum		Serum treated with anti-IgE ^b			Control serum		Serum treated with anti-IgE ^b		
	W ^c	E ^c	W ^c	E ^c	W ^c	W ^c	E ^c	W ^c	E ^c	W ^c
	(mm)		(mm)			(mm)		(mm)		
Undiluted	5	10	0	0	0	8	30	0	0	0
1:3	3	6	0	0	0	5	10	0	0	0
1:10	0	3	0	0	0	3	8	0	0	0

^a Serum (or dilution) 0.1 ml was injected at each site; antigen was injected at each site 72 hr later. The G-200 fraction 4 (1/250 dilution) of seminal plasma antigen and mixed ragweed (1000 PNU/ml) were used. The reactivity of skin sites which had received the anti-IgE-treated serum 72 hr earlier was demonstrated by challenge with anti-IgE at the time of skin testing. Sites were reactive by wheal-erythema reactions indicating that the lack of reactions was not due to depletion of skin histamine.

^b Serum treated with anti-human IgE (71 μ g AbN/ml) 30 min at 37°C and 18 hr at 4°C. Control serum was diluted with buffer and treated similarly.

^c W = Wheal, E = Erythema.

Heparinized whole blood from 20 different donors including normals and individuals with allergic diseases was used to test for histamine release with seminal plasma fraction 4. There was no significant histamine release from the blood of any of these individuals.

Nature of the antibody. Attempts to sensitize leukocytes from normal donors with the patient's serum were unsuccessful. An inherent control in these experiments was the fact that the patient also has ragweed hayfever. Normal leukocytes were sensitized in a reaction mixture containing 20% of the patient's serum, EDTA, and heparin (8). There was 52% histamine release on the addition of ragweed antigen but less than 2% when seminal plasma antigen was used in the presence of 10% plasma.

Prausnitz-Kustner (P.K.) tests were then performed on the patient's husband. The results are shown in Table 4. At 72 hr after transfer of the patient's serum the skin sites were challenged with either ragweed or seminal plasma antigen. The serum titer was 1/3 for seminal plasma and 1/10 for ragweed. The activity was lost by prior treatment of the serum with anti-human IgE.

DISCUSSION

Studies of the antigen responsible for this allergic reaction have shown that it is ubiquitous in seminal plasma. It is not associated with the spermatozoa and has a molecular weight of 20,000 to 30,000 daltons (2). The present experiments demonstrated that it is present in prostatic tissue obtained surgically (from a patient with benign prostatic hypertrophy). Human prostatic fluid contains specific antigens not present in the circulation and is a mixture of heterogeneous macromolecules including enzymes (9,10). The major protein in prostatic fluid was found by ultracentrifugation to be 3.3–3.8S (9). Comparing the previous antigenic studies of prostatic tissue with the present results does not clearly in-

dicating which of the prostatic proteins is the one responsible for the allergic reaction.

The seminal plasma fraction with the allergic activity was treated with different enzymes (Fig. 1 and Tables 1, 2). There was complete loss of histamine-releasing activity when it was treated with proteolytic enzymes. However, incubation with carbohydrate-digesting enzymes caused little loss in activity. The results indicate that the antigen is a protein and that carbohydrate residues (digestible with these enzymes) are not important antigenic determinants in this reaction.

Histamine release from the leukocytes of this patient could be demonstrated with the seminal plasma antigens. Histamine release was not observed when blood from 20 different individuals was tested with seminal plasma fraction 4. Histamine release was a very useful tool for studies on the purification of the antigen and its modification by enzymatic treatment. However, there was little histamine release from washed leukocytes with the seminal plasma antigens except in the presence of serum (Table 3). This effect was observed only for the seminal plasma antigen and not for ragweed-induced histamine release. Serum from normal donors was equally effective and this phenomenon was not observed in histamine release studies of a second patient with seminal plasma allergy (7).

The antibody which mediates this seminal plasma reaction appears to be IgE. This was demonstrated by P. K. testing. The activity could be removed by prior treatment of this patient's serum with anti-human IgE (Table 4). The concentration of antibody present in the serum, however, appears to be low since the titer at which the serum was positive was only 1:3. Total serum IgE was 140 and 190 units/ml in two different radioimmunoassay determinations. The ultimate proof for the role of IgE in this allergic syndrome would be the passive transfer of the antibody to a normal donor. The appearance of the same symptoms in the normal recipient would demonstrate the role of this antibody. Such a test is clearly not feasible in this case.

All the reported cases of allergic reactions after intercourse seem quite similar. The antigen appears in the seminal plasma, the antibody is IgE, and the individuals have the same history of atopy. The symptoms are those of generalized urticaria, periorbital swelling, and abdominal cramps. Asthma is reported in some of the patients (1-3).

As the antigen which causes this allergic reaction appears to be ubiquitous, it is not clear why this disease is so rare. It would be difficult to attribute its rarity to a lack of its recognition. The temporal occurrence of the symptom within minutes of sexual intercourse would immediately suggest the diagnosis. Therefore, it can be assumed that the disease is not underreported but truly a rare event. Probably a number of factors have to coexist for the individual to become allergic to seminal plasma. These might include: the genetic predisposition of the individual to atopic disease and the rate of exposure to the allergen. The rate at which an individual is exposed to allergen is probably determined by several factors including local factors which affect the systemic absorption of the antigen. Perhaps all these different factors must coexist for an individual to become allergic to seminal plasma.

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