

Allergy to human seminal fluid: Characterization of the allergen and experience with immunotherapy

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A 20-year-old woman was observed with a history of a severe generalized systemic reaction after topical contact with seminal fluid. A prick test with undiluted seminal fluid produced a 5.0 mm wheal-and-flare response with pseudopods. Prick tests with saliva and serum from the same source as the seminal fluid were negative. Measurement of IgE antibody to seminal-fluid allergen with a Biotin-Avidin ELISA technique yielded strong activity. No IgG antibody could be detected. Significant prick test reactivity could be found in Sephadex G-100 fractions that had a molecular weight range of 12,000 to 75,000 daltons and that contained approximately 5% of the total protein in the starting material. Isoelectric focusing fractions with strong skin test reactivity had a pI range of 5.4 to 6.6. These fractions contained one major protein band. Immunotherapy was conducted with a Sephadex fraction of seminal fluid during a 24-month period. A cumulative dose of 32 mg of protein was administered. No side effects other than local swelling occurred. Ten months after the start of immunotherapy, IgE antibody became unmeasurable, an effect that was demonstrated not due to the inhibitory effect of IgG antibody. IgG antibody rose progressively in this period. Clinically, the patient became less sensitive to topical contact. Although the natural history of seminal-fluid allergy is not known, immunotherapy may be effective. (J ALLERGY CLIN IMMUNOL 1990;85:103-7.)

Immediate hypersensitivity reactions to human seminal fluid in women have been increasingly recognized since the first article published in English in 1967.¹ These reactions range from local swelling^{2, 3} to generalized systemic reactions.^{3, 4, 6} There is strong evidence that these reactions are IgE mediated. Seminal fluid has been demonstrated to produce a typical wheal-and-flare response in clinically sensitive individuals,^{1, 2, 5, 7-11} as well as measurable IgE antibody.^{3, 4, 6, 12} It is not known whether a single component was involved in the reported cases or whether multiple allergenic components were involved. There is some similarity in the molecular weight estimates of seminal-fluid allergen in different studies, as discussed below. The evidence to date would suggest

Abbreviation used

IEF: Isoelectric focusing

that the allergen is derived from the seminal vesicle or prostate¹³ and that the allergen is not widely distributed in male-derived fluids. Vasectomy did not affect the allergenicity of seminal fluid.^{13, 14} The allergen appears to be present in multiple samples of seminal fluid from different sources.^{1, 10, 13} Male saliva and serum were not allergenic in several studies by passive transfer and direct skin test,^{5, 10} although slight skin test reactivity to male serum was reported in one study.⁴

There have been five published articles of attempts to reduce allergic sensitivity by immunotherapy with whole seminal fluid^{1, 4, 15} or with a fraction of seminal fluid^{14, 16} with various protocols. In two of the studies, immunotherapy for 1 to 2 years failed to produce convincing clinical benefit.^{1, 4} In the other three studies, there was believed to be some evidence of efficacy.¹⁴⁻¹⁶

We relate an additional case of IgE-mediated seminal-fluid allergy. The allergen involved was partially characterized, and a course of immunotherapy

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Supported by the Veterans Administration Medical Research Service. Received for publication Nov. 1, 1988.

Revised March 20, 1989.

Accepted for publication March 22, 1989.

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1/1/17122

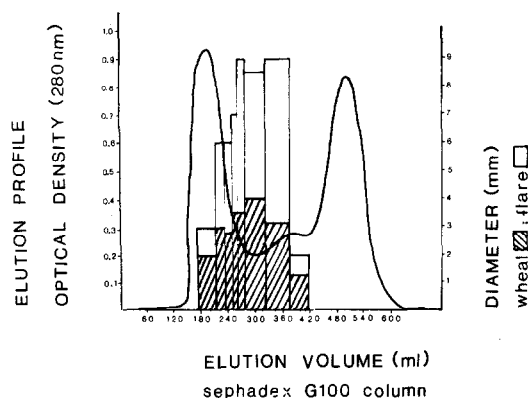


Fig. 1. Five milliliters of seminal fluid was fractionated on a Sephadex G-100 column. The elution profile at 280 nm optical density is indicated by the solid line. Fractions were pooled as indicated by bars, and each fraction was concentrated to 5 mL. Prick tests were conducted with each of the fractions, and results are reported as wheal (striped bars) and flare (open bars) measured after 15 minutes.

was performed with a low-molecular-weight fraction. A reduction in IgE antibody and an increase in IgG antibody was associated with evidence of decrease in clinical sensitivity.

Although this study does not provide conclusive proof of the efficacy of this form of therapy, it is of significance because this form of allergy is rare and perhaps often overlooked. It presents the treating physician with unusual difficulties. Because the properties of the allergen and the response to immunotherapy are better defined in this study than in previous studies, this experience should provide valuable guidelines for similar treatment attempts in the future.

METHODS

Seminal-fluid collections

Seminal fluid was collected exclusively by condom from the individual to whom the patient was exposed. Samples were immediately frozen and kept frozen until further processing.

Gel filtration

Sephadex G-100 was prepared in phosphate-buffered saline (0.01 mol/L) and poured into a 2.5 by 61 cm column that was then calibrated with blue dextran, α -chymotrypsinogen, cytochrome c, and tyrosine. A calibration curve was prepared as previously described.¹⁷ Crude seminal fluid was spun for 2 minutes at 11,000 rpm, and 5 mL of supernatant was fractionated on a Sephadex G-100 column.

IEF

A 2 mm thick agarose IEF gel, pH range 3.5 to 10, was prepared with 2% ampholytes, 10% sorbital, and 1% aga-

rose EF (LKB Instruments, Bromma, Sweden). Samples of crude and Sephadex G100-fractionated seminal fluid were applied directly to the surface of the gel, and flatbed IEF was carried out for 1.25 hr at 4° C with constant power with a Multiphor apparatus (LKB). On completion of IEF, gel tracts were either fixed and stained as per manufacturers instructions or sliced and eluted in distilled water overnight at 4° C. The pH gradient was read from the solutions of eluted slices.

ELISA

The ELISA was performed with a standard protocol, as previously described.¹⁸ Briefly, microtiter plates were coated with 10 μ g per well of Sephadex G-100-fractionated seminal fluid followed by patient serum collected at intervals during immunotherapy or with nonallergic sera. Biotin-labeled antihuman IgG and IgE was added followed by Avidin-horseradish peroxidase and substrate. The reaction was terminated by the addition of 2 N HCl and read at 492 nm with a microtiter-plate reader (Titertek Multiscan, Flow Laboratories, McLean, Va.). Intensity of color is directly proportional to bound IgG or IgE. The seminal fluid and serum samples were stored frozen until immediately before conducting the assays. These assays were all performed after the collection of the final serum sample, thus testing all of the serum samples under identical conditions.

Skin testing

Prick tests were performed as previously described¹⁷ with crude seminal fluid, Sephadex G-100 fractions of seminal fluid, and eluted IEF fractions of seminal fluid. The average diameters of the wheal and flare in millimeters were noted after 15 minutes.

Intracutaneous tests were performed with 0.03 mL of solution used for immunotherapy. After 12 minutes the reactions were graded on a scale of 1+ to 4+.

All specimens for skin tests were freeze-dried in several aliquots and reconstituted in buffered saline diluent with 0.4% phenol (Allergy Laboratories of Ohio, Columbus, Ohio) immediately before testing.

Immunotherapy protocol

Immunotherapy was conducted with a Sephadex G-100 fraction as described in RESULTS. An initial subcutaneous injection of 0.05 mL of a 10 μ g/mL solution, as determined by 280 nm optical density comparison with bovine serum albumin standard,¹⁷ was administered. Doses were continued initially at weekly intervals with the same or increasing amounts of the allergen as tolerated by the patient. Serum samples were collected at intervals as indicated in the RESULTS section.

Case report

A 20-year-old woman was first observed with a history of severe generalized periorbital angioedema and pruritic rash immediately after topical contact with seminal fluid not related to intercourse. This was initially treated with a course of systemic corticosteroids that required an emergency room

visit for the administration of adrenalin. Her past history was positive for mild intermittent flexural eczema and mild rhinitis. A previous evaluation by an allergist reportedly produced positive skin test reactions to dog, cat, and house dust, but no reactions to pollen or mold. The patient had never experienced vaginal intercourse. At the time of our initial evaluation, 2 months after her allergic reaction, a prick test with undiluted seminal fluid yielded a 5.0 mm wheal-and-flare reaction with pseudopods. Undiluted serum and saliva from her male partner produced negative prick test reactions.

Nine months later, after a series of immunochemical studies were performed, the patient was reevaluated. Skin testing with the undiluted seminal fluid yielded a 6.0 mm reaction with pseudopods. A decision was made at the time to initiate immunotherapy with a fraction of seminal fluid from her partner. This decision was made after the patient exhibited considerable anxiety about the dangers of intercourse. The possible risks and benefits were discussed with the patient.

RESULTS

Gel filtration of seminal fluid

Five milliliters of seminal fluid was applied to a Sephadex G-100 column, as described in METHODS. The results of one such fractionation are illustrated in Fig. 1. Pools were collected and concentrated to the volume of the original sample. Prick tests were performed with each pool. As indicated, the bulk of the allergenic activity was obtained at elution volumes of 200 to 360 ml containing protein with a molecular weight range of 12,000 to 75,000 daltons. The active fraction was collected and prepared for use in immunotherapy.

IEF

Fifty micrograms of a protein obtained by Sephadex gel filtration, as indicated above, was applied to gel tracts and subjected to IEF. Fractions were eluted and prepared for skin testing as described in METHODS. The results are summarized in Fig. 2. Skin test reactivity was observed in fractions with a pI range of 5.4 to 6.6. An optical scan of the stained gel revealed three peaks in this range with a central predominant peak having a pI of approximately 6.2. Skin test reactivity was most intense with the fraction that contained this peak.

Immunotherapy and clinical response

The patient was started with immunotherapy at a concentration of 10 μ g of protein fraction per milliliter. During the course of 24 months, the patient received a cumulative dose of 32 mg of protein. The only side effect experienced was local swelling at the injection site that peaked at approximately 36 hours and, at its maximum, was approximately 3 inches in

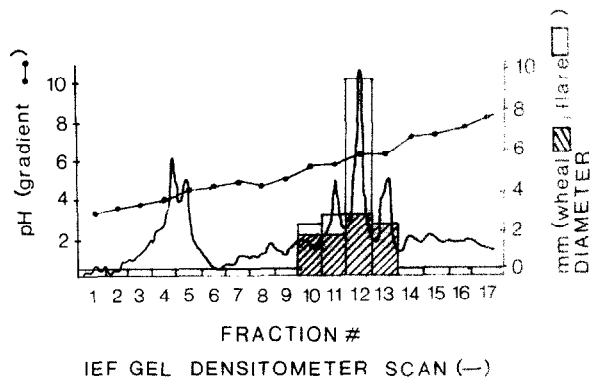


Fig. 2. IEF was carried out on Sephadex G-100 fractionated seminal fluid. A densitometer scan of the stained gel is illustrated by the solid line. Fractions of a parallel unstained gel tract were excised and eluted for prick testing. The pH gradient measured from eluted slices is illustrated by the solid line with closed circles. Prick test responses were measured after 15 minutes and reported as wheal (striped bars) and flare (open bars) diameters.

diameter. Serum specimens were collected at intervals and frozen for subsequent testing for IgG and IgE antibody.

Immunotherapy has now been conducted during a 24-month period. Although intercourse has not been attempted without the use of a condom, the patient has had a number of deliberate topical contacts with seminal fluid. At first this was associated with much less local reaction than what had been experienced before immunotherapy. More recently, no reactions were reported. No systemic reactions occurred on topical contact.

Intracutaneous skin testing with the treatment preparation was performed at the start of treatment and at 1, 3, 5, 12, and 18 months of therapy. All sites revealed 4+ reactions at all dilutions down to 10 μ g of protein per milliliter with local pruritus at the skin test sites. At 18 months, although similar visible reactions were present, the patient indicated that she had no sensation of pruritus. Control tests with saline diluent were consistently negative.

IgG and IgE antibody responses to immunotherapy

The use of crude seminal fluid for coating the wells of the microtiter plates did not result in significant IgE antibody binding, probably because the presence of large amounts of nonallergenic protein in the crude material. Therefore, the purified fraction was used. In the baseline blood sample, values were consistently three times higher than values obtained with control sera. The results of sequential measurement of IgE and IgG antibody in the two baseline samples and four

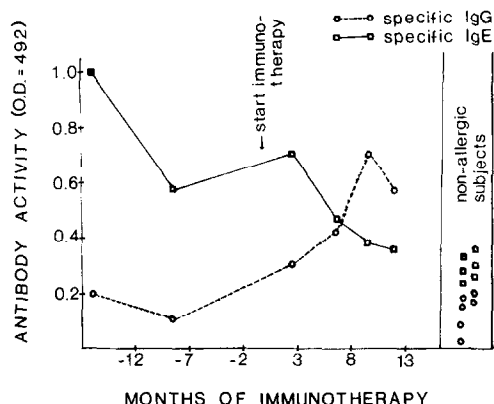


Fig. 3. Specific IgE (open squares) and IgG (open circles) antibody to seminal fluid was measured in serum samples from the patient before and during immunotherapy with ELISA. Control sera from nonallergic subjects were also measured.

samples during the course of immunotherapy are illustrated in Fig. 3. The IgE and IgG assays were all performed on the same day, and serum samples were thawed immediately before the assay. The results were confirmed on another day with the same set of serum samples, and no apparent diminution of activity occurred on freezing and thawing a second time. The second baseline measurement of IgE performed 6 months after the first was somewhat lower than the first measurement. During immunotherapy, there was a drop in the IgE values; measurements of the last two samples, obtained at 9 and 12 months, did not differ from the values obtained with nonallergic control sera. IgG antibody, which did not differ from that in control sera at the two baseline intervals, demonstrated a progressive increase at 2, 5, and 9 months, with a slight drop at 12 months.

Because high levels of IgG antibody can inhibit the binding of IgE antibody to allergen in solid-phase assays, we conducted a series of tests to determine if this was the cause of the decreased IgE antibody after immunotherapy. An IgG fraction prepared from a postimmunotherapy patient's serum by diethylaminoethyl cellulose ion exchange chromatography¹⁹ was concentrated to the volume of the serum initially applied to the column. Dilutions of this fraction were then added in equal volume to the initial patient's serum in which IgE could readily be measured. No inhibitory effect was observed when ELISA values were compared to a control in which saline or normal human gamma globulin was added. This was considered strong evidence that the IgE decrease observed was not artifactual.

DISCUSSION

This study represents another case of human allergy to seminal fluid. We do not know the frequency for this type of allergy. To our knowledge, the first case report in English was published in 1967. Since that time, a number of articles have dealt with this problem, suggesting that an awareness of the problem increases recognition and frequency of diagnosis. The nature of this kind of allergy would make it logical to suspect that it is frequently not recognized, especially when it causes only a bothersome local reaction^{2,3} and not a life threatening, systemic reaction.

The occurrence of anaphylaxis to an antigen of human origin is of biologic interest. IgE-mediated reactions are subject to genetic influences and are readily suppressed by cellular factors, the feedback effects of antibody, and by excessive immunizing doses. Allergy to seminal fluid would suggest the presence of a strong antigen (probably derived from tissue of the seminal vesicle or prostate) that is "foreign" to the allergic individual.

There are some similarities in the physicochemical properties of seminal-fluid allergen that have been reported in the literature. In this study, the allergen appeared to have a molecular weight of 12,000 to 75,000 daltons, which is in general agreement with a number of other studies^{6, 10, 13, 20} and slightly higher than that observed in several other studies.⁷⁻⁹ The pI of allergenic activity that we observed (5.4 to 6.6) is lower than pI estimates in several other studies.^{7, 20} Whether these studies deal with a single allergen in polymorphic form or a number of different antigens cannot be determined until the immunologic properties of the allergen(s) involved are better defined.

As with most kinds of anaphylaxis, avoidance is the best treatment. The use of condoms would reduce or eliminate exposure during intercourse. The occurrence of severe anaphylaxis after topical contact not related to intercourse in our patient of our study suggests that smaller degrees of contact may be significant.

In our patient, immunotherapy was undertaken only after demonstration of persistent sensitivity during a 9-month period. We had communication with two other investigators who had had apparent success with this kind of therapy. The use of a purified fraction enabled us to remove the bulk of the nonallergenic protein from the sample of seminal fluid. Twelve months of immunotherapy resulted in the reduction of serum IgE to unmeasurable levels and a progressive increase in IgG antibody from unmeasurable levels. Qualitative skin test reactivity did not change mark-

edly, although the patient reported clinically less sensitivity on topical contact.

The results of one case history can in no way be construed as evidence for the efficacy of this kind of therapy. However, because of the rarity of this kind of allergy, we believe that the experience of even one case history is worth reporting. This is the only case report that combines a characterization of the allergen with a careful analysis of IgG and IgE antibody responses. The antibody changes that we observed are associated with clinical benefit in more defined kinds of immunotherapy.²¹ Future attempts at immunotherapy in seminal-fluid allergy can be rationally guided by the methodology and results presented in this article.

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