

Successful Long-term Immunotherapy for Human Seminal Plasma Anaphylaxis

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● Anaphylactic sensitivity to her husband's seminal fluid occurred in a 46-year-old woman six weeks after a hysterectomy. Her husband was azoospermic as a result of an elective vasectomy. A pooled sample of his seminal fluid was used for skin and laboratory tests. Fractionating (Sephadex G100) chromatography was also performed on his seminal fluid. The patient showed positive intracutaneous skin tests to his unfractionated seminal plasma and a Sephadex G100 fraction designated No. 3. This fraction was also passively transferable to a nonallergic volunteer and it stimulated histamine release from the patient's leukocytes. The dose-response effects of this fraction compared with unfractionated seminal plasma in these assays showed greater bioactivity. After informed consent was obtained, the patient was immunized to fraction 3 in a classic desensitization protocol. Two weeks after the final dose of immunotherapy, intercourse was performed without condoms in a medical facility and under the supervision of a physician. No adverse symptoms were experienced by the patient on this occasion. Regular and frequent sexual activity is required to maintain her tolerant state.

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ALLERGIC reactions to seminal fluid have been recognized and documented by independent investigators on a worldwide basis.^{1,3} Systemic symptoms often progress to life-threatening anaphylaxis caused by classic IgE-mediated pathophysiological mechanisms. A comprehensive immunologic evaluation of four women with adverse reactions to seminal fluid confirmed both the specificity and diversity of these reactions.³ Evidence of in vitro cell-mediated reactivity without concomitant humoral antibody was obtained in two patients with severe localized symptoms occurring after intercourse. In contrast, two patients who were initially seen with anaphylactic symptoms exhibited reaginic antibodies specific for allergens in pooled and single specimens of human seminal plasma. Moreover, similar to previous reports,

it was also determined that the sensitizing principle(s) was present in partially purified fractions of human seminal plasma obtained by gel exclusion chromatography.^{2,4} One of these patients also manifested specific reaginic activity to sonicated extracts of her husband's sperm. Also, two of these couples showed an unusual degree of shared histocompatibility locus antigens.

This report concerns another woman who experienced seminal plasma-induced anaphylaxis with distinctive features. Severe systemic symptoms were first observed six weeks after a recent hysterectomy. The patient's husband had undergone a vasectomy without complications, and azoospermia of his seminal fluid was documented by a microscopic examination at the time his wife experienced symptoms. This couple, therefore, provided a unique opportunity of evaluating the anaphylactogenic potential of sperm-free seminal fluid. When this had been confirmed and further localized to one of the purified protein components of her husband's seminal plasma, immunother-

apy was suggested as a therapeutic alternative. After giving informed consent, the patient received specific immunotherapy, which either abolished or markedly attenuated systemic symptoms occurring after intercourse.

Clinical History

A 46-year-old woman was referred for evaluation of anaphylactic symptoms that were temporally associated with intercourse. She had been in good health until two years ago, when she began to experience episodes of dry cough after intercourse with her husband. Six months later, she required a hysterectomy for uterine fibroids. Sexual intercourse was resumed six weeks after surgery. Thirty minutes after ejaculation on this occasion, rhinorrhea, cough, and dyspnea developed. Repeated episodes of intercourse were associated with progressive increase of respiratory symptoms and generalized pruritus, eventually requiring medical intervention with epinephrine hydrochloride and other bronchodilators. She had never experienced pruritus of the vagina or perivaginal skin. The use of condoms during intercourse became mandatory, and the patient no longer experienced adverse symptoms. Apart from an elective vasectomy performed on the husband eight years ago, findings from complete medical, gynecologic, and urologic examinations of the couple were normal. Review of the patient's medical history showed a family and personal atopic background. She described symptoms of allergic rhinitis occurring regularly during the tree pollination season.

Materials and Methods

Five daily ejaculates of the husband were collected, allowed to liquify, and

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Table 1.—Intracutaneous Threshold Skin-Test Responses* of Unfractionated Human Seminal Plasma and Sephadex G100 Fractions

	Threshold Concentration, mg/mL	Skin Test Score†
Human seminal plasma	1.0	4+
Sephadex G100 fraction		
1	0.49	0
2	0.195	0
3	0.32	2+
4	0.22	0
5	0.05	0
Tree mixture	1.0	3+

*A positive skin-test threshold response in this system is defined as that skin-test concentration eliciting a 2+ or greater skin-test score when the tenfold weaker dilution of the same antigen causes a skin-test score of less than 2+.

†Skin-test scores: 2+ indicates a wheal 3 times the size of control; 3+ indicates a wheal 4 times the size of control; and 4+ indicates a wheal 5 times the size of control or any wheal with pseudopodia.

pooled. Each specimen was examined microscopically for spermatozoa and none were found. The azoospermic pooled sample of seminal fluid was diluted with an equal volume of 0.05M phosphate-buffered saline (PBS) (pH 7.4) and centrifuged at 4 °C for 30 minutes at 1,000 rpm. The particulate-free supernatant sample was used either as a fresh testing reagent on the same day or stored at -70 °C for subsequent in vitro tests.

Separative chromatography was performed as previously reported.⁴ Briefly, the sample was applied to a fractionating (Sephadex G100) column, elution was performed with 0.05 M of phosphate buffer, and 5-mL fractions were collected. Protein contents of unfractionated seminal plasma, as well as the component fractions, were determined by the method of Folin et al.⁵ Five major protein peaks were identified and subsequently used as test reagents. Further characterization by sodium dodecyl sulfate-polyacrylamide disk gel electrophoresis showed that fraction 3 contained five low-molecular-weight proteins in the range of 25,000 daltons, while fraction 4 contained a single dominant low-molecular-weight protein and only traces of the other four protein bands observed in fraction 3.

Scratch and intracutaneous skin tests were performed and interpreted according to previously standardized criteria.³ In addition, intracutaneous skin-test threshold responses were determined for unfractionated and chromatographic components of seminal plasma. An intracutaneous skin test of an aqueous tree mixture (1:1,000 w/v) was also performed. All skin tests were performed simultaneously in a healthy volunteer.

Unfractionated seminal plasma of the

husband was coupled to microcellulose disks, and the radioallergosorbent (RAST) procedure was performed as previously reported.³

After it was ascertained that the patient's serum was negative for hepatitis and syphilis screening, passive transfer tests were performed on the back of a normal volunteer who had previously given informed consent for this procedure.³ Wheal-area results were interpreted in relation to the wheal size of a site challenged with unfractionated seminal plasma (1 mg/mL). Since protein concentrations of all chromatographic fractions were less than 1 mg/mL, a dose equivalence factor (DEF) for these reagents was calculated as follows: 1 mg of protein human seminal plasma divided by protein of respective fractions. To determine comparative bioequivalence between seminal plasma and seminal plasma fractions in this assay, a bioactivity index (BI) was calculated as follows:

$$BI = \frac{WA \text{ of Experimental Site} - WA \text{ of PBS Control Site}}{WA \text{ of USP} - WA \text{ of PBS Control Site}} \times DEF \times 100$$

where WA indicates wheal area (sq mm) and USP, unfractionated seminal plasma; PBS pH, 7.4

Leukocyte histamine-release tests were performed by the method of Lichtenstein and Osler.⁶ Dose-response assays were conducted with both unfractionated and chromatographic fractions of seminal plasma. Each assay was accompanied by control experiments on leukocytes from a healthy volunteer. In this laboratory, a notable response is considered to be that antigen concentration that elicits 20% or greater histamine release.

Lymphocyte tissue typing and ABO antigenic analysis were performed. The tissue-typing procedure was a microcytotoxicity assay as previously described.³

Immunotherapy was carried out with the seminal plasma fraction showing the highest degree of skin reactivity. Subcutaneous injections were given three times weekly, starting at an allergen concentration (0.0032 mg/mL) at least 100 times below the positive skin-test threshold concentration. The maximum dose attained was 0.50 mL of the allergenic fraction concentration (3.2 mg/mL), tenfold higher than the positive skin-test threshold dose.

Results

Scratch tests with undiluted seminal plasma and chromatographic fraction reagents were negative. Results of skin intracutaneous threshold responses are summarized in Table 1. The positive threshold skin concentration for unfractionated seminal

plasma was 1 mg/mL. Only Sephadex G100 chromatographic fraction 3 gave a positive threshold skin response at a concentration of 0.32 mg/mL. This suggested that the biologic skin reactivity of fraction 3 was approximately three times greater than that contained in the unfractionated seminal plasma sample. A positive skin-test reaction to a standard test dose of aqueous tree extract was compatible with the clinical history of early-spring upper respiratory tract symptoms.

The RAST assays of patient's serum incubated with microcellulose disks coupled with unfractionated seminal plasma and various Sephadex G100 fractions, including fractions 3 and 4, did not differ from the serum samples of normal control subjects.

Table 2 lists results of the passive transfer experiment. This study was designed to confirm the specificity of direct skin-test results and to demonstrate dose response, heat lability, neutralization effects, and the relative potencies of chromatographic fractions in eliciting the reaginic response. Calculation of a passive transfer BI facilitated interpretation of the raw wheal-area data obtained in this experiment. Experimental sites 1, 2, and 3 clearly illustrated that reaginic activity was dose dependent. The passive transfer antibody response was reduced greater than 50% after heating the patient's serum at 56 °C for four hours (experimental site No. 4, Table 2). The neutralization passive transfer site (experimental site No. 5, Table 2) was prepared by incubating 0.05 mL of seminal plasma containing 0.05 mg of protein with 0.05 of the patient's serum. When this site was challenged 48 hours later by unfractionated human seminal plasma reagent, there was moderate attenuation of wheal size but this was not greater than that caused by a simple dilution effect (experimental site No. 2). This indicated that 0.05 mg of seminal plasma protein was too small a dose for effective neutralization. Unfortunately, the appropriate neutralization dose could not be determined because the total number of passive transfer sites was limited. Elicitation of the reaginic response in these passive transfer experiments by several fractional components of seminal plasma was noteworthy. Fraction 3 demonstrated the highest BI ob-

Table 2.—Passive Transfer Tests of Unfractionated Human Seminal Plasma (HuSePl) and Sephadex G100 Fractions (Fr)

Experiment No.	Passive Transfer Sites			Challenged With			Wheal Size	
	Serum, 0.05 mL	Control Diluent, 0.05 mL*	HuSePl, 0.05 mL	Allergen	Protein, mg/mL	DEF†	sq mm	Bioactivity Index‡
1	+	0	0	HuSePl	1	1	37.9	100
2	+	+ (1:1)§	0	HuSePl	1	1	15.9	33
3	+	+ (1:10)	0	HuSePl	1	1	7.0	6
4	+	+ (1:1)	0	HuSePl	1	1	9.6	14
5	+	0	+ (1:1)	HuSePl	1	1	19.6	44
6	+	+ (1:1)	0	Fr 1	0.49	2	0	0
7	+	+ (1:1)	0	Fr 2	0.18	5.6	3.0	0
8	+	+ (1:1)	0	Fr 3	0.32	3.1	28.3	220
9	+	+ (1:1)	0	Fr 4	0.22	4.5	9.6	64
10	+	+ (1:1)	0	Fr 5	0.05	20.0	3.1	0
11	0	+	0	Control*	1	1	4.9	0

*Phosphate-buffered saline (pH 7.4).

†DEF indicates dose equivalent factor (see text).

‡See text for definition and calculation.

§Parentheses in the diluent and HuSePl columns contain final dilutions of all samples.

||Heated at 56 °C for four hours.

Table 3.—Leukocyte Histamine Release by Unfractionated Human Seminal Plasma and Sephadex G100 Fractions

Elicitation Conditions		Leukocyte Histamine Release, %	
Agent	mg/mL	Patient	Control
Human seminal plasma	1.0	11	0
	0.1	11	0
Sephadex G100 fraction			
1	4.9	11	8
	0.49	0	15
2	1.85	7	9
	0.185	11	5
3	3.2	22	6
	0.32	29	5
4	2.2	37	6
	0.22	15	8
5	0.5	7	5
	0.05	7	3

tained in this experiment. This was more than double that manifested by the reference undiluted serum site challenged with unfractionated seminal plasma. Fraction 4 also exhibited a moderate degree of reaginic bioactivity but this was less than the reference site and considerably less than fraction 3. Fractions 1, 2, and 5 did not elicit any reaginic response.

Data on human leukocyte release are summarized in Table 3. Both fractions 3 and 4 induced substantial histamine release from the patient's leukocytes at concentrations shown in this table. Similar to passive transfer results, fractions 1, 2, and 5 did not show allergenic activity in this assay system. Unfractionated seminal plasma failed to induce substantial histamine release.

Histocompatibility leukocyte antigen (HLA) typing of husband and wife showed a B7 common alloantigen, but this did not constitute a notable degree of shared histocom-

patibility locus antigens, as had been demonstrated previously in two other couples.³ Typing of red blood cell ABO alloantigens in this couple was also dissimilar.

Since direct skin tests, passive transfer experiments, and human leukocyte release data had all suggested that fraction 3 contained the highest allergenic potency, this fraction was selected as the most suitable reagent for immunoprophylaxis. The possible hazards of this procedure were carefully explained to the patient, and at the beginning of treatment, proper emergency equipment was available. In the initial stages of this program, the patient experienced small, local reactions on two separate occasions, but she was able to tolerate all other injections up to and including the maximal dosage without exaggerated local or systemic responses. Two weeks after the final dose of immunotherapy, intercourse was performed without condoms in a medical facility

and under the supervision of a physician. No adverse symptoms were experienced, and she has not experienced intercourse-associated symptoms since that time, with one exception. One year after completion of immunotherapy, respiratory tract symptoms, chiefly dry cough, occurred during the first sexual contact after an abstinence period of two weeks. On resumption of regular sexual activity—two to three times per week—her symptoms once again disappeared.

Comment

The diagnosis of clinical hypersensitivity to seminal plasma in this case was corroborated by (1) positive direct skin tests, (2) demonstration of heat-labile, passively transferable antibody, and (3) induction of leukocyte histamine release by several chromatographic fractions derived from seminal plasma. Evidence for the exclusive anaphylactogenicity of seminal plasma constituents was unequivocal because sperm antigens could not have been present in the patient's vasectomized husband. Failure to demonstrate circulating reaginic activity by the RAST technique was, in fact, consistent with the negative scratch test in this case and other clinical data about the sensitivity constraints of this method in other well-defined allergen-antibody systems.⁷ The paradox between leukocyte histamine releasability by unfractionated seminal plasma and two of its component fractions may be more apparent than real. This interpretation is supported in part by the

modest degree of histamine release exhibited by this patient's leukocytes (maximum release, 37%). Since the 0.32-mg/mL concentration of fraction 3 was known to have substantially greater allergenic bioactivity than unfractionated seminal plasma, it is postulated that the allergenically active component in seminal plasma was below the threshold dose required to trigger histamine release from the patient's relatively sluggish basophils. Presumably, the highest activity demonstrated by the 2.2-mg/mL concentration of fraction 4 (37%) was also a dose-response effect in this system, but this was not proved by prior passive transfer results at this particular concentration.

A unique constellation of events that occurred in this couple may lead to a better understanding of possible susceptibility factors in this disease. The most explicable predisposing factor in this patient was an atopic background, which had also been observed in the majority of previous case reports.³ Not as well understood are associations between the development of anaphylactic symptoms and major events involving the female reproductive tract. In this case, for example, even though mild premonitory symptoms had been present for several months, the initial appearance of severe symptoms occurred during the first intercourse six weeks after a hysterectomy. It is of interest that a substantial number of previous patients with seminal plasma-mediated anaphylaxis either experienced initial reactions after the first sexual experience or during the first intercourse of the immediate postpartum period.³ Although these clinical associations do not seem to have a common basis, it is possible that they upset the homeostatic balance of female immunosuppressive factors or interfere with cellular processing and/or normal clearance of seminal plasma allergen(s).³ In particular, immunoregulatory mechanisms may be dampened in the short time span immediately after delivery or, as in this case, hysterectomy.

Another possible determinant of susceptibility may relate to biochemical differences of seminal plasma generated by vasectomized males. Although pooled samples of seminal plasma are antigenically similar in normal and vasectomized males, re-

cent experimental evidence suggests that the immunosuppressive activity of seminal plasma from vasectomized males may be diminished.⁹ Immunosuppressive factors have been isolated from seminal plasma by independent investigators and it is purported that such factors may regulate sensitivity responses to sperm or seminal plasma allergens.^{9,10} Proteins derived from bovine, murine, and human seminal plasma are known to suppress lymphocyte blastogenesis induced by mitogens, antigens, and allogeneic cell surface antigens in mixed lymphocyte culture experiments. Animal experimental data also indicate that specific protein fractions of seminal plasma are synthesized at different locations within the male reproductive tract and that these protein factors exert differential suppression of lymphocyte subpopulations.¹⁰ Taken together, the combination of exposure to seminal plasma from a vasectomized male in an atopic and recently hysterectomized female may have provided the ideal milieu for development of anaphylactic sensitivity in this case.

Immunologic evaluation of chromatographic fractions of seminal plasma provided additional clues about the pathogenesis of this disease. Using similar methods, major allergenic activity in other patients was found in fractions 1, 2, and 4.³ In the current case, allergenic bioactivity was limited to fractions 3 and 4. However, the criteria for partial purification—ie, augmentation of biologic activity—were only fulfilled for fraction 3. Therefore, it is possible that the allergenic activity of fraction 4 could represent common or overlapping proteins from the adjacent fraction 3 peak.

The most important aspect of this case was the achievement of successful and long-term desensitization to one of the major allergenic determinants in the husband's seminal plasma. In two previous immunotherapeutic interventions with parenteral injections of unfractionated seminal plasma, one was completely unsuccessful¹ and the other partially successful.¹¹ Compared with these previous reports, the almost total success of immunotherapy in this case is unparalleled. This successful outcome is attributed to use of a partially purified and more potent immunogenic reagent for the immunotherapy

procedure, although it should be stressed that immunization involves the known risk of anaphylaxis as well as potential risks of desensitizing with an unknown human biologic component. Thus far, the patient has shown no evidence of an adverse clinical effect that could be related to the latter possibility. However, at least one exacerbation occurred in this patient when exposure to seminal plasma was interrupted for a period of two weeks. Complete remission of symptoms was again noted after reinstitution of sexual intercourse at regular intervals. It therefore seems that a regimen of sustained "natural immunotherapy with seminal plasma" is required to maintain her tolerant state. Serial immunologic studies are planned to determine how this fine immunoregulatory balance prevents recurrence of seminal plasma anaphylaxis.

James Farr, MD, referred the patient, Jeffrey Doughman prepared the seminal plasma fractions, and Jan Shulman provided secretarial assistance.

References

1. Halpern BN, Ky T, Robert B: Clinical and immunological study of an exceptional case of reaginic type sensitization to human seminal fluid. *Immunology* 1967;12:247-258.
2. Levine BB, Siraganian RP, Schenkein I: Allergy to human seminal plasma. *N Engl J Med* 1973;288:894-896.
3. Bernstein IL, Englander BE, Gallagher JS, et al: Localized and systemic hypersensitivity reactions to human seminal fluid. *Ann Intern Med* 1981;94:459-465.
4. Marcus ZH, Freisheim JH, Houk JL, et al: *In vitro* studies in reproductive immunology: I. Suppression of cell-mediated immune response by human spermatozoa and fractions isolated from human seminal plasma. *Clin Immunol Immunopathol* 1978;9:318-326.
5. Folin O, Ciocalteu V: Estimation of protein with the Folin-Ciocalteu phenol reagent, in Kabat EA, Mayer MM (eds): *Experimental Immunochimistry*. Springfield, Ill, Charles C Thomas Publisher, 1948, pp 321-323.
6. Lichtenstein LM, Osler AG: Studies on mechanisms of hypersensitivity phenomena: IX. Histamine release from human leukocyte by ragweed pollen antigen. *J Exp Med* 1964;120:507-530.
7. Perera M, Bernstein IL, Michael JG, et al: Predictability of the radioallergosorbent test (RAST) in ragweed pollenosis. *Am Rev Respir Dis* 1975;111:605-610.
8. Beer AE, Neaves WN: Antigenic status of semen from the viewpoints of the female and male. *Fertil Steril* 1978;29:3-22.
9. Hess EV, Marcus ZH: The inhibitors in seminal plasma. *J Lab Clin Med* 1980;96:577-581.
10. Anderson DJ, Tarter TH: Immunosuppressive effects of mouse seminal plasma components *in vivo* and *in vitro*. *J Immunol* 1982;128:535-539.
11. Mathias CGT, Frick OL, Caldwell TM, et al: Immediate hypersensitivity to seminal fluid and atopic dermatitis. *Arch Dermatol* 1980;116:209-212.