

Selective desensitization to seminal plasma protein fractions after immunotherapy for postcoital anaphylaxis

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A 24-year-old white woman reported sexual intercourse-related pruritus, hives, wheezing, and dyspnea within 5 minutes after ejaculation. Systemic reactions (SRs) were prevented by use of condoms. Prick testing confirmed sensitization to five Sephadex G-100-separated fractions of her husband's seminal plasma. The intradermal end point threshold concentrations (ETC) were 10^{-4} and 10^{-1} μ g of protein per milliliter to fractions 2 and 3, respectively. Leukocyte histamine release studies exhibited 100% release to fraction 2 and 37% release to fraction 3. A 2-day protocol of rapid immunotherapy (IT) was performed with subcutaneous incremental doses of human seminal plasma (HuSePl) fractions 2 and 3. The patient experienced an SR after receiving a cumulative dose of 38.55 μ g of fraction 2 on day 1. On day 2, rapid IT with fraction 2 was administered until the patient experienced a mild SR after having received a cumulative dose of 102.8 μ g. There was a one-log₁₀ increase in the intradermal ETC to both fractions 2 and 3 at the end of day 2. IT was continued three times weekly for 4 months until the patient tolerated 100 μ g doses of both fractions 2 and 3. At 4 months, coitus was resumed without SRs, and HuSePl IT was stopped. The intradermal ETC to fractions 1, 3, 4, and 5 was increased 6 months after cessation of HuSePl injections, but there was a one-log decrease in the ETC to fraction 2. Our experience demonstrated that systemic tolerance can be achieved by parenteral administration of selected HuSePl fractions. Partial immunologic desensitization of patients with anaphylactic sensitivity can be achieved. This appears to be selective for most, but not all, allergenic fractions contained in HuSePl. (J ALLERGY CLIN IMMUNOL 1990;86:954-60.)

Anaphylaxis to HuSePl was first described by Halpern et al.¹ in 1967. In 1973, Levine et al.² reported cutaneous reactivity and LHR to allergens contained in HuSePl in a woman who experienced anaphylaxis after sexual intercourse.² In 1981, we³ described four patients with two distinct types of clinical reactions to HuSePl associated with sexual intercourse consisting of either anaphylaxis or localized vaginal reactions. Interestingly, there was an unexpected high degree of histocompatibility leukocyte antigen sharing between the male and female partners of these cou-

Abbreviations used

HuSePl:	Human seminal plasma
PBS:	Phosphate-buffered saline
LHR:	Leukocyte histamine release
IT:	Immunotherapy
ETC:	End point threshold concentration
TH:	T helper cell
SR:	Systemic reaction

ples. Elevated levels of serum-specific IgE antibodies to HuSePl were detected in two of these patients who experienced postcoital anaphylaxis. Five protein fractions were purified on a Sephadex G-100 column from whole HuSePl. The allergen activity was detected in fractions 1, 2, and 3 with the most potent allergens contained in fraction 2. In the two other patients who experienced postcoital vaginal swelling and dyspareunia, specific in vitro cell-mediated responses to HuSePl and spermatozoa antigens were demonstrated.

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Treatment of women diagnosed with postcoital anaphylaxis resulting from immediate hypersensitivity to HuSePl is problematic. In general, avoidance of sexual intercourse or use of protective barriers, such as condoms, are effective measures for preventing further anaphylactic reactions. In 1984, Friedman et al.⁴ reported the first case of successful subcutaneous desensitization with a fraction of HuSePl. Subsequently, two other women have been desensitized in a similar fashion with specific allergenic fractions of HuSePl.⁵ Efficacy of this treatment was demonstrated by toleration of sexual intercourse after completion of HuSePl IT. Ohman et al.⁶ recently reported successful desensitization of a woman with allergy to human seminal fluid with an allergenic fraction of HuSePl. In this article, we describe our experience with HuSePl IT in one patient with postcoital anaphylaxis in which true desensitization was accomplished, but appeared to be restricted to some, but not all, allergenic fractions of HuSePl.

CASE HISTORY

In August 1987, a 24-year-old white woman was referred for evaluation of an 18-month history of recurrent anaphylaxis occurring during sexual intercourse. In March 1986, generalized urticaria and pruritus began to occur inconsistently after sexual intercourse. After the birth of a second child in March 1987, generalized urticaria, vaginal burning, wheezing, and dyspnea occurred within 5 minutes after ejaculation. This required treatment with epinephrine in an emergency room. Thereafter, intercourse-related anaphylaxis recurred consistently, and each time required treatment with subcutaneous epinephrine. Only the use of condoms prevented the occurrence of anaphylactic episodes. There was no history of use of vaginal ointments, of ingestion of oral medications, or foods before sexual intercourse that would suggest an exogenous cause for these reactions. There was no prior history of food- or drug-induced allergic reactions in either the patient or her husband. The patient had tolerated coitus with her present mate for 3 years before the onset of anaphylactic episodes. A small ventricular septal defect was diagnosed during childhood. There was no history of atopic dermatitis. Physical examination was remarkable for an IV/VI, harsh holosystolic murmur along the left sternal border. Prick skin testing to common inhalant aeroallergens was negative. The patient was referred for evaluation and treatment of anaphylactic sensitivity to HuSePl. Documentation of specific IgE-mediated sensitivity was demonstrated by a positive skin prick test to whole HuSePl and three of five Sephadex G-100-separated fractions at protein concentrations of 10 to 100 µg/ml.

METHODS

Preparation of HSP fractions

Five ejaculates were collected from the husband, allowed to liquify, and then pooled. The pooled specimen of semi-

TABLE I. Rapid IT treatment protocol demonstrating doses of fractions 2 and 3 of HuSePl administered on days 1 and 2

Time	Fx 2 dose*	Fx 3 dose
Day 1	0.00005	—
	0.0005	—
	0.005	0.005
	0.1	0.10
	0.2	0.20
	0.4	0.40
	0.8	0.80
	2.0	2.00
	5.0	5.0
	10.0	10.0
	20.0	20.0
Total dose	38.55	36.5
Day 2	0.8	—
	4.0	—
	8.0	—
	10.0	—
	20.0	—
	20.0	—
	40.0	—
Total 2-day cumulative dose	141.35	36.5

Fx, Fraction.

*Micrograms of HuSePl protein.

nal fluid was diluted with an equal volume of 0.05 mol/L of PBS (pH 7.4) and centrifuged at 4° C for 30 minutes at 10,000 rpm. The presence of spermatozoa was excluded by microscopic examination of the seminal-fluid specimen. The whole HuSePl was dialyzed with Spectrapor (American Scientific Products, McGaw Park, Ill.) dialysis membrane tubing that excluded 6000 to 8000 kd molecules for 24 hours against phosphate buffer at 4° C 1 ml, and then layered onto a Sephadex G-100 column (Pharmacia, Piscataway, N.J.) that had previously been equilibrated with PBS at pH 7.4. The column was eluted with 0.05 mol/L of PBS, and approximately 100 ml of each of five fractions was collected.⁷ The elution profile demonstrating five peaks of absorbance is illustrated in Fig. 1. The protein content of unfractionated HuSePl, as well as the five collected fractions, were determined by the Folin method.⁸ All fractions were sterilized after separation by Millipore filtration with a 22 µm filter (Millipore Corp., Bedford, Mass.).

Desensitization protocol

The potential risks and benefits involved with HuSePl IT were carefully explained, and informed consent was obtained from both the patient and her spouse. Active infection with hepatitis A and B, HIV, and syphilis were excluded by serologic screening of the husband before initiation of treatment. During treatment, an intravenous line

TABLE II. Intracutaneous sensitivity to HuSePl antigen before, immediately after completion of 2-day rapid IT, and 6 months after completion of slow desensitization with HuSePl fractions 2 and 3

HuSePl fx	Before RIT	After RIT	6 Mo after IT
1	10 ⁻¹	ND	10 ⁰
2	10 ⁻⁴	10 ⁻³	10 ⁻⁵
3	10 ⁻¹	10 ⁰	10 ²
4	10 ⁰	ND	NR*
5	10 ⁰	ND	NR

fx, Fraction; RIT, rapid immunotherapy; ND, not done.

Results are expressed as the ETC (micrograms of protein per milliliter).

*No response at highest concentration of 1×10^2 μ g/ml.

was in place, and full emergency resuscitation equipment was available.

Two HuSePl fractions (2 and 3) that demonstrated the highest degree of allergenicity based on in vitro LHR studies (see Table III) were selected for treatment. Fraction 1 was excluded from treatment because previous data suggested that fraction 1 possessed in vitro immunosuppressive properties.⁷ Both fractions 4 and 5 possessed relatively less reaginic activity based on direct LHR results (see Table III). The rapid IT treatment schedule used on days 1 and 2 is presented in Table I. The treatment was begun with subcutaneous injections of HuSePl fraction concentrations that were one tenfold dilution higher than the intradermal ETC. The second major fraction (fraction 2) was administered at a beginning dose of 5×10^{-5} μ g of protein and increased up to the maximal tolerated dose. The same doses of HuSePl fraction 3 were administered with the exception that the beginning dose was 5×10^{-3} μ g, and the final dose was 20 μ g. Injections were alternated between fractions 2 and 3 every 15 minutes until a maximal single dose of between 20 to 40 μ g of HuSePl was achieved or until an SR occurred. After completion of the initial treatment, injections of fractions 2 and 3 were continued and administered three times weekly for the next 4 months. Dosages of both fractions were gradually increased until a target dose of 100 μ g of both HuSePl fractions was achieved. At the end of 4 months, sexual intercourse was resumed after HuSePl injections were stopped.

Immunologic studies

Before treatment, intracutaneous titration testing was performed with whole HuSePl and each of the five major fractions. Tenfold increasing concentrations of each HuSePl fraction, ranging between 10^{-7} and 10^2 μ g/ml of protein, were used for intracutaneous testing. Skin testing was repeated immediately after completion of rapid IT on day 2 as well as 10 months after initiation of HuSePl treatment.

Specific IgE was measured by a modification of the RAST.⁹ Unfractionated and concentrated fractions

of HuSePl were coupled to cyanogen bromide-activated methylcellulose disks. After stepwise incubation of antigen-coupled disks with the patient's serum and ¹²⁵I-labeled anti-IgE, radioactivity bound to the paper disks was measured in a gamma counter. The radioactivity bound in the patient's serum was compared both to a known positive control serum (kindly provided by Dr. John Ohman) with proven HuSePl sensitivity and to a negative control panel of sera from 10 asymptomatic female volunteers.

Specific IgG to HuSePl, as well as to fractions 1, 2, 3, 4, and 5, was determined by a modified ELISA method.¹⁰ Optical density was read at 405 nm on a micro-ELISA reader, MR 590 (Dynatech Laboratories, Alexandria, Va.).

LHR tests were performed according to the method of Lichtenstein and Osler.¹¹ Dose-response assays were conducted with both unfractionated and Sephadex G-100 fractions of the husband's HuSePl. Results were expressed as percent of histamine release.

RESULTS

Results of the intracutaneous titration and LHR demonstrated that the most potent allergenic activity resided in fraction 2. Intradermal reactivity (Table II) was detected to all five fractions with the greatest sensitivity to fraction 2. Results of LHR studies performed in the patient and an age-matched control subject after stimulation with HuSePl fractions are summarized in Table III. There was 100% release in response to unfractionated HuSePl and fraction 2. Fractions 1, 3, 4, and 5 demonstrated less, but nevertheless significant, histamine release in response to all the fractions tested. The control subject exhibited 100% release to whole HuSePl but negligible release after stimulation with the individual fractions. Thus, the whole HuSePl appeared to cause nonspecific release of histamine. As presented in Table III, an elevation in serum-specific IgE was detected to fraction 2 but not to whole HuSePl or the other four fractions. Before treatment, RAST binding to fraction 2 was 7.5%, which was >3 SD above the mean ($2.2\% \pm 0.3\%$) of 10 nonsensitive female control subjects.

Rapid IT was performed with HuSePl fractions 2 and 3 because they contained the highest allergenic activity based on results of LHR studies. As represented in Table I, on day 1 of treatment, the patient tolerated a total cumulative dose of 36.5 μ g of fraction 3. On day 1, the patient experienced diffuse urticaria and wheezing beginning 10 minutes after administration of a 20 μ g dose of fraction 2 and after having received a total cumulative dose of 38.55 μ g. IT with fraction 3 was stopped because clinical tolerance was achieved, but IT with fraction 2 was continued the next day. On day 2, a higher cumulative dose of 102.8 μ g was achieved before the patient experienced cough and chest tightness after the final 40 μ g dose was administered. As presented in Table II, there was a

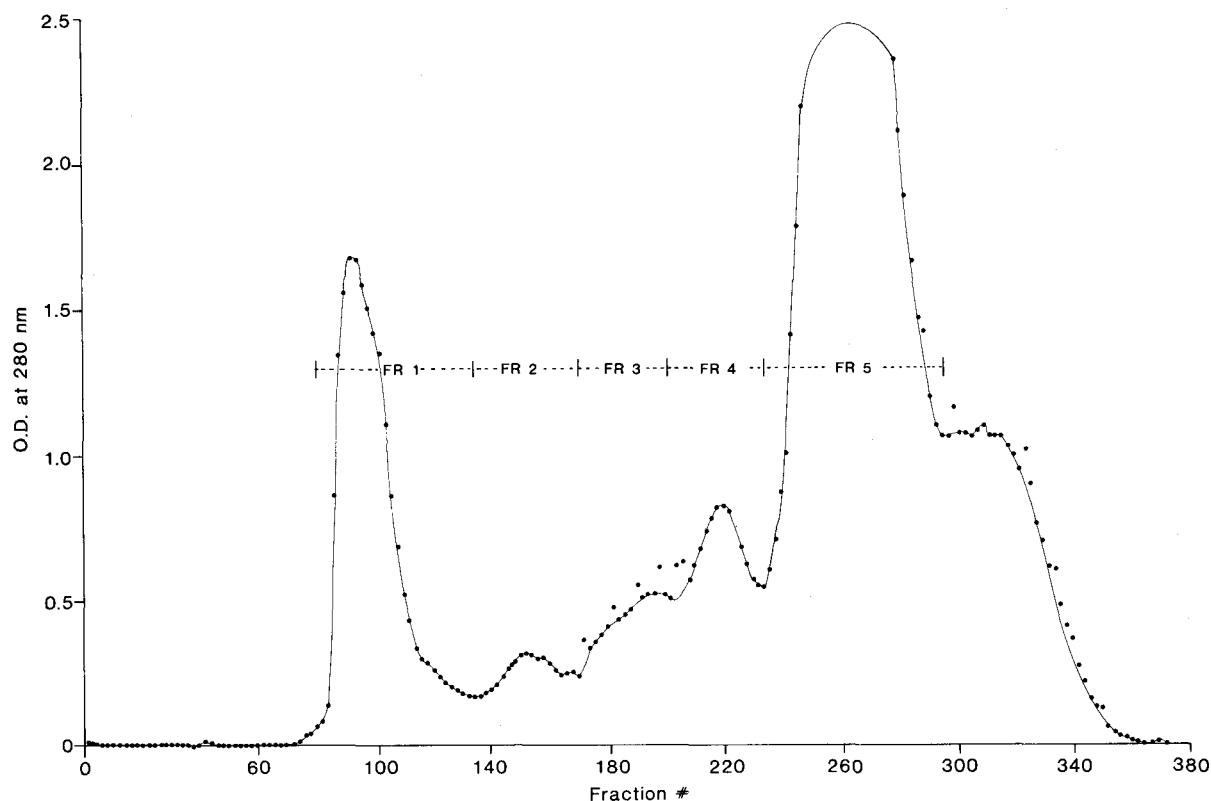


FIG. 1. Sephadex G-100 column chromatography of HuSePI. HuSePI from the husband, diluted in 0.05 in phosphate buffer (pH 7.4) was applied to a Sephadex G-100 column equilibrated with the same buffer, and 6 ml fractions were collected. Absorbance of each is illustrated and expressed as optical density (O.D.) Based on the major identifiable peaks, samples were pooled into five large fractions.

TABLE III. Baseline pretreatment studies of in vitro LHR after stimulation with whole HuSePI and five Sephadex G-100 fractions in the patient and an age-matched control subject, and serum-specific IgE to whole HuSePI and five fractions determined by the RAST assay

Antigen	% Histamine release		RAST (% binding)	
	Patient	Control subject	Patient	Control subjects*
Whole HuSePI	100	100	2.1	1.7 ± 0.1
Fx 1	32	0	2.0	2.1 ± 0.2
Fx 2	100	0	7.5	2.2 ± 0.3
Fx 3	37	13	1.9	1.8 ± 0.2
Fx 4	21	6	2.0	2.0 ± 0.2
Fx 5	32	19	2.1	1.8 ± 0.1

Fx, Fraction.

*Results expressed as mean ± standard deviation of 10 asymptomatic female subjects.

one-log₁₀ increase in the intracutaneous ETC to both fractions 2 and 3 after completion of the second day of rapid IT.

Because clinical tolerance to fraction 2 was not evident at the end of day 2, routine IT was given

three times weekly for 4 months with both fractions 2 and 3. After 4 months of treatment, the patient tolerated 100 µg doses of both fractions 2 and 3 without reactions. At that time, sexual intercourse was resumed without the use of condoms. No local vaginal

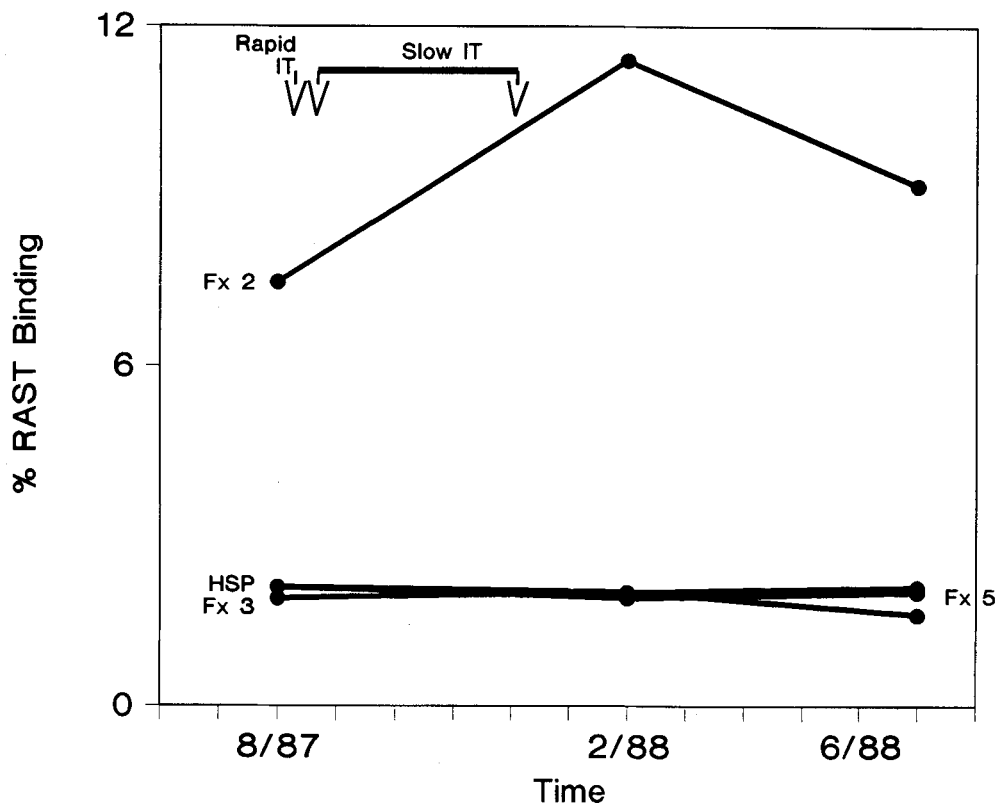


FIG. 2. Specific IgE determined by RAST to whole HuSePl and to fractions 2, 3 and 5 before, as well as 2 and 6 months after cessation of subcutaneous treatment with fractions 2 and 3.

or anaphylactic reactions were reported after ejaculation. Parenteral HuSePl treatment was stopped, and the patient was instructed to maintain a regular schedule of sexual intercourse every 2 to 3 days. The couple resumed sexual intercourse, and no recurrence of anaphylactic episodes occurred during a 6-month period after cessation of HuSePl injections.

Immunologic evaluation after treatment

At 6 months after cessation of HuSePl IT, intracutaneous ETCs to fraction 3 had increased by three $\log_{10}$ concentrations. In contrast, a decrease in ETC to fraction 2 by one $\log_{10}$ concentration was observed 6 months after completion of HuSePl treatment. Intradermal ETC of fraction 1 was increased by one log concentration, and cutaneous reactivity had completely disappeared to fractions 4 and 5.

As presented in Table III, serum-specific IgE to fraction 2 was elevated before treatment. That the RAST binding remained elevated at 2 and 6 months after cessation of HuSePl IT is illustrated in Fig. 2. Serum-specific IgG (Fig. 3) to fractions 1, 2, 3, 4, and 5 rose after IT but decreased toward baseline values months after HuSePl injections were stopped.

There were no serum-specific IgG antibody responses detectable to whole HuSePl.

DISCUSSION

Postcoital anaphylaxis is a rare clinical entity that is most often caused by IgE-mediated sensitization to HuSePl protein allergens. Other rare causes of postcoital anaphylaxis have been identified, including allergic responses to drugs or food antigens transferred via the HuSePl of male to female partners with pre-existing sensitivities.¹² Neither food- or drug-induced hypersensitivity responses could be implicated in this patient or in previous patients that we have studied.^{3, 4} In our patient, the presence of IgE-mediated sensitization to HuSePl allergens was clearly demonstrated by both in vivo and in vitro methods. As in previous studies, Sephadex G-100-separated fractions 2 and 3 were found to contain the most potent HuSePl allergens based on data obtained from RAST and LHR, and intracutaneous testing with fraction 2 exhibited the highest degree of allergenicity.^{3, 4}

Because barrier treatment by use of condoms was unacceptable to this couple, a 2-day protocol of rapid IT with HuSePl was used based on our previous fa-

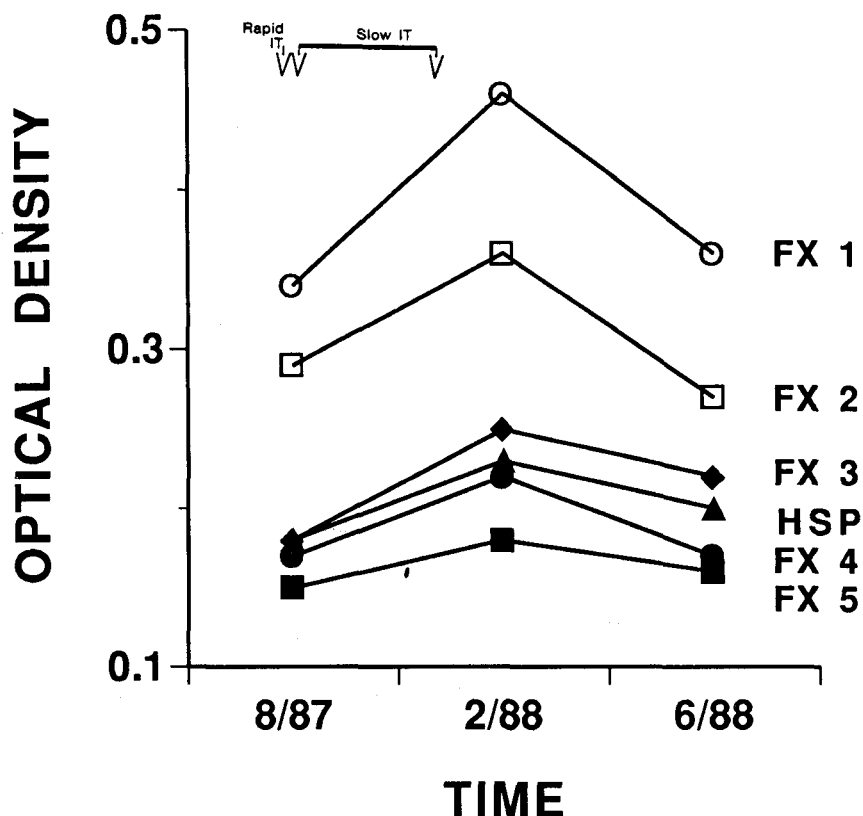


FIG. 3. Specific IgG determined by ELISA to whole HuSePl and to fractions 1, 2, 3, 4, and 5 before, as well as 2 and 6 months after, cessation of treatment with fractions 2 and 3.

avorable experience with this technique. However, in contrast to previous cases, successful desensitization to fraction 2 was not achieved after the initial 2 days of treatment, as evidenced by the occurrence of systemic reactions after fraction 2 injections. Because of failure to achieve clinical tolerance to fraction 2 by rapid IT, frequent injections with HuSePl fractions 2 and 3 were continued for a period of 4 months until doses of 100 μ g of each were tolerated without adverse reaction. At that time, sexual intercourse was resumed without difficulty. Intracutaneous titration testing performed months after completion of HuSePl injections revealed that there was evidence of immunologic desensitization to fractions 1, 3, 4, and 5, but not to fraction 2. Successful desensitization to fractions 1, 4, and 5, which were not used as treatment, could reflect the existence of cross-reactivity with antigens in fractions 2 and 3. Fraction 2-specific IgE levels also failed to decrease 2 and 6 months after completion of HuSePl IT. Thus, despite clinical tolerance to all HuSePl fractions, immunologic desensitization was not demonstrated to allergens in fraction 2. Recently, Ohman et al.⁶ reported a case of successful clinical desensitization to an allergenic fraction of HuSePl that resulted in a decrease in serum-specific

IgE to seminal fluid, but no parallel decrease in intracutaneous sensitivity was observed.

The unusual selectivity of immunologic desensitization to various fractions is difficult to explain. First, it is unlikely that induction of specific IgG or "blocking" antibodies were responsible because specific IgG to all the fractions had already begun to decrease 6 months after the cessation of HuSePl injections and at the same time that cutaneous desensitization was apparent. It is possible that desensitization to fractions 1, 3, 4, and 5 could have been maintained by local exposure to HuSePl antigens as a result of frequent sexual contact after completion of HuSePl injections. Selectivity of desensitization by fractions 1, 3, 4, and 5 compared to fraction 2 could be consistent with selective proliferation of TH subsets. It has been proposed that proliferation of TH₁ subsets result primarily in secretion of interleukin-2 and interferon- γ , both of which down regulate IgE synthesis.^{13, 14}

Conversely, stimulation of TH₂ subsets causes enhanced IgE production. The antigen contained in fractions 1, 3, 4, and 5, but not the antigen in fraction 2, might preferentially stimulate TH₁ subsets that could effectively inhibit specific IgE production. Whatever the mechanism of clinical protection may be, we have

demonstrated that anaphylaxis to HuSePI can be successfully treated by desensitization to HuSePI fractions.

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