

Asthma and urticaria induced by seminal plasma in a woman with IgE antibody and T-lymphocyte responsiveness to a seminal plasma antigen

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Summary

A patient with asthma urticaria and angioedema induced by allergy to seminal plasma was examined at intervals for 10 years. Before treatment her anaphylactic susceptibility to seminal plasma was manifested by very strong prick-test responses, IgE antibody to an allergenic fraction of seminal plasma determined by RAST, and by antigen-induced histamine release from her blood leucocytes. The skin test and *in vitro* lymphocyte tests indicated concomitant delayed hypersensitivity to the same allergen. The patient's lymphocytes treated with seminal plasma allergen fraction showed much increased incorporation of thymidine, and also synthesis of a product (NIF) that inhibited migration of neutrophils from a normal donor. The allergen fraction of seminal plasma had about five components in the range of 20 000–40 000 daltons molecular weight; the major fraction binding IgE appeared to be a glycoprotein.

The patient was successfully desensitized by injections of her husband's seminal plasma. Desensitization was not associated with persistent amounts of antigen-specific IgG antibodies.

Introduction

Since the first report by Halpern, Ky and Robert (1967) of a case of anaphylactic allergy to seminal plasma, further occasional cases have been reported. They tend to have several features in common, in that the patient is atopic, the clinical manifestations are severe, prick-test responses are elicited to seminal plasma or to fractions thereof, and IgE antibodies are detected to these antigens.

We report a case followed for 10 years, who presented with acute urticaria and asthma induced by anaphylactic sensitivity to seminal plasma. She was extremely sensitive to skin-prick or intradermal tests, had large amounts of IgE antibody to an allergen-containing fraction of seminal plasma from her husband or from a pool

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sample, and release of histamine from her blood leucocytes when treated with the same antigen fraction.

There were also rarely reported features, with evidence of delayed hypersensitivity based on: (i) the persistent skin test reactions, (ii) the incorporation of tritiated thymidine which was 105-fold above background in lymphocyte transformation tests, and (iii) on antigen-treated lymphocytes synthesizing a product that inhibited migration of a normal donor.

The other unusual feature is that the patient was successfully desensitized by injections of her husband's seminal plasma, and no further treatment for this allergy was necessary.

Materials and methods

Preparation of antigen and allergenic fraction

Seminal plasma was centrifuged at $250 \times g$ for 15 min, dialysed against 0.9% NaCl overnight and centrifuged at $5000 \times g$ for 30 min at 4°C . Samples intended for agglutination tests were dialysed against 0.001 EDTA in 0.5 M Tris buffer in 0.9% NaCl to remove the red-cell clumping factor (Jeffery & Parish, 1972). Dilutions were prepared by homogenizing the seminal plasma in 0.9% NaCl with phosphate buffer pH 7.5. Protein concentrations were determined of the starting material and the dilutions. The allergenic fraction was prepared by passing the cell-free plasma down a Sephadex G200 column with 0.7 M NaCl plus phosphate buffer pH 7.5. The fraction from different samples in the range of 20 000–40 000 daltons molecular weight were concentrated, dialysed against saline, and freeze-dried. Other fractions collected were in the range of about 5000–20 000, 40 000–160 000 and the remainder above 160 000. The allergenic fraction was reconstituted in the appropriate diluent for the test and centrifuged at $250 \times g$ for 20 min. Antigen for the lymphocyte tests was sterilized with a millipore syringe filter before use.

Immunoglobulin assays and antibody determinations

Total immunoglobulins G, M and A were assayed by radial immunodiffusion plates. Total IgE was assayed by Phadebas PRIST.

Antibodies to suspensions of dead, washed, spermatozoa, and to seminal plasma by the tanned-cell agglutination test were done by the methods of Jeffery and Parish (1972).

Agglutinating antibody to the seminal-plasma allergenic fraction was determined by the red-cell-linked antigen-antiglobulin reactions (RCLA-AR) of Coombs *et al.* (1965) and Hunter and Coombs (1969) as modified by Parish (1969). Rabbit IgG anti-human group O red cells was reduced and reconstituted in a univalent 7S form. The antibody was mixed with the seminal-plasma antigen fraction in the approximate ratio of 1:2 mol, and coupled with bis-diazo-benzidine (BDB) in the ratio of 1 mol total protein:4–5 mol BDB. After treatment for 6 hr at 0°C , the coupled antibody was dialysed against saline pH 7.2 and purified by filtration down a Sephadex G200 column. The antibody-coupled antigen preparation was diluted 1/32 to sensitize human group O red cells for optimal binding. Before the agglutination test the sera to be examined were absorbed at 4°C by the same washed, non-antigen coupled red cells to remove any antibody to the cells.

The agglutination tests were examined for direct agglutination, and then after

washing and treatment with anti-IgG₁ and anti-IgG₄ and anti-IgM (prepared by us) by the method of Coombs *et al.* (1965).

IgE antibody assays to seminal-plasma allergen fraction (samples 1972–1974) and to seminal plasma (samples 1976 and 1981) follow the RAST method of Wide, Bennich and Johansson (1967).

Antigen was coupled by cyanogen bromide to microcrystalline cellulase (Sigma) using previously determined optimum conditions of coupling. For seminal-plasma saline extract the optimum was 4.20 µg protein bound to each mg microcrystalline cellulase with 0.15 M phosphate buffer pH 6.4, and for the seminal-plasma allergen fraction 130 µg protein bound to each mg cellulase with 0.15 M disodium buffer pH 8.6. The coupled particles were washed 5 times with 0.85% saline containing 1% Tween 20.

The control serum sample was a pool of young males, blood groups A, B and O, and total IgE of 126 IU/ml, and without agglutinins to spermatozoa. In 1972–1974, the tests were done in triplicate and are expressed as the ratio of disintegrations per minute for the control pool serum to test serum samples; maximum variation in results between the triplicate samples was 4.7%. In 1976 and 1981 the ratio is expressed as counts per minute (cpm). The results of individual tests as samples were received did not differ greatly from those on a final test comparing all samples together 1972–1974.

IgE antibody to whole milk was detected by Pharmacia reagents. IgG subclass antibodies to individual milk proteins were examined by a slightly modified ELISA using monoclonal anti-IgG subclass antibodies (Seward) (method to be published by Scholes and Goodwin).

Antigen-induced histamine release from blood leucocytes

Peripheral-blood leucocytes were separated by dextran sedimentation (Goldsmith, 1965), and the histamine-release procedure was essentially that of Osler, Lichtenstein and Levy (1968). No serum was added to the washed leucocytes. The amount of histamine after 20 min incubation with seminal plasma, or its allergenic fraction, in amounts stated in Table 3, was assayed by a sensitive fluorometric method against histamine acid phosphate standards. The results are expressed as percentage net histamine base released after subtraction of the nil antigen control amounts. The allergenic fraction slightly promoted histamine release in the controls.

Lymphocyte thymidine (TdR) incorporation and neutrophil migration inhibition factor

Lymphocytes were separated from venous blood by the method of Boyum (1968). Blood treated with EDTA was mixed with an equal volume of 0.9% saline and layered over an isopaque (sodium metrizoate)-Ficoll solution. No attempt was made to remove any monocytes in the lymphocyte suspension. Lymphocytes were washed in RPMI-1640 medium containing either 10% heat-activated pooled human group AB serum, or 10% heat-inactivated antologous serum according to the test. No antibiotics were added. If any culture was seen to be contaminated, or at the end of the test when one loop was transferred to peptone water and subcultured onto agar, the test was discarded.

Mitogenic susceptibility was tested with PHA at a suboptimal 2 µg/ml (optimum for batch was 4 µg/ml); 0.5 ml of lymphocyte suspension containing 2×10^6 cells/ml was treated with 0.5 ml of PHA in medium with HEPES buffer, at 37°C in an atmosphere of 5% CO₂ in air for 64 hr. Then 1.0 µCi [³H]TdR was added for 4 hr before centrifugation, cell lysis extraction, and counting.

The test with candida antigen extract was made by adding 10 μ g antigen to cells in 10% autologous serum, and incubation for 5 days.

Tests with seminal-plasma antigen, or with fractions thereof, were made as for candida antigen with amounts stated in the test, and pulsed with [^{3} H]TdR for 4 hr. Tests were made in triplicate.

Leucocyte migration inhibition was examined essentially by the method of Clausen (1972; 1973) using the indirect, two-step procedure, and examining the lymphocyte culture fluids on purified neutrophils in capillary tubes instead of agar wells. Lymphocytes, 2×10^6 /ml, were tested with seminal-plasma allergen fraction at 20 μ g/ml in medium containing a final concentration of a non-toxic human serum, blood group AB, and rhesus D positive, from a male donor. No antibiotics were added, and no fetal calf serum to avoid any stimulation by these substances to which an atopic person may have had sensitivity. The lymphocytes were incubated for 2 days, centrifuged, and the supernatant fluid passed through a millipore filter. Before the test, one sample of lymphocytes from each donor (test and three controls) was treated with 20% puromycin for 1 hr, the puromycin-containing solution was then removed before addition to the antigen. Lymphocyte viability for the preparation with and without antigen by dye exclusion was 78–91%; for the puromycin treated culture it was 69–74%. The cell-free supernatant fluids were used to treat aliquots of purified neutrophils from a normal male. Each culture fluid was added to a tube of neutrophils, incubated 1 hr, then the neutrophils were dispensed into capillary tubes, three per culture fluid sample, centrifuged and cultured, horizontally in dishes in an atmosphere of 5% CO₂ in air by standard methods. After 16–18 hr the distance of migration, along two diameters at right angles, was measured.

Results

Clinical history

AK, Spanish, born 1939. Breast fed for 2 years. She was the fifth of nine children of a family with no atopic history.

AK has lived in England since 1960, married in 1964. She has had five pregnancies; the first a miscarriage at 2 months, the second a normal delivery, the third an incomplete abortion at 3 months. At the fourth pregnancy, which went to term she had a post partum haemorrhage. She was transfused with 3 pints of blood and 2 pints of plasma on 23 June 1968. The infant died at 2 months of a chest infection. The fifth pregnancy in 1971 was a full-term normal delivery, but the infant is now asthmatic.

AK developed asthma for the first time in October 1968 (4 months after her fourth pregnancy). The trigger factors were exertion, exposure to dust or coke fumes and respiratory infection.

Her first admission to hospital for asthma was in November 1968—chest X-ray at that time was normal. Sputum contained *Streptococcus pneumoniae*. She was discharged 7 days later after a course of antibiotics and bronchodilators. She was readmitted in April 1969 for 3 days—chest X-ray still normal.

In December 1969 she developed her first attack of urticaria, angioedema and asthma with coitus. There were no prodromal symptoms or signs. She was perfectly well for 2 hr after coitus with no local or vaginal irritation or swelling. She then developed a blotchy rash on her abdomen and chest, spreading to involve the whole body within 30–60 min. Her eyes, lips and tongue were swollen, also her face. A cough developed with tightness in her chest and wheezing. The signs increased in severity over

the next 2 hr. Subcutaneous 1/1000 adrenaline gave partial relief, greatly improving for a further 24 hr, the rash and swollen face clearing within 3 days.

There were four such incidents, each time after coitus. The wearing of a condom by the husband prevented these signs.

She was first seen in December 1970. She was 4'11" in height, weight 9 st. 7 lb. No abnormal signs in pelvis or chest. Peak flow 350 l/min. She was skin prick tested to all common allergens and foods. She and her husband would not agree for her to be tested with her husband's semen.

Her husband had a vasectomy which they thought would prevent the reaction. Unfortunately this was not so.

In June 1972, April and May 1973 she showed strong prick-test responses to seminal plasma and subsequently a course of desensitization carried out with a preparation of her husband's seminal plasma (details in next section).

AK was not seen in 1975 to April 1976. For the past year she had unprotected coitus without adverse effects. However, she was now being investigated in another hospital for a suspected peptic ulcer following recurrent attacks of vomiting. Skin prick tests gave strong positive reactions to milk and milk products, egg yolk and meat. She was recommended to avoid milk and dairy products. The investigations for peptic ulcer were negative, and she accepted in 1981 that milk and dairy products induced the vomiting. This was confirmed by repeated elimination and challenge by different foods: she is still intolerant of milk and dairy products.

Prick-test results and desensitization to seminal plasma

In December 1970, when first seen, prick tests were strongly positive to dust mite *Dermatophagoides pteronyssinus* (18 mm) and *D. farinae* (10 mm), animal hair (cat 10 mm, dog 10 mm) but negative to horse, rabbit, wool, feathers, grass pollen, trees, moulds and foods (milk, nuts, eggs, wheat, yeasts, fish and fruits).

In June 1972, a post-vasectomy semen sample from her husband diluted in two volumes of saline, Seitz-filtered, and further diluted, elicited positive prick tests at 1:20 000 dilution, and an intradermal test response at $1:1 \times 10^6$. The reactions developed within 2–3 min, and subsided within 4 hr. Some blotchiness of the face and arms occurred within 20 min; those on the arms clearing within 4 hr, those on the face persisting 24 hr. There was delayed swelling of the forearm. A drop of the seminal plasma placed on the skin surface, on the vulva and on the vagina elicited no local swelling or irritation.

In April 1973, further prick tests were made with two post-vasectomy seminal plasma samples from two other males. Both induced large reactions immediately which were positive at 1:20 000 dilution. The prick-test weals cleared within 2 hr, but

A further seminal plasma sample from her husband was obtained in May 1973. This was the preparation used to prepare the desensitizing solution. This sample also elicited a prick-test response at 1:20 000, and an intradermal response at $1:1 \times 10^6$ dilution. The local reaction cleared within 2 hr but swelling of the forearm to the wrist persisted for 12 hr. One and a half hours after the skin test, she started to cough and wheeze, and was given adrenalin subcutaneously. The chest signs cleared within 20 min.

Prick test with dilutions of her husband's seminal plasma on three other atopic persons induced no positive response.

The desensitizing preparations of her husband's seminal plasma were prepared

with the help of Dr W. Frankland of St Mary's Hospital. The strengths of the solutions prepared were 1, 10, 100, 1000, 10 000 and 100 000 Noon units. The doses given were 0.1, 0.2, 0.4, 0.6 and 0.9 ml from each preparation, injected subcutaneously twice weekly, for 6 months; then each week for a further 6 months, and monthly for a further 6 months. The maximum dose was 0.15 ml of the 100 000 Noon unit concentration. When AK was seen in April 1976 she reported that she was 'normal' and unprotected coitus no longer induced allergic signs; this state continues in 1984.

Preliminary preparation of the allergen-containing fraction of seminal plasma

The preparation of the allergen fraction followed the examination of the patient previously examined for anaphylactic allergy to seminal plasma (Frankland & Parish, 1974). The IgE antibody from that patient was used as the reagent to detect which fractions of seminal plasma contained the allergen.

Fractions from Sephadex G200 columns were further fractionated by electrophoresis on cellulose-acetate membranes, treated with a globulin fraction of the patient's serum, and after thorough washing with ^{125}I -labelled anti IgE (IgG fraction).

A fraction of 20 000–40 000 daltons molecular weight, contained a component with the greatest binding for IgE. A major and minor band had most of the binding activity; three other components had little or no binding activity. The major band stained with ninhydrin, and with periodic acid Schiff, which was interpreted to indicate a glycoprotein. The band had little binding activity for IgG anti-seminal plasma, and almost no affinity for a non-antiglobulin IgG fraction, therefore the binding was not due to Fc. The fraction of 40 000–160 000 daltons also bound some IgE, but this was weaker than the sample termed the allergenic fraction.

The allergenic fractions used to test serum of the new patient K, obtained from donor pools of seminal plasma, or from her husband, were fractions of about 20 000–40 000 molecular weight, and tested to contain allergen by binding IgE from the previous patient. Fractions of greater molecular weight were not tested.

Assays of each immunoglobulin class

The amounts of IgG, IgA, IgM and IgE from 1972 to 1981, with two or three samples per year for 3 of the years were all within the normal range. There is a tendency for the amounts of IgA to be within the lower range of normal (1.28–1.81 mg IgA/ml) and for the IgM to be significantly in the higher range of normal (normal amount about 0.9 ml IgM/ml). The amounts of IgM were consistently large (160–200 IU/ml).

The amounts of IgE were within the normal range throughout.

Desensitization did not greatly influence total immunoglobulins apart from a slight increase of IgG in the sample taken in 1974.

Tests for IgG and IgE anti-seminal plasma antibodies

There were no significant titres of agglutinating antibody to suspensions of washed spermatozoa (Table 1).

The very discriminating tanned-cell agglutination test with pooled whole seminal plasma as antigen, showed very little evidence of anti-seminal plasma antibody (Table 1).

The red-cell-linked antigen-antiglobulin test using the allergen fraction of seminal plasma showed no significant amount of agglutinins in the direct test, but there were significant, though low, titres of non-agglutinating IgG antibody detected by anti-total

Table 1. Agglutinins to washed spermatozoa and to seminal plasma, and RAST IgE anti-seminal plasma in serum of patient K (1972-1981)

Year	Sample	Sperm aggl'n	TCT	Seminal-plasma antibody RCLA-AR			RAST ratio
				Direct	anti γ	anti G ₁	
1972	i	8	16	2	64	16	19.2
	ii	8	8	2	32	32	21.6
1973	i	32	8	4	32	8	22.0
	ii	—	—	—	—	—	20.4
Desensitization							
	iii	128	512	—	—	—	23.8
1974	i	—	—	—	—	—	17.1
1976	i	—	16	—	—	—	1.1
	ii	—	4	—	—	—	1.0
1981	i	—	32	—	—	—	1.2

— signifies no test done; TCT: tanned-cell agglutination test; RCLS-AR: red-cell-linked antigen-antiglobulin reaction; RAST ratio: counts (dpm or cpm) test serum:control normal serum; maximum variation of tests in duplicate or triplicate 4.7%. RAST 1972-1974 tested against seminal-plasma allergen fraction; samples of 1976 and 1981 against whole seminal plasma.

IgG (γ) antiglobulin. Much of this appeared to be of the IgG₁ subclass (Table 1). No IgG₄ subclass antibodies were detected, and tests for IgM antibody were negative or inconclusive.

Sera of twenty-three young adults tested in the same month by RCLA-AR with the same allergen fraction of seminal plasma were all negative.

Large amounts of IgE antibody to the allergen fraction of seminal plasma were detected between 1972 and 1974. The results expressed as the ratio of dpm for the serum of K/dpm of a pool of normal sera showed that there was between 19.2 and 22.0 times the amount of IgE antibody in serum samples of patient K than the background binding in the normal sera (Table 1). There was a slight, though possibly insignificant increase after desensitization had started, and a lesser amount in 1974. The results of different samples taken each year show similar results, and the maximum variation in counts in tests on any one sample of serum against the allergen fraction was 4.7%. The RAST on samples taken in 1976 and 1981 differed in that the tests were made against diluted whole seminal plasma, and the ratio determined by comparing counts per minute (cpm) instead of disintegrations per minute.

Desensitization was associated with higher titres of sperm and seminal plasma agglutinins, but later serum samples showed that seminal-plasma agglutinins determined by TCT returned essentially to their first observed titres.

Histamine release from peripheral-blood leucocytes

One sample of the patient's blood leucocytes taken before desensitization, was tested for histamine release. The blood sample contained 1.7% basophils of the total leucocytes.

The cells of K released 21% histamine (19.8 and 21.9% in duplicate samples) when tested with the saline extract of whole seminal plasma at 5 µg/ml. The optimal concentration of the allergen extract was 2 µg/ml which released 27% histamine (26.1 and 28.3% in duplicate samples) though at 20 µg/ml the amount of histamine released, 23%, was not much less than that of the optimum concentration (Table 2).

Leucocytes of four married normal women and of two males tested at the same time with the same antigen preparations, showed no significant release of histamine.

The cells of K did not release more histamine spontaneously than did the cells of the other donors all in the range of 2.8–5.3%.

Seminal-plasma stimulation of lymphocyte transformation (TdR incorporation)

The lymphocytes taken before desensitization, of patient K, tested with a suboptimal amount (2 µg/ml) and a slightly above optimal amount (5 µg/ml) of PHA incorporated similar amounts of [³H]TdR as lymphocytes of a normal male and two normal females tested at the same (Table 3) and within the usual range determined in this laboratory. Patient K also responded to the suboptimal (5 µg/ml) and optimal (10 µg/ml) amounts of *Candida albicans* antigen though this response was slightly greater than normal; nevertheless it was still below that of donor F2.

Lymphocytes of patient K were strongly stimulated by the seminal-plasma allergen fraction at 2 and 20 µg/ml. At 2 µg/ml the allergen fraction induced 47.7-fold, and the 20 µg/ml fraction 105.4-fold greater response than background. Two of the control donor cells showed increases from 1.6- to 2.0-fold above their background, non-antigen treated, resting lymphocytes (Table 3). Donor F2 showed a maximum response of 19.4-fold, significantly less than that of the cells of K.

The response to the saline extract of whole unfractionated seminal plasma, was much less than that observed for the allergen fraction. Furthermore the more concentrated preparation (50 µg/ml) of whole plasma stimulated less incorporation of TdR than did the weaker (2 µg/ml) preparation of the allergen fraction. There was a weaker response to the 50 µg/ml seminal-plasma antigen than to the 5 µg/ml.

Table 2. Histamine release from blood leucocytes of patient K and of seven control persons on treatment of cells with seminal plasma, or its allergenic fraction

Test antigen	Dilution	% net histamine release						
		Cell donors						
		K	F1	F2	F3	F4	M1	M2
Seminal plasma	1/100	21	6	4	5	4	7	6
Saline extract	1/1000	14	4	5	3	6	5	6
500 µg/ml								
Allergen	1/10	23	5	6	4	5	7	8
fraction	1/100	27	6	5	6	3	4	7
200 µg/ml	1/1000	18	3	4	4	3	2	4
	1/10 000	9	7	6	7	5	5	6

Leucocytes from patient K. Four normal female donors F1–4 and two male donors M1–2.

Table 3. Tritiated-thymidine incorporation by lymphocytes of patient K and of three normal donors when cells treated with PHA, *Candida albicans*, whole seminal-plasma and its allergenic fraction

Disintegrations per min following tests with											
Lymphocyte donor	Nil 0*	PHA		<i>Candida albicans</i>		Seminal-plasma whole extract		Seminal-plasma allergen fraction		Seminal-plasma antigen ratio/nil antigen	
		2	5	5	10	5	50	2	20	2	20
K	284 ± 19	22 010 ± 986	31 233 ± 1248	17 832 ± 721	23 205 ± 938	3837 ± 172	1092 ± 58	13 536 ± 792	29 927 ± 1024	× 47.7	× 105.4
M1	191 ± 17	18 468 ± 540	27 434 ± 723	6391 ± 298	9242 ± 455	212 ± 19	289 ± 22	309 ± 24	326 ± 28	× 1.6	× 1.7
F1	261 ± 28	12 837 ± 301	21 928 ± 497	14 812 ± 634	17 261 ± 788	296 ± 13	210 ± 16	478 ± 31	512 ± 35	× 1.8	× 2.0
F2	429 ± 38	28 437 ± 1240	61 211 ± 3812	22 313 ± 906	38 629 ± 1872	772 ± 48	816 ± 63	6212 ± 412	8315 ± 639	× 14.5	× 19.4

* µg/ml.

Lymphocytes from patient K, normal male M1, normal females F1 and F2.

PHA counts made after total 72-hr incubation. Antigen counts after 5 days. All tests pulsed for 4 hr with [³H]TdR.

All tests were done in triplicate.

Responses induced in the lymphocytes of the control donors showed little difference between the two concentrations of plasma antigen. Nevertheless lymphocytes of K showed a significant stimulation to the whole antigen, compared to donors M1 and F1. The cells of F2 appeared hyper-responsive to the mitogen and all antigens.

Seminal-plasma stimulation of lymphocyte synthesis of neutrophil migration inhibition factor

The cell-free culture fluids following incubation of lymphocytes of patient K, before desensitization, and of normal donors M1, F1 and F2 with the allergen-containing fractions of seminal plasma at a concentration of 20 µg/ml, were tested for inhibition of migration of neutrophils taken from another male. Two seminal-plasma allergen fractions were prepared, one from the husband (H) and one from the pool used in the previous tests (M).

The lymphocytes of K, incubated with antigen from her husband and from the pool sample, synthesized a product that much inhibited migration of neutrophils. The inhibition was consistent with little variation in results of tests made in triplicate. Prior treatment of the lymphocytes with puromycin before exposure to antigen abolished the inhibition, indicating that active cell synthesis was necessary for the generation of the migration inhibitor. The value for migration of neutrophils exposed to fluid from puromycin-treated lymphocytes was approximately the same as that of neutrophils which received no lymphocyte-culture fluid treatment. Lymphocytes not stimulated by seminal plasma released no substance inhibiting migration of neutrophils, and possibly weakly stimulated migration, because the neutrophils untreated by fluid from lymphocyte cultures did not migrate as far as those incubated with medium from unstimulated lymphocytes (Table 4).

Table 4. Migration inhibition tests on purified neutrophils from a normal donor with supernatant culture fluids from lymphocytes treated with seminal-plasma allergenic fraction

Antigen source of seminal plasma	Lymphocyte test	K lymphocytes		M1 lymphocytes		F1 lymphocytes		F2 lymphocytes	
		Migration index	Well dif. %	Migration index	Well dif. %	Migration index	Well dif. %	Migration index	Well dif. %
H	+	0.42	3.2	0.92	4.3	1.15	5.2	1.20	5.8
M	+	0.38	2.8	1.10	3.8	1.26	4.6	1.24	5.2
H	+ puromycin	0.97	4.7	0.91	2.1	0.94	2.6	1.00	2.1
M	+ puromycin	0.95	3.8	0.93	3.3	0.91	1.8	0.92	2.3
O	+	1.00	4.6	1.00	4.1	1.00	2.2	1.00	2.1
O	+	1.16	4.1						
O	O	0.91	5.0						
O	O	0.88	5.3						

Test on lymphocytes from patient K and normal donors male 1 and females 1 and 2.

Seminal-plasma antigen fraction of husband H, or normal pool M + lymphocyte in culture with puromycin; addition of puromycin to lymphocyte culture. O, no antigen or no lymphocytes.

Well dif. %: difference in diameter in three wells of greatest and least migration.

Lymphocytes from normal donors M1, F1 and F2 treated with seminal-plasma antigen fraction generated no product-inhibiting migration of the neutrophils (Table 4). In tests on lymphocytes from these donors there was slight stimulation compared to the activity of media of lymphocytes pre-treated with puromycin.

Lymphocytes of donor F2 generated no product-inhibiting migration of neutrophils, though incorporating more TdR when stimulated by seminal-plasma antigen in the previous test.

There was no evidence of carry-over of puromycin from the treated lymphocytes affecting the subsequent test of the culture fluid on the neutrophils.

IgE and IgG antibodies to milk antigens

By 1976, K had developed allergy to milk and gave strong prick-test responses to milk. Samples of her sera taken in 1976 and 1981 were examined for antibodies to milk.

Tests for IgE using Pharmacia milk discs in two serum samples taken in 1976 and one in 1981 showed all three to have a RAST score of 3, indicating a high level of allergen-specific IgE.

Tests on the same samples by ELISA with monoclonal anti-IgG subclass reagents showed that in 1976 antibody was present in all subclasses to bovine serum albumin, and with the exception of IgG₂, also to α lactalbumin. In 1981 antibody to these antigens was found only in two subclasses, IgG₂ and IgG₄ to BSA, and IgG₃ and IgG₄ to α -lactalbumin. No antibody was found to β lactoglobulin or to human serum albumin in concomitant tests on all three samples.

Discussion

Despite the many antigens in semen, some of which are potent when present in other body fluids and introduced by non-genital routes (e.g. blood groups A and B antigens in secretors), and despite the frequency of exposure, anaphylactic allergy to semen is rare, even in atopic subjects. However, it may occur, and most reports have several

features in common. The patient is atopic, the response tends to be severe with generalized urticaria, angioedema, asthma and occasionally shock, the antigen is present in seminal plasma of any male and is variously described as a basic protein or glycoprotein with a molecular weight of about 20 000–30 000 daltons. The patients show prick-test-positive reactions to the seminal plasma, and IgE anti-seminal plasma antibody has been demonstrated by a variety of techniques (Halpern, Ky & Robert, 1967, one case; Levine, Siraganian & Schenkein, 1973, one case; Frankland & Parish, 1974, one case; Schultz, Shirren & Kueppers, 1974, one case; Mikkelsen *et al.*, 1975, one case; Reunala *et al.*, 1977, two cases; Mumford *et al.*, 1978, one case; Kroon, 1980, six cases; Mathias *et al.*, 1980, one case; Bernstein *et al.*, 1981, four cases).

Our patient K had IgE antibody to seminal plasma and was extremely allergic to this antigen, her lymphocytes were sensitized to the same antigen. Despite the extreme sensitivity she was successfully desensitized by injection of her husband's seminal plasma.

The seminal-plasma allergenic fraction, that bound the greater part of the IgE antibody of our previous patient (Frankland & Parish, 1974) had a molecular weight in the range of 20 000–40 000 daltons, and on the results of the staining tests appeared to be a glycoprotein, but the carbohydrate could have been co-eluted with the protein. The allergen used in our investigation appears similar to that reported by Levine *et al.* (1973), Siraganian *et al.* (1975) and Mikkelsen *et al.* (1975), with a molecular weight in the region of 20 000–30 000 daltons, and by Schultz *et al.* (1974) between 14 000 and 18 000 daltons. Halpern *et al.* (1967) considered the antigen to be a glycoprotein, staining strongly with periodic-acid Schiff reagent and containing much sialic acid. Siraganian *et al.* (1975) reported the antigen to be unaffected by enzymes degrading carbohydrates, but susceptible to proteases, and to be of prostatic origin. A preliminary examination of the fractions of higher molecular weight indicated some weaker binding, but the specificity of this was not examined further.

The responses to the skin tests with seminal plasma, were on occasion intense and persistent, with more generalized signs, indicating a high degree of sensitivity. This was confirmed by the detection of IgE antibody to the seminal-plasma allergen fraction by RAST, with 19–23 times binding than the control pool serum, which contained approximately the same amount of total IgE as did that of the patient K. The IgE antibody-mediated sensitivity was further confirmed by the much greater amounts of histamine net released from the peripheral-blood leucocytes than detected from six control cell preparations examined at the same time (Table 2). Seminal-plasma-stimulated histamine release from blood leucocytes was also reported in the one case described by both Levine *et al.* (1973) and Siraganian *et al.* (1975), in one case examined by Mathias *et al.* (1980) and two of the four cases reported by Bernstein *et al.* (1981).

The severity and persistence of the skin-test responses of K to seminal plasma prompted examination of her lymphocytes for evidence of delayed hypersensitivity to this substance. Her lymphocytes showed strong responses to seminal-plasma antigens as determined by incorporation of TdR and by generation of neutrophil inhibition of migration factor (NIF). The culture conditions using an acceptable human serum in the medium, without fetal calf serum, horse serum or antibiotics, avoided the potential for stimulation by these substances for the cells of an atopic person. The allergenic fraction that bound the IgE anti-seminal plasma antibody, also stimulated the lymphocytes of patient K. As tests with other fractions were not done, it cannot be

concluded that this fraction contained the most potent, or the only, plasma antigen capable of stimulating K's lymphocytes. The lymphocytes of K showed a normal response to the mitogen PHA, and a slightly above normal reactivity to the common commensal antigen of *Candida albicans*.

Lymphocytes of K stimulated by plasma fraction antigen, synthesized a product that inhibited migration of neutrophils from a normal donor. Generation of this product was abolished by treatment of the lymphocytes with puromycin before addition of the antigen. Lymphocytes from three other donors tested at the same time showed no such activity for the neutrophils, and the similarity of the migration of the neutrophils tested with these culture fluids, and those of the non-puromycin, non-antigen controls indicated no transfer of puromycin (Table 4).

The cells from normal donor F2 (a non-allergic married woman with two children) showed a greater responsiveness to PHA and to *Candida albicans* than normally seen in this laboratory in tests with the same batches of reagents. It would appear that the cells of F2 were generally hyper-responsive to stimulation, though she was apparently clinically normal.

In previous reports on lymphocyte responses to semen, Mumford *et al.* (1978) detected lymphocyte transformation to spermatozoa but not to seminal plasma in one patient, and Mathias *et al.* (1980) found no evidence of lymphocyte transformation in cells of one patient tested with dilutions of whole semen. In another study, two of four patients showed lymphocyte responsiveness; the cells of one had positive lymphocyte transformation tests to extracts of sperm to whole seminal plasma and fractions thereof, the cells of the other responded only to extracts of sperm; the cells of the first patient treated with the spermatozoal extract formed a product that inhibited migration of guinea-pig macrophages (Bernstein *et al.*, 1981). It was not stated whether the lymphocyte stimulation was greater or less in tests with whole plasma than with its fractions.

In our tests on antigen-stimulated TdR incorporation, the response of lymphocytes to patient K to saline extract of whole seminal plasma was much less than that to the seminal-plasma allergen fraction. Furthermore, stimulation was less at 50 $\mu\text{g/ml}$ concentration than at 5 $\mu\text{g/ml}$. The cells of the control donors also showed less response to the whole plasma than to the allergenic fraction, but for donors M1 and F1 there was no significant difference between the background, nil antigen cell preparations and those treated with the seminal plasma. This phenomenon was not further examined.

Subsequent to our investigation it was reported that seminal plasma contains a substance that inhibits *in vitro* lymphocyte activation by mitogens and by antigens (Stites & Erickson, 1975; Prakash, Coutinho & Möller, 1976; Lord, Sensabaugh & Stites, 1977; Marcus *et al.*, 1978). It is considered that this substance contributes significantly to the immune tolerance of women to semen. The substance is believed to have a molecular weight of greater than 100 000 daltons (Prakash *et al.*, 1976) or greater than 200 000 daltons (Lord *et al.*, 1977) and to be active at a concentration of 1/50 or greater dilutions of seminal plasma.

Based on the above evidence, it is probable that use of the allergen fraction that particularly binds IgE and which contains substances in the range of 20 000–40 000 daltons molecular weight, enabled us to detect the strong lymphocyte responsiveness to seminal plasma in cells of K, and that the weaker activity in tests with whole extract of seminal plasma was due in part to the much larger weight inhibitor. The failure to

detect lymphocyte transformation to whole semen or seminal plasma in the tests of Mumford *et al.* (1978), Mathias *et al.* (1980) and in at least one patient of Bernstein *et al.* (1981) may have been due to this inhibitor. The lymphocytes of two patients are reported to show transformation on tests with spermatozoa, which do not contain inhibitor, though not with semen or seminal plasma, which would contain inhibitor (Mumford *et al.*, 1978; Bernstein *et al.*, 1981).

The successful desensitization of our patient K made it impossible to investigate further the possibility that her lymphocytes were less receptive to the inhibitor than those of women not allergic to seminal plasma, the possibility that the inhibitor modified T-helper cells, and the possible relation between the lymphocyte susceptibility to the inhibitor and IgE synthesis. It is of interest that this patient K, and the previous patient allergic to seminal plasma examined in this laboratory (Frankland & Parish, 1974) both had IgE antibodies to the same seminal-plasma antigen, fraction, but insignificant amounts of anti-semen IgG or IgM antibodies.

Our patient K was successfully desensitized by a course of injections of diluted samples of her husband's seminal plasma, and thereafter practised unprotected coitus without adverse effect. No maintenance desensitizing injections were required. Another successful desensitization has been reported, but at the time of the report, a maintenance intradermal injection of seminal plasma was required once every 3 weeks (Mathias *et al.*, 1980).

Following the successful desensitization by injections of seminal plasma, patient K presented again 2 years later with clinical food allergy, and positive prick tests to milk, eggs and meat. She had a high RAST score for IgE antibody to milk and IgG subclass antibodies to two of four milk proteins. It is possible that this was a spontaneous late manifestation of her atopic state. It is, however, conceivable that the milk sensitivity resulted from the desensitization. A monoclonal antibody against a seminal-plasma antigen detected the same antigen in human milk (Shigeta *et al.*, 1980), and reference is made to lactoferrin also being present in seminal plasma and human milk. If similar antigens also occur in cow's milk, it is possible that the injection of human seminal plasma induced, or predisposed, to the sensitivity to milk. We did not examine any antigenic similarities between the seminal plasma of cow's milk protein, and if it did occur the desensitization would not have induced the sensitivity to egg and meat.

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