Inhibition of human seminal fluid DNA polymerase by an IgG fraction of seminal plasma from vasectomized men

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Summary. Immunoglobulin G (IgG) was isolated from ejaculates of intact and vasectomized men by precipitation with ammonium sulphate and DEAE-cellulose ion-exchange chromatography. Velocity centrifugation revealed that all of the IgG from intact males was 7S protein while <40% of the seminal IgG of vasectomized men co-sedimented with the 7S marker; the remaining, immunologically unidentifiable, protein was considerably smaller and heterogeneous in size. Only the 7S IgG from the post-vasectomy ejaculates inhibited the activity of a DNA polymerase from the seminal fluid of an intact male. These results suggest that formation of antibody reactive with the seminal fluid DNA polymerase is one manifestation of a vasectomy-associated autoimmune response in man.

Introduction

In men, humoral and cell-mediated autoimmune responses are known to result from exposure to autologous spermatozoa (Cochran, 1974; Hekman & Rumke, 1976; Mettler, Gradl & Scheidel, 1976; Katsh & Katsh, 1977). Although such auto-sensitization to sperm antigens may occur under pathological conditions, vasectomy, a procedure widely employed as a method of fertility control, produces similar effects. Morphological alterations within the male reproductive tract, including granuloma formation and macrophage infiltration (Edwards, 1976), as well as sperm-directed auto-immunological reactions, are frequent consequences of vasectomy (Ansbacher, 1971; Coombs, Rumke & Edwards, 1973; Shulman, 1974; Alexander, 1975, 1976, 1977; Alexander & Schmidt, 1977). Antibodies to sperm nuclear antigen(s) can develop after vasectomy (Tung, 1975) and at least one sperm auto-antigen has been identified as protamine (Kolk, Samuel & Rumke, 1974). Auto-antibodies may be produced to previously sequestered sperm antigens because of the accumulation and phagocytosis of spermatozoa after vasectomy (Edwards, 1976), or perhaps in response to extravasation and granuloma formation.

Human seminal fluid and membrane-free sperm nuclei contain DNA-synthesizing complexes which are antigenically related (Witkin & Higgins, 1978), ribonuclease-sensitive and actinomycin D-resistant (Witkin, Korngold & Bendich, 1975; Witkin & Bendich, 1977). Analysis of semen from the same donors before and after vasectomy revealed a 3–4-fold post-operative decrease in the DNA-synthesizing activity of cell-free seminal fluid (Witkin & Higgins, 1978). Since the enzymic properties of the seminal fluid complex could be inhibited by antiserum to purified sperm nuclei (Witkin & Higgins, 1978), this suggested that the decrease in seminal fluid DNA-synthesizing activity might be due to the presence of DNA polymerase auto-antibodies. To test directly whether immunoglobulins capable of inhibiting the activity of the seminal fluid complex were present in the semen of vasectomized men, immunoglobulin G (IgG) was purified from the cell-free seminal fluid of intact and vasectomized males, and its effect on DNA polymerase activity was tested.

Materials and Methods

Preparation of seminal IgG

Multiple ejaculates were obtained from 10 healthy medical student donors. Seminal fluid from vasectomized men was obtained from the Margaret Sanger Center, Planned Parenthood of New

York City, Inc. An equal volume of glycerol was added to each fresh specimen before storage at -20°C . After thawing, 30 samples of each type of semen were pooled to give about 100 ml semen-glycerol.

Cellular elements were removed from the seminal fluid of vasectomized men (V) and whole semen (W) by centrifugation at 2000 *g* for 20 min. The supernatants were further clarified by re-centrifugation at 15 000 *g* for 20 min. The resulting supernatants were brought to 33 % saturation with neutral ammonium sulphate (Campbell, Garvey, Cremer & Sussdorf, 1970), stirred for 4 h at 5°C , and centrifuged at 5000 *g* for 20 min. The pellets were resuspended in 5 ml 0.01 M-sodium phosphate buffer, pH 7.5, and dialysed exhaustively against this buffer. The dialysed preparations were centrifuged at 10 000 *g* for 20 min at 4°C and subjected to chromatography on DEAE-cellulose (Campbell *et al.*, 1970). Fractions contributing to the single peak recoverable by ion-exchange chromatography were pooled, concentrated to 1 mg protein/ml with the use of Millipore Immersible Separators and dialysed against 0.1 M-Tris-HCl buffer, pH 7.5. Protein concentration was determined by the method described by Shatkin (1969). Immunological identification of IgG was by agar double-diffusion in Immunoplates (Pattern D: Hyland Laboratories, Costa Mesa, California) and immunoelectrophoresis in 1 % (w/v) agarose with Beckman B-2 barbiturate buffer, pH 8.6, at 20 mA and 200 V for 50 min. Antisera raised in goats to whole human serum and human IgG were purchased from Cappel Laboratories, Cochranville, Pennsylvania. Antisera to human IgM and IgA were obtained from Behring Diagnostics (Woodbury, New York). Precipitin patterns were stained with amido-black 10B (Lardinois & Page, 1969) after incubation at 37°C for 72 h. The isolated IgGs were layered on 4 ml 10–40 % (w/v) sucrose/phosphate-buffered saline gradients, and centrifuged at 165 000 *g* at 4°C for 18 h. The bottom of each tube was punctured with a 21-gauge needle and 5-drop fractions were collected. The optical density of the fractions at 280 nm was determined with a Beckman DU spectrophotometer. Peak absorbance fractions were pooled, dialysed against 0.1 M-Tris-HCl, pH 7.5, and examined for DNA polymerase inhibitory activity. Aliquots of these fractions were adjusted to 0.4 mg protein/ml Tris buffer for immunochemical analysis.

Seminal fluid DNA polymerase isolation

Purification of the seminal fluid DNA polymerase from a single normal ejaculate has been described in detail elsewhere (Witkin & Higgins, 1978). Briefly, the semen was diluted 3-fold in 0.01 M-Tris-HCl, pH 7.5, 0.15 M-NaCl, 2 mM-EDTA, and the cell-free seminal fluid was obtained by successive centrifugations at 1500 *g* and 16 000 *g*. A particulate fraction was isolated from the last supernatant by sedimentation through an 18 % (w/v) Metrizamide solution (Accurate Chemical and Scientific Corp., Hicksville, New York). DNA polymerase in the Metrizamide pellet fraction was solubilized with 0.5 % Triton X-100, 0.8 M-KCl, 0.01 M-dithiothreitol and fractionated by gel filtration on Ultrogel Aca 44 (12 000–130 000 molecular weight fractionation range; LKB Instruments Inc., Rockville, Maryland). One peak of DNA polymerase activity, assayed with the synthetic template dT_{12–18}·poly rA (P-L Biochemicals, Milwaukee, Wisconsin), was identified just after the void volume. The peak fractions were diluted with an equal volume of glycerol and stored at -20°C .

Inhibition of DNA polymerase activity

The effect of IgG on the seminal fluid DNA polymerase activity was assessed in two ways. In the first, IgG isolated from the seminal fluid of vasectomized males was mixed in various proportions with IgG derived from the serum of a normal male donor, with no previous history of surgery, so that each sample tested contained a total of 50 µg IgG. In the second test, various concentrations of IgG derived from the seminal fluid of intact or vasectomized males were tested in the absence of serum IgG from a normal male. The seminal fluid enzymes were partly purified by gel filtration (see above) and were incubated with IgG, 0.15 M-KCl and 0.01 M-dithiothreitol at 0°C in a total volume of 75 µl. After 150 min, the samples were diluted with an equal volume of the DNA polymerase reaction mixture (0.32 mM-dATP; 0.04 M-Tris-HCl, pH 7.5; 2 mM-MnCl₂; 0.75 µg dT_{12–18}·poly rA; 7 µCi [³H]dTTP (sp. act. 46 Ci/mmol: New England Nuclear, Boston, Massachusetts)) and incubated at 30°C for 20 min. The reactions were terminated by the addition of a 100-fold excess of ice-cold 5 %

(v/v) trichloroacetic acid containing 1% (w/v) sodium pyrophosphate. After 15 min at 0°C, acid-insoluble products were collected on glass-fibre filters and the radioactivity was determined (Witkin & Bendich, 1977).

Virus and viral disruption

Simian sarcoma virus (SSV) was generously given by Dr Harry Snyder. To release the reverse transcriptase, purified virus (2 µg protein) was disrupted in 380 mM-KCl, 0.3% Triton X-100 and 10 mM-dithiothreitol by incubation at 4°C for at least 60 min. To test for IgG-mediated inhibition of viral reverse transcriptase activity, the disrupted viral suspension was diluted 2.5-fold in solutions of 0.05 M-Tris-HCl, pH 7.5, containing various IgG concentrations. After incubation at 4°C for 150 min, exogenously templated (dT₁₂₋₁₈·rA) polymerase activity was assayed as described for seminal fluid.

Results

Isolation of IgG

The results of sucrose velocity sedimentation of aliquots of the IgG fractions obtained from Samples W and V are shown in Text-fig. 1. The IgG derived from the seminal fluid of intact males (Sample W) sedimented as a single 7S peak, but that from the vasectomized men (Sample V) gave a 7S peak and another peak of more slowly sedimenting material. Immunoelectrophoretic and agar double-diffusion analyses of the pooled 7S fractions of each velocity sedimentation and the more slowly sedimenting fraction of Sample V confirmed the identification of the 7S material in both samples as IgG (Pl. 1, Figs 1 and 2). No additional precipitin arcs or spurs were seen after staining the precipitin patterns and there was no reaction with anti-IgM or anti-IgA. The material in fractions 7-9 of Sample V could not be identified by our immunological tests.

A total of 1.5 mg protein, all apparently 7S IgG, was isolated from approximately 50 ml Sample W. Of the 1.1 mg protein obtained from the corresponding fraction of a similar volume of Sample V, only 0.4 mg was 7S IgG; the remaining 0.7 mg was protein of a considerably smaller S value.

Inhibition of DNA polymerase activity

IgG derived from normal male serum and Sample V were combined in various proportions to a total of 50 µg IgG present in each assay and tested for their effect on the seminal fluid DNA polymerase. The degree of enzyme inhibition was clearly proportional to the amount of Sample V IgG in the reaction mixture (Table 1).

Table 1. Inhibition of seminal fluid DNA polymerase activity by IgG derived from the semen of vasectomized men

Conc. of IgG (µg)		DNA polymerase activity*
From seminal fluid of vasectomized men	From serum of intact men	
0	50	880
10	40	721
20	30	176
30	20	158
40	10	132
50	0	88

* As c.p.m. of acid-insoluble product (see text). The value of 100 c.p.m. for the polymerization of [³H]TTP in the absence of added enzyme has been subtracted from each value.

Text-fig. 1. Velocity sedimentation of the IgG isolated, by ammonium sulphate precipitation and DEAE-cellulose ion-exchange chromatography, from the semen of intact (○) and vasectomized (▲) men.

To test whether the inhibition of seminal fluid DNA polymerase activity was specific to the seminal fluid IgG of vasectomized males or was also present in that from intact men, the purified 7S IgGs from both sources, as well as the <7S fraction of Sample V (see Text-fig. 1), were used individually. Because of the small amount of 7S IgG present in Sample V, concentrations up to 10 µg only were studied.

The 7S IgG of sample W and the <7S fraction of Sample V IgG did not inhibit the seminal fluid enzyme at concentrations of up to 15 µg (Table 2). In contrast, 10 µg of the 7S IgG from Sample V was sufficient to cause a 50% reduction in seminal fluid DNA polymerase activity.

Discussion

Increased serum titres of apparently naturally occurring antisperm antibodies and the appearance of an antibody directed against sperm nuclear antigen have been observed after vasectomy (Tung, 1975). Since the demonstration of the nuclear antigen required prior trypsin and dithiothreitol treatment of the spermatozoa, it is unlikely that the antigen was derived from seminal fluid or

EXPLANATION OF PLATE

Fig. 1. Immunoelectrophoretic analysis of fractions isolated from the seminal fluid of intact and vasectomized men by ion-exchange chromatography on DEAE-cellulose and sucrose velocity centrifugation. The antigen wells contained 10 µl of a solution containing 0.4 mg protein/ml of the following: (S) cell-free seminal fluid; (1) reference 7S serum IgG; (2) 7S IgG isolated from the semen of intact males (Sample W); (3) IgG-containing fraction of semen from vasectomized males (Sample V); (4) the 7S IgG fraction of Sample V; (5) the <7S fraction of Sample V. Electrophoresis was carried out at 20 mA and 200 V for 50 min and then 50 µl antiserum to (A) whole human serum or (B) human IgG were added to the troughs and the plate was incubated at 37°C for 72h.

Fig. 2. Agar double-diffusion test of the homology among the IgG isolates obtained from human seminal fluid. Designations are as for Fig. 1. Peripheral sample wells contained 10 µl IgG-containing fractions or the <7S component of Sample V. Central wells contained the antiserum to (A) whole human serum or (B) human IgG. The precipitin line of identity which formed among all the IgG-containing fractions attests to the antigenic similarity of the semen isolate relative to 7S serum IgG.

Fig. 1

Fig. 2

Table 2. The effect of various purified fractions of IgG from the semen of intact and vasectomized ram on seminal fluid DNA polymerase activity*

Conc. of IgG fraction (μ g)	IgG fraction		
	Intact men 7S	Vasectomized men	
		7S	<7S
0	724	701	735
8	716	692	796
9	—	470	—
10	—	315	—
13	742	—	765
15	721	—	773

* As c.p.m. of acid-insoluble product (see text). Duplicate experiments performed on an independent enzyme isolate indicated that 8 and 10 mg concentrations of the 7S seminal fluid IgG from vasectomized men reduced the level of incorporated [3 H]TTP from 1500 c.p.m. to 930 and 525, respectively, whereas the 7S IgG fraction of semen from intact men was without effect.

represented microbial semen contaminants and/or their constituent antigens absorbed onto the spermatozoon. It appears, therefore, that the acquisition after vasectomy of antibodies to sperm nuclear antigen(s), and possibly also to certain acrosomal antigens (Tung, 1976), may reflect true auto-immunization to sperm-specific antigens. Because the DNA polymerases of the sperm nucleus and the seminal fluid are related antigenically (Witkin & Higgins, 1978), the IgG class of antibodies inhibitory to the seminal fluid polymerase may have arisen as a result of a vasectomy-induced immune response to the sperm nuclear enzyme.

The immunoglobulins in seminal plasma consist mostly of IgG (Edwards, 1976; Friberg & Tilly-Friberg, 1976), are obtainable in reasonable quantity, and probably enter the seminal plasma by transudation, mainly in the prostate (Rumke, 1974). The serum antibodies directed to sperm nuclear components (Kolk *et al.*, 1974; Tung, 1975) have been identified as IgG and might therefore be expected to find their way into the seminal fluid. Semen rather than serum was selected as the antibody source in the present study because seminal fluid may contain locally produced antibodies (Husted & Hjort, 1975; Friberg & Tilly-Friberg, 1976) in addition to serum-derived immunoglobulin (Quinlivan & Sullivan, 1972; Friberg & Tilly-Friberg, 1976). Semen-derived immunoglobulins, therefore, should reflect both the general systemic IgG response to sperm antigens following vasectomy and any locally produced IgG which may not be represented in the peripheral circulation.

All of the IgG derived from the semen of intact males sedimented as 7S protein. In contrast, approximately 60% of the protein recoverable from the semen of vasectomized men was considerably smaller than 7S. This fraction did not inhibit the seminal fluid DNA polymerase activity and was not identifiable in the immunological tests. It may, however, represent IgG fragments degraded to the point of antigenic non-reactivity. Friberg & Tilly-Friberg (1976) have shown that, despite the considerable proteolytic activity intrinsic to seminal fluid, no degradation of seminal immunoglobulins occurs. Our results with IgG isolated from semen of intact men supports this observation. Whether the formation of this smaller material is an in-vivo consequence of vasectomy or a degradation product of storage *in vitro* is unknown.

The IgG from Sample V at concentrations of up to 50 μ g had no effect on the dT₁₂₋₁₈-rA-templated DNA polymerase activity of SSV (data not shown). This indicates that inhibition of the seminal fluid polymerase was antibody mediated and not due to the presence of contaminating protease in the IgG preparations.

Our results indicate that vasectomy leads to the formation of auto-antibodies, not detectable in the semen of the intact males studied, that are capable of inhibiting the activity of the seminal fluid

DNA polymerase. This inhibition may represent one consequence of auto-immunity after vasectomy. The effect of IgG from the seminal fluid and serum of vasectomized men on the DNA polymerases of spermatozoa and somatic cells is currently being studied.

The specific identity of the seminal fluid DNA polymerase activity is still uncertain. Its ability to utilize the exogenously supplied template-primer dT₁₂₋₁₈·rA suggests that it is similar to polymerase γ and retroviral reverse transcriptase.

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