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Research Article

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Sperm-related Antigens, Antibodies, and Circulating Immune Complexes in Sera of Recently Vasectomized Men

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ABSTRACT Sera from 35 men were collected before and at timed intervals subsequent to vasectomy and examined for the presence of (*a*) antibody reactive with human spermatozoa, (*b*) sperm-related antigen, and (*c*) circulating immune complexes (CIC). Fewer than 10% of the men examined were ever positive for antisperm antibodies. However, sperm-related antigens were elevated in the sera of 18, 18, and 26% of the mean at 2 wk, 2 mo, and 4 mo postvasectomy, respectively. CIC were detected in the sera of some vasectomized men by three different assays. The CIC in patients' sera were precipitated with polyethylene glycol, dissociated, and the individual CIC components identified by an enzyme-linked immunosorbent assay. Most, but not all, of the CIC contained antigen reactive with antisperm immunoglobulin (Ig)G and some also contained complement components C3 and/or Clq. IgA was identified in some of the CIC positive for IgG and sperm antigen and two men had IgM-containing CIC. Analysis of the CIC by sucrose gradient centrifugation revealed them to be heterogeneous in size.

INTRODUCTION

The severing of the vas deferens in healthy males (vasectomy) as a means of fertility regulation is currently a popular procedure. It is estimated that nearly 1 million vasectomies are performed annually in the United States (1). Spermatozoa continue to be produced after vasectomy (2) and since the normal outlet is now blocked, spermatozoa accumulate in the epididymis and proximal vas deferens (3). Eventually, sperm antigens leak out of the genital tract where they come into contact with immunologically competent cells. Because spermatozoa are autoantigenic (4) it is not surprising that ~50% of men eventually develop antibodies to spermatozoa after vasectomy (5).

A chronic leakage of sperm antigens into the circulation would also favor the formation of circulating immune complexes (CIC)[1] since CIC predominate when serum antigen is present in excess of its corresponding antibody. Clear evidence has been presented by Bigazzi and co-workers (6, 7) that rabbits develop immune complex-associated orchitis after vasectomy. Sperm antigen, antibody and complement were identified in the basement membrane of rabbit seminiferous tubules. A similar consequence of vasectomy may also occur in rhesus macaques (8), although in this case, sperm antigens could not be identified and lesions were sometimes also present in nonvasectomized controls. In another study to measure CIC formation in vasectomized rabbits it was found that immunization 1 wk after vasectomy with complete Freund's adjuvant was necessary in order to elicit CIC that could be detected by a solid phase Clq CIC assay (9). No CIC were identified in vasectomized rabbits not given adjuvant or in sham-operated controls given adjuvant.

Several groups of investigators have examined whether vasectomy in humans leads to the appearance of immune complexes in the circulation. In one study, using the Raji cell radioimmunoassay and solid phase Clq assay, CIC were found in 12% of 160 men who had been vasectomized 2–4 yr previously (9). Although this incidence was not significantly higher than that

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[1] *Abbreviations used in this paper:* AHG, aggregated human IgG; CIC, circulating immune complex; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline.

found in their control population, the CIC levels measured in the vasectomy group were much higher than in the controls. In a prospective study, sera from 41 vasectomized men were examined by a Clq precipitation assay for CIC over a 2-yr period (10). No CIC were detected at any time. In a third study, sera from 29 men obtained 5–6 yr postvasectomy were evaluated for CIC by three assays (11). All sera were negative for CIC by a Clq inhibition test, 4 were positive by a modified Clq deviation test and 14 were positive by an indirect phagocytosis immunofluorescence assay. In still another study, 3.8% of 156 men tested 2 yr postvasectomy were found to have immune complexes in their sera by a Clq binding assay (12). The composition of the CIC was not analyzed for immunoglobulin class, sperm antigen, or complement components in any of these human studies.

We now present the results of a prospective study to assess CIC formation after vasectomy using newly developed assays. Immune complexes were identified in the sera of men following vasectomy. Subsequent analysis revealed that some of the CIC contained IgG, sperm antigen, and complement components.

METHODS

Material. Blood was collected without anticoagulants from 35 men prior to vasectomy and at 2 wk and 2 mo postvasectomy. A fourth sample was collected from 16 of these men at 4 mo postvasectomy. The blood was allowed to clot for 60 min at room temperature, centrifuged, and the sera stored in aliquots at −70°C. CIC analyses were performed on previously unthawed aliquots.

Bull semen was generously provided by the Eastern Artificial Insemination Cooperative, Inc., Ithaca, NY.

Raji cells, a human lymphoblast tumor cell line, were grown as described (13). Only 48-h cultures with >90% viability were used in the CIC assay.

Alkaline phosphatase-conjugated swine antisera to human IgG, IgA, or IgM were purchased from Medical Diagnostics, Inc. (Hackensack, NJ) as was swine antihuman C3. Rabbit antihuman Clq was from Miles Laboratories (Elkhart, IN).

Sperm antibody enzyme-linked immunosorbent assay (ELISA). The sera were assayed for antibody to spermatozoa by an ELISA (14), using human spermatozoa fixed to wells of a microtiter plate. Sera were assayed in duplicate at a dilution of 1:20 and values differing by >10% were reassayed.

ELISA for circulating sperm antigen. Visibly pure populations of motile spermatozoa were isolated (15) from a single ejaculate of a male of proven fertility and washed by four cycles of centrifugation and resuspension in phosphate-buffered saline (PBS). The spermatozoa were counted in a hemocytometer and 2×10^7 spermatozoa in 1 ml were admixed with an equal volume of Freund's complete adjuvant. The mixture was injected subcutaneously into multiple sites on the neck of a rabbit. At week 1 and 2, booster injections were given subcutaneously along the back using 2×10^7 spermatozoa from the same donor in Freund's complete adjuvant. At week 3, 2×10^7 spermatozoa in incomplete adjuvant was injected along the back. At week 5, a fourth booster was also given along the back. At week 7, blood was collected and IgG was isolated from the serum by $(NH_4)_2SO_4$ precipitation and DEAE cellulose chromatography (16). The IgG was concentrated by ultrafiltration and chromatographed twice through a column of human serum bound to Sepharose by CNBR. After this procedure the rabbit IgG was unreactive with human serum as judged by double immunodiffusion. A fraction of this absorbed IgG was then conjugated to alkaline phosphatase by a standard procedure (17).

To assay sera for antigen reactive with the absorbed rabbit antibody, the unconjugated antisperm IgG was fixed to wells of a microtiter plate (5 μg/ml) by overnight incubation in carbonate buffer at 4°C (17). The wells were then washed three times with 0.2 ml PBS containing 0.05% Tween 20 (PBS-Tween) and aliquots of sera diluted 1:10 in PBS-Tween containing 1 mg/ml IgG from an unimmunized rabbit (0.2 ml final vol) were added in duplicate to the IgG-containing wells and to blank wells. The addition of preimmune soluble rabbit IgG at this concentration to the PBS-Tween was shown in pilot experiments to fully compete with the bound rabbit antibody for binding to other human serum antigens. After 120 min at room temperature the liquid was shaken out, the wells were washed three times in PBS-Tween, and 0.2 ml of alkaline phosphatase-conjugated rabbit antisperm IgG (diluted 1:200 in PBS-Tween) was added to the wells. After an additional 120 min at room temperature the wells were again washed three times with PBS-Tween and incubated with 0.2 ml of *p*-nitrophenyl phosphate (1 mg/ml) in 10% diethanolamine, pH 9.8. After 30–60 min, the intensity of the yellow color that formed was quantitated by determining the absorbance of the wells at 405 nm using a Titertek Multiscan (Flow Laboratories, McLean, VA). Duplicates differing by >10% were reasssayed. Samples without fixed rabbit IgG always exhibited a final absorbance of <0.06.

Raji cell ELISA for CIC. The Raji cell radioimmunoassay for CIC detection (13) was adapted to an ELISA. Raji cells (1×10^5/well) were fixed to wells of a microtiter plate with gluteraldehyde (18), incubated with sera diluted 1:10 in PBS-Tween (0.2 ml final vol) and assayed as described for the sperm antigen ELISA except that detection was with alkaline phosphatase-conjugated swine antihuman IgG. In pilot experiments it was determined that CIC binding to the fixed Raji cells was via complement receptors. CIC incubated in heated serum (56°C, 30 min) did not bind to the cells. Parallel analysis of 25 sera by the Raji radioimmunoassay and Raji ELISA were highly correlated (Spearman rank correlation, $r = 0.46$, $P < 0.001$).

Bull sperm ELISA for CIC. The bull sperm ELISA for CIC detection was as previously published (18), using sera diluted 1:10 and 2×10^5 sperm per well. Detection was with alkaline phosphatase-conjugated swine antihuman IgG.

Anti-Clq F (ab')$_2$ ELISA for CIC. Goat antiserum to purified human Clq was previously prepared in our laboratory by a published procedure (19). IgG was purified from the antiserum as described above and F(ab')$_2$ fragments were prepared by pepsin digestion (16) and isolated by gel filtration on Ultragel ACA 34 (LKB Instruments, Rockville, MD). For the assay, (F(ab')$_2$ fragments of anti-Clq were fixed to microtiter plate wells (5 μg/well) by overnight incubation in carbonate buffer. After washing with PBS-Tween, sera diluted 1:10 in PBS-Tween were added to the wells and the incubations continued as described for the other ELISA.

For each of the three CIC assays standard curves consisting of gradated concentrations of soluble aggregated human IgG (AHG) in human serum prepared as previously described (18) were assayed in parallel to the test sera. The majority of the sera yielded values in the CIC assays that were approximately equivalent to that obtained with 20 μg/ml AHG.

This is in agreement with data obtained in our laboratory (18) and in others (13) on the value of AHG equivalents present in sera of healthy adults. All sera with ELISA values less than or equal to 20 μgeq of AHG/ml were, therefore, designated as lacking CIC.

Sucrose gradient centrifugation. The sizes of the CIC were determined by centrifugation of serum aliquots at 165,000 *g* for 16 h through 5 ml 10–40% (wt/wt) sucrose gradients in PBS. Purified human IgG was run in a parallel gradient as a 7S marker and identified by absorbance at 280 nm. The gradients were fractioned by collecting 15-drop fractions, aliquots were diluted 1:10 in PBS-Tween, and 0.2 ml of each fraction was added to fixed bull spermatozoa (2×10^5 per well) in microtiter plate wells. Identification of CIC in the fractions was by the bull sperm ELISA (18).

Analysis of CIC composition. The composition of CIC was determined by a modification of a recently published method (20). Sera were diluted 1:20 in PBS and 0.1-ml aliquots were added to wells of a polystyrene microtiter plate. An equal volume of 4.8% (wt/vol) polyethylene glycol (PEG 6000, Fisher Scientific, St. Louis, MO), containing 0.04 M EDTA was then pipetted into each well and the plates incubated at 4°C for 18 h. This procedure precipitates CIC but not free immunoglobulins (21). The plates were then centrifuged at 2,000 *g* for 30 min, the supernates discarded and the pellets resuspended in 0.2 ml of 3% PEG plus 0.02 M EDTA. After an additional 30-min centrifugation the pellets were resuspended in 0.2 ml of 0.1 M glycine-HCl buffer, pH 2.5, and incubated at 4°C for 18 h. This treatment dissociates the CIC into its components, which then adsorb to the polystyrene. The plates were then washed three times with PBS-Tween, incubated with alkaline phosphatase-conjugated anti-IgG, IgM, IgA, C3, Clq, or sperm antibodies, and reactivity measured as described above. Controls consisted of adding the reagents to wells in which sera was replaced with PBS.

Statistical methods. Various assays were correlated pairwise at each time point using both the Kendall rank correlation and the Spearman rank correlation methods. The results were very similar and so *P* values are reported for the Kendall statistic only. In addition, a randomized block analysis of variance was performed on each assay to ascertain if there was a time effect or a subject effect.

RESULTS

Antibodies reactive with spermatozoa. The presence of antibodies in pre- and postvasectomy sera reactive with human spermatozoa was quantitated by an ELISA (14). To more accurately reflect individual changes in reactivity, each postvasectomy assay value was converted to a percentage of the preimmune assay value obtained from that same man (100% equals prevasectomy value). The results (Fig. 1) demonstrate that only a small percentage of the men have begun to produce sperm antibody over the interval tested. Only 2/34 (5.9%), 3/34 (8.8%), or 1/15 (6.6%) of the men developed at least a 40% increase in antibody reactive with spermatozoa at 2 wk, 2 mo, or 4 mo postvasectomy, respectively.

The sera highly reactive in this assay were shown to contain an IgG antibody that was specific for spermatozoa; they yielded values comparable to control

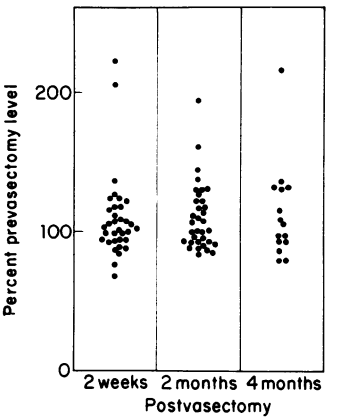

FIGURE 1 Antibody reactive with human spermatozoa in the sera of vasectomized men. Sera from men obtained before or at various times following vasectomy were assayed by an ELISA for antibody reactive with human spermatozoa. The postvasectomy ELISA values are plotted for each individual as a percentage of his prevasectomy value. 100% equals the prevasectomy level.

sera in ELISA using either human peripheral blood T lymphocytes or cell-free seminal fluid fixed to the wells (data not shown).

Circulating sperm antigens. The specificity of our alkaline phosphatase-conjugated rabbit antisperm IgG for spermatozoa was analyzed by an ELISA using spermatozoa or extracts of human brain, liver, spleen, or kidney fixed to the wells (Table I). The conjugate reacted exclusively with the spermatozoa; the other values obtained were the same as observed when the conjugate was incubated with blank wells. In addition, antibody reactivity with seminal fluid obtained from a vasectomized male or with sera from females was at control levels.

The presence of antigens in the sera of vasectomized men that were reactive with this antibody was

TABLE I
Specificity of Rabbit IgG Antibody to Human Spermatozoa

Substrate	Antibody binding (absorbance at 400 nm)
Spermatozoa	0.493
Brain	0.102
Liver	0.095
Spleen	0.119
Kidney	0.103
Buffer	0.096

Substrates were fixed to wells of a microtiter plate, alkaline phosphatase-conjugated rabbit antisperm IgG, previously absorbed with human serum, was added and IgG binding was measured by an ELISA.

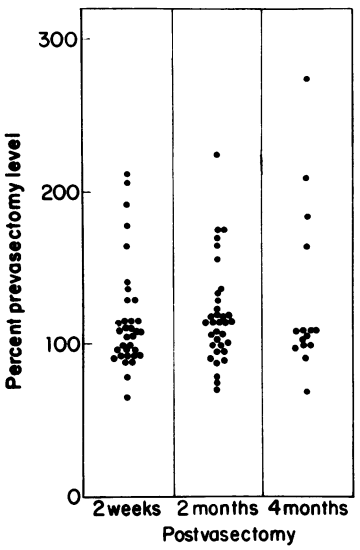

FIGURE 2 Antigens reactive with antisperm antibody in the sera of vasectomized men. Pre- and postvasectomy sera were assayed by an ELISA for antigens reactive with a purified rabbit antibody to human spermatozoa. The values for each individual are plotted as described in Fig. 1.

then measured by an ELISA using the antisperm antibody fixed to wells (Methods). Once again, for ease of comparison, the postvasectomy values were converted to a percentage of the prevasectomy value obtained from each individual. The results are presented in Fig. 2. By 2 wk postvasectomy, 6/34 men (17.6%) had at least a 40% increase over the prevasectomy level of serum antigen reactive with the rabbit antisperm antibody. By 2 mo postvasectomy 6/34 (17.6%) were positive in this assay and at 4 mo 4/15 (26%) of the men exhibited elevated levels of this component(s) in their sera.

Circulating immune complexes. The apparent increase in circulating sperm-related antigens in the sera of some men after vasectomy, coupled with a much lower incidence of sperm-related antibody, prompted us to examine the sera for evidence of CIC. To maximize our possibilities for identifying CIC, the sera were analyzed by three assays that would detect CIC containing Clq (anti-Clq F $[ab']_2$ assay), CIC containing C3 (Raji cell assay) and CIC that did not bind complement (bull sperm assay).

The distribution of CIC at various times after vasectomy, as detected by the three assays, is presented in Table II. Using the Raji cell assay, it was found that four men had CIC (280–580 μg AHG equivalents/ml) before vasectomy that persisted after surgery; no men were positive for CIC only in their prevasectomy sample. An additional six men exhibited CIC (340–660 μg AHG equivalents/ml) only transiently at one of the postvasectomy time points. Most importantly, five men first developed CIC (340–1,420 μg AHG equivalents/ml) after vasectomy that persisted over time. The results of the bull sperm ELISA were very similar to the Raji data: five men had CIC (360–1,920 μg AHG equivalents/ml) prevasectomy that persisted after surgery; eight men had CIC (402–1,730 μg AHG equivalents/ml) in only one assay postvasectomy, whereas six men developed persistent CIC (423–1,200 μg AHG equivalents/ml) after vasectomy. The individual patient results using the Raji and bull sperm assays were highly correlated (Kendall rank correlation, $P \leq 0.018$).

TABLE II
Distribution of CIC after Vasectomy

CIC	Number of men		
	Raji assay	Anti-Clq assay	Sperm assay
A 16 men observed for 4 mo postvasectomy:			
None	8	10	5
2 wk postvasectomy only	1	1	3
2 mo postvasectomy only	0	1	2
4 mo postvasectomy only	2	0	1
2 wk, 2 mo, 4 mo postvasectomy	2	0	2
2 mo and 4 mo postvasectomy	1	0	1
pre- and all postvasectomy times	2	4	2
B 18 men observed for 2 mo postvasectomy:			
None	11	10	9
2 wk postvasectomy only	1	0	2
2 mo postvasectomy only	2	2	1
2 wk and 2 mo postvasectomy	2	1	3
pre- and all postvasectomy times	2	3	3

Using the anti-Clq F $(ab')_2$ assay, seven men were positive for CIC (860–6,400 μg AHG equivalents/ml) in their prevasectomy sera and four men had CIC (400–900 μg AHG equivalents/ml) only at one postvasectomy time. Just one patient had measurable CIC (1,600, 3,600 μg AHG equivalents/ml) at two time points postvasectomy (Table II). The anti-Clq assay results correlated with the other two CIC assays ($P \leq 0.053$, Raji; ≤ 0.004, sperm).

The increases in patient CIC levels after vasectomy are shown in Fig. 3. The postvasectomy CIC level for each individual was again converted to a percentage of the prevasectomy level. Using the Raji assay, 19%, 22%, and 25% of the patients (6/32, 7/32, 4/16) had higher CIC levels at 2 wk, 2 mo, and 4 mo postvasectomy, respectively, as compared with their prevasectomy level. Similar results were obtained with the bull sperm assay. Fewer patients had increased CIC levels postvasectomy by the anti-Clq assay than by the other assays.

Analysis of variance. A randomized block analysis of variance (ANOVA) was performed for each assay to ascertain whether time differences and/or subject differences were present when the 35 subjects were considered together. No statistically significant time effect was found ($P \geq 0.096$). All subject effects were highly significant ($P \leq 0.008$), except for sperm antibody, in which no significant subject effect was exhibited ($P > 0.3$).

For the CIC assays, a close look at the data clarified the presence of subject effect and the absence of time effect. As shown in Table II, a number of subjects appear to have CIC at all times and others have CIC only occasionally. As time goes on, the patterns of different individuals vary. Approximately half of the subjects never have detectable CIC, and others first show elevations of CIC 2 wk or 2 mo postvasectomy. There is also a group of subjects who have elevated levels of CIC initially and exhibit no particular pattern over the four time points. All-in-all, several different CIC patterns are exhibited.

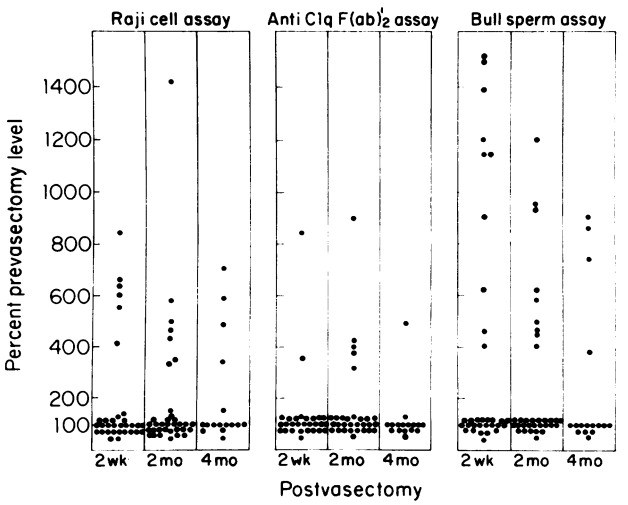

FIGURE 3 Circulating immune complexes in the sera of vasectomized men. Pre- and postvasectomy sera were assayed for CIC by the Raji cell ELISA, anti-Clq $F(ab')_2$ ELISA and bull sperm ELISA. The values for each individual are plotted as described in Fig. 1.

Analysis of CIC components. The composition of the CIC that appeared following vasectomy was analyzed directly by a procedure in which the CIC were selectively precipitated from sera with 2.4% PEG, dissociated with glycine-HCl, pH 2.5, and bound to a solid phase. Detection of the components was by an ELISA using alkaline phosphatase-conjugated anti-human IgG, IgA, IgM, C3, Clq, and antisperm IgG. 33 sera from 9 patients with CIC were analyzed by this assay. The prevasectomy sera from each patient served as the control for nonspecific binding of serum components to the wells. The results of the analyses are shown in Table III. In two men (2 and 16) there were no increases in reactivity of any of the conjugated antibodies over the prevasectomy level. Two other men (4 and 7) had an increased level of bound IgG only at 2 mo postvasectomy but not at a later time. In one of these cases (4) sperm antigen was also present only at the 2-mo period; the other man first developed demonstrable sperm antigen at 4 mo postvasectomy concomitant with increased IgM reactivity. The other five men all had persistently higher levels of IgG and IgA (3 and 18), IgG (21 and 25), or IgM (22) postvasectomy. The results suggest that complexes containing each of the three immunoglobulin classes are present in the patient's sera after vasectomy.

Since rabbit IgG may have reactivity for human immunoglobulins (22) the sera were also assayed for reactivity with alkaline phosphatase-conjugated rabbit IgG antibody to a human breast cyst fluid particulate component (23). No postvasectomy increase in reactivity to this conjugate was detected in any of the sera (data not shown). In addition, sera from men with free circulating sperm antibody or sperm-related antigen, but lacking CIC, were also negative in this assay.

The concomitant detection of sperm antigen (3, 18 and 25; 4 mo), C3 (3 and 18; 2 mo), and Clq (3, 18, 22; 2 mo and 25; 4 mo) in these CIC-containing sera provides evidence the CIC contain sperm antigen and that some of them are capable of complement activation.

Sucrose gradient centrifugation of CIC. To assess whether the CIC-containing sera contained IgG in high molecular weight complexes, aliquots of sera (0.2 ml) were layered on top of 10–40% sucrose, centrifuged for 16 h, fractionated, and aliquots assayed for

TABLE III
Analysis of CIC Components

Patient	Time postvasectomy	Increase from prevasectomy level					
		IgG	IgA	IgM	Clq	C3	Sperm Ag
2	2 wk	−	−	−	−	−	−
	2 mo	−	−	−	−	−	−
	4 mo	−	−	−	−	−	−
3	2 wk	+	+	−	−	+	+
	2 mo	+	+	−	+	+	+
4	2 wk	−	−	−	−	−	−
	2 mo	+	−	−	−	−	+
	4 mo	−	−	−	−	−	−
7	2 wk	−	−	−	−	−	−
	2 mo	+	−	−	−	−	−
	4 mo	−	−	+	−	−	+
16	2 wk	−	−	−	−	−	−
	2 mo	−	−	−	−	−	−
	4 mo	−	−	−	−	−	−
18	2 wk	+	+	−	−	−	+
	2 mo	+	+	−	+	+	+
21	2 wk	−	−	−	−	−	−
	2 mo	+	−	−	−	−	−
	4 mo	+	−	−	−	−	−
22	2 mo	−	−	+	−	−	−
	4 mo	−	−	+	+	−	−
25	2 wk	+	−	−	−	−	−
	2 mo	+	−	−	−	−	−
	4 mo	+	−	−	+	−	+

Sera were precipitated with 2.4% PEG, 0.02 M EDTA, washed and dissociated in microtiter wells with 0.1 M gly-HCl, pH 2.5. Components bound to the wells were detected by an ELISA with the appropriate alkaline phosphatase-conjugated antiserum. −, no increase over prevasectomy level; +, an increase of 30% or more over prevasectomy level.

CIC by the Raji cell and bull sperm ELISA. Results obtained with the prevasectomy and 2 wk and 2 mo postvasectomy sera from one individual are shown in Fig. 4. All reactivity with the fixed bull sperm in the prevasectomy sample was in the 7S IgG region of the gradient. We typically find that after sucrose gradient centrifugation monomeric IgG from all sera reacts with bull sperm (or Raji cells). The reason for this is unknown, but may involve conformational shifts in the IgG Fc region in the sucrose solution. At 2 wk postvasectomy, a large area of reactivity with bull spermatozoa was present at the very bottom of the gradient. By 2 mo, smaller sized CIC of heterogenous size were present. In four other men, CIC of various sizes

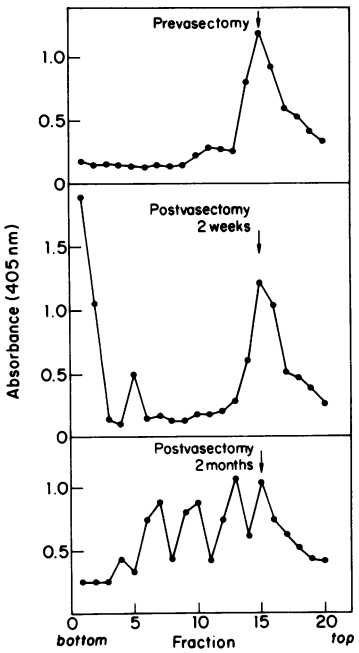

FIGURE 4 Sucrose gradient centrifugation of sera for determining CIC size. Sera (0.2 ml) from one individual obtained prior to vasectomy and at 2 wk and 2 mo postvasectomy were layered onto 5 ml 10–40% (wt/wt) sucrose gradients and centrifuged for 16 h at 165,000 *g*. The gradients were fractionated and aliquots assayed by the bull sperm CIC ELISA. The arrow marks the position of a 7S IgG marker.

were also evident postvasectomy (data not shown). No one immune complex size seemed to predominate in the circulation of vasectomized men, nor was there a consistent pattern of change in CIC size over time.

DISCUSSION

We have presented data demonstrating that in the first months following vasectomy, antigen(s) reactive with antisperm antibody appeared in the circulation of ~20% of the 35 men tested. Circulating antibody to spermatozoa was present to a lesser extent. These conditions of antigen excess favor the formation of CIC containing sperm components. CIC were identified in some of these men. Analysis of the CIC revealed that IgG, IgA, or IgM were sometimes present along with sperm-related antigen and complement components. It is apparent, therefore, that an early consequence of vasectomy is the appearance in some men of CIC consisting of sperm-related antigen and corresponding antibody.

The observation that more men have CIC than sperm antibody indicates that the incidence of sperm antibody following vasectomy may have been underdetected. Antibody that was bound to circulating

sperm antigen would not react with bound spermatozoa and so would be undetected in our assay for sperm antibody.

It is unlikely that our results are due merely to a surgical incision rather than to a specific severing of the vas deferens. The difficulty, however, in finding a suitable control group of healthy men who have undergone a minor outpatient surgical procedure under local anesthesia is evident. Experimental studies have shown, however, that antisperm antibodies do not appear in sham-vasectomized monkeys (24) or rats (25) and that immune complex deposition is only evident in vasectomized monkeys (24).

The majority of previous studies to detect CIC following vasectomy did not test samples obtained in the first few months following surgery and/or used assays based on Clq binding (9–12). A comparison of the three CIC assays used in this report revealed that the anti Clq F $(ab')_2$ assay was least effective in detecting CIC that first appeared after vasectomy. This would partially explain why only a small percentage of vasectomized men tested in the other studies had detectable CIC. In the one instance where the Raji cell assay was used to measure CIC in sera of vasectomized men (9) only sera from men vasectomized 2–4 yr previously were studied. In addition, since no prevasectomy sera from the same men were tested in this latter study, it is impossible to determine if the CIC that were detected appeared *de novo* or were exacerbations of previous conditions that favored CIC formation.

It has been proposed that vasectomy fosters development of atherosclerosis in captive subhuman primates. Naturally occurring atherosclerosis in rhesus macaques (26) and diet-induced atherosclerosis in cynomolgus monkeys (24) were more severe in vasectomized animals than in sham-operated controls. Whether this may also occur in humans is still completely unsubstantiated. The reported results may be unique to monkeys, since immune complex-associated orchitis also develops in nonvasectomized monkeys (8). In fact, in a recent epidemiological study it was determined that vasectomized men had a lower incidence of nonfatal myocardial infarctions than did nonvasectomized men (27).

Whether the CIC that we detected in some men at 2–4 mo postvasectomy persist over time or are of medical consequence remain to be determined. Since sperm antibody levels in sera of vasectomized men increase over at least the first 2 yr after surgery (12), it would be reasonable to assume that in most cases sperm antigen-antibody complexes formed at later times postvasectomy would be of sufficient size to be readily cleared from the circulation by the reticuloendothelial system. Possibly, individuals with a diminished capacity to mount an immune response to spermatozoa may constitute a subpopulation at increased risk following vasectomy.

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