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Autoantibodies against Leydig cells in patients after spermatic cord torsion

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SUMMARY

This study is aimed at searching for the presence of circulating antibodies against frozen sections of human testis, ovary and trophoblast in patients that had spermatic cord torsion. Sixty-eight sera samples were studied. Nine patients (13·2%) were positive for organ specific anti-testis autoantibodies. Six patients were positive for antibodies against Leydig cells: five were positive only with the indirect immunofluorescence technique of complement fixing (ITT/CF), the sixth patient was positive only with the indirect immunofluorescence technique (ITT). The other three patients were positive for antibodies against germ line cells: two patients were positive with both techniques, the third was positive only with indirect immunofluorescence technique. Eight of these patients were negative for antibodies against adrenal cortex while only one case was positive with indirect immunofluorescence technique both on adrenal cortex and Leydig cells. Human lyophilized testis absorbed the reactive antibodies against Leydig cells and germ line cells, while adrenal cortex and lyophilized testosterone were ineffective. This study shows the identification of a specific antibody against Leydig cells and germ line cells in patients after spermatic cord torsion.

Keywords testicular autoimmunity Leydig cells autoantibodies germ-line cells autoantibodies spermatic cord torsion sperm autoantibodies

INTRODUCTION

Men often become sterile following spermatic cord torsion. Krarup (1978) found sterility in 60% of these patients while Austoni *et al.* (1980) assert that only 10% become sterile. Bartsch *et al.* (1980) and Mastrogiacomo *et al.* (1982) observed a sterility rate of 40%, particularly in patients with atrophic testis. Furthermore a recent editorial in The Lancet (July 1981) reported that only one of 19 patients whose testis have been 'saved' after spermatic cord torsion had a normal seminal analysis.

The pathogenic mechanism of infertility as a consequence of spermatic cord torsion remains unknown. Some authors (Chakraborty *et al.*, 1980; Dondero *et al.*, 1980; Mastrogiacomo *et al.*, 1982) suggested an autoimmune pathogenesis. Dondero *et al.* (1980) found that lymphocytes of patients with atrophic testis produced macrophage inhibitor factor (MIF) *in vitro* in the presence of spermatozoa and postulated the involvement of a cell-mediated immunity. Mastrogiacomo *et al.* (1982) found statistically significant correlation between serum spermioimmobilizing antibodies and sterility.

Harrison *et al.* (1981) described characteristic histological alterations in rats after monolateral experimental spermatic cord torsion in controlateral testis. They proposed two possible mechanisms. Either an increase in the amount of naturally occurring antibody that might penetrate

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or damage the membrana propria, thus gaining access to the seminiferous tubule, or a change in the specificity of the immunoglobulin that might be direct against the membrana propria, damaging it, and thus allowing entrance into the seminiferous tubule.

The increased levels of gonadotrophins (Bartsch *et al.*, 1980) could also explain a notable involvement of Leydig cells in this pathological process.

The present study was undertaken to research the presence of circulating organ specific antibodies and their correlation with testicular atrophy and the levels of gonadotrophins and testosterone in serum and seminal analysis alterations in patients after spermatic cord torsion.

MATERIALS AND METHODS

Patients. Sixty-eight patients, negative for family history of autoimmune diseases, ranging from 9 and 28 years ($17\cdot6 \pm 3\cdot5$, mean $\pm$ s.d.) who had monolateral spermatic cord torsion within the last 7 years were studied.

Sera were obtained between 5 days and 7 years after spermatic cord torsion and stored at $-20°$C until use. The sera of 19 patients were obtained and studied repeatedly.

The sera of 13 healthy women and 21 healthy men, without autoimmune diseases, were studied as controls.

Immunofluorescence technique. Organ specific autoantibodies were investigated with the following immunofluorescence methods. (a) Indirect immunofluorescence technique (IIT), according to Roitt & Doniach (1969), on frozen thin (3·5 μm) unfixed sections of the human stomach, thyroid and pancreas; rat kidney and liver; normal human testis obtained from two patients with prostate carcinoma (not treated with oestrogens); normal human ovaries, obtained from two patients submitted to oophorectomy for carcinoma of the breast; normal human trophoblast, drawn at the 11 week of gestation; normal ox and human adrenal gland. The following anti-gammaglobulins conjugated with fluorescein were used: $F(ab')_2$ sheep serum kindly sent to us by Dr G. F. Bottazzo (Middlesex Hospital, London) diluted 1:20 and commercially available (Behringwerke) $F(ab')_2$ rabbit serum, undiluted and 1:10–1:20–1:40–1:80 diluted and an IgM serum (Behringwerke) diluted 1:20. (b) Indirect immunofluorescence complement fixing technique (IIT/CF) (Roitt & Doniach, 1969) was performed using a normal human serum as source of complement (II layer) and a serum anti-human C3 conjugated with fluorescein (Behringwerke) diluted 1:40 on normal human testis, normal human ovaries, normal human trophoblast, normal ox and human adrenal gland.

The human sera were tested undiluted and those positive were titrated in serial dilutions until the extinction of reaction.

Each slide was mounted with buffered glycerol and studied under a Leitz Orthoplan microscope with a 100W HBO lamp and K 455/K 490 filters.

Thyroglobulin antibodies. Thyroglobulin antibodies were detected by haemoagglutination using Thymume-T kit (Wellcome Reagents Ltd.).

Sperm antibodies. The Kibrick's technique (Kibrick, Belding & Merrill, 1952) for agglutinating antibodies and the Isojima's technique for spermioimmobilizing antibodies (Isojima, Li & Ashitaka, 1968) were used. To detect antibodies by indirect immunofluorescence, the spermatozoa were washed twice in saline and centrifuged for 10 min at 1,000 r/min. Smears made from sperm were air dried and then fixed in methanol for 30 min. The technique of Hjort & Hansen (1971) was then used.

Absorptions. All positive sera for antibodies against human gonadal tissue antigens were absorbed respectively with 20 mg/ml of homogenated and lyophilized ox adrenal gland, with 20 mg/ml of lyophilized human testosterone (Sigma, lot 113-c 1460, No. T-1500) and with 40 mg/ml of homogenated and lyophilized human testis.

The sera were incubated 1 h at 37°C and then at 4°C overnight with lyophilized antigens and ultracentrifuged at 10,000 r/min for 30 min. The serum that reacted both with some Leydig cells and with ox and human adrenal gland was not absorbed.

The specificity of lyophilized testis was investigated by similarly absorbing human sera that were

positive for adrenal cortex, parietal cells and thyroid microsomal antibodies; residual activity was tested with the IIT and IIT/CF on human testis, ovary, thyroid, stomach and ox and human adrenal cortex.

Semen analysis. Semen of six patients with antibodies against gonadal structures was collected from 3 months to 7 years after the acute episode, analysed and classified as described by Dondero & Isidori (1980). We considered as criteria for fertility a minimum of 30×10^6/ml of spermatozoa and motility of at least 40% after 2 h. Seminal analysis was not performed on three patients: one because of his young age and the other two did not consent.

Hormonal study. Gonadotrophins (LH and FSH) and testosterone levels were determined with radioimmunoassay, using RIA kits from Biodata.

RESULTS

The serum of nine of 68 patients (13·2%) that had spermatic cord torsion revealed antibody activity against testicular and spermatozoa antigens. Particularly, six sera (8·8%) reacted with cytoplasmic Leydig cells antigens and three sera (4·4%) with cytoplasmic antigens of the intratubular cells (germ line and Sertoli cells) of the testis (Table 1).

None of control sera reacted with testicular antigens.

Antibodies against Leydig cells

Of the six positive sera against Leydig cells, four demonstrated intense cytoplasmic fluorescence with all the Leydig cells of the human testis only with IIT/CF (Fig. 1). The serum of one patient (F.G.) was found positive only in the first sample analysed. The following samples (after 6–9 months) of the same patient were negative. The fifth serum (C.O.) demonstrated intense cytoplasmic fluorescence with some Leydig cells only with IIT/CF. The reactions of all these sera

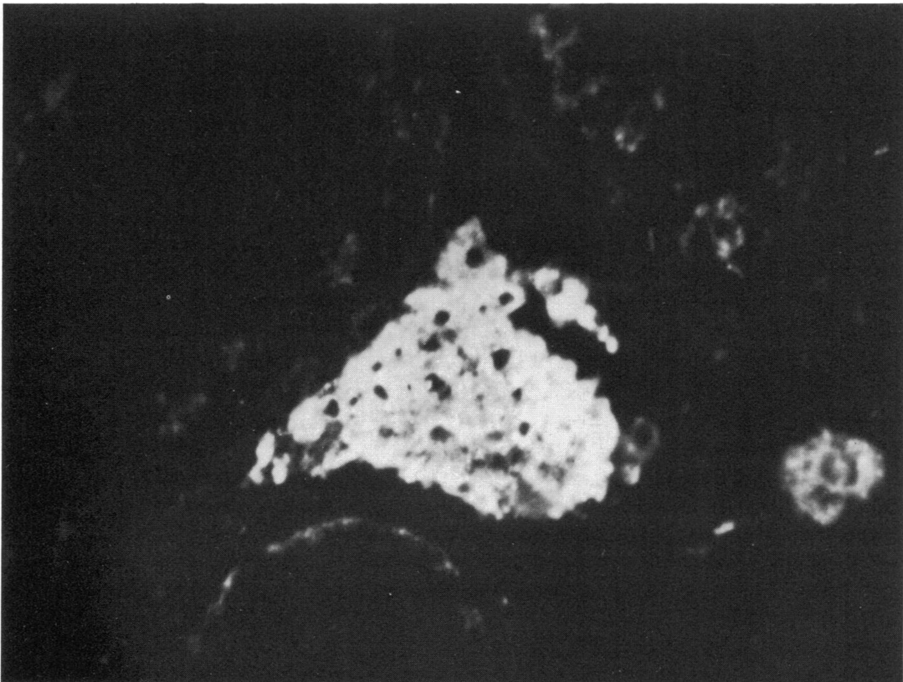

Fig. 1. Indirect immunofluorescence complement fixing technique on cryostat section of human testis (magnification ×400). Autoantibodies on human testis showing cytoplasmic staining in Leydig cells situated in the space between four seminiferous tubules.

Table 1. Immunological and clinical features in patients after spermatic cord torsion with autoantibodies against testicular structures

						IIT		IIT/CF		Sperm antibodies			
Name	Age	Testis	Semen analysis	Date of torsion	Date of samples	Intratub. cells	Leydig cells	Intratub. cells	Leydig cells	SA	SI	IIT	Other antib.
C.A.	20	normal	normospermic	1977	1980	1:4*	neg	neg	neg	neg	neg	neg	neg
					1981	1:4	neg	neg	neg	neg	neg	neg	
P.C.	19	atrophic	necrospermic	1980	after 3 months	1:16	neg	1:16	neg	neg	neg	neg	neg
B.D.	18	normal	n.d.	1978	1980	1:16	neg	1:4	neg	neg	neg	E 1:128	neg
F.G.	17	atrophic	oligo/ astenospermic	1979	after 3	neg	neg	neg	1:1	neg	neg	neg	SMA ANA
					6–9 months	neg	neg	neg	neg	neg	neg	neg	
M.A.	22	orchid	oligospermic	1977	1978	neg	neg	neg	1:8	pos	neg	MTP	neg
					1980	neg	neg	neg	1:8	pos	neg	1:256	
B.A.	23	normal	necrospermic	1979	after 3–6–9–12 months	neg	neg	neg	1:8	neg	pos	neg	SMA ANA
T.S.	14	atrophic	n.d.	1979	after 3	neg	neg	neg	1:4	neg	neg	Ac MTP	SMA ANA
					6–12 months	neg	neg	neg	1:8	neg	neg	1:128	
C.O.†	28	atrophic	n.d.	1979	1980	neg	neg	neg	1:4	neg	neg	Ac MTP 1:64	ANA
M.C.	21	atrophic	necrospermic	1973	1980	neg	1:16	neg	neg	neg	pos	neg	neg
					1981	neg	1:16	neg	neg	neg	pos	neg	

* Only some intratubular cells.
† This patient also showed antibodies against adrenal cortex.
n.d. = not determined.
SA = spermioagglutinating antibodies; SI = spermioimmobilizing antibodies.
IIT = indirect immunofluorescence technique; IIT/CF = indirect immunofluorescence technique of complement fixing.

showed a titre from 1:1 to 1:8. This pattern is not present with IIT using $F(ab')_2$ IgG and IgM fluorescein conjugated at different dilutions.

The sixth serum (M.C.) showed cytoplasmic fluorescence against all the Leydig cells only with the indirect immunofluorescence technique. The reaction showed a titre of 1:16.

Antibodies against germ and Sertoli cells

Two sera reacted with the cytoplasm of the intratubular cells (germ and Sertoli cells) with both the IIT and IIT/CF (Fig. 2). The patient B.D. showed a titre of 1:16 with IIT and 1:4 with IIT/CF

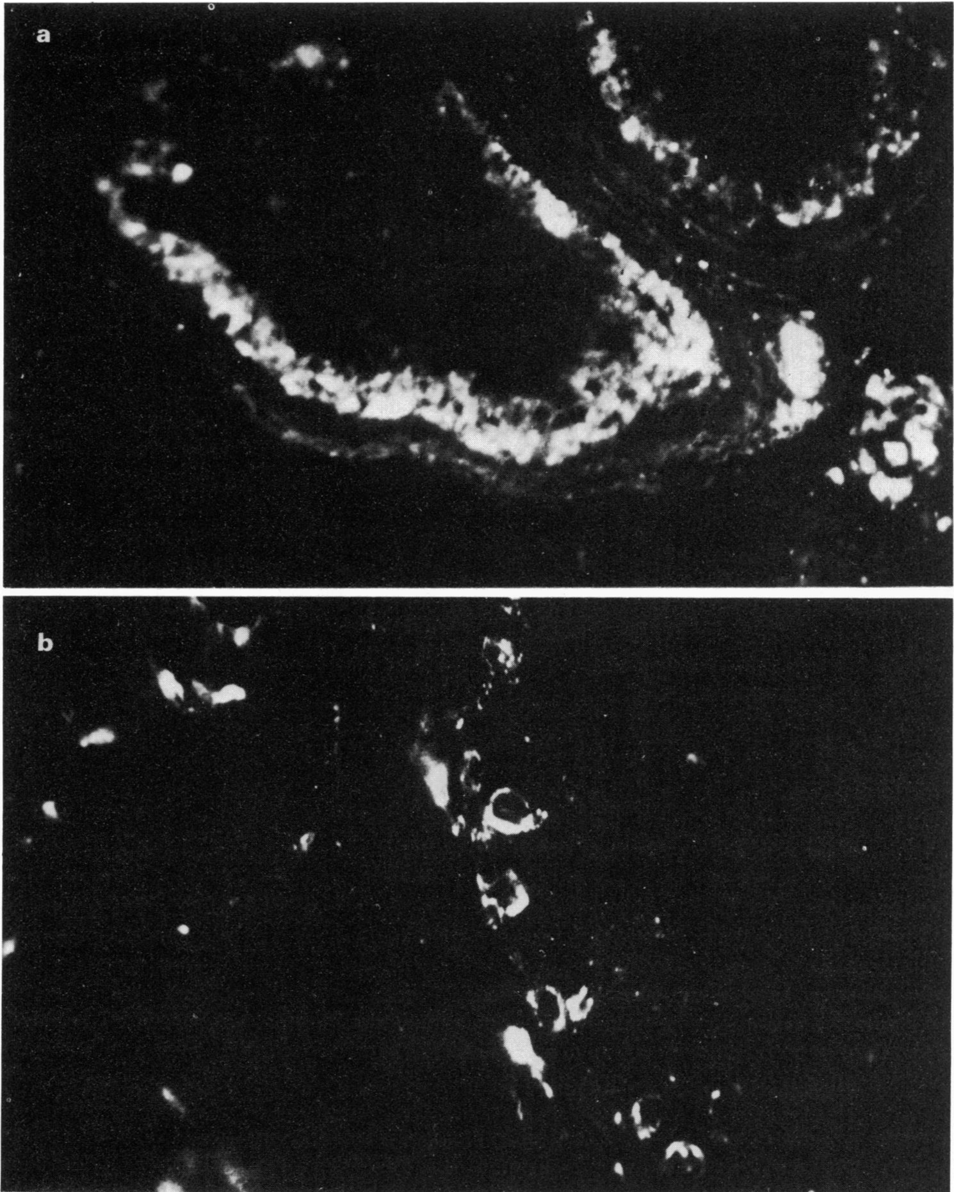

Fig. 2. Some indirect immunofluorescence technique patterns involving the intratubular cells (magnification ×400). (a) Human testis showing immunofluorescent pattern of all intratubular cells (Sertoli and germ line cells), (b) human testis showing immunofluorescent pattern of some intratubular cells.

respectively. The patient P.C. showed a titre 1:16 with both techniques. A third serum (C.A.) was positive against the intratubular cells only with IIT (titre 1:4).

Antibodies against adrenal cortex

The serum of the patient C.O. that reacted with some Leydig cells with the IIT/CF reacted also with ox and human adrenal gland with the same technique, but it was negative using IIT with $F(ab')_2$, IgG and IgM conjugated with fluorescein at different dilutions.

All of the sera of the other patients and all controls did not react against adrenal antigens with either method.

Antibodies against human ovary and trophoblast

None of the patients' and controls' sera showed reactivity against human ovary and trophoblast cells with either method.

Sperm antibodies

Eight patients (11·7%) were positive for the presence of spermioagglutinating antibodies (SA) and seven (10·2%) for spermioimmobilizing antibodies (SI). Twenty-eight (41·1%) patients were positive for the presence of anti-sperm antibodies detectable by indirect immunofluorescence: 17 (25%) were positive for acrosomal, 11 (16·1%) for equatorial segment and eight (11·7%) for main tail piece antigens. Six patients were positive for acrosomal and main tail piece antigens, one for acrosomal and equatorial segment antigens and one for equatorial segment and main tail piece antigens.

Other antibodies

The incidence of ANA and SMA antibodies was not raised above that in normal controls. All patients were negative for other organ specific antibodies.

Absorptions

Absorption of the sera containing antibodies against testicular structures with lyophilized testis removed all the reactivity. On the other hand the reactivity against testicular structures after absorption with adrenal cortex and testosterone was maintained.

Similarly the absorption with lyophilized adrenal and testis antigens removed the reactivity of the positive serum with antibodies against adrenal cortex; while no change was observed after absorption with testosterone. On the contrary the reactivity of the sera positive for parietal cells and thyroid microsomes antibodies was maintained after absorption.

Semen analysis of patients with anti-testicular antibodies

Three patients with atrophic testis showed altered seminal analysis: one was oligoasthenospermic, the other two were necrospermic. One patient that was orchidectomized was oligospermic but with

Table 2. Hormonal levels in patients after spermatic cord torsion with antibodies against testicular antigens

Name	LH (iu/ml)	FSH (iu/ml)	Testosterone (ng/ml)
C.A.	5·1	8·1	367
P.C.	7·1	6·7	708
B.D.	10·4	13	460
F.G.	7·4	6·7	726
M.A.	9·2	10·1	482
B.A.	5·4	6·9	539
T.S.	8·6	5·7	318
C.O.	9·6	14·4	700
M.C.	9·7	5·4	744

normal motility. Another patient with normal testis was necrospermic. Only one patient with normal testis showed normal seminal analysis.

Hormonal levels

The gonadotrophins and testosterone levels were within the normal range: LH from 5·4 to 10·4 iu/ml (7·7 ± 1·8, mean ± s.d.), FSH from 5·4 to 14·4 iu/ml (8·5 ± 3·2) and testosterone from 318 to 744 ng%ml (560 ± 164) (Table 2).

DISCUSSION

Antibodies reacting against steroid producing cells (StCA) (adrenal cortex, Leydig, hilus, luteal, thecal, trophoblast) have been identified by Anderson *et al.* (1968) in two out of 20 patients with idiopathic Addison's disease. These studies were confirmed by Irvine *et al.* (1968) who observed a correlation between the StCA and clinical ovarian deficiency in patients with Addison's disease. These results demonstrated the presence of antibodies cross-reacting with adrenal cortex, ovary, testis and placenta antigens.

Few cases were described concerning the presence of antibodies reacting only with the ovarian structures and not with the adrenal cortex. Ruehesen *et al.* (1972) described one patient with primary ovarian deficiency associated with Addison's disease, and Coulam & Lufkin (1981) described another patient with primary ovarian failure and diabetes mellitus with antibodies against ovary. Both patients lacked antibodies reacting against the adrenal cortex.

In male pathology (Anderson *et al.*, 1968; Irvine *et al.*, 1968; Irvine & Barnes, 1975) antibodies against Leydig cells were present only in patients who concomitantly exhibited circulating antibodies against the adrenal cortex.

We now report the observation of circulating autoantibodies reacting only against Leydig cells in patients that have suffered spermatic cord torsion. These antibodies reacted with specific testicular antigens as demonstrated by immunofluorescent and absorption studies and did not cross-react with other steroid producing structures. The reactivity was maintained following absorption with lyophilized adrenal cortex however this positivity completely disappeared after absorption with lyophilized testis.

This reactivity was against intracytoplasmic antigens and not against hormone produced by these cells, since lyophilized testosterone was not able to absorb the positive sera.

Four sera were positive only with the IIT/CF. This observation is uncommon in autoimmune diseases, however, this was not the only described example in the literature. Some authors have described the detection of organ specific antibodies with the IIT/CF only. In fact, sera of patients with Herpes gestationis contain IgG that bind to the basement membrane and that are detectable only with anti-C3 sera (Jordon, Mitchell Sams & Beutner, 1969; Katz, Hertz & Yaoita, 1976; Carruthers & Rosemary Ewins, 1978). Similarly in vitiligo, circulating antibodies to melanin producing cells were detected only with the IIT/CF (Hertz *et al.*, 1977; Betterle, Peserico & Bersani, 1979; Peserico *et al.*, 1981).

An acceptable explanation, according to Bottazzo *et al.* (1980), for the variable complement fixing capacity of some IgG antibodies is that these react with very few epitopes so that the IgG molecules are too far apart to fix complement, whereas patients with a stronger hereditary predisposition (Galli & Edelman, 1972) react with a sufficient number of sites to initiate the complement cascade. Another possibility is that an unrecognized non complement fixing allotype may exist within an Ig subclass normally known to be complement fixing, as recently shown in the mouse (Ey, Prowse & Jenkin, 1979).

From a pathogenic point of view the presence of circulating complement fixing autoantibodies can serve as lesion markers. Subjects without diabetes mellitus but with circulating complement fixing antibodies against pancreatic islet cells can develop diabetes mellitus (Bottazzo *et al.*, 1980; Betterle *et al.*, 1980). At the present time our patients with complement fixing antibodies against testicular structures did not reveal abnormal levels of gonadotrophins and testosterone. This fact

could be due either to the low titre of antibodies unable to damage the Leydig cells and/or to the high resistance of these cells to the action of the antibodies.

In male pathology autoantibodies reacting against intratubular structures of the testis (Sertoli and germ cells) have already been identified. Indeed, Wall, Stedronska & Lessof (1974) reported antibodies against Sertoli cells in one hypogonadic man with gynaecomastia and in another infertile woman with Sjögren's syndrome. Sotsiu, Bottazzo & Doniach (1980) have found similar antibodies in two patients with Addison's disease, in three infertile patients and in one case of amenorrhea.

In our case, three patients showed circulating autoantibodies against intratubular cells. One patient with these antibodies and a normal testis had normal seminal analysis; another with atrophic testis was necrospermic. The third with normal testis did not consent to seminal analysis. At the present time the conclusion of Mancini (1976) that these antibodies can alter spermatogenesis can not be sufficiently supported by us.

In conclusion our observations indicate that acute ischaemic damage to testicular structures following spermatic cord torsion can lead to the production of antibodies against various gonadal antigens but, at the present time, to what degree autoimmunity contributes to the ultimate failure of gonadal function is still a matter of speculation.

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