Testicular antispermatogenic antibodies in obstructive azoospermia

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High titres of antisperm antibodies have been found in the sera of patients suffering from obstruction in the sperm passages (Shulman, 1971; Abdel Aal, El Atribi, Abdel Hafiz & Aidaros, 1975). The presence of antibodies in the interstitial spaces and in damaged basement membranes in the testicular tissue of infertile men was reported by Isidori, Dondero & Garufi (1968) and Isidori, Dondero & Lombardo (1970), who used an immunofluorescence technique, and confirmed by Le Lorier, Salat & Rotman (1970). The incidence of immunological lesions in the testes of infertile men was about 22.3% (Le Lorier *et al.*, 1970; Isidori *et al.*, 1973). In normal men, testicular immunoglobulins mainly occur in the interstitial cells and their concentration in the germinal cells is very low (Abdel Aal, Abdel Hafiz, Kandil & El Tonsy, 1976). The aim of the present work was to study the pattern and concentration of testicular immunoglobulins in patients with obstructive azoospermia.

Materials and methods

Anti-human gamma globulin antiserum, raised in rabbits, was purchased freeze-dried (Miles Laboratories, Inc., Kankakee, Illinois, U.S.A.) and conjugated to horse radish peroxidase (Sigma type VI) by the method of Nakane & Pierce (1966). The potency of the antiserum was 5.5 mg antibody/ml. The contents of two vials of antiserum (55 mg antibody) and 55 mg peroxidase were dissolved in 2.1 ml 0.5 M cold carbonate buffer (pH 10) and 0.27 ml 0.5% *p,p'*-difluoro-*m-m'*-dinitrodiphenyl sulphone in acetone was added. The mixture was agitated for 6 hr at 4°C and was dialysed against phosphate-buffered saline (PBS) overnight. A precipitate was removed by centrifugation. After precipitation with an equal volume of saturated ammonium sulphate in distilled water, the precipitate was collected, washed twice in a 50% solution of saturated ammonium sulphate in PBS, and dissolved in and dialysed against PBS. The conjugate was absorbed with liver powder.

Testicular biopsies were surgically obtained from 9 patients who were admitted to Al-Hussein Hospital for infertility investigations. All the patients had obstructive azoospermia which followed bilateral epididymo-orchitis. The testicular tissue was fixed in Bouin's fluid, and embedded in paraffin wax. For each biopsy, three slides of 6 µm sections were prepared. One slide was stained with haematoxylin and eosin; the second (test) was washed with PBS and incubated with the enzyme-labelled antiserum for 30 min. The third (control) slide was washed in PBS, and incubated with an unlabelled portion of the antiserum for 30 min. After incubation, test and control sections were washed in three changes of PBS.

The method of Graham & Karnovsky (1966) was used to determine the sites of localization of testicular immunoglobulins (test sections). The sections were incubated at room temperature for 30 min in a solution composed of 75 mg 3,3'-diamino benzidine hydrochloride and 0.001% peroxide in 100 ml 0.05 M-tris-HCl buffer, pH 7.6. The sections were washed in tris buffer and osmicated in 2% OsO₄ in distilled water, washed, dehydrated and cleared in xylol and mounted. The peroxidase enzyme label produced a light to dark-brown colour according to the concentration of the antibodies. The stain was considered specific in the test section when it was reduced in density in the corresponding control section.

Results and discussion

Six of the nine cases exhibited variable degrees of maturation arrest, mainly at the spermatid stage (Pl. 1, Fig. 1). Disorganization, sloughing of immature cells and Sertoli cell vacuolization were also seen in some specimens. In five of these specimens, the germinal cells contained very high concentrations of immunoglobulins (Pl. 1, Fig. 2). All the control sections for these specimens (Pl. 1, Fig. 3) showed that the stain was specific for immunoglobulins.

The abnormal findings in the present study can reasonably be attributed to the presence of cell-bound antibodies in the germinal cells, since no immunoglobulins were found in the germinal cells of normal men (Abdel Aal *et al.*, 1975). The maturation arrest associated with the presence of immunoglobulins may be due to the cell-bound antibodies being antispermatogenic. The mechanism of production of such antispermatogenic testicular antibodies may be autoimmunization against spermatozoa extravasating behind the obstruction. Epididymo-orchitis, which is usually the cause of obstruction, can enhance the immune response and produce serum antisperm antibodies. A cellular type of immunity seems to be needed for cell-bound antibodies to pass the blood-testis barrier and become fixed to the germinal cells. Raff (1973) has explained how T and B lymphocytes can interact in humoral and cell-mediated immunity and how each type of immune response can potentiate the action of the other. Shulman (1971) has stated that sperm autoantibodies often occur in patients who have normal testicular histology, but the present findings suggest that cell-mediated immunity is the more important immune response which affects the testicular histology.

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PLATE 1

Fig. 1. Section of testis from a man with obstructive azoospermia showing maturation arrest. H & E, $\times 73$.

Fig. 2. Testicular section from the same patient as Fig. 1 showing the high concentrations of peroxidase-labelled antihuman gamma-globulins in the germinal cells. $\times 80$.

Fig. 3. Testicular section from the same patient as Fig. 1 showing the lack of staining by peroxidase-labelled antihuman gamma-globulins after incubation with unlabelled antiserum. $\times 80$.