

EFFECT OF HOMOLOGOUS ANTISTREPTOCOCCAL ANTIBODIES ON TESTICULAR TISSUE

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In guinea pigs and mice autoimmune orchitis (AIO) has been shown to develop against a background of streptococcal sensitization [1]. Injury to the testes is due to the development of an immune reaction to streptococcal antigens and cross-reacting testicular autoantigens [1]. The ability of antibodies arising in streptococcal sensitization to exert a pathological action on testicular tissue has not hitherto been studied although this is important for determining the role of cross-reacting antistreptococcal antibodies in the induction of the pathological process.

The object of this investigation was to study the ability of sera from guinea pigs immunized with streptococcal antigen to exert a cytotoxic action on cells of the spermatogenic epithelium in experiments *in vitro* and to cause injury to the homologous testis when injected into animals with disturbed permeability of the blood-testis barrier (BTB).

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature male noninbred guinea pigs weighing 400 g. Guinea pigs in which AIO was induced by a single intradermal injection of 5 mg dry weight of heat-killed group A type 12 streptococcal cells into the hind limb foot pad together with Freund's complete adjuvant, the total volume of mixture injected being 0.5 ml, served as donors of the sera. The animals were killed 7, 14, 21, 30, and 60 days after immunization and the testes and blood were removed for investigation. Antibodies against testicular tissue and streptococcus in the sera were determined by the immunoluminescence method, using cryostat sections of homologous testis and also films of washed spermatozoa and streptococci [1]. Complement-dependent cytotoxicity of immune sera against testicular cells was determined by Hutna's method [3]. Sensitivity of the serum antibodies to cysteine hydrochloride was determined by Chernokhvostova's method [2]. The guinea pig serum globulins were separated into IgM and IgG by gel-filtration on columns with DEAE-cellulose and Sephadex G-200 [4].

The recipients received intradermal injections into the hind-limb footpads of 0.25 ml Freund's complete adjuvant 14 days before transfer in order to increase the permeability of the BTB. AIO was transferred by intravenous injection of 3 ml serum per animal. The recipients were killed 2 h and also 2, 4, 7, and 14 days after transfer and the testes were removed for investigation. The degree of injury to the testis was determined from the change in weight of the gland and by histological examination in paraffin sections stained by Hotchkiss' method. In cryostat sections of the recipients' testis washed beforehand with 0.05 M phosphate buffer (pH 7.2) fixation of globulin was determined by the direct immunoluminescence method [1].

EXPERIMENTAL RESULTS

Immunization of guinea pigs with streptococcus together with Freund's complete adjuvant led to the development of AIO, detectable histologically with effect from the 14th day after sensitization. Antibodies against testicular and streptococcal antigens were determined in the serum in the same period of time by the immunoluminescence method and the spermatocytotoxic test (Table 1). Antibodies detectable by the immunoluminescence method stained the membranes

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TABLE 1. Dynamics of Antibody Formation in Guinea Pigs Immunized with Streptococci

Antibodies discovered	Time after immunization, days				
	7	14	21	30	60
Sperm-cytotoxic *	$3,2 \pm 0,1$	$14,3 \pm 0,7$	$39,7 \pm 3,5$	$50,7 \pm 5,2$	$7,8 \pm 0,5$
Luminescent †	0	$4 \pm 0,6$	$5,2 \pm 0,9$	7 ± 2	$8,6 \pm 2$

*Index of cytotoxicity (in percent).

†log₂ of reciprocals of titers.

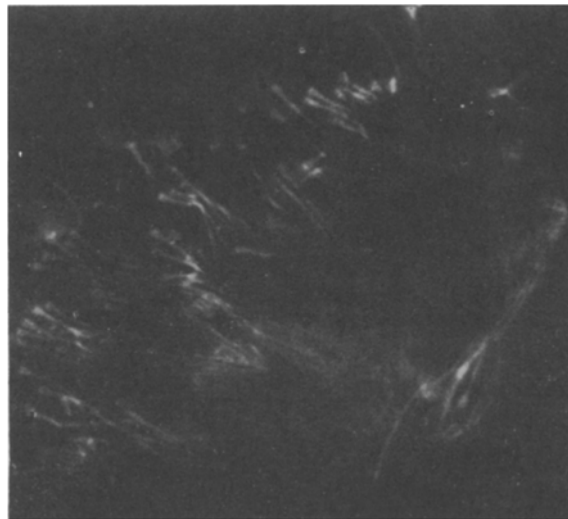


Fig. 1. Fixation of globulin on spermatozoa of convoluted tubules of testis on second day after injection of homologous antistreptococcal serum into guinea pigs. Direct immunoluminescent method, 400 ×.

and cytoplasmic structures of the spermatids in sections through the testes, and the acrosome and tail of the spermatozoa in sperm films. Cytotoxic antibodies were discovered until the 30th day and luminescent antibodies until 30 months inclusive after streptococcal immunization, with a peak at 30 days and 60 days respectively. Antistreptococcal antibodies were discovered up to 3 months after immunization, with a peak on the 30th day.

Treatment of the immune sera with cysteine abolished their spermacytotoxic activity while preserving their ability to react in the indirect immunoluminescence test with testicular structures. This shows that the spermacytotoxic antibodies belong to the IgM group. When immunoglobulins from serum obtained on the 30th day after immunization were fractionated by the gel-filtration method cytotoxic activity of the antibodies was found in the IgM fraction, whereas luminescent antibodies against testicular antigens were found only in the IgG fraction.

Passive transfer experiments using guinea pig sera obtained 7, 14, 21, 30, and 60 days after immunization with streptococci showed that only injection of serum obtained on the 30th day after immunization caused injury to the testis (Table 2). This was found from 2-4 days after transfer and was characterized by a decrease in weight of the testes and disappearance of mature spermatozoa from the convoluted tubules. In the first 2 days after injection of 30-day serum fixation of globulins on spermatozoa of the convoluted tubules was observed in the recipients' testes (Fig. 1). By the seventh day after transfer, atrophy of the spermatogenic epithelium was observed in the testes as far as the spermatocyte-spermatogonium stage, with proliferation of Sertoli cells in the convoluted tubules (Fig. 2).

Testing the activity of IgM (2.5 mg/ml protein) and IgG (5.1 mg/ml protein) preparations from 30-day guinea pig sera showed that only the IgM preparations were able to cause injury to the testis (Fig. 3).

TABLE 2. Transfer of AIO by Antistreptococcal Guinea Pig Sera to Homologous Recipients

Time after in- jection of serum, days	Sera injected into recipients							
	antistreptococcal						normal	
	14-day		30-day		60-day		n	percent of tubules with ripe sperm
	n	percent of tubules with ripe sperm	n	percent of tubules with ripe sperm	n	percent tubules with ripe sperm		
1	6	42±3,5	8	38,4±3,5	6	43,7±4	6	44±4
2	7	36±3	8	28±2,5	7	42,6±4,6	6	40,8±4
7	6	26,7±2	9	7±0,5 ¹	6	38,3±4,3	6	37,6±3,5
14	8	23,6±2	10	0*	8	35,8±3,4	6	37,7±3,5

*P < 0.01.

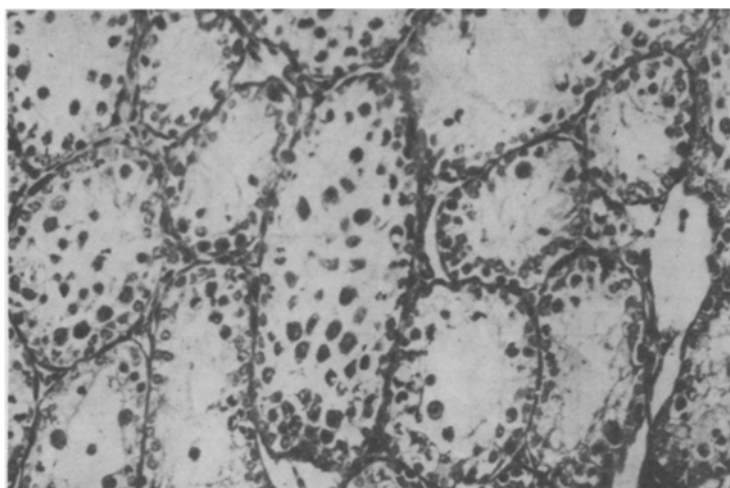


Fig. 2

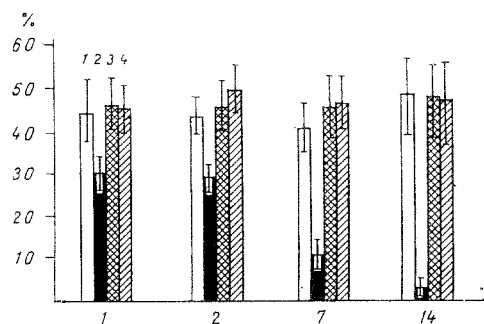


Fig. 3

Fig. 2. Convoluted tubule of testis of guinea pig seven days after injection of homologous antistreptococcal serum. Stained by Hotchkiss' method, 200 ×.

Fig. 3. Comparative activity of antistreptococcal sera containing IgM and IgG in experiments to transfer injury to the testes in guinea pigs. Antistreptococcal sera: IgG (1) and IgM (2); normal sera: IgG (3) and IgM (4). Abscissa, days after transfer; ordinate, number of convoluted tubules with mature spermatozoa (in percent).

Antibodies against testicular antigens arising in streptococcal AIO thus reacted in the indirect method with the cytoplasm of spermatids, and also with the acrosome and tail part of the spermatozoa, containing autoantigen [5]. An autoantigen cross-reacting with streptococcus also is probably located on the surface of spermatids and mature spermatozoa, for antistreptococcal sera can exert a cytotoxic action on a suspension of spermatogenic epithelial cells in the presence of complement. Only sera containing spermatotoxic antibodies (IgM) were active in AIO transfer. The injected antibodies were fixed on the surface antigens of the spermatozoa and caused injury to them, which spread also to the underlying layer of spermatogenic epithelium.

These results are evidence of the ability of antistreptococcal antibodies of the IgM type to exert a pathological action on the spermatogenic epithelium of the testis in experiments *in vitro* (the spermatocytotoxic test) and that injury to the testes can be transferred to homologous recipients in which permeability of the BTB is disturbed.

LITERATURE CITED

1. R. P. Ogurtsov and Yu. N. Zubzhitskii, Arkh. Patol., No. 12, 23 (1978).
2. E. V. Chernokhvostova, Lab. Delo, No. 6, 323 (1965).
3. T. Hutna and M. Richlikova, Folia Biol. (Prague), 10, 188 (1964).
4. Z. Pokorna, Folia Biol. (Prague), 16, 320 (1970).
5. F. Toullet, G. A. Voisin, and M. Nemerovsky, Immunology, 24, 635 (1973).