IMMUNOLUMINESCENCE DETECTION OF ANTIBODIES AGAINST TESTICULAR ANTIGENS IN EXPERIMENTAL AUTOIMMUNE ORCHITIS

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Antibodies reacting in the indirect immunoluminescence test with structures of spermatids in sections of the testis and with the acrosome and tail part of the spermatozoa in sperm films, were detected in the sera of guinea pigs and mice with experimental autoimmune orchitis. They were found in mice from the 14th to the 30th day and in guinea pigs from the 14th to the 60th day after immunization respectively. The period of circulation and titer of luminescent antibodies were significantly less in immunized female guinea pigs and mice. Luminescent antibodies had no cytotoxic activity and belonged to the IgG fraction. Luminescent guinea pig antibodies did not give cross reactions with mouse testicular antigens, nor did mouse antibodies cross-react with guinea pig antigens.

KEY WORDS: orchitis; testicular antigens; antibodies.

The development of injury to the spermatogenic epithelium in experimental autoimmune orchitis (EAIO) is accompanied by the appearance of antibodies against testicular antigens in the blood. The relationship of these antibodies to the various cells of the spermatogenic epithelium of the testis has not yet been adequately studied, although the sera of animals with EAIO have been shown to react with isolated testicular cells in cytolysis tests [5, 6].

In the present investigation an immunoluminescence method was used to study the location of antibodies formed in guinea pigs and mice with EAIO on testicular cells. In parallel experiments the ability of antibodies in the sera of animals with EAIO to exert a cytotoxic action on a suspension of testicular cells was compared.

EXPERIMENTAL METHOD

Experiments were carried out on male and female noninbred guinea pigs weighing 250-300 g and on CC57Br mice weighing 16-18 g. To induce EAIO, guinea pigs and mice were immunized with testicular homogenate in Freund's complete adjuvant in the footpads. A single dose of 60 mg of homogenate of allogeneic testis was given to guinea pigs, whereas mice received two injections, each of 40 mg of homogenate of syngeneic testes, at an interval of 2 weeks. On the 7th, 14th, 21st, 30th, and 60th days after the last injection of antigen the animals were killed and blood and the testes were taken for investigation. Animals of the control group received injections of complete adjuvant with physiological saline according to the same scheme. The extent of injury to the testes was assessed by examination of histological sections stained with hematoxylin and eosin, by determining the decrease in the number of convoluted tubules containing mature spermatozoa [6]. Antibodies against testicular antigens in the sera of guinea pigs and mice were determined by the indirect immunoluminescence method. Sections of normal testes fixed with 96° ethanol for 10 min and also films of sperm expressed from the vas deferens and fixed for 5 min with absolute methanol, washed three times with phosphate buffer (pH 7.2), were used as antigens. Rabbit sera against guinea pig and mouse globulins (from the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR) were used as fluorescein isothiocyanate-labeled sera. The activity of cytotoxic antibodies against a suspension of guinea pig and mouse testicular cells was determined by Hutna's method [5]. Isolation of IgG and IgM from the guinea pig sera, followed by purification and concentration, were carried out by Pokorna's method [6]. To abolish IgM activity the animals' sera were treated with cysteine hydrochloride [2].

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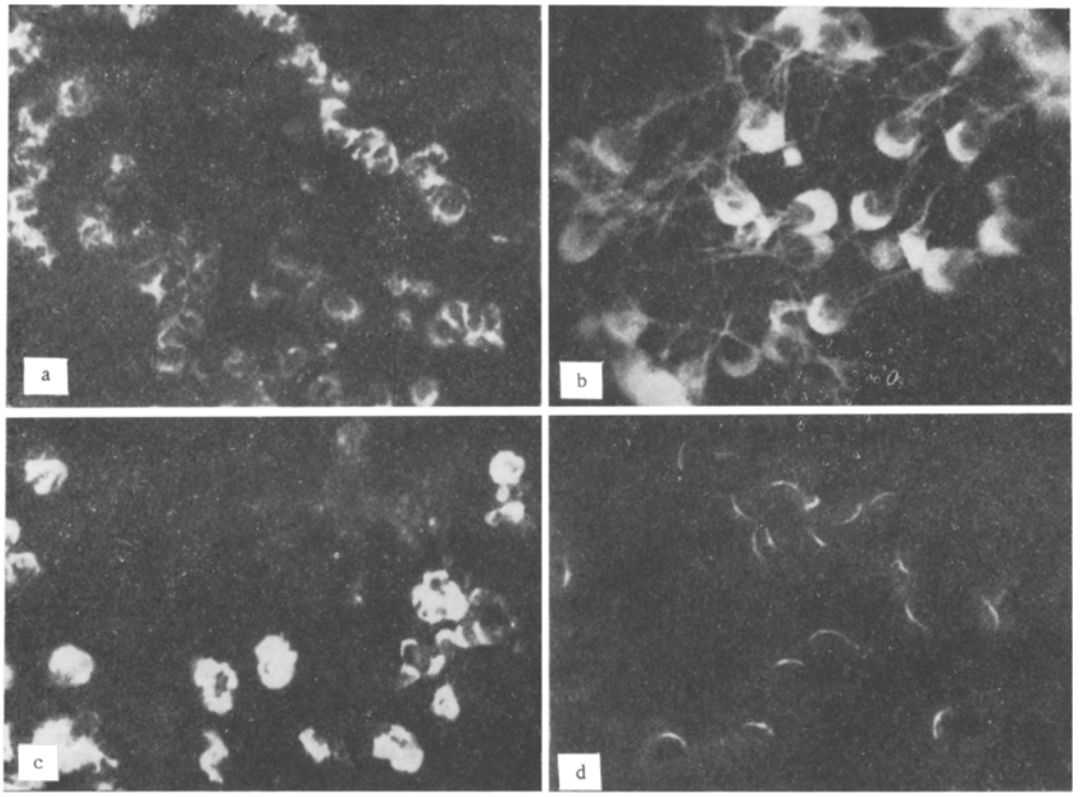

Fig. 1. Localization of serum antibodies of animals with EAIO in structures of the testis and spermatozoa of guinea pigs and mice revealed by the indirect immunoluminescence method. a) Luminescence of spermatids in section of guinea pig testis (210×); b) staining of acrosomes and tail part of spermatozoa in guinea pig sperm film (420×); c) luminescence of spermatids in section through mouse testis (210×); d) luminescence of acrosomes in mouse sperm film (210×).

EXPERIMENTAL RESULTS

Injury to the testes developed in guinea pigs and mice immunized with testicular homogenate. In guinea pigs it was characterized by a reduction in weight of the testes and by atrophy of the spermatogenic epithelium, except cells in the spermatogonium stage, whereas in mice mature spermatozoa disappeared from the convoluted tubules. Injury to the testes in guinea pigs was found from the 14th through the 60th day and in mice from the 14th through the 30th day after immunization. No injuries were found in the testes of guinea pigs and mice of the control groups.

Antibodies capable of staining the cell wall and cytoplasm of spermatids in the convoluted tubule in sections of normal testes were found in sera of male guinea pigs from the 14th through the 60th day after immunization (Fig. 1a). These same sera stained the acrosome of the head and the tail of spermatozoa in guinea pig sperm film (Fig. 1b). In mice immunized by the same method antibodies reacting with structures of spermatids in a section of normal mouse testis and antibodies staining only acrosomes in films of syngeneic spermatozoa were detected from the 14th through the 30th day (Fig. 1c, d). Antibodies were found both in male guinea pigs and mice and in females immunized with testicular antigens. In the females, however, the titer of antibodies was considerably lower and they circulated for a shorter time (Table 1). These differences were particularly marked in mice. On the 30th day, for instance, the antibody titer in the males reached a maximum, whereas in the females it was zero. The titer of antibodies reacting with structures of spermatids found in sections of the testis was always higher than the titer of antibodies staining spermatozoa in sperm films in these same sera (Table 1).

Cytotoxic activity against allogeneic testicular cells was discovered in the sera of male guinea pigs from the 14th through the 30th days after immunization (Table 1).

In mice, cytotoxic activity against syngeneic testicular cells was discovered in the serum on the 14th day after immunization only. The level of cytotoxic activity of the sera was the same in males and females and in guinea pigs and mice.

TABLE 1. Characteristic of Immune Response of Animals Immunized with Testicular Homogenate

Species of animals	Sex	Days after immunization	No. of animals in experiment	No. of animals with orchitis	Log_2 of reciprocals of titers of luminescent antibodies		Cytotoxic activity of sera, %
					reacting with sections of testis	reacting with sperm films	
Mice	Males	14-th	37	23	5±0,9	3,6±0,2	54±3,6*
		30-th	39	23	6±2	4,8±0,9	19±1,6
		Not immunized	24	0	0	0	14,2±1,5
	Females	14-th	20	—	3,6±0,2	2±0,1	52±6,5*
		30-th	25	—	0	0	17±2,1
		Not immunized	21	—	0	0	12,5±1,6
Guinea pig	Males	14-th	18	12	4±0,6	3,3±0,2	60±5,3*
		30-th	21	20	7±3	5,4±1,5	64,2±6,8*
		Not immunized	10	0	0	0	10,2±1,3
	Females	14-th	12	—	3,6±0,2	3±0,2	61,4±7,5*
		30-th	15	—	3,4±0,2	2,6±0,1	63±6,4*
		Not immunized	11	—	0	0	11±1,5

*$P < 0.01$.

No cytotoxic activity and no antibodies staining spermatids in sections of the testis or structures of spermatozoa in sperm films were found in sera of guinea pigs and mice of the control group.

The sera of immunized male guinea pigs did not react in the cross immunoluminescence test with mouse testicular sections or sperm films; in the same way, immune sera of mice did not react with guinea pig testicular antigens. Similar results also were obtained in the cytotoxic test.

Fractionation of a mixture of immunoglobulins from five sera of male guinea pigs obtained on the 30th day after immunization on columns with DEAE-cellulose and Sephadex G-200 yielded two fractions: IgM and IgG. Cytotoxic activity was localized in the IgM fraction (0.6 mg/ml) whereas antibodies staining structures of spermatids and spermatozoa in testicular sections and sperm films were present only in the IgG fraction (0.8 mg/ml).

Treatment of immune sera of male guinea pigs and mice with cysteine hydrochloride, which destroys IgM, removed only the cytotoxic activity of the sera without reducing their ability to stain testicular sections and sperm films in the indirect immunoluminescence test.

Immunization of guinea pigs and mice with testicular antigens thus leads to injury of the spermatogenic epithelium of the testis. The onset of injury to the testis in the animals correlates with the appearance of cytotoxic antibodies against testicular cells and of antibodies reacting in the immunoluminescence test with structures of spermatids and spermatozoa in the serum. It has been suggested that spermatids and spermatozoa contain autoantigens, i.e., antigens capable of inducing the development of EAIO on immunization [2, 6].

Differences found in the staining of spermatogenic epithelial cells in testicular sections and in sperm films with antibodies can be explained by differences in the antigenic composition of the sperm in the convoluted tubules of the testes and sperm contained in the vas deferens, and also by differences in the density and localization of autoantigen on spermatids (deep location) and spermatozoa (superficial location).

When the role of luminescent and cytotoxic antibodies is discussed it must be remembered that although they appear simultaneously at the beginning of the process in the testes, the nature of these antibodies and the structures against which they act are different. Cytotoxic antibodies (IgM) are directed against the surface structures of the testicular cells, they can cause destruction of these cells, and they disappear rapidly from the blood stream. The titer of luminescent antibodies (IgG) without cytotoxic activity rises gradually during development of injury to the testis. The difference observed in the titer of luminescent antibodies in females and males suggests that the higher titer and the longer duration of circulation of antibodies in the latter can be attributed to additional sensitization of the animal with autoantibodies entering the blood stream from the injured testis. Since the cytotoxicity of the sera does not increase under these circumstances, but the intensity of staining of the spermatogenic epithelial cells increases on account of antibodies containing IgG, it can be tentatively suggested that the presence of luminescent antibodies is evidence of a pathological process.

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TRANSFORMING AND ONCOGENIC ACTIVITY OF SIMIAN SA 7 ADENOVIRUS DNA

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Transforming and oncogenic activity of whole Simian adenovirus SA 7 DNA (Ad SA 7) and of a mixture of its fragments produced by restriction endonucleases R. Bam HI and R. Sal I, was studied. Whole virus DNA was shown to transform rat kidney cells and rat embryonic fibroblasts and to induce tumor formation in newborn hamsters. Ad SA 7 DNA, hydrolyzed by R. Bam HI, possesses transforming activity; a mixture of DNA fragments produced by R. Sal I was oncogenic for hamsters.

KEY WORDS: DNA; transformation; oncogenic activity; restriction endonucleases.

Simian adenovirus SA 7 (Ad SA7) is known to possess high oncogenic and transforming activity [7, 8]. Physical mapping of the DNA of this virus by the use of several restriction endonucleases [3] has improved the chances of discovery of the region of the genome carrying the "oncogen."

The object of this investigation was to study the transforming and oncogenic activity of whole Ad SA 7 DNA and of DNA obtained by hydrolysis with restriction endonucleases R. Bam HI and R. Sal I.

EXPERIMENTAL METHOD

Ad SA 7 was grown on green guenon kidney cells [1]. The virus was purified by treatment with Freon-113 and centrifugation in a cesium chloride density gradient [6]. Ad SA 7 DNA was isolated by the detergent-phenol deproteinization method using pronase [4]. Isolation of the endonucleases and hydrolysis of the DNA by the enzymes were carried out as described previously [2].

A primary culture of kidney cells of 5-7-day WAG rats (KC) and a culture of WAG rat embryonic fibroblasts during the second half of pregnancy (EFC) were used for transformation. The EFC was used in the first subculture. The cells were cultured in 50-ml flasks with a seeding density of 180,000 cells/ml for KC and 150,000 cells/ml for EFC. The cells were grown in medium No. 199 with the addition of 10% calf serum. The cell culture was usually used on the 2nd day (70-80% monolayer). Transformation of the cells was carried out by Graham's method [5].

To study oncogenic activity 3-5 μg DNA in a volume of 0.03-0.05 ml was injected into day-old hamsters subcutaneously into the region of the dorsal surface of the neck.

EXPERIMENTAL RESULTS

On the addition of Ad SA 7 DNA to the flasks with the primary culture KC and EFC from WAG rats foci of transformation were formed. The foci appeared as white spots on the cell monolayer and became visible

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