SPECIFIC INHIBITION OF ADHESION OF MACROPHAGES FROM ANIMALS WITH POSTTRAUMATIC AUTOALLERGIC ASPERMATOGENESIS

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Inhibition of adhesion of peritoneal macrophages obtained from guinea pigs with induced posttraumatic aspermatogenesis at various times after injury to the testis was studied. Significant inhibition of macrophage adhesion was observed in the presence of organ-specific testicular antigen. The inhibition of adhesion reflects the autoimmune character of the testicular lesion.

KEY WORDS: *injury to the testis; macrophages; autoallergic aspermatogenesis.*

Injury to the testis in sexually mature mammals is followed by progressive orchitis and ultimately terminates in atrophy of the organ [2, 4]. The developing process is directed against antoantigens of the spermatogenic epithelium and it leads to total annihilation of the generative cells [5]. The process is of a well-marked autoimmune character, and according to most investigators its basic component is increased sensitivity of delayed type to autoantigens of the spermatogenic epithelium.

The object of this investigation was to study the reaction of the afferent system of the immune response (A cells) during the development of posttraumatic orchitis.

EXPERIMENTAL METHOD

Noninbred male guinea pigs weighing 450-550 g, kept on the ordinary animal house diet with daily addition of products containing vitamin C (fresh cabbage or carrots), were used. Trauma consisted of making an incision in the scrotum, withdrawing one testis (usually the right), then puncturing it with a hot probe about 3 mm thick in the avascular zone [1]. The wound was sutured in layers. Combined mechanical and thermal trauma was thus inflicted.

A suspension of peritoneal cells was obtained on the 2nd, 4th, 6th, 8th, 10th, 12th, and 14th day after injury. The macrophage adhesion inhibition test (MAIT) was carried out by the method of Holan et al. [9] in 10-cm glass tubes in the presence of organospecific testicular antigen isolated by the method of Freund et al. [7]. The ASPM* fraction was used. Four tubes with peritoneal cells from each animal were used in each experiment and two tubes in the control. Each tube contained 0.15 ml of the cell suspension ($4 \cdot 10^6$ cells/ml) and 0.15 ml of antigen, both diluted with Eagle's medium. The following controls were set up: 1) macrophages of intact animals and organ-specific testicular antigen; 2) macrophages of intact aimals and 0.15 ml medium; 3) macrophages of the experimental animals and bovine serum albumin (BSA); 4) macrophages of the experimental animals and 0.15 ml medium. The degree of inhibition was assessed from the percentage of cells not adherent to the glass. In parallel experiments the morphology of the injured and intact testes was studied (fixation in Carnoy's fluid, paraffin sections stained with hematoxylin–eosin). The degree of development of autoallergic aspermatogenesis was estimated from the weight of the injured and intact testes and also from the number of tubules with active spermatogenesis. In each testis 400 tubules were counted in each of 2 sections taken arbitrarily [11].

*Ammonium sulfate precipitate material (translator's note).

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TABLE 1. State of Spermatogenesis and Number of Nonadherent Macrophages at Various Times of Development of Posttraumatic Orchitis (M ± m)

Time after trauma, days	Per cent of nonadherent cells in presence of vesicular antigens	Per cent of tubules with spermatogenesis	Weight of testis, mg		
			injured	intact	P
2	52,4±4,8	25,0±3,5	1200±15	1500±10	>0,01
4	69,7±13,3	20,1±4,1	1380±10	1400±13	>0,01
6	70,1±7,2	12,4±8,3	1000±18	1500±15	<0,001
8	70,5±7,4	5,6±5,1	730±20	1380±18	<0,001
10	75,4±5,1	5,2±3,0	600±32	1400±20	<0,001
12	60,1±7,0	3,8±5,6	620±16	1370±12	<0,001
14	51,2±8,4	3,0±1,2	500±16	1390±15	<0,001

EXPERIMENTAL RESULTS

After trauma to the testis the anatomical integrity of the structure composing the blood-testis barrier was disturbed and immunocompetent cells obtained access to the spermatogenic epithelium, individual areas of which were destroyed as a result of trauma. In the present experiments mechanical disturbance of the blood–testis barrier was combined with thermal injury to the testicular tissue and for that reason autoantigens were set free not only in the native but also in the denatured state.

The results reflecting the dynamics of development of posttraumatic aspermatogenesis and the results of the MAIT at the corresponding times are given in Table 1. Comparison of the weights of the injured and intact testes shows a progressive fall in weight of the injured testis. Whereas during the first 4 days after injury this could be explained by resorptive processes in the wound canal, the later fall in weight could only be the result of the development of autoallergic changes in the injured testis. This was confirmed by the dynamics of the basic criterion of the process, the degree of preservation of spermatogenesis. In the intact organ it was held steadily at 25–30% throughout the period of observation, whereas in the injured testis the greatest disturbance of spermatogenesis took place from the 6th to the 12th day of development of the process inclusive (by the 12th day after injury spermatogenesis was observed in only 3.8% of tubules). Pathological changes in the injured testis were observed as early as on the 2nd day after trauma, not only in the focus of injury but also in more remote areas. Desquamation of cells of the spermatogenic epithelium into the lumen of the tubules, sometimes (rarely) accompanied by polynuclear spermatids was observed. Later during development of the process foci of infiltration consisting of lymphocytes appeared in the stroma of the organ. By the 12th-14th day sex cells were almost completely absent in the testes and the lumen of the tubules contained only Sertoli cells with vacuolated cytoplasm; the weight of the injured testes was reduced to 500 mg. The picture observed was in general agreement with data in the literature [3, 6].

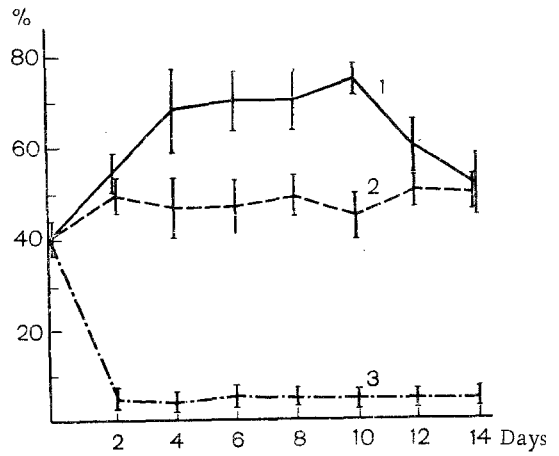

Fig. 1. MAIT at various times after injury to testis: 1) macrophages of experimental animals and organ-specific testicular antigen; 2) macrophages of experimental animals and BSA: 3) macrophages of experimental animals without addition of antigen. Abscissa, days after trauma; ordinate, percentage of nonadherent cells.

The development of the MAIT in animals with posttraumatic aspermatogenesis in the presence of organ-specific testicular antigens is illustrated in Fig. 1. During the first 2-4 days after trauma a sharp increase was observed in the number of nonadherent macrophages, but later, as the process developed, their number increased slowly up to a maximum on the 10th day, i.e., rather sooner than the changes in the spermatogenic epithelium reached the peak of severity (12-14 days after trauma). By that time the number of nonadherent macrophages had fallen almost to its initial level.

Inhibition of macrophage adhesion in the experiments with organ-specific testicular antigen was specific in character (the differences from the control — adhesion of macrophages in the presence of BSA — were significant). The large number of nonadherent cells in the presence of BSA (about 50%) could be explained by nonspecific delay of adhesion in medium containing large quantities of any protein [8]. The number of nonadherent cells increased from 6 to 40% on the addition of 15% calf serum to the medium and to 50% on the addition of tissue antigens. In the control (in Eagle's medium) the number of nonadherent cells did not exceed 6% in the absence of antigen.

The mechanism of inhibition of macrophage adhesion is not yet clearly understood. The most likely explanation is direct interaction between receptors of the sensitized macrophage and the antigen [9]. The origin of these receptors also is not clear. However, the dynamics of the reaction (in the present experiments) shows that the macrophagal receptors cannot be cytophilic antibodies, for production of the latter continues for a relatively long time after the antigenic stimulus [10], whereas the intensity of the MAIT was declining by the 14th day of the process.

Comparison of the dynamics of the MAIT in the presence of vesticular antigen and the development of posttraumatic orchitis reveals the important role of macrophages in the inductive phase of the autoimmune process and in the development of enhanced sensitivity of delayed type in relation to the testicular autoantigens.

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