6. J. F. A. P. Miller, M. A. Vadas, A. Witlow, et al., Proc. Natl. Acad. Sci. USA, 72, 5095 (1975).
7. S. D. Miller, M. S. Sy, and H. N. Claman, J. Exp. Med., 145, 1071 (1977).
8. J. W. Moorhead, J. Exp. Med., 150, 1432 (1979).
9. S. S. Rich, C. S. David, and R. R. Rich, J. Exp. Med., 149, 114 (1979).
10. D. H. Katz and B. Benacerraf,eds., The Role of Products of the Histocompatibility Gene Complex in Immune Responses, New York (1976).
11. G. M. Shearer, Eur. J. Immunol., 4, 527 (1974).
12. T. Tada, M. Taniguchi, and C. S. David, J. Exp. Med., 144, 713 (1976).
13. R. Zinkernagel, J. Exp. Med., 144, 776 (1976).

DELAYED TYPE AUTOIMMUNE REACTION AFTER LIGATION OF THE VASCULAR BUNDLE OF THE TESTIS

V. S. Sazonov and V. V. Endolov

UDC 616.137.72+616.146.6/-089.814.-07:
616-008.9-097.3-079.73

KEY WORDS: cellular immunity; ischemia of the testis; inhibition of adhesion of peritoneal cells.

The ability of autoantigens of the spermatogenic epithelium to induce autoimmunity after injury to the blood-testis barrier has not been proved [1, 3, 6]. However, injury to the testis after ligation of the vascular bundle supplying the organ is usually attributed predominantly to the after-effects of hypoxia, and autoimmune processes are disregarded [2, 13]. Nevertheless, there is evidence in the literature of a humoral response to testicular antigens after crushing of individual components of the testicular vascular bundle [7, 9, 14]. The development of cellular immunity under these circumstances can be judged by the infiltration of monocytes found in some cases into the testicular tissue [2, 5], but only in one investigation [7] was it assessed by the rosette formation test with spermatozoa.

The object of this investigation was to study the delayed type immune reaction to autoantigens of the testis after ligation of its vascular bundle.

EXPERIMENTAL METHOD

Experiments were carried out on 35 noninbred mature male rats weighing 150-300 g. All painful manipulations including euthanasia were carried out under ether anesthesia. The vascular bundle of the testis was crushed by a ligature applied unilaterally for 40 min, which was repeated from 1 to 5 times at intervals of 1-30 days. The results were assessed after 7, 30, or 50-100 days. The difference in weight of the experimental and contralateral (intact) testes, expressed as a percentage of the weight of the contralateral testis, was used as an index of testicular atrophy.

Cellular immunity was assessed by the test of inhibition of adhesion of peritoneal exudate cells to glass in the presence of antigen (the adhesion inhibition test – AIT) [10]. The degree of a positive response was judged from the percentage of nonadherent cells after incubation of the cell suspension with antigen. The AIT of a mixture (1:1) of peritoneal cells with lymphocytes from the inguinal lymph node, the regional node for the experimental testis, also was investigated. The supernatant of a homogenate of intact autologous testis with a protein concentration of 2.5-3 mg/ml served as the autoantigen. Peritoneal cells were obtained without preliminary injection of irritants into the peritoneal cavity.

The experimental animals were divided into five groups: 1) ligation once, 2) twice, 3) three times, 4) ligation five times with an interval of 7 days, and 5) ligation five times with an interval of 1 day. The animsl were killed not less than 25 days after the first ligation (except in two cases), and in groups 4 and 5 they were killed after at least 50 days. The controls were intact animals and also: a) adhesion of cells without antigen; b) adhesion in the presence of bovine serum albumin (BSA) in a concentration of 2.5-5 mg/ml.

Department of Biology, Academician I. P. Pavlov Ryazan Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 11, pp. 579-581, November, 1981. Original article submitted December 16, 1980.

 0007-4888/81/9211-1536$07.50

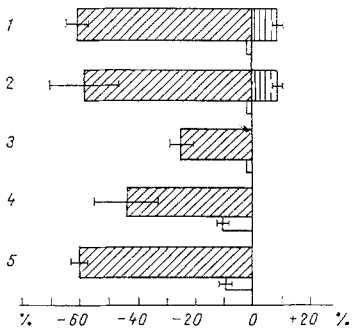

Fig. 1

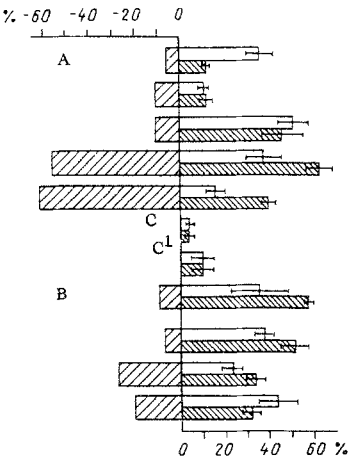

Fig. 2

Fig. 1. Atrophy of rat testis after repeated ligation of its vascular bundle. Abscissa, change in weight of experimental testis (in % of weight of contralateral testis). Oblique shading denotes severe atrophy (over 25%); vertical shading denotes increase in weight of experimental testis; unshaded columns denote slight atrophy (under 15%). 1, 2, 3, 4, 5) Groups of animals.

Fig. 2. Inhibition of adhesion of peritoneal cells during atrophy of the testis following ligation of its vascular bundle. A) Ligation five times (interval 1 day); B) ligation two and three times (interval 7 days). Abscissa: on left – loss of weight of experimental testis (in % of weight of contralateral testis); on right – percentage of nonadherent cells in presence of antigen relative to number of cells before beginning of incubation. Narrow shading – positive response of mixture (1:1) of peritoneal cells and lymphocytes from regional inguinal lymph node; no shading – positive response of peritoneal cells; wide shading – atrophy of testis. C) Control without antigen (done for each test); C_1) control of BSA.

EXPERIMENTAL RESULTS

When the results were analyzed the great variability of all indices was noted, even in the case of a single ligation. Comparison of the groups showed that severe strophy of the testis (loss of weight of 25-60%) was observed in all groups (Fig. 1). After ligation once and twice a small increase in weight of the experimental testis was found in some of the animals. In each group, atrophy was slight in 30-60% of cases, even after ligation five times. It is interesting to compare the results of weekly and daily ligation (Fig. 1). In both groups there were animals with over 60% of atrophy of the testis, but there were more cases of severe atrophy after weekly ligation (in 65% of animals compared with 40%). After weekly ligation the degree of atrophy varied considerably, whereas after daily ligation atrophy only two variants were found: either slight atrophy ($10 \pm 5\%$) or severe ($60 \pm 3\%$; $P < 0.01$).

A study of cellular immunity after ligation gave the following results. The level of the positive response in the AIT to testicular autoantigens varied considerably in different animals (Fig. 2). The cellular response of the peritoneal suspension did not correlate with the degree of testicular atrophy. Addition of lymphocytes from the regional lymph nodes for the affected testis to the peritoneal suspension, however, in some cases significantly raised the level of the positive reaction ($P < 0.01$). In group 5 this strengthening of the positive response on the addition of lymphocytes was observed only for cases with severe testicular atrophy (Fig. 2A), and it was not observed when atrophy was slight. The phenomenon of strengthening of the positive response to autoantigens on the addition of lymphocytes also was observed in the other groups at all times of observation (up to 68 days after ligation; Fig. 2B).

In the controls for AIT without antigen the number of nonadherent cells did not amount to 10% and was usually $4 \pm 2\%$. The level of the positive response in the AIT with BSA did not exceed $10 \pm 5\%$. The level of the positive response in the intact animals as a rule did not reach 30%. Addition of lymphocytes did not change the values obtained in the control. An additional series of experiments showed, moreover, that the level of the positive response in the AIT in the intact animals was more dependent on antigen concentration than in the experimental animals.

Contrary to the view that testicular atrophy develops as a result of the successive development of hypoxic injury to the germinative cells [2, 13], no correlation was found in the present experiments between the degree of atrophy and the time elapsing after ligation, in agreement with data obtained by other workers [13]. If it is accepted that autoimmune mechanisms participate in the development of atrophy, the scatter of the results finds a full explanation. It may be due to differences in the individual immune reactivity of the animals, in agreement with data showing interlinear differences in the ability of rats to autoimmune orchitis [12]. The more frequent atrophy following delayed repeated ligation at weekly

intervals points indirectly to the possible involvement of autoimmune processes in a response of secondary type. The presence of an autoimmune component in "pure" hypoxic injury to the testes after repeated "ascents" in a pressure chamber, which also confirms these suggestions, was described previously [4].

Reactions of delayed type play an important role in autoimmune injuries to the testis [3, 6, 15]. The inhibition of cell adhesion test in the presence of antigen is an adequate test of increased sensitivity of delayed type to testicular antigens [3, 8]. Recent investigations have shown [11] that the positive response in the AIT is due to T lymphocytes producing a soluble factor in the presence of antigens. From this point of view the phenomenon of potentiation of the positive response which we found is evidence of the appearance of lymphocytes sensitized to testicular tissue after ligation. Potentiation of the cellular response was observed not only in the early period of injury, but also 2 months after ligation. Under these circumstances a significant degree of potentiation was observed only when atrophy of the testis was severe.

The results indicate that an autoimmune reaction of delayed type participates in the development of atrophy of the testis after ligation of its vascular bundle.

LITERATURE CITED

1. N. S. Gladkova, "Electron-microscopic investigation of the blood-testis barrier and the spermatogenic epithelium of rats under normal conditions and after experimental procedures," Author's Abstract of Candidate's Dissertation, Moscow (1976).
2. O. A. Gotsiridze, N. A. Onishchenko, R. M. Kurlinyan, et al., Krovoobrashchenie, No. 12, 48 (1979).
3. V. V. Endolov and N. G. Artsimovich, Byull. Éksp. Biol. Med., No. 3, 324 (1977).
4. Yu. A. Medvedev and Zh. T. Turganbaev, Arkh. Patol., No. 7, 56 (1973).
5. E. P. Mel'man, S. M. Mints, B. V. Gripulyak, et al., Arkh. Patol., No. 5, 71 (1969).
6. S. S. Raitsina, Trauma of the Testis and Autoimmunity [in Russian], Moscow (1970).
7. S. S. Raitsina, A. V. Kurnosov, I. M. Yarovaya, et al., Byull. Éksp. Biol. Med., No. 1, 44 (1979).
8. B. G. Dorsman, A. G. Tumboh-Oeri, and T. K. Roberts, J. Reprod. Fertil., 53, 277 (1978).
9. J. Friberg, A. Fritjofsson, Arch. Androl., 2, 317 (1979).
10. V. Holan, M. Hasek, J. Bubenik, et al., Cell. Immunol., 13, 107 (1974).
11. T. A. Koppi, A. E. Maluish, and W. J. Halliday, J. Immunol., 123, 2255 (1979).
12. H. L. Lipscomb, P. J. Gardner, and G. J. Sharp, J. Reprod. Immunol., 1, 209 (1979).
13. A. G. Oettle and R. C. Harrison, J. Path. Bact., 64, 273 (1952).
14. P. Rümke, Proc. Roy. Soc. Med., 61, 275 (1968).
15. K. S. K. Tung, C. Leong, and T. McCarty, J. Immunol., 118, 1774 (1977).