VARIABILITY OF THE IMMUNE RESPONSE IN MICE IMMUNIZED AT DIFFERENT TIMES AFTER TOTAL AND PARTIAL REMOVAL OF THE TISSUE OF ORGANS DIFFERING IN REGENERATIVE POWER

A. G. Babaeva and V. V. Arsent'eva

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The immunoreactivity of animals is known to vary. Its changes have been analyzed in most detail in studies of the antibody-forming capacity of splenocytes after their adoptive transfer. These data have been reviewed on several occasions [2]. It follows from a number of investigations in which the adoptive transfer was used to study the immune response that this model can explain many aspects of relations between immune and repair processes and the character of changes in the immune response in animals during regeneration of certain organs. Meanwhile, many aspects of the problems remain unstudied. In particular, we have no systematic information about changes in the immunoreactivity of animals depending on the period of the recovery process, or how it affects the general state of the organism. It will be quite evident that the cascade process, developing immediately after injury, must affect the possibility of implementation of the various properties of lymphoid cells. Yet this effect and the dependence of immunoreactivity on the stage of the process have not received their due attention.

The aim of this investigation was to determine the immunoreactivity of mice after operations on various organs, when the animals were immunized at different times after the operation.

EXPERIMENTAL METHOD

In accordance with the aim of the investigation, tissue of one of the following organs was partly or completely removed from male CBA mice: liver, kidney, salivary gland, testis. This choice of organs was determined by the difference in their powers of recovery: from high (liver, kidney) to very weak, or even absent (salivary gland, testis). All operations were performed under ether anesthesia: removal of two-thirds of the liver (by the usual method), of one kidney, of one or two submandibular glands (a single complex with the sublingual glands), and one or two testes. Animals undergoing these operations or mock operations and intact animals were immunized by a single injection of sheep's red blood cells (in a dose of $5 \cdot 10^8$ cells per mouse in 1 ml of medium 199) immediately after the operation and 1, 3, 5, 7, and 14 days later. The mice were killed 5 days after immunization by cervical dislocation and the number of antibody-forming cells (AFC) was determined in the spleen by the local hemolysis in gel method [5]. From seven to 10 animals were used at each time. The numerical results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Removal of two-thirds of the liver increased the ability of the immunocompetent cells to respond by an increase in the number of AFC compared with their number in intact animals and animals undergoing a mock operation, in cases when immunization coincided in time with the operation, or was carried out 24 h after it. When mice were immunized in the later stages (3, 5, 7, and 14 days) after the operation, the level of antibody production did not differ significantly from

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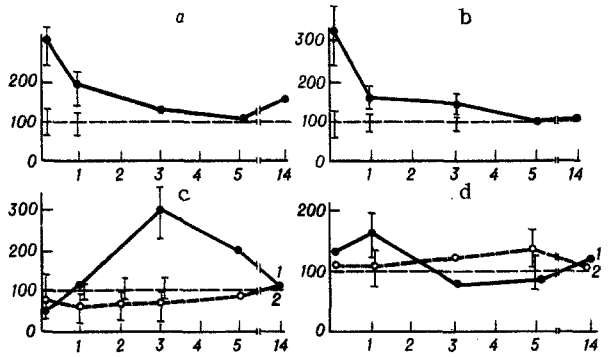

Fig. 1. Changes in antibody-forming capacity of splenocytes in mice immunized at different times after partial hepatectomy (a), unilateral nephrectomy (b), unilateral sialadenectomy (a, 1), bilateral sialadenectomy (c, 2), unilateral castration (d, 1), and bilateral castration (d, 2). Abscissa, time after operation and beginning of immunization with sheep's red blood cells (in days); ordinate, number of AFC (in % of number in control).

that in the control (Fig. 1a). In the case of unilateral nephrectomy, similar changes were observed in the level of antibody production (Fig. 1b), the only difference being that the duration of the postoperative period during which immunization induced a strengthened immune response was significantly greater than after partial hepatectomy. These data are in agreement with those obtained in a study of this process under conditions of adaptive transfer of splenocytes of animals undergoing the operation [3].

Unilateral sialadenectomy caused completely different changes in antibody production from operations on the liver and kidney. In the case when the animals were immunized immediately and 24 h after the operation the number of AFC in the spleen of the experimental mice was significantly less than in the control. When the mice were immunized on the 3rd-7th days after the operation, antibody production by the experimental animals, on the other hand, was at a higher level than in the control, i.e., in intact mice and mice undergoing a mock operation (Fig. 1c).

Unexpected results were obtained by immunization of mice subjected to unilateral and bilateral castration. None of the observed deviations of the level of antibody production from the control was significant (Fig. 1c, d). after removal of two salivary glands (bilateral removal of the submandibular and sublingual salivary glands) changes in immunoreactivity of the animal were virtually absent (Fig. 1c).

The results are evidence that different organs interact differently with the immune system. On resection of organs with high regenerative capacity, increased ability of the immunocompetent cells to give an immune response is observed in the early period after the operation. After operations on organs with weak powers of regeneration, the response of the immune system differed. Unilateral sialadenectomy, not accompanied by any compensatory growth of the residual gland, leads to inhibition of the immune response. It can be tentatively suggested that the unilateral operation is accompanied by the release of certain biological substances, in which the submandibular gland of mice is rich and which have a suppressor effect on the immune response [1], into the bloodstream from the residual gland.

As regards the testes, we know that they are barrier organs, whose protein substances are autoantigenic [4]. The impression is created that it is for these reasons that the immune system is "uninformed" regarding the presence or absence of this organ. It is noteworthy that the change in immunoreactivity of the animals undergoing the operations is phasic in character. This is due to the operation itself and is independent of the vector of the initial change in the level of antibody production.

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ACTION OF ANTICHOLINERGIC RECEPTOR ANTIBODIES ON THE FROG HEART

G. V. Burlakov and V. A. Kamysheva

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It has been shown that anticholinergic receptor (ACR) antibodies, obtained by immunization of rabbits with cholinergic receptors isolated from motor-denervated skeletal muscles of Balb/c mice, were able to block the cholinergic receptors of mouse splenic lymphocytes by preventing the action of cholinergic drugs on them [1, 2]. This blockade indirectly modified the functions of the immune receptors [1]. It was also found by the direct immunofluorescence method that specific action by ACR antibodies against cholinergic receptors of B lymphocytes led to the virtually total suppression of interaction of the labeled antiglobulin serum with antigen-binding receptors of B lymphocytes, which confirmed previous data relating to changes in the functional state of the immune receptors of lymphocytes during blockade of mediator receptors [2]. Blocking of cholinergic receptors by ACR antibodies also was found in our experiments on the dorsal muscle of *Hirudo medicinalis* [3].

In the investigation described below, the action of ACR-antibodies was studied on the frog heart — an organ with a high density of cholinergic receptors (mainly of the M type) [5].

EXPERIMENTAL METHOD

Experiments were carried out on 85 autumn—winter frogs of the species *Rana temporaria*, male and female, weighing 60-80 g. The test object was the isolated heart, which was removed and cannulated in the usual way. Ringer's solution for cold-blooded animals was used. Cardiac contractions were recorded under isometric conditions on the revolving drum of an electric kymograph. Experiments on the surviving organ were carried out within a period of 2.5-3 h at room temperature. To denervate the hind limb muscles the sciatic nerve of 350 adult frogs and 500 Balb/c mice was divided. The number of cholinergic receptors in denervated muscles is much greater than in nondenervated muscles [4, 9, 10, 12]. Protein of acetylcholine receptors in its membrane-bound form was isolated and purified by the method in [11]. Chinchilla rabbits were immunized with the preparation thus obtained. The antibody titer at which the sera were used in the experiments in the complement fixation test was 1:1280. The following ACR-antibodies were used: antibodies obtained by immunization of rabbits with cholinergic receptor protein isolated from the motor-denervated muscles of the frogs' hind limbs, and also (for the control) form nondenervated frog hind limbs; antibodies against cholinergic receptors of motor-denervated hind limb muscles of Balb/c mice. Serum of unimmunized intact rabbits also was used as the control. The sera were used in dilutions of 1:10, 1:5, and 1:2, and also undiluted. The experimentally selected incubation time of the test

Central Research Laboratory for Synthesis and Testing of New Drugs, N. I. Pirogov Second Moscow Medical Institute. Institute of Immunology, Ministry of Health of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 8, pp. 170-174, August, 1991. Original article submitted February 6, 1991.

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