EFFECT OF UNILATERAL NEPHRECTOMY IN MICE ON HUMORAL IMMUNE RESPONSE LEVELS TO T-INDEPENDENT ANTIGEN

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It was shown previously that unilateral nephrectomy induces activation of the immune system during the first 2 days after the operation. In this period the spleen cells of nephrectomized mice, unlike splenocytes of intact animals or after a mock operation, had increased ability to induce a regional graft versus host reaction [4] and responded by enhancement of antibody formation to a foreign antigen in an adoptive transfer system [5].

It was postulated that enhancement of the humoral immune response in this system was not associated with direct activation of B lymphocytes, but was mediated by helper T cells [3, 5].

To test this hypothesis it was decided to study the immune response to a class 2 T-independent antigen, not possessing mitogenic activity [1], in intact and unilaterally nephrectomized animals.

EXPERIMENTAL METHOD

Experiments were carried out on 220 male (CBA×C57BL/6)F_1 mice weighing 18-20 g, obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR.

The experiments were carried out under adoptive transfer conditions. Both intact and unilaterally nephrectomized animals were used as splenocyte donors. The operation was performed 17-20 h before removal of the spleen, for that is the time when splenocytes of unilaterally nephrectomized animals have increased ability to produce antibodies to T-dependent antigen [5]. For the operation the donors were anesthetized with pentobarbital, which was dissolved in physiological saline and injected intraperitoneally in a dose of 60 mg/kg body weight and in a volume of 0.2 ml per mouse.

Syngeneic animals, lethally irradiated in a dose of 11 Gy on a GUBE-1500 apparatus served as recipients. Splenocytes were injected into the retro-orbital sinus in a dose of 5×10^7 per mouse.

Together with spleen cells, some of the recipients also received antigen. As the class 2 T-independent antigen the mice were injected with 5×10^{-8} μg polyvinylpyrrolidone with mol. wt. of 350 kD (PVP_{350}), and as T-dependent antigen, with 5×10^8 sheep's red blood cells (SRBC).

The number of cells forming IgM-antibodies (AFC) and the number of immunoglobulin-forming cells (IGFC) were determined at various times after adoptive transfer and immunization, in the recipients' spleens, by the method of single [9] and reverse [11] hemolysis in gel.

As the test antigens for determination of AFC to SRBC and PVP_{350} we used the corresponding native SRBC or red blood cells sensitized by PVP with mol. wt. of 24 kD [10], and for determination of IGFC we used SRBC sensitized with rabbit antibodies to mouse immunoglobulins [11]. The number of cells producing nonspecific immunoglobulins (NIGFC) was calculated as the difference between the numbers of IGFC and AFC per 10^6 cells.

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TABLE 1. Time Course of Specific (AFC) and Nonspecific (NIGFC) Components of Immune Response to PVP_{350} under Conditions of Adoptive Transfer of Syngeneic Intact Splenocytes

Days after transfer and immunization with PVP_{350}	Number of AFC and NIGFC per 10^6 splenocytes (M ± m)	
	AFC	NIGFC
0	33±4,5	5 135±1 052
2	22±4,0	13 068±1 630
5	355±45,0	39 719±5 320
7	397±58,0	48 749±12 934
9	279±105,0	10 601±2 219

Legend. Ten animals were used in each group.

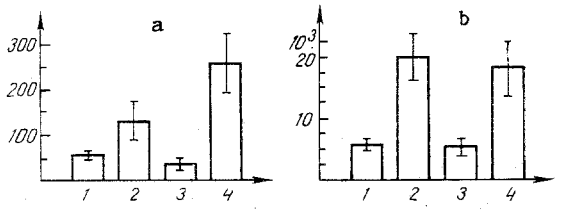

Fig. 1. Effect of unilateral nephrectomy on specific (a) and nonspecific (b) components of immune response to T-independent (I) and T-dependent (II) antigen. Columns represent groups of lethally irradiated recipients receiving: 1) splenocytes of unilaterally nephrectomized mice, 2) splenocytes of unilaterally nephrectomized mice and antigen, 3) splenocytes of intact animals, 4) splenocytes of intact animals and antigen. Vertical axis — Number of cells per 10^6 splenocytes.

The results were subjected to statistical analysis by the Fisher—Student method with determination of the error of the mean and the level of significance.

EXPERIMENTAL RESULTS

Before embarking on the main series of experiments it was necessary to determine the optimal times of recording the immune response to PVP_{350} in the adoptive transfer system. The dose of antigen was chosen allowing for data obtained previously, according to which injection of $5 \cdot 10^{-1}$ μg PVP_{350} into mice leads to maximal antibody formation [2]. Changes in specific (number of AFC) and nonspecific (number of NIGFC) components of the immune response in the recipients' spleen were determined successively on the 2nd, 5th, 7th, and 9th days after immunization and transfer of the splenocytes. As Table 1 shows, immunization of recipients with PVP_{350} leads to induction of both AFC and NIGFC. A maximum of AFC and NIGFC formation was observed on the 5th-7th days, but the rise of the nonspecific component of the immune response was observed earlier than that of the specific response. The NIGFC/AFC ratio on the 5th and 7th days was 111.9 and 123.5 respectively. Thus in the adoptive transfer system, just as in vivo [2], PVP_{350} induced the formation not only of AFC, but also of antigen-dependent NIGFC. Subsequent experiments were carried out to determine the number of AFC and NIGFC on the 5th day after transfer and immunization of the mice.

In the main series of experiments the effect of unilateral nephrectomy on the immune response to PVP_{350} was studied. Since the operation was performed under pentobarbital anesthesia, recipients receiving splenocytes from intact animals which also were anesthetized 17-20 h before splenectomy were used as the control. It will be clear from Fig. 1, that unilateral nephrectomy had virtually no effect on induction of the antigen-dependent immune response. For instance, splenocytes of the nephrectomized animals responded to T-independent antigen by the formation of 20,351 ± 3261 NIGFC whereas splenocytes of intact mice responded

by the formation of 18,577 ± 4172 NIGFC. The background number of NIGFC, moreover, did not depend on the operation (Fig. 1, I). Meanwhile removal of one kidney caused a decrease in the specific component of the immune response by 1.9 times compared with the intact control (132 ± 38 AFC in the nephrectomized animals compared with 256 ± 66 AFC to PVP_{350} per 10^6 splenocytes in the controls).

Thus unilateral nephrectomy had virtually no effect on the antigen-dependent nonspecific component of the immune response, but caused slight inhibition of the specific component. Although this inhibition was not statistically significant, it was repeated from one experiment to another. The results with T-independent antigen differed sharply from those obtained by the use of T-dependent antigen, which enhances the humoral immune response after unilateral nephrectomy [3, 5]. These data indicate that T lymphocytes of nephrectomized animals differ functionally from the T lymphocytes of intact animals. Consequently, the very slight reduction of the immune response observed in the present experiments with PVP_{350} (Fig. 1, I) can evidently also be explained by its action on T lymphocytes, specifically on suppressor T cells, which participate in regulation of the humoral immune response to PVP_{350} [8, 10].

The effect of unilateral nephrectomy on enhancement of only the specific immune response to T-dependent antigen was demonstrated previously [3, 5]. The results obtained with T-independent antigen led us to study the effect of the operation of nephrectomy on the nonspecific component of the immune response to a T-dependent antigen (SRBC) also. The results of these experiments are shown in Fig. 1, II. Clearly unilateral nephrectomy leads not only to a twofold increase in the number of AFC to SRBC, but also to a threefold increase in the number of antigen-dependent NIGFC.

The results obtained with T-independent and T-dependent antigens are thus evidence that after unilateral nephrectomy activation of helper T cells and also, perhaps, of suppressor T cells, takes place in the spleen of animals undergoing the operation. In that case the data obtained with T-independent antigen can be explained on the grounds that helper T cells are activated more strongly than suppressor T cells, or that activation of helper T cells takes place before activation of suppressor T cells. All these problems can be solved by the use of pure subpopulations of T and B lymphocytes from the nephrectomized animals.

It must be specially emphasized that unilateral nephrectomy causes changes in the spleen which lead to a disturbance of humoral immunity at the level not only of the specific, but also of the nonspecific component of the immune response. Data obtained by the use of T-dependent antigen are evidence that these two processes (the specific and antigen-dependent nonspecific response) pursue a parallel course in the body, in agreement with results obtained on other experimental models [6, 7].

LITERATURE CITED

1. M. G. Agadzhanyan, Usp. Sovrem. Biol., 104, No. 1 (4), 55 (1987).
2. M. G. Agadzhanyan, N. I. Smirnova, and E. V. Sidorova, Byull. Éksp. Biol. Med., No. 8, 206 (1986).
3. A. G. Babaeva and E. A. Zotikov, Immunology of Adaptive Growth, Proliferation, and Their Disturbances [in Russian], Moscow (1987).
4. A. G. Babaeva and E. I. Gimmel'farb, Byull. Éksp. Biol. Med., No. 3, 336 (1985).
5. A. G. Babaeva and E. I. Gimmel'farb, Ontogenez, No. 1, 42 (1988).
6. A. A. Mikhailova, A. E. Gurvich, and R. V. Petrov, Vopr. Med. Khimii, 15, 128 (1969).
7. M. G. Agadzhanyan (M. G. Agadjanian), V. G. Nesterenko, T. B. Megrabian, et al., Immunol. Lett., 9, 307 (1985).
8. P. J. Baker, N. D. Reed, P. W. Stashak, and B. Prescott, J. Exp. Med., 137, 1431 (1973).
9. R. E. Jerne and A. A. Nordin, Science, 140, 405 (1963).
10. J. P. Lake and N. D. Reed, Cell. Immunol., 21, 364 (1976).
11. N. R. Ling, S. Bishop, and R. Jefferis, J. Immunol. Meth., 15, 279 (1977).