CORRELATION BETWEEN CELLULAR AND HUMORAL IMMUNE RESPONSES TO DIFFERENT DOSES OF SHEEP'S RED BLOOD CELLS IN MICE

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The principal forms of cellular and humoral immune response, namely the delayed-type hypersensitivity reaction (DTHR) and antibody production (ABP), are interconnected both with each other and with the process of nonspecific phagocytosis (NSP). Mediators of effector T cells of the DTHR (T_e) not only induce immune inflammation, but also increase the specific activity of macrophages and other phagocytic cells [1, 7], but antibodies, because of their opsonizing effect, facilitate antigen-specific phagocytosis [3]. Meanwhile activity of the phagocytes largely determines the threshold of sensitivity of the lymphoid tissue to antigen [4, 6]. whereas DTHR and AGP, which complement each other, may in some cases compete with one another [2, 4]. Under these circumstances the biological significance, genetic determination, and immune mechanisms of these interactions are still largely unclear. This is particularly true of the local immune process, when the main site of T_e formation and of antibody-forming cells (AFC) is the regional lymph nodes.

In the investigation described below correlation between DTHR and ABP was studied during the local and generalized immune response to different doses of sheep's red blood cells (SRBC) in inbred and noninbred mice.

EXPERIMENTAL METHOD

Mice weighing 18-22 g were used: 160 CBA, 170 C57BL/5, 190 (CBA × C57BL/6)F_1 hybrids, and 140 noninbred albino mice.

The intensity of the immune response was assessed 5 days after subcutaneous (into the foot) or intraperitoneal immunization with various doses of SRBC, suspended in 0.05 or 0.5 ml physiological saline respectively. The reacting dose of SRBC ($5 \cdot 10^7$) was injected into the foot of the mice 24 h before sacrifice. The number of AFC in the regional (popliteal) lymph nodes or in the spleens was determined by the direct local hemolysis method [8], whereas DTHR in the same animals was assessed as the degree of thickening of the foot of the experimental mouse compared with the control (a difference of 0.1 mm was taken as one conventional unit). In individual experiments the intensity of edema of the limb without preliminary sensitization and also the number of spontaneous AFC in the spleen and the titer of normal serum hemagglutinins were determined [5]. Antigen-specific suppressor T cells of the DTHR were selectively blocked by subcutaneous injection of 20 mg/kg cyclophosphamide 2 days before immunization [2, 9].

The numerical results were subjected to statistical analysis by Student's test; the value of m relative to M did not exceed 20% in all experimental groups (except the hemagglutinin titer).

EXPERIMENTAL RESULTS

The results support the original view that the spleen facilitates a generalized immune response, lymphocytes a local response. For instance, after intraperitoneal injection of $1 \cdot 10^8$ SRBC (the optimal dose for a generalized immune re-

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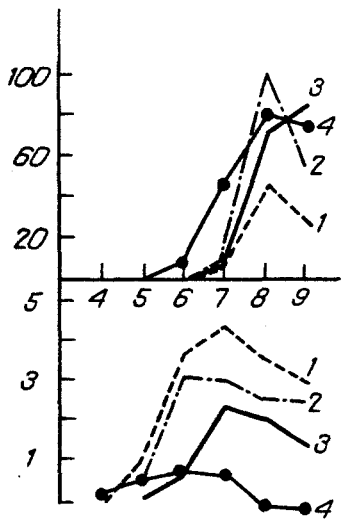

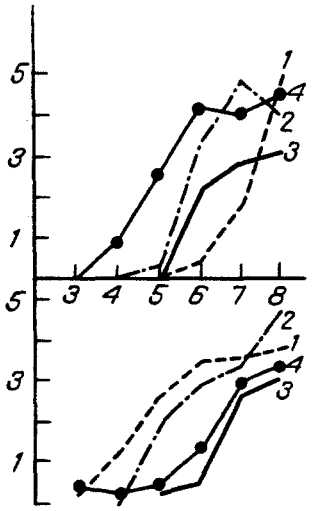

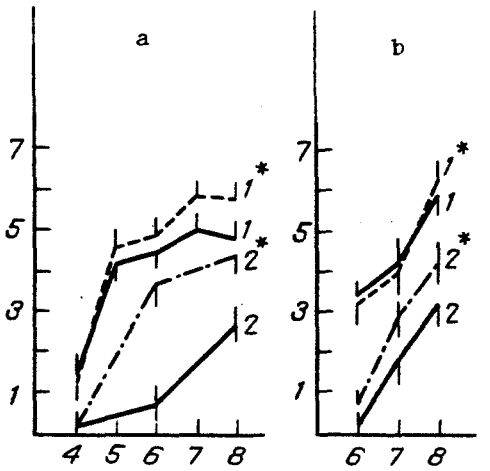

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Intensity of ABP in spleen and of DTHR after intraperitoneal immunization with different doses of SRBC in mice. Here and in Fig. 2: 1) C57BL/6 mice, 2) (CBA × C57BL/6)F_1 hybrids, 3) noninbred mice, 4) CBA mice; abscissa, log of dose of SRBC; ordinate; above — number of AFC, $\cdot 10^3$ per organ, below — intensity of DTHR in relative units. In all cases average level of nonspecific edema of the foot (Table 1) in response to the first injection of SRBC was taken as the zero marker of DTHR.

Fig. 2. Intensity of ABP in regional lymph node and of DTHR in response to subcutaneous immunization with different doses of SRBC in mice.

Fig. 3. Effect of cyclophosphamide on intensity of DTHR in mice after subcutaneous sensitization with different doses of SRBC. Abscissa, log of dose of SRBC; ordinate, intensity of DTHR in relative units. A: 1) C57BL/6 mice, 2) CBA mice; B: 1) (CBA × C57BL/6)F_1 hybrids; 2) noninbred mice. * denotes effect of cyclophosphamide.

sponse) into CBA mice, AFC were virtually absent in the popliteal lymph node. In response to subcutaneous immunization with a similar dose of SRBC the intensity of ABP in the spleen of the CBA mice was negligible (5600 ± 720 AFC per organ), whereas in noninbred animals and C57BL/6 mice the number of AFC in the organ did not exceed their average spontaneous level. If the data in Fig. 1 are taken into account, to induce a generalized immune response after subcutaneous immunization, the dose of SRBC which must be used is evidently at least two orders of magnitude greater than in the case of intraperitoneal injection.

The higher threshold of antigenic sensitivity of the immune response (DTHR + ABP) in noninbred mice than in inbred mice (see Figs. 1 and 2) is evidently connected with the greater activity of NSP in them [7]. Under these circumstances, the level of normal antibodies (in noninbred mice it is even lower) and the more significant (in CBA mice) value of the nonspecific inflammatory edema in response to subcutaneous injection of SRBC (Table 1), probably have no significant effect on the level of this threshold.

Analysis of the data in Figs. 1 and 2 reveals three different types of immune response: I) with predominance of the cellular component (in C57BL/6 mice); II) of the humoral component (in CBA and noninbred mice); III) intermediate, observed in hybrids.

These types of immune response were particularly well differentiated in the case of immunization with suboptimal doses of the antigen. The type I response was characterized by an antigenic threshold of DTHR that was one (intraperitoneal immunization) or two (subcutaneous immunization) orders of magnitude lower than that of ABP. In the type II response, the opposite effect was observed (subcutaneous immunization) or the level of sensitivity to the antigen for cellular and humoral components of the immune response was comparable. These differences, in our view, are based on the

TABLE 1. Intensity of Nonspecific Edema, Number of Spontaneous AFC in Spleen, and Titer of Normal Agglutinins, Specific for SRBC in Mice (M ± m)

Mice	Degree of edema of foot (conventional units)	Number of AFC	Titer of hemagglutinins
Noninbred	2,5±0,41	91±13	1:(16±3,3)
CBA	5,1±0,45	320±54	1:(30±6,6)
C57BL/6	2,1±0,25	162±25	1:(25±6,0)
F_1(CBA×C57BL/6)	2,7±0,36	311±47	1:(24±5,9)

character of NSP in animals of different lines. For instance, in CBA mice, unlike C58BL/6, specific activity of the macrophages was resistant to the action of T_e mediators [1], and for that reason ABP in these mice, which replaces the DTHR, must be included in the immune response even to small doses of antigen, whereas after its delocalization of DTHR in the animal it is completely ineffective. The situation is different in noninbred mice, in which NSP initially is high, the need for its additional stimulation by T_e is not so evident, and the antigenic threshold for DTHR is raised relative to ABP.

If large immunogenic doses of SRBC are used, differences between types I and II of immune response are not in such great contrast. In this case, on intraperitoneal immunization, with mutual competition between DTHR and ABP at the stage of mature effector cells, there is a characteristic increase in specificity of the immune response due to priority of ABP over DTHR. This applies to a lesser degree to C57BL/6 mice in which, as was shown previously [2], the lower level of AFC in the spleen predetermines the greater intensity of the DTHR. Conversely, after subcutaneous immunization with large doses of SRBC computation between DTHR and ABP is not so evident, for with an increase in dose of the antigen, in all cases the intensity of the two types of immune response increases.

There can be no doubt about the role of regulatory subpopulations of lymphoid tissue in the formation of one or other types of immune response. For instance, the somewhat lower level (subcutaneous sensitization) of DTHR in CBA mice and to some extent also in noninbred mice compared with that in C57BL/6 mice is connected with the greater activity of cyclophosphamidesensitive antigen-specific suppressor T cells of DTHR in the former (Fig. 3).

The character of the immune response is thus largely determined by activity of phagocytic cells and by their sensitivity to factors of the immune response, and it is formed both at the stage of mature effector immunocompetent cells and at the stage of their induction. In each concrete case of the immune response, the body strives not for maximal intensity of all of its components (the potential ability to generate AFC and T_e both in the spleen and in lymph nodes of mice with different genotypes is about the same), but to the choice of their optimal ratio. Naturally, the greater diversity of the genotype broadens the possibility of a solution to this problem. For instance, in (CBA × C57BL/6)F_1 mice, in response to large doses of SRBC the strong type of immune response is inherited as a dominant or codominant type, and this is at least partly linked with the recessive type of inheritance of high activity of suppressor T cells of the DTHR (Fig. 3). In response to intraperitoneal injection of suboptimal doses of SRBC the strong cellular and weak humoral type of immune response is dominant; in this situation ABP is utilized evidently by the body as the second "echelon" of the immune response.

Assessment of the immune response in each concrete case is thus an exceptionally delicate task and requires analysis of possible contradictions between the factors composing it.

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FUNCTIONAL ACTIVITY OF NATURAL KILLER CELLS AND KILLER T CELLS IN MICE AFTER OVARIAN TRANSPLANTATION

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Prevention of the reaction of immunologic incompatibility during transplantation of foreign organs and tissues is a complex and still incompletely studied region of transplantology [1, 7]. A particularly urgent aspect is the study of the function and role of cytotoxic effector systems and, in particular, of natural killer (NK) cells and of killer T cells in transplantation.

The writers previously studied general and special aspects of the function of these lymphocyte subpopulations during transplantation of hematopoietic tissues [3]. In this paper we give data on the study of NK cells and killer T cells after allografting of the ovaries in mice.

EXPERIMENTAL METHOD

Mice of inbred lines CBA ($H-2^k$), C57BL/6 ($H-2^b$), and first-generation hybrids (CBA × C57BL/6)F_1, obtained from the "Stolbovaya" Laboratory Animals Nursery, Academy of Medical Sciences of the USSR, were used. Subcutaneous transplantation of CBA ovaries into C57BL/6 mice was carried out under aseptic conditions and hexobarbital anesthesia [8]. Some animals underwent a mock operation (without transplantation) under similar conditions. C57BL/6 mice of the same litter were used as the control group.

NK activity was determined in the membranotoxic test with ^{3}H-uridine [6]. Human erythromyeloid leukemia K-562 cells, maintained in culture [9], were used as target cells. Killer T cells were tested in the experimental system suggested by R. V. Petrov and co-workers [4]. Intact (CBA × C57BL/6)F_1 hybrid mice, after irradiation in a dose of 650 R, received transplanted lymph node cells ($1 \cdot 10^6$) from C57BL/6 mice: intact, subjected to a mock operation, or receiving an allograft of the ovaries (CBA). Lymph node cells were transplanted at different times (3, 7, 15, and 30 days) after the operation.

EXPERIMENTAL RESULTS

The experiments showed that in the early period after ovarian transplantation the membranotoxic activity of the recipients' NK cells was significantly (by 1.5-2 times) depressed compared with the control values. It can be tentatively suggested that suppression of NK activity at these times was due to stress, which is known to cause acute immunosuppres-

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