MECHANISM OF ENHANCEMENT OF THE IMMUNE RESPONSE TO STAPHYLOCOCCI IN MICE BY TRANSPLANTATION OF SPLENOCYTES FROM PRIMED DONORS

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Injection of splenocytes from syngeneic immune donors into intact animals is known to cause the development of an immune response to the homologous antigen [5, 9]. However, the writers have shown that if heat-inactivated staphylococci are used as antigen, transplantation of splenocytes of immune mice not only does not inhibit the immune response of syngeneic recipients but, on the contrary, significantly increases the number of antibody-forming cells (AFC) in the spleen and the serum antibody titers [2, 3]. It has also been shown that the effect we discovered is antigen-specific, and its magnitude is proportional to the dose of antigen taken to immunize the donors and the number of transplanted splenocytes. However, it is still not clear whether the transplanted spleen cells (SC) exert a regulatory influence on the recipient's immune system or whether, in the absence of suppressor action of specific antibodies, the transplanted cells give a response of secondary type to the antigen, and thereby considerably intensify the immune response. The present investigation was conducted to study these problems.

EXPERIMENTAL METHOD

CBA, C57BL/6, and (CBA×C57BL/6)F_1 hybrid mice weighing 18-20 g were used. Staphylococcal corpuscular antigen (SCA) was prepared as described previously [2], using a 24-h culture of *Staphylococcus aureus* strain Wood-46. Donor mice were immunized intravenously in a dose of $5 \cdot 10^9$ staphylococcal cells in 0.5 ml of physiological saline. After 5 days a suspension of immune splenocytes was prepared from the spleens of the primed mice with a concentration of $6 \cdot 10^7$ cells to 1 ml of physiological buffered saline (PBS, pH 7.2). The number of dead cells, revealed by staining with trypan blue, usually did not exceed 5%.

Mice of the experimental group (5-6 intact syngeneic recipients) were given an intravenous injection of 0.5 ml of the cell suspension ($3 \cdot 10^7$ SC), whereas the control animals received 0.5 ml of PBS. After 1 h the mice were immunized intravenously with SCA in a dose of $5 \cdot 10^9$ microbial cells.

To eliminate B lymphocytes the immune CS were incubated in Eagle's medium (Difco, USA) with rabbit antiserum against mouse immunoglobulins in the presence of fresh guinea pig complement for 45 min at 37°C. The concentration of splenocytes was $2 \cdot 10^7$-$3 \cdot 10^7$ cells in 1 ml and the final dilutions of antiserum and complement were 1:20 and 1:8, respectively. The control suspension of immune CS was treated with normal rabbit serum and complement under the same conditions. After incubation and washing in PBS twice the splenocytes were transplanted into intact recipients as described above, and the animals were then immunized with SCA.

When the role of the transplanted SC in the recipient's immune response was investigated, splenocytes obtained from primed CBA mice were transplanted into (CBA × C57BL/6)F_1 first generation hybrids, which were then immunized with SCA. Before the number of AFC in the recipient's spleen was determined, the lymphoid cells of the hybrid were eliminated in some experiments with the aid of CBA-anti-C57BL/6-serum and fresh guinea pig complement. The anti-C57BL/6-serum was obtained after sixfold intraperitoneal immunization of CBA mice with splenocytes of C57BL/6 mice, and it was used in a final dilution of 1:4. In the cytotoxic test the CBA-

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TABLE 1. Effect of Anti-Immunoglobulin Serum in Presence of Complement on Ability of Splenocytes of Primed Mice to Enhance Immune Response to Staphylococci in Syngeneic Recipients

Treatment of CS in vitro	Number of AFC in recipients' spleen	Number of AFC per 10^6 splenic karyocytes	Anitbody titer ($-\log_2$)
—	35 250±11 048 (5)	225±66	5,00±0,30
Complement	87 100±15 779* (6)	479±56**	5,80±0,19*
Anti-immunoglobulin serum + complement	39 500±4 568	234±27	5,05±0,11

Legend. Here and in Table 2: *P < 0.05, **P < 0.01; number of mice given in parentheses.

TABLE 2. Effect of Splenocytes of Primed CBA Mice on Height of Immune Response to Staphylococci in Hybrids

Donors of SC	Number of AFC in recipients' spleen	Antibody titer ($-\log_2$)
—	17 933±809 (6)	4,10±0,06
Intact	16440±2,463 (5)	4,25±0,25
Immune	49 667±13 522* (6)	5,51±0,24**

anti-C57BL/6-serum caused death of 75-80% of nucleated CS of (CBA × C57BL/6)F_1 hybrids but did not affect the viability of splenocytes of CBA mice.

The animals were killed by decapitation. The number of AFC specific for SCA in the spleen of the mice was determined 4 days after immunization by the immunofluorescent replicas method [1]. Serum antibody titers were determined by the agglutination test using Takachi's microtitrator. The numerical results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Transplantation of splenocytes from primed mice into syngeneic animals significantly increased the number of AFC (P < 0.01) in the spleen and the agglutinin titers (P < 0.05) in the recipients' blood serum (Table 1). However, preliminary treatment of immune splenocytes with anti-immunoglobulin serum and complement abolished the effect observed previously, evidence that immunoglobulin carrying lymphocytes play a definite role in the stimulating action of CS of primed mice in this particular experimental system.

It was also shown that complete identity of donor and recipients with respect to H-2 antigens is not an essential condition for enhancement of the immune response of mice receiving immune splenocytes. For instance, transfer of primed CS of the parental genotype into first generation hybrids, just as in the case of a syngeneic donor-recipient pair, leads to marked enhancement of the immune response to SCA. Transplantation of splenocytes of immune (but not intact) CBA mice increased the number of AFC in the spleen of (CBA × C57BL/6)F_1 recipients threefold compared with the control (Table 2). The increase in titers of antibodies to SCA in the serum of the hybrid recipients also was statistically significant (P < 0.01).

In view of these results it was possible to use the method of discriminative analysis to study the origin of the cells on account of which the humoral immune response was enhanced in mice receiving primed splenocytes. It was found that antibody-producers, discovered in the spleen of (CBA × C57BL/6)F_1 hybrids, into which CS from immune CBA mice, resistant to the ac-

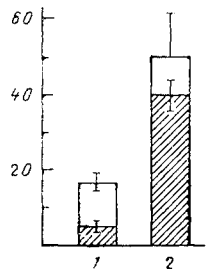

Fig. 1. Effect of CBA-anti-C57BL/6-serum in presence of complement on number of AFC in spleen of (CBA × C57BL/6)F_1 hybrid mice, immunized with staphylococci and receiving (2) and not receiving (1) splenocytes of immune CBA mice. Ordinate, number of AFC ($\times 10^3$) specific for SCA in mouse spleen. Shaded part of column, the same after treatment of test splenocytes with CBA-anti-C57BL/6-serum and complement.

tion of CBA-anti-C57BL/6-serum in the presence of complement, were transplanted before immunization (Fig. 1). It will be clear from Fig. 1 that the anti-C57BL/6-serum which was used eliminated fewer than 20% of the AFC in the spleen of recipients of semiallogeneic cells. Meanwhile similar treatment of splenocytes from control mice led to a significant decrease (about 70%) in the number of AFC against staphylococci. Differences in sensitivity to the action of the alloantiserum between the control and experimental versions were statistically significant ($P < 0.01$).

It can be concluded from these results that enhancement of the immune response to SCA by transplantation of syngeneic splenocytes from primed mice is evidently due to an anamnestic response of the donors' cells as a result of their repeated antigenic stimulation in the recipient's body. Meanwhile we know that immunologic memory can be adoptively transferred only to newborn or irradiated syngeneic recipients, i.e., to animals whose immune system is not yet formed or is depressed. Otherwise lymphoid tissue of an intact recipient usually inhibits the realization of immunologic memory by transplanted lymphocytes from a primed donor, due to the so-called isogeneic barrier [6], and which has repeatedly been demonstrated in various experimental systems with the use of many antigens [4, 7, 8].

The possibility cannot be ruled out that the effect observed in the present experiments is a special feature of the immune response to SCA (and also, perhaps, to certain other antigens of microbial origin), and is connected with manifestations of the immunobiological activity of the staphylococcus, which have no direct relationship to realization of its antigenic properties. However, the final solution to this problem requires further research.

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