MIGRATION AND INTERACTION OF T- AND B-LYMPHOCYTES IN MICE OF DIFFERENT GENOTYPES

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Interaction between bone marrow cells and thymus cells in CBA, C57BL, and F_1(CBA × C57BL) mice was investigated during the production of antibody-forming cells against sheep's erythrocytes when the thymus and part of the bone marrow was screened during lethal irradiation. Genetically determined differences between CBA mice, reacting strongly to erythrocytes, and C57BL mice, with a low level of reaction, were shown to be associated with differences in the intensity of cooperative interaction between the B- and T-cells; under the conditions used this difference in intensity evidently depends on differences in the intensity of migration of the B-lymphocytes from the bone marrow or T-lymphocytes from the thymus.

KEY WORDS: genotype; antibody formation; immunocompetent cells; interaction between cells; migration of cells.

Ability to react (or not to react) to a given antigen, as well as the intensity of the immune response, are genetically determined [2, 4]. Genetic differences in antibody formation are manifested at the level of lymphocyte population [5]. It has also been shown that the development of an immune response to most antigens requires interaction between at least two types of cells: cells of bone marrow (B-lymphocytes) and thymus (T-lymphocytes) origin [3, 6, 8, 10]. As a rule, interaction between B- and T-cells has been studied during adaptive transfer of cells in vivo. The study of processes leading to interaction between T- and B-cells in situ is thus of great interest [7].

Migration and interaction of B- and T-lymphocytes depending on the genotype of mice during the development of an immune response to sheep's erythrocytes were investigated. Lines of mice with opposite patterns of response – CBA, with a high intensity of response, and C57BL, with a low intensity – were chosen [2, 4, 5].

EXPERIMENTAL METHOD

Mice of lines CBA and C57BL/6 and F_1(CBA × C57BL/6) hybrids aged 4-5 months were used. Interaction between T- and B-lymphocytes in situ during the production of antibody-forming cells (AFCs) was investigated by means of the model developed previously [17]. For this purpose, during irradiation of the mice the region of the thymus and part of the bone marrow of the hind limbs up to the level of midway along the femur were protected by a screen (6 mm Pb + 1 mm Al). In control mice either the thymus or part of the bone marrow was screened. After irradiation the animals received an intravenous injection of $2 \cdot 10^8$ sheep's erythrocytes, and the number of AFCs in the spleen was determined on the 5th, 7th, and 9th days by Jerne's method [9]. To determine the magnitude of the cooperative immune effect of the T- and B-lymphocytes, the index of stimulation (IS) was calculated by the equation:

$$IS = \frac{A}{B+C} \cdot 100,$$

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TABLE 1. Number of AFCs Accumulating in Spleen of Lethally Irradiated Mice after Screening of Thymus and Part of Bone Marrow and Immunization with Sheep's Erythrocytes ($\bar{X}$ and confidence limits for $P < 0,05$)

Line of mice	Area screened	No. of AFCs calculated per spleen							
		No. of mice	5th day	No. of mice	7th day	No. of mice	9th day
CBA	BM	9	9,6 (6,8—12,4)	10	8,2 (6,4—10,0)	10	64,9 (42,3—87,5)
	T	9	15,9 (8,8—23,0)	12	232,7 (154,2—311,2)	14	98,8 (68,1—129,5)
	BM + T	12	172,6 (111,0—234,2)	13	2436 (1836—3034)	19	982,6 (533,2—1432,0)
C57BL/6	BM	8	19,5 (14,1—24,6)	10	148,7 (101,2—196,2)	7	215,4 (88,0—342,8)
	T	7	15,5 (7,3—23,7)	10	108,2 (68,2—148,2)	9	303,4 (192,5—414,3)
	BM + T	12	36,9 (27,2—46,6)	13	1484 (1188—1780)	12	1284 (624—1944)
F_1(CBA×C57BL/6)	BM	10	9,1 (7,7—10,5)	10	42 (27,8—56,2)	12	106,2 (83,8—128,6)
	T	10	12,8 (10,1—15,5)	10	126 (103,9—145,1)	11	153,4 (134,6—192,2)
	BM + T	10	156,5 (133,5—179,5)	14	2157 (1722,8—2591,2)	14	908,4 (676,4—940,4)

Note: BM) bone marrow; T) thymus.

where A is the number of AFCs after screening of the bone marrow and thymus; B and C are the number of AFCs after screening of the bone marrow or thymus respectively.

The mice were irradiated on the RUM-17 apparatus (dose rate 215R/min) in a dose of LD 100/14, which amounted to 750R for whole-body irradiation of C57BL/6 mice and 800R for CBA and F_1(CBA × C57BL/6) mice. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Screening the region of the thymus or bone marrow in mice irradiated with a lethal dose and immunized with sheep's red cells was followed by the formation of comparatively few AFCs in the spleen (Table 1). Screening of both the thymus and the bone marrow during lethal irradiation led to a sharp increase (especially on the 7th day) in the production of AFCs in the spleen; i.e., synergism was shown between the thymus and bone marrow in the immune response to sheep's erythrocytes. Interaction (cooperation) between thymus and bone marrow cells observed in this particular model was manifested by the fact that if both the sources of the T- and B-cells were screened the number of AFCs formed from precursors immigrating into the spleen was 3-12 times greater than the number expected (the sum of the AFCs found after screening of the thymus and bone marrow separately).

However, interaction between bone marrow and thymus cells when both were screened was manifested to different degrees depending on the genotype. In CBA mice a marked cooperative effect of interaction between T- and B-cells of the thymus and bone marrow was manifested at all times of the investigation, with a maximum (IS of AFC production 1000%) on the 7th day after irradiation and immunization (Table 1). A similar cooperative immune response also was observed in the F_1(CBA × C57BL/6) hybrids. However, in C57BL/6 mice the cooperative effect was completely absent on the 5th day after irradiation and immunization. On the 7th day, the cooperative effect reached 570% but the value of IS under these circumstances was considerably less than in CBA (1000%) and F_1(CBA × C57BL/6) mice (1250%). On the 9th day, the cooperative immune response was lowered in all mouse genotypes studied, most of all in C57BL/6 mice.

The fact that in CBA and F_1(CBA × C57BL/6) mice an equally high cooperative immune response was observed, but in the C57BL/6 mice the response was low indicates that ability to give a high cooperative immune response is inherited as a dominant. This conclusion is in agreement with data showing that the character of "strength" of the immune response to different antigens is inherited as a dominant [2, 4, 5].

Screening of the bone marrow alone led to the production of more AFCs at all times of the investigation in C57BL/6 mice than in CBA or F_1(CBA × C57BL/6) mice (Table 1). This was evidently connected with the fact that the bone marrow of CBA and F_1(CBA × C57BL/6) mice contains virtually no T-cells, whereas the bone marrow of C57BL/6 mice is contaminated with a number of T-lymphocytes [10].

It can be concluded from the results of these experiments that genetically determined differences between CBA mice, with a high level of response to sheep's erythrocytes, and C57BL/6 mice, with a low level of response, are linked with differences in the intensity of cooperative interaction between T- and B-cells, possibly based on differences in the intensity of migration of the B-lymphocytes from the bone marrow or T-lymphocytes from the thymus.

This interpretation will become clear if it is recalled that there is evidence that all AFCs in the spleen, if the thymus and bone marrow are screened, arise entirely from precursor cells immigrating from these organs into the spleen [6, 7]. The number of AFCs formed also is known to depend on the relative numbers of B- and T-lymphocytes [10, 11]. In other words, an increase or decrease in the number of T-cells in the interacting mixture (the number of B-cells remaining constant), or an increase or decrease in the number of B-cells (the number of T-cells remaining constant) is accompanied by an increase or decrease respectively in the number of AFCs produced.

There is thus every reason to suppose that the inability of C57BL/6 mice to exhibit a high level of immunologic reactivity is due to the low intensity of migration of T- or B-cells in these animals. This fact is manifested particularly demonstratively on the 5th day after screening of the thymus and bone marrow. However, on the 7th-9th day, when a sufficient number of T- and B-cells for the cooperative effect has evidently accumulated in the spleen, the immune response of the C57BL/6 mice increases significantly. During adaptive transfer of a known excess of bone marrow and thymus cells stimulated by sheep's erythrocytes, differences in the immune response of CBA and C57BL/6 mice largely disappear [1]. Further investigations will be carried out to study the concrete differences between ability of the T- and B-cells to migrate in mice of different genotypes and, in particular, in CBA and C57BL/6 mice.

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