PARTICIPATION OF HEMATOPOIETIC STEM CELLS IN FORMATION OF THE IMMUNE RESPONSE

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The effect of different doses of sheep's red blood cells (SRBC) on endogenous colony formation and immunogenesis was studied in CBA and C57BL mice. The absolute and relative numbers of antibody-forming cells (AFC) was shown to increase with an increase in the dose of SRBC from 2×10^7 to 2×10^8, and to decrease with a dose of 2×10^9 in mice of both lines. With an increase in the dose of antigen, the number of endogenous splenic colonies (ESC) of hematopoietic cells also increased. Interlinear differences in the number of ESC persisted. It is suggested that the mechanism of stimulation of the hematopoietic series is based on a unique type of blockade of the reticuloendothelial system by foreign red blood cells, leading to increased proliferative activity of the stem cells.

KEY WORDS: immunogenesis; endogenous splenic colonies; macrophages; genotype; sheep's red cells.

The ability of animals and man to give an immune response to antigens is known to be under genetic control [3]. In particular, it has been found that C57BL mice give a weak response to sheep's red blood cells (SRBC), whereas CBA mice are highly responsive to this antigen. According to the generally accepted view, three types of cells participate in the formation of the immune response: T- and B-lymphocytes and macrophages [4]. It has been shown that, depending on the antigen, the effect of the genotype of animals on their antibody-producing capacity can be exerted at the level of either T-cells, B-cells, or macrophages [5]. It is evidently the macrophages which largely determine the difference in antibody formation against SRBC in mice of the two different strains [7].

The number of endogenous splenic colonies (ESC) of hematopoietic cells formed by hematopoietic stem cells also depends on the genotype of the mice [1, 13]. For example, the number of ESC observed in C57BL mice is about twice that found in CBA mice. A stimulating effect of antigens on the number of ESC has been found in animals [8, 11]. There is convincing evidence in the literature that the "hematopoietic microenvironment" has a regulatory effect on proliferation and differentiation of hematopoietic stem cells [13, 14].

The object of this investigation was to study the effect of antigens on endogenous colony formation and on immunogenesis in CBA and C57BL mice, responding strongly or weakly, respectively, to SRBC. The aim was to discover the possible linking role of macrophages in colony formation and immunogenesis.

EXPERIMENTAL METHOD

Experiments were carried out on CBA and C57BL mice obtained from the "Stolbovaya" Nursery of Laboratory Animals, Academy of Medical Sciences of the USSR. Experiments were carried out on female mice aged 3.5-4.5 months. In order to determine the number of stem cells forming ESC, the animals were irradiated in a dose of 500 R. The animals were killed 9 days after irradiation, and the number of endogenous colonies of hematopoietic cells was counted in their spleens, fixed in a mixture of acetic acid and ethyl alcohol (1:3).

Irradiation was carried out on the RUP 150/30-1 apparatus (dose rate 50 R/min, tube voltage 180 kV, current 10 mA, filter Al-3).

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TABLE 1. Number of AFC and Endogenous Hematopoietic Colonies in Spleen of Mice after Injection of Different Doses of SRBC ($M \pm mt$, $P < 0.05$)

Line of mice	Dose of SRBC	No. of AFC per 10^6 spleen cells	No. of AFC in spleen	No. of ESC
CBA	Control			3,3±0,6
	2.10^7	140±31	23 145±9 996	5,5±2
	2.10^8	255±41	31 617±6 860	10,4±4
	2.10^9	97±26	22 843±5 488	>40
C57BL	Control			8,3±4
	2.10^7	9±2	14 499±412	13,34
	2.10^8	23±4	4 978±1 960	24,4±10
	2.10^9	11,3±3	1 814±470	>40

An intravenous injection of SRBC in doses of 2×10^7, 2×10^8, and 2×10^9 was given to the mice 24 h before irradiation. These doses also were used to immunize the animals. On the 4th day after immunization the animals were killed and the number of antibody-forming cells (AFC) in the spleen was determined by a modified method of local hemolysis in semi-liquid medium [9].

The significance of differences was estimated by Student's t-test.

EXPERIMENTAL RESULTS

The results given in Table 1 confirm those obtained by other workers who showed that CBA and C57BL mice are opposite in their immune response to SRBC [6]. The difference between them was 9-10-fold, regardless of the dose of antigen, although in both cases development of the immune response to different doses of SRBC obeyed a general rule: an increase in the relative and absolute numbers of AFC with an increase in the dose of SRBC from 2×10^7 to 2×10^8 and a decrease in their number in mice of both lines in response to SRBC in a dose of 2×10^9.

It will be clear from Table 1 that if SRBC were injected 24 h before sublethal irradiation the number of ESC was increased in the CBA and C57BL mice. With an increase in the dose of SRBC, the number of ESC also increased, but differences between the two strains for the number of ESC still remained.

As already stated, the "hematopoietic microenvironment" plays a decisive role in processes of proliferation and differentiation of hematopoietic stem cells. It has been suggested that phagocytic cells of the reticulo-endothelial system (RES) are a component part of this microenvironment. Investigations have shown that blocking the RES by injection of various inert particles (carbon, carmine) into mice causes an increase in the number of ESC and leads to inhibition of antibody formation [10, 12]. There is reason to suppose that differences in functional activity of the RES, together with other factors, may lie at the basis of interlinear differences in the number of ESC. Under normal conditions phagocytic cells of the RES evidently exert an inhibitory influence on the powers of proliferation and differentiation of the stem cells. It can tentatively be suggested that in the present experiments there was also, evidently, a "unique blockade of the RES" by foreign red blood cells, leading to escape of the stem cells from the controlling influence of macrophages and to an increase in the proliferative activity of the hematopoietic stem cells.

There is evidence of stimulation of lymphoid, erythroid, and myeloid branches in the hematopoietic organs in response to antigenic action [2]. It has been suggested that one mechanism of the influence of the animal's genotype of antibody formation may be differences in the intensity of the response of the different branches of hematopoiesis. Most probably this difference is based upon the unequal numbers of hematopoietic stem cells that proliferate and differentiate during antigenic stimulation. This hypothesis is confirmed by the results of the present investigations. Very probably the stronger response of the erythroid and myeloid series, as a result of competitive relations with the lymphoid series for the area within the spleen, leads to inhibition of formation of the clone of AFC in C57BL mice. Finally, there is every reason to suppose that the strengthening of the powers of proliferation and differentiation of the stem cells after antigenic stimulation, followed by a quantitative increase in the erythroid and myeloid series of hematopoiesis, is one of the feedback mechanisms that inhibit the spatial "expansion" of clones of immunocompetent cells and

determine the decrease in antibody production with time after injection of an antigen. The decrease in the number of AFC observed in response to SRBC in a dose of 2×10^9, against the background of marked stimulation of proliferative activity of the hematopoietic stem cells, can evidently be taken to indicate that the above mechanism plays a definite role in the development of tolerance to this particular antigen.

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CHANGES IN THE PROLIFERATIVE RESPONSE OF HUMAN LYMPHOCYTES IN VITRO UNDER THE INFLUENCE OF SUBCELLULAR COMPONENTS OF *Bordetella pertussis*

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Different subcellular components of *Bordetella pertussis* were found to have a similar inhibitory action on incorporation of thymidine-^{3}H by lymphocytes stimulated by phytohemagglutinin. Lymphocytes were obtained from donors immunized with tetanus toxoid. However, the same components of *B. pertussis* had a differential action on lymphocyte proliferation in the presence of tetanus toxoid: murein-containing membranes increased incorporation of thymidine-^{3}H, the RNA-containing fraction inhibited it, and the water-soluble components of the homogenate had no effect on lymphocyte proliferation.

KEY WORDS: *Bordetella pertussis*; proliferation of lymphocytes; tetanus toxoid.

During contact between mammalian cells and subcellular components of *Bordetella pertussis* in vitro various changes take place in the surface membrane structures and, in particular, the architectonics of the surface of tumor cells in culture is modified [5], macrophages lose their ability to form erythrocytic rosettes [7], and lymphocytes are stimulated toward nonspecific blast transformation [3]. Consequently it might be expected that after exposure to the action of subcellular components of *B. pertussis* the ability of human lymphocytes to respond by proliferation to lectins and specific antigens in vitro would also be changed.

The object of this investigation was to study the character of these changes during the response to phytohemagglutinin (PHA) and tetanus toxoid.

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