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CORRELATION BETWEEN IMMUNOLOGIC RESPONSIVENESS TO HAPTENS AND STIMULATION OF STEM CELL MIGRATION BY IMMUNIZATION WITH HAPTEN-CARRIER CONJUGATES

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CBA and C57BL mice were immunized intraperitoneally with conjugates (2,4-dinitrophenyl–bovine γ-globulin, 2,4,6-trinitrophenyl–bovine serum albumin, diazotized p-aminobenzoic acid–bovine serum albumin, and sulfanilic acid–bovine serum albumin) and migration of hematopoietic stem cells from bone marrow into spleen was investigated. Immunization with hapten-protein conjugates in most cases was shown to stimulate migration of hematopoietic stem cells in mice of a line responding weakly to that particular hapten and, at the same time, as a rule, it lowered the intensity of migration in mice of a weakly reacting genotype. It is concluded that ability to respond by a change in stem cell migration to immunization is evidently linked with genetically determined differences in the strength of the immune response in mice of different genotypes.

KEY WORDS: immunologic reactivity; migration; stem cells; haptens.

Migration of stem cells from the bone marrow is an important stage in immunopoiesis which is essential for the constant repopulation of the central organs of the immune system where populations of T- and B-lymphocytes are formed [4]. Immunization is known to increase the intensity of migration and of recirculation of stem cells tenfold [5]. The writers showed previously that genetically determined differences in the strength of the immune response between mice of line C57BL, with a weak response to sheep's red blood cells (SRBC) and CBA mice, responding strongly to SRBC, were associated with unequal intensity of migration of hematopoietic stem cells and T- and B-lymphocytes [3, 6].

The object of the present investigation was to study migration of stem cells following immunization of mice belonging to lines responding oppositely to various haptens, with hapten–carrier conjugates.

EXPERIMENTAL METHOD

Experiments were carried out on CBA and C57BL mice aged 2-4 months and weighing 20-22 g, obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR. The following conjugates were used for immunization: 2,4-dinitrophenyl–bovine γ-globulin (BGG), containing 48 DNP-groups per protein molecule ($DNP_{48}BGG$); 2,4,6-trinitrophenyl–bovine serum albumin ($TNP_{32}BSA$); diazotized p-aminobenzoic acid–bovine serum albumin ($PAB_{20}BSA$), and sulfanilic acid–bovine serum albumin ($Sulf_{18}BSA$).

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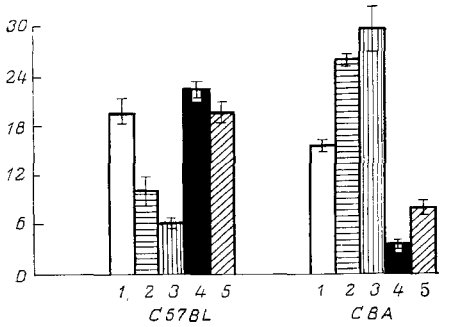

Fig. 1. Effect of immunization with hapten-carrier conjugates on stem cell migration from bone marrow in CBA and C57BL mice. 1) Migration control (without immunization); 2) immunization with $DNP_{48}BGG$; 3) immunization with $TNP_{32}BSA$; 4) immunization with $PAB_{20}BSA$; 5) immunization with $Sulf_{18}BSA$. Short vertical lines denote confidence intervals of arithmetic means with 95% probability ($P < 0.05$). Ordinate, number of hematopoietic colonies in spleen. Results of three to five experiments pooled. From 15 to 30 mice in each group.

$DNP_{48}BGG$ was obtained by the method described previously [1]. $TNP_{32}BSA$ was obtained as follows: 300 mg BSA (from Serva, West Germany) was dissolved in 10 ml of 0.05 M borate buffer, pH 9.2-9.4. After addition of 2,4,6-trinitrobenzenesulfonic acid (from BDH, England) to the solution the reaction mixture was stirred at room temperature for 4 h. At the end of trinitrophenylation the product was chromatographed twice on Sephadex G-25 (medium). The conjugate thus prepared was poured into ampuls and stored at −20°C. The number of TNP-groups introduced per molecule of carrier was determined spectrophotometrically at 348 nm in 0.05 M phosphate buffer, pH 7.0-7.2 [9]. $PAB_{20}BSA$ and $Sulf_{18}BSA$ were obtained by the method of Tabachnik and Sobotka [10], followed by determination of the number of PAB-groups introduced spectrophotometrically at 500 and 460 nm in 0.1N NaOH [11]. The conjugates were injected into the animals in Freund's complete adjuvant (from Calbiochem, USA) in a dose of 100 μg per mouse immediately after irradiation.

To determine the intensity of migration of hematopoietic stem cells from bone marrow the method of Petrov and Khaitov [2] was used; the basis of this method is that during irradiation of mice the hind limb is protected by a screen (6 mm Pb+1 mm Al) to halfway up the leg. The number of macroscopically visible colonies in the animals' spleen was counted 7 days after irradiation by the method of Till and McCulloch [12]. Each colony was formed from a hematopoietic stem cell which migrated into the spleen from the screened area of bone marrow. The mice were irradiated on the RUM-17 apparatus (dose rate 206 R/min) in a dose of $LD_{100/14}$, which was equivalent to 750 R for totally irradiated C57BL mice and 850 R for CBA mice.

Plaque-forming cells secreting antibodies against haptens were determined by a modified method of Jerne and Nordin [7], and rosette-forming cells and serum agglutinins were determined by Osoba's method [8] and a micromethod. SRBC conjugated with the appropriate hapten were used as the indicator system.

The numerical results were subjected to statistical analysis with calculation of the arithmetic mean and confidence inteval ($P \leq 0.05$).

EXPERIMENTAL RESULTS

As the preliminary experiments showed, as a result of immunization with conjugates $DNP_{48}BGG$ or $TNP_{32}BSA$, CBA mice developed an immune response of the high type, whereas C57BL mice developed a response of low type. Conversely, CBA mice gave a low response to immunization by conjugates $PAB_{20}BSA$ and $Sulf_{18}BSA$, whereas C57Bl mice gave a high response.

During immunization with $DNP_{48}BGG$ and $TNP_{32}BSA$ migration of stem cells from the bone marrow was considerably stimulated in CBA mice (Fig. 1). Injection of $DNP_{48}BGG$ or $TNP_{32}BSA$ into these animals, however, led to approximately twofold stimulation of migration. Meanwhile immunization of C57Bl mice with these conjugates led to a decrease in the intensity of stem cell migration by 50-67% respectively.

In response to immunization with conjugates $PAB_{20}BSA$ and $Sulf_{18}BSA$ migration of stem cells from the bone marrow in CBA mice was sharply reduced (by almost 80% and by 50% respectively). Immunization of C57BL mice with $Sulf_{18}BSA$ had practically no effect on the intensity of stem cell migration, but injection of $PAB_{20}BSA$ stimulated migration of stem cells.

The experiments showed that immunization with various hapten-protein conjugates in most cases stimulated migration of hematopoietic stem cells in mice belonging to a line responding strongly to that particular hapten and, consequently, as a rule the intensity of migration was reduced in mice with a weakly responding genotype. This rule for stimulation of migration did not apply only in one case – in C57BL mice immunized with $Sulf_{18}BSA$, to which they gave a high type of response. However, this does not infringe the general rule, for there was no inhibition of migration in this case. On the whole, therefore, just as after immunization with SRBC [3], in the case of response to hapten groups genetically determined differences in the strength of the immune response in mice of different genotypes are associated with differences in the intensity of migration of stem cells in response to immunization. Changes in stem cell migration in response to immunization may evidently play a definite role in the realization of interlinear differences in antibody formation as one stage of immunogenesis at which genetic control of the immune response is manifested.

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