ROSETTE FORMATION IN A SYSTEM OF ADOPTIVE TRANSFER OF CELL POPULATIONS FROM DONORS WITH AN ALTERED SEROTONIN LEVEL

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UDC 612.112.94.017.1.014.46:577.175.823

The formation of the primary immune response to injection of cells of various immunocompetent organs of nonimmune intact donors and also of animals receiving serotonin into sublethally irradiated recipients was studied. Considerable changes were found in the development of the immune response, mainly on account of IgM-rosette-forming cells, both after transfer of spleen cells alone and after combined injection of spleen cells with bone marrow and thymus cells from the same donors. The results are evidence of a possible effect of serotonin on changes in the relative proportions and migration of T- and B-cells in different organs and also on the action of serotonin on T- and B-suppressor cells.

KEY WORDS: serotonin; rosette-forming cells; T- and B-cells.

During a study of the action of serotonin on immunogenesis the writers showed that an increase in its concentration leads to inhibition of humoral antibody production and rosette formation [2, 8]. Cooperation between subpopulations of T- and B-cells, the relative proportions of which in immunocompetent organs differ and have an important bearing on the development of the immune response, is known to be essential for the induction of immunogenesis. This has been demonstrated mainly for humoral antibodies, whereas the principles of cooperation for the phenomenon of rosette formation remain virtually unstudied. Meanwhile, since rosette-forming cells (RFC), as a heterogeneous population, include both antibody-forming and antigen-binding cells [10, 13], the phenomenon of rosette formation gives a more complete picture than humoral antibodies of the state of the immune system.

It was accordingly decided to study rosette formation in a system of adoptive transfer of cells from different immunocompetent organs of donors with a normal and raised serotonin level.

EXPERIMENTAL METHOD

Experiments were carried out on 460 adult male CBA mice weighing 20-25 g.

The recipients were irradiated in a dose of 600 R. An intravenous injection either of spleen cells (4×10^7 per mouse) or of spleen cells together with bone marrow or thymus cells (1×10^7 or 2×10^7 per mouse) was given to the animals 24 h later, with simultaneous immunization with sheep's red blood cells (SRBC) in a dose of 5×10^6.

Control experiments showed that spleen cells of irradiated recipients cannot form rosettes. Rosette formation took place at the background level observed in intact animals both after injection of antigen alone and after transfer of cell populations without antigen.

Nonimmune intact animals or mice receiving serotonin (from Reanal, Hungary) 24 h before removal of the organs in a dose of 100 mg/kg subcutaneously in Freund's incomplete adjuvant, served as donors.

On the 5th and 7th days after transfer of cells the number of RFC was counted in the recipients' spleens; IgM- and IgG-RFC were differentiated by the method described by the writers previously [2], based on the different sensitivity of these cells to 2-mercaptoethanol.

Laboratory of Physiology of Immunity, Institute of Physiology, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 12, pp. 688-691, December, 1979. Original article submitted February 7, 1979.

 0007-4888/79/8812-1438$07.50

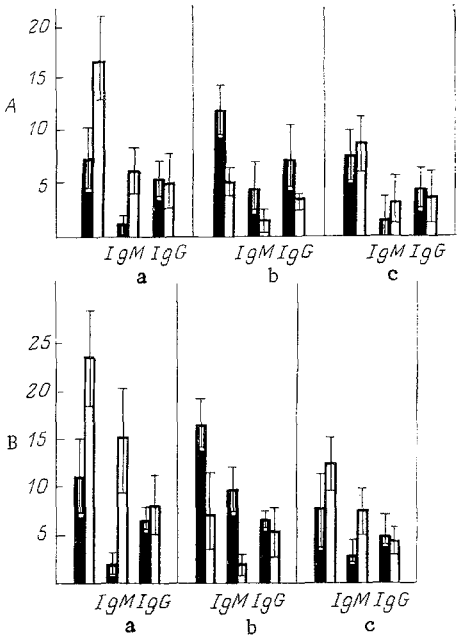

Fig. 1. Primary immune response in recipients on 5th (A) and 7th (B) days after immunization and transfer of cells from intact donors (black columns) and animals receiving serotonin in a dose of 100 mg/kg subcutaneously in Freund's incomplete adjuvant (unshaded columns). Ordinate, number of RFC per 1000 cells. a) Transfer of spleen cells; b) simultaneous transfer of spleen and bone marrow cells; c) simultaneous transfer of spleen and thymus cells.

EXPERIMENTAL RESULTS

After immunization and simultaneous injection of spleen cells from donors receiving serotonin, a marked increase in the number of RFC was observed in the recipients compared with the control – recipients receiving cells from intact animals (17.0 ± 3.1 compared with 7.1 ± 1.1 on the 5th day and 23.4 ± 3.4 compared with 11.2 ± 1.7 on the 7th day). The effect was due mainly to an increase in IgM-RFC at both times of investigation (Fig. 1).

Instead of the effect of inhibition of the immune response by serotonin observed in situ [2, 8], after transfer of spleen cells from nonimmune donors with an increased serotonin concentration marked stimulation of the immune response was observed in the recipients after immunization.

In this experiment rosette formation in the recipients took place only on account of the donors' spleen cells, but in the intact organism interaction takes place between cells of various organs. Accordingly, in the next experiments, combined transfer of spleen cells and cells from other immunocompetent organs (thymus, bone marrow) was carried out.

Combined injection of spleen and bone marrow cells of the control animals was shown to lead to an increase in the number of RFC by almost 100% on the 5th day and by 50% on the 7th day compared with the response of recipients receiving only spleen cells from the control animals (Fig. 1).

This stimulation in the control animals can probably be explained, on the one hand, by the fact that by adding bone marrow cells, mainly B-cells, to the spleen (in which there are about equal numbers of T- and B-cells [12]), we increased the number of B-cells in the transferred fraction and created a more favorable ratio of B- and T-cells for the effective interaction. On the other hand, in the presence of bone marrow cells

the inhibitory function of the T-suppressors could not be exhibited, as has been shown [4] in experiments with combined cultures of cells from immune lymph nodes, intact bone marrow, and T-suppressors.

We now know that the RFC population is heterogeneous and includes antigen-binding and antibody-forming cells, their precursors and, possibly, memory cells [10, 13]. The data described above indicate that definite interaction between different cell populations is necessary for the formation both of rosettes and of humoral antibodies.

Meanwhile addition of bone marrow cells of animals receiving serotonin to the spleen cells considerably inhibited the immune response compared both with the response of recipients into which only a suspension of spleen cells from the same donors had been transferred (5.3 ± 0.6 compared with 17.0 ± 3.1 on the 5th day and 7.8 ± 1.6 compared with 23.4 ± 3.4 on the 7th day), and the response of animals receiving spleen and bone marrow cells of control mice (5.3 ± 0.6 compared with 12.2 ± 1.1 on the 5th day and 7.3 ± 1.6 compared with 16.8 ± 1.2 on the 7th day; see Fig. 1).

It can tentatively be suggested that elevation of the serotonin level in animals changes the ratio between subpopulations of functionally different cells in different organs, as a result of which injection of a suspension of "spleen cells + bone marrow cells" into animals creates an excess of B-cells, and this acts as the signal for activation of T-suppressors, or the B-cells themselves may induce a suppressive effect [5, 11, 14].

Another possibility is that when the serotonin concentration is raised the effect of that very small population of T-suppressor cells found in the bone marrow of CBA mice, and which can be activated only under definite conditions such as, for example, during hyperactivation of histocompatibility antigen, when they suppress the immune response to SRBC [1], is exhibited.

Transplantation of spleen cells into irradiated recipients simultaneously with thymus cells of control animals caused virtually no significant change in rosette formation compared with transfer of a suspension of spleen cells alone. As regards combined injection of these cells from donors receiving serotonin, comparison with the control revealed a tendency toward an increase in the number of RFC, although the response was considerably lower than that to transfer of spleen cells alone (Fig. 1).

The data are thus evidence that a decrease in the immune response with an increase in the serotonin concentration, usually observed in situ [2, 8], is exhibited in the transfer system only after combined injection of spleen cells and bone marrow cells into the recipients, whereas after addition of thymus cells to the spleen cell suspension and, in particular, after transfer of spleen cells alone, more intensive rosette formation was found than in the control. The inhibition and stimulation thus observed were due, it should be noted, chiefly to IgM-RFC.

The results show that for rosette formation, just as for antibody formation, definite interaction between T- and B-cells is necessary. The action of serotonin is most probably associated with redistribution of subpopulations of T- and B-cells. This argument is supported by data [3] showing that the effect of the serotoninergic system on rosette formation can be exerted through the adrenal system, which is known [6] to take part in the control of migration of T- and B-lymphocytes. Allowing for the possibility of migration of T-cells into the bone marrow under the influence of adrenal hormones [7] it seems a likely suggestion that serotonin, by its action through the adrenal system, reduces the number of T-suppressors in the thymus and, in particular, in the spleen of unimmunized donors, so that transfer of cells of these organs induces stimulation of the immune response in the recipients compared with the control. Meanwhile the suspension of bone marrow cells of these donors is enriched with T-suppressors, so that their combined transfer with spleen cells leads to inhibition of the recipients' immune response. The possibility cannot be ruled out that the effect obtained under the influence of serotonin may be due to the formation of suppressors in the B-cell population. This hypothesis is supported by data showing that B-cells of mouse bone marrow depress the IgM-response both in vitro and in vivo [5, 9, 14].

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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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UDC 615.37.015.4:612.419

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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