INCREASING THE NUMBER OF BONE MARROW SUPPRESSOR T CELLS DURING INHIBITION OF IMMUNOGENESIS BY MODIFYING NEUROTRANSMITTER ACTIVITY

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It was shown previously that activation of the serotoninergic system and blockade of the dopaminergic system, stimulating immunogenesis, leads to depression of the immune response [13]. In investigations conducted in a system of syngeneic cell transfer [4, 7] definite correlation was found between inhibition of the immune response and the increase in suppressor activity of bone marrow cells in these animals, whose regulatory role in the development of the immune response has been demonstrated by many investigators [5, 6, 11].

Transfer of bone marrow cells from donors with increased activity of their serotoninergic system and blockade of their dopaminergic system leads to abrupt weakening of the immune response in irradiated recipients, immunized with sheep's red blood cells, whereas transplantation of spleen and lymph node cells, on the other hand, led to some increase in the immune response [4, 7].

It was accordingly decided to study the relative numbers of T and B lymphocytes in various immunocompetent organs and to undertake a phenotypic characterization of bone marrow cells causing inhibition of immunogenesis. For this purpose monoclonal anti-Lyt2.2 antibodies were used, for with them it is possible to distinguish T cells with a suppressor function [9, 13].

EXPERIMENTAL METHOD

Experiments were carried out on 65 CBA and C57B1/c mice weighing 20-23 g. To enhance activity of the serotoninergic system, serotonin (Reanal, Hungary) was injected in a dose of 50 mg/kg 2 h before removal of the organs, or sertraline (Pfizer, USA), a blocker of serotonin reuptake, was injected in a dose of 5 mg/kg 1 h before removal of the organs. Activity of the dopaminergic system was depressed by blocking postsynaptic dopamine receptors with haloperidol (Gedeon Richter, Hungary), 1 mg/kg, or by activating dopamine autoreceptors with (−)-3-PPP (Astra Lakemedel AB, Sweden), 3.4 mg/kg, which were injected twice a day, the last injection being 30 min before removal of the organs. All preparations were injected intraperitoneally, control mice receiving physiological saline, the solvent for all the drugs used.

The number of T lymphocytes in the bone marrow cell population was determined in the cytotoxic test with the aid of rabbit antiserum against cerebral cortical tissue of CBA mice, absorbed with mouse liver homogenate, mouse erythrocytes, and sheep's erythrocytes.

The number of B lymphocytes was tested in the cytotoxic reaction with anti-B-serum (anti-MBLA), obtained by the method in [15]. The number of T and B lymphocytes was determined in the bone marrow, spleen, and lymph nodes of intact animals and in animals with activation of serotoninergic and dopaminergic systems.

The phenotype of the T lymphocytes was identified by the two-stage cytotoxic test using anti-Lyt2.2 (ascites) monoclonal antibodies in a dilution of 1:100 and fresh rabbit complement in a dilution of 1:10 [2].

In each test no fewer than 300 cells were counted; their viability was estimated with the aid of a 0.5% aqueous solution of trypan blue. In each test the cells were counted twice.

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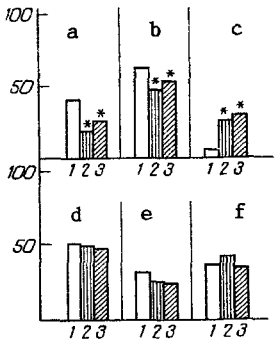

Fig. 1

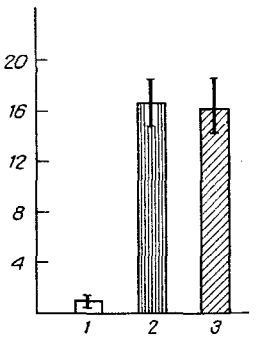

Fig. 2

Fig. 1. Effect of serotonin and haloperidol on number of T (a, b, c) and B (d, e, f) lymphocytes in spleen (a, d), lymph nodes (b, e), and bone marrow (c, f). 1) Control; 2) serotonin; 3) haloperidol. Ordinate, content of lymph nodes (in per cent).

Fig. 2. Increase in number of cells with Lyt2.2 phenotype in bone marrow of C57B1/c mice with activation of serotoninergic system. 1) Control; 2) serotonin; 3) (−)-3-PPP. Ordinate, number of cells (in per cent).

EXPERIMENTAL RESULTS

Determination of the relative percentages of the lymphocytes showed that their number was reduced on activation of the serotoninergic system (injection of serotonin) to 18.5 ± 1.5 in the spleen, and after blockade of the dopaminergic system by haloperidol, to 20.8 ± 4.8 compared with 40.1 ± 0.8 in the control, whereas in the lymph nodes it fell to 45.3 ± 3.5 and 51.0 ± 2.3 compared with 64.1 ± 3 in the control respectively. In the bone marrow the number of Thy-1-positive lymphocytes rose significantly. Whereas in the control the number of B lymphocytes was insignificant, namely 2.6 ± 0.7, after injection of serotonin it rose to 24.4 ± 2.2 and after injection of haloperidol to 22.3 ± 2.1 (Fig. 1). So far as B lymphocytes are concerned, their number in all the immunocompetent organs (spleen, lymph nodes, and bone marrow) remained virtually the same as their number in the control mice, in which activity of the neurotransmitter systems was unchanged (Fig. 1).

The investigation thus showed that increased activity of the serotoninergic system and blockade of the dopaminergic system in unimmunized animals lead to a change in the distribution of T and B-cell populations in different immunocompetent organs, characteristic of the control animals. It is a noteworthy fact that in the bone marrow in animals receiving serotonin and the blocker of postsynaptic dopamine receptors, haloperidol, the number of T-cells was increased. Since transfer of the bone marrow population, rich in T cells, from these animals caused depression of the immune response in immunized irradiated recipients [10], it might be supposed that suppressor T cells accumulate in the bone marrow in response to activation of the serotoninergic system and blockade of the dopaminergic system.

It is now clear that the suppressor T-cell population is a large and varied population of suppressor T cells with varied degrees of maturity, which depress proliferation or differentiation of other subclasses of T cells. As a rule, however, suppressor T cells differ in their phenotype from T-cells with other functional properties, such as helpers. Suppressor T cells induced by antigen in vivo or activated by con A in culture, or natural suppressors have the $Lyt1^{-}2^{+}$ phenotype, distinguishing them from helpers which have the $Lyt1^{+}2^{-}$ phenotype. Next, Lyt2.2 suppressor T cells in populations removed from animals receiving sertraline (a blocker of serotonin reuptake) were tested with monoclonal antiserum. Incidentally, the cells were removed 1 h after injection of the drug, i.e., when it caused maximal reduction of serotonin reuptake (to 53%) [12].

As many as 16.6 ± 1.8 cells with the Lyt2.2 phenotype were found in the bone marrow of these C57B1/c mice, whereas it was shown that in intact animals the number of these cells in the bone marrow is very small, not more than 0.9 ± 0.4 (Fig. 2). Blockade of the dopaminergic system by specific activation of dopamine autoreceptors with (−)-3-PPP also leads to an increase in the number of T lymphocytes with the Lyt2.2 phenotype in the bone marrow, their number rising to 16.1 ± 2.5 (Fig. 2).

It can be concluded from all the data obtained previously [4, 7, 10] and presented in the present publication, that inhibition of immunogenesis in response to activation of the serotoninergic system and blockade of the dopaminergic system is the result of redistribution of suppressor T cells in immunocompetent organs, with accumulation of suppressor cells in the bone marrow. The first evidence of this is given by the functional properties of the T-cells in the bone marrow of these animals, namely their ability to induce weakening of the immune response when transferred to irradiated recipients, by contrast with T-cells of the spleen and lymph nodes, which enhance the immune response of recipients [4, 7, 10]. Second, the effect of an increase in the number of cells in the bone marrow with the Lyt2.2 phenotype, which according to data obtained by many investigators, is a feature of suppressor T cells [9, 13], and the decrease in the number of T lymphocytes in other immunocompetent organs.

Immunogenesis is regulated by complex interaction between T- and B-lymphocytes, which possess different functions, of macrophages, of mediators secreted by them, and also of factors produced by the thymus and bone marrow.

So far as the action of the neurotransmitter systems of the brain is concerned, their action on immunogenesis is a modulating one and it is determined by their effect on certain mechanisms of regulation characteristic of the immune system itself. It will be clear from the results of this investigation that weakening of the immune response due to activation of the serotoninergic system or to blockade of the dopaminergic system, which are in reciprocal relations with one another [3], is effected through the same mechanism, namely an increase in the number of suppressor T cells with the Lyt2.2 phenotype in the bone marrow, We know that the suppressor T cells of the bone marrow block differentiation of clones of B-cells and depress proliferation of all actively dividing cells [6]. If we consider that the bone marrow is a supplier of polypotent stem cells, and that the organ in which differentiation of the B-cells takes place, the biological significance of realization of the immunomodulating effect of the neurotransmitter systems through this mechanism becomes comprehensible.

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