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EFFECT OF SEROTONIN ON ANTIGEN-NONSPECIFIC THYMUS SUPPRESSORS

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There is now considerable evidence to show that the serotoninergic system is involved in neuroimmunomodulation. It has been found that the biogenic amine serotonin has an inhibitory influence on the immune response due to the participation of suppressor cells of the thymus, spleen, and bone marrow [1]. The antigen-nonspecificity of suppressor T and B cells in these same organs has been demonstrated during activation of the serotoninergic system with the aid of two antigens: corpuscular (sheep's red blood cells — SRBC) and soluble (human serum albumin — HSA) [2].

An important role in autoregulation of immunogenesis is played by antigen-specific and antigen-nonspecific suppressor cells; the contribution of these suppressor clones, moreover, can vary considerably depending on the action of modulating agents such as hormones, peptides, and chalones [10]. Accordingly, to elucidate the mechanisms of serotoninergic regulation of the formation of antigen-nonspecific components of suppression, it is important to study the specific component of thymic suppressors in the inductive phase of the immune response.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice weighing 20-25 g, aged 2-3 months, in a syngeneic adoptive cell transfer system. Six groups of mice were used in the experiments, in which the donors of thymocytes were the following groups of animals: 1) mice receiving serotonin in a dose of 100 mg/kg, once, subcutaneously, in Freund's incomplete adjuvant, 30 min before immunization with SRBC (5×10^8 cells); 2) mice immunized intravenously with SRBC (5×10^8) and receiving serotonin in a dose of 100 mg/kg 30 min before immunization, and also 2-deoxyguanosine (dGUA) in a dose of 1 mg per mouse [7] from

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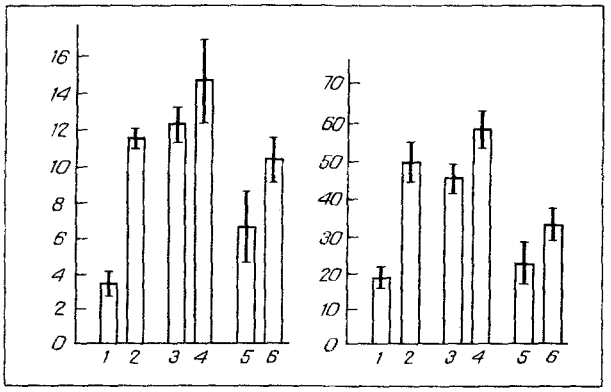

Fig. 1 Fig. 2

Fig. 1. Number of RFC (10^3 cells) in irradiated recipient mice after transfer of thymocytes together with SRBC and spleen cells from the 3rd day of development of the immune reaction from normal donors. Here and in Fig. 2: 1) immunized with SRBC, receiving serotonin, 2) immunized with SRBC, receiving serotonin, and treated with dGUA, 3) immunized with SRBC, 4) immunized with SRBC, treated with dGUA, 5) intact, 6) intact, treated with dGUA.

Fig. 2. Number of IgM-PFU (10^6 cells) of irradiated recipient mice after transfer of thymocytes together with SRBC and spleen cells from donors after the 3rd day of development of the immune response.

day 0 (day of immunization) until the 2nd day inclusive; 3) mice immunized with SRBC (5×10^8); 4) mice immunized intravenously with SRBC (5×10^8) and receiving dGUA daily from day 0 until the 2nd day; 5) intact animals; 6) intact mice receiving dGUA in a dose of 1 mg/mouse daily for 3 days.

Thymocytes were removed from the donors on the 3rd day after immunization. Thymus cells in a dose of 10^7 were injected together with spleen cells of an intact mouse in a dose of 4×10^7, and also with antigen (5×10^8 SRBC) into recipients irradiated in a dose of 8 Gy on the 1st day.

The level of the immune response was determined in the recipients' spleen on the 5th day after injection of the antigen, in the rosette-formation test [1] and the direct plaque formation test [4].

The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Analysis of the experiments showed that transfer of thymus cells from donors receiving serotonin led to marked suppression of the immune response in the irradiated recipients (Fig. 1). Injection of the purine nucleoside substrate dGUA into the donors of the thymus cells was shown to modify the level of the recipients' immune response. dGUA is known not to affect activation of B cells, and it is effective only against T-cell proliferation [9]. The selective toxicity of dGUA against antigen-nonspecific suppressor T cells [7] is connected with accumulation of toxic metabolic products: in the presence of dGUA growth of T lymphoblasts, PHA-induced proliferation, and suppressor T-cell activity are inhibited [5]. Daily injection of dGUA into donors receiving serotonin (group 2) during 3 days of formation of the immune response prevented inhibition of the immune response observed in a series of the same animals but not receiving dGUA (group 1), in the recipients. Incidentally, the weight of the thymus in the series of immunized donors receiving serotonin, and treated with dGUA, was significantly lower (24.1-2.02 compared with 40.4-1.53 mg) than in the same mice, but not receiving dGUA, evidently because of a reduction in the number of thymocytes due to inhibition of cell proliferation. Consequently, suppression recorded during RFC testing in the group of animals receiving thymocytes from donors treated with serotonin is transmitted to a considerable degree by a clone of antigen-nonspecific suppressor T cells sensitive to inhibition of DNA synthesis by dGUA.

Comparison of levels of rosette formation in the control groups (3 and 4) demonstrated a tendency for the number of RFC to increase in animals in which a population of thymocytes was transplanted from immune donors, treated with dGUA (group 4).

In the case of "expulsion" by dGUA of antigen-nonspecific suppressor cells in groups 2 and 4, it was shown that injection of serotonin into immunized donors led to a significant reduction of RFC in the recipients (group 2) compared with the immune response in mice receiving thymocytes from immunized donors, not treated with serotonin (group 4).

This fact may be evidence in support of inhibition of cell proliferation under the influence of serotonin, as confirmed by data in the literature, indicating reduction of mitogen-induced cell proliferation under the influence of serotonin [6]. Inhibition of the immune response in group 2 may be the result of intensified migration of suppressor T cells from the thymus in response to injection of serotonin into the spleen, where suppressor T cells accumulate during this same period (the 3rd day) [1]. Thus, the results of these investigations are evidence that on the 3rd day after immunization with SRBC the nonspecific suppressor T-cell response to elevation of the serotonin level was 71%, but only 37% in the intact animal.

Analysis of direct plaque-forming cells (IgM-PFU; Fig. 2) revealed changes characteristic on the whole of those observed during investigation of rosette-forming cells. It must be pointed out, however, that due to the number of IgM-PFU, the percentage restoration of the immune response after treatment with dGUA was rather different than during analysis of total RFC, immunized with mouse SRBC, namely 21.9, for the same animals after treatment with serotonin it was 62.5, and for intact animals 29.7. Consequently, if values of IgM-AFC with rosette-forming cells are compared, the suppressor T-cell response was higher in the immunized animals when IgM-PFU were recorded, and it was lower in groups of immunized donors receiving serotonin and of intact animals. These results suggest that under conditions of immunization, the preferred target cell in the spleen for antigen-specific suppressor thymocytes is the rosette-forming and IgM-antibody-forming cell population.

In the experiments the fraction of nonspecific suppression by thymocytes increased in the following order: the immune animal — intact — immune + activation of the serotoninergic system. This situation appears not to be accidental. In the present investigation, during an early immune response to SRBC, the level of antigen-nonspecific suppression in the thymus based on the number of IgM-AFC reached 21.9%, whereas in the spleen at the same time during immunization with horse red blood cells, antigen-nonspecific suppressor T cells accounted for 20%, according to data in [11]. It seems that the nonspecific component of T-suppression is a relatively stable value and reflects the existence of a population of a clone of suppressors with identical differences of affinity for different epitopes. The high percentage of antigen-nonspecific suppressor T thymocytes in the intact animal, namely 37 (total RFC) and 21.7 (IgM-AFC) may be evidence of the existence of a large number of resting clones, isolated from the antigenic stimulus, capable of suppression, and forming a natural network of reactive specificity. With elevation of the serotonin level in animals immunized with SRBC a sharp rise of nonspecific suppressors of the thymus was recorded on the 3rd day of the immune response. In the early stages of immunogenesis (by the 3rd day) maximal expression of mouse receptors for interleukin-2 occurs on the T-lymphocyte clone [12], inhibiting the formation of nonspecific suppressor T cells [8]. In view of data on suppression of cell proliferation by serotonin [6], it seems probable that serotonin may abolish the inhibitory effect of the interleukin-2-dependent T-cell clone by inhibiting proliferation of antigen-nonspecific suppressors.

On activation of the serotoninergic system the regulatory mechanisms of the early phase of immunogenesis are thus joined by a quite considerable clone of thymic antigen-nonspecific suppressor cells, and this ultimately determines the nonspecific nature of the quantitative basis of the immune response.

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USE OF A PLASMA CLOT TO ASSESS FIBROBLAST PRECURSORS IN HUMAN BONE MARROW

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One method of studying the stromal microenvironment of bone marrow is to study the clonal capacity of fibroblasts. As a rule culture of hematopoietic cells from animals, healthy blood donors, or patients with various diseases in liquid media is used for this purpose [3-5]. To increase cloning efficiency, it has been suggested that irradiated hematopoietic bone marrow cells from laboratory animals be added to the cells in culture [2]. The positive effect is probably connected with additional stimulation by platelet-secreted growth factor (PSGF), contained in the platelets and megakaryocytes of the added cells [10, 14], or by other stimulators of hematopoietic cells [12]. Incidentally, comparable used richer culture media without the addition of a feeder [17]. The method of culturing bone marrow cells in methylcellulose medium [22] does not differ likewise from this system in principle. In that system fibroblast colonies are formed from precursor cells which settle on the bottom of the culture vessel and adhere to it.

Methods of culturing fibroblasts in semisolid media are alternatives. Under ordinary conditions fibroblasts do not proliferate in semisolid agar medium. Colony formation in semisolid agar medium by untransformed fibroblasts can be obtained only by the use of transforming growth factors, or with the aid of high concentrations of PSGF and epidermal growth factor [18]. Another possibility, in principle is to use as semisolid medium a collagen gel, in which fibroblasts, proliferating in the semisolid medium, are able to adhere to the substrate [16]. It seems that a semisolid medium containing fibrin threads (plasma clot) may also be used to assess proliferation of connective-tissue cells. An attempt was made to use such a system to culture precursor cells of the bone marrow and hematopoesis [13].

The aim of this investigation was to culture bone marrow fibroblasts and to evaluate their relationship with hematopoietic cells in semisolid medium, using a plasma clot.

EXPERIMENTAL METHOD

Bone marrow was obtained from the sternum of 42 patients with somatic diseases in a stage of remission or compensation. Before culture the bone marrow was carefully treated mechanically and washed to remove plasma factors [4, 5]. The bone marrow from some subjects was treated not only mechanically, but also with collagenase [14]. The bone marrow cells were cultured in medium containing 70% of McCoy' s medium with the addition of 10% placental blood serum from healthy women in labor [16], 20% citrated blood plasma from normal blood donors, and 0.7 ml of 10% calcium chloride solution to 100 ml of medium. Another version of culture was the combined use of plasma and methylcellulose (final concentration 0.8%), made up in double Dulbecco's medium. Culture was carried out in plastic dishes from the "Medpolimer" Factory (diameter 40 mm) under conditions of absolute humidity and with CO_2 concentration of 7%. For morphological study the plasma clot was dried and stained with Wright's stain. For comparison, a bone marrow cell suspension was cultured in Carrel's flasks [3-5]. In some cases, during a change of medium the nonadherent cells were re-explanted into a Carrel's flask with nutrient medium. Marrow from

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