EFFECT OF B-ACTIVIN ON ROSETTE-FORMING ABILITY OF MOUSE LYMPHOCYTES AT PEAK IMMUNE RESPONSE TO SHEEP'S RED BLOOD CELLS

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The study of mechanisms of regulation of the immune response by bone marrow cells is an important task in modern immunology. The ability of bone marrow cells to synthesize substances involved in the formation of the immune response was first established in [2]. At the present time, a pharmacological preparation known as B-activin, which possesses immunocorrective properties [11], has been produced on the basis of low-molecular-weight peptides isolated from supernatants of bone marrow cell cultures. Injection of B-activin into mice at the peak of the secondary immune response into the region of injection of the antigen (sheep's red blood cells — SRBC) doubles the number of antibody-forming cells (AFC) in the regional lymph nodes [3]. However, development of the immune response to SRBC is known to be characterized by an increase in the number both of AFC and of cells carrying surface antigen-recognizing receptors and capable of forming immune rosettes (iRFC) [9, 13].

The aim of this investigation was to study the effect of B-activin on the number of iRFC at the peak of the secondary immune response in mice and to estimate quantitatively changes in the relative numbers of AFC and iRFC arising under the influence of B-activin.

EXPERIMENTAL METHOD

Experiments were carried out on hybrid (CBA × C57BL/6)F_1 mice weighing 18-22 g. The mice were immunized twice with a 10% suspension of SRBC either subcutaneously in a dose of 0.1 ml into the footpads of both hind limbs, or intraperitoneally in doses of 0.3 ml with an interval of 2-4 weeks. The B-activin was obtained from supernatants of pig bone marrow cell cultures by the method described previously [1]. The active dose of B-activin was calculated from the amount of protein contained in it, which was determined by Lowry's method [8].

To study the effect of B-activin on the number of iRFC in tests in vitro and in vivo, the preparation was added on the 3rd day of the secondary immune response to a cell culture from immune lymph nodes (50 μg to $5 \cdot 10^6$ cells in 1 ml) or injected subcutaneously into the footpad of one hind limb (100 μg per mouse) respectively. Physiological saline was used as the control. Cells of immune lymph nodes were cultured in penicillin flasks in 1 ml of medium 199, containing 2 mM of L-glutamine and 10% fetal calf serum (FCS) for 18 h at 37°C. The number of immune rosettes in the lymphocyte culture or in cell suspensions from the control and experimental lymph nodes was counted next day. For this purpose, cells washed off in medium 199 ($2 \cdot 10^6$ cells in 1 ml) were mixed in equal volumes with a 0.5% suspension of SRBC, centrifuged for 5 min at 100g, and incubated for 1 h at 37°C [5]. The residue was then resuspended accurately and the number of cells forming rosettes with SRBC (RFC) was calculated by the equation:

$$RFC = \frac{A \cdot 100}{B \cdot 22.5},$$

where A is the number of RFC in two grids of a Goryaev's chamber; B the number of cells in five large squares; 22.5 is a coefficient.

To determine the ratio between the numbers of iRFC and AFC and changes in that ratio under the influence of B-activin, immune rosettes were sedimented by centrifugation on a Ficoll—Verografin density gradient of 1.09 g/cm^3, and the relative number of mononuclear cells

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and antibody-producers was determined in each of the fractions obtained on the day after intraperitoneal injection of B-activin. For this purpose cells from immune spleens ($5 \cdot 10^7$ in 1 ml) were mixed in equal volumes with a 10% suspension of SRBC containing 20% FCS. The resulting mixture was centrifuged for 5 min at 100g and incubated for 1.5 h at 37°C [7]. The residue was then accurately resuspended, diluted twice with medium 199, layered above Ficoll, and centrifuged for 15 min at 2000g [10]. The resulting cell fractions were harvested, washed to remove Ficoll, and the red blood cells of the sedimented fraction were hemolyzed by hypotonic shock. During control fractionation cells of immune lymph nodes and spleens were centrifuged in a Ficoll density gradient without SRBC. The number of AFC in each of the fractions obtained was determined by local hemolysis in gel [6]. The significance of the results was estimated by Student's test.

EXPERIMENTAL RESULTS

Data showing that the presence of B-activin in a cell culture from immune lymph nodes, isolated on the 3rd day of the secondary immune response to SRBC reduces by half the number of iRFC compared with the control. For instance, the average number of iRFC in the control lymphocyte culture was 0.6%, and in the experimental 0.34%. The difference is significant when t is calculated by the method of paired comparison of control with experiment ($p < 0.02$). However, it was shown previously [4] that in the course of culture cells may lose their surface receptors spontaneously. To rule out any possible effect of the conditions of cell culture on the decrease in the number of iRFC under the influence of myelopeptides, B-activin was injected in vivo and the number of iRFC in cell suspensions from control and experimental lymph nodes was determined next day. It follows from the results given in Fig. 1 that injection of B-activin into mice in the productive phase of the immune response also led to a fall in the number of immune rosettes (control 1.0 ± 0.1%, experiment 0.7 ± 0.1%; $p < 0.05$), which is evidently the result of the action of B-activin and unconnected with the conditions of cell culture.

According to one hypothesis [2], the increase in number of AFC taking place under the influence of B-activin is connected with involvement of "silent" plasma cell precursors with a properly formed system for antibody biosynthesis, but in a state of suppression, in antibody production. Under the influence of immunoregulatory myelopeptides, further differentiation of precommitted B lymphocytes may perhaps take place in plasma cells, a feature of which is the absence of surface antigen-recognizing receptors [12].

To study the connection between the effect of B-activin on expression of antigen-recognizing receptors and on the number of AFC in the productive phase of the immune response, iRFC from lymph nodes and spleen were sedimented by centrifugation on Ficoll and the number of nucleated cells and of antibody producers was determined in each of the fractions obtained, the B-activin having been injected into the mice 24 h before fractionation.

As a result of fractionation (Table 1) we obtained two cell fractions: residue, containing iRFC and rich in antibody producers, and interphase, deprived of both iRFC (by definition) and of AFC compared with the original cell suspension. The data in Table 1 show that among the comparatively small number of cells in the residual fraction there was a considerable number of AFC and, conversely, among the larger number of interphase cells, there were far fewer antibody producers. Control fractionation, excluding contact between lymphocytes and SRBC,

TABLE 1. Results of Fractionation of Mouse iRFC at the Peak of the Secondary Immune Response to SRBC (M ± m)

Source of cells	Fraction	AFC/10^6 cells	Nucleated cells, %	AFC, %
Lymph nodes	Original	645±135	100	100
	Interphase	152±48	32±6	6,6±0,8
	Residue	11 806±4 043	1,2±0,1	35,4±11,1
Spleen	Original	1 158±72	100	100
	Interphase	307±65	28,7±4,7	7,5±1,5
	Residue	4 690±1 744	13,0±2,9	49,3±11,4
Spleen after injection of B-activin	Original	1 066±69	100	100
	Interphase	616±134	26±0,5	14,7±2,1
	Residue	5 477±2 600	11,3±2,6	49,6±15

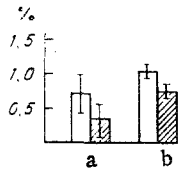

Fig. 1. Decrease in number (in %) of iRFC in cell suspensions from immune lymph nodes after addition of B-activin in vitro (a) and its injection in vivo (b). Unshaded column — control, shaded — experiment.

led to the conclusion that immune rosette formation, preceding fractionation, leads to a twofold increase in the number of mononuclear cells in the residue and an increase of several times in the number of AFC in it.

Fractionation of iRFC after intraperitoneal injection of B-activin showed that the number of cells in the residue was statistically significantly reduced, whereas the number of antibody producers in the interphase was doubled (Table 1). The observed effect can evidently be explained on the grounds that under the influence of B-activin some cells capable of forming immune rosettes with SRBC lost their ability to form rosettes on account of transition into the phase of active antibody synthesis, accompanied by "shedding" by the cell of its antigen-recognizing receptors [12]. This leads to a decrease in the number of cells in the residue and to the appearance of an additional number of antibody producers in the interphase.

The results of fractionation of immune rosettes on Ficoll thus agree with data showing a reduction in the number of iRFC under the influence of B-activin in the system both in vitro and in vivo. They are evidence that at the peak of the secondary immune response the decrease in the number of iRFC and the increase in the number of AFC observed under the influence of B-activin are interconnected phenomena resulting from the conversion of precommitted B lymphocytes carrying antigen-recognizing receptors into plasma cells.

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