system which may lead to functional disorders. The most marked structural-functional changes in the thymus were revealed in animals with a generalized herpetic infection. These findings justify the includsion of immunomodulating preparations such as Thymogen, Tactivin, and Timalin, created on the basis of active peptides of the thymus, into complex etiopathogenetic therapy of herpetic infection, in particular, of its generalized form.

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ONCOLOGY

Suppressor Activity of Nonadhesive Bone Marrow Cells is Determined by the Cells Bearing Erythroblast Antigen

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Key Words: erythroblast antigen; supressor cells; bone marrow

Bone marrow (BM) of humans and animals is known to have so-called natural supressor cells (NSC), which are able to inhibit mitogen- and antigen-stimulated proliferation of lymphocytes, proliferation of T cells and formation of cytotoxic T lymphocytes in MLC, as well as proliferation of tumor cells *in vitro* [5,7-10]. NSC have been shown to have receptors to the wheat germ agglutinin; however, they have no markers of mature immunocompetent cells [9]. Elimination of the cells bearing erythroblast antigen from BM pro-

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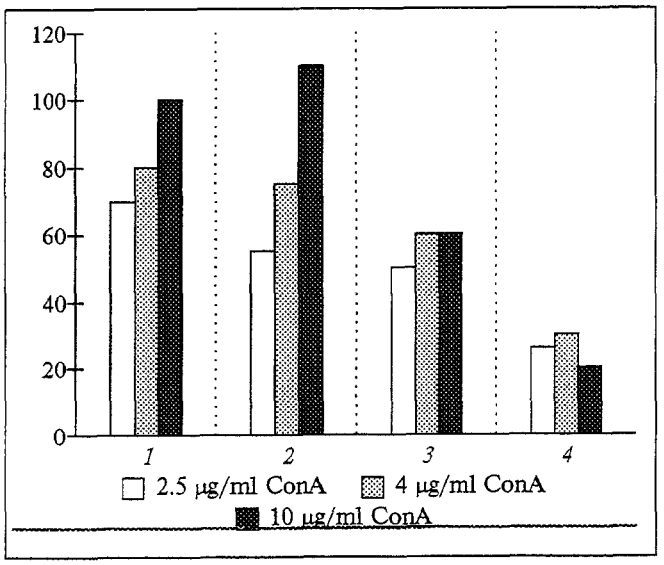

Fig. 1. Activity of BM natural suppressor cells as a function of ConA concentration and the ratio of BM cells and splenocytes. ^{3}H-thymidine×10 (cpm/well) incorporation into splenocytes stimulated by ConA. The ratio of splenocytes to BM cells is 0:1 (*1*), 1:2 (*2*), 1:1 (*3*), and 2:1 (*4*).

moted a considerable decrease in suppressor activity studied on the model of syngeneic adaptive transfer *in vivo* [2-4].

To elucidate the mechanisms of the suppressive effect of BM we assayed the dependence of NSC activity on the presence of Ag-Eb-positive cells, as well as the influence of monoclonal antibodies to Ag-Eb on NSC activity.

MATERIALS AND METHODS

Experiments were performed on C57Bl/6 mice (8-12-weeks old), which were fed a standard diet. Cells were cultured in RPMI-1640 medium containing 2 mM HEPES buffered saline, 2 mM L-glutamine, 10% fetal calf serum (Flow), 5×10^{-5} M 2-mercaptoethanol (Fluka), 100 U/ml penicillin, and 100 μg/ml streptomycin.

Natural suppressor activity of BM cells was determined by the degree of inhibition of ConA-stimulated proliferation of normal syngeneic splenocytes. For this 3×10^5 spleen cells and 3×10^5 Bm cells were placed in a round-bottom 96-well dish and incubated for 72 h in the presence of 2.5 μg/ml ConA at 37°C in a humidified atmosphere of 5% CO_2. We added 1 μCi ^{3}H-thymidine into every well 18 h prior to the termination of culturing. ^{3}H-thymidine incorporation was determined by a standard technique using a Flow harverster, and then activity was measured on a Mark-III β-counter (Delta). The level of suppression was calculated by standard formula.

We used P815 mastocytoma to determine the degree of inhibition of tumor cell proliferation. For this 2×10^4 P815 mastocytoma cells and 2×10^5 BM cells were placed in each of the 96 round-bottomed wells and incubated for 16 h under the conditions indicated above. We added 1 μCi ^{3}H-thymidine (specific activity) to each well 4 h prior to the termination of culturing, and then ^{3}H-thymidine incorporation was determined on a Mark-III β-counter (Delta) using a standard technique. The cytostatic index was calculated using the formula:

$$CI=\left(1-\frac{\text{pulse frequency in the well (effector cells++target cells)}}{\text{pulse frequency in the well containing target cells}}\right)\times100\%$$

To determine the suppressor factor, 4×10^6 cells/ml BM were placed in a 24-well dish (Costar) and incubated in the culture medium for 48 h at 37°C. Cell sediment was removed by centrifugation (5 min, 1000 rpm) and various dilutions of collected supernatant were added to 3×10^5/well normal splenocyte cells in the presence of 2.5 μg/ml ConA.

Adhesive cells were eliminated from BM by the incubation of 4-6 mln/ml BM cells in a Petri dish (Costar) for 90 min at 37°C, after which BM was used in the experiments.

Ag-Eb positive cells of BM were eliminated by panning [6]. Monoclonal antibodies to Ag-Eb were isolated using MAE-15 hybridoma cells [2]. Mice monoclonal antibodies H417.3 (IgG1) used in the control experiments revealed the antigen typical for the cells of small cell lung cancer, some tumors, and normal tissues [1].

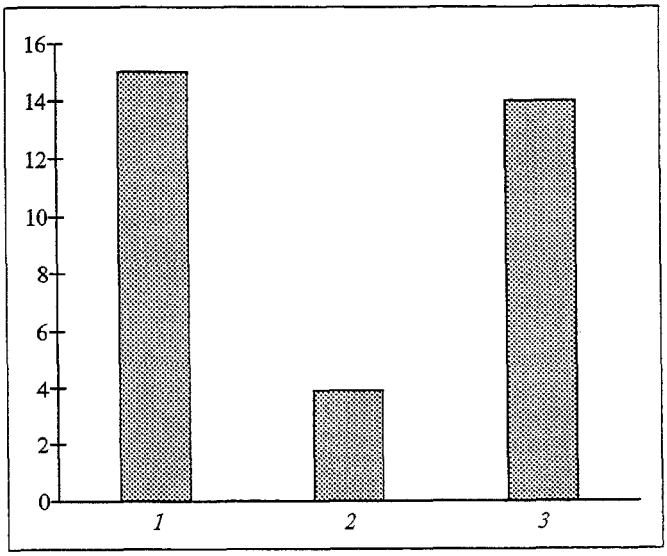

Fig. 2. Effect of Ag-Eb-positive cells on suppressor activity of natural suppressor BM cells. ^{3}H-thymidine×10 (cpm/well) incorporation into splenocytes stimulated by ConA: *1*) in the absence of other cells; *2*) for addition of nonadhesive fraction of BM cells; *3*) for addition of nonadhesive BM cells after elimination of Ag-Eb-positive cells.

TABLE 1. Effect of Preincubation of BM Cells with Monoclonal Antibodies on NSC Activity

Cells per well		Preincubation with antibodies at a concentration, μg/ml		^{3}H-thymidine incorporation, cpm
Spleen cells stimulated by ConA (2.5 μg/ml), $\times 10^5$	nonadhesive BM cells, $\times 10^5$	MAE-15	H417.3	
3	–	–	–	24364±3762
3	3	–	–	13528±1974
3	3	10	–	16665±2096
3	3	75	–	16304±407
3	3	150	–	23103±3779
3	3	300	–	23585±1146
2	–	–	–	89434±7498
2	4	–	–	7503±1587
2	4	–	225	9898±2345
2	4	225	–	44057±4789
2	2	–	–	24899±3421
2	2	–	225	25342±4576
2	2	225	–	64000±6756

BM cells were preincubated with various concentrations of MAE-15 antibodies in the culture medium for 1 h at room temperature. The cells were rinsed twice after incubation and used for determination of suppressor activity as described above.

Statistical analysis of the data was performed using Student's *t* test.

RESULTS

We observed a pronounced inhibition of ConA-induced splenocyte proliferation by normal BM cells at a 2:1 ratio of BM cells to responder cells (Fig. 1). The suppressor effect was either considerably less pronounced or disappeared at other ratios (1:1 and 0.5:1). It should be noted that the suppressor effect of BM cells was observed at all ConA concentrations used, the maximum effect being observed with the mitogen at a concentration of 10 μg/ml. However, ConA at a concentration of 2.5 μg/ml revealed an inhibitory effect of BM cells when the minimal amount of cells was added. Therefore, further investigations were carried out at this concentration level of ConA (2.5 μg/ml).

Evidence in the literature suggests that cells bearing Ag-Eb on their surface may play a certain role in the mechanism of the suppressor effect of BM cells [4]. To specify the role of Ag-Eb in the action of NSC we studied the influence of MAE-15+ BM cells on the proliferation of spleen cells stimulated by ConA and P815 tumor cells. Figure 2 demonstrates that elimination of Ag-Eb-positive cells by panning reliably decreased BM inhibitory effect on ConA-induced proliferation of splenocytes.

BM cells prior to their addition to splenocytes were preincubated with various concentrations of MAE-15 monoclonal antibodies to assess the direct participation of erythroblast antigen in the studied mechanisms of natural suppressor activity. Suppressor activity of the cells tested was measured after the elimination of nonbound antibodies at a 1:1 and 2:1 ratio of BM cells to splenocytes.

^{3}H-thymidine incorporation in the splenocyte culture stimulated by mitogen in the absence of BM cells was 23,360+3760 cpm. We noted a 44% reduction in ^{3}H-thymidine incorporation upon the addition of nonadhesive BM cells (up to 13,530+1970 cpm). Preliminary incubation of BM cells with MAE-15 antibodies decreased the suppressor effect observed. Table 1 shows that the suppressor effect of BM cells decreased with an increasing concentration of monoclonal antibodies in a dose-dependent manner and nearly disappeared at concentrations of 150 and 300 μg/ml.

At the same time, and under the same conditions, we conducted control experiments on BM cell preincubation with H417.3 monoclonal antibodies, which are immunoglobulins of class G like MAE-15. Table 1 demonstrates that pretreatment of BM cells with H417 antibodies has no effect on their suppressor activity and, therefore, it follows that reduction of suppressor activity after treatment with MAE-15 antibodies is a specific function of antibodies toward Ag-Eb.

Thus, these findings allow us to conclude that, first, elimination of cells sensitive to MAE-15 monoclonal antibodies results in a reliable decrease in the natural suppressor activity of BM due to diminished production of inhibitory factor by BM cells, and, second, it is possible to regulate NSC activity by the antigen sensitive to MAE-15.

The possibility of regulating NSC activity by using MAE-15 may prove to be useful in the study

of some processes, such as tumor growth, BM transplantation, and graft versus host disease, where NSC can play an important role.

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EXPERIMENTAL GENETICS

Genetic Regulation of Conjugative Properties of pAP18 Plasmid Complex in *E.coli*

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Key Words: *plasmid complex; genetic transfer regulation system*

Cells of natural bacterial populations often harbor complexes of various plasmids providing genetic control over drug resistance, virulence, and other properties of these organisms [1]. However, in many cases the regularities of the relationship between individual plasmids of the complex which determine their most important properties (such as the manifestation of conjugative properties) remain unclear. The objective of the present study was to analyze the interplay between the systems of genetic regulation in the conjugational transfer of plasmids of the pAP18 complex that was found earlier in an *E.coli* strain isolated from an ill animal [2].

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MATERIALS AND METHODS

The plasmids under study were pAP18-1 (TcColV) and pAP18-2 (Sm) comprising the plasmid complex pAP18. As test plasmids we used the standard plasmid Flac and a collection of derepressed F-like plasmids with known types of sensitivity to transfer inhibitors [3]. As host cells or recipient cells we used a serologically untyped *E.coli* strain K-12

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