3. T. A. Danilova and N. M. Fedorova, Byull. Éksp. Biol. Med., No. 4, 462 (1976).
4. N. M. Fedorova, T. A. Danilova, and F. E. Novikov, Vopr. Revmat., No. 2, 36 (1976).
5. T. Feizi, Med. Biol., 58, 123 (1980).
6. K. Kano, J. M. Merrick, and F. Milgrom, Int. Arch. Allergy, 73, 373 (1974).
7. N. G. Rogentine and B. A. Plocinik, J. Immunol., 113, 848 (1974).
8. G. F. Springer, P. R. Desai, M. S. Murthy, et al., Prog. Allergy, 26, 42 (1979).
9. L. Svennerholm, Biochim. Biophys. Acta, 24, 604 (1957).
10. E. Suzuki and M. Naiki, J. Biochem., 95, 103 (1984).
11. O. Tönder, J. B. Natvig, and R. Matre, Immunology, 12, 629 (1967).
12. W. M. Watkins, Adv. Genet., 10, 1 (1980).

EFFECT OF B-ACTIVIN ON HUMAN T SUPPRESSOR CELLS

S. S. Gambarov, A. M. Khzardzhyan,
N. V. Adamyan, A. V. Shakhsuvarov,
A. A. Suzdal'tseva, and G. A. Rakhmanova

UDC 612.112.94.017.1.014.46:615.276.4

KEY WORDS: T supressor cells; concanavalin A; B-activin.

A new mediator produced by bone marrow cells, and known as stimulator of antibody producers (SAP), has recently been described. If added to a cell culture or injected into an animal in the productive phase of antibody formation, SAP doubles or trebles the number of antibody producers [3, 4, 7]. Previous investigations on mice showed that one possible mechanism of action of SAP is inhibition of the effect of T suppressor cells by this mediator [1-3]. On the basis of SAP, the immunoregulatory agent B-activin has now been produced [5, 6].

The aim of this investigation was to study the influence of B-activin on the effect of human concanavalin A (con A)-induced T suppressor cells and also on the process of induction of T suppressor cells by con A and stimulation of proliferative activity of lymphocytes by phytohemagglutinin (PHA).

EXPERIMENTAL METHODS

Con A-induced suppression and the effect of B-activin on it were studied in a system in which the test cell culture and the culture for induction of suppressors were prepared simultaneously.

Peripheral blood was obtained from blood donors. Mononuclear cells were isolated on a one-step Ficoll–Urografin density gradient. After isolation the cells were distributed among three penicillin flasks for subsequent culture. The cells, in a concentration of 10^6/ml, were cultured in 3 ml of medium RPMI-1640 (Gibco, USA) with the addition of up to 10% inactivated human group IV (AB) serum, 2 μM glutamine, 10 μM HEPES, 100 U/ml penicillin, and 100 μg/ml streptomycin.

PHA (from Difco, USA), in a dose of 10 μg/ml was added to the first flask (preparation of the test culture), con A (Difco) in a dose of 40 μg/ml was added to the second flask (induction of suppressor cells), and no mitogens were added to the third flask (control of spontaneous proliferation).

After 48 h cells from the first flask were distributed among nine flasks, $3 \cdot 10^5$ cells into each flask. Lymphocytes from the second flask (inhibited with con A) were washed, resuspended in medium RPMI-1640 with the above-mentioned additives, and incubated for 40 min with mitomycin C (40 μg/ml). After incubation the cells were washed twice and transferred into six of the nine flasks containing PHA-stimulated cells, in the ratio of 1:1, and also into three empty flasks, as the control of treatment with mitomycin ($3 \cdot 10^5$ cells

Erevan Branch, All-Union Science Center for Surgery, Academy of Medical Sciences of the USSR. (Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 5, pp. 598-600, May, 1986. Original article submitted June 19, 1985.

 0007-4888/86/0005-0660$12.50

TABLE 1. Effect of B-Activin on Proliferative Activity Induced by PHA

Experimental conditions	No. of expts.	StI	
		control	experiment
PHA + B-activin simultaneously	10	25,6 (12,6—38)	30,3 (19,7—40,9)
PHA followed 48 h later by B-activin	10	32,8 (19,0—46,6)	27,6 (15,7—39,5)

TABLE 2. Influence of B-Activin on Effect of Con A-Induced Suppressors and on Their Induction

Experimental conditions	Number of experiments	SpI	
		control	experiment
A	12	56,2 (48,2—64,2)	—14 (6.2 to 20.8)
B	6	48,4 (33,9—62,9)	44,2 (28,3—60,1)

Note. A) B-Activin was added together with con A-induced supressors to the test culture; B) B-activin was added together with con A to a culture for induction of supressors.

into each flask). B-Activin (50 μg per culture) was added to three of these six flasks. Finally, cells ($3 \cdot 10^5$) incubated without the mitogen for 48 h (control of spontaneous proliferation) were introduced into three flasks. ^{3}H-thymidine with specific radioactivity of 2 μCi was added to all the flasks. The results were read 24 h later. In this model, therefore, the suppression effect was determined 72 h after the beginning of the experiments.

The action of B-activin on proliferative activity of PHA-stimulated lymphocytes also was studied in separate experiments. For this purpose, B-activin was added to some cultures 0 or 48 h after incubation with PHA in a dose of 50 μg per culture.

The supression index (SpI) was calculated by the formula:

$$\text{SpI} = \left[1 - \frac{I\,(\text{II, III}) - I\,(\text{IV}) - I\,(\text{I})}{I\,(\text{I}) - I\,(\text{V})}\right] \cdot 100\,\%,$$

where I denotes the number of counts, I-V denote in the test culture with PHA, in the culture containing a mixture of cells incubated with PHA for 48 h and con A-induced suppressor cells in the ratio of 1:1, in a culture containing the same cells as the previous system, plus B-activin, in a culture containing lymphocytes stimulated by con A and treated with mitomycin, and in the culture respresenting the spontaneous proliferation control, respectively.

The stimulation index (StI) was determined by the formula:

$$\text{StI} = \frac{I\,(\text{I}) - I\,(\text{V})}{I\,(\text{V})}.$$

The numerical results were subjected to statistical analysis with determination of the arithmetic mean and its confidence interval at the P = 0.05 level.

RESULTS

It will be clear from Table 1 that StI, in cultures stimulated by PHA to which B-activin was added after 48 h virtually did not differ from that in cultures not containing B-activin. Similar results were obtained on the addition of B-activin to the culture together with PHA on day 0 of the experiment. Thus, B-activin does not affect proliferative activity of lymphocytes induced by PHA.

The results of investigation of the influence of B-activin on the effect of con A-induced supressors and also on the process of their induction are given in Table 2.

It will be clear from Table 2 that suppressors induced by con A and added 48 h after the beginning of culture to a test culture of PHA-stimulated lymphocytes inhibited the proliferative activity of the target cells by about 50% (SpI = 56%). Meanwhile, if B-activin was added to the test culture together with con A-induced suppressors in a dose of 50 μg per culture, the proliferative activity of the target cells was not inhibited. SpI became "negative." Consequently, B-activin blocks the effect of con A-induced human suppressor cells.

To study the effect of B-activin on induction of T suppressor cells by con A, a series of experiments was undertaken in which B-activin was added to the suppressor cell induction

system (together with con A). It will be clear from Table 2 that B-activin did not block accumulation of suppressor cells after 48 h in cultures containing con A. The ability of cells incubated with con A in the presence of B-activin, and treated with mitomycin C, to inhibit proliferation in the test culture was virtually indistinguishable from that of lymphocytes incubated with con A alone. Consequently, B-activin has no effect on the process of induction of suppressor cells by con A.

B-activin thus blocks the effect of con A-induced human suppressor cells but does not affect their induction.

There are two possible mechanisms of this effect of B-activin on T suppressor cells: through blocking of T suppressor cells and at the level of mediators of these cells.

The results widen the scope for the use of B-activin in clinical practice, and, in particular, in pathological states accompanied by increased T suppressor cell activity.

The B-activin was obtained from the Laboratory of Mediators of the Immune System, Institute of Immunology, Ministry of Health of the USSR, for which the authors are sincerely grateful.

LITERATURE CITED

1. S. S. Gambarov, A. A. Mikhailova, and A. M. Khzardzhyan, in: Mediators of the Immune Response in Experimental and Clinical Medicine [in Russian], Moscow (1983), p. 39.
2. S. S. Gambarov, A. M. Khzardzhyan, A. A. Mikhailova, et al., in: Problems of Molecular-Cellular Biology [in Russian], Erevan (1983), p. 41.
3. A. A. Mikhailova, "Cell cooperation at the level of mature antibody producers," Author's Abstract of Dissertation for the Degree of Doctor of Biological Sciences, Moscow (1978).
4. A. A. Mikhailova, R. V. Petrov, and R. N. Stepanenko, Dokl. Akad. Nauk SSSR, 229, 247 (1976).
5. R. V. Petrov, A. A. Mikhailova, L. A. Zakharova, et al., Inventor's Certificate No. 1005790 (USSR).
6. Yu. O. Sergeev, P. G. Deryabin, E. M. Novikova, and S. V. Kibza, in: Mediators of the Immune Response in Experimental and Clinical Medicine [in Russian], Gor'kii (1983), p. 144.
7. R. V. Petrov, A. A. Mikhailova, R. N. Stepanenko, and L. A. Zakharova, Cell. Immunol., 17, 342 (1975).