EVALUATION OF THE ACTION OF TACTIVIN ON DIFFERENT STAGES OF T CELL DIFFERENTIATION

M. G. Mikhna, I. V. Miroshnichenko,
M. S. Nikonova, R. Kh. Aknazarova,
A. A. Yarilin, V. Ya. Arion,
and I. V. Sanina

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Adequate evaluation of the activity of humoral preparations from the thymus is a difficult task because thymus hormones have many points of application of their action and the immunologic disturbances which are indications for the therapeutic use of thymus preparations are varied in character. One of the most active preparations of this kind is the Soviet preparation tactivin [1], which has proved an effective therapeutic agent in immunodeficiency states.

The aim of this investigation was to evaluate the manifestations of action of tactivin on T cells at certain stages of development. The aim of the investigation was to determine the precise points of application of tactivin, which is essential in order to identify the exact indication for its therapeutic use.

EXPERIMENTAL METHOD

T lymphocytes isolated from the bone marrow and thymus of CBA mice (from the Stolbovaya nursery, Academy of Medical Sciences of the USSR) served as the test object. The bone-marrow T-lymphocyte precursors (TLP) were enriched by freeing a suspension of bone marrow cells from erythrocytes, adherent cells, Fc-receptor-carrying cells, and T and B lymphocytes [3]. PTC were identified on the basis of their expression of the SC-1 antigen, detected in the cytotoxic test using rabbit immune serum against mouse brain, exhausted with mouse liver and thymocytes [4]. The number of $SC\text{-}1^+$ cells in the enriched suspension was about 20%. Functionally immature cortical thymocytes were separated from mature medullary thymocytes by agglutination of the thymus cells in the presence of peanut lectin, followed by rinsing out with galactose [5]. Expression of the receptor for peanut lectin was determined by enzyme immunoassay in suspension. Preparations of peanut lectin, unlabeled and labeled with peroxidate, were generously provided by M. D. Lutsik. The number of cells carrying the receptor for peanut lectin in the agglutinated fraction was more than 95% (PNA^+ cells).

The action of tactivin of PTC was assessed by studying disappearance of SC-1 antigen, expression of Thy-1,2-antigen, and induction of short-term proliferation (in a 20-h culture). Thy-1,2-antigen was determined in the cytotoxic test with AKR-anti-CBA alloantiserum and guinea pig complement. The action of tactivin on PNA^+ thymocytes was estimated as reduction of the proportion of PNA^+-cells in the unfractionated thymocyte suspension and acquisition by purified PNA^+ thymocytes of the ability to respond by proliferation to the action of phytohemagglutinin (PHA, from Difco) in a 72-h cell culture. The action of tactivin on PNA^- lymphocytes was determined by its effect on spontaneous proliferation of cells and their response to PHA. The proliferative reaction in all cases was estimated by counting incorporation of ^3H-thymidine. Tactivin was present in the cultures throughout the period of culture. When its effect on the phenotype of the cells was determined, they were incubated with tactivin for 1 h.

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

Institute of Immunology, Ministry of Health of the USSR. Institute of Physicochemical Medicine, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 2, pp. 189-191, February, 1988. Original article submitted March 20, 1987.

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TABLE 1. Action of Tactivin on Surface Phenotype and Proliferative Activity of T Precursor Cells and Thymocytes

Concentration of tactivin, μg/ml	Per cent of cells carrying markers			Incorporation of ^{3}H-thymidine, cpm (index of stimulation)				
	Thy-1,2$^+$-PTC	SC-1$^+$-PTC	PNA$^+$ thymocytes	PTC	PNA$^+$ thymocytes		PNA$^-$ thymocytes	
					no PHA	with PHA	no PHA	with PHA
0	0	18,3±2,1	85,7±0,3	1690±500	892±191	539±8,9 (0,6)	323±112	588±11 (1,8)
10^{-3}	0	19,0±1,5	87,0±1,4					
10^{-2}	10,1±3,0	16,0±4,1	83,5±1,8	1709±688	928±467	1431±901 (1,54)	211±30	513±48 (2,4)*
10^{-1}	15,2±2,7	5,0±3,8	69,2±2,4	4780±804	220±64	864±187 (3,9)*	285±23	2130±962 (7,5)*
10^{0}	8,3±3,6	0	63,6±1,7	1737±184	256±40	639±123 (2,5)*	763±200	1645 (2,2)

Legend. Results of typical experiments are shown. Each point corresponds to 5-10 tests. Values differing significantly from corresponding values in the absence of tactivin ($p < 0.05$) are underlined. Asterisk indicates significant stimulation of proliferation in the presence of PHA.

EXPERIMENTAL RESULTS

Data on the effect of tactivin in the test systems used are summarized in Table 1. By the action of the preparation of PTC the surface markers of the cells were changed: the PTC marker SC-1-antigen was lost and the T cell marker Thy-1,2 appeared. Just as through the action of other thymus preparations, to induce Thy-1,2 antigen a lower concentration of the factor was required than to remove the SC-1-antigen [2, 3]. As a result, within a certain interval of hormone concentrations on the surface of PTC, both markers were present simultaneously. In the presence of high tactivin concentrations (like other thymus factors also), no expression of the Thy-1,2-antigen took place. Under the influence of tactivin on the thymocytes the fraction of cells expressing the receptor for peanut lectin was reduced, and this corresponds phenotypically to the transition into more mature PNA- cells. Phenotypic changes in PTC and PNA$^+$ thymocytes take place with very low concentrations of the preparation (10^{-5}-10^{-4} mg/ml), evidence of its very high activity.

In some concentrations tactivin stimulated the outflow of PTC into the S phase of the cycle. A similar effect also has been recorded for other thymus hormone preparations [2, 3]; it is exhibited only briefly (after culture for 20 h) and only weakly. Tactivin itself did not affect the level of proliferation of PNA$^+$ thymocytes or lowered it a little. At the same time, it had a weak mitogenic action on PNA$^-$ thymocytes.

The data in Table 1 are evidence that as a result of treatment with tactivin PNA$^+$ cells acquire the ability to espond to PHA by proliferation, and the response of PNA$^-$ thymocytes to this mitogen is intensified. These two effects of tactivin, in our opinion, are the most important, for acquisition of the ability to respond to mitogen by immature thymocytes can be interpreted as a manifestation of functional maturation, whereas potentiation of the response of mature PNA$^-$ thymocytes to PHA can be regarded as evidence of an immunostimulating effect, for proliferative expansion of lymphocytes is an essential condition for the immune response, and largely determines its intensity. However, it must be emphasized that the influence of tactivin on the response of cortical thymocytes to PHA was distinguished by some degree of variability. A condition for its reproduction, in particular, is effective removal of all mature thymocytes from the PNA$^+$ cells and absence of any response of the test suspension itself to PHA. The stimulating action of tactivin on the proliferative responses of cells of the T series also is exhibited within the same range of concentrations as its effect on cell phenotype.

It can thus be concluded from these results that tactivin acts on all three stages of maturation of T cells studied. The effect is manifested as changes in cell phenotype coinciding with those observed during maturation, acquisition of ability to respond by proliferation to mitogenic stimuli (a feature of functional maturation), and potentiation of the response of mature T cells to PHA, which can be regarded as a manifestation of its immunostimulating action. All the effects observed were reproduced by tactivin in very low concentrations.

Experience of the evaluation of numerous natural thymus preparations and of their synthetic fragments and analogs [2, 3] shows that the nature, specificity, and importance of their phenotypic and functional effects differ. Changes in the phenotype of cells stimulat-

ing their maturation are induced by many factors of nonthymic origin, capable of raising the intracellular cAMP level [6]; a change in the phenotype of cells by no means invariably implies functional maturation. Ability to induce this is a much more specific property of thymus hormones. For instance, the acquisition by PNA^{+}- thymocytes of the ability to respond to PHA, of all the preparations which we tested, was induced by tactivin and thymoptin, but not by thymostimulin, synthetic fragments of thymus hormones, or several other peptides. It can be tentatively suggested that the change in phenotype of the cells under the influence of thymus hormones reflects modification of their surface, so that it can receive a more specific signal, determining their true functional differentiation.

On the basis of data showing that tactivin acts on different stages of T-cell differentiation and potentiates the response of mature T-lymphocytes to mitogen, it can be recommended for clinical use in disturbances of T-lymphocyte maturation at different levels and in disturbances of T-cell function.

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