MITOGENIC ACTIVITY OF PURIFIED THYMOCYTE GROWTH FACTOR

V. Yu. Talaev, A. A. Yarilin, V. P. Shichkin, and T. M. Shuvaeva

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Processes of growth and differentiation of intrathymic precursors of T lymphocytes (PTL) are regulated by different humoral factors, namely thymic hormones and cytokines; some cytokines, moreover, are produced by PTL themselves. One such cytokine, in particular, is thymocytic growth factor (TGF), whose activity was discovered previously in the supernatant of cells of lines of transformed mouse PTL TC.SC-1.1/1 and TC.SC-1.2/0. The supernatant of these cells (SNC) stimulated growth of inactivated mouse thymocytes, splenocytes, and lymph node cells. It has been shown that the target for the action of SNC in the thymus consists of $PNA^+SC\text{-}1^+L3T4^-$ $Lyt2^-$PTL, which are radioresistant and cortisone-resistant, but sensitive to the action of α_1-thymosin [1, 2, 4].

In this paper we give characteristics of the biological activity of TGF from SNC TC.SC-1.2/0, purified to homogeneity.

EXPERIMENTAL METHOD

Experiments were carried out on CBA mice aged 4-5 weeks (from the "Stolbovaya" Nursery, Academy of Medical Sciences). Activity of TGF was assessed by a test involving stimulation of proliferation of inactivated mouse thymocytes. For this purpose, cells in a concentration of $2 \cdot 10^6$ cells/ml in medium RPMI 1640 with 10% fetal calf serum and dilutions of TGF were cultured in 96-well planchets (0.1 ml suspension per well). On the 4th day of culture, 0.5 μCi of ^{3}H-thymidine was added to each well in the planchets. The contents of the wells were removed after 20 h on to glass fiber filters and their radioactivity determined on a scintillation counter. Activity of the samples was expressed in cpm and activity units (1 unit corresponds to the dose of TGF causing half of the maximal incorporation of label by thymocytes in the test). Separation of the splenocytes into Ig^+- and Ig^--populations was carried out by immunoadsorption on plastic dishes, covered with proteins of the Ig-fraction of antiserum to mouse Ig [3]. During the study of TGF-induced proliferation, monoclonal antibodies of hybridomas 7D4 and 11B11, specific to the receptor for interleukin-2 and to interleukin-4, were used. The hybridomas were generously provided by B. D. Brondz.

EXPERIMENTAL RESULTS

TGF, a glycoprotein with mol. wt. of 22 kD was used after purification to homogeneity by gel-filtration and reversed phase HPLC. The purified TGF stimulated, dose-dependently, growth of thymocytes inactivated by the mitogen. The minimal concentration of TGF stimulating proliferation of thymocytes was 0.002 ng/ml, and the maximal response of the thymocytes was observed with TGF in a concentration of 0.016 ng/ml (Fig. 1). The specific activity of the purified TGF, according to the results of testing on thymocytes, was $2.89 \cdot 10^9$ U/ml. Concanavalin A, in the doses used from 0.5 to 2 μg/ml, and phorbol-13-myristate-12-acetate, in doses of 5 and 10 ng/ml, did not enhance proliferation induced by purified TGF (Table 1).

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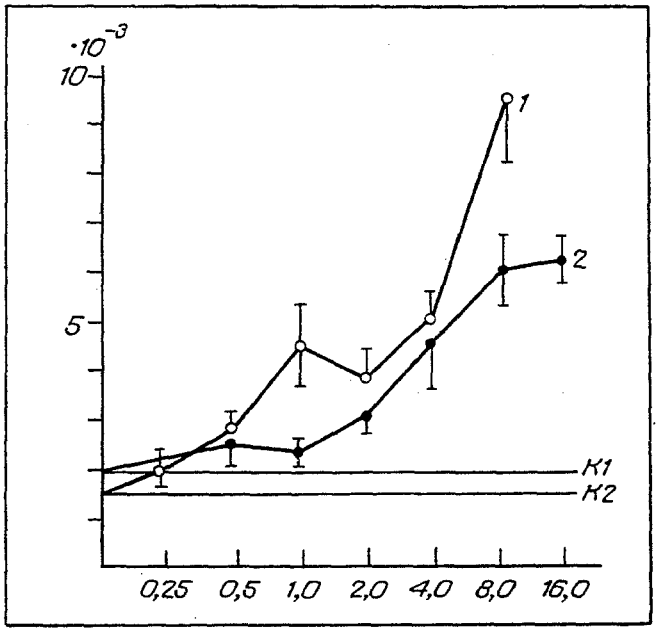

Fig. 1. Dose-dependence of response of thymocytes (1) and splenocytes (2) to purified TGF. Abscissa, TGF concentration (in pg/ml); ordinate, incorporation of ^{3}H-thymidine (in cpm). K1 and K2) levels of spontaneous proliferation for thymocytes and splenocytes respectively.

TABLE 1. Growth-Inducing Action of Purified TGF on Thymocytes and Splenocytes

Growth-inducing action on thymocytes	Activity of samples, cpm	
	TGF	medium
Constimulator	5585±125	1725±21
Concanavalin A, 2 μg/ml	5514±459	3255±231
Concanavalin A, 1 μg/ml	6187±234	2995±154
Phorbol-myristate acetate, 10 ng/ml	5132±143	1234±122
Supernatant of hybridoma 7D4	5934±258	1893±123
Supernatant of hybridoma 11B11	5123±153	1785±136
Growth-inducing action on splenocyte Cells	TGF	Medium
Unseparated splenocytes	1632±123	231±14
Ig^--splenocytes	1512±261	407±32
Ig^+-splenocytes	1259±67	392±28

Purified TGF effectively stimulates growth of inactivated splenocytes: the minimal TGF concentration stimulating splenocyte proliferation was 0.0015 ng/ml, and maximal incorporation of ^{3}H-thymidine was observed with TGF in a concentration of 0.01 ng/ml (Fig. 1). On separation of the splenocytes into Ig^+- and Ig^--populations, cells of both populations responded by proliferation to TGF (Fig. 1). Separation of the cells adherent to plastic did not affect the growth action of TGF.

Purified TGF did not sustain long-term growth and did not stimulate incorporation of the label by cells of the interleukin 2/interleukin 4 (IL-2/IL-4)-dependent CTLL-2 line. Antibodies specific for the receptor for IL-2 and to IL-4 and blocking their functions, did not abolish, in effective concentrations, the growth-inducing response of thymocytes and splenocytes to TGF (Table 1). Thus TGF not only differs from IL-2 and IL-4, but its growth-inducing action, in the test which we used, is not mediated by production and reception of these lymphokines.

The combined growth-inducing action of TGF, partly purified by gel-filtration, and of the IL-2-containing supernatant of EL-4 cells on the thymus cells significantly exceeded (by 7.7 times) the level of summation of the effects of the growth stimulators taken separately (Fig. 2). Antibodies blocking the receptor for IL-2 observed synergism of action of the IL-2-containing supernatant and of TGF. It is interesting to note that the greatest synergic

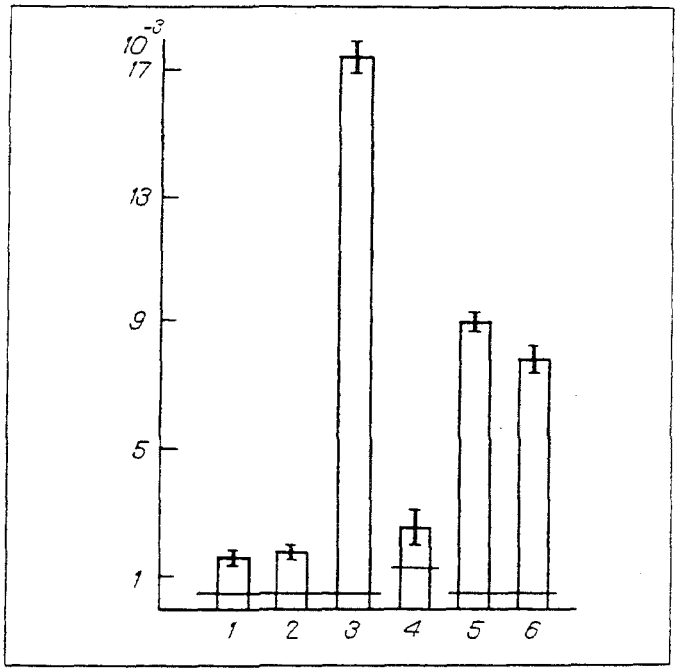

Fig. 2. Synergic growth-inducing action of partly purified TGF and IL-2. 1) Proliferation of thymocytes in response to TGF. 2) Proliferation of thymocytes in response to IL-2-containing supernatant of line EL-4. 3) Combined growth-inducing action of TGF and of IL-2-containing supernatant. 4) Disappearance of combined growth-inducing action by supernatant of hybridoma 7D4. 5) Proliferation of thymocytes in response to original SNC. 6) Combined growth-inducing action of SNC and of IL-2-containing supernatant. Horizontal lines indicate level of cell proliferation without growth stimulators. Ordinate, level of incorporation of label (cpm).

effect was caused by low doses of TGF, stimulating growth of thymocytes only weakly without IL-2. When the original SNC was used instead of the purified TGF the effect of syngergism with IL-2 was not observed, evidently because of the presence of contaminating IL-2 in SNC, masking the effect of the exogenous IL-2. The peak of the growth-inducing action of TGF on thymocytes jointly with IL-2-and without it was observed on the 3rd-4th days after stimulation of the cells.

The character of the growth-inducing action of purified TGF differs from activity of the known cytokines: TGF is able to stimulate growth of mouse lymphoid cells when inactivated by mitogen or by phorbol-myristate acetate; moreover, concanavalin A and phorbol-myristate acetate do not potentiate TGF-induced proliferation. It can be concluded from a general review of the results that purified TGF stimulates dose-dependently growth of non-activated thymocytes and splenocytes (both Ig^+ and Ig^-). The TGF preparation does not contain IL-2 and IL-4 activities, and the growth-inducing action of TGF, in the test which we used, is not mediated through production and reception of these lymphokines, but at the same time, TGF can stimulate growth of thymocytes synergistically with IL-2.

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