PRECURSORS OF T LYMPHOCYTES ARE TARGET CELLS FOR THYMOCYTE GROWTH FACTOR

E. A. Protsak, V. P. Shichkin, and A. A. Yarilin

UDC 612.112.94.017.1.08

KEY WORDS: T lymphocytes; thymocytes; target cells of thymocyte growth factor.

The writers showed previously that cultural supernatants of TC.SC-1/1.1 and TC.SC-1/2.0 cells, which are transformed precursors of mouse T lymphocytes (PTL) [5, 6], contain a factor which induces proliferation of thymocytes, without any preliminary activation, and known as thymocyte growth factor (TGF) [13], TGF has a molecular weight of 30 kD; it does not maintain growth of IL-2- and IL-3-dependent lines. The properties and biological effects of TGF are currently being studied. The aim of this investigation was to identify the target cells of TGF.

EXPERIMENTAL METHOD

Cultural supernatants of TC.SC-1/2.0 cells were the source of TGF. Cells of this line were maintained on complete nutrient medium, namely medium RPMI-1640 ("Amimed") with the addition of 10% embryonic calf serum ("Gibco"), L-glutamine (300 μg/ml), gentamycin (100 μg/ml), glucose (4 mg/ml), and 10 mM HEPES buffer ("Serva"). To obtain the supernatant containing TGF, the cells were suspended in Eagle's medium with the addition of L-glutamine and gentamycin in the above concentrations, and cultured for 24 h at 37°C in a concentration of 10^6 cells/ml. The cells were then sedimented by centrifugation and the supernatant was used. A supernatant of EL-4 cells, stimulated under similar conditions by phorbol myristate acetate (5 ng/ml; "Sigma") for 24 h, was used as the source of IL-2.

Activity of TGF- and IL-2-containing supernatants was assessed on the basis of induction of a proliferative response of unfractionated CBA mouse thymocytes. The thymocytes were cultured in a concentration of 10^6 cells/ml in a volume of 200 μl in 96-well round-bottomed panels (Leningrad Medical Polymers Factory) in the presence of serial dilutions of the test supernatants for 5 days at 37°C in an atmosphere of 5% CO_2 in complete nutrient medium. ^{3}H-Thymidine ($1.8 \cdot 10^6$ Bq) was added to the wells 18 h before the end of culture. The cells were collected on filters by means of a "Titertek" ("Flon") automatic cell harvester and their radioactivity measured on a Mark III β-scintillation counter. Indices of stimulation (IS) were calculated as the ratio of incorporation of the label in the experimental and control samples. Each sample was tested in three repetitions. To separate fractions of thymocytes carrying and not carrying receptors for peanut lectin (PNA^+ and PNA^-), the cells were incubated in a solution of peanut lectin (2 mg/ml), provided by M. D. Lutsik (L'vov Branch, A. V. Palladin Institute of Biochemistry, Academy of Sciences of the Ukrainian SSR), after which they were allowed to stand on a layer of medium RPMI 1640 with 5% embryonic calf serum, and washed with a 0.5 M solution of D-galactose ("Reakhim") and with medium [11]. More than 95% of PNA^+ cells (PNA^+ fraction) were identified in the bottom fraction by the immunoperoxidase method [4], and about 10% of PNA^+ cells in the suspended fraction (PNA^- fraction). Cortisone-resistant thymocytes were isolated from the thymus of CBA mice 2 days after receiving an injection of hydrocortisone ("G. Richter") in a dose of 125 mg/kg body weight. To remove $SC\text{-}1^+$- and $Thy\text{-}1.2^+$-cells, suspensions of thymus or bone marrow cells ($2 \cdot 10^7$ cells/ml) were subjected to mass cytolysis by treatment with rabbit antiserum to SC-1 antigen [7, 10] or monoclonal antibodies to Thy-1.2 antigen, produced by clone G4 [1] respectively in a working dilution, together with guinea pig complement, tested for absence of toxicity. Cytolysis was monitored by staining the cells with 0.1% eosin. To assess the

Gor'kii Institute of Epidemiology and Microbiology, Ministry of Health of the RSFSR. Institute of Immunology, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR I. N. Blokhina.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 4, pp. 462-464 April, 1989. Original article submitted July 22, 1988.

 0007-4888/89/0004-0524$12.50

TABLE 1. Proliferative Response of Thymocytes and Their Fractions to Supernatants Containing TGF and IL-2

Thymocyte fraction	Content of PNA^+ cells, %	Response (IS) to action of supernatants containing	
		TGF	IL-2
Unfractionated thymocytes	86	20,7±2,7	17,2±1,5
Cortisone-resistant thymocytes	3	22,6±4,2	4,9±1,1
PNA^+ thymocytes	96	23,3±0,3	4,8±1,0
PNA^- thymocytes	10	8,1±0,9	25,5±2,4

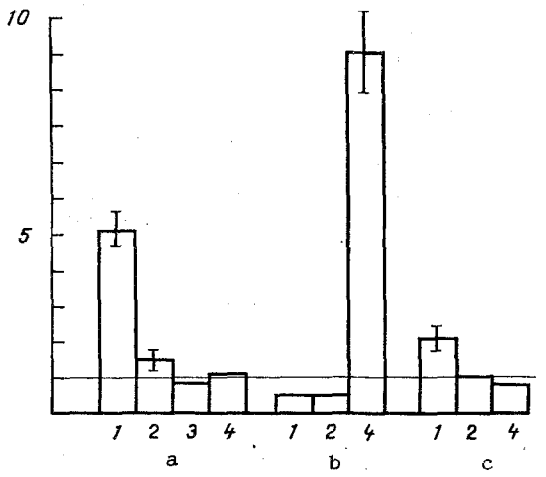

Fig. 1. Effect of removal of cells carrying SC-1 and Thy-1.2 antigens and treatment with thymotropin on proliferative response of mouse thymus and bone marrow cells to the action of TGF. a) Thymocytes; b) CBA mouse bone marrow cells; c) nude mouse bone marrow cells: 1) intact cells; 2) cells treated with anti-SC-1 serum and complement; 3) cells treated with monoclonal anti-Thy-1.2 antibodies and complement; 4) cells treated with thymotropin; ordinate, IS.

effect of the thymus hormonal preparation thymotropin (obtained from the Institute of Blood Substitute and Hormone Technology, Ministry of the Medical Industry of the USSR), this preparation in a concentration of $1 \cdot 10^{-4}$ μg/ml, was added to the test systems for estimation of the proliferative response of the thymocytes to TGF.

EXPERIMENTAL RESULTS

The connection of the target cells of TGF with fractions of cortisone-resistant PNA^+, PNA^-, $SC\text{-}1^+$, and $Thy\text{-}1.2^+$ thymocytes was assessed by noting the proliferative response of the corresponding fractions to the action of TGF in cell culture.

Table 1 shows that unfractionated thymocytes gave a quite high response to the action of both TGF-containing material and the supernatants of activated EL-4 cells, containing several lymphokines including IL-2, which is evidently primarily responsible for induction of the proliferative response. Cortisone-resistant thymocytes completely preserved their ability to respond to TGF, although their response to the IL-2-containing supernatant was much weaker than that of the unfractionated thymocytes. Ability to respond to TGF- and IL-2-containing supernatants was distributed reciprocally in the fractions of PNA^+ and PNA^- thymocytes: PNA^+ cells responded strongly to TGF and weakly to IL-2 and accompanying lymphokines, whereas PNA^- cells, on the contrary, responded weakly to TGF (possibly on account of the contaminating PNA^+ cells) and strongly to IL-2.

At first glance the results appear paradoxical, for we know that the PNA-receptor is associated with cortisone-sensitive thymocytes [11]; this state of affairs is reflected in Table 1: only 3% of cortisone-resistant cells express the PNA-receptor. In the subcapsular zone of the cortex, however, there is a minor PTL fraction, characterized by resistance to the action of cortisol [12] and, at the same, by the presence of PNA receptors [8]. Evidence in support of the small number of target cells of TGF in the thymus is given by the slow development of the proliferative response to its action.

Many PTL in the thymus are known to have SC-1$^+$ Thy-1$^+$ phenotype [3]. To verify the connection between target cells of TGF and PTL, the action of this factor on a suspension of thymocytes from which the SC-1$^+$ or Thy-1.2$^+$ cells had been removed by complement-dependent cytolysis, was therefore assessed. As Fig. 1 shows, such treatment completely abolished the response of the thymus cells to TGF. The targets for TGF in the thymus are thus cortisone-resistant PNA$^+$ SC-1$^+$ Thy-1$^+$ cells, most probably subcapsular PTL.

It follows from these results that more mature forms of thymocytes than PTL, and lacking the SC-1 antigen, do not respond to TGF. If this is so, treatment of thymocytes with thymotropin, a thymus hormone preparation, leading to transition of SC-1$^+$ Thy-1$^+$ cells into the SC-1$^-$ Thy-1$^+$ stage [3], should be accompanied by loss of their ability to respond to TGF. This is in fact so, as will be clear from Fig. 1.

PTL within the thymus arise from PTL of the bone marrow, migrating into the thymus. In bone marrow PTL are present in two stages, one following the other – SC-1$^+$ Thy-1$^-$ and SC-1$^+$ Thy-1$^+$ [2]; the switch from stage to stage evidently takes place under the influence of circulating hormones of thymus origin, for SC-1$^+$ Thy-1$^+$ cells are not present in the bone marrow of nude mice. Estimation of the action of TGF on mouse bone marrow cells gave negative results (Fig. 1). However, their treatment with thymotropin led to acquisition of the ability to give an intensive proliferative response to TGF by bone marrow cells of normal euthymic mice. Cells of nude mice do not acquire this ability. Induction of responsiveness to TGF is evidently possible during the action of thymotropin on bone-marrow SC-1$^+$ Thy-1$^+$, but not SC-1$^+$ Thy-1$^-$ cells. The latter must evidently be subjected to the preliminary action of thymus hormones. The results of this experiment indicate that despite the similarity of their membrane phenotype, SC-1$^+$ Thy-1$^+$ PTL of the bone marrow and thymus differ in their response to TGF and they evidently correspond to different stages of maturation of cells of the T series.

Thus intrathymic PTL are both the source and the target for TGF, i.e., TGF is an autocrine growth factor for these cells.

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