SOME PROPERTIES OF T CELL GROWTH FACTOR OBTAINED FROM HUMAN LYMPHOCYTES STIMULATED BY DIUCIPHON

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UDC 612.112.94:612.6].014.46:615.276.4

KEY WORDS: T cell growth factor, receptors, diuciphon

Data obtained by the writers previously suggested that the immunostimulating activity of diuciphon (Di) is linked with its ability to induce synthesis of T cell growth factor (TCGF) [2]. It was shown that Di, while not inducing lymphocyte proliferation, nevertheless induces the appearance of costimulating activity in the supernatant (SN) of the cells, corresponding to TCGF activity [1]. These data are interesting because cells which have received a signal inducing proliferation are nowadays used as the source of TCGF, which has been identified as interleucin-2 (IL-2) [7]. It can be postulated that, although its biological action is similar to that of IL-2, TCGF obtained from nonproliferating cells differs in its properties from IL-2.

The aim of the present investigation was to study some properties of TCGF obtained from cells stimulated by Di (TCGF-Di).

EXPERIMENTAL METHOD

Human mononuclear cells (MC) were obtained from the peripheral blood of healthy volunteer blood donors by centrifugation on a one-step Ficoll–Urotrast gradient with density of 1.080 g/cm^3. A 2.7% solution of EDTA was used as anticoagulant (1 ml to 10 ml of blood). To obtain the SN containing TCGF-Di, the cells (10^6 in 1 ml) were incubated with Di in a concentration of 10 μg/ml for 6 h at 37°C. In all cases human MC were cultured in complete medium 199 containing 10% embryonic calf serum (from the N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Moscow), 5 mM HEPES (from Sigma, USA), 20 mM glutamine (from Serva, West Germany), and 300 U/ml of gentamicin, at 37°C, 95% humidity, and 5% CO_2. At the end of incubation the cells were washed off with phosphate-buffered physiological saline (PBP) and cultured for a further 24 h in flakes (area of bottom 4.1 cm^2) in a volume of 2 ml and with a density of MC of 10^6 cells/ml. To obtain the SN intended for fractionation complete medium 199 not containing embryonic calf serum was used. The resulting SN was drawn off and frozen at -70°C. When 500 ml of SN had accumulated, the biological activity of the TCGF in it was determined on the basis of the constimulating action of SN on the proliferative response of MC induced by phytohemagglutinin (PHA). For this purpose, the test SN was added to wells in 96-well plastic plates (Falcon, USA), at the rate of 1:1 by volume, and with a cell density of 10^6/ml, after which this system was stimulated by PHA. The response was considered to be positive if the level of proliferation in the culture with SN was significantly higher than in the control. The resulting SN were then subjected to treatment with 80% ammonium sulfate. The residue was centrifuged and rediluted in distilled water, after which dialysis was carried out against physiological saline in the cold. The resulting solution, in a volume of 3 ml, was applied to a column with Sephadex G-100 superfine (from Pharmacia, Sweden), measuring 70 × 1.6 cm. Blue dextran with mol. wt. of 10^6–2 × 10^6 daltons and cytochrome c, with mol. wt. of 12,500 daltons were used as markers. Fractionation took place at the rate of 3 ml/h. Fractions, each 2 ml in volume, were tested in a system of human MC and mouse thymocytes, activated by the mitogen, and also in a system of human MC pre-activated by concanavalin A (con A, from Sigma, USA). For this purpose human MC were stimulated by PHA (from Difco, USA) with a cell density of 10^6 cells/ml, under the conditions above, in plates, in the absence or presence of fractionated material. To obtain thymocytes the thymus from CBA mice was homogenized. The mouse thymocytes were washed three times with PBP. The cells thus obtained were cultured in plates in medium RPMI-1640, containing 10% embryonic calf serum, 5 mM HEPES, 20 mM glutamine,

Laboratory of Cytology, Institute of Immunology, 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. 100, No. 12, pp. 738-740, December, 1985. Original article submitted January 18, 1985.

0007-4888/85/0012-1737$09.50

and 300 U/ml of gentamicin, under the conditions indicated above, with a cell density of 5×10^6 cells/ml in the absence of presence of fractionated material, and with PHA in a concentration of 3 μg/ml, Con A-blast cells were obtained by activation of MC with con A in a concentration of 10 μg/ml for 24 h, followed by washing and further incubation for 72 h (96-h con A blast cells). At the end of culture the cells were again washed and used in the experiments. For this purpose the cell density was adjusted to 10^6 cells/ml, the cells were transplanted into plates, and the level of their proliferation was studied in the absence of the fractions, without additional stimulation by the mitogen. To study adsorption of TCGF, freshly isolated MC were incubated for 4 h in the flasks mentioned above in the absence or presence of SN or of the 49th fraction. The cells were then washed and transferred into plates, where they were activated by PHA.

In all the experiments the cells were cultured in plates for 72 h. Into each well 1 μCi of ^{3}H-thymidine was introduced 4 h before the end of the culture time. Radioactivity was counted on liquid β-scintillation counters.

Ficoll was obtained from Pharmacia, ^{3}H-thymidine from Czechoslovakia, medium RPMI-1640 and ammonium sulfate from Serva, USA, and the EDTA and gentamicin were of Soviet origin.

EXPERIMENTAL RESULTS

Addition of fractions Nos. 41-44 and 47-54 of the SN from MC treated with Di significantly intensified the PHA-induced response of freshly isolated cells from a healthy subject to a suboptimal dose of mitogen. On addition of these same fractions, thymocytes of CBA mice became able to respond to PHA. Fractions Nos. 41-44 and 47-54 also were able to intensify proliferation of 96-h con A blast cells significantly. In other words, in all the systems tested fractions of SN Nos. 41-44 and 47-54 exhibited the biological activity characteristic of IL-2 [7]. The two peaks of biological activity (fractions Nos. 41-44 and 47-54) are evidence that TCGF-Di molecules are probably heterogeneous for molecular weight. The fact that fractions Nos. 47-54, possessing the biological activity of TCGF, were eluted after the marker protein with mol. wt. of 12,500 daltons suggests that some molecules of TCGF-Di have a molecular weight of under 12,500 daltons. Incidentally, IL-2 isolated from cells which have received the signal inducing proliferation, have a molecular weight of over 12,500 daltons [8].

It has been shown [6] that IL-2 is fixed to activated lymphocytes exclusively. It has been shown that receptors appear on lymphocytes 6-18 h after stimulation with mitogen in the G_1 phase of the cell cycle [3, 5]. Accordingly we studied the ability of TCGF-Di, isolated (unlike IL-2) from cells which had not received the signal inducing proliferation, and with a molecular weight probably less than that of IL-2, to undergo adsorption on intact, freshly isolated human lymphocytes. For this purpose intact cells were incubated in medium containing TCGF-Di, and the response of cells incubated in the presence of TCGF-Di and washed, to the mitogen was determined. In parallel experiments the costimulating activity of medium containing TCGF-Di, in which the intact lymphocytes were incubated, was assessed in parallel tests. It was assumed that if the cells adsorbed TCGF-Di their response to the mitogen would be increased, and medium containing TCGF-Di, after incubation of the cells in it, would lose its costimulating activity. Incidentally, in experiments conducted under analogous conditions, activity of IL-2 was removed from SN only by activated lymphocytes [4, 7]. Freshly isolated intact MC from a healthy blood donor, preincubated for 4 h in the presence of fraction No. 49, and then washed, gave a significantly higher response to PHA than in the control. Medium to which fraction No. 49 was added lost its constimulating activity after incubation of lymphocytes in it. The ability of TCGF-Di to be adsorbed on unstimulated MC also was demonstrated in experiments with unfractionated SN. The proliferative response of MC, preincubated in SN and then washed, was shown to be considerably intensified, but SN lost its costimulating activity after incubation of MC in it.

The results of this series of experiments suggest that TCGF-Di, unlike IL-2 isolated from cells which have received the signal inducing proliferation, can be fixed to intact lymphocytes.

Incubation of intact MC with TCGF-Di, incidentally, did not induce proliferation. In other words, fixation of TCGF-Di is probably not a sufficient condition for induction of the proliferative response of intact lymphocytes. At the same time, as Fig. 1c shows, addition of the factor to activated lymphocytes (96-f con A blast cells) significantly increased proliferation of these cells without additional stimulation by the mitogen, i.e., fixation of TCGF-Di to activated cells induces proliferation. Possibly by analogy with other cell recep-

tors (Fcγ, Fc_ε) the receptor for TCGF-Di consists of two sites — fixing and "triggering." Activation of the cells by mitogen facilitates manifestation of the latter. IL-2 can evidently be fixed only to activated receptors for TCGF-Di with a developed "triggering" site. The fact that TCGF-Di can fix itself to intact lymphocytes suggests that TCGF-Di-like substance is constantly present on the membrane of lymphocytes and determines their readiness for proliferation.

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