T cell growth factors from adult T cell leukemia virus-transformed cell lines

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Some characteristics of T cell growth factors derived from adult T cell leukemia virus (ATLV)-transformed cell lines, MT 1 and MT 2 were analyzed. MT 1 cells release significant interleukin 2 (IL 2) activity into the culture medium, which showed the same elution pattern of gel filtration and isoelectric focusing of IL 2 from lectin-stimulated normal human lymphocytes. This activity was also detected in the cell extract of MT 1. In contrast, MT 2 cell line did not produce IL 2 activity, but non-IL 2 type growth factor was observed. The significance of these factors from MT cell lines is discussed from the viewpoint of 'autokine' in ATLV-transformed cells.

ALTV-transformed cell *T cell growth factor*

1. INTRODUCTION

Human T-cell leukemia virus (HTLV) has been isolated from several cases of cutaneous T cell lymphoma in the US [1]. Subsequently, adult T-cell leukemia virus (ATLV) was also isolated from cases of adult T-cell leukemia (ATL) in Japan [2]. It was later shown that HTLV and ATLV have closely related DNA sequences [3].

On the other hand, normal lymphoid cells are stimulated by T cell growth factor (TCGF), which is produced from lectin-stimulated normal human mononuclear cells [4]. TCGF was later designated interleukin 2 (IL 2). Previously, authors in [5] analyzed the production and response of TCGF in HTLV-bearing cell lines and found that one of these cloned cells, HUT 102, produces significant TCGF. However, IL 2 activity cannot yet be detected in ATLV-bearing cell lines [6].

Here, we have analyzed growth factors from ATLV-bearing cell lines, MT 1 [7] and MT 2 [2]. IL 2 activity is produced by the MT 1 cell line and detected in the culture medium and cell extract. However, MT 2 cells do not produce IL 2, but other non-IL 2 type growth factors were observed. The significance of these growth factors from ATLV-transformed cells is discussed.

2. MATERIALS AND METHODS

2.1. *Cell culture and preparation of T cell growth factors*

MT 1 and 2 cell lines were cultured in RPMI 1640 medium (Nissui Seiyaku, Tokyo) supplemented with 10% fetal calf serum (FCS, Gibco). 20 ml of the cell suspension (5×10^7 cells) was centrifuged at $800 \times g$ for 10 min and the culture medium was further centrifuged at 30000 rpm for 80 min in a Beckman SW-50 rotor. The supernatant was recovered, mixed with equal volume of cold saturated ammonium sulphate (pH 7.5) and kept for 30 min at 4°C. After centrifugation at 16000 rpm for 15 min in a Hitachi RP20 rotor, the precipitate was dissolved into 2.5 ml of 10 mM phosphate-buffered saline (PBS) (pH 7.2), applied on a Sephadex G-100 column (1.8 × 24 cm) and eluted with PBS at 2.5 ml per fraction. The active fractions of Sephadex G-100 chromatography were combined and diluted with equal volume of deionized water and applied on an LKB isoelectric

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focusing column with a pH gradient from pH 3.0 to 10.0 by the use of Ampholine (Pharmacia). TCGF from normal human lymphocytes was prepared as in [4]. Human peripheral blood lymphocytes (5×10^7 cells) were washed and incubated in 20 ml of RPMI 1640 medium–10% FCS containing phytohemagglutinin (PHA, Sigma, 10 μg/ml) at 37°C in 5% CO_2/95% air. After 2 days, the cells were removed by centrifugation and the conditioned medium was followed by the same Sephadex G-100 chromatography. Purified human IL 2 was purchased from Electro-Nucleonics (lot no.1464/74, MD). For the preparation of the cell extract of MT 1, MT 1 cells (2×10^8 cells) were frozen at −80°C, thawed at 37°C, mixed with 10 mM phosphate buffer containing 1 M NaCl and kept for 1 h at 0°C. The cell lysate was centrifuged at 16000 rpm for 10 min in a Hitachi RP20 rotor and the supernatant was recovered, which was followed by Sephadex G-100 chromatography as described above.

2.2. *Assay for T cell growth*

Thymocyte DNA synthetic activity was assayed as in [8]. 2.5×10^5 thymocytes of C3/He mice were suspended in 0.1 ml RPMI 1640 medium–5% FCS with concanavalin A (Con A, Sigma, 2.5 μg/ml) in a microtiter well and 0.1 ml of each fraction was added. After incubation at 37°C in 5% CO_2/95% air for 2 days, the cells were labeled with 1 μCi [^{3}H]thymidine (5 Ci/mmol, Radiochemical Center, England) for 1 day and the incorporation of [^{3}H]thymidine into cells was trapped with a GF/C membrane filter (Whatman) with the aid of a cell harvester. Thereafter the membrane was washed with deionized water and ethanol successively. The radioactivity of the membrane was counted with a Beckman scintillation counter.

2.3. *Assay for interleukin 2*

IL 2 activity was assayed by an IL 2-dependent cytotoxic T cell killer clone, 'QE', which was a generous gift from Professor Hamaoka (Osaka University, School of Medicine). 2.5×10^4 cells were suspended in 0.1 ml RPMI 1640 medium–5% FCS and 0.1 ml of test solution was added. After culturing for 18 h, the cells were labeled with 1 μCi [^{3}H]thymidine for 10 h. Incorporated radioactivity was counted as described in the assay for Con A-induced thymocyte DNA synthetic assay.

3. RESULTS

We have studied T cell growth activities in the medium conditioned by the ATLV-transformed leukemia cell line, MT 1. As shown in fig.1, major Con A-induced DNA synthetic activities were observed at the positions of 60–100 kDa and a small amount of activity was detected at about 30 kDa or 10–15 kDa in Sephadex G-100 chromatography (closed circles in fig.1). In contrast, lectin-stimulated peripheral blood lymphocytes (PBL) showed the activities at 60–100, 20 and 10–15 kDa (open circles in fig.1). Since the assay method mentioned above reflects the various T cell growth activities such as interleukin 1 (IL 1) [9] and 2 (IL 2) [4], IL 2 activities in these T cell DNA synthetic activities were analyzed with an IL 2-dependent killer T cell clone. The major IL 2 activities of MT 1 cells were detected at 60–100 kDa and lesser activities were observed at 30 and 10–15 kDa (closed circles in fig.2). Although MT 1 cells showed low activity for IL 2-dependent killer T cell clone compared to

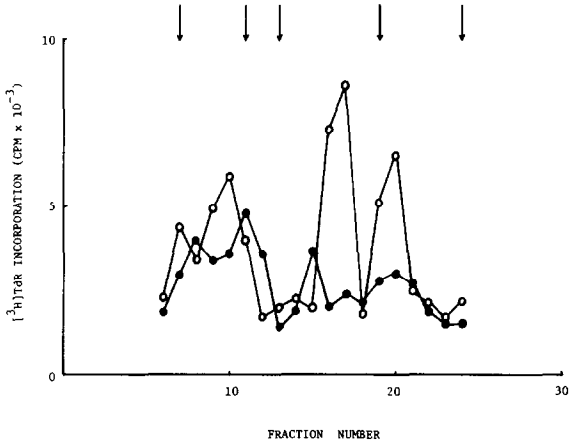

Fig.1. Con A-induced thymocyte DNA synthetic activity in the medium conditioned by MT 1 and lectin-stimulated PBL in Sephadex G-100 chromatography. Sephadex G-100 chromatography was performed as described in section 2. (○, ●) Activities of Con A-induced thymocyte DNA synthesis of lectin-stimulated PBL and MT 1 cells, respectively. Arrows indicate positions of molecular mass markers: blue dextran 2 kDa (void volume), bovine serum albumin (67 kDa), horseradish peroxidase (40 kDa), cytochrome *c* (13 kDa) and bacitracin (1.5 kDa) from left to right.

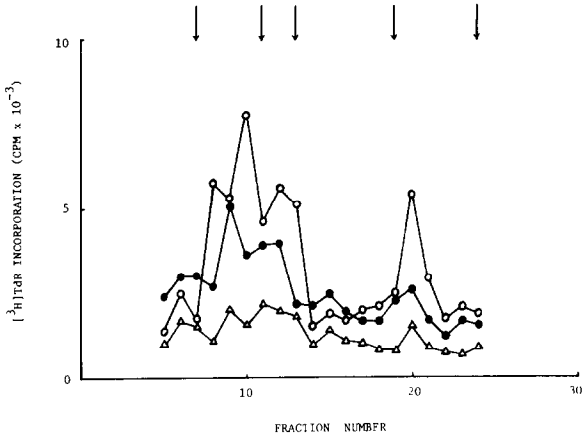

Fig.2. IL 2 activities of the medium conditioned by MT 1 and lectin-stimulated PBL in Sephadex G-100 chromatography. Each fraction of the chromatography was assayed by a killer T cell clone 'QE' as described in section 2. (○, ●) Activities from lectin-stimulated PBL and MT 1 cells. (△) Background activities of RPMI 1640 medium supplemented with 10% FCS. Arrows as in fig.1.

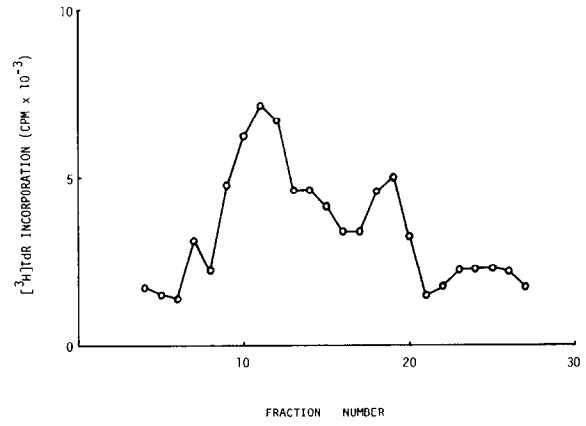

Fig.3. IL 2 activities in the cell extract of MT 1 in Sephadex G-100 chromatography. The cell extract was prepared from MT 1 cells and applied on a Sephadex G-100 column as described in section 2. Arrows as in fig.1.

those of lectin-stimulated PBL, their elution pattern is very similar to that of lectin-stimulated PBL (open and closed circles in fig.1). In addition, since IL 2 activities of MT 1 cells were higher than those of FCS used for cell culture (closed circles and triangles in fig.2), it seems that MT 1 cells produce significant IL 2. On the other hand, it was reported [5] that considerable TCGF activity is localized in a cell membrane fraction of TCGF-producing cells. Then, with respect to the result mentioned above, MT 1 cells were freeze-thawed as described in section 2 and the cell extract was subjected to Sephadex G-100 chromatography. As shown in fig.3, IL 2 activities were detected at similar elution positions of the chromatography to MT 1 and lectin-stimulated PBL in fig.2. In addition, the polymorphism of IL 2 activities in fig.2 and 3 seems to be due to oligomerism of IL 2 molecules as shown in [10].

Next, we compared the charge properties of IL 2 from MT 1 and lectin-stimulated PBL as indicated in fig.4. Both IL 2 exhibited a similar elution pattern in isoelectric focusing chromatography, positioned at p*I* 4.5–5.2, 6.0–6.5 and 7.0–7.5. This result suggests that IL 2 from MT 1 has similar charge properties to those of lectin-stimulated PBL.

On the other hand, MT 2 cells showed a differnt chromatographic pattern. Significant IL 2 activity could not be detected (closed circles in fig.5), but the highest Con A-induced T cell DNA synthetic

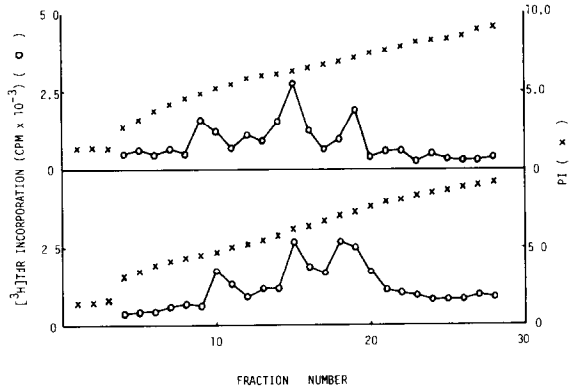

Fig.4. IL 2 activities of MT 1 and lectin-stimulated PBL in isoelectric focusing chromatography. (a) Purified IL 2 from lectin-stimulated PBL was purchased from Electro-Nucleonics. 2 ml of original IL 2 solution and 48 ml of deionized water were mixed and applied on an LKB isoelectric focusing column. (b) The active fractions of Sephadex G-100 chromatography of MT 1 (fig.2) were combined, diluted with equal volume of deionized water (final volume: 50 ml) and applied to the same column.

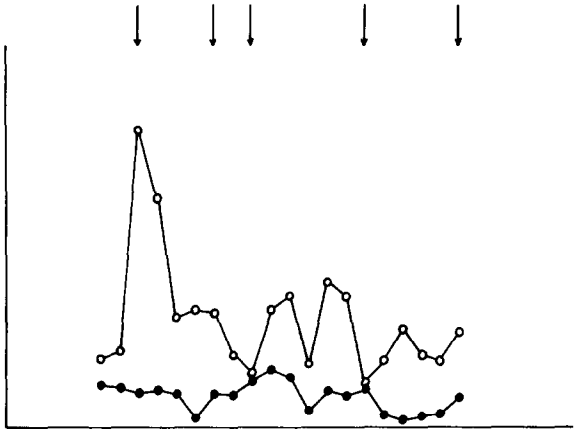

Fig.5. Con A-induced T cell DNA synthetic and IL 2 activities of the conditioned medium of MT 2 in Sephadex G-100 chromatography. The medium conditioned by MT 2 was prepared and applied on a Sephadex G-100 column as described in section 2. (○, ●) Con A-induced T cell DNA synthetic and IL 2 activities, respectively. Arrows as in fig.1.

activity was observed at the void volume of Sephadex G-100 chromatography (open circles in fig.5). These results show that MT 2 cells do not produce IL 2, but that non-IL 2 type growth factors are produced.

4. DISCUSSION

Our results suggest that MT 1 and 2 produce different T cell growth activities. MT 1 cells produce IL 2, which was detected in the culture medium and cell extract (fig.2,3). In contrast, MT 2 did not produce IL 2, but non-IL 2 type growth factor was observed (fig.5). As shown in fig.1 and 2 (closed circles), Con A-induced T cell DNA synthetic activities of MT 1 cells correspond to the positions of IL 2 activities. This suggests that MT 1 selectively produce IL 2. Authors in [11] reported that the HTLV-bearing cell line, HUT 102, releases differently charged IL 2. However, IL 2 from MT 1 has similar charge properties to IL 2 from normal stimulated PBL (fig.4). The reason for such a difference between the present result and that in [11] remains unclear.

However, MT 1 and 2 cell lines can be cultured for a long period in the medium supplemented with high concentrations of FCS (10–20%). However, this culturing fails at lower concentrations of FCS (not shown). In addition, it is known that proliferation of MT cell lines is considerably stimulated by exogenously added IL 2 from lectin-stimulated PBL, which suggests that MT cells have IL 2 receptors [6]. Then, the following possibility can be considered concerning self-proliferation of MT 1 cells. MT 1 cells may proliferate by self-producing IL 2 under a high serum condition. At low serum concentration, MT 1 cells become dormant, which seems to be associated with a decrease in IL 2 production. In this hypothesis, serum-derived helper factor for IL 2 production is postulated.

In contrast, MT 2 cells do not produce significant IL 2, but do release the other strong T cell DNA synthetic activity at the void volume of the Sephadex G-100 chromatography (fig.5). As a possibility, this factor may be responsible for the proliferation of MT 2 cell line.

In future, detailed study of T cell growth factors from MT cell lines may provide clues to the elucidation of the transformation by ATLV.

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REFERENCES

[1] Poiesz, B.J., Ruscetti, F.W., Reitz, M.S., Kalyanaramanan, V.S. and Gallo, R.C. (1981) Nature 294, 268–271.
[2] Miyoshi, I., Kubonishi, I., Yoshimoto, S., Akagi, T., Ohtsuki, Y., Shiraishi, Y., Nagata, K. and Hinuma, Y. (1981) Nature 294, 770–771.
[3] Popovic, M., Reitz, M.S. jr, Sarngadharan, M.G., Robert-Guroff, M., Kalyanaraman, V.S., Nakao, Y., Miyoshi, I., Minowada, J., Yosida, M., Ito, Y. and Gallo, R.C. (1982) 300, 63–66.
[4] Morgan, P., Ruscetti, F. and Gallo, R.C. (1976) Science 193, 1007–1008.
[5] Gootenberg, J.E., Ruscetti, R.W., Mier, J.W., Gazdar, A. and Gallo, R.C. (1981) J. Exp. Med. 154, 1403–1418.

[6] Sugamura, K. (1983) Metabolism (Tokyo) 20, 1567–1573.
[7] Miyoshi, I., Kubonishi, I., Sumida, M., Hiraki, S., Tsubota, T., Kimura, I., Miyamoto, K. and Sato, J. (1980) 71, 155–156.
[8] Okai, Y., Tashiro, H. and Yamashita, U. (1982) FEBS Lett. 142, 93–95.
[9] Mizel, S.B., Oppenheim, J.J. and Rosenstreich, D.L. (1978) J. Immunol. 120, 1497–1503.
[10] Mier, J.W. and Gallo, R.C. (1982) Lymphokines 6, 137–163.
[11] Gootenberg, J.E., Ruscetti, F.W. and Gallo, R.C. (1983) in: Interleukins, Lymphokines and Cytokines (Oppenheim, J.J. and Cohen, S. eds) pp.3–10, Academic Press, New York.