INTERLEUKIN 2 INHIBITS IN VITRO GROWTH OF HUMAN T CELL LINES CARRYING RETROVIRUS

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Human T cell leukemia virus (HTLV), which we previously called adult T cell leukemia virus (ATLV), is a possible causative agent of human adult T cell leukemia (ATL) (1). Since the HTLV genome was demonstrated not to contain a typical v-onc gene (2) and the HTLV proviral genome is integrated into random sites in cellular DNA of leukemia cells in each ATL patient (3), the mechanism of HTLV-induced oncogenesis is still unknown. The interleukin 2 (IL-2) autocrine hypothesis can be excluded as the mechanism of ATL leukemogenesis, because the IL-2 receptor (IL-2R) was detected in all ATL leukemia cells and HTLV-transformed human T cells that were examined (4), but the IL-2 gene was not transcribed in most of these cells (5). However, it was reported (6) that expression of the IL-2R is abnormal in ATL leukemia cells because it is not down-regulated by anti-IL-2R (Tac) antibody, and is constitutive, while IL-2R expression in peripheral blood leukocytes (PBL) stimulated with concanavalin A is down-regulated. This observation resulted in another hypothesis, that the constitutive expression of the IL-2R may play a crucial role in ATL leukemogenesis. Our recent observation (7) that HTLV can induce expression of the IL-2R on human B cell lines, strongly supports the notion that HTLV directly contributes to the constitutive production of the IL-2R on ATL leukemia cells and other HTLV-carrying human T cells. However, it is still unknown how the cell growth signal can be transduced constitutively from the IL-2R expressed on ATL leukemia cells and HTLV-transformed cell lines. Similarly, it has been demonstrated (8) that the A431 human epidermoid carcinoma cell line has a large number of receptors for the epidermal growth factor (EGF) that could be products of the c-erbB gene, a cellular oncogene, and it is suggested (8) that overproduction or uncontrolled expression of the EGF receptors could be related to the appearance of the transformed phenotype in the A431 cell line.

We previously (4) obtained HTLV-carrying human T cell lines of two distinct types with respect to their IL-2 dependency. One type (ILT) is IL-2 dependent; the other (TL) is IL-2 independent for in vitro growth. Most of the IL-2-independent TL cell lines arose spontaneously from IL-2-dependent ILT cell lines in vitro. This change from IL-2 dependence to IL-2 independence could be due to immortalizing transformation of the cells induced by HTLV (9, 10).

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In the present study, to clarify the difference, if any, between the IL-2R in ILT and TL cell lines, we examined whether the TL cell lines could be stimulated further by IL-2. Results showed that in vitro growth of TL cell lines was markedly inhibited by IL-2, suggesting that the IL-2R of TL cell lines mediates directly in transducing the signal for continuous cell growth. The roles of IL-2R in HTLV-induced transformation and leukemogenesis of human T cells are discussed.

Materials and Methods

Cell Lines. The HTLV-carrying cell lines used were established as reported previously (4). Cell lines, TL-Mor, TL-TerI, and TL-Su, were selected for IL-2-independent growth from the IL-2-dependent cell lines ILT-Mor, ILT-TerI, and ILT-Su, respectively. The TL-OmI cell line was also an IL-2-independent cell line established directly from PBL of an ATL patient. TL cell lines were maintained in RPMI 1640 medium supplemented with 20% fetal calf serum (FCS). ILT cell lines were maintained in RPMI 1640 medium supplemented with 20% FCS and 50 U/ml of recombinant IL-2. The other HTLV-carrying T cell lines used were MT-1 (11), MT-2 (9), and TCL-As2 (4). An HTLV-carrying B cell line, LCL-KanCl, was prepared as reported previously (7).

Cell Growth Curves. Samples of $1 \times 10^6$ cells were suspended in 5 ml of appropriate medium in flasks with 7% CO$_2$. Viable cells were counted by dye exclusion staining, and, when the medium was changed every 2 to 3 d by centrifugation, the cell number was readjusted to $1 \times 10^6$ or less in 5 ml of fresh medium. Cell growth curves are represented as total cell numbers of cultures.

IL-2. Recombinant human IL-2 was obtained from Shionogi Co., Japan, and IL-2 prepared from a concanavalin A-treated Jurkat cell culture was obtained from Ajinomoto Co., Japan. Native IL-2 was prepared from the culture fluid of phytohemagglutinin-treated human spleen cells as reported previously (12). IL-2 activities were assayed and units of activity were calculated by the method reported by Suzuki et al. (13).

Antibodies. Mouse monoclonal antibodies H-31 and F10 were used. H-31 (IgG1) antibody is against human IL-2R and can completely block the binding of IL-2 to IL-2R-positive cells. F10 (IgG1) antibody is against HTLV gp21 (14).

Results

Four HTLV-carrying cell lines, TL-Mor, TL-OmI, TL-Su, and TL-TerI, were cultured in the presence or absence of 50 U/ml of recombinant IL-2. Their growth curves are shown in Fig. 1A. Growth of TL-Mor cells was completely inhibited in the IL-2-containing culture after cultivation for 4 d, and no viable cells were detectable on day 10. Growth inhibition of TL-OmI cells was also observed after culture with IL-2 for 7 d. Moreover, the growth rates of TL-Su and TL-TerI cells were lower in the presence than in the absence of IL-2 after cultivation for 5 d. TL-OmI, TL-Su, and TL-TerI cells maintained viability for at least a further 2 wk in the presence of IL-2, although they did not grow. The dose response of the growth inhibitory effect of IL-2 was examined. The growth curves of TL-Mor cells and ILT-Mor cells, which are the parental strain of TL-Mor cells, were measured in the presence of various doses of recombinant IL-2 (Fig. 2A). The proliferation of ILT-Mor cells depended on the dose of IL-2, with the critical dose being 5 U/ml. Growth of TL-Mor cells was markedly inhibited by 5 U/ml IL-2 after cultivation for 6 d, and growth inhibition was

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observed after only 4 d with IL-2 at >50 U/ml. Growth inhibition of TL-TerI cells was also dependent on the IL-2 dose, whereas that of their parental ILT-TerI cells was not affected by IL-2 (Fig. 2 A). >50 U/ml of IL-2 also seems to be effective for growth inhibition of TL-TerI cells. Growth inhibition of TL-Mor cells was also induced by medium containing native IL-2 derived from human spleen cells treated with phytohemagglutinin or Jurkat cells (data not shown). On the other hand, several HTLV-transformed T cell lines tested were resistant to growth inhibition by IL-2. Three HTLV-transformed T cell lines, MT-1, MT-2, and TCL-As2, and an HTLV-carrying B cell line, LCL-KanCl, were cultured in the presence or absence of IL-2, and their growth curves are shown in Fig. 1 B. Up to 500 U/ml of IL-2 did not affect growth of these cell lines.

The specificity of the cell growth inhibition by IL-2 was examined with monoclonal antibody to the IL-2R. Fig. 2 B shows that the inhibition of TL-Mor cell growth in the presence of IL-2 was blocked by addition of anti-IL-2R antibody, H-31, which can inhibit binding of IL-2 to receptors, while anti-gp21 antibody F10, as a control, did not block the growth-inhibiting effect of IL-2. These results indicate that cell growth inhibition is caused by specific binding of IL-2 to the IL-2R on the TL cells.

Discussion

The present study demonstrates that growth of four HTLV-carrying TL cell lines was inhibited in the presence of IL-2. TL-Mor cells seemed to be the most sensitive of these lines to growth inhibition by IL-2. The inhibiting mechanism was based on IL-2 binding to IL-2R on the cell surface, since anti-IL-2R, which completely blocks IL-2 binding to the IL-2R, also prevented the growth-inhibiting effect of IL-2. As three of the TL cell lines were derived from IL-2-dependent
Figure 2. (A) Dose response effects of IL-2 on cell growth. TL-Mor, ILT-Mor, TL-Terl, and ILT-Terl cells were cultured in the presence of 0 (○), 0.5 (●), 5 (▲), 50 (●), or 500 (△) U/ml of IL-2. (B) Effect of anti-IL-2R (H-31) monoclonal antibody on inhibition of growth by IL-2. TL-Mor cells were cultured in medium containing 50 U/ml of IL-2 and 25% of H-31 supernatant (●) or F10 supernatant (○) as a control.

Parental ILT cells by selection for autonomous growth without IL-2, the growth signal is probably continuously transduced from the IL-2R, without IL-2 binding in these TL cells. However, the mechanism of this continuous transduction is unknown. The growth inhibition of TL cells by IL-2 could be due to disturbance of the ability of the IL-2R to transduce the growth signal. On the other hand, we did not observe significant growth inhibition mediated by IL-2 in cultures of HTLV-transformed MT-1, MT-2, and TCL-As2, or of an Epstein-Barr virus-transformed HTLV-carrying B cell line, LCL-KanCl, which also expressed the IL-2R. In those cell lines that are resistant to the growth-inhibiting effect of IL-2, cell growth signals could bypass the IL-2R or could start beyond the IL-2R. Thus, we suppose that there are at least three phases of cell transformation induced by HTLV in vitro. In the first phase, cells infected with HTLV are induced to express the IL-2R, as demonstrated previously (7) in human B cell lines, and they respond to the growth-stimulating effect of IL-2, as in ILT cell lines. In the second phase, cells acquire autonomous growth ability, as in TL cell lines that are sensitive to the growth inhibiting effect of IL-2. In the third phase, cells such as MT-1 become able to proliferate despite the presence of IL-2. We found previously (unpublished data) that ATL leukemia cells can hardly proliferate in the presence of IL-2 during short-term cultivation, and we found by karyotyping that all the IL-2-dependent T cell lines established from PBL of ATL patients during long-term cultivation were derived from normal T cells (unpublished data). Thus, the situation of ATL leukemia cells seems to be similar to that of the TL cell lines in which growth was inhibited by IL-2.

The growth of epidermoid carcinoma cell lines such as A431 is also suppressed by EGF (15, 16), although EGF promoted the growth of various EGF receptor-
bearing primary epidermal cells (17). These observations seem very similar to the present observations on the interaction between IL-2 and TL cells. The mechanism of cell growth inhibition by EGF is not yet fully understood. It has been suggested (18) that the growth-inhibiting effect of EGF on A431 cells results from the large number of EGF receptors that show tyrosine-specific protein kinase activities, because variants of A431, which were resistant to the growth-inhibiting effect of EGF, had fewer EGF receptors and these receptors showed lower EGF-stimulated protein kinase activity than those of the parental A431 cells. It was also reported (19) that EGF at low (picomolar) concentrations, which possibly bind to high affinity EGF receptors, stimulated A431 proliferation, whereas higher (nanomolar) concentrations of EGF inhibited A431 proliferation. However, in the present study, although high concentrations of IL-2 (>50 U) inhibited growth of TL cells, low concentrations (<0.5 U) did not stimulate cell growth. Moreover, we could not detect any significant difference in the affinities or numbers of the IL-2R on HTLV-transformed T cell lines that were sensitive and resistant to the growth-inhibiting effect of IL-2.2 Furthermore, we have not found IL-2R to be associated with protein kinase activity. Thus, we have no critical evidence to explain the inhibiting mechanism of IL-2 on cell growth. Since HTLV-carrying T cell lines such as HUT102 are known to express more IL-2R than normal activated T cells (20), high concentration of IL-2R may be necessary for growth inhibition induced by IL-2 in certain types of HTLV-transformed T cell lines, such as TL. We have obtained a variant of TL cells that became resistant to growth inhibition by IL-2 during cultivation in IL-2-containing medium. These various HTLV-carrying, IL-2R-positive cell lines and their variants should be useful in studies on the mechanism of transduction of the cell growth signal from the IL-2R.

Summary

Four human T cell lines, TL-Mor, TL-Su, TL-TerI, and TL-OmI, carrying human T cell leukemia virus (HTLV), were established previously. TL-Mor, TL-Su, and TL-TerI were derived from interleukin 2 (IL-2)-dependent parental cell lines cloned from peripheral blood leukocytes (PBL) of three healthy HTLV carriers, while TL-OmI was directly established from PBL of a patient with adult T cell leukemia. These four TL cell lines grow autonomously without IL-2. When they were cultured in the presence of IL-2, their growth was inhibited after a few days. This growth inhibition depended on the dose of IL-2, and the effective dose significantly promoted growth of their parental IL-2-dependent cell lines. The growth inhibition is demonstrated to be due to specific binding of IL-2 to receptors on the TL cells.

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