Autocrine Growth of Interleukin 2-Producing Leukemic Cells in a Patient With Adult T Cell Leukemia

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Leukemic cells in the peripheral blood of a patient with adult T cell leukemia (ATL), which expressed the Tac antigen/interleukin 2 (IL 2) receptor, were investigated in vitro for autocrine growth by IL 2. The cells showed spontaneous proliferation in mitogen-free medium. The spontaneous proliferation of the cells was inhibited by monoclonal anti-IL 2 or anti-Tac antibody. These cells were found to produce messenger RNA for IL 2 and secrete IL 2 during short-term culture in the same medium. Recombinant IL 2 and IL 2 secreted by the cells enhanced the proliferation of the cells in a dose-dependent manner when added to the initial culture. These findings demonstrate that an autocrine mechanism by IL 2 is involved in the proliferation of ATL cells during short-term culture.

A N AUTOCRINE MECHANISM was demonstrated in the growth of malignant cell lines in vitro, and it was suggested that this mechanism could play a role in vivo tumor growth. Therefore, interleukin 2 (IL 2), an in vitro growth factor of activated T cell line, is a possible candidate for an in vivo growth factor of some neoplastic T cells. The present study provides the evidence for the autocrine mechanism involved in IL 2, which was revealed in the leukemic cells of a patient with adult T cell leukemia (ATL). To our knowledge, the ATL cells of this patient demonstrate the first case of human leukemic cells that proliferate under this mechanism.

MATERIALS AND METHODS

Patient. A 55-year-old man from Kagoshima was admitted to our hospital with fever and leukocytosis (46,100/mm³) with 62% abnormal lymphocytes possessing lobulated and convoluted nuclei. ATL (ATL-associated) antibody in serum was positive (320 x).

Characterization of the ATL cells. Peripheral blood was obtained from the patient, and mononuclear cells were separated by centrifugation on LSM (Ficoll-sodium diatrizoate) solution (Biotechnics, Kensington, Md). One million cells were stained with monoclonal anti-Leu-1, -2a, -3a antibodies, anti-DR antibody, and anti-Tac antibody by indirect immunofluorescence, and fluorescein isothiocyanate (FITC)-conjugated goat antihuman immunoglobulin (Cappel) by direct immunofluorescence. The human T cell leukemia virus (HTLV) proviral genome was examined by the Southern blotting method with the use of a viral cDNA (U(R)) as a probe (provided by Dr M. Maeda and Dr J. Yodoi of Kyoto University).

Cell culture—IL 2 production and thymidine incorporation. The cells were suspended in RPMI-1640 (Flow Laboratories, Rockville, Md) medium containing 10% fetal calf serum (FCS, Gibco, Grand Island, NY); 5 x 10⁻³ mol/L of 2-mercaptoethanol and antibiotics were placed at 2 x 10⁶/mL in plastic culture dishes (Falcon 3002, Becton Dickinson, Oxnard, Calif) and cultured for varying time periods at 37°C in 5% CO₂. The culture supernatant was harvested daily and IL 2 activity was assayed. The cells were also cultured at 2 x 10⁵/200μL for varying time periods, using 96-well flat bottom tissue culture plates (Costar, Cambridge, Mass) and pulsed with 0.5 μCi per well of ³H-thymidine (Tdr) (6 Ci/mmmol; New England Nuclear, Boston) for the last six hours, and the ³H-Tdr incorporation was measured after harvesting the cells.

Response of ATL cells to IL 2. The cells were cultured at 1 x 10⁵/200μL with varying doses of IL 2 for 24 hours. The cultures were pulsed with 0.2 μCi of ³H-Tdr per well for the last six hours, and ³H-Tdr incorporation was measured. Dr. J. Hamuro of Central Research Laboratories, Ajinomoto Co, kindly donated the human recombinant IL 2. The culture medium of ATL cells containing IL 2 activity was prepared by culturing the ATL cells at 5 x 10⁶/mL in the medium for five days. The sample used contained 56.0 μg/mL of IL 2 activity.

Inhibition of cell proliferation by monoclonal anti-IL 2 and anti-Tac antibodies. The cells were cultured at 5 x 10⁵/200 μL with varying doses of anti-IL 2 (DMS2) or anti-Tac antibody for 72 hours. The cultures were pulsed with 0.2 μCi of ³H-TdR for the last six hours and then harvested.

IL 2 assay. The IL 2 assay was done using an IL 2-dependent mouse T cell line as described previously. One tenth unit per milliliter of IL 2 gave 33% of the maximum response under the culture conditions in our study.

Detection of IL 2 mRNA by the Northern blotting analysis. IL 2 mRNA was detected as described previously. Briefly, RNA was extracted from the ATL cells in the presence of guanidine isothiocyanate, and the glyoxalated RNA was electrophoresed through agarose gel and transferred to nitrocellulose as described. The RNA dot was hybridized with ³²P-labeled IL 2 cDNA (Pst-Stul fragment) as described previously. In control studies, tonsillar cells obtained from a patient with tonsillitis were used.

RESULTS

Characterization of the ATL cells. The cells expressed Leu-1 (96.3%), Leu-3a (86.0%), Tac (79.6%), and DR (69.9%) antigens but not Leu-2a antigen (1.2%) and surface...
immunoglobulin (1.0%). The cells had HTLV proviral genome.

IL 2 production and proliferation of the cells without mitogen. As shown in Fig 1, the \(^{3}H\)-Tdr incorporation of the cells was detected on the first day and reached a maximum on the third day. The IL 2 activity was detected in the medium on the second day and reached a maximum on the fifth day. Decrease of the TdR incorporation after the fifth day is probably due to depletion of the medium because the medium was not renewed during the culture period.

Detection of mRNA. The IL 2 mRNA was detected in the cells harvested on the fourth day of the culture (Fig 2). In control studies, a large amount of IL 2 mRNA was detected in tonsillar cells when stimulated with PHA-M and TPA, but not in tonsillar cells cultured in mitogen-free medium, indicating that normal lymphocytes do not produce detectable amounts of IL 2 mRNA when cultured without stimulation. These results indicate that these ATL cells are producing IL 2 without any mitogenic stimulation.

Response to IL 2. As shown in Fig 3, the proliferation of the cells was enhanced in a dose-dependent manner by both the recombinant IL 2 and IL 2 produced by the patient's own ATL cells. These results suggest that these ATL cells respond to IL 2 produced by themselves during culture.

Inhibition of proliferation by monoclonal anti-IL 2 and anti-Tac antibodies. The proliferation of the cells in mitogen-free medium was inhibited about 85% of the \(^{3}H\)-Tdr incorporation by both anti-IL 2 and anti-Tac antibodies but not by anti-DR antibody used as a control, as shown in Fig 4. These results definitively demonstrate that the in vitro growth of the ATL cells of our patient depends on their production of IL 2, and the proliferation is mediated through the interaction of the IL 2 receptors on the cells with IL 2 molecules.
AUTOCRINE GROWTH OF ATL CELLS BY IL 2

Fig 4. The proliferation of the ATL cells was inhibited by either anti-IL 2 and anti-Tac antibody but not by anti-DR antibody. The ATL cells were cultured at $1 \times 10^7/200 \mu l$ in the medium described in Fig 1 with varying doses of anti-IL 2 (DMS2), anti-Tac, or anti-DR antibody for 72 hours. The cultures were pulsed with 0.2 mCi of $^3H$-Tdr per each well for the last six hours and then harvested. Monoclonal anti-IL 2 antibody used was purified by protein A (Bio-Rad, Richmond, Calif) affinity chromatography from the culture supernatant of DMS2 hybridoma. Anti-Tac and anti-DR antibodies used in this experiment were the ascites produced by hybridoma clones. Data represented means ± SD of triplicate cultures. (A) Effect of anti-IL 2 antibody (DMS2). (B) Effect of anti-Tac (○—○) and anti-DR (○—○) antibodies.

DISCUSSION

ATL is associated with HTLV. However, in contrast to other RNA tumor viruses, HTLV lacks oncogene and the integration sites of the HTLV in ATL cells are not common. This indicates that the transcriptional activation of protooncogene of host cells by the insertion of viral genome at a common site, which appears to work in the case of avian leukosis virus, seems to be unlikely in the case of ATL. On the other hand, it has been proposed from recent tissue culture studies with malignant cell lines that malignant cells respond to growth factors that they produce. The possibility has also been raised that growth factors might be associated with the continuous growth of malignant cells in leukemia. In fact, Gordon et al reported that the Epstein-Barr virus-transformed B cells respond to growth factor that they produce. Duprez et al also recently reported that Tac-positive and RNA virus-negative human T cell line, IARC 301, proliferates by autostimulation, with IL 2 secreted by itself. As ATL cells also possess Tac antigen/IL 2R on the cell surface, it is possible that these cells might continue their growth with the aid of an autostimulation mechanism by IL 2. Arya et al and Yodoi et al suggest that the continuous proliferation of ATL cells may result from IL 2-independent activation of the cells. However, our results support an autocrine mechanism, at least in our patient. It appears that this disagreement represents a heterogeneous leukemogenesis by HTLV, or the leukemogenesis follows the progression model proposed by Gordon et al and Schrader, ie, initial utilization of a growth factor, followed by production of the growth factor in the next stage and the ultimate emancipation from the growth factor of tumor cell growth. In any case, a number of ATL patients should be studied before sweeping conclusions can be reached.

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REFERENCES

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