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A SOLUBLE-FACTOR(S) SECRETED BY A HUMAN SKIN CANCER CELL LINE SUPPORTS CLONAL GROWTH OF ADULT T-CELL LEUKEMIA CELLS

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Leukemic cells from four out of eight patients with adult T-cell leukemia (ATL) were successfully grown by cocultivation with HSC-I cells, a human skin cancer cell line, in the presence of interleukin-2. Three of these four cultures of growing cells showed rearrangement of the T-cell receptor β-chain gene like the original leukemic cells *in vivo*, and also showed conservation of the patterns of HTLV-I integration of the original leukemic cells *in vivo*. Cell-to-cell contact between HSC-I cells and leukemic cells was not necessary for growth of the leukemic cells. The results indicate that some soluble growth factor secreted by HSC-I cells and interleukin-2 are required for the *in vitro* growth of leukemic cells from some patients with adult T-cell leukemia.

Key words: Adult T cell leukemia — Growth factor — Clonality — Human skin cancer cell line

Human T-cell leukemia virus type I (HTLV-I) is closely associated with adult T-cell leukemia/lymphoma (ATL).[1] Although HTLV-I producing cells are known to immortalize normal T-cells when cocultivated with them,[2] little is known about the mechanism of leukemogenesis of ATL or the maintenance of leukemic cell growth *in vivo*.

Many cell lines have been established from peripheral blood or lymph nodes of ATL patients in the presence or absence of interleukin-2 (IL-2), but most of these established cell lines have been found to be different clones from the original leukemic cells *in vivo*.[3,4] These findings prompted us to search for some other factor(s) in addition to IL-2 that facilitated growth of the leukemic cells from ATL *in vivo*.

In patients with ATL, leukemic cells have a helper/inducer T-cell phenotype, and these cells frequently infiltrate the skin. Keratinocytes of mouse skin are known to secrete a soluble factor(s)[5,6] that plays crucial roles in immunological reactions involving T lymphocytes[7] and growth of mouse helper T-cells.[8] Thus, some factor(s) secreted by human keratinocytes in the skin may be involved in the skin lesions in ATL patients. Therefore, we examined whether a human skin cancer cell line provides a favorable environment for proliferation of leukemic cells from patients with ATL. Here, we report that a soluble factor(s) derived from a human skin cancer cell line plus IL-2 could support the *in vitro* growth of the original leukemic cell populations from some ATL patients.

About 10^7 peripheral blood mononuclear cells from each of the 8 patients with acute ATL were co-cultivated in a 25 cm^2 culture flask with subconfluently grown human skin cancer cell line, HSC-I cells,[9] as feeder cells in RPMI 1640 medium supplemented with 20% FCS and 100 U/ml of recombinant IL-2 (Shionogi Co., Osaka). The medium was changed twice a week and the feeder cells were changed when they had grown confluently. After about 2 months, mononuclear cells from 4 patients with ATL grew continuously and in all 4 cases the cell number doubled within 3 days. Moreover, in all 4 cases, the growing cells expressed HTLV-I antigens, detected by enzyme immunoassay by the avidin-biotin-peroxidase complex method. The growing cells had the surface markers of inducer/helper T-cells that are typical of ATL leukemic cells. About 3 months after the start of co-cultivation, the clonality of the growing cells was examined.

In cases 1 and 2, the patterns of the T-cell receptor β-chain gene rearrangement, as revealed by *Eco*RI or *Hin*dIII digestion, and HTLV-I proviral integration, as revealed by *Pst*I digestion, were the same in the growing

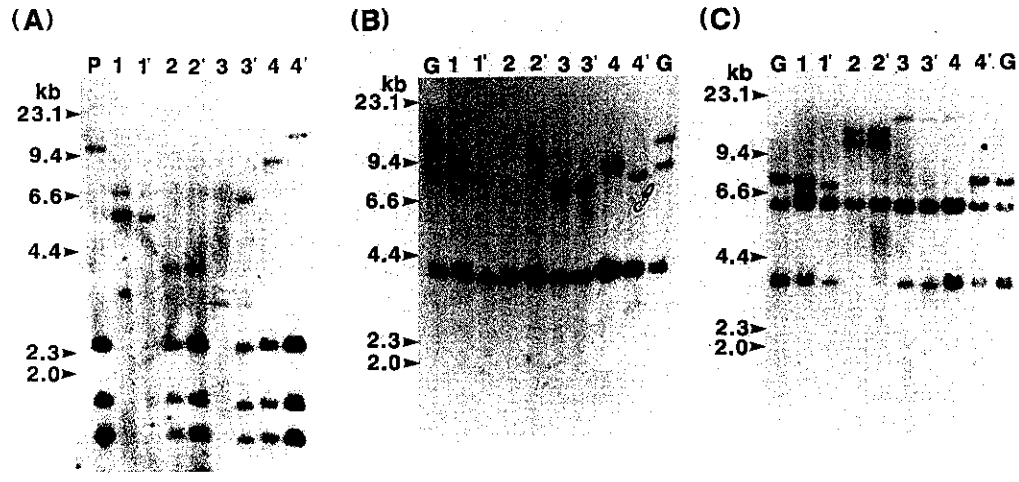

Fig. 1. Patterns of proviral integration and T-cell receptor β-chain gene rearrangement. Mononuclear cells from fresh peripheral blood were separated on a Ficoll-Conray gradient. High-molecular-weight DNAs were extracted by the phenol-$CHCl_3$ method, and samples of 10 μg of DNA were digested with *Pst*I (Fig. 1A), *Eco*RI (Fig. 1B) or *Hin*dIII (Fig. 1C). The fragments were separated by electrophoresis in 1% (A) or 0.8% (B and C) agarose gel, transferred to nylon membrane filters, and hybridized with ^{32}P-labeled probes. (A) Detection of proviral integration site with probes [^{32}P]pATK 32 and [^{32}P]pHT-I(M)3.9.[10] Lane P is the ATL 1K cell line,[11] used as a control, which has one copy of the HTLV-I genome per cell. Lanes 1, 2, 3 and 4 correspond to DNAs of the leukemic cells of cases 1, 2, 3 and 4, respectively. Lanes 1′, 2′, 3′ and 4′ correspond to DNAs of the growing cells of cases 1, 2, 3 and 4, respectively. (B) and (C) Patterns of T-cell receptor β-chain gene rearrangement revealed by digestions with *Eco*RI (B) and *Hin*dIII (C) with a probe containing the constant region of the T-cell receptor β-chain gene.[12] In each panel, G is the germ line pattern of normal human placental DNA. Descriptions of lanes 1, 1′, 2, 2′, 3, 3′, 4 and 4′ are the same as for (A).

cells as in the original leukemic cells *in vivo*, as shown in Fig. 1. Therefore, in cases 1 and 2, these growing cells were concluded to be the same clones as the leukemic cells in the peripheral blood of the respective patients. In case 3, the patterns of T-cell receptor β-chain gene rearrangement of DNAs of the growing cells and leukemic cells were the same, but an additional proviral band was observed in the growing cells. Thus, in case 3, during *in vitro* cultivation the growing cells might have been reinfected by HTLV-I produced by a minor population of cells with the complete provirus. An alternative explanation could be an expansion during cultivation of a minor population of cells, which was the same clone but had the additional proviral band, already present in the patient. In case 4, the patterns of T-cell receptor β-chain gene rearrangement and proviral integration of the growing cells and leukemic cells were different, so the growing cells were probably derived from a different clone from that of the original leukemic cells. In case 4 the growing cells could grow in the presence of IL-2 without HSC-I cells, but in cases 1, 2 and 3, the growing cells required both IL-2 and HSC-I cells.

For examination of whether proliferation of these clones was due to a soluble-factor(s) secreted from HSC-I feeder cells or to cellular interaction between the leukemic cells and HSC-I cells, the growing cells of case 3 were cultured in Transwell (Costar, Cambridge, MA), in which the growing cells in the upper well were separated by a Nucleopore membrane (pore size 0.4 μm) from the HSC-I feeder cells, which were attached to the lower well. Figure 2 shows that the growing cells of

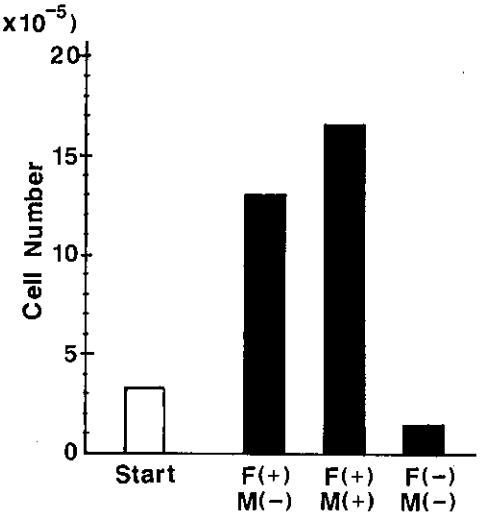

Fig. 2. Support of growth of ATL leukemia cells by a soluble-factor(s) secreted by HSC-I cells. Inocula of 3.3×10^5 growing cells from case 3 were cultured in a 4.7 cm^2 Transwell with a Nucleopore membrane in a 9.6 cm^2 culture dish containing subconfluently grown HSC-I cells in 5 ml of freshly prepared RPMI-1640 medium with 20% FCS and 100 U/ml of recombinant IL-2. In the control dishes, there was no Transwell with or without HSC-I cells. The number of viable cells were counted after 7 days of cultivation. F(−) and F(+) indicate conditions without and with feeder HSC-I cells, respectively. M(−) and M(+) indicate conditions without and with a Nucleopore membrane, respectively.

case 3 could grow equally well in the presence and absence of the separating Nucleopore membrane, but they did not grow at all in the absence of HSC-I cells (Fig. 2) or IL-2 (data not shown). These observations indicate that the growth of the cells is supported by a soluble growth factor(s) secreted by HSC-I cells.

Our experiments suggest that a growth factor(s) produced by the HSC-I cell line is important for supporting *in vivo* proliferation of leukemic cells of some, but not all, ATL patients. Clarification of the nature of this factor(s) with reference to the factors secreted by keratinocytes[5, 6, 8] seems important for understanding the regulation of leukemic cell growth *in vivo*.

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