CONCISE REPORT

The Expression of the p75 Subunit of Interleukin 2 Receptor in Tac Negative Leukemic Cells of Two Patients With Large Granular Lymphocytic Leukemia

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The expression of interleukin 2 receptor[1] (IL-2R) on leukemic cells of natural killer (NK) and T cell lineages in two patients with large granular lymphocytic (LGL) leukemia was examined. The p55 Tac IL-2R was not detected by the indirect immunofluorescence method and it did not participate in the IL-2 binding to the surface of these cells. However, these leukemic cells proliferated in a IL-2 dose-dependent manner and expressed p55. A p75 IL-2 receptor (IL-2-R) subunit was detected on the LGL leukemic cells of both NK and T lineages in a crosslink assay. Thus, it is suggested that the primary signal of IL-2 is mediated by the p75 alone. A study of the inhibitions of the proliferative response of LGL leukemia cells by anti-Tac revealed that both p75 and secondarily induced p55 are required for the cell proliferation.

FOLLOWING OUR previous demonstration[2] that Tac negative leukemic natural killer (NK) cells from a patient with large granular lymphocytic (LGL) leukemia could proliferate and expand their cytotoxic spectrum following stimulation with recombinant interleukin 2 (rIL-2), similar results have also been reported by others.[3-5] However, it is unclear how the IL-2 receptor negative (ie, Tac antigen negative) leukemic NK cells responded to exogeneous IL-2 in vitro.

Data on receptor reconstitution[6] and chemical crosslinking experiments suggest that high affinity IL-2 receptors (IL-2-R) constitute multimeric membrane components, and are themselves composed of at least two polypeptide chains[1,7-9] (p55 and p75), each of which is independently capable of binding IL-2.[7-9] The p55 chain corresponds to the well characterized Tac antigen,[10] while the p75 chain has been demonstrated to be an element required for IL-2 signal transduction.[8]

We examined the IL-2R of Tac negative leukemia cells of two patients with LGL leukemia whose cells showed proliferative response to IL-2 in vitro.

MATERIALS AND METHODS

Patients. The details of patient YS who had NK lineage LGL leukemia were described previously.[2] Patient MH, a 69-year-old male, was transferred to our hospital in May, 1985, due to incidentally identified leukocytosis. His hemoglobin level was 15.5 mg/dL, WBC count was 23,900 cells/μL, platelet count 14.1 $\times$ 10^4/μL, and lymphocyte count, 21,000 cells/μL, most of which were morphologically LGL.

Phenotypic studies and IL-2 response. Peripheral blood mononuclear cells (PBMCs) were isolated from leukapheresis blood by Ficoll-Paque density gradient centrifugation. Cell staining with the monoclonal antibodies OKT3, OKT4, OKT8, OKM1, OKIa1, OKT11 (Ortho Pharmaceutical Corp, NJ), Leu 7, Leu 11 (Becton Dickinson, Mountain View, CA), anti-Tac, and the H-48 monoclonal antibody which recognizes an epitope of p55 distinct from the IL-2 binding site[11] was done by indirect immunofluorescence methods. Cell response to rIL-2 was examined as described previously.[2] Samples of 3 $\times$ 10^5 PBMCs were suspended in 150 μL of 10% fetal calf serum (FCS; Hyclone Laboratories, UT)/RPM1640 (The Research Foundation for Microbial Disease of Osaka University, Osaka, Japan). The suspension was incubated for 48 hours, and pulsed with [^{3}H] thymidine four hours before harvest. Before the experiments, the cells were stored in liquid nitrogen after being suspended in medium containing 50% FCS, 40% RPMI1640, and 10% dimethyl sulfoxide (DMSO; Wako Pure Chemical Industries, Osaka, Japan) and frozen with a Cell Freezer (Planer Biomed, Middlesex, England).

IL-2 Binding and chemical crosslinking. Scatchard plot analysis was done as described previously.[6] The final concentration of anti-Tac ascites in the IL-2 binding inhibition experiments ($\times$ 200) was based on a preliminary study that showed an effective inhibitory effect of ^{125}I-rIL-2 binding to the phytohemagglutinin (PHA) blasts. The IL-2 binding and chemical crosslinking study was performed as described by Sharon et al.[1] The control cells consisted of normal PBMCs isolated and cultured for 72 hours in RPMI 1640 medium containing 10% FCS and 50 μg/mL PHA, washed once with 10% FCS/RPMI 1640 and cultured for a additional two hours at 4°C or at 37°C to deplete the cells of bound endogeneous IL-2. Leukemic cells, thawed and washed three times, and control cells were resuspended at 10^7 cells/mL in RPMI 1640 containing 25 mM Hepes and 1% bovine serum albumin (BSA; Wako Pure Chemical Industries) at pH 7.4. One group of cells was preincubated with an excess amount of rIL-2 (1.2 μM) at 4°C for one hour. The other group was also preincubated at this temperature without rIL-2. Binding was subsequently carried out with ^{125}I-labeled rIL-2 under low affinity condition (5 nM) and cell agitation for one hour at 4°C. Cells were then pelleted at 300 $\times$ g, and resuspended in 10 mL of phosphate-buffered saline (PBS), pH 8.3, containing 1 mM $MgCl_2$. Crosslinking was carried out according to a modification of the method of Brenner et al.[12] The crosslinker, disuccinimidyl suberate (DSS) (Pierce Chemical, IL) was dissolved in 10 mg/mL of DMSO and added to cell suspensions to a final concentration of 100 μg/mL. The cells were agitated at 4°C for 20 minutes and then the reaction was stopped by the addition of 5 mL of 20 mM Tris and 2 mM of ethylendiaminetetraacetic acid disodium salt (EDTA), pH 7.5. Cells were pelleted at 600 $\times$ g, and then lysed and extracted in a mixture of 150 mM NaCl, 10 mM Tris, and 1% NP40, 1 mM phenyl methanesulfonyl fluoride, pH 7.4. The results of the crosslinking

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studies were analyzed by 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

The concentration of anti-Tac ascites for the inhibition experiments of IL-2 induced proliferation was determined by the following methods: normal PHA blasts were stimulated with 10 μg/mL of PHA for 7 days, washed three times, then cultivated for more than 7 days with medium alone to reduce the level of spontaneous proliferation. The final concentration of anti-Tac × 50 ascites was found to be the optimum concentration that effectively inhibited the IL-2 induced proliferation of the PHA blasts.

RESULTS

The surface markers analysis of PBMCs from patients YS and MH revealed that 82% of the PBMCs from patient YS were positive for OKT 11, 81% for Leu 11, 81% for EAγ but 0% for p55 IL-2R Tac antigen. Ninety seven percent of the PBMCs from patient MH were positive for OKT3, 78% for OKT8, and 93% for OKM1, but 0% for EAγ and Tac. Leukemic cells from patient MH showed rearrangements in both T cell receptor β and γ chain genes but cells from patient YS did not (data not shown).

Following stimulation with rIL-2 (7.6 nM) for 48 hours, 8% of leukemic cells from patient MH and 36% from patient YS became Tac positive, but when examined with H-48, 32% and 30% of them were positive for p55, respectively.

Figure 1 shows the IL-2 dose response curve for both types of leukemic cells. Half maximum proliferation was observed at a concentration of approximately 80 pM for both types.

As shown in Figure 2, crosslinking analysis, followed by autoradiography of SDS-PAGE gels, of normal PHA-stimulating blasts preincubated at either 4°C (lane 2) or at 37°C (lane 3) usually revealed two broad bands, in addition to the IL-2, which migrated with a molecular weight of 15.5 kilodalton (kD). The lower band migrated at a molecular weight position of 72 kD corresponding to a summation of the molecular weight of IL-2 (15.5 kD) and the p55 protein. A weak second band with an apparent molecular weight of 85 kD was also detected in the lysates of normal PHA-stimulated blasts, probably corresponding to the p75 subunit of a novel IL-2 receptor. Lysates of the leukemic cells from patients YS (lane 5) and MH (lane 7) contained only this second band, the level of which was significantly higher than that detected in the PHA blasts. Neither the p75 detected in the leukemic cell lysates of either patients, nor the p55 and

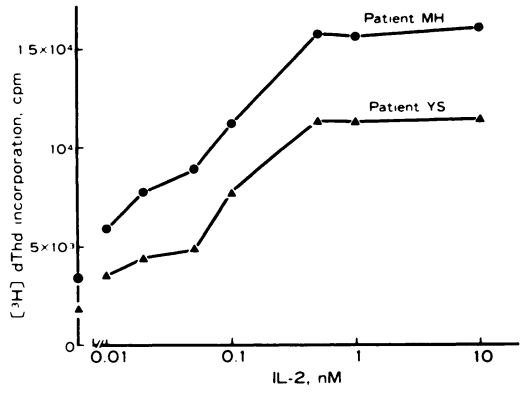

Fig 1. Proliferative response to IL-2 of LGL leukemic cells.

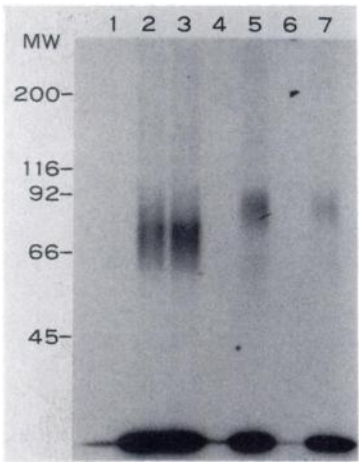

Fig 2. SDS polyacrylamide gel electrophoresis of cells extracts of affinity labeled cells from two patients, and from normal PHA-activated lymphocytes with ^{125}I-labeled IL-2. DSS-mediated crosslinking of PHA-activated normal lymphocytes depleted of endogenous IL-2 from their receptor by preincubation at 4°C for two hours (lane 2) or at 37°C for two hours (lane 3); cells from patient YS (lane 5), and cells from patient MH (lane 7). DSS-mediated crosslinking of PHA-activated cells (lane 1); cells from patient YS (lane 4) and cells from patient MH (lane 6) preincubated with excess unlabeled IL-2.

p75 of the normal PHA-stimulated blasts were detected when cells were pretreated with excess rIL-2 before the addition of ^{125}I-labeled rIL 2 (lanes 1, 4, and 6, respectively) demonstrating that both were involved in the specific binding of IL-2 to its receptors.

Table 1 summarizes the results of Scatchard analysis of the leukemia cells from the two patients. The affinity (Kd) was 6.08 nM in the PBMCs from patient MH and 1.14 nM in those from patient YS, both of which were of low affinity compared to the high affinity sites reported.[13] This difference in the affinities to IL-2 may result in the difference in the density of p75 band of these leukemia cells shown in Fig 2. In contrast, the amount of IL-2 binding sites was almost the same in both of the leukemia cells being about 1,500 molecules/cell.

Figure 3 shows the results of the inhibition of the IL-2 binding to the surface of these leukemic cells by anti-Tac. The results demonstrate that p55 Tac did not participate in the binding of IL-2 to the surface of unstimulated LGL leukemic cells.

The results of the proliferation block by anti-Tac are shown in Fig 4. Anti-Tac markedly inhibited the proliferation of PHA blasts and leukemic cells from patient MH of T cell origin, especially at a low concentration of IL-2. On the

Table 1. Receptor Number and Affinities

Source	^{125}I-IL-2 binding	
	Sites (molecules/cell)	Kd (nM)
MH cells	1,520	6.08
YS cells	1,570	1.14

Binding sites numbers and dissociation constants (Kd) were determined by Scatchard analysis of IL-2 binding data.

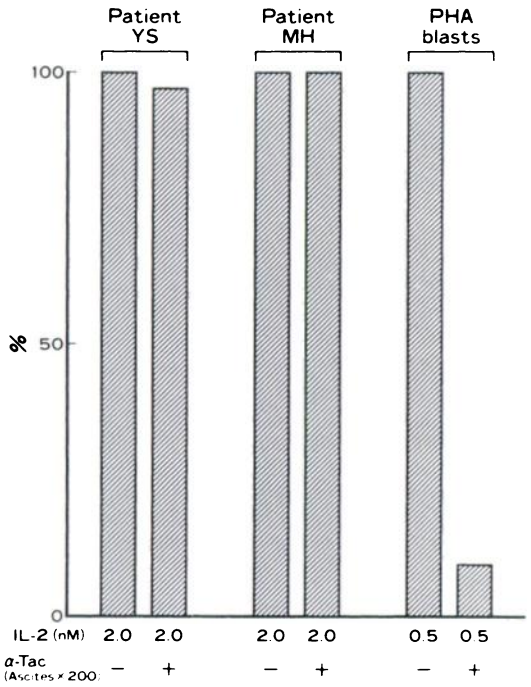

Fig 3. Inhibition by anti-Tac of the IL-2 binding to the surface of LGL cells. The cpm of the ^{125}I-rIL-2 bound to the cells under these different conditions were examined: (A) the cpm of the bound ^{125}I-rIL-2 to the cells (4 × 10^6 per tube) incubated at 37°C for 30 minutes with ^{125}I-rIL-2 in the indicated concentrations in the presence of anti-Tac ascites; (B) the cpm of ^{125}I-rIL-2 bound to cells in the presence of control monoclonal antibody in ascites form (W6/32, Sera-Lab, Sussex, England) with the same isotype but against an irrelevant antigen, major histocompatibility complex, class 1, common framework at the same concentration; and (C) in the presence of 300-fold molar excess of cold rIL-2. The proportion of (A-C/B-C) × 100 (%) was calculated to estimate the specific binding-inhibitory effect of anti-Tac.

other hand, the IL-2 concentration at which anti-Tac caused maximal inhibition was rather high in the leukemic cells from patient YS (500 pM). However, in either patient's leukemic cells, it is possible to conclude that the IL-2 dependent proliferation was blocked by anti-Tac.

DISCUSSION

IL-2 has been reported[2-5] to induce a proliferative response and Tac antigen on LGL leukemia cells. We have extended these observations by identifying the exclusive expression of p75 IL-2R on unstimulated LGL leukemic cells of both NK and T lineages, this expression may mediate the IL-2 signal in the primary p55-independent phase of proliferation. Teshigawara et al[14] and Robb et al[15] independently identified and characterized the p75 binding protein in a NK-like leukemia cell line designated YT.

Robb and Greene demonstrate that the p75 protein is capable of mediating internalization of bound ligand.[8] Unlike p75, p55 is present on activated T and NK cells and is not known to mediate any biological activity or internalization IL-2.[8] The precise role of the p55 in high affinity IL-2R function remains uncertain.

We thus investigated the functional significance of this

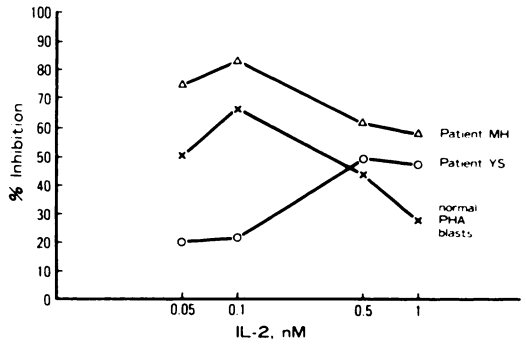

Fig 4. Effects of anti-Tac on IL-2 induced proliferation of LGL leukemia cells. After 30 minutes of incubation with anti-Tac, LGL leukemia cells were stimulated with rIL-2 at the concentrations indicated, in the presence of anti-Tac. Data are expressed as % inhibition of the response obtained in cultures receiving IL-2 alone. W6/32 was used as a control, but had no detectable effects.

induced p55. We evaluated the role of p55 by eliminating the contribution of p55 with anti-Tac. Anti-Tac had a significant inhibitory effect on the IL-2–induced proliferation of the leukemic cells of both patients.

It seems likely that the primary signal of IL-2 is mediated by p75 originally expressed on LGL leukemic cells, but together with the p55 secondarily induced, initiates the proliferation of LGL leukemia cells. It remains unresolved whether the affinities of unstimulated LGL leukemic cells negative with the p55 are regulated by the p75 alone because apparent affinities of both leukemia cells of NK and T cell lineages were different.

Since this manuscript was submitted, Tsudo et al[16] has also reported the expression of p75 on LGL leukemic cells and Siegel et al[17] reported the role of p75 in mediating proliferative signal in normal LGL.

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