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FUNCTIONAL INTERLEUKIN-2 RECEPTOR ON A TAC NEGATIVE HUMAN LEUKAEMIA T-CELL LINE

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Abstract—Cell line PER-423 was derived from the cells of a patient with an immature acute T-lymphoblastic leukaemia and the growth of this human cell line is strictly dependent on interleukin-2 (IL-2). PER-423 cells express the p75 (β) subunit of the IL-2 receptor (IL-2Rβ), while the p55 chain (IL-2Rα) is not detectable by immunofluorescence. The analysis of the IL-2R revealed that it is of intermediate affinity and the median effective IL-2 concentration for PER-423 cells (EC_{50} value) was determined to be 1.44 ± 0.29 nM. Chemical crosslinking studies showed that the receptor consists of one polypeptide of approximately 95 kDa as well as a doublet of 70 kDa and 60 kDa and does not include the IL-2R α-chain. The steady-state mRNA level for the p75 subunit was similar to that present in a cell line expressing an IL-2R$\alpha^+\beta^+$, while only traces for the α-chain were detectable. PER-423 cells can be induced to express the α-chain of the IL-2R on the cell surface, concomitant with a much reduced EC_{50} level.

Since cell line PER-423 is functionally dependent on IL-2, it provides an ideal model for IL-2 signal transduction studies and for investigations focusing on the requirements for ligand binding vs activation.

Key words: Acute lymphoblastic leukaemia, T-cell phenotype, IL-2 receptor complex.

INTRODUCTION

INTERLEUKIN-2 (IL-2) plays a pivotal role in the generation and regulation of the immune response. Antigen activation of mature T-cells results in the synthesis and secretion of IL-2 and surface expression of functional IL-2 receptors (IL-2Rs). Subsequently, binding and endocytosis of IL-2 leads to T-cell growth. High-affinity functional IL-2Rs are composed of at least two distinct subunits, the extensively characterized α subunit of 55,000 Da (p55 or IL-2Rα) and a β subunit of 75,000 Da (p75 or IL-2Rβ), initially identified in ^{125}I-labelled IL-2 crosslinking studies [reviewed in 1, 2]. The p55 subunit lacks a significant intracytoplasmic domain and is nonfunctional in the absence of p75, suggesting that the major function of p55 is in IL-2 binding. In contrast, p75 not only binds IL-2 but is also involved in mediating IL-2 signal transduction in the absence of p55.

cDNA cloning of the human p75 has demonstrated that this protein consists of 525 amino acids with an extracellular region of 214 amino acids, a single hydrophobic transmembrane region and a large cytoplasmic domain of 286 amino acids that most likely functions in IL-2 signal transduction [3]. When this cDNA was expressed in T-cells, the encoded p75 IL-2R bound and internalized IL-2 and formed high-affinity IL-2Rs in the presence of p55 [3]. One unexpected and interesting result from this study was that transfection of p75 in non-lymphoid cells resulted in cell surface expression of this protein that bound anti-p75 monoclonal antibodies but did not bind IL-2. This finding suggests that either p75 is differently modified in lymphoid vs non-lymphoid cells or that it associates functionally with accessory molecules which are preferentially expressed in lymphocytes. A number of candidate molecules that associate with the two known IL-2R chains have been reported [4–7].

The p75 chain appears to be essential for growth signal transduction since it is capable of internalising receptor-bound ligand [8] and induces cytolytic activity in NK cells [9]. However, the biochemical mechanism underlying IL-2 signal transduction remains obscure. Recent studies have suggested that one or more protein tyrosine kinases are activated during the early phase of intracellular signal transduc-

Abbreviations: IL-2, interleukin-2; *IL-2R*, interleukin-2 receptor.

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tion following the binding of IL-2 with its receptor [reviewed in 10], although the cytoplasmic portion of the IL-2Rβ polypeptide lacks an obvious kinase domain [3]. These findings have raised the distinct possibility that additional receptor subunits may be required for signal transduction via the IL-2Rβ chain.

Here we describe an immature T-cell line, PER-423, which is strictly dependent on IL-2 for continuous growth and expresses an IL-2R complex consisting of three chains one of them the well characterized IL-2Rβ chain. Because this line is dependent on IL-2 it provides a model system for the further examination of signal transduction via the IL-2Rβ chain.

MATERIALS AND METHODS

Establishment of cell line PER-423

Cell line PER-423 was established from the leukaemia cells of a five-year old boy diagnosed with T-cell acute lymphoblastic leukaemia. Bone marrow cells obtained at the time of first diagnosis were separated on Ficoll–Hypaque (Pharmacia Fine Chemicals AB, Uppsala, Sweden) and used to establish permanent cell lines by a method described previously [11]. The cells were cultured for three months in RPMI 1640 (Grand Island Biological Company, Grand Island, NY) supplemented with L-glutamine (2 mM final concentration), 2-mercaptoethanol (10^{-5} M), pyruvate (1 mM), non-essential amino acids (Flow Laboratories, Irvine, Scotland) and supplemented with 20% foetal calf serum (FCS, Flow Laboratories). They were subsequently grown in medium supplemented with FCS and recombinant human IL-2 (rIL-2, kindly supplied by Cetus Corporation, Emeryville, U.S.A.). The concentration of rIL-2 in the culture medium was 200 U/ml [4.4 nM]. The cells have been in continuous culture for more than 16 months and were repeatedly shown to be free of *Mycoplasma* using an agar culture technique [12].

Two leukaemia cell lines established from other patients were utilised as controls. Analysis by immunofluorescence showed that both lines belong to the T-cell lineage and that PER-420 cells express the IL-2Rα chain on 25% of cells (using the Tac antibody), while 6% of cells stained with Mik-β1 (IL-2Rβ chain). PER-427 cells express the IL-2Rα chain on 40% of cells, while the IL-2Rβ chain is not detectable by this method. Both lines are dependent on IL-2 for growth in tissue culture. In addition, cell line PER-423-9, derived from the same leukaemia sample as PER-423, was used for comparison. PER-423-9 was established from the same initial culture of the patient's leukaemia cells as gave rise to PER-423. However, PER-423-9 cells were transferred into medium supplemented with IL-2 one month from the start of the culture, while PER-423 cells were transferred after three months (see above). PER-423-9 cells express the IL-2Rα chain on 90% of cells and 9% of cells stain with Mik-β1. PER-423-9 cells are also dependent on IL-2 for growth, however TCR rearrangement studies show they represent a different leukaemic clone from PER-423. The PER-423 line shows the same TCR rearrangements as were present in the dominant leukaemia cells from the patient, with both alleles for TCR β rearranged.

Characterization of PER-423 cells by immunofluorescence

The immunofluorescence assay has been described previously [11]. The analysis was performed on a FACscan II flow cytometer, Becton Dickinson, U.S.A. WT-31 (detecting common determinants on TCRα/β), anti-TCR-γ/δ-1, Leu7 (recognising NK cells) and Leu11 (CD16) were purchased from Becton Dickinson, U.S.A. All OKT and the OKM-1 hybridoma cell lines were obtained from the American Type Culture Collection, Rockville, Maryland, U.S.A. We gratefully acknowledge the gift of the following: mAb RFT-1 (CD5), RFT-12 (CD6), RFAL-1 (CD10) and RFDR-1 (against HLA-DR common determinants) from Dr G. Janossy, London. The anti-Tac antibody (specific for the p55 chain of the IL-2R) was generously provided by Dr T. Waldmann, Bethesda, U.S.A., the Mik-β1 mAb by Dr Tsudo, Tokyo, the HD-237 (CD19) by Dr G. Moldenhauer, Heidelberg; LeoA1 [13] (present on activated T-cells) by Dr G. Burns, Newcastle, Australia and UCHT-1 (CD3) by Dr P. Beverley, London. The cytoplasmic CD3 expression was detected using the UCHT-1 antibody. The characterization of the cell lines by immunofluorescence and the RNA analyses (see below) were performed on cells cultured for the same length of time.

Proliferation response to IL-2

Cells were suspended in culture medium with or without rIL-2 and concentrations from 7 pM to 23 nM rIL-2 were tested. For each concentration triplicate cultures of PER-423 cells (5×10^4 cells in 0.2 ml medium) in round-bottom microtitre wells (Linbro Cat. No. 76-042-05) were incubated for 48 h. ^{3}H-thymidine incorporation (^{3}H-TdR, 0.5 μCi/well, 15–30 Ci/mmol) was determined for a 4 h culture period as previously described [14]. Means of the triplicate cultures were determined, and as standard errors were less than 5% of the mean values they were omitted in the figure.

Cytotoxicity assay

The cytotoxic activity was measured in a standard ^{51}Cr release assay using cell line K562 as target cells. Assays were run at 37°C for 4 h as previously described [15]. PBL were prepared by Ficoll–Hypaque separation.

IL-2 receptor binding assay

Receptor numbers and dissociation constants (K_d) were measured by binding studies using ^{125}I-IL-2 (Cat. No. NEX-229, specific activity 29–38 μCi/μg, from NEN Research Products, Du Pont, Australia) as described previously [15].

Chemical crosslinking

10^7 cells were washed three times in HEPES buffered RPMI medium, with incubations of 30 min between washes. Cells were then incubated with ^{125}I-IL-2 (final concentration 5 nM) at 4°C for 1 h with shaking. Crosslinking was carried out in the presence of 0.5 mM disuccinimidyl suberate (DSS, Pierce, Rockford, U.S.A.) which was dissolved in DMSO and diluted in PBS immediately before use. Cells were extracted in lysis buffer (PBS containing 1% Triton X 100) in the presence of protease inhibitors (1 mM phenylmethyl sulfonyl fluoride and 2×10^{-3} TIU aprotinin) and analysed on SDS-polyacrylamide gels under reducing conditions. Competitive crosslinking experiments were performed by incubating with 500-fold excess of unlabelled IL-2 or anti-Tac mAb.

Isolation and analysis of nucleic acids

Genomic DNA was prepared by standard phenol-chloroform extraction and ethanol precipitation. Digestion with restriction enzymes, electrophoresis and Southern blotting were carried out as previously described [16], except that probes were labelled by random priming and Hybond N+ membranes (Amersham International, U.K.) were utilised. RNA was extracted using the method by Chomczynski and Sacchi 1987 [17]. Samples were denatured by incubation with glyoxal, electrophoresed on 1% agarose gels and transferred to Hybond N+ membrane.

DNA probes and analysis of autoradiographs

The probe for the IL-2Rα chain [18] was a gift from Dr T. Honjo, Kyoto, the probe for the IL-2Rβ chain [3] was kindly provided by Dr M. Hatakeyama, Osaka and a cDNA clone of the TCRβ chain [19] was kindly provided by Dr T. Mak, Toronto. A probe for the rat glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) [20] was utilised to monitor the amount of mRNA on Northern and slot blots. All probes were labelled using the random priming technique [21]. Densitometric scanning of autoradiograms from RNA blot analyses were only performed on autoradiograms in which the band intensity was in the linear range as a function of time of exposure.

TABLE 1. IMMUNOFLUORESCENCE ANALYSIS OF CELL LINE PER-423 AND PATIENT'S LEUKAEMIA CELLS

MAb	CD[a]	% cells staining[b] PER-423	% cells staining[b] Patient's cells
OKT-6	1	<1	15
OKT-11	2	<3 (21)	<1
OKT-3	3	<3	<1
OKT-4	4	<3	<1
RFT-1	5	<1	<1
RFT-12	6	<1	<1
RFT-2	7	93–99	92
OKT-8	8	76–88 (6)	3
Tac	25	<1	<1
Mik β-1		24–32 (1)	ND
WT-31		<1	ND
TCRγ/δ-1		<1	ND
LeoA1		82–93 (2)	ND
RFAL-1	10	<1	<1
OKM-1	11b	9–20 (1)	<1
HD-237	19	<2	<1
RFDR-1		<2	<1

[a] CD cluster of differentiation as defined at the Workshops on Leukocyte Differentiation Antigens.

[b] % cells staining (range determined in four experiments over a four month culture period of PER-423 cells). Background staining with isotype controls was subtracted. In parenthesis is the percentage reactivity after 3 months in culture without IL-2.

RESULTS

Characterization of cell line PER-423

PER-423 cells were initially cultured in medium not supplemented with lymphokines. After three months in culture, cell growth could not be sustained and rIL-2 was added to the medium. The surface markers present on the patient's leukaemia cells and on PER-423 cells are summarized in Table 1. The comparison showed that mAb RFT-2 (CD7) reacted with more than 92% of both cell populations and with high fluorescence intensities (mean fluorescence: 17.6). Fifteen percent of the patient's cells stained weakly with OKT-6 (CD1) (mean fluorescence: 3.4), while no staining was observed on PER-423 cells. Other mAb specific for cells of the T-cell lineage (CD2, CD3, CD4, CD5, CD6) did not react with PER-423 cells cultured in IL-2 supplemented medium. In particular, there was no expression of the CD2 and the Tac antigen on the patient's cells nor on cell line PER-423. Moreover, the cell line was CD3/TCR negative and did not react with the NK cell specific mAb Leu7 and Leu11. Polymorphic HLA-DR determinants and B-lineage antigens were not expressed on either cell population. PER-423 cells showed cytoplasmic CD3 expression, providing further evidence that the line belongs to the T-cell lineage.

The addition of rIL-2 to the medium also resulted in the expression of several surface markers, including OKM1 (CD11b) on 9–20% of cells, OKT8 (CD8) on 76–88% of cells (weak intensity, mean fluorescence: 3.7), LeoA1 (present on activated T-cells) on 82–93% of cells and Mik-β1 on 24–32% of cells. It should be noted that the Tac antigen (CD25) was never detectable on PER-423 over the entire culture period of 16 months. In contrast to the observed upregulation of the four markers listed above, OKT-11 (CD2) stained 21% of PER-423 cells after the initial three months culture period (without IL-2) and did not react with cells at later stages.

In order to examine whether PER-423 cells have the capacity to express the Tac antigen on the cell surface, cells were incubated for 24 h in the presence of increasing concentrations of phorbol myristate acetate (PMA). Upon incubation in the presence of 0.1 ng/ml PMA, 6% of cells expressed the Tac antigen, while 1 ng/ml and 10 ng/ml PMA induced the Tac antigen on 52% and 67% of cells, respectively.

The T-cell phenotype and clonality of PER-423 cells were further confirmed using probes for the TCRβ gene. Southern blot analysis showed that the patient's leukaemia cells and PER-423 cells are monoclonal populations and are identical regarding this gene rearrangement, with both alleles for TCRβ rearranged (not shown). As illustrated in Fig. 1, PER-423 cells show strong MHC-nonrestricted cyto-

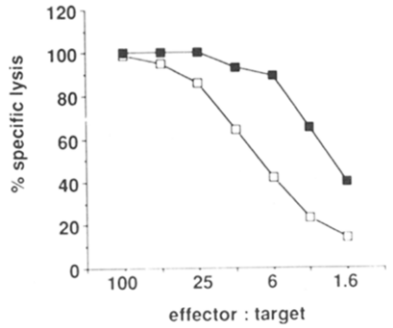

FIG. 1. Cytotoxic activity of PER-423 cells (■) and PBL (□) on K562 target cells. % specific ^{51}Cr release obtained for a range of effector:target ratios is shown.

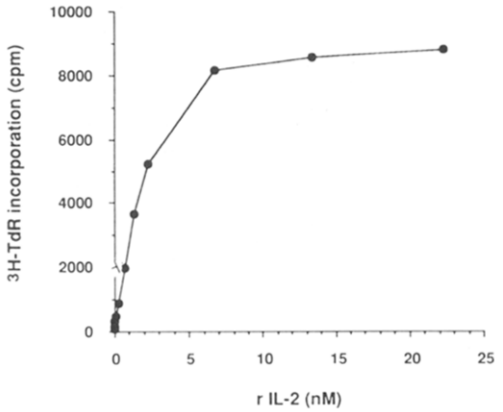

FIG. 2. Proliferation of PER-423 cells in the presence of rIL-2 (7 pM to 23 nM) measured as ^{3}H-TdR incorporation after culturing the cells for 48 h. The ED_{50} value was determined to be 1.44 ± 0.29 nM from four independent experiments.

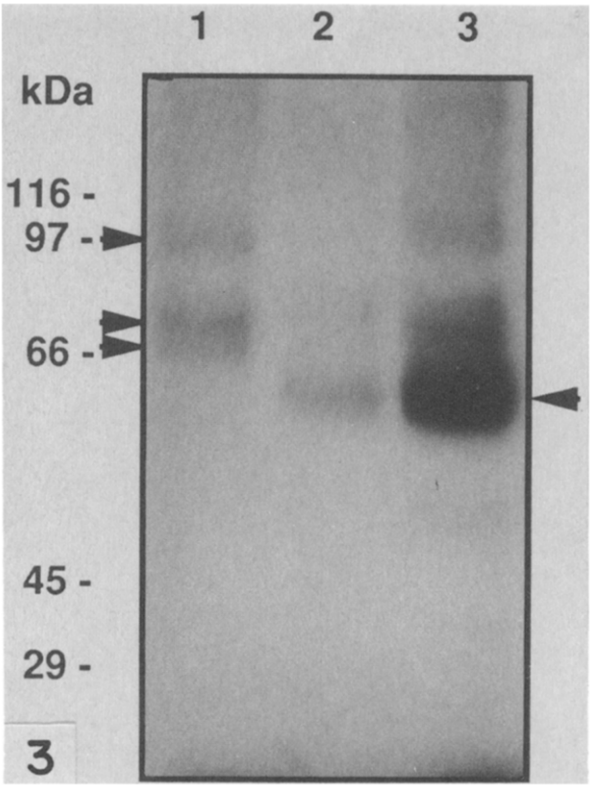

FIG. 3. DSS-mediated crosslinking of ^{125}I-labelled IL-2 (5 nM) using various cell lines. Detergent lysates of crosslinked cells were analysed on a 7.5% SDS-PAGE gel under reducing conditions. Lane 1: PER-423 cells (Tac negative) revealing three chains of approximate sizes 95 kDa, 75 kDa and 60 kDa (arrowheads); lane 2: PER-427 cells (Tac on 40% of cells); lane 3: PER-423-9 (Tac on 90% of cells) showing strong band of approximate size 55 kDa (arrowhead).

toxic activity when assayed on K562 target cells. We were not able to assess the activity of PER-423 cells cultured without IL-2.

Surface expression of the IL-2R on PER-423 cells

Equilibrium binding studies were performed using ^{125}I-IL-2. The Scatchard plots revealed that PER-423 cells express 4100–5300 IL-2 binding sites per cell with a single intermediate affinity (K_d = 2.3 nM) (not shown). The growth dependence of PER-423 cells on IL-2 was measured in proliferation assays carried out over a 48 h culture period (Fig. 2). The median effective IL-2 concentration (EC_{50}) was determined to be 1.44 ± 0.29 nM.

In order to further characterise the IL-2R on PER-423 cells, chemical crosslinking experiments utilising disuccinimidyl suberate (DSS) to covalently link ^{125}I-IL-2 to various cells were performed. These experiments were carried out under intermediate affinity binding conditions. PER-423-9 cells, expressing Tac on 90% of cells, were used as controls and six IL-2 binding polypeptides with approximate sizes of 150 kDa, 95 kDa, 70 kDa, 55 kDa, 32 kDa and 20 kDa were revealed. It should be noted that in this cell line, the band corresponding to the Tac protein is much stronger than the other bands, consistent with the high surface expression of p55 and the low surface expression of the p75 chain (Fig. 3, lane 3). PER-427 cells which express Tac on 40% of cells produced a much weaker band corresponding to the IL-2Rα chain when compared with PER-423-9 (viz. lanes 2 and 3, Fig. 3). In contrast, cell line PER-423 expressed two polypeptides with sizes corresponding to the 95 kDa and 70 kDa chains, detected also in PER-423-9 cells, and in addition a polypeptide chain of approximate size 60 kDa (Fig. 3, lane 1). This smaller size band was detectable in four out of six experiments. These studies confirmed the absence of the IL-2Rα chain on this immature T-cell line, and

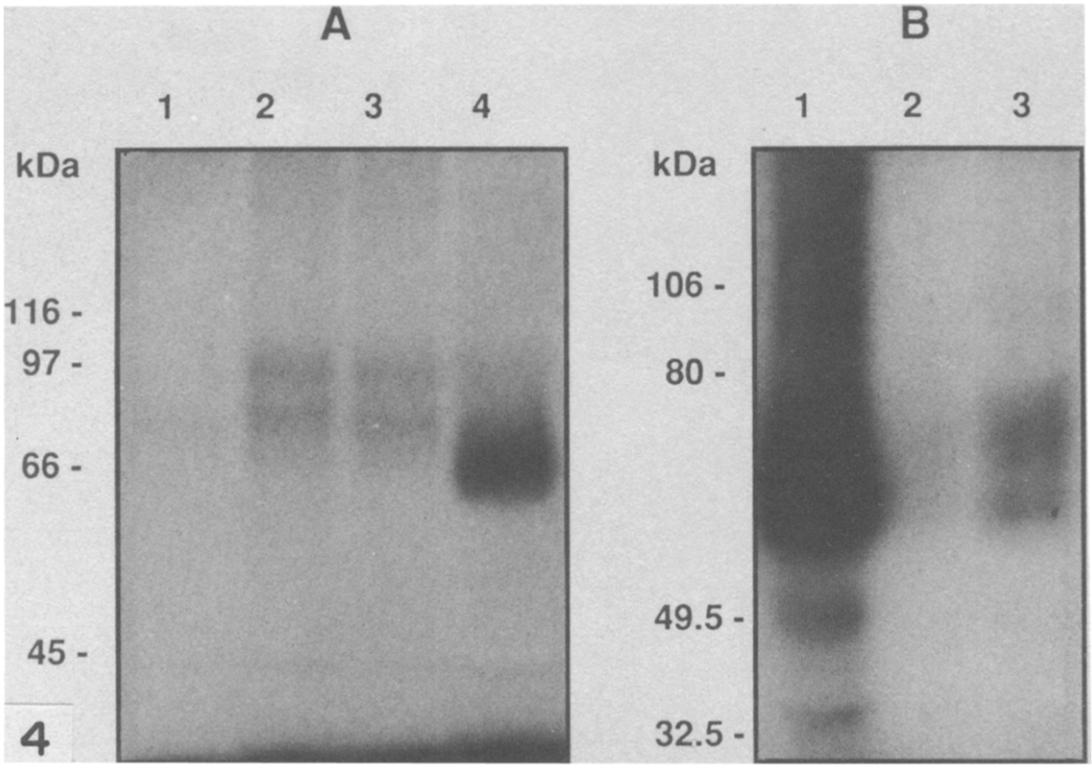

FIG. 4. Inhibition of crosslinking of ^{125}I-labelled IL-2 to PER-423 cells using the DSS-mediated crosslinking technique. Conditions for assay as in Fig. 3. **Panel A**: lane 1: PER-423 cells pre-incubated with unlabelled IL-2; lane 2: PER-423 cells pre-incubated with medium control; lane 3: PER-423 cells pre-incubated with excess Tac antibody; lane 4: PER-423-9 control cells (Tac positive) pre-incubated with medium control. **Panel B**: lane 1: PER-423-9 control cells (Tac positive); lane 2: PER-423-9 cells pre-incubated with unlabelled IL-2; lane 3: PER-423-9 cells pre-incubated with excess Tac antibody.

these findings agree with results from the immunofluorescence analysis.

We next demonstrated that the bands present after crosslinking PER-423 cells to ^{125}I-IL-2 arose from specific binding and crosslinking of IL-2 to IL-2Rs. PER-423 cells were incubated for 1 h with excess unlabelled IL-2 or with the Tac antibody before binding and crosslinking with ^{125}I-IL-2. Incubation with excess unlabelled IL-2 blocked the appearance of the crosslinked species (Fig. 4A, lane 1), while pre-incubation with the Tac antibody did not cause any blocking, confirming that the IL-2R chains on PER-423 cells do not carry the Tac determinants, and that the chains demonstrated by crosslinking do indeed specifically bind to IL-2. The experiment shown in Fig. 4A is an example where the 70 kDa band did not separate into a doublet (see above). Figure 4B shows that binding of IL-2 to the p55 chain on PER-423-9 cells was blocked by the same concentration of Tac antibody.

IL-2Rα and β mRNA levels in PER-423 cells

Southern blot hybridization revealed that the genes for the IL-2Rα and β chains in PER-423 cells are present in the same configuration as reported for normal placental DNA (Fig. 5, Panels A and B) [3, 18]. This finding excludes the possibility that the genes for the known IL-2R chains are present in aberrant form in this cell line of leukaemic origin. Northern blot hybridizations showed that PER-423 cells express IL-2Rβ mRNA, while only traces of IL-2Rα mRNA are present (Fig. 5, Panel C). Panel b in Fig. 5C shows the hybridization using a probe for IL-2Rβ and 4.0 kb bands were detectable in all three cell lines (PER-427, PER-420 and PER-423 in lanes 1, 2 and 3, respectively). In contrast, hybridization with a probe for IL-2Rα revealed strong bands of 3.5 kb and 1.5 kb sizes for PER-427 and PER-420 (both lines are Tac positive), while only a very faint band was present for the Tac negative PER-423 cell line (Panel a).

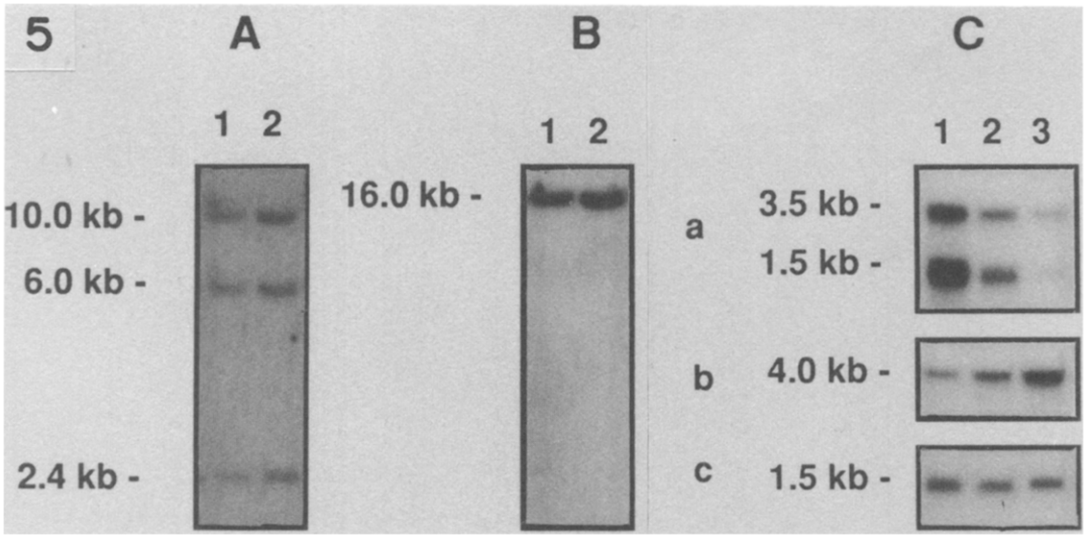

FIG. 5. Southern blot hybridizations and mRNA levels in cell lines analysed with probes for IL-2Rα and IL-2Rβ chains. **Panel A**: DNA samples (5 μg/lane) digested with *Eco* RI, lane 1: PER-420 (Tac on 25% of cells); lane 2: PER-423 and hybridized with probe for IL-2Rα. **Panel B**: Same samples as in panel A hybridized with probe for IL-2Rβ. **Panel C**: mRNA samples (10 μg/lane) from cell lines PER-427 (Tac on 40% of cells) (lane 1), PER-420 (Tac on 25% of cells) (lane 2) and PER-423 (lane 3) hybridized with probes for IL-2Rα (a), for IL-2Rβ (b) and for GAPDH (c).

Densitometric analysis of the results obtained for the three cell lines shown in Fig. 5C were compared with the surface expression of p55 and p75. The comparison of steady-state mRNA levels was carried out by measuring the respective amounts of mRNA for each chain, normalised to the amount of RNA loaded per lane, as determined by hybridization with the probe for GAPDH, shown in Panel c, Fig. 5C. PER-427 cells (Tac: 40%, Mik-β1: 0%), PER-420 cells (Tac: 25%, Mik-β1: 6%) and PER-423 cells gave signals of the expected magnitude, i.e. corresponding to the cell surface expression, when analysed with the two probes for the IL-2R chains. Although faint, the bands detectable for IL-2Rα mRNA in PER-423 cells are of the expected sizes, i.e. 3.5 kb and 1.5 kb, respectively [18]. However, compared to the densitometric reading for PER-427 cells (assigned the value of 100%), the signals in PER-423 cells amount to only 17.5% (average from two experiments). Since Northern blot analysis is not the optimal method for quantitation of mRNA, the steady-state levels of mRNA for the two known chains of the IL-2R were further examined using slot blot analysis (Fig. 6). This method revealed that the mRNA levels for IL-2Rα and IL-2Rβ chains present in PER-423 cells amount to 7% and 86%, respectively, compared again to PER-427 cells (average from two experiments).

DISCUSSION

The results presented here show that PER-423 cells display the unique features of strict dependency on IL-2 for continuous growth and expression of an IL-2R complex of intermediate affinity which is composed of chains of approximate sizes of 95 kDa, 70 kDa and 60 kDa (whereby the latter two did not always separate into two bands). These chains could be shown to specifically bind the IL-2 molecule, while not showing any reactivity with the Tac antibody. The absence of the p55 chain of the IL-2R was demonstrated (i) by immunofluorescence, (ii) by chemical crosslinking experiments and (iii) by competition analysis.

Whether the 95 kDa and the 60 kDa chains are identical with associated molecules (some of them also variously called γ subunit) of the IL-2R described by other authors remains to be determined [4–7]. In four of six crosslinking experiments utilising PER-423 cells, two chains of approximate sizes 70 kDa and 60 kDa were detectable as separate bands. Other investigators have reported that double or triplet

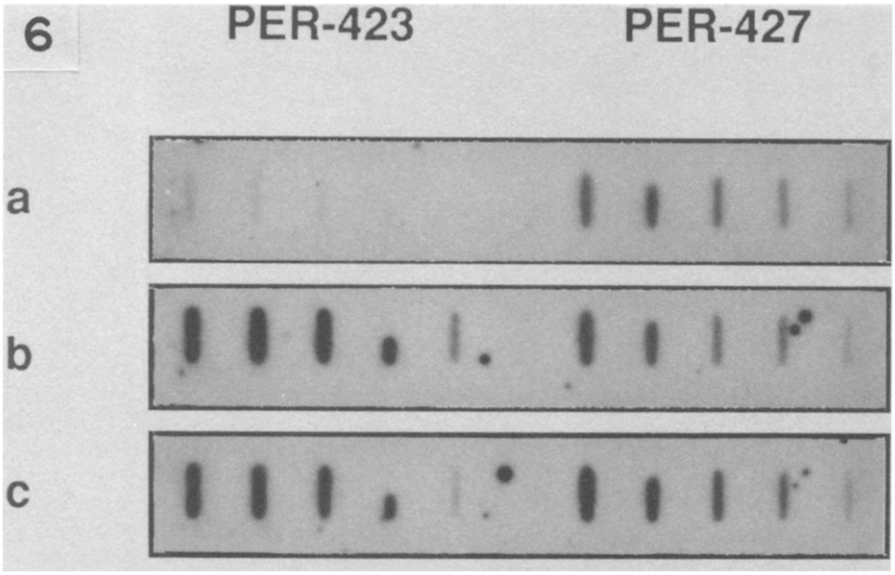

FIG. 6. Quantitation of mRNA for IL-2Rα and IL-2Rβ chains present in PER-423 cells (Tac negative) and PER-427 cells (Tac on 40% of cells) by slot blot analysis. In each panel mRNA samples (5 μg to 0.06 μg in serial three-fold dilutions) were hybridized with the probes for IL-2Rα (a), for IL-2Rβ (b) and for GAPDH (c).

bands were present in this molecular mass range [22–24]. The further elucidation of the composition of the IL-2R on PER-423 may determine whether the IL-2Rβ gene yields products of apparently different sizes or whether the 70 kDa and the 60 kDa polypeptides are two distinct molecules.

The Northern and slot blot analyses clearly showed that PER-423 cells express mRNA for the p55 chain, although at much reduced levels compared with cell lines expressing surface IL-2Rα chain. Despite the continuous presence of IL-2 at 200 U/ml in the culture medium, PER-423 cells do not upregulate the IL-2Rα chain on the cell surface. The IL-2 concentration present in the medium corresponds to 4.4 nM, which is much higher than the EC_{50} concentration of 1.44 nM determined for the cells. However, upon incubation of PER-423 cells in PMA, the cells express the p55 chain on the surface, concomitant with a much reduced EC_{50} level (not shown), indicating that the cells have the capacity to express a functional IL-2Rα chain on the cell surface.

The p75 subunit only is widely expressed among acute leukaemias and T-cell lymphomas, while mature, chronic or well differentiated neoplasms predominantly express both chains of the IL-2R [25, 26]. These findings led to the hypothesis that IL-2 may play a role in the early stages of haematopoiesis. The leukaemia cells which gave rise to cell line PER-423 was of the phenotype CD7+, CD3− CD4− CD8−. Although showing early T-cell markers this phenotype is postulated to represent a syndrome of malignant pluripotent lymphohaemopoietic cells, based on their capacity of multilineage differentiation *in vitro* [27, 28]. Experiments are in progress to determine whether PER-423 can be induced to express markers of nonlymphoid lineages.

The deregulated IL-2R expression found in adult T-cell leukaemia (ATL) have led to the notion that autocrine release of stimulatory factors like IL-2 may play a role in the expansion of leukaemic T cells [29]. Accordingly, the production of regulatory factors by PER-423 cells, both constitutively and upon induction, will be assessed.

The constitutive expression of the p75 chain of the IL-2R occurs on resting T-cells, NK and large granular lymphocytes [2, 3]. Double negative (CD4−, CD8−) foetal thymocytes and some T-cell leukaemias have been reported to display MHC-nonrestricted cytotoxic activity after stimulation with IL-2 and this effect was mediated by the p75 subunit of the IL-2R [2]. It appears likely that the observed cytotoxic activity of PER-423 is due to the same mechanism.

The salient finding reported here is the presence of a *functional* IL-2R not incorporating the p55 subunit, on a CD3−/TCR− cell line. These features set PER-423 cells apart from other lines which are either IL-2 dependent and express both chains of the IL-2R [28, 29] or display exclusively the IL-2Rβ chain, but are not functionally dependent on IL-2 [1, 2, 30].

The only similar line is cell line PER-315 which was derived from the same leukaemia cells as PER-423 [15]. PER-315 have comparable characteristics to PER-423 cells, except that the IL-2 binding affinity is different. The detailed investigation of the IL-R complex reported here for PER-423 cells is not feasible on PER-315 cells since the long term propagation (>1 yr) of these cells could not be achieved.

The absence of the p55 chain of the IL-2R and the functional dependence on IL-2 of cell line PER-423 make it an ideal model for IL-2 signal transduction studies in a TCR negative T-cell line. Although several potential pathways have been described, the physiological consequences of the signalling processes through the IL-2R complex remain unresolved. Investigations to examine the molecular nature of the ligand-receptor interaction will also be feasible, in particular for studies focusing on the question as to whether binding and activation requirements can be separated [31].

Additional note: Since submission of this paper, T. Takeshita *et al.* [32] have reported the cloning of the γ chain of the human IL-2 receptor. The intermediate-affinity receptor appears to consist of $\beta\gamma$ heterodimers.

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REFERENCES

1. Smith K. A. (1988) Interleukin-2: inception, impact, and implications. *Science* **240,** 1169.
2. Waldmann T. A. (1989) The multi-subunit interleukin-2 receptor. *Ann. Rev. Biochem.* **58,** 875.
3. Hatakeyama M., Tsudo M., Minamoto S., Kono T., Doi T., Miyata T., Miyasaka M. & Taniguchi T. (1989) Interleukin-2 receptor β chain gene: generation of three receptor forms by cloned human α and β chain cDNA's. *Science* **244,** 551.
4. Herrmann T. & Diamantstein T. (1988) The high affinity interleukin 2 receptor: evidence for three distinct polypeptide chains comprising the high affinity interleukin 2 receptor. *Molec. Immun.* **25,** 1201.
5. Burton J., Goldman C. K., Rao P., Moos M. & Waldmann T. A. (1990) Association of intercellular adhesion molecule 1 with the multichain high-affinity interleukin 2 receptor. *Proc. natn. Acad. Sci. U.S.A.* **87,** 7329.
6. Colamonici O. R., Neckers L. M. & Rosolen A. (1990) Putative γ-subunit of the IL-2 receptor is detected in low, intermediate and high affinity IL-2 receptor-bearing cells. *J. Immun.* **145,** 155.
7. Takeshita T., Asao H., Suzuki J. & Sugamura K. (1990) An associated molecule, p64, with high-affinity interleukin 2 receptor. *Int. Immun.* **2,** 477.
8. Robb R. J. & Greene W. C. (1987) Internalization of interleukin 2 is mediated by the β chain of the high-affinity interleukin 2 receptor. *J. exp. Med.* **165,** 1201.
9. Phillips J. H., Takeshita T., Sugamura K. & Lanier L. L. (1989) Activation of natural killer cells via the p75 interleukin 2 receptor. *J. exp. Med.* **170,** 291.
10. Mills G. B., Zhang N., Schmandt R., Fung M., Greene W., Mellors A. & Hogg D. (1991) Transmembrane signalling by interleukin 2. *Biochem. Soc. Transactions* **19,** 277.
11. Kees U. R., Ford J., Price P. J., Meyer B. F. & Herrmann R. P. (1987) PER-117: a new human ALL cell line with an immature thymic phenotype. *Leukemia Res.* **11,** 489.
12. Schneider E. L. & Stanbridge E. J. (1975) Comparison of methods for the detection of *Mycoplasma* contamination of cell cultures: a review. *In Vitro* **11,** 20.
13. Burns G. F., Triglia T., Werkmeister J. A., Begley C. G. & Boyd A. W. (1985) TLisA1, a human T-lineage-specific activation antigen involved in the differentiation of cytotoxic T-lymphocytes and anomalous killer cells from their precursors. *J. exp. Med.* **161,** 1063.
14. Kees U. R. (1987) Resistance to 1-β-D-arabinofuranosyl-cytosine after high-dose treatment in childhood lymphoblastic leukemia: isolation of a drug resistant and a sensitive cell line. *Cancer Res.* **47,** 3088.
15. Kees U. R., Ford J., Ranford P. R. & Dawson V. M. (1990) Characterization of an interleukin-2 dependent human leukemic cell line, PER-315, with an immature T-cell phenotype which does not express the Tac antigen. *Leukemia* **4,** 292.
16. Kees U. R. (1988) Phorbol ester induces expression and function of interleukin-2 receptors on a human leukemic cell line with a T-cell precursor phenotype. *Blood* **72,** 1524.
17. Chomczynski P. & Sacchi N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Ann. Biochem.* **162,** 156.
18. Nikaido T., Shimizu A., Ishida N., Sabe H., Teshigawara K., Maeda M., Uchiyama T., Yodoi J. & Honjo T. (1984) Molecular cloning of cDNA encoding human interleukin-2 receptor. *Nature* **311,** 631.
19. Yanagi Y., Yoshikai Y., Leggett K., Clark S. P., Aleksander I. & Mak T. W. (1984) A human T-cell specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains. *Nature* **308,** 145.
20. Fort P., Marty L., Piechaczyk M., Sabrouty S. E., Dani C., Jeanteur P. & Blanchard J. M. (1985) Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate dehydrogenase multigenic family. *Nucleic Acids Res.* **13,** 1431.
21. Feinberg A. P. & Vogelstein B. (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Ann. Biochem.* **132,** 6.
22. Herrmann T. & Diamantstein T. (1988) The human intermediate-affinity interleukin 2 receptor consists of two distinct, partially homologous glycoproteins. *Eur. J. Immun.* **18,** 1051.
23. Dukovich M., Wano Y., Bich-Thuy Le T., Katz P., Cullen B. R., Kehrl J. H. & Greene W. C. (1987) A second human interleukin-2 binding protein that may be a component of high-affinity interleukin-2 receptors. *Nature* **327,** 518.
24. Robb R. J., Rusk C. M., Yodoi J. & Greene W. C. (1987) Interleukin-2 binding molecule distinct from the Tac protein: analysis of its role in formation of high-affinity receptors. *Proc. natn. Acad. Sci. U.S.A.* **84,** 2002.

25. Rosolen A., Nakanishi M., Poplack D. G., Cole D., Quinones R., Reaman G., Trepel J. B., Cotelingam J. D., Sausville E. A., Martin G. E., Jaffe E. S., Neckers L. M. & Colamonici O. R. (1989) Expression of interleukin-2 receptor β subunit in hematopoietic malignancies. *Blood* **73,** 1968.
26. Ichikawa M., Kawai H., Komiyama A., Tsudo M., Miyasaka M., Kinoshita A. & Nakazawa S. (1991) Functional p75 interleukin-2 receptor expression on the fresh blast cells in childhood acute lymphoblastic leukemia with natural killer cell properties. *Am. J. Hemat.* **36,** 259.
27. Kurtzberg J., Waldmann T. A., Davey M. P., Bigner S. H., Moore J. O., Hershfield M. S. & Haynes B. F. (1989) $CD7^+$, $CD4^-$, $CD8^-$ acute leukemia: a syndrome of malignant pluripotent lymphohematopoietic cells. *Blood* **73,** 381.
28. Santoli D., O'Connor R., Cesano A., Phillips P., Colt T. L., Lange B., Clark S. C. & Rovera G. (1990) Synergistic and antagonistic effects of IL-1α and IL-4, respectively, on the IL-2-dependent growth of a T-cell receptor-$\gamma\delta$+ human T-leukemia cell line. *J. Immun.* **144,** 4703.
29. Yodoi J. & Uchiyama T. (1986) IL-2 receptor dysfunction and adult T-cell leukemia. *Immun. Rev.* **92,** 135.
30. Starkebaum G., Loughran T. P., Waters C. A. & Ruscetti F. W. (1991) Establishment of an IL-2 independent, human T-cell line possessing only the p70 IL-2 receptor. *Int. J. Cancer* **49,** 246.
31. Zurawski S. M., Imler J. & Zurawski G. (1990) Partial agonist/antagonist mouse interleukin-2 proteins indicate that a third component of the receptor complex functions in signal transduction. *EMBO J.* **9,** 3899.
32. Takeshita T., Asao H., Ohtani K., Ishii N., Kumaki S., Tanaka N., Munakata H., Nakamura M. & Sugamura K. (1992) Cloning of the γ chain of the human IL-2 receptor. *Science* **257,** 379.