Phorbol Ester Induces Expression and Function of Interleukin-2 Receptors on a Human Leukemic Cell Line With a T-Cell Precursor Phenotype

By Ursula R. Kees

We show here that a human leukemic cell line, PER-117, bearing the markers of a T-cell precursor phenotype, can be induced to express receptors for interleukin-2 (IL-2). These IL-2 receptors could be demonstrated to mediate a physiologic response to the lymphokine for which the high-affinity form of the IL-2 receptor appears to be essential. The phenotype of PER-117 cells corresponds to the earliest identifiable stage of T-cell differentiation, which is defined by the lack of the T3-T-cell receptor complex and the presence of the 40 Kd protein recognized by monoclonal antibodies of the CD7 group. Further evidence for the clonality and T-cell lineage of this cell line was obtained by analysis of rearrangements of genes for the T-cell receptor (TCR) β chain and for the immunoglobulin heavy-chain (IgH) genes. PER-117 cells could be shown to have rearranged TCRβ genes but no rearrangement of the IgH genes. Cell line PER-117 provides a model to investigate the requirements for induction of IL-2 receptors in a cell expressing the first T-cell-specific marker and may help to elucidate the role of IL-2 during thymic differentiation and in the uncontrolled proliferation of T-cell leukemias.

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MATERIALS AND METHODS

Cell lines. Cell line PER-117 was established from bone marrow cells of an 18-month-old boy with acute lymphoblastic leukemia. Surface marker analysis of the subline used in this study revealed the following phenotype: RFB-1*, 3A1* (CD7), OKT9*, OKT10* and HLA-DR*. The cells did not react with monoclonal antibodies belonging to CD1, CD6, CD8, and CD25 (IL-2 receptor). The cells are maintained in RPMI 1640 medium supplemented with L-glutamine (2 mmol/L final concentration), 2-mercaptoethanol (10⁻³ mol/L), pyruvate (1 mmol/L), nonessential amino acids (Flow Laboratories, Scotland) and 10% heat-inactivated fetal calf serum (FCS) (Flow Laboratories). Cells are cultured in a humidified 37°C incubator containing 5% CO₂ in air. The IL-2-dependent cell line CTLL was maintained under the same conditions.

Southern blot hybridization. DNA was isolated from PER-117 and peripheral blood mononuclear cells by standard phenol-chloroform extraction and ethanol precipitation. Twelve micrograms of DNA from each sample was digested overnight with restriction enzymes suitable to differentiate TCRβ and IgH gene rearrangements from germline configuration (EcoRI, HindIII, BamHI). The DNA fragments were separated by electrophoresis on 0.8% agarose gels and the DNA was transferred to a nylon membrane (Zeta Probe, Bio Rad Laboratories, Australia) by the method of Southern. Hybridization was carried out using 32P-labeled nick translated probes. After washings at high stringency, membranes were exposed to Fuji x-ray film for two to four days at −70°C with intensifying screens. Rearrangements of the TCRβ chain was detected using a cDNA clone of the TCRβ chain containing 770 base pairs cloned into the PstI site of pBR-322 (kindly provided by Dr T. Mak, University of Toronto). The immunoglobulin (Ig) gene configuration was determined using an IgH probe consisting of a 2.4 kilobase Sau 3A fragment (kindly provided by Dr P. Leder, Harvard Medical School, Boston).

Induction experiments. PER-117 cells were incubated for 24 hours at 37°C at 2 × 10⁶ cells/mL in the presence of various concentrations of phorbol 12-myristate 12-acetate (PMA; Sigma, St Louis) and phytohaemagglutinin (PHA; Wellcome Laboratories, Sydney, Australia) in culture medium supplemented with 5% FCS. Cells were washed three times and then subjected to further analysis.

Proliferation assay. PER-117 cells were suspended in culture medium supplemented with 10% FCS with or without recombinant IL-2 (Batch LP-315, kindly supplied by the Cetus Corporation, CA). A concentration of 1 Cetus U/mL of the preparation equals 22.2 pmol/L. Quadruplicate cultures of cells in round-bottom microtitre wells (Linbro, No. 76-042-05) contained 5 × 10⁶ cells in 0.2 mL culture medium. ³H-thymidine incorporation (0.5 μCi/well, 15 to 30 Ci/mmol) was determined for a 17-hour culture period or was added

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17 hours before the termination of cultures incubated for a total of 65 hours (at 37°C).

**Immunofluorescence.** Cells were incubated for 30 minutes at 4°C with saturating concentrations of the anti-Tac antibody (a kind gift from Dr T. Waldmann, National Institutes of Health) or other antibodies, washed, and then incubated with biotin-coupled sheep anti-mouse immunoglobulin (30 minutes at 4°C). After further washing the cells were incubated with fluorescein isothiocyanate-conjugated streptavidin (products no. 1001 and 1232 from Amer sham, Sydney, Australia) for 30 minutes at 4°C. Samples were analyzed on a Cytofluorograph (Model 50-H; Ortho Diagnostic System, Raritan, NJ). Cell populations were gated according to light scatter to exclude dead and aggregated cells.

**Radiolabeling and immune precipitations.** PER-117 cells induced by PMA or uninduced were separated on Ficoll-Hypaque (Pharmacia Fine Chemicals AB, Uppsala, Sweden) and cell surface iodinations were performed as described by Knowles. After lysis of labeled cells in detergent lysis buffer, cellular debris was removed by centrifugation, and the clarified supernatants were preclared with fixed *Staphylococcus aureus* Cowan Strain I (Commonwealth Serum Laboratory, Melbourne, Australia). Precipitation with anti-Tac antibody or normal mouse serum was then carried out overnight at 4°C, followed by *S aureus* (one hour at 4°C). Proteins (reduced) were separated on sodium dodecyl sulfate (SDS) polyacrylamide slab gels using the buffer system of Laemmli.

**RESULTS**

A recently established human acute lymphoblastic leukemia cell line, PER-117, was used in this study. The surface marker analysis reveals that this cell line expresses the RFB-I and OKT10 antigens, both found on immature hematopoietic cells in the bone marrow and on cortical thymocytes. PER-117 cells react strongly with three monoclonal antibodies of the CD7 group (RFT-2, 3A1, WT1), which selectively react with cells of the T lineage. No staining was obtained with the monoclonal antibodies OKT3, OKT4, OKT6, OKT8, and OKT11, nor was the intracellular marker terminal deoxynucleotidyl transferase (TdT) detectable. This pattern of surface markers defines a leukemic cell population with the phenotype of an immature T-cell precursor, similar to the recently described DU.528 cell line and HSB-2 cells.

In order to further characterize PER-117 cells, Southern blot analysis was used to investigate the IgJ and TCRβ chain configuration. DNA from PER-117 cells was digested with restriction enzymes EcoRI, HindIII, and BamHI and hybridization was performed with probes for the TCRβ chain and IgJ. The analysis showed that the IgJ genes are in germline configuration, while the TCRβ chain genes were found to be rearranged in PER-117 cells (Fig 1). Digestion of DNA with the enzyme BamHI revealed two fragments indicating that both alleles are rearranged and that the leukemic cells are monoclonal with respect to TCRβ gene rearrangement. Phorbol esters, including PMA, which activate protein kinase C are capable of inducing, in leukemic cell lines, cellular responses that may reflect normal differentiation steps. Initial experiments examined the effect of PMA on PER-117 cells. 2 x 10⁶ cells/mL were incubated for 24 hours in the presence of 10 ng/mL PMA. The cells were subsequently analyzed for the expression of T-cell markers using a panel of monoclonal antibodies that covered all CD specificities of the T lineage, including the anti-Tac antibody. The immunofluorescence tests revealed a striking change in staining with the anti-Tac antibody after PMA induction: 78% to 96% of PER-117 cells expressed the antigen, which was not detectable on uninduced cells (Fig 2). In all experiments, cells induced by PMA and stained with anti-Tac antibodies displayed a broad spectrum of fluorescence intensities, indicating a varying degree of receptor expression.

PER-117 cells were subsequently incubated with various PMA concentrations, ranging from 0.01 to 10 ng/mL. In order to determine whether the T-cell mitogen PHA has an additional effect, these experiments were carried out in the presence or absence of PHA. The results from a representative experiment (Table 1) show that PMA concentrations of 1 ng/mL or higher induced the IL-2 receptor on a majority of the cells. PHA (at 1 μg/mL) does not increase the IL-2 receptor expression nor does it induce IL-2 receptor expression by itself or at suboptimal PMA concentrations (≤0.1 ng/mL). The time course of Tac expression after induction

![Fig 1. Southern blot analysis of DNA samples from PER-117 cells (labeled 117) and control cells (labeled gl). DNA was analyzed as described in Materials and Methods. The probes and restriction enzymes used are indicated above each autoradiograph. The sizes in kilobases of the germline fragments are indicated (dash marks). Arrows indicate rearrangements in PER-117 cells.](www.bloodjournal.org)
with 1 ng/mL PMA shows it was clearly detected after an incubation time of six hours, at which time 21% of cells were staining with the antibody (Fig 3). The maximal number of cells expressing Tac was reached after 15 hours.

Tac induction was further characterized by cell surface labeling, immune precipitation, and analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The results of these immunoprecipitations are shown in Fig 4. As expected, no precipitate is formed using lysates from uninduced PER-117 cells incubated with anti-Tac antibody (Fig 4, lane 1). In contrast, lysates from PMA-induced PER-117 cells incubated with the anti-Tac precipitated a protein with a molecular weight of 55,000 daltons (Fig 4, lane 2), which is the molecular weight reported for the Tac antigen isolated from lymphocytes activated with PHA.2

To establish whether PER-117 cells induced by PMA express functionally competent IL-2 receptors, such cells were incubated in medium containing 4 or 20 U/mL of recombinant human IL-2. The proliferation of PER-117 cells to IL-2 was determined after a 65-hour incubation and is summarized in Table 2. A 24-hour induction period in the presence of PMA without addition of IL-2 caused a marked inhibition of the DNA proliferation (measured over 17 hours), amounting to 16.6%, 18.9%, and 9.6% of the control cultures in medium, for experiments 1, 2, and 3, respectively, an effect that has been reported by other investigators.26

**Table 1. The Proportions of IL-2 Receptor-Positive PER-117 Cells on Induction With PMA and PHA**

<table>
<thead>
<tr>
<th>Stimulant in Culture*</th>
<th>Tac-positive Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PMA</td>
</tr>
<tr>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>10 ng/mL</td>
<td>None</td>
</tr>
<tr>
<td>1 ng/mL</td>
<td>None</td>
</tr>
<tr>
<td>0.1 ng/mL</td>
<td>None</td>
</tr>
<tr>
<td>None</td>
<td>1 µg/mL</td>
</tr>
<tr>
<td>10 ng/mL</td>
<td>1 µg/mL</td>
</tr>
<tr>
<td>1 ng/mL</td>
<td>1 µg/mL</td>
</tr>
<tr>
<td>0.1 ng/mL</td>
<td>1 µg/mL</td>
</tr>
</tbody>
</table>

*PER-117 cells were incubated for 24 hours in the presence of the indicated stimulators.

Control cultures (not induced) incubated in the presence of recombinant IL-2 showed a proliferation rate comparable with cells incubated in medium not containing IL-2. In contrast, PER-117 cells induced by PMA showed 2.7- to 14.3-fold higher proliferation rates in the presence of 20 U/mL recombinant IL-2 (experiments 3 and 1, Table 2) over medium control. It could be demonstrated that IL-2 concentrations of 4 U/mL were sufficient to obtain these proliferation rates. CTLL cells incubated for 65 hours in the presence of 20 U/mL recombinant IL-2 showed proliferation rates of 17.2- to 29.6-fold above medium controls. These results clearly show that PER-117 cells were induced to express functionally competent IL-2 receptors.

**DISCUSSION**

The results presented here show that a leukemic cell line of T-cell precursor phenotype can be induced to express functionally competent receptors for IL-2. It was previously
reported that the acute lymphoblastic T cell lines Jurkat and HSB-2 can be induced to express the Tac antigen.\(^{10}\) In contrast to these two cell lines, PER-I \(_{17}\) cells can be induced to express functionally competent IL-2 receptors. In addition, Jurkat cells represent mature thymocytes and HSB-2 cells have the features of the immature cortical thymocyte, according to the subdivision proposed by Furley et al.\(^{27}\) In contrast to these two cell lines, PER-\(_{17}\) cells belong to an earlier compartment of the T-cell lineage and correspond to the earliest identifiable stage of T cell differentiation, the precursor cortical thymocyte cell, which is defined by the lack of the T3-T-cell receptor complex and the presence of the 40,000 dalton transmembrane phosphoglycoprotein recognized by monoclonal antibodies of the CD7 group.\(^{30,21,22}\) Furthermore, PER-\(_{17}\) cells do not express the 55,000 dalton TL-like glycoprotein recognized by antibodies of the CD1 group, which is present on immature cortical thymocytes. Since this antigen can be detected on HSB-2 cells (our own observations),\(^{28}\) these cells correspond to the immature cortical thymocyte cell.

The analysis of the TCR and Ig\(_\gamma\) rearrangements in PER-\(_{17}\) cells confirmed that the cell line belongs to the T-cell lineage and is monoclonal. Thus, PER-\(_{17}\) cells correspond to the earliest developmental compartment for the T-cell lineage and have rearranged TCR\(_\beta\) genes, which is in agreement with published observations.\(^{27}\)

Our studies on the requirements for IL-2 receptor induction on PER-\(_{17}\) cells show that PMA concentrations of 1 ng/mL or higher induce the IL-2 receptors on a majority of cells. IL-2 has been shown to induce the expression of IL-2 receptors in vitro on human thymocytes\(^{30}\); however, IL-2 alone does not trigger the expression of the IL-2 receptor on PER-\(_{17}\) cells (data not shown). It is conceivable that other lymphokines or a combination of factors are required. Our finding that PHA does not increase the IL-2 receptor expression on PER-\(_{17}\) cells is in agreement with the observation that PHA activates Jurkat cells at least in part, by binding to the T3-T-cell receptor complex.\(^{30}\) PER-\(_{17}\) cells do not express the T3 antigen nor do they stain with the antibody WT31 (unpublished results), which recognizes common determinants on the T-cell receptor.\(^{31}\)

The proliferation responses of PER-\(_{17}\) cells induced by PMA showed considerable variation between experiments (Table 2). It should be noted that it has not been possible to clone the PER-\(_{17}\) cell line and it is conceivable that PER-\(_{17}\) cells contain subpopulations that preferentially express IL-2 receptors of high or low affinity. The interaction of IL-2 with the high-affinity receptors on activated T cells has been directly correlated with the physiologic response of these cells to IL-2.\(^{32,33}\) Furthermore, it has been determined that high-affinity saturable binding occurs at IL-2 concentrations <1 nmol/L. The proliferation responses of PER-\(_{17}\) cells reported here were detectable at IL-2 concentrations in the picomolar range (88.9 to 444 pmol/L), which is consistent with the expression of high-affinity IL-2 receptors on PER-\(_{17}\) cells. It has been reported recently that high-affinity IL-2 receptors consist of at least two subunits, the Tac antigen, 55,000 daltons, and a second subunit of 75,000 daltons.\(^{24}\) Further analysis revealed that internalization of IL-2 can be mediated by the 75 Kd chain alone or by the heterodimeric high-affinity IL-2 receptor.\(^{24}\) It is thus possible that in individual experiments a variable proportion of PER-\(_{17}\) cells that could be triggered to express the elements necessary for ligand internalization was present. Successful cloning of PER-\(_{17}\) cells may help to resolve this issue in conjunction with direct binding assays.

The induction of IL-2 receptors by PMA is not restricted to leukemic cell lines and can be found in fresh leukemic T cells.\(^{33}\) Thus, the possibility that IL-2 contributes to the uncontrolled proliferation of T-cell leukemias in vivo must be considered, particularly since on induction with PMA, PER-\(_{17}\) cells secrete small amounts of IL-2 (data not shown). Similarly, it is not known whether the constitutive expression of IL-2 receptors on ATL cells plays a key role in the initial stages of leukemogenesis or whether it is involved in the continued proliferation of leukemic cells.\(^{7}\)

The observation that leukemic cells of T-cell precursor phenotype can express functionally competent IL-2 receptors raises the question whether the normal counterpart of this cell line has the same capacity. A small population of lymphoblasts in the human thymus has been found to express IL-2 receptors, without in vitro activation.\(^{36,37}\) Hofman et al recently reported that fetal thymocytes express the Tac antigen,\(^{38}\) which was detectable as early as 12 weeks gestation. In addition, IL-2 has been shown to be produced by 17-week and 21-week human fetal thymocytes.\(^{39}\) These results indicate that normal fetal thymocytes, corresponding in phenotype to the leukemic cell line PER-\(_{17}\), appear to secrete IL-2 and express the receptor for this lymphokine. Whether the IL-2 receptor is expressed at particular stages

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**Table 2. Proliferative Response of PMA-induced PER-117 Cells in the Presence of Recombinant IL-2**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Stimulant in Culture*</th>
<th>17-h Culture</th>
<th>65-h Culture</th>
<th>65-h Culture</th>
<th>65-h Culture</th>
<th>65-h Culture</th>
<th>65-h Culture</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No IL-2</td>
<td>No IL-2</td>
<td>IL-2 4 U/mL†</td>
<td>IL-2 20 U/mL†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>16,388 ± 855</td>
<td>33,738 ± 2,137</td>
<td>ND</td>
<td>38,031 ± 4,546</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>PMA</td>
<td>2,720 ± 186</td>
<td>2,145 ± 130</td>
<td>ND</td>
<td>30,575 ± 3,842</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>13,695 ± 570</td>
<td>18,007 ± 2,328</td>
<td>21,521 ± 1,648</td>
<td>21,352 ± 2,267</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*PER-117 cells were incubated for 24 hours in the presence of PMA (1 ng/mL) or in medium alone.
†Mean cpm ± SEM of quadruplicate samples.
‡Purified recombinant IL-2 from Cetus Corporation.

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of differentiation without prior stimulation or whether antigenic triggering by self antigens is required is at present not known. The appearance of the IL-2 receptor at this developmental stage might be related to the self-nonself discrimination that occurs in the thymus. Thus, PER-117 cells may provide a convenient experimental system to further elucidate the regulation of IL-2 receptor expression at an early stage of T-cell development.

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Phorbol ester induces expression and function of interleukin-2 receptors on a human leukemic cell line with a T-cell precursor phenotype

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