Acute Lymphoblastic Leukemia and Non-Hodgkin’s Lymphoma of T Lineage: Colony-Forming Cells Retain Growth Factor (Interleukin 2) Dependence

By Ivo Touw, Ruud Delwel, George van Zanen, and Bob Löwenberg

The regulatory role of interleukin 2 (IL 2) in the proliferation of T acute lymphoblastic leukemia (T-ALL) and T non-Hodgkin’s lymphoma (T-NHL) cells from six individual patients was analyzed in a colony culture system where pure recombinant IL 2, and the lectin phytohemagglutinin (PHA) or the phorbol ester 12-O-tetradecanoyl phorbol-13-acetate (TPA), had been added. The proliferative response was correlated with the inducibility of receptors for IL 2 on the surface membrane of T-ALL and T-NHL cells by incubation with TPA or PHA for 18 hours. Leukemic T cell colonies, identified by immunophenotyping or cytogenetic analysis, appeared in vitro following TPA and IL 2 stimulation in all six cases. Accordingly, receptors for IL 2, initially absent from the cell surface, were found on high proportions of the T-ALL and T-NHL cells after in vitro exposure to TPA. In contrast, colony formation stimulated by PHA and the induction of IL 2 receptors by PHA were limited to the one case of T-NHL with the mature thymocyte immunophenotype. The cells from the other patients, expressing common or prothymocyte phenotypes, did not respond to PHA. No colonies were formed in any of these cases when PHA or TPA was withheld from the IL 2-containing cultures. Although colony growth depended absolutely on exogenous IL 2 in three cases (ALL), in the other three cases (one ALL, two NHL) some colonies grew also when no IL 2 had been added to the culture. Upon further analysis of the cells of one of the latter patients, it was found that the cells produced IL 2 and proliferated in response to this endogenous IL 2. The results from this study indicate that the requirements of endogenous IL 2 and IL 2 receptor activation by PHA and TPA vary from patient to patient. In addition, they support the notion that T-ALL and T-NHL cells have not lost dependence on IL 2 and IL 2 receptor activation for in vitro growth.

Attempts at understanding the underlying mechanisms of tumor growth are concerned with the possibility that neoplasms are disturbed in their response to growth factors. Their uncontrolled outgrowth may be caused by an altered sensitivity to growth regulators (eg, as a result of aberrant expression or function of membrane receptors) or to the acquired ability to self-produce factors. These phenomena have been described for a variety of tumors and in a number of cases were found to correlate with the expression of oncogenes. Alternatively, proliferation of tumor cells may become completely independent of normal growth regulators.

The availability of interleukin 2 (IL 2) gene probes, pure recombinant IL 2 preparations, and monoclonal antibodies (MoAb) reactive with cell surface membrane receptors for IL 2 allows for the analysis of the growth requirements of human T cell leukemias. These approaches have been pursued mainly in leukemias of mature T cell type, in particular, those associated with the human T cell lymphotropic virus I (HTLV I). Until now, however, scarce information was available on the control of proliferation by IL 2 in immature T cell neoplasms, ie, T acute lymphoblastic leukemia (T-ALL) and certain T cell non-Hodgkin’s lymphomas (T-NHL).

In the present study, we have examined T-ALL and T-NHL cells from six individual patients for their proliferative response to IL 2. We applied a colony culture technique in which pure recombinant IL 2 (r-IL 2) was added as the single growth factor. The lectin phytohemagglutinin (PHA) or the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) was supplemented simultaneously for activation of the cells.

Our findings indicate that distinct patterns of IL 2 and activation requirements can be recognized in individual cases of T-ALL and T-NHL. However, like normal T cells, the leukemia cells remain dependent on PHA or PHA activation and IL 2 for proliferation. The proliferating neoplastic cells do not acquire new surface antigens indicative of maturation to later cell stages, suggesting that their capacity to differentiate in vitro is very limited. We conclude that proliferation of T-ALL and T-NHL cells cannot be simply explained by the loss of control by IL 2 or by the aberrant (ie, independent of activation) expression of membrane receptors for IL 2.

Materials and Methods

Patient data and immunologic phenotypes of leukemic T cells. Selected clinical and immunologic data of the patients entered into this study are summarized in Table 1. Patients with prothymocyte-like (stage I) leukemic cells (TdT+, WT1+, E+/-, T3-, T4-, T6-, T8-) had been diagnosed cytohistopathologically as cases of T-ALL (patients 1 to 3) and patients carrying thymocyte-like (stage II, stage III) malignant T cells (T3-, T6+, T4/T8+ or T3+, T4+, T10+, T6-, T8-) as T-ALL (patient 4) or leukemic T-NHL (patients 5 and 6).

Isolation of leukemic T cells. Leukemic cells were isolated from peripheral blood or bone marrow at diagnosis, after informed consent, using Ficoll-Isopaque separation. Further separation was applied to remove residual normal (E+, T3+) T lymphocytes because, in most cases, these could interfere with leukemic growth in the IL 2-supplemented cultures. In one case of E rosette-negative T-ALL (ie, patient 1), normal T cells were eliminated by E rosette depletion using 2-aminothiouronium bromide (AET)-treated sheep erythrocytes as described.

In the other case (ie, patients 2 through 5), residual normal T lymphocytes were removed from the leukemic cell fractions on the basis of differential T3 antigen expression on normal and neoplastic
T cells by fluorescent staining of the T3 antigen (see the subsequent sections) followed by fluorescence-activated cell sorting (FACS 440, Becton Dickinson, Sunnyvale, Calif) (Fig 1). The cells of patient 6 were studied without further separation because both cytogenetic and immunologic analysis of fresh and cultured cells indicated that contaminating growth of normal T cells did not occur.

Isolation of E rosette-forming lymphocytes from normal peripheral blood samples. E rosette-forming lymphocytes were recovered from the sediment after centrifugation of AET-treated E rosetting cells through Ficoll(Isopaque as described. Sheep erythrocytes were removed from these fractions by hypotonic lysis (incubation for five minutes at 37°C in 0.7% wt/vol NH4Cl in 3 mmol/L Tris-HCl, pH 7.2).

Immunofluorescence. Surface membrane antigens on fresh and cultured cells were assayed by indirect immunofluorescence microscopy using murine MoAb and goat antimouse Ig immunoglobulins coupled to fluorescein isothiocyanate (GAM/FITC; Nordic Immunology, Tilburg, The Netherlands). The presence of TdT in the cell nuclei was assessed on cytocentrifuged cells using an indirect immunofluorescence kit (GIBCO, Ghent, Belgium).

MoAb. MoAb reactive with the T3, T4, T6, T8, and T10 antigens and with the Td antigen were obtained from Ortho Pharmaceutical Corp (Raritan, NJ) and all used in a 1:40 dilution. MoAb WT1, reactive with a pan-T cell antigen, was supplied by Dr W.J.M. Tax (Nijmegen, The Netherlands) and used in a titer of 1:100. MoAb anti-IL 2 receptor (aIL 2r) obtained from Becton Dickinson (Mountain View, Calif) was used in a 1:20 dilution. The reactivity of this antibody is identical to that of anti-Tac MoAb, detecting a myelocytic antigen, was a gift from Dr P. Lantsdorp (Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam).

Colony culture. Colony cultures were performed basically as described for normal T cell colony formation with the following modifications: (1) Feeder leukocytes were not included in the system as a source of growth stimuli. Instead, pure r-IL 2 (Biogen SA, Geneva) was added to the culture upper layer. (2) Either PHA (reagent grade, Wellcome Reagents, Darford, England) at a concentration of 0.7% vol/vol or the phorbol ester TPA at a concentration of 7 ng/mL (Sigma Chemical Co, St Louis) were used as mitogens in culture.

Colony cells were mass harvested with a Pasteur pipette, washed three times with phosphate-buffered saline (PBS), and prepared for indirect immunofluorescence and E rosetting.

In one case (patient 6), the colony cells were analyzed cytogenetically (Dr A. Hagemeyer, Department of Cell Biology and Genetics, Erasmus University, Rotterdam).

Induction of IL 2 receptors. Short-term (18 hours) suspension cultures supplemented with PHA or TPA to induce membrane receptors for IL 2 were performed as described. After culture, the cells were washed three times with PBS and prepared for indirect immunofluorescence with the aIL 2r antibody.

Microcytose of NHL cells and in vitro blocking of IL 2 receptors. Proliferation of NHL cells in culture as measured by 3H-thymidine (Tdr) uptake was examined with and without blocking of IL 2 receptors. Parallel cultures were established to which MoAb aIL 2r (IgGl, noncytotoxic), control MoAb (OKT3, B13.9) or no MoAb had been added at their initiation. A quantity of 1 x 106 cells were cultured in triplicate in round-bottom microtiter wells (Greiner, Alphen aan den Rijn, The Netherlands) for three days in 100 μL RPMI 1640 medium (GIBCO) with 10% heat-inactivated fetal calf serum supplemented with 5 x 10−5 mol/L beta-mercaptoethanol.

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Table 1. Selected Data of Patients With T-ALL and T-NHL

<table>
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<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Blood Leukocyte Count (x 109/L)</th>
<th>WT1†</th>
<th>T3</th>
<th>T4</th>
<th>T6</th>
<th>T8</th>
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<td>94</td>
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Abbreviation: ND, not determined.

*Assessed by indirect immunofluorescence and E rosetting (200 cells counted).
†Weak expression (see Fig 1).
‡Eighthy-eight percent of the cells expressed T10 antigens.
§Diffuse, well-differentiated lymphocytic lymphoma.

Patients 1 to 5 are terminal deoxynucleotidytransferase (TdT)-positive; patient 6 is TdT-negative. In none of the cases did the leukemic cells express Ia antigens.

Fig 1. FACS histograms of Ficoll-Isopaque isolated cells of patient 3 (T-ALL) after OKT3 + GAM FITC (——) and GAM/FITC control (—) staining. The ALL cells of this patient showed a weak T3 antigen expression, whereas residual normal T lymphocytes (interfering strongly with leukemic colony growth) express the T3 antigens in a high density on their cell surface. To eliminate the normal T cells from the ALL cell sample of this patient (and of patients 2, 4, and 5) cell sorting was performed on the basis of T3 fluorescence intensity. The vertically drawn interrupted line indicates the level at which the cells were separated; ALL cells were recovered left from this line (the fraction with low fluorescence intensity).
and L-glutamine (referred to as complete RPMI medium) supplemented with 0.1% PHA.

Sixteen hours before harvesting of the cells (Titertek Cell Harvester 550) 0.1 μCi of Tdr (Amersham Corp, Amersham, UK; specific activity, 2 Ci/mmol) was added to each microwell. Radioactivity was measured by liquid scintillation counting (Beckman LS 3800, Beckman Instruments, Inc, Fullerton, Calif). All cultures were performed in triplicate. Data are expressed as mean ± SD of triplicate cultures.

**IL 2 assay.** Murine IL 2-dependent cytotoxic T lymphocyte cell line (CTLL) cells (a gift from S. Knaan-Shanzer, Radiobiological Institute TNO, Rijswijk, The Netherlands) were cultured in triplicate after three washings with Hanks’ balanced salt solution (HBSS) in complete RPMI medium at 1 x 10⁶ cells/100 μL in microtiter wells. At 20 hours, cultures were supplemented with 0.1 μCi Tdr, and four hours later, the cells were harvested and assayed for TdR incorporation by liquid scintillation counting.

To estimate IL 2 concentration in T-NHL culture supernatant, the proliferation of CTLL cells in the presence of these supernatants was compared with proliferation in response to titrated r-IL 2 (specific activity, 2 Ci/mmol) was added to each microwell. Radioactivity was measured by liquid scintillation counting (Beckman LS 3800, Beckman Instruments, Inc, Fullerton, Calif).

**Preparation of T-NHL culture supernatant.** A quantity of 2 x 10⁶ cells T-NHL cells/mL complete RPMI medium with or without 1% vol/vol PHA in 6-mL tubes (Greiner) was placed in the incubator for three days. Supernatants were then recovered, filter sterilized through a 0.22-μm filter (Millipore, Bedford, Mass), and stored at -20 °C.

## RESULTS

**Induction of IL 2 receptors.** Untreated T-ALL and T-NHL cells, like normal peripheral blood T lymphocytes, did not express membrane receptors for IL 2 as measured in indirect immunofluorescence using MoAb aIL 2r (Table 2). Studies on cells from short-term (18 hours) suspension cultures then disclosed that IL 2 receptors appeared on significant proportions of the cells when TPA or PHA had been added to these cultures (Table 2). In the absence of TPA/PHA, IL 2 receptor induction did not occur. A striking discrepancy with respect to PHA sensitivity was evident between the prothymocyte or immature thymocyte-like leukemia cells (ie, patients 1 to 5) and the normal T lymphocytes. These leukemias, in contrast to the normal T cells, did not respond to PHA. In contrast, the cells of patient 6 carrying a mature thymocyte phenotype were sensitive to activation by both TPA and PHA, thus sharing this property with normal peripheral T cells.

**Colony stimulation.** To assess the proliferative response of the normal T and the T-NHL/T-ALL cells to IL 2, the cells were plated in colony culture under selected stimulatory conditions (Table 2).

Colony formation by the normal T lymphocytes depended on the presence of both IL 2 and mitogen (either PHA or TPA). When the addition of mitogen (ie, PHA or TPA) was omitted from IL 2–containing cultures, no colonies were formed, which is in agreement with the finding that nonactivated T cells lack membrane receptors for IL 2. On the other hand, suboptimal colony formation was noted in the presence of TPA or PHA when no IL 2 had been added to the cultures. The fact that some normal T cell colonies develop in non–IL 2–supplemented cultures is most likely to be explained by endogenous production of IL 2 by the T cells stimulated by the IL 1–like activity of TPA27 and/or some residual monocytes present among the cells.

The colony-forming abilities of the cells from patients 1 to 5 were clearly different from those of normal blood T lymphocytes. The cells did not form colonies in response to PHA even when IL 2 had been added to culture. In contrast, when combined with TPA, IL 2 stimulated the formation of considerable numbers of colonies in all of these cases. The capacity of the cells to form colonies in response to TPA but not to PHA was entirely in accordance with, respectively, the ability and inability of these compounds to induce IL 2 receptors in the individual cases. The cells of patient 6 showed a divergent response pattern: colony formation and IL 2 receptor induction occurred with either TPA or PHA.

To define the IL 2 dependence of T-ALL and T-NHL colony-forming cells, IL 2 dose titration experiments were performed (Fig 2). These experiments show that the T-ALL cells of patients 1 and 3, activated by TPA, like normal T lymphocytes require the addition of IL 2 to the cultures for optimal colony formation (panels A, B, and D). Patient 1 is representative of the pattern in which the cells are typically absolutely dependent on the exogenous supply of IL 2 to culture. This type of response also applies to that in patients 2

### Table 2. Induction of IL 2 Receptors and Colony Formation

<table>
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<tr>
<th>Patient*</th>
<th>Percentage of Untreated TPA†</th>
<th>PHA‡</th>
<th>TPA + IL 2 25</th>
<th>PHA</th>
<th>TPA + PHA</th>
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*See Table 1 for a description of the patients.
†Colony numbers per 2 x 10⁶ plated cells.
‡Determination of IL 2 receptors following 18 hours of TPA/PHA-supplemented suspension culture (see Materials and Methods).
§§-positive lymphocytes purified from normal donor peripheral blood.
PHA costimulation.

purified from normal peripheral blood; + TPA costimulation;
general reflected similar stages of maturation as the cells
percentages of maximal colony stimulation. (A) T-ALL patient
+ activated normal helper T cells (T3+ T10+). The fact that these colonies were indeed leukemia
ation of IL2 colonies (although in suboptimal numbers) without the addi-
and 4. Plateau IL 2 requirements for leukemic colony formation were variable, ie, 25 units per culture dish for patient 1 and 0.1 units per dish for patient 3. Further increases in IL 2 concentrations (up to 500 units per culture dish) did not significantly alter the numbers of (normal and

The TPA-activated T-ALL cells of patient 3 produced colonies (although in suboptimal numbers) without the addition of IL 2. A similar phenomenon was evident with the T-NHL cells of patient 6 cultured with PHA (panel C). These findings raise the possibility that certain T-ALL and T-NHL cells are capable of IL 2 release and autostimulation (see the following).

Colony typing. Membrane marker analysis was performed after colony culture (Table 3), and the immunophenotypes of colony cells were compared with those of the fresh cells.

Immunologic phenotypes of the leukemic colony cells in general reflected similar stages of maturation as the cells before culture. For example, colony cells from patients 1, 2, and 3 still expressed the surface antigens characteristic of their preculture immaturity (Table 1).

Colony cells of patients 4 and 5 appeared slightly more mature than the cells before culture, since they had largely lost the T6 antigen. In neither of these two cases, however, had maturation progressed toward the T3-positive mature thymocyte differentiation stage. Normal T cell colonies (Table 3) contained mature activated T lymphocytes with a blastic appearance and a mature T cell immunophenotype, ie, T3+, T4 or T8+, T6−, WT1+, and E+. Colony cells of patient 6 expressed the normal phenotype. It was identical to that of the preculture cells, ie, resembling that of mature activated normal helper T cells (T3+, T4+, T6−, T8−, T10+). The fact that these colonies were indeed leukemic was apparent from cytogenetic analysis. It was found that 91% of the colony cells in metaphasia had the complex cytogenetic abnormality [46, XY, 6q−, 7p+, t(8p−; 11p−q?), 12 q+, −14, 15p+, + Mar (14q−?)] that had been demonstrated in 76% of the fresh NHL cells in metaphasia.

Analysis of growth requirements of leukemic T cells that do not depend on the addition of IL 2 for prolifera-
tion. Colony formation in three cases occurred in spite of the absence of exogenous IL 2 (ie, in patients 3 and 5 with TPA alone and in patient 6 with PHA alone) (Table 2, Fig 2). Possible explanations for these findings are that these leukemias either proliferated independently on IL 2 or that IL 2 was needed but provided by the neoplastic cells themselves. To clarify this question, the cells of patient 6 (NHL) were tested in TdR incorporation microculture assays (Fig 3). First, it was investigated whether the cells were capable of IL 2 production. Culture supernatants conditioned by PHA-activated T-NHL cells (T-NHL−CM) were tested for IL 2 activity by inducing proliferation of CTLL cells (murine IL 2-dependent cell line). From Fig 3A it can be seen that increasing concentrations of T-NHL−CM stimulated CTLL proliferation progressively. IL 2 activity of the T-NHL−CM was estimated at approximately 5 u/mL by comparison with dose response relationships of pure r−IL 2. Without PHA

Table 3. Surface Membrane Antigens on Colony Cells

<table>
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<tr>
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<td>90</td>
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*Preinculture phenotypes of the leukemic cells are given in Table 1.
†Assessed by indirect immunofluorescence (200 cells counted).

Fig 2. Induction of ALL/NHL (A, B, C) and normal (D) T colony formation with titrated doses of r−IL 2. The data are expressed as percentages of maximal colony stimulation. (A) T-ALL patient 1. (B) T-ALL patient 3. (C) T-NHL patient 6. (D) E+ lymphocytes purified from normal peripheral blood; v—v, TPA costimulation; o—o, PHA costimulation.

Fig 3. Microculture analysis of IL 2 production (A) and the effect of IL 2 receptor blocking on proliferation (B) (T-NHL patient 6). A. Dose-dependent proliferation induction of fully IL 2-dependent murine CTLL cells by increasing concentrations of culture medium conditioned by the leukemic T cells (T-NHL−CM). B. Inhibition of proliferation of T-NHL cells in cultures with PHA and without exogenous IL 2 following the addition of MoAb anti-IL2 receptor (5ug/ml).
activation, the culture media conditioned by the NHL cells contained only 1 U/mL IL-2 activity.

The elaboration of IL-2 in the culture medium by the NHL cells of patient 6 and the appearance of IL-2 receptors on these cells following PHA stimulation suggested that their proliferation depended on the release of endogenous IL-2. Therefore, we investigated the effect of blocking IL-2 membrane receptors (with the anti-IL-2 receptor MoAb aIL-2r) on the proliferation of the T-NHL cells in the absence of exogenous IL-2 (Fig 2B). Culturing of the cells in the presence of MoAb aIL-2r resulted in a 60% to 70% inhibition of TdR incorporation. On the other hand, (1) the incubation of T-NHL cells with control MoAb OKT3 (IgG2a) or B13.9 (IgG1) or (2) the incubation of control (acute myelogenous leukemia) cells with MoAb aIL-2r did not result in a significant suppression of TdR incorporation. Taken together, these data confirm that the T-NHL cells of patient 6 indeed require IL-2 for proliferation. Notably, however, exogenous IL-2 is not required because the cells elaborate their own IL-2 at effective concentrations.

DISCUSSION

The induction of proliferation of normal T lymphocytes in vitro involves a dual-step mechanism in which an activator (eg, antigen, lectin, phorbol ester) and the lymphokine IL-2 (previously designated as T cell growth factor) are both essential components.14 Activation is required to induce receptors for IL-2 on the T cell membrane, thereby rendering the cells sensitive to the growth factor. The discovery that certain (HTLV-infected) leukemias of mature T cell type continuously express IL-2 receptors without prior activation15 and the finding that some cutaneous T cell leukemia/lymphoma cell lines produce and respond to IL-216 supported the idea that the growth of leukemic T cells depends on a permanent responsiveness to IL-2 in association with the ability to self-produce this growth factor. Such a model of autostimulation driven by IL-2 was initially thought to be crucial in the development of HTLV-associated neoplasms.14 However, this suggestion was later considered less likely because it was found that not all HTLV-induced leukemias are capable of IL-2 production. In addition, the cells of the latter neoplasms do not always require (endogenous or exogenous) IL-2 for proliferation.20

In another class of mature T cell leukemia, ie, malignant cutaneous T cell lymphoma (Sézary syndrome), the cells were found to produce and respond to IL-2 in vitro following stimulation with PHA and TPA.21 Sézary cells, with few exceptions, are not infected with HTLV.22 They generally lack spontaneous IL-2 receptor expression and are therefore dependent on mitogenic stimulation. The autocrine secretion of IL-2 by Sézary cells, however, is not a tumor-associated characteristic because normal T helper lymphocytes express the same ability.22,23

The role of IL-2 in the regulation of the growth of T-ALL and T-NHL has as yet remained a rather unexplored area. The present study was undertaken to establish the in vitro culture requirements of these T cell neoplasms. The results from colony culture and TdR incorporation experiments indicate that T-ALL and T-NHL cells from six individual patients had remained dependent on IL-2 (either added to the cultures or endogenously produced) for proliferation (Table 2, Figs 2 and 3). Besides IL-2, T-ALL and T-NHL cells require activation by TPA for colony growth. In all cases, colonies were formed in TPA- plus IL-2-supplemented cultures (Table 2). TPA is needed for the induction of IL-2 surface membrane receptors on the T-ALL and T-NHL cells. Fresh leukemic T cells lacked these receptors, whereas exposure to the phorbol ester resulted in their appearance on a high proportion of the cells (Table 2). The induction of receptors for IL-2 by TPA on certain leukemic T cell lines initially lacking such receptors has also been reported.23 Thus, T-ALL and T-NHL cells share with normal T lymphocytes the requirement of activation before they are capable of responding to IL-2.

Although it is clear that the interaction of foreign antigen with antigen receptors on the cell surface is the in vivo activation signal for T lymphocytes and stage III thymocytes, no in vivo mechanism underlying activation of immature T cell types (ie, not expressing the T3 antigen receptor complex on the cell membrane) is known. Therefore, no conclusions can as yet be drawn from our in vitro experiments as regards the role of IL-2 in the proliferation of stage I and stage II thymocyte like T-ALL and T-NHL cells in vivo. However, it was recently shown that IL-2 receptors are present on a population of freshly isolated murine thymocytes with an immature (Ly2−, L3T4−) immunophenotype,27,28 and on this basis, it was suggested that thymocytes can be activated by a stimulus intrinsic to the thymus.27 If this hypothesis is correct, it raises the possibility that, in vivo, the IL-2 responsiveness of leukemic T cells with stage I or stage II thymocyte immunophenotypes can be induced in situ in the thymus. Notably, thymic involvement is frequently observed in T-ALL and T-NHL.

Unlike TPA, PHA exerted an effect only in the one case of T-NHL expressing a mature thymocyte (stage III) immunophenotype (Table 2, patient 6). The expression of IL-2 receptors and colony formation were induced by PHA (as well as by TPA) in this mature (stage III thymocyte) positive NHL. The other, prothymocytelike or immature (stage 1, stage II) thymocytelike tumors lacked PHA responsiveness. They share this inability with their putative normal thymic counterparts.29 With Jurkat cells and a variety of mutants derived from this cell line it was recently shown that the PHA responsiveness of T cells is directly related to the expression of the T3 antigen receptor complex on the cell surface.30

In three cases (ie, patients 1, 2, and 4), colony formation was strictly dependent on the addition of IL-2 to culture. The fact that IL-2 had to be added suggests that the neoplastic cells were not capable of IL-2 production. However, on the basis of our experiments we cannot exclude the possibility that some IL-2, insufficient for autostimulation, was actually released by the cells of these patients. On the other hand, in the three other cases (ie, patients 3, 5, and 6) colony growth was apparent with TPA or PHA as a single additive to culture and did not require exogenous IL-2. It was documented in one of these cases (patient 6) that the neoplastic cells produced considerable amounts of IL-2. Culture media
conditioned by the T-NHL cells of patient 6 contained IL 2 activity on the order of 5 U/mL. Thus, we propose that T-ALL and T-NHL may be subdivided into categories on the basis of in vitro proliferation characteristics, ie, (1) responsive to both TPA and PHA, (2) responsive to TPA but not to PHA, (3) capable of IL 2 production in sufficient amounts for self-stimulation in colony culture, and (4) not capable of significant IL 2 production.

Normal T cells at different stages of maturation may express identical features. Therefore, the results of our in vitro studies as yet do not provide indications for a disordered response to IL 2 underlying the uncontrolled proliferation in T-ALL and T-NHL. However, it is at present not known whether critical abnormalities exist in these T cell leukaemias as far as the detailed requirements of activation of IL 2 synthesis, activation and down-regulation of IL 2 receptors, and function of these receptors are concerned. Such abnormalities have been described for adult T cell leukemia and certain leukemic T cell lines.5,6,11

Results from immunophenotyping provided no positive evidence for differentiation of the T-ALL and T-NHL cells during colony formation in cultures supplemented with TPA. No acquisition of new differentiation antigens (eg, E, T3, T6) indicative of in vitro maturation of the leukemic T cells was apparent (Table 1, Table 3). These data contrast with those of experiments with certain leukemic T cell lines (Jurkat, CEM, and Molt-3) in which TPA was found to induce the expression of E rosette receptors and T3 antigens.7,8

Cloning efficiencies of T-ALL/T-NHL cells in IL 2 plus mitogen-containing cultures were significantly lower than those of normal peripheral T cells (Table 2). This raises the question as to whether culture conditions were suboptimal for T-ALL and T-NHL, for example, because T-ALL and T-NHL cells require other lymphokines for proliferation besides IL 2. The finding that the aforementioned subpopulation of mouse thymocytes (ie, early Ly2−/L3T4− thymocytes) do not respond in vitro to IL 2, although the cells express functional (ie, high affinity) IL 2 membrane receptors,27,28 suggests that other growth factors in combination with IL 2 act on early stages of (murine) T cell maturation and possibly on the leukemic counterparts of the analogous cell types.

Results from previous work on non-T-ALL indicated that in vitro colony formation by common and pre-B-ALL cells depends on the combined exposure to IL 2 and a second factor elaborated by feeder leukocytes.16 However, we could not demonstrate a significant effect of the same crude source of stimulation on colony-forming cells of ALL/NHL of T lineage (results not shown). To further elucidate the critical requirements additional to IL 2 for in vitro colony growth of T-ALL and T-NHL cells, the availability of pure (recombinant) growth factor preparations will be essential.

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REFERENCES

16. Touw I, Delwel R, Bolhuis R, van Zanen G, Löwenberg B: Common and pre-B acute lymphoblastic leukemia cells express...
interleukin 2 receptors and interleukin 2 stimulates in vitro colony formation. Blood 66:556 1985


34. Ryffel B, Henning CB, Huberman E: Differentiation of human T-lymphoid leukemia cells into cells that have a suppressor phenotype is induced by phorbol 12-myristate 13-acetate. Proc Natl Acad Sci USA 79:7336, 1982
Acute lymphoblastic leukemia and non-Hodgkin’s lymphoma of T lineage: colony-forming cells retain growth factor (interleukin 2) dependence

I Touw, R Delwel, G van Zanen and B Lowenberg