Microenvironment and Immunology

IL-7 Contributes to the Progression of Human T-cell Acute Lymphoblastic Leukemias

Ana Silva¹,³, Angelo B.A. Laranjeira⁴, Leila R. Martins¹, Bruno A. Cardoso¹, Jocelyne Demengeot², J. Andrés Yunes³, Benedict Seddon⁵, and João T. Barata¹

Abstract

The importance of microenvironmental factors for driving progression in leukemia has been debated. Previous evidence has pointed to interleukin-7 (IL-7), a fundamental cytokine to normal T-cell development and homeostasis, as an important determinant of the viability and proliferation of T-cell acute lymphoblastic leukemia (T-ALL) cells in vitro. In this study, we report that IL-7 is also a critical determinant of T-ALL progression. T-ALL cell lines and primary T-ALL samples initiated leukemia more slowly when engrafted into immunocompromised Bag2−/−Il2rg−/− mice lacking IL-7. This effect was not related to reduced engraftment or homing of transplanted cells to the bone marrow. Instead, IL-7 deficiency diminished expansion of leukemia cells in the bone marrow and delayed leukemia-associated death of transplanted mice. Moreover, infiltration of different organs by T-ALL cells, which characterizes patients with advanced disease, was more heterogeneous and generally less efficient in IL-7–deficient mice. Leukemia progression was associated with increased Bcl-2 expression and cell viability, reduced p27Kip1 expression, and decreased cell-cycle progression. Clinical measurements of IL-7 plasma levels and IL-7 receptor (IL-7R) expression in T-ALL patients versus healthy controls confirmed that IL-7 stimulates human leukemia cells. Our results establish that IL-7 contributes to the progression of human T-cell leukemia, and they offer preclinical validation of the concept that targeting IL-7/IL-7R signaling in the tumor microenvironment could elicit therapeutic effects in T-ALL. Cancer Res; 71(14); 4780–9. ©2011 AACR.

Introduction

Interleukin-7 (IL-7) is essential for normal T-cell development and homeostasis. Paradoxically, several lines of evidence indicate that the IL-7/IL-7 receptor (IL-7R) axis may also play a significant role in promoting leukemogenesis. IL-7 can act as an oncogene in vivo, as IL-7 transgenic mice develop B- and T-cell lymphomas (1, 2), and murine thymocytes spontaneously overexpressing IL-7R have a selective advantage that contributes to proliferation and leukemogenesis (3). IL-7 is produced by bone marrow and thymic stroma (4–7), suggesting that IL-7 is present in the microenvironments where leukemia cells develop, thus having the potential to directly modulate leukemia growth. In vitro studies have shown that thymic epithelium and bone marrow stromal cells promote survival of T-cell acute lymphoblastic leukemia (T-ALL) cells via production of IL-7 (8, 9). Importantly, IL-7 induces in vitro proliferation of the majority (>70%) of primary T-ALL samples (10, 11). IL-7 promotes cell-cycle progression and viability of T-ALL cells via activation of the phosphoinositide-3-kinase (PI3K)/protein kinase B (PKB)/Akt signaling pathway (12), resulting in downregulation of the cdk inhibitor p27Kip1 and upregulation of Bcl-2 (13). Remarkably, IL-7 may contribute to resistance to treatment with rapamycin in ALL in general (14) and to imatinib in Bcr/Abl+ALL (15, 16). However, these observations are counterweighted by the understanding that most malignant cells proliferate independently of external cues. Furthermore, although IL-7 may have a significant impact on some primary T-ALL cells cultured in vitro under conditions of growth factor paucity, it is possible that IL-7 has only a redundant effect on leukemia expansion in vivo, given the diversity of other survival and proliferative signals present in the tumor milieu.

In the present study, we sought to clarify the importance of IL-7 for the progression of human T-ALL in vivo, using a combination of xenotransplant mouse models of human leukemia and analysis of primary T-ALL specimens collected directly from patients. Our results indicate that IL-7 is consumed by and stimulates human T-ALL cells in vivo. IL-7 induces p27Kip1 downregulation and Bcl-2 upregulation in T-ALL cells, promotes their viability and proliferation, and...
contributes to leukemia progression. Our data support the notion that microenvironmental cues, produced in a nonautocrine fashion, can partake in leukemia development.

Material and Methods

Cells
Primary T-ALL cells were collected from peripheral blood or bone marrow of patients at diagnosis as described previously (17). Normal thymocytes were isolated from thymic tissue obtained from children undergoing cardiac surgery. Informed consent and Institutional Review Board approval were obtained for all primary leukemia and thymic sample collections. The IL-7–dependent T-ALL cell line TAIL7 was established by our group, and it is regularly tested by flow cytometry and PCR (18). TAIL7 cells share significant similarities with primary leukemic samples (18). The growth factor–independent T-ALL cell line HPB-ALL expresses the IL-7R and responds to IL-7. P12-ICHI-KAWA is a growth factor–independent IL-7R–negative T-ALL cell line. Both cell lines were from a cell bank and characterized as specified (19). HPB-ALL cells were stably transduced with lentiviral vectors, kindly provided by Dr. Luigi Naldini (San Raffaele Scientific Institute, Milan, Italy; 20), driving the expression of luciferase and GFP (HPB-ALL.Luc.GFP). Primary T-ALL samples and cell lines were immunophenotyped using standard techniques (21) and their maturation stage was classified according to the European Group for the Immunological Classification of Leukemias (EGIL) criteria (ref. 22; Table 1).

Xenotransplantation
Protocols and studies involving animals were conducted in accordance with the UK Home Office regulation and local guidelines. Rag2<−/−>,Il2rg<−/−>, and Il7<−/−> strains were obtained from The Jackson Laboratory and intercrossed to generate compound knockouts required for this study. Il7<−/−>Il2rg<−/−>Rag2<−/−> and Il2rg<−/−>Rag2<−/−> strains were both on C57Bl6/j background. Strains were bred in specific pathogen-free facilities at the National Institute for Medical Research (London, UK), Instituto Gulbenkian de Ciência (Oeiras, Portugal), and Instituto de Medicina Molecular (Lisbon, Portugal). HPB-ALL, Luc.GFP, TAIL7, P12, and primary T-ALL cells (5 × 10⁶ to 2 × 10⁷ cells) were injected i.v. in a final volume of 250 μL per injection. Mice were sacrificed when moribund or at the indicated time points. Organs were collected for postmortem analysis. Mice were classified as leukemic when T-ALL cells represented more than 20% of the bone marrow cellularity, according to the clinical definition of human leukemia (23). We further classified mice as preleukemic (<20% and >0.1% malignant cells in the bone marrow) and nonleukemic (>0.1%).

In vivo bioluminescence imaging
Mice were intraperitoneally (i.p.) injected with 150 μg luciferin/g, anaesthetized, and scanned with an IVIS Lumina bioimaging device (Caliper Life Sciences). Mice were imaged after 7 minutes and total flux (photons per second) was calculated using Living Image software (Caliper Life Sciences).

Blood and organ analysis
For flow cytometric analysis, collected blood was depleted of erythrocytes by using red blood cell lysis buffer (BD Pharmingen). Bone marrow was extracted by flushing off bones. Spleen, liver, and kidneys were mechanically disintegrated into single-cell suspensions, which were then washed in PBS, stained for 45 minutes at 4°C with monoclonal antibody for human CD2 or CD5 (Becton Dickson), and analyzed using a FACScalibur flow cytometer (Becton Dickinson) and FlowJo software (Tree Star). For histology analysis, small representative portions of the collected organs (spleen, liver, and kidneys) and at least 1 complete femur were preserved in formalin at 4°C for 24 to 48 hours with hematoxylin/eosin staining.

CFSE labeling for analysis of engraftment efficiency
TAIL7 cells (10 × 10⁶ cells/mL) were incubated for 10 minutes at 37°C with 2 μmol/L of carboxylfluorescein succinimidyl ester (CFSE; Sigma-Aldrich). Cells were washed 3 times in culture medium, resuspended as 10⁷ cells/250 μL in Iscove's modified Dulbecco's medium/bovine serum albumin, and injected i.v. into mice.

Intracellular staining
Bone marrow cells were stained with anti-human CD2-PE- or CD5-fluorescein isothiocyanate (FITC)-conjugated antibodies, fixed in 1 × Fix/Perm solution (eBioscience) for 30 minutes at 4°C, washed in PBS, and resuspended in 1 × Permeabilization Buffer (eBioscience) for 10 minutes at 4°C. Samples were

Table 1. Immunophenotype and maturation stage of primary T-ALL samples and cell lines

<table>
<thead>
<tr>
<th>CD1a</th>
<th>CD2</th>
<th>CD3</th>
<th>cCD3</th>
<th>CD5</th>
<th>CD7</th>
<th>CD4</th>
<th>CD8</th>
<th>IL-7Rα</th>
<th>Maturation stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt#1</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>III (cortical)</td>
</tr>
<tr>
<td>Pt#2</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>IV (mature)</td>
</tr>
<tr>
<td>TAIL7</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>II (pre-T)</td>
</tr>
<tr>
<td>HPB-ALL</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>III (cortical)</td>
</tr>
<tr>
<td>P12</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>III (cortical)</td>
</tr>
</tbody>
</table>

NOTE: Maturation stages were defined according to the EGIL criteria (see Materials and Methods). Plus sign indicates >30% positive cells; minus sign indicates <30% positive cells, except for IL-7Rα, where minus sign indicates <3% positive cells. Abbreviations: cCD3, cytoplasmic CD3; Pt, patient.
stained for 30 minutes at 4°C with anti-Bcl-2-FITC antibody (Dako), anti-Ki-67-PE antibody (Becton-Dickinson), or with irrelevant isotype-matched antibody. For viability analysis, bone marrow cells were surface stained for human CD5-FITC, washed and resuspended in 1× binding buffer, and stained with Annexin V–phycoerythrin (PE). Samples were washed and analyzed by flow cytometry. Results were expressed as the percentage of positive cells in comparison with the negative control and as specific mean intensity of fluorescence (MIF).

**Immunoblotting**

Cell lysates were resolved by 10% SDS-PAGE, transferred onto nitrocellulose membranes, and immunoblotted with antibodies against ZAP-70 (Upstate) or p27kip1 (Santa Cruz Biotechnology). Immunodetection was carried out by incubation with horseradish peroxidase–conjugated anti-mouse or anti-rabbit IgG (Promega) and developed by enhanced chemiluminescence (Amersham-Pharmacia).

**Quantitative reverse transcriptase PCR**

Total RNA (1 μg) was reverse transcribed using the ImProm II Reverse Transcription enzyme (Promega) and random hexamers. The quantitative assessment of IL7R transcripts was made by SYBR green-based quantitative reverse transcriptase PCR (qRT-PCR) on an ABI PRISM 7500 (Applied Biosystems) with the following primers: ABL, TGGAGATAACACTCTAAG; IL7R, GGATTAAGCCTATCGTAT-GGC (exon 7) and GCTTGA-CATAACTAAAGGT and GATGTAGTTGCTTGGGACCCA; and GAPDH, ATGTAGTTGCTTGGGACCCA and GATGTAGTTGCTTGGGACCCA; and IL7R, GGATTAAGCCTATCGTAT-GGC (exon 8). Standard curves were obtained by serial dilutions of PCR products, and IL7R transcript values were normalized with respect to the number of ABL transcripts.

**IL-7 plasma quantifications**

Human IL-7 levels were quantified in plasma, using the high sensitivity IL-7 QuantiKine HS Elisa kit (R&D) according to the manufacturer’s instructions. Samples were assayed in duplicate.

**Ex vivo assessment of IL-7Ra expression in primary T-ALL cells**

Primary T-ALL cells were cultured in 24-well plates as 2 × 10^5 cells/mL at 37°C with 5% CO_two in RPMI-1640 medium supplemented with 10% FBS (Invitrogen Corporation), with or without 10 ng/mL hIL-7 (Peprotech EC). Immediately ex vivo (0 hour) and at 24 hours of culture, cells were stained with IL-7Ra-PE (R&D) analyzed by flow cytometry.

**Statistical analysis**

Log-rank test, Student t test, Mann–Whitney test, and 2-way ANOVA were used to compare data, as appropriate (P < 0.05 was considered significant).

**Results**

**IL-7 accelerates leukemia expansion and leukemia-related death in mice xenotransplanted with human T-ALL cell lines**

Although transgenic mouse models of leukemia provide valuable insights into the mechanisms of leukemogenesis, in vivo studies using primary human leukemia cells may help elucidate processes unique to human disease. Both recombinant IL-7 and endogenously produced murine IL-7 are bioactive upon human lymphocytes, including T-ALL cells (24, 25). Therefore, we sought to test the in vivo effects of microenvironmental IL-7 on human T-ALL development by xenogeneic transplantation of leukemia cells into Rag2^−/−Il2rg^−/− hosts differing exclusively in the capacity to produce IL-7. In the absence of Rag2 recombinese and the IL-2 common γ-chain, these hosts lack T, B, and natural killer (NK) cells and therefore tolerate T-ALL transplantation. HPB-ALL T-ALL cells stably expressing luciferase and eGFP (HPB-ALLLuc.GFP) were transplanted i.v. into Rag2^−/−Il2rg^−/− [IL-7 wild-type (WT)] and Rag2^−/−Il2rg^−/−Il7^−/− [IL-7 knockout (KO)] mice. Five weeks (35 days) posttransplantation, mice were injected with luciferin to assess tumor burden and localization by whole-body bioluminescence imaging. IL-7 WT animals displayed strikingly stronger and more disseminated luciferase activity (Fig. 1A, left). Quantification of bioluminescence intensity confirmed that the tumor burden was significantly higher in IL-7 WT mice (Fig. 1A, right). Analysis of sacrificed animals showed that the lungs, spleen, liver, kidneys, and bone marrow were infiltrated with HPB-ALLLuc.GFP cells (data not shown). These data suggested that absence of IL-7 delayed disease progression and dissemination in vivo.

To confirm these observations, we used TAIL7 cells, which are IL-7-dependent in vitro and constitute a model for primary T-ALL (18). First, we transplanted CFSE-labeled TAIL7 cells into IL-7 WT and IL-7 KO mice and evaluated engraftment 6 hours after i.v. injection. Analysis of the bone marrow revealed the presence of very few leukemia cells, and no difference between IL-7 WT (36.5 ± 26.7) and IL-7 KO (31.6 ± 26.7) mice (Supplementary Fig. S1). Spleen and several nonlymphoid organs (lung, liver, and kidney) were also analyzed and all presented very few cells with no differences between the 2 mouse strains (data not shown). Our data indicate that IL-7 did not affect the engraftment and initial homing capacity of T-ALL cells.

We next evaluated the effect of IL-7 on disease progression. The percentage of TAIL7 cells circulating in the peripheral blood increased with time and was consistently higher in IL-7 WT mice at all time points analyzed (Fig. 1B). These differences may have resulted from compromised leukemia expansion in the bone marrow of IL-7-deficient mice. To test this possibility, mice were sacrificed 84 days posttransplantation, before disease symptoms were apparent. All IL-7 WT animals (n = 9) presented clear bone marrow involvement. Most mice (6 of 9; 67%) displayed overt leukemia according to the clinical definition (23), presenting more than 20% leukemia cells in the bone marrow. All the remaining IL-7 WT mice showed detectable disease in the bone marrow and were thus considered preleukemic (3 of 9; 33%), as defined in Materials and Methods (Fig. 1C). In contrast, only 4 of 10 (40%) IL-7 KO mice were leukemic and 2 of 10 preleukemic (20%; Fig. 1C). The remaining 4 IL-7 KO mice did not show detectable signs of bone marrow involvement at this time point or displayed extremely low leukemia cellularity (<0.1%). All IL-7 WT and most IL-7 KO mice, except those without signs of leukemia, displayed splenomegaly (data not shown) due to infiltration...
Figure 1. IL-7 accelerates leukemia expansion and leukemia-related death in mice xenotransplanted with human T-ALL cell lines. A, Rag2−/−/Il2rg−/− mice, either Il7+/+(IL-7 WT; n = 3) or Il7−/− (IL-7 KO; n = 3), were injected i.v. with 10⁷ HPB-ALL.Luc.GFP cells and analyzed 5 weeks posttransplantation. Animals were injected i.p. with 150 μg luciferin/g 7 minutes before imaging and ventrally scanned for total-body luminescence (total flux), with a medium binning 3-minute exposure, using IVIS Lumina and Living Image software. Images on the left are overlays of the luciferase signal with photographs of each animal. Corresponding bioluminescence values are represented as mean ± SEM in the graph on the right (P = 0.0245; Student t test). B–E, IL-7 WT (black; n = 9) or IL-7 KO (red; n = 10) mice were injected i.v. with 10⁷ TAIL7 cells. B, human leukemia cells were monitored in the blood through time by flow cytometric analysis with an anti-human CD2 antibody. Differences between IL-7 WT and IL-7 KO groups were statistically significant (P = 0.0047; 2-way ANOVA). C, animals were sacrificed 84 days posttransplantation, and bone marrow infiltration of TAIL7 cells was evaluated as described in the Materials and Methods. Animals were classified as leukemic if presenting more than 20% T-ALL cells in the bone marrow, preleukemic if 20% or less, and nonleukemic if less than 0.1%. D, infiltration of TAIL7 cells in the spleen was analyzed at the same time point (P = 0.0782; Mann–Whitney test). Lines indicate median values of each group. E, survival of TAIL7-transplanted IL-7 WT (n = 8) and IL-7 KO mice (n = 14) was compared. Kaplan–Meier survival curves are indicated. Disease progression was significantly accelerated in IL-7 WT mice (P = 0.0023; log-rank test). Experiments in A and E were carried out once. Results in B–D are representative of 3 independent experiments.
with malignant blasts (Fig. 1D). The nonlymphoid organs analyzed (liver, kidney, and lungs) also presented leukemia infiltration (Supplementary Fig. S2). However, IL-7 KO mice consistently displayed a more heterogeneous distribution and lower median values of malignant infiltration in all lymphoid and nonlymphoid organs analyzed (Fig. 1C and D; Supplementary Fig. S2). Importantly, we found that the median survival of TAIL7-transplanted IL-7 WT mice (97 days) was significantly shorter than that of IL-7 KO mice (138 days; \( P = 0.0023 \); log-rank test; Fig. 1E). Postmortem analysis showed that all animals died of leukemia with multiple organ infiltration (Supplementary Fig. S3). Hence, although our data

Figure 2. IL-7 accelerates leukemia expansion in mice xenotransplanted with human primary T-ALL cells. IL-7 WT \(( n = 3)\) and IL-7 KO \(( n = 4)\) mice were injected i.v. with \( 5 \times 10^6 \) primary T-ALL cells. A, human T-ALL cells were monitored in the blood through time by flow cytometric analysis, using an anti-human CD2 antibody. Differences between IL-7 WT and IL-7 KO groups were statistically significant \(( P = 0.0259; 2\)-way ANOVA\). Representative results of 1 of 2 independent experiments are shown. Animals were sacrificed 112 days posttransplantation and (B) bone marrow \(( P = 0.0002; \text{Student } t \text{ test})\), (C) spleen \(( P = 0.0014)\), liver \(( P = 0.0040)\), kidney \(( P = 0.0873)\), and lung \(( P = 0.0044)\) were disrupted into a cell suspension and analyzed for primary T-ALL cell infiltration by flow cytometry with an anti-human CD5 antibody. Results indicate mean \( \pm \) SEM for each organ and represent a pool from 2 independent experiments using 2 different patient samples for a total of 4 IL-7 WT and 6 IL-7 KO mice.

Figure 3. IL-7 promotes p27Kip1 downregulation, Bcl-2 upregulation, proliferation, and viability of xenotransplanted human primary T-ALL cells. IL-7 WT and IL-7 KO mice were injected i.v. with \( 5 \times 10^6 \) primary T-ALL cells from 2 distinct patients in independent experiments. Animals were sacrificed 112 days posttransplantation and primary T-ALL cells in the bone marrow were discriminated by flow cytometry with anti-human CD5 antibodies. A, Bcl-2 protein levels were assessed by flow cytometry after intracellular staining of primary T-ALL cells (patient 1) with FITC-conjugated anti-Bcl-2 antibody. Left, results are representative of both patients analyzed. Specific MIF, as described in Materials and Methods, is indicated in the histogram for each condition. Right, Bcl-2 levels in T-ALL cells collected from IL-7 WT and IL-7 KO mice \(( P = 0.0006; \text{Student } t \text{ test})\). Results indicate mean \( \pm \) SEM and represent a pool from the 2 different patient samples for a total of 4 IL-7 WT and 6 IL-7 KO mice. B, primary T-ALL cells (patient 1) recovered from IL-7 WT \(( n = 3)\) and IL-7 KO \(( n = 2)\) mice were stained with Annexin V-PE and viability was determined by flow cytometry. Left, a representative histogram overlay is shown with the percentage of Annexin V-negative cells indicated. Right, percentage of Annexin V-negative T-ALL cells collected from IL-7 WT and IL-7 KO mice \(( P = 0.0514)\). Results represent a pool from the 2 different patient samples for a total of 4 IL-7 WT and 2 IL-7 KO mice. C, total bone marrow cells from IL-7 WT \(( n = 3)\) and IL-7 KO \(( n = 6)\) mice were lysed, resolved by 10% SDS-PAGE, and immunoblotted with anti-p27Kip1 antibody. Left, membrane was stripped and reprobed with malignant blasts (Fig. 1D). The nonlymphoid organs analyzed (liver, kidney, and lungs) also presented leukemia infiltration (Supplementary Fig. S2). However, IL-7 KO mice consistently displayed a more heterogeneous distribution and lower median values of malignant infiltration in all lymphoid and nonlymphoid organs analyzed (Fig. 1C and D; Supplementary Fig. S2). Importantly, we found that the median survival of TAIL7-transplanted IL-7 WT mice (97 days) was significantly shorter than that of IL-7 KO mice (138 days; \( P = 0.0023 \); log-rank test; Fig. 1E). Postmortem analysis showed that all animals died of leukemia with multiple organ infiltration (Supplementary Fig. S3). Hence, although our data

Published OnlineFirst May 18, 2011; DOI: 10.1158/0008-5472.CAN-10-3606
IL-7 Accelerates Human T-ALL In Vivo

A

B

C

D

with actin to confirm equal loading. Patient 1, lanes 1, 2, 6, and 7; patient 2, lanes 3 to 5 and 8 to 11; total lysates of TAIL7 cells were used as a positive control. Right, corresponding densitometric analysis of p27Kip1 levels, normalized to actin loading control and expressed in arbitrary units (a.u.), in IL-7 KO and IL-7 WT mice (P = 0.0260). D, Ki-67 protein levels were assessed by flow cytometry after intracellular staining of primary T-ALL cells with PE-conjugated anti-Ki-67 antibody. Left, a representative histogram overlay is shown. Right, percentage of Ki-67−positive T-ALL cells collected from IL-7 WT and IL-7 KO mice (P = 0.0121). Results represent a pool from the 2 different patient samples for a total of 4 IL-7 WT and 6 IL-7 KO mice.
indicate that lack of IL-7 is not sufficient to prevent leukemia progression and leukemia-related death, IL-7 clearly contributed significantly to leukemia acceleration in vivo.

Both IL-7 KO and IL-7 WT mice lack γ-chain, which is essential for efficient IL-7–mediated signaling by forming the IL-7R together with IL-7Rα (26, 27). Thus, the differences observed in leukemia expansion should largely reflect the direct impact of IL-7 on T-ALL cells, which display both IL-7Rα and γ-chain (11, 28). For confirmation, we transplanted growth factor–independent, IL-7Rα-negative P12 T-ALL cells and analyzed IL-7 KO and WT mice at 4 weeks. Both strains showed similar signs of leukemia (Supplementary Fig. S4).

**IL-7 accelerates leukemia expansion in mice xenotransplanted with human primary T-ALL cells**

To ask whether the influence of IL-7 was a more general feature of T-ALL and not restricted only to leukemia cell lines, we xenotransplanted primary T-ALL cells collected from 2 patients at diagnosis, which differed in their developmental maturation block (Table 1). According to the EGIL criteria (22), the samples were arrested at the cortical (patient 1) and mature (patient 2) thymocyte stage, whereas TAIL7 cells are pre-T and HPB-ALL are cortical but differ from patient 1 in the expression of CD3 (Table 1). Similar to the cell lines, we observed a longitudinal increase in the percentage of circulating T-ALL cells, with systematically higher levels in the presence of IL-7 (Fig. 2A). In addition, IL-7 WT mice culled 112 days posttransplantation presented significantly higher leukemia involvement in the bone marrow than IL-7 KO animals (Fig. 2B). Furthermore, absence of IL-7 clearly impaired infiltration of organs such as spleen, liver, kidney, and lungs (Fig. 2C).

**IL-7 promotes p27Kip1 downregulation, Bcl-2 upregulation, proliferation, and viability of xenotransplanted human primary T-ALL cells**

We previously showed that IL-7 promotes survival and cell-cycle progression of T-ALL cells in vitro via Bcl-2 upregulation and p27Kip1 downregulation (12, 13). We sought to understand whether the same mechanisms and functional effects were responsible for the accelerated leukemia progression in the presence of IL-7 in vivo. We collected T-ALL cells from the bone marrow of mice sacrificed at 112 days and analyzed them for viability and Bcl-2 expression. Primary leukemia cells collected from IL-7 WT animals displayed significantly higher Bcl-2 levels (Fig. 3A) and increased percentage of viable cells (Fig. 3B), in agreement with the observation that IL-7 transgenic mice present upregulation of Bcl-2 protein (29). Conversely, p27Kip1 levels were downregulated in T-ALL cells recovered from IL-7 WT mice (Fig. 3C), which presented higher Ki-67 expression, indicative of increased in vivo proliferation (Fig. 3D).

**Leukemia cells in T-ALL patients appear to respond to and consume IL-7**

IL-7 circulating levels are regulated by consumption (30) and IL-7 signaling leads to the transcriptional downregulation of IL-7Rα (31). Although cancer cells may grow independently of external stimuli, our data argue that a significant fraction of human T-ALL cells respond to and benefit significantly from the presence of IL-7 in vivo. We collected evidence directly from patient specimens at diagnosis supporting this notion. We found that T-ALL cells had significantly lower IL7RA mRNA levels than normal T-cell precursors (Fig. 4A) and that IL-7 levels were decreased in the plasma of T-ALL patients as compared with healthy age-matched controls (Fig. 4B), suggesting that T-ALL cells consume IL-7 and thereby downregulate IL-7Rα. In support of this notion, we found that 4 of 6 patient samples cultured ex vivo in medium without IL-7 showed upregulation of IL-7Rα surface expression, which was prevented by the addition of IL-7 (Fig. 4C). Taking into account that more than 70% of T-ALL patient samples respond to IL-7 ex vivo and express functional IL-7Rs (11, 28), our current observations suggest that human T-ALL cells from a majority of patients might be sensitive to IL-7 signaling in vivo in the actual disease setting.

**Discussion**

There is mounting evidence that microenvironmental factors can contribute to tumor progression. IL-7 is produced in the thymus and bone marrow, the microenvironments where T-ALL develops and expands. Interestingly, IL-7 has been shown to have oncogenic potential in vivo. Transgenic mice expressing IL-7 in lymphocytes develop B- and T-cell lymphomas (1) as a consequence of an autocrine loop involving IL-7 stimulation via IL-7R (2). However, there is no evidence that human T-ALL cells express IL-7, largely excluding the existence in this malignancy of an IL-7–dependent autocrine loop that has been suggested to play a role in other hematologic cancers (32, 33). Thus, in the present study, we explored the alternative hypothesis that IL-7 produced by the microenvironment may promote human T-ALL expansion in vivo.

Our data indicate that IL-7 does not affect the engraftment and initial homing capacity of T-ALL cells to the bone marrow or other organs. However, it remains to be evaluated whether the late differences between IL-7–deficient and IL-replete mice regarding leukemia infiltration into organs such as spleen and liver reflect modulation of leukemia cell trafficking from the bone marrow to these organs and/or of malignant cell proliferation within each infiltrated organ.

We found that lack of IL-7 is not sufficient to prevent leukemia progression and leukemia-related death, such that all animals died of leukemia irrespective of IL-7. This may reflect the acquisition of growth factor independence or the effect of other microenvironmental stimuli (e.g., γc–signaling cytokines (11), Notch ligands (34), or chemokines such as CCL25 and CXCL13 (35, 36) that may provide T-ALL cells with alternative growth signals in the absence of IL-7. This notwithstanding, IL-7 clearly contributes significantly to leukemia acceleration in vivo. Curiously, despite the evidence that mouse and human IL-7 have similar in vitro bioactivity toward human lymphocytes (24, 25), including T-ALL cells (25), it was recently shown that in vivo IL-7 present in the mouse is less active on human cells (37). This suggests that the effect of IL-7 on human leukemia progression in the RAG KO xenotransplant models we used may constitute an underestimation of
the impact of IL-7 on human T-ALL patients. Moreover, because cancer cells are generally viewed as nonreliant on growth factor signals (38), the fact that IL-7 accelerated disease progression is especially remarkable. The related questions of whether IL-7 promotes survival of a putative leukemia stem cell population and participates in T-ALL initiation remain to be scrutinized.

At the molecular level, we found that IL-7–related disease progression was associated with decreased p27Kip1 expression and upregulated Bcl-2. Given our previous demonstration that regulation of p27Kip1 and Bcl-2 is mandatory for IL-7–mediated antipapoptotic and proliferative effects on T-ALL cells in vitro (13), it is reasonable to infer that the same mechanisms underlie T-ALL expansion in vivo in response to IL-7. In support of this possibility, it is noteworthy that T-ALL patient samples frequently display p27Kip1 downregulation (39) and Bcl-2 upregulation (40). Moreover, most primary T-ALL samples show PI3K pathway activation (17), which may be the consequence not only of cell autonomous mechanisms (17, 41, 42) but also of microenvironmental stimuli such as IL-7 (12). The importance of IL-7/IL-7R signaling is further illustrated by the observations that in vitro IL-7 responsiveness (28) and IL7RA gene expression (43) appear to have prognostic value in T-ALL. Notably, our studies indicate that the in vivo impact of IL-7 on T-ALL cells is not restricted to a particular maturation stage or CD4/CD8 immunophenotype (Table 1). These observations are reminiscent of our in vitro findings, in that T-ALL patient samples proliferated in response to IL-7 irrespective of the stage at which they were developmentally blocked (11).

Altogether, our data provide the first clear evidence that human T-ALL cells utilize the IL-7/IL-7R axis for expansion in vivo, providing experimental support to the hypothesis that responsiveness to a γc-signaling cytokine contributes to T-ALL development in vivo (5, 44). Because the majority of T-ALL cases are known to respond to IL-7 in vivo (11) and thus possibly benefit from IL-7 stimulation in vitro, therapeutic approaches that integrate targeting IL-7, IL-7R, or key elements in IL-7/IL-7R–mediated signaling into current protocols may prove beneficial for the treatment of T-ALL. In addition, our observations confirm the need for caution when administering IL-7 in the context of cancer immunotherapy (45) and suggest that the promise that IL-7 holds as an immunorecombinant (46, 47) and in enhancing immunologic responses against cancer (48, 49) should be further evaluated strictly in patients suffering from cancers that are well characterized as refractory to the cytokine.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Dr. Paulo Lúcio for kindly providing the immunophenotypic analysis of primary patient samples, Dr. Miguel Abecasis for generously providing the thymic specimens, and Daniel Ribeiro, Nádia Correia, Júlia Ferreira de Vasconcellos, and Priscila P. Zenatti for their invaluable help. We especially acknowledge the contribution of patients and their families, and Dr. Mário Chagas and all the physicians and nurses at the Pediatric Service of Instituto Português de Oncologia de Lisboa, in providing primary samples.

Grant Support

This work was supported by grants from Associação Portuguesa Contra a Leucemia, FCT (POCI/SAN-UBI/58913 and PTDC/SAN-UBI/104816), and...
References


36. Guipponi Z, Jie X, Youxin J, Qun W, Wei J, Chun L, et al. Selectively frequent expression of CXCR5 enhances resistance to apoptosis in...
IL-7 Contributes to the Progression of Human T-cell Acute Lymphoblastic Leukemias

Ana Silva, Angelo B.A. Laranjeira, Leila R. Martins, et al.

Cancer Res 2011;71:4780-4789. Published OnlineFirst May 18, 2011.

Updated version: Access the most recent version of this article at: doi:10.1158/0008-5472.CAN-10-3606

Supplementary Material: Access the most recent supplemental material at: http://cancerres.aacrjournals.org/content/suppl/2011/05/18/0008-5472.CAN-10-3606.DC1

Cited articles: This article cites 48 articles, 27 of which you can access for free at: http://cancerres.aacrjournals.org/content/71/14/4780.full#ref-list-1

Citing articles: This article has been cited by 7 HighWire-hosted articles. Access the articles at: http://cancerres.aacrjournals.org/content/71/14/4780.full#related-urls

E-mail alerts: Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions: To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions: To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.