Interleukin 7 requirement for survival of T-cell acute lymphoblastic leukemia and human thymocytes on bone marrow stroma

We explored the role of interleukin-7 (IL-7) in the bone marrow (BM) stroma-mediated survival of primary T-cell acute lymphoblastic leukemia (T-ALL) cells and normal thymocytes. We present evidence that IL-7 has a major role in the enhanced survival mediated by BM stroma both in T-ALL cells and thymocytes.

Table 1. Immunophenotype and classification of T-ALL patients.

<table>
<thead>
<tr>
<th>T-ALL (case)</th>
<th>CD1a</th>
<th>CD2</th>
<th>CD3</th>
<th>CD4</th>
<th>CD5</th>
<th>CD7</th>
<th>CD8</th>
<th>Maturation stage</th>
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</thead>
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<td>III</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>III</td>
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</table>

*T-ALL samples from patients were considered positive for a defined antigen if at least 30% of the leukemic cells were positive compared to an isotype matched control. *In all cases, greater than 90% of blasts were positive for TdT, and cytoplasmic CD3. **T-ALL maturation stages of primary samples were defined as previously described. Stage II: pre-T-ALL; stage III: cortical T-ALL; stage IV: mature T-ALL.

derived from normal thymuses of children (<5 years of age) undergoing cardiac surgery. BM stroma induced a significant increase of survival in both T-ALL cells and thymocytes (Figure 1A and 1B), as measured by annexin V-fluoroscein isothiocyanate/propidium iodide staining (Bender Med System, Vienna, Austria) and flow cytometry (FACSCalibur, Becton Dickinson, Palo Alto, CA, USA). The enhanced survival was maintained throughout the period of observation (Figure 1C and D). BM stroma also induced proliferation in all T-ALL cases, but not in thymocytes, as assessed by thymidine-incorporation microassay after culture with 4000-rad irradiated stromal cells (Figure 1E). The proliferative response of T-ALL was maintained
IL-7 enhances T-ALL and thymocyte survival. Primary T-ALL cells (B) or thymocytes (C) were cultured in medium containing IL-7 (10 ng/mL). At the indicated time, T-ALL or thymocyte apoptosis was measured by annexin V/PI staining and flow cytometry. Results are expressed as percentage of annexin V/PI negative cells. Values represent the mean ± SD of three independent experiments with cells from the representative T-ALL case no. 3 (B) and four independent experiments performed with thymocytes from different donors (C). (D) Co-cultures between T-ALL or thymocytes and BM stromal cells were performed in the presence of anti-IL-7 (BMS/IL-7) or anti-IL-7R (BMS/IL-7R) antibodies. Rabbit polyclonal (BMS/pAb) or mouse isotype-matched monoclonal antibodies (BMS/mAb) were used as controls for anti-IL-7 or anti-IL-7R antibodies, respectively. After 1 day of culture, T-ALL or thymocyte apoptosis was measured by annexin V/PI staining and flow cytometry analysis. Cells from T-ALL cases no. 3 and 5, which had a higher number of cells, were used in this experiment. Results are expressed as percentage of annexin V/PI negative cells. Values represent the mean ± SD of three independent experiments for T-ALL cases and the mean±SD of three independent experiments performed with thymocytes from different donors (thy).

We next investigated the functional role of the interaction between IL-7 and its receptor (IL-7R) in the BM-induced survival of T-ALL cells or thymocytes. BM stroma produced IL-7, as evaluated by analyzing supernatants from four independent cultures by an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) (0.8±0.3, 1.4±0.8, 2.1±0.8, 3.3±1.2 pg/mL±SD at days 1, 3, 5, and 7, respectively). All T-ALL cells and thymocytes expressed the IL-7Rα chain (CD127) (Figure 2A) and showed a significant response to recombinant human IL-7 (10 ng/mL, Calbiochem, Merck Biosciences, Darmstadt, Germany), as determined by increased survival revealed by annexin V/propidium iodide analysis (Figure 2B and 2C). Functional blockade of IL-7 or IL-7Rα with antibodies (10 µg/mL, rabbit polyclonal, Biosource International, Camarillo, CA, USA; and monoclonal R34.34, Immunotech, Marseille, France, respectively) during co-cultures significantly decreased the BM-induced survival in both T-ALL cells and thymocytes with respect to control isotype-matched antibodies (Figure 2D). Time course analysis revealed that maximum inhibition of survival occurred after 1 day of co-culture and the effects decreased with time (data not shown). Furthermore, IL-7 or IL-7Rα blockage reduced BM-induced T-ALL proliferation (30% reduction, data not shown). However, as proliferation also depends on cell viability, it is difficult to dissect out the direct effects of IL-7/IL7R blockage from the consequences of reduced cell viability.

In summary our data indicate that (i) BM stromal cells inhibited apoptosis in both human T-ALL and thymocytes, thus extending previous data; (ii) BM stromal cells induced proliferation in T-ALL cells but not in normal thymocytes; (iii) the enhanced survival mediated by BM stroma in both T-ALL cells and thymocytes required the IL-7/IL-7R interaction. However, IL-7/IL-7R blockade only partially inhibited survival, thus suggesting the existence of other mechanisms acting in co-operation with IL-7. Interestingly, it has been reported that LFA-1 and E-selectin are required, although not alone sufficient, for BM-mediated T-ALL survival. Furthermore, the effects of anti-IL-7/IL-7R antibodies on T-ALL and thymocyte survival decreased with time, which could suggest that other cellular events, triggered by IL-7 but acting later on independently of it, could be involved. Taken together, these findings suggest that a complex signaling pathway involving IL-7 activity mediates BM-induced enhanced survival of T-ALL and thymocytes.

In conclusion, our findings support the notion that IL-7 can contribute to the expansion of T-ALL within the BM microenvironment. Furthermore, they highlight a property of BM stromal cells that could be relevant during early human T-cell lymphopoiesis.
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Key words: T-ALL, thymocytes, BM stroma, IL-7, survival, proliferation.

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References

Job offer

Our Hematology department at Hopital Avicenne/Paris 13 University will have an Assistant Professor position available during 1, 2, 3 or 4 years starting in November this year (2007), which is accessible to all MDs who have graduated in Hematology, Oncology, Internal medicine or pediatrics (for the last two, some experience in hematology would be required), and are EU citizens. The job includes clinical work in our department, and clinical and possibly lab research on our favorite subjects: MDS, AML, MPD other than CML, and CLL. However, we have also a large recruitment of myelomas and lymphomas, especially T lymphomas, and because there is a large African population in this Paris area, a lot of patients with sickle cell anemia are also followed. The monthly net wages are about 2700 euros. The idea is obviously to help the candidate publish as many papers as possible in peer-reviewed journals. A permanent, tenured position may be available at the end of the 4 years, but this is still being negotiated.

Our hospital is very close to Charles de Gaulle airport, and about 30 mn by subway from the center of Paris (Gare du Nord, etc…). If you have a candidate, please contact me at the address below, preferably by e-mail.

May I thank you in advance.

Yours

Pierre Fenaux

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