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.

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T-cell acute lymphoblastic leukemia (T-ALL) cells and thymocytes, their normal counterpart, undergo spontaneous apoptosis when cultured *in vitro*. Co-cultures with bone marrow (BM)[1,2] or thymic stroma[3] can induce T-ALL survival and proliferation. However, the molecular mechanisms promoting these effects in the microenvironments remain poorly elucidated. Interleukin-7 (IL-7), produced by BM and thymic stroma, plays a crucial role in the development of normal T cells[4] and contributes to the pathogenesis of T-cell leukemia.[5] IL-7 induces survival[6] and proliferation of early thymocytes[7] and regulates survival, cell cycle, and growth of primary T-ALL cells.[5]

To explore the mechanisms involved in the enhanced survival mediated by BM stroma, we cultured primary T-ALL cells or normal human thymocytes *in vitro* with either human BM stromal cells obtained from seven healthy donors, as previously described,[8] or the murine M2-10B4 fibroblast-like cell line of BM stromal origin (kindly provided by Dr Connie J Eaves, Terry Fox Laboratories, Vancouver, Canada). T-ALL cells were derived from enriched leukemic cells isolated from adult patients and classified according to their maturation stage by immunophenotypic analysis (Table 1), as previously described.[9] T-ALL cells were considered positive for a defined antigen if at least 30% of cells were positive compared to the isotype-matched control. Thymocytes were 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

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

	Immunophenotype*º							
T-ALL (case)	CD1a	CD2	CD3	CD4	CD5	CD7	CD8	Maturation stage^
1	+	+	–	+	+	+	+	III
2	+	+	+	+	+	+	+	III
3	+	+	+	+	+	+	+	III
4	–	+	–	–	+	+	–	II
5	+	+	–	+	+	+	+	III

**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. ºIn 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.[9] Stage II: pre-T-ALL; stage III: cortical T-ALL; stage IV: mature T-ALL.*

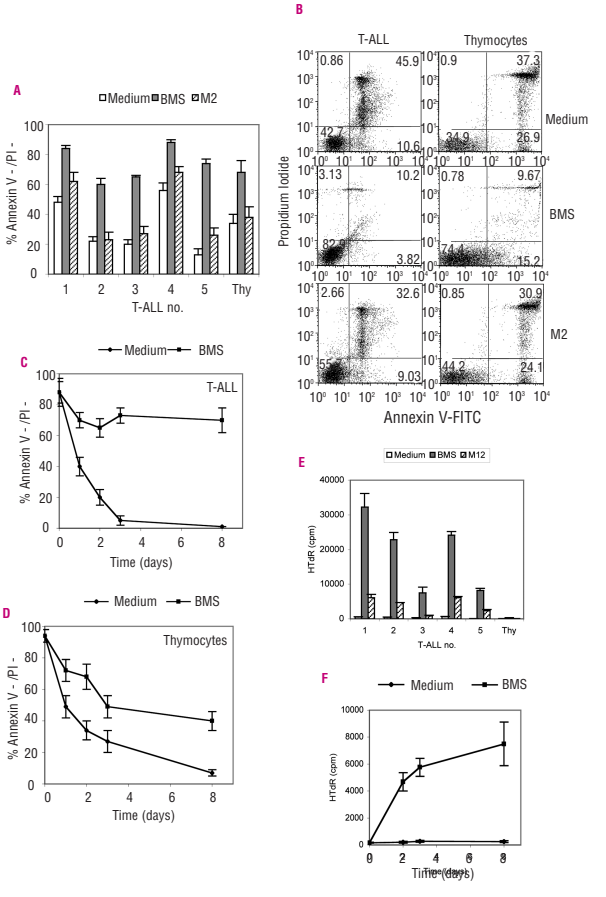

Figure 1 (right). BM stromal cells enhance T-ALL-cell and thymocyte survival and promote proliferation of T-ALL cells. T-ALL cells or thymocytes were cultured in medium alone, or in the presence of normal BM cells or M2.10B4 stromal cells. Cell apoptosis was measured by annexin V/PI staining and flow cytometry and proliferation was examined by thymidine incorporation. (A) Percentage of annexin V/PI-negative cells after 2 days of co-culture with stromal cells. Values represent the mean±SD of three independent experiments for T-ALL cases or the mean±SD of four independent experiments performed with thymocytes from different donors (thy). (B) Flow cytometry analysis of annexin V/PI-stained cells from the representative T-ALL case n. 1 (left panel) and one representative thymocyte preparation (right panel), after 2 days of co-culture with stromal cells. (C) Time course analysis of annexin V/PI-negative T-ALL-cells cultured with BM stromal cells. Values represent the mean±SD of three independent experiments with cells from the representative T-ALL n. 3. (D) Time course analysis of annexin V/PI-negative thymocytes cultured with BM stromal cells. Values represent the mean±SD of four independent experiments performed with thymocytes from different donors. (E) BM stromal cells, T-ALL cells or normal thymocytes were cultured for 8 days in medium alone, in the presence of BM stromal cells or M2.10B4 cells. Results are expressed as cpm. Values represent the mean±SD of three independent experiments for T-ALL cases and the mean±SD of four independent experiments performed with thymocytes from different donors (thy). (F) Time course analysis of thymidine incorporation of T-ALL cultured with BM stromal cells. Results are expressed as cpm. Values represent the mean±SD of three independent experiments with cells from the representative T-ALL case n. 3.

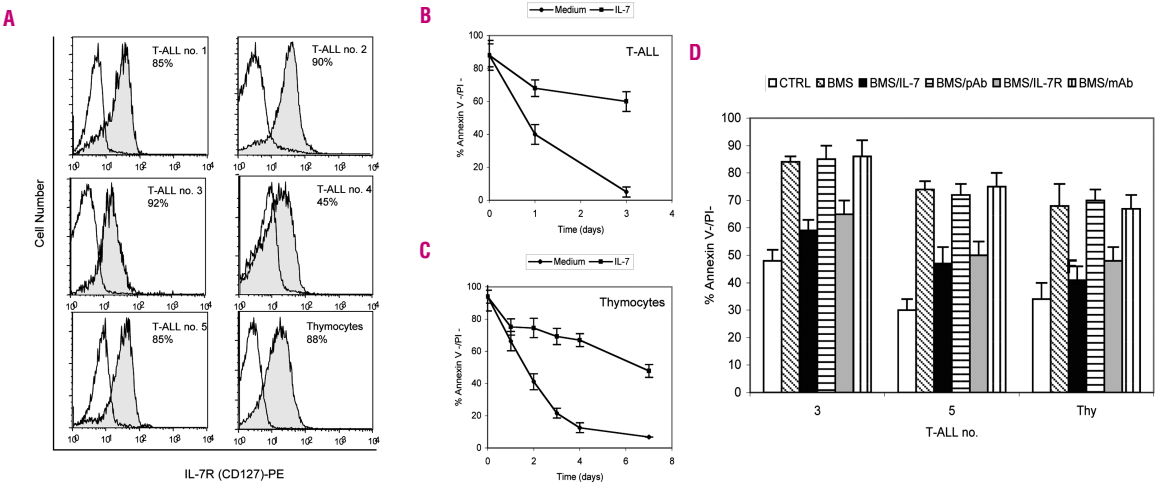

Figure 2. The IL-7/IL-7R interaction is required for BM stroma-mediated increased survival. (A) Surface expression of IL-7R (CD127) in T-ALL cells and thymocytes. The filled histogram curve corresponds to the CD127 profile and is superimposed over an open histogram curve corresponding to the control isotype-matched monoclonal antbody. The percentage of CD127-positive cells is indicated for each histogram. (B-C) 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 n. 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 n. 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).

throughout the period of observation (Figure 1F). In contrast, neither BM stroma nor M2-10B4 induced significant proliferation in normal thymocytes at any time point considered (days 2, 3, and 8; *data not shown*) and at different thymocyte:stromal cell ratios (ranging from 0.1:1 to 10:1). Comparable levels of survival and proliferation were observed when cells from the same patient were cultured with BM stromal cells from different donors (*data not shown*).

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 blockage 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;[1,2,10] (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.[2] 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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