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ORIGINAL ARTICLE

Analysis of Normal Hematopoietic Stem and Progenitor Cell Contents in Childhood Acute Leukemia Bone Marrow

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Background and Aims. Childhood acute leukemias (AL) are characterized by the excessive production of malignant precursor cells at the expense of effective blood cell development. The dominance of leukemic cells over normal progenitors may result in either direct suppression of functional hematopoiesis or remodeling of microenvironmental niches, contributing to BM failure and AL-associated mortality. We undertook this study to investigate the contents and functional activity of hematopoietic stem/progenitor cells (HSPC) and their relationship to immune cell production and risk status in AL pediatric patients.

Methods. Multiparametric flow cytometry of BM aspirates was performed to classify AL on the basis of lineage and differentiation stages and to analyze HSPC and immune cell frequencies. Controlled co-culture systems were conducted to evaluate functional lineage potentials of primitive cells. Statistical correlations and inter-group significant differences were established.

Results. Among 113 AL BM aspirates, 26.5% corresponded to ProB, 19.5% to PreB and 32% contain ProB and PreB differentiation stages, whereas nearly 9% of the cases were T- and 13% myeloid-lineage leukemias. We identified ProB-ALL as the subtype endowed with the highest relative contents of HSPC, whereas T-ALL and PreB-ALL showed a critically reduced size of both HSC and MLP compartments. Notably, lower cell frequencies of HSPC in ProB-ALL correlated to high-risk prognosis at disease debut.

Conclusions. HSPC abundance at initial diagnosis may aid to predict the clinical course of ALL and to identify high-risk patients. A clearer understanding of their population dynamics and functional properties in the leukemia setting will potentially pave the way for targeted therapies. © 2016 IMSS. Published by Elsevier Inc.

Key Words: Acute lymphoblastic leukemia, Stem/progenitor cells, Lymphoid immune cells, Bone marrow, ProB-ALL high risk.

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Introduction

The most important disease endangering the hematopoietic system in pediatric patients is acute leukemia (AL), which constitutes the major cause of death worldwide (1,2). Particularly, childhood AL in Mexico shows among the highest incidence rates with extraordinary heterogeneity and inferior outcomes (3). Almost 85% of the cases compromise the lymphoid linage, whereas 15–20% are from myeloid origins (2). Historically, T-lineage leukemias have been considered the subtype endowed with the most adverse scores (4). However, accumulating evidence of cytopenias and impaired hematopoiesis in some B- and myeloid-AL, often resulting in bone marrow (BM) failure and fatal outcomes has suggested that ineffective hematopoiesis is the clinical hallmark of all AL (4). The high proliferative activity and oligoclonal dominance of leukemic cells over the normal hematopoietic stem and progenitor cells (HSPC) displace them in a dynamic competition for the BM niches (1). As leukemic-initiating cells (LICs) coexist with normal primitive cells within the $CD34^+$ cell compartment, no surface markers can be used so far to distinguish normal HSPC from LICs, making the normal vs. malignant population dynamics still uncertain (5).

Hematopoietic primitive cells fulfill their functional production of blood cells in the context of microenvironmental regulation. Emerging research sustains the notion that neoplastic cells are also dependent on the surrounding microenvironment and point to the influence of specialized niches for leukemia progression (6–9). Hematopoietic suppression as a result of excessive leukemic growth may be contributed by direct inhibition of developing cells by soluble factors, by the outcompetition for stem cell niches and by leukemic cell-induced microenvironment remodeling, creating a leukemic sanctuary unsuitable for normal hematopoiesis (10).

The biological and molecular mechanisms involved in the displacement of normal hematopoiesis are currently under investigation by a number of groups. Our recent findings suggest that primary B-lineage ALL cells are capable of producing pro-inflammatory cytokines and abnormal amounts of growth factors that interfere with regular hematopoietic proliferation and differentiation processes (11). Moreover, exhaustion of the HSPC compartment has been demonstrated in chronic myeloid leukemia (CML) in a leukemic cell-derived IL-6 dependent manner (12). Elegant animal models have been useful to confirm that leukemic cells hijack and remodel normal mouse niches displacing normal hematopoiesis to favor leukemic progression concomitant to down-regulation of the primary chemokine CXCL12 in BM (13,14). Accordingly, we have reported that cell frequencies of functional HSPC are critically reduced in B-ALL patients (15), with our recent data suggesting that mesenchymal reticular niche within the leukemic BM is not able to sustain *ex vivo* normal lymphopoiesis due to a diminished expression of CXCL12, but facilitating instead the maintenance and progression of primary ALL cells (Balandrán JC et al., submitted). Of note, abnormal BM mesenchymal stromal cell (MSC) niches in a T-ALL mice model were shown to be unable to support HSPC due to their accelerated senescence (16).

Strikingly, minimal residual disease (MRD) detection has been the most important prognostic indicator of treatment success or failure, with the supposed concomitant emergence of normal hematopoiesis responsible for repopulating the BM following remission (17). To date, no reports monitoring HSPC numbers and function in childhood AL or correlation studies with clinical outcomes are recorded. Rebounding of normal hematopoiesis should be reflected in blood and BM cell frequencies close to homeostatic values. Interestingly, experimental data from a competitive repopulation in a pre-clinical model showed a good correlation of HSPC with good prognosis, with increasing doses of normal HSPC delaying the LIC establishment (18). Similar observations have resulted from experimental models of AML (19).

Mature B, T, NK and dendritic cells as well as macrophages are a substantial part of the BM microenvironment. As all immune cells derive from HSPC, the evaluation of alterations in such a primitive compartment is of high relevance. In solid tumor settings, immune cell contents constitute a prognosis factor where global outcomes depend on immunoregulatory cell subsets abundance. Further immunity evaluation in cancer will provide better information with prognostic value (20). A novel immunoscore allows classification of malignancies within an immune context where, remarkably, infiltration of subsets of T cells has been associated with good prognosis in several solid tumors (21), whereas activation of T cells in leukemias contribute to tumor surveillance and control (22).

In this work the contents and functional activity of HSPC and their relationship to immune cell production and risk status in acute leukemia have been investigated. Among five subtypes of AL (ProB-ALL, PreB-ALL, ProB/PreB-ALL, T-ALL and AML), we found that ProB-ALL has the highest contents of HSPC, whereas T-ALL and PreB-ALL showed scarce HSC and MLP cells. Notably, lower cell frequencies of HSPC in ProB-ALL correlated to high-risk prognosis. To the best of our knowledge, this is the first report describing BM normal hematopoietic content in the immune context in childhood AL.

Materials and Methods

Patient Characteristics and Sample Collection

One hundred thirteen children diagnosed with ALL were included in this study: 63 were referred to the "Hospital para