CORRESPONDENCE

Quality of Repopulation in Nonobese Diabetic Severe Combined Immunodeficient Mice Engrafted With Expanded Cord Blood CD34+ Cells

To the Editor:

In a recent issue of *Blood*, Guenechea et al[1] reported that ex vivo cytokine-expanded CD34+ cord blood cells retained nonobese diabetic/severe combined immunodeficient (NOD/SCID) repopulating activity of fresh cells, although there was a significant delay in the time to achieve similar levels of engraftment. They also found that there was little change in the quality of engraftment in terms of represented lineages 120 days after inoculation, although they suggest a trend toward a reduction of the myeloid (CD33+) compartment.

To develop a clinically applicable protocol, we have recently examined the potential for expansion of cord blood progenitors under serum-free conditions. As part of this study, we analyzed the characteristics of NOD/SCID engraftment of cells cultured ex vivo for 14 days (Fig 1). In contrast to the findings reported above, we found that culture with interleukin-6 (IL-6) (10 ng/mL), IL-11 (10 ng/mL), Flt3-ligand (FL) (50 ng/mL), and thrombopoietin (TPO) (10 ng/mL) was associated with a switch from dominant B lymphopoiesis (CD19+) toward myelopoiesis (CD13+) in animals analyzed after 40 days in the absence of in vivo cytokine support. Furthermore, in other studies using alternative combinations of cytokines (IL-3 [20 ng/mL], IL-6 [20 ng/mL], stem cell factor [SCF] [100 ng/mL], and FL [100 ng/mL]), we found that the same switch in engraftment quality occurred after 48 hours of ex vivo culture (Demaison et al, in press). These findings can be interpreted in two ways. Firstly, this may reflect a forced commitment of the mutipotent SCID-repopulating cell (SRC) to myelopoiesis, and/or selective inhibition of B-lymphoid commitment, and is similar phenotypically to the patterns of engraftment observed in NOD/SCID mice supplemented in vivo with FL either alone or in combination with IL-7 and SCF, or a combination of SCF, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-3.[2] If this is the case, our findings suggest that these changes in commitment are initiated by exposure to cytokines in vitro and, more importantly, do not revert spontaneously in vivo. Secondly, there may be a selective expansion or retention of SRCs intrinsically restricted to the myeloid lineage. This possibility is supported by previous studies that used retroviral marking or radiation-induced chromosomal aberrations to track distinct cell lineages in mice, and our own recent experiments that indicate that murine retroviral vectors can selectively mark ex vivo–cultured human CD34+ cells that repopulate NOD/SCID mice in a myeloid-restricted pattern[3,4] (Demaison et al, in press). Furthermore, good evidence has emerged for a murine clonogenic common lymphoid precursor (CLP) population that possesses lymphoid-restricted (T, B, and natural killer) repopulating activity.[5] Heterogeneity within the SRC compartment is not surprising. For example, a small population of CD34−lin− cells has the capacity to repopulate in a pattern similar to that of cord blood and adult CD34+ populations.[6] Conversely, second-gestation human fetal blood samples engraft in the absence of cytokine support with a pronounced erythroid bias when compared with cord blood or adult samples (Pahal G and Thrasher AJ, unpublished observations, 1999).

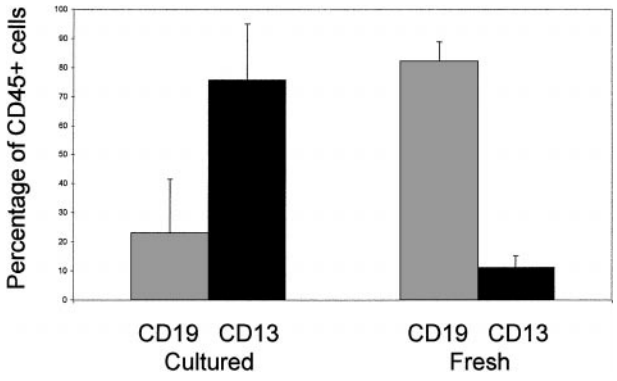

Fig 1. Lineage distribution in NOD/SCID mice engrafted with fresh cord blood CD34+ cells or cord blood CD34+ cells cultured for 14 days in serum-free medium supplemented with IL-6 (10 ng/mL), IL-11 (10 ng/mL), FL (50 ng/mL), and TPO (10 ng/mL). Nine mice engrafted with 2 × 10^5 to 10^6 CD34+ cells from 2 cord samples were analyzed in each group.

As clearly shown in another recent publication, the time for reconstitution after cord blood transplantation, which is directly related to the cell dose, may be a significant limiting factor.[7] Selective expansion of alternative repopulating cell populations, such as those restricted to the myeloid lineage, may therefore be of significant benefit for hematopoietic support in the early time period after cord transplantation. At the same time, potential changes in the commitment of repopulating cells initiated by ex vivo culture need to be clarified. The reasons for the differences in engraftment quality noted in Guenechea's study and our own are not entirely clear but could relate to the timing of analysis (120 *v* 40 days), or to differences in ex vivo culture conditions. Further studies on the quantitation, kinetics, and quality of repopulating cell engraftment after ex vivo culture of cord blood cells are therefore warranted if stem cell expansion protocols are to be effectively optimized for clinical use.

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API1-MALT1/MLT Is Involved in Mucosa-Associated Lymphoid Tissue Lymphoma With t(11;18)(q21;q21)

To the Editor:

Dierlamm et al[1] reported in the June 1 issue of *Blood* that an apoptosis inhibitor gene, API2 at 11q21, and a novel gene, MLT at 18q21, are involved in t(11;18)(q21;q21) associated with mucosa-associated lymphoid tissue (MALT) lymphoma.[1] We recently reported the results of a breakpoint analysis of an MALT lymphoma with t(11;18)(q21;q21) by means of fluorescence in situ hybridization (FISH) analysis using YAC contig at 18q21, which identified a YAC clone, y789F3, encompassing the breakpoint region.[2] Our further analysis of t(11;18)(q21;q21) with the YAC clone has identified a novel gene at 18q21, which was found to be derived from the same gene as the MLT gene.[3] This gene was designated as MALT1 in accordance with the recommendation by the genome nomenclature committee.

We first would like to point out that there is some confusion regarding the name of the 11q21 gene. Dierlamm et al[1] described the 11q21 gene as API2 in their report, but the sequence shown in their article represents that of API1 rather than API2 as assessed by the GenBank registry (GenBank Accession No. NM001165). The other three names already designated to the 11q21 gene, namely c-IAP2,[4] HIAP1,[5] and MIHC,[6] were correctly used in Dierlamm's article and, again, the sequence of these genes is identical to that of API1. We have also confirmed the presence of API1(c-IAP2)-MALT1/MLT fusion transcripts by using reverse transcription-polymerase chain analysis (RT-PCR) for our series of MALT lymphomas with t(11;18) as noted in our report.[3] To avoid any further confusion, we strongly suggest that API2 should be corrected to API1.

We next would like to point out a few but significant differences between Dierlamm et al's results[1] and ours[3] regarding the analysis of the MALT1/MLT gene. In the former, it is mentioned that the MLT transcript is approximately 3.0 kb and the predicted MLT protein consists of 729 amino acids (data not shown in the article).[1] Our analysis, on the other hand, indicated that the MALT1 transcripts are 4.5 kb and 3.1 kb and the open reading frame of MALT1 predicted a protein of 813 amino acids.[3] The Northern blot analysis with MALT lymphoma with t(11;18)(q21;q21) demonstrated additional aberrant signals in the range from 6.4 kb to 9.4 kb.[3] Furthermore, the deletion of 11 amino acids has been noted in our MALT1 sequence (API2-MLT amino acids 625 to 635; GRTDEAVECTE).[1,3] In our analysis, 3 out of 5 MALT1 cDNA clones did not contain the 11 amino acids, whereas the remaining 2 cDNA clones did (unpublished results, March 1999). We tentatively chose the cDNA sequence lacking the 11 amino acids for the MALT1 sequence,[3] and consider it most likely that the difference between the two sequences is due to alternative splicing of the MALT1/MLT gene. Although the true size of MALT1/MLT amino acids remained to be determined, awareness of the above-mentioned differences should be helpful for readers who plan to perform molecular genetic analysis of the t(11;18) translocation. Further studies are warranted to clarify the essential role of API1-MALT1/MLT fusion protein in the molecular pathogenesis of MALT lymphomas.

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