Commentary

The Development of B Cells in the Bone Marrow Is Controlled by the Balance Between Cell-autonomous Mechanisms and Signals from the Microenvironment

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B cells are generated from hematopoietic stem cells (HSCs) in the liver during the fetal life, and in the bone marrow in the adult. The differentiation pathway from HSC to mature B cell can be divided into several stages, based on the phenotype and on functional properties that cells of the B lineage progressively acquire. Progenitor B (pro-B) cells can be identified by cell surface expression of B220, CD43, and c-kit. Differential expression of heat-stable antigen (HSA) and of the maturation marker BP-1 discriminates four fractions of pro-B cells (fractions A, B, C, and C′ [1]). At this stage of development, DNA rearrangement begins in the Ig H chain locus. Most pro-B cells of fraction A carry Ig genes in germline configuration. $D_H \rightarrow J_H$ rearrangements are found in almost all cells of fraction B. $V_H \rightarrow D_H J_H$ rearrangement occurs in fractions C and C′ (2). Cells in fractions B to C′ are also called pre-B I cells. As soon as μH chain proteins appear in the cytoplasm and can be assembled into a functional precursor B cell receptor (pre-BCR), pre-B I cells develop into large pre-B II cells that are c-kit and CD43 negative (3). Successful rearrangement of the H chain and a correctly assembled pre-BCR associated with a functional signaling machinery allow pre-B II cells to proliferate. How is this proliferation controlled, what stimulates it, and what terminates it? When proliferation stops, pre-B II cells become smaller (so-called small pre-B II) and rearrangement starts again, this time in the L chain locus. Immature B cells subsequently express a complete IgM molecule on their surface. B cells leave the bone marrow at the transitional B cell stage and complete their final development into mature B cells in the periphery (4).

Analysis of mice with mutations of genes involved in the rearrangement process and in BCR signaling has shown that the pre-BCR (2) and the BCR (4) regulate the progression of cells of the B lineage along their differentiation pathway at earlier and later stages of development, respectively. However, the microenvironment in the bone marrow also plays a role of fundamental importance. When HSCs are injected intravenously, long-term B cell poiesis is established only in the bone marrow, suggesting that only this microenvironment supports the growth and differenti-

ation of cells of the B lineage in the adult. The liver functions as the site of hematopoiesis only transiently, during the fetal life. Although stromal cells are the key elements of the microenvironment of the bone marrow, very little is known about their biological diversity and functional characteristics (5). Stromal cells form a network in the intersinusoidal spaces of the bone cavity that extends from the endostium to the endothelial cell basement membrane of the sinusoids. Hematopoietic cells differentiate and proliferate in the interstices of this network in close contact with long cytoplasmic processes of stromal cells (6, 7). One major function of stromal cells is the production of IL-7. IL-7 was originally cloned from a stromal cell line as a factor supporting the growth of pre-B cells in vitro. Because pre-B cells do not grow on stromal cells that fail to produce IL-7, this cytokine is an indispensable requirement for B cell development (8). The receptor for IL-7 (IL-7R) on lymphocytes is a heterodimer composed of an α and a γc chain. The α chain is shared by the IL-7R and by the receptor for the recently identified cytokine thymic stromal lymphopoietin (TSLP [9]). The common γc chain is employed for signaling purposes by receptors for IL-2, IL-4, IL-9, and IL-15 in addition to IL-7 (10). The generation of mice deficient for individual elements of the IL-7/IL-7R system has shed light on the in vivo function of this cytokine. Mice that lack IL-7 (11), the IL-7R α chain (12), or the common γc chain (13) all show a block of B cell development at the pro-B stage. This results in a strong reduction of the pre-B cell population and, consequently, of the mature B cell pool in the periphery. The defect is more severe in mice lacking the IL-7R α chain than in mice lacking IL-7 or the γc. In the IL-7R$\alpha^{-/-}$ mice, D_H-J_H recombination is not affected, but germline transcription from distal unrearranged V segments does not occur. This essentially blocks recombination of distal V_H segments (14). It has been shown that transcription of V_H gene segments is controlled by the transcription factor Pax-5. The analysis of Pax-5$^{-/-}$ mice has shown recently that Pax-5 is indispensable for the irreversible commitment of hematopoietic precursors cells to the B lineage. B cell development is arrested at the pro-B cell stage in Pax-5$^{-/-}$ mice. Like normal pro-B cells, Pax-5$^{-/-}$ pro-B cells undergo $D_H J_H$ rearrangement, express B cell-specific genes, and grow in vitro in the presence of IL-7. Surprisingly, however, these pro-B cells are not committed to the B lin-

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eage, and maintain the ability to develop into other hematopoietic cell types in vitro (15) and in vivo (16). Interestingly, expression of Pax-5 and of Pax-5–dependent genes is reduced in IL-7Rα–deficient mice (14). By augmenting Pax-5 expression, IL-7 may facilitate the commitment of hematopoietic cells to the B lineage.

B cell development is also blocked at the pro-B cell stage in IL-7$^{-/-}$ mice (11). However, in this case, rearrangement occurs, all fractions of pro-B cells develop, and a few mature B cells populate the spleen. This shows that although differentiation of B cells proceeds unimpaired, the proliferative expansion of pre-B cells is abolished. This phenotype is similar to that of γc$^{-/-}$ mice (13). Because $V_H \rightarrow D_H J_H$ rearrangement is impaired only in IL-7Rα–deficient mice, we have to postulate that another factor at least partially replaces the function of IL-7 in pro-B cells of mice deficient for IL-7 or the γc chain. TSLP is a good candidate for this function, because it supports the differentiation of pre-B cells into membrane-bound (m)IgM$^+$ B cells in long-term bone marrow cultures (LTBMCs) without inducing proliferation. In addition, its receptor uses the IL-7R α chain, but not the γc chain (9).

A recent study has dissected the function of different domains of the α chain of the IL-7R (17). Proliferation of pro-B cells in response to IL-7 was abrogated by a single mutation (Tyr449→Phe) in the cytoplasmic tail of the IL-7R α chain, which prevents the recruitment of phosphatidylinositol-3 kinase (PI-3 kinase) upon ligand binding. PI-3 kinase is indispensable for the induction of proliferation by many growth factors. The importance of PI-3 kinase for the expansion of the pre-B cell pool was confirmed by the analysis of mice deficient for the p-85α subunit of PI-3 kinase. In these mice, pro-B cells are present, but pre-B cells are reduced, and only a few B cells can be found in the periphery. The mutated IL-7R α chain retains the ability to induce $V_H \rightarrow D_H J_H$ rearrangement in pro-B cells, showing that these two functions of the IL-7R are physically separated in the cytoplasmic tail of the α chain.

In conclusion, IL-7 expands the diversity of the antibody repertoire by inducing $V_H \rightarrow D_H J_H$ rearrangement in pro-B cells. The proliferation of the pre-B cells leads to the selective expansion of the cells, which have successfully rearranged the H chain and express a functional pre-BCR.

However, recent data have demonstrated that the expansion of the pre-B cell pool is not only controlled by IL-7. Two papers from the group of C. Paige at the Ontario Cancer Institute have shown that IL-7 plays different roles in different stages of B cell development, and that the expression of the BCR modulates the response to IL-7 (18, 19). Both pro-B and pre-B II cells can be induced to proliferate in culture in the presence of IL-7. However, pre-B cells proliferate in response to picogram amounts of IL-7, whereas only nanogram amounts can induce pro-B cells to divide. The extreme sensitivity of pre-B cells to IL-7 depends on the expression of the pre-BCR, because the ability of recombination activating gene (RAG)$^{-/-}$ pro-B cells to proliferate is increased by an H chain transgene (18). It has been shown that stromal cells contain IL-7 in the cyto-

plasm but release only minimal amounts of it, too small to be detectable in the supernatant. Pre-B cell lines proliferate only when they are in direct contact with primary stromal cells, probably because sufficient concentrations of IL-7 can be supplied only at the surface of the stromal cells (7). In vivo, therefore, pre-B cells are provided with low amounts of IL-7 via intimate contact to stromal cells. It is possible that under these conditions only pre-B II cells proliferate, because their threshold of response is lower. Although IL-7 concentrations may be insufficient to induce proliferation of pro-B cells, they nevertheless suffice to induce $V_H \rightarrow D_H J_H$ rearrangement in pro-B cells. This implies that either the signaling machinery used by the IL-7R is different in pro- and pre-B cells, or that the signal emanating from the IL-7R is integrated with signals that are simultaneously generated by the pre-BCR. Thus, the eventual outcome of IL-7 signaling would be dependent on the "signaling context" in which the signal is given. The signaling context is predicted to vary with the absence or presence of additional receptors and, therefore, with the differentiation stage of the cell. Biochemical studies are necessary to clarify this point.

Rolink et al. (20) in this issue address the question of the interplay between IL-7 and the pre-BCR and demonstrate that they have distinct and overlapping functions in B cell development. Sorted pro-/pre-B I cells undergo two to five rounds of proliferation in vitro in the absence of IL-7 and stromal cells. This proliferative expansion requires the expression of the pre-BCR, because pro-/pre-B I cells of λ$_5$$^{-/-}$ mice do not proliferate under these conditions. After 3–5 d in culture, >95% of the recovered cells were cytoplasmic μH chain (cμ)$^+$, and most of them expressed mIgM, indicating that they had successfully rearranged the L chain genes. These data demonstrate that the presence of the pre-BCR is indispensable and sufficient for the proliferation and differentiation of pre-B cells in vitro, and that the microenvironment of the bone marrow is not necessary for these developmental steps. However, Rolink et al. also show that IL-7 does have a function, because 10–15 times higher numbers of cells can be recovered after culture in saturating concentrations of IL-7. This is mainly due to the rescue of pro-/pre-B I cells that failed to produce a functional pre-BCR. The number of cells that express cμ and mIgM is increased to a lesser extent. Therefore, the expression of a functional pre-BCR is sufficient to direct a developmental program that includes few rounds of proliferation followed by L chain rearrangement and differentiation to mIgM B cells. IL-7 increases proliferation, but does not induce differentiation.

A final interesting point is addressed by this paper. Single pro-/pre-B I cells sorted and cultured in microwell plates proliferate with the same frequency and at the same extent as in bulk culture. This strongly suggests that the signal initiated by the pre-BCR does not depend on an external ligand. The pre-BCR, similarly to the BCR, organizes a complex of signaling molecules in proximity of the cell membrane (21). The presence of this complex may be sufficient to generate a constitutive signal, which changes the

signaling context of the cell and causes the last proliferation and differentiation steps.

It is important to realize that IL-7 also plays a role in the development of T cells. Its function is different in γ/δ and α/β T cells. IL-7 controls germline transcription and rearrangement in the TCR-γ locus (22). In α/β T cells, IL-7 instead promotes survival and proliferation. Interestingly, it has been shown recently that the development of α/β T cells is driven by independent and overlapping signals generated by IL-7 and by the pre-TCR (23). Therefore, the interplay between their lineage-specific receptors (pre-BCR, pre-TCR) and IL-7 regulates development in both B and T cells.

The development of pro- and pre-B cells is a clear example of how the microenvironment and the expressed receptors cooperate to decide upon the fate of a cell (Fig. 1). Pro-B cells receive signals from the IL-7R, which induces $V_H \rightarrow D_H J_H$ rearrangement. At the pre-B II cell stage, these signals are then integrated with those generated by the pre-BCR complex, resulting in extensive cell proliferation. As soon as the pre-B II cells are deprived of the IL-7 signal, either because contact to stromal cells is lost or because the expression of the IL-7R is downregulated (18), only the pre-BCR guides its development. Proliferation stops, rearrangement is induced on the L chain locus, and the BCR is assembled. The BCR also generates signals in the absence of antigen; it is indispensable for the survival of mature B cells in the periphery (24).

The work described above demonstrates that in B cells, commitment, proliferation, differentiation, survival, and all developmental steps are regulated by the finely tuned balance between cell-autonomous mechanisms (pre-BCR, BCR) and signals provided by the microenvironment (IL-7, TSLP). This leads to the selective development of B cells, which are fit for their function because they express a correctly assembled pre-BCR, and are fit for their environment because they respond to cytokines. Stromal cells produce and release IL-7. Therefore, they play an important role in the regulation of B cell development in the bone marrow, mainly controlling the size of the B cell compartment (from commitment to the pre-B cell stage). In the bone marrow, different types of hematopoietic cells develop. It is reasonable to hypothesize that different types of stromal cells exist and organize lineage-specific microenvironments (5). The structure created by the stromal cells is often considered a fixed characteristic of the bone marrow that reproducibly regulates the production of a defined number of cells of each lineage. However, the number of B cells produced from the bone marrow changes with age (7) and appears to be regulated by the size of the peripheral B cell pool (25). Therefore, a mechanism must be postulated that controls the plasticity of the bone marrow microenvironment and regulates the function or the number of stromal cells able to produce IL-7 and, eventually, the number of newly produced B cells.
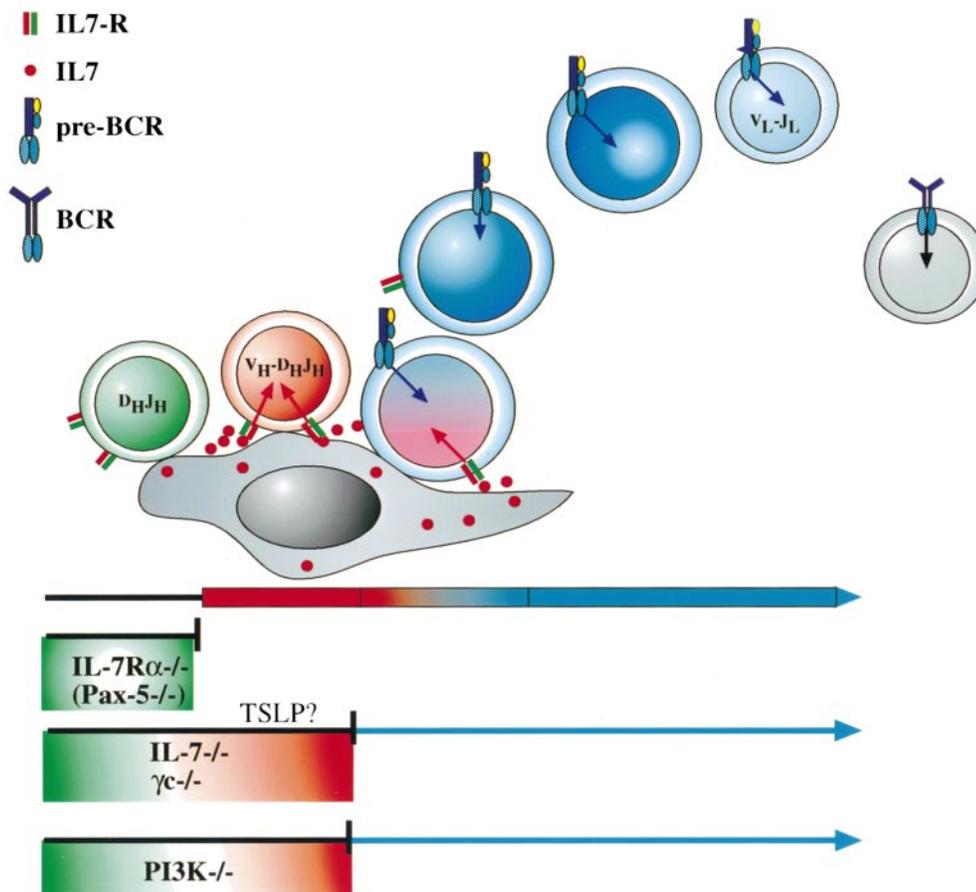

Figure 1. The interplay between IL-7 and the pre-BCR in the bone marrow. Stromal cells produce and release IL-7. Pro-B cells of fraction A (green) express the IL-7R, and their Ig H chain genes are in germline or D_H-J_H configuration. $V_H \rightarrow D_H J_H$ rearrangement is induced in pro-/pre-B I cells of fraction B–C after an IL-7–generated signal (red). At the large pre-B II cell stage, the expression of the pre-BCR generates a constitutive signal (blue), which is integrated with the signal induced by IL-7 (red). Pre-B cells progressively lose the contact to stromal cells and downregulate the IL-7R. At this point, they exclusively depend on the pre-BCR for the last phases of development. Immature B cells (light gray) express a complete BCR. The top line represents the B cell developmental pathway in normal mice. The color code indicates the developmental phases that depend on IL-7 (red) or on the BCR (blue). The other lines show the developmental block seen in mutant mice. The thin blue line shows that in the indicated mutant mice pre-BCR–dependent development can proceed, but without extensive proliferation.

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