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Commentary

B lymphopoiesis: Global factors, local control

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The bone marrow is the only site in adult mammals where B lymphocytes differentiate from stem cell precursors. Much effort has therefore been devoted to learning what is unique about this highly specialized tissue. While a number of factors influence the replication and differentiation of lymphohemopoietic precursors (1–3), none is restricted in its distribution to bone marrow. This situation can now be generalized to five more molecules that have recently been found to influence the survival and proliferation of B-cell precursors. Indeed, some of them are hormones previously known for their effects on other biologic processes. It appears likely that B-lineage differentiation in the bone marrow microenvironment is determined by a unique combination and concentration of molecules rather than by tissue-restricted factors.

Interleukin 7 (IL-7), which is made by keratinocytes and stromal cells in bone marrow, spleen, thymus, and kidney, is the best established replication factor for pre-B cells (4). Especially compelling are two recent studies showing that pre-B cells are virtually absent from mice injected with antibodies to either IL-7 or its receptor (5, 6). However, while IL-7 is necessary for B-lymphocyte formation, it is clearly not sufficient. Additional signals, presumably present in the bone marrow microenvironment, are required for efficient recruitment to, and differentiation of cells within, this lineage (3, 7, 8). It is these signals that recent studies have sought to define in molecular terms.

Kishimoto and colleagues (9) used the stromal cell-dependent pre-B-cell clone DW34 as a biological indicator to clone a soluble mediator, which they designated pre-B-cell growth stimulatory factor (PBSF) (9). The 89-amino acid protein stimulated proliferation of DW34 cells and enhanced their response to IL-7. Moreover, while PBSF markedly augmented the proliferative effect of IL-7 on freshly isolated bone marrow cells, it had little effect when used alone.

PBSF has structural homology to members of the intercrine α family, which includes proinflammatory cytokines already known to influence replication of stem cells and myeloid progenitors (10). A PBSF clone, termed SDF-1α, had previously been isolated by Honjo and colleagues (11), who devised a unique strategy. They prepared cDNA libraries enriched for genes with consensus signal sequences of transmembrane and secreted proteins and used a vector that places an epitope tag on expressed proteins. Selection of transfected COS-7 cells with an antibody to that epitope increased the probability of isolating open reading frames. Although PBSF/SDF-1α was cloned from a stromal cell library, its expression is widespread.

The route to discovery is often as interesting as the discovery itself. This is certainly the case for another pre-B-cell costimulatory factor, BST-1 (12). Hirano and colleagues prepared numerous transformed bone marrow stromal cell lines from normal individuals and patients with rheumatoid arthritis. Some of these human cell lines, and especially those from arthritis patients, supported substantial proliferation of the murine DW34 pre-B-cell line. A monoclonal antibody that preferentially recognized stromal cells with high support capability was prepared and used to isolate the gene encoding this antigen. The BST-1 gene encoded a glycosyl phosphatidylinositol-anchored protein that was, as expected, highly expressed on rheumatoid stromal cells. Moreover, when introduced by transfection, the BST-1 gene conferred the DW34 support capability upon otherwise nonsupporting fibroblasts. Finally, as was true for PBSF, BST-1 was expressed in many normal tissues (T. Hirano, personal communication).

A third factor was isolated from stromal cells because of its ability to enhance the emergence of B-lineage cells in culture and was identified as insulin-like growth factor I (IGF-I) (13). There is no indication that this ubiquitous hormone alone stimulates growth of B-cell precursors. However, it does promote proliferation of IL-7-dependent lymphocyte clones and normal marrow cells, which have an early phenotype (14, 15). Furthermore, lymphocytes, including pre-B cells in bone marrow, increased substantially in mice infused with recombinant IGF-I (ref. 16; K. Dorshkind, personal communication).

RNA from activated lymphocytes was the starting point for isolation of another cytokine gene (17). Samal and colleagues used degenerate oligonucleotides corresponding to signal peptidase processing sites found in a number of cytokines. These were used to screen a cDNA library. One of several clones encoded a 52-kDa protein that stimulates growth of pre-B cells in the presence of IL-7 and stem cell factor. Transcripts for this protein, pre-B-cell colony-enhancing factor (PBEF), were found in many tissues.

IL-7, PBSF, BST-1, IGF-I, and PBEF all appear to be positive regulators of B-cell formation. While negative regulators have been less extensively studied, they may also enjoy a wide tissue distribution. A potentially important regulatory mechanism for B lymphopoiesis was unexpectedly discovered in this laboratory (18). B-cell precursors, including IL-7-responsive cells, markedly and selectively declined in bone marrow of pregnant mice. Recent immigrants in the spleen also declined, but mature B-cell numbers were unaffected. Suspecting that these changes related to hormonal fluctuations during pregnancy, we found that estrogen was particularly effective for depletion of B-lineage precursors in nonpregnant mice (19). Progesterone alone had no effect, but it dramatically reduced the effective dose of estrogen when the two hormones were given together. Glucocorticoids also deplete pre-B cells (20), but initial culture experiments suggest an important difference in mechanisms of action. While glucocorticoids may act directly to elicit apoptotic pre-B-cell death, estrogen is suppressive only in the presence of stromal cells (21, 22).

Additional studies with hypogonadal animals suggest that sex steroids contribute to normal steady state regulation (G. Smithson, W. G. Beamer, K. L. Shultz, S. W. Christianson, L. D. Schultz & P.W.K., unpublished data). The mice are genetically incapable of synthesizing these hormones and have greatly elevated B-lineage precursors in bone marrow. Sex steroids must therefore be added to a relatively small list of potential negative regulators of B lymphopoiesis (22). There are interesting parallels to studies of the thymus, where the same hormones suppress proliferation of immature T lymphocytes, and age-related involution of the thymus is partially restored by castration (reviewed in ref. 22).

All of these results argue against the hypothesis that B-cell differentiation in

marrow is determined by tissue-specific cytokines. What are the alternatives? The effects of hormones such as erythropoietin are primarily restricted to marrow because erythroid progenitors there are among the very few cell types with receptors for it. In contrast, estrogen and IGF-I receptors are found on many cell types in many tissues. Bone marrow could be the unique site of B-cell emergence because the combination and concentrations of essential cytokines are unique, even though none of those factors is individually restricted to that location. In this respect, marrow might resemble the gonads, which also provide unique microenvironments for differentiation of highly specialized cells. Local production of sex steroids in the gonads depends on sequential conversion of blood-borne precursors by two closely coordinated cell types (Sertoli/Leydig and theca/granulosa cells) (23). Similarly, aromatases made by fat cells in marrow might permit local production of estrogen in excess of systemic levels (24). Immobilization of cytokines on extracellular matrices or via transmembrane anchoring domains could also achieve high local concentrations of essential signals.

Locally produced enzymes might contribute to a unique bone marrow microenvironment in other ways. Stromal cells synthesize both IGF-I and several of the six known IGF binding proteins (IGF-BPs) (25). IGF-BPs can either enhance or inhibit the cellular effects of IGF-I, with the outcome determined by posttranslational modifications catalyzed by kinases and proteases (26). Proteolysis of IGF-BPs can be triggered by IGF-I binding (27). A number of membrane-bound ectoproteases are known to be expressed on stromal cells and/or B-cell precursors (reviewed in ref. 28). Secreted proteases might also contribute to the bone marrow microenvironment through effects on cytokines. For example, cathepsins released by breast stromal cells in response to estrogen are capable of activating latent type β transforming growth factor (TGF-β) (29). Other studies suggest a connection between sex hormones and TGF-β activity in the thymus and ovaries (23, 30).

It is unlikely that all of the local and widely disseminated components of the bone marrow microenvironment have been identified, and discovery of these cytokines/interaction molecules raises a number of questions. With the exceptions of IL-7 and sex hormones, we have little idea of their importance *in vivo* and it is probable that a degree of redundancy will be demonstrated. Indeed, overlapping functions at the level of cytokines or their receptors provide hemopoietic cells with the potential for highly sophisticated responses (31). All five of the recently described molecules influence the survival and/or proliferation of IL-7-responsive cells. There is a pressing need to learn about other cellular targets and whether these newly discovered mediators also influence differentiation of B-lymphocyte precursors. While B cells are well established as models for selective gene rearrangement and expression, we know comparatively little about the environmental cues that trigger these events.

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