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Immune-Cell Lineage Commitment: Translation from Mice to Humans

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Much of the current understanding in hematopoietic stem cell differentiation into immune-cell lineages comes from mouse studies, but how well does it translate to the human system?

The pathways through which hematopoietic stem cells (HSCs) generate lineage-committed progenitors and ultimately the mature cells of the blood and immune system have been progressively defined during more than 40 years of investigation. Most of the fundamental concepts on which we base our understanding of hematopoietic differentiation have relied on the analysis of specific murine mutants and/or the use of experimental murine transplantation models. The power and elegance of these experimental models have allowed a progressively more-detailed dissection of murine lymphohematopoietic development; however, comparatively few similar studies exist in humans.

The reasons for the predominance of murine over human studies in hematopoiesis and immunology have been technical, logistical, and ethical. The ability to manipulate the expression of single genes in the mouse allows targeted gain- and loss-of-function studies within the developing animal, often leading to well-defined, mechanistic conclusions. Competitive-repopulation models allow quantitation of murine stem and progenitor cells with defined patterns of repopulation. Within each inbred murine strain, data are highly reproducible, a stark contrast to the marked variability inherent in immunophenotypic and functional studies with human cells. It should be noted, however, that substantial differences in immunophenotype and function can be seen between murine strains (de Haan et al., 2000; Spangrude and Brooks, 1992). It is hardly surprising then, that even greater biological differences exist between murine and human species.

Species differences range from detailed technical considerations to fundamental biological processes. Immunophenotypic differences between mice and humans are particularly striking in the study of hematopoietic stem and progenitor cells. For example, with the exception of a rare subset, the majority of human HSCs express the CD34 antigen; further fractionation of $CD34^+$ cells reveals that lineage commitment and loss of generative and repopulating capacity are marked by the onset of CD38 expression (Hao et al., 1996; Larochelle et al., 1996; Terstappen et al., 1991). In contrast, the expression of CD34 and CD38 is reversed in murine hematopoiesis, with long-term repopulating capacity contained within the $CD34^-CD38^+$ subset and short-term repopulation in the $CD34^+CD38^-$ subset (Osawa et al., 1996; Randall et al., 1996).

Functional proof of a HSC population is generally accepted to require the use of a transplantation model that allows long-term engraftment of cells with full lymphohematopoietic potential. In murine studies, this is most often accomplished by competitive repopulation of donor cells into lethally irradiated congenic hosts. The use of xenogeneic in vivo models to study engraftment and differentiation of isolated human cells, although considered the gold standard for human HSC studies, has obvious limitations. The most commonly used model for human studies, the NOD-Scid immune-deficient mouse, generates predominantly B cell progenitors, with little myeloid and no T cell engraftment. It is assumed that the lineage skewing seen in this, as well as the related NOD-SCID-β_2-microglobulin-deficient mouse, is from selective lineage differentiation due to incomplete species cross-reactivity of signals from the microenvironment. It is also possible that these models have selective defects in the type of human cells able to home and engraft successfully. Recently described models with more-profound blocks in thymopoiesis, such as the Rag2 and IL2Rγ double-deficient mouse and the NOD-SCID IL2Rγ-deficient mouse, provide an environment for more-complete lineage (including T lymphoid) differentiation for human cells and have substantially extended the kinds of questions investigators can now ask in vivo with human cells. Nevertheless, it remains unclear exactly how each of these immune-deficient xenogeneic models reflects the biology either revealed by the assays of congenic murine transplant or seen in the clinical bone marrow (BM) transplantation setting. An example of such confusion is how long-term versus short-term repopulation should be defined in xenogeneic models, a concept that is one of the classic tests for assigning stem versus progenitor status. Can we necessarily expect a human HSC to exhibit normal clonal activation and self-renewal patterns in a mouse environment?

The logistical and regulatory difficulties in obtaining human tissue for research also act to discourage such studies. Largely as a response to this problem, umbilical cord blood, which is discarded as waste after most

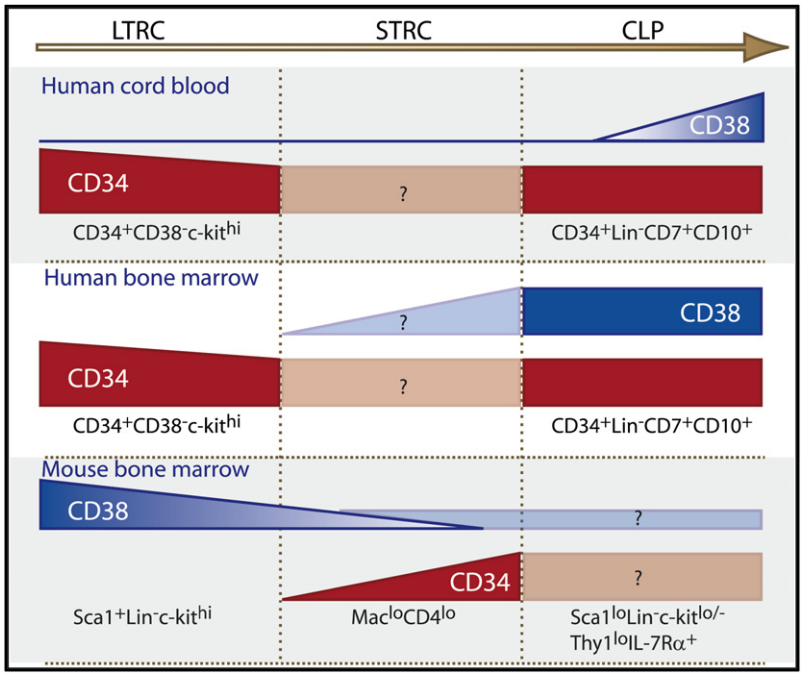

Figure 1. Reciprocal Expression of CD34 and CD38 between Human and Murine Stem and Lymphoid Progenitors

In humans, CD34 is expressed on both HSCs (LTRC, long-term repopulating cells; and STRC, short-term repopulating cells) and lymphoid progenitors. A subset of cord-blood CLPs does not express either CD38 or IL-7Rα; these appear to be the precursors of $CD38^+$ CLPs in CB. No such $CD38^-$ CLP exists in human BM, i.e., CD38 is expressed on all BM CLPs. No phenotype has yet been defined that discriminates between human STRC and LTRC. CD34 and CD38 expression on murine CLP has not been reported.

deliveries and can be collected from donors without risk or discomfort, has become the predominant tissue source for the study of human hematopoietic stem and progenitor biology. However, similar cautions as those described above should be exercised in extrapolating data from one human hematopoietic source to another. The potential confusion this can cause is illustrated in more detail in two examples that involve IL-7 regulation of lymphopoiesis: the identification of the early stages of lymphoid commitment and the role of IL-7 in B lymphopoiesis.

For many years, lymphoid and myeloid cells have been assumed to be generated from the hematopoietic stem cell through mutually exclusive pathways of differentiation that pass initially through either the common lymphoid progenitor (CLP) or a myeloid-erythoid progenitor, respectively. The murine CLP was identified in 1997 by Kondo et al., using expression of the IL-7 receptor α (IL-7Rα) to mark lymphoid-restricted progenitors within the $Sca\text{-}1^{lo}$, $Thy\text{-}1^-ckit^{lo}$, lin^- population of BM (Kondo et al., 1997). This finding fit well with the known phenotype of IL-7Rα-deficient mice, i.e., a lack of B, T, and natural killer (NK) cells in combination with normal HSC function and myeloid differentiation. Two years earlier, Galy et al. (1995) had identified in the human BM a $CD34^+lin^-CD10^+$ population that satisfied the criteria of CLP, i.e., full lymphoid (T, B, and NK) but no myeloid or erythroid differentiation potential; the expression of IL-7Rα on the $CD10^+$ CLP in the human BM was not explored at that time. However, more recently a primitive multilymphoid (B, NK, and recently T cell) progenitor was identified in human umbilical cord blood, on the basis of expression of CD7 on a subpopulation of $CD34^+CD38^-$ cells (Hao et al., 2001; Hoebeke et al., 2007). In contrast to the murine CLP, this $CD34^+CD38^-CD7^+$ cord-blood progenitor does not express IL-7Rα. One hypothesis from these findings is that IL-7 acts at the CLP stage in murine, but not human lymphopoiesis, a theory that would fit with the finding that children born with mutations in IL-7R, either in the ligand binding (α) chain or the signaling (γ) domain, have less-severe lymphoid defects than mice with similar mutations (B cell numbers are normal) (Fischer et al., 2005). However, it should be noted that the $CD34^+CD38^-CD7^+$ immunophenotype of cord blood is not found in BM. The previously described $CD34^+Lin^-CD10^+$ lymphoid-restricted population in human BM expresses CD38 and is in fact heterogeneous, including both $CD7^+$ B and NK cell progenitors and $CD7^-$ B cell progenitors (Rossi et al., [2003] and G.M.C., unpublished data) (Figure 1). The expression of IL-7Rα on human CLP from BM would be a more-relevant comparison with the murine studies, but has not yet been elucidated.

The potential for confusion in overlaying data from different stages of ontogeny and from different species is also seen in studies of IL-7 and B cell differentiation. Human B cell production has long been thought to differ from that in the mouse with respect to the requirement for IL-7 (Milne and Paige, 2006). Initial data from in vitro models of murine B cell production and from IL-7- and IL-7Rα-deficient mice indicated that B cell production in the mouse is dependent on IL-7. Subsequent experiments showed that fetal and neonatal B cell production was spared in mice with IL-7 defects, providing evidence that murine B cell production at early points in ontogeny was not dependent on IL-7 (Milne and Paige, 2006). This finding was important because the assessments of patients with IL-7R signaling defects ($T^-NK^-B^+$) as described above occurred in newborns or very young children. In addition, the initial in vitro studies of human B cell development relied on fetal BM as a hematopoietic source. Thus, initial conclusions about differences in mouse and human B cell development were based on comparisons of adult murine B cell production and fetal or neonatal human B lymphopoiesis. It is possible that at least some of the differences in B cell development that have been attributed to evolutionary differences between the

mouse and human immune systems are due to differences in fetal and adult B lymphopoiesis.

More-recent studies of in vitro human B cell development have used CB and adult BM as hematopoietic sources. However, these studies employed cocultures that included murine stromal-cell lines that have been selected for their ability to support mouse B lymphopoiesis. These cell lines produce high amounts of murine IL-7 (Johnson et al. [2005] and K.J.P. et al., unpublished data), and, contrary to initial reports, murine IL-7 has now been shown to stimulate human IL-7R signaling (Johnson et al., 2005). Thus, the requirement for exogenous human IL-7 and its effects on human B cell development have likely been obscured in most studies to date. Experiments using a human stromal-cell coculture model are providing evidence that in humans, as in mice, the production B cells from adult BM is dependent on IL-7 (K.J.P. et al., unpublished data).

If B cell production in adult humans is dependent on IL-7, as it is in the mouse, it will be important to determine whether IL-7 plays identical roles and targets similar B cell precursor populations at equivalent points in ontogeny in both species. Studies by Hardy et al. (Hardy et al., 1991) with subsequent refinements and extensions have correlated immunoglobulin gene rearrangement and intracellular-protein expression with changes in surface-marker expression during mouse B cell differentiation. The resulting model of B cell development that identified precisely defined, developmentally sequential populations of B lineage cells on the basis of surface immunophenotype became the standard used in assessing B cell development in genetically engineered mice. This model provided a valuable tool because it allowed not only the identification, but also the isolation of living B lineage cells at precise points in differentiation. Isolated precursors could then be assessed for transcription-factor expression or function, or they could be placed in culture so that subsequent stages of development could be examined. A number of human studies have identified a variety of surface markers that are helpful in identifying progressive stages in human B lymphopoiesis. However, no single model of human B cell development has emerged as an initial standard for subsequent refinement as it has for the mouse. This has made it difficult to compare results from different laboratories and to fit published data into a comprehensive model of human B cell development that can be used to compare B cell development at different points in ontogeny.

The importance of equivalent tissue sources in comparative studies of murine and human immunity is underscored by the recent identification of a distinct B-1 cell progenitor that selectively predominates during the fetal period in the mouse (Montecino-Rodriguez et al., 2006). Murine studies that go back almost three decades have identified B-1 ($CD5^+$) and B-2 cells ($CD5^-$) as distinct B lymphocyte subsets that perform complementary immune functions (Hardy, 2006). B-1 cells predominate early in life, participate in T independent responses, and generate polyreactive "natural" antibodies that provide protection from common bacterial pathogens. B-2 cells are the "conventional" B lymphocytes that form the majority of circulating B cells in the adult and give rise to the diverse, highly specific, hypermutated antibodies typically associated with the adaptive immune response and immunological memory. Murine B-1 immunoglobulin specificities have been described as relatively "hard-wired." They arise from the selective use of a limited number of immunoglobulin heavy-chain variable (IgH V) gene segments. In addition, the mechanisms for generating diversity that are present in conventional B-2 cells (N nucleotide insertions and somatic hypermutation) are either greatly reduced or absent in murine B-1 cells.

Whether humans produce distinct B lymphocyte subsets that are functionally comparable to murine B-1 and B-2 cells is unclear. In humans, $CD5^+$ B cells do predominate early in life—approximately 85% of B cells in umbilical cord blood express $CD5^+$, whereas the absence of CD5 characterizes ~85% of the B cells in adult peripheral blood (K.J.P. et al., unpublished data). However, the expression of CD5 may not be an indicator of B-1 cells in all species. $CD5^+$ and $CD5^-$ B cells are present in pigs, but they do not give the B-1–B-2 functional dichotomy observed in mice (Wilson and Wilkie, 2007). In addition, CD5 can be upregulated during B cell activation (Hardy, 2006), and there is evidence to suggest that CD5 is transiently expressed during post-BM stages of human B cell development (Sims et al., 2005). Thus, in humans, CD5 may be a marker of B cell lineage, B cell activation, and/or B cell development. Clearly, experiments that seek to identify and functionally assess putative B-1 and B-2 cells in humans will need to be designed to take into account these possibilities.

Mechanisms that function in determining antibody specificities may also vary between mice and humans. B-1 cells, if present in humans, may be more diverse than those in mice because of terminal deoxynucleotide transferase (TdT)-mediated, N nucleotide additions during the immunoglobulin gene-rearrangement process. In humans, TdT is expressed in B cell development during the fetal period and throughout life. In contrast, the expression of TdT in mice is limited to adult B lymphopoiesis. During IgH gene rearrangement in mice, B-1 cells selectively incorporate V gene segments from IgH families that are proximal to the D and J segments, whereas B-2 cells use specificities encoded by distal IgH V gene segments (Hardy, 2006). Whether differential V gene usage distinguishes $CD5^+$ and $CD5^-$ B cells in humans is controversial. Should IgH V gene segments used in human B1 and B2 cells show structural similarity to those in the mouse, the mechanisms involved in specification of IgH V gene usage are likely to be quite different. It is believed that most IgH V gene segments arose from gene-duplication events that occurred after divergence of mice and humans, including a fairly recent expansion of the distal half of the murine IgH V gene locus (de Bono et al., 2004). This is important because the V gene segments that are selectively incorporated in mouse B-2 cells, the V gene segments that selectively require Ezh2-mediated methylation of histone H3 for rearrangement, (Su et al., 2003), the V

gene segments that selectively undergo STAT5-regulated histone H4 acetylation (Bertolino et al., 2005), and the sites that direct nuclear compartmentalization (Yang et al., 2005) are located in the distal portion of the murine IgH V locus. Thus, divergent genetic and epigenetic factors probably impact IgH V gene usage in mice and humans.

Differences in mouse and human centromeric DNAs suggest that there may also be at least subtle variations in epigenetic regulation between mice and humans. Studies of the Ikaros DNA-binding protein provide evidence that this could impact mechanisms that regulate gene expression in the immune system. Pericentromeric heterochromatin in the mouse is relatively homogeneous, including γ satellite repeats with Ikaros binding sites at all centromeres (Cobb et al., 2000). However, multiple unique centromeric DNAs that vary from chromosome to chromosome have been identified in humans, and recent evidence suggests that Ikaros is able to maintain its regulatory function in humans through mechanisms conferred by the selective expression of a unique Ikaros splice variant in human cells (Ronni et al., 2007). This variation in the Ikaros regulatory mechanism between mice and humans is likely to be a factor in B cell development because Ikaros regulates expression of TdT and the λ5 component of the pre-B cell receptor, molecules important in very early stages of B lymphopoiesis.

Thus, critical comparisons between the species require close attention to many variables including those of ontogeny, tissue of origin, and the potential limitations of both in vitro and in vivo assays. Given the fairly obvious risk in assuming that data can be extrapolated from one species to another, it is surprising how rarely this caveat is provided in presentations and publications of data from murine studies. A common tendency is to view data from mice as "generic," rather than specific, with the identity of the species mentioned only in the methods sections of published papers. Clearly, comprehensive models of human lymphoid commitment and differentiation are difficult to establish given the experimental and logistical obstacles. The elegant experimental approaches available only for the analysis of murine biology will remain an essential first step in the process of understanding human systems. However, human models based on fine-tuned definitions of surface-marker expression during progressive stages of ontogeny and in different tissues will be essential tools to define the mechanisms by which human lymphoid commitment and differentiation is regulated.

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