

Lymphocyte development

Editorial overview

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Abbreviations

GC germinal center
pHSC pluripotent hematopoietic stem cell

Lymphohematopoietic cells have to clear many hurdles on the path from pluripotent hematopoietic stem cells (pHSCs) to their ultimate fates as mature members of either the innate or the adaptive immune systems. In addition, lymphocytes of the adaptive immune system must develop into effector cells upon antigenic stimulation. One such pHSC is capable of generating all cells of the innate and the adaptive immune systems, with an estimated 2×10^9 cells in the mouse and approximately 1000-fold more in human. However, only a minority of all the cells that are created as the cells differentiate and proliferate actually then survive to be useful members of these defense systems. As in previous years, this year's articles on lymphocyte development focus on different stages of this wasteful, but in the end very effective, process that builds, and continuously rebuilds, our immune system. The articles address the earliest hematopoietic stem cells and the formation of lymphoid organs, the subsequent molecular events that control progressive fate decisions in lymphoid development and the formation of lymphocyte repertoires. Also addressed are the selective events that couple the specificity of newly formed lymphocyte precursors to their maturation or death, antigen driven differentiation of lymphocyte subtypes, memory cell formation and the homeostatic events that regulate the pools of mature lymphocytes.

The earliest identifiable pHSCs with capacity for long-term reconstitution of the innate and adaptive immune systems and for the erythroid/megakaryocyte/platelet lineages arise in the mouse embryo in the aorta/gonad/mesonephros area just prior to the establishment of blood circulation. The few hundred pHSCs that can be detected in a mouse embryo between day 8.0 and 12.5 after gestation are thought to use the newly established circulatory routes to migrate to competent sites (yolk sac, fetal liver, omentum and bone marrow), where they subsequently establish hematopoietic development. Cumano and Godin (pp 166–171) review the experimental evidence for the roles of adhesion molecules and chemokines in the control of this migration. Also discussed are the transcription factors that guide early

hematopoiesis and impart a flexible program that allows these pHSCs to develop along the alternative pathway leading to the vascular endothelium.

The key roles of chemokines and their receptors in regulating the distribution of early progenitor cells to hematopoietically competent sites, such as fetal liver and bone marrow, and the subsequent retention of these cells within these primary hemato-lymphopoietic organs are described by Ansel and Cyster (pp 172–179). For the differentiation of pHSCs and their early descendants along the B lymphocytic or myeloid lineages, SDF-1 (now called CXCL-12) and its monogamous receptor, CXCR-4, are pivotal whereas T cell development appears unaffected by defects in the genes encoding either CXCL-12 or CXCR-4. The situation appears to be more complex in the thymus, where both in the cortical as well as the medullary regions, multiple chemokine–chemokine-receptor interactions appear to guide cell migration and retention. Ansel and Cyster also review the current knowledge of how chemokines and cytokines control organogenesis of secondary lymphoid organs such as lymph nodes and Peyer's patches, and the centers of immune reactions, the germinal centers, which develop as a consequence of responses to foreign antigens.

Our already detailed knowledge of the cellular stages from early progenitors to B lymphocyte lineage precursors, immature and mature B cells, and to the different types of myeloid cells has allowed the identification of nonredundant transcription factors that control some of the transitions from one to the next stage of cellular differentiation. Kee and Murre (pp 180–185) review the effects of targeted disruption of PU.1, E2-A, EBF, PAX-5 or LEF-1, which lead to arrests of cellular development in myeloid/lymphoid or B lymphoid progenitors. Since E2-A and EBF control the expression of PAX-5, they appear to act upstream of the latter gene. A pattern emerges wherein a hierarchy of pivotal transcription factors appears to control a hierarchical cellular development process. Little is yet known of the target genes that convey properties to cells that are necessary for subsequent differentiation along a given pathway. Since transcription factor complexes are composed of a multitude of proteins, interacting with each other and with promoter and enhancer elements in DNA, it is safe to predict that this analysis of molecular hierarchies of transcription factors controlling expression of critically functional genes will not be simple.

Perhaps the most unexpected phenotype of a targeted disruption of a transcription factor gene is that seen in the PAX-5^{-/-} mouse. The mutation arrests B cell development at the D_HJ_H-rearranged pre-B-I cell stage but introduces, at the same time, a flexibility that allows the cells to dedifferentiate

and redifferentiate to all known myeloid cell lineages, to NK cells, and to thymocytes and T cells. This is probably just the first of more examples to be discovered where commitment of a given cell to a given lineage can become less fixed, more flexible. Whether earlier progenitors, such as pHSCs, maintain a less committed state under normal circumstances, to be flexible for demands that ask them to either become hematopoietic or vascular endothelial cells, needs to be investigated. Similar flexibility might be expected from stem cells in bone marrow which can enter into regeneration of the nervous system or from stem cells in muscle which can be induced to hematopoietic differentiation.

In PAX-5^{-/-} precursor B cells, D_H-to-J_H rearrangements do not irreversibly commit these cells to the B lineage pathway whereas the same cells from normal mice have an overwhelming tendency to enter further B cell differentiation. Hence, they begin to rearrange V_H-to-D_HJ_H segments on the H chain locus, followed by V_L-to-J_L rearrangements on the κL and λL chain gene loci. Grawunder and Harfst (pp 186–194) describe the factors that mediate V(D)J recombination: those that initiate the process by cleavage of both DNA strands and those that process the DNA ends that are formed as signal ends or hairpinned coding ends. A multiprotein postcleavage complex is formed and the cleaved DNA is prepared for end rejoining, which happens by ligation of signal and coding ends. It is suspected that additional, novel components of this multiprotein complex will be discovered since an increasing number of mutations have now been identified that alter the capacity for V(D)J recombination. Obviously V(D)J recombination, in its biochemical features, borders on nonhomologous recombination, which is the basis of genetic variation in evolution and, hence, is one of the central molecular mechanisms that we need to understand.

Once an H- and an L-chain allele have been productively rearranged in a B lineage cell and the resulting μH chain and L chain can combine to form a BCR on the cell surface, an immature B cell is made. The fate of the immature B cell depends on the specificity of its BCR. If that receptor recognizes an autoantigen in the environment with high avidity, it is negatively selected — deleted by accelerated apoptosis. If it has a BCR with low avidity for autoantigens, it can be positively selected. Such cells undergo activation, secrete the BCR as cross-reactive antibody and appear in follicular and marginal zones of secondary lymphoid organs. At least some of such cells are called B1 cells. Their induction, selection, antigen specificities, survival in the periphery and molecular markers are described by Martin and Kearney (pp 195–201).

Mature, conventional B cells are derived from immature B cells that do not recognize autoantigens with any detectable avidity. The migration of these immature cells from the bone marrow to the spleen, their maturation in the spleen and some of the genes that apparently control this two-step development process are the subject of the article by Rolink *et al.* (pp 202–207). Although it is apparent that

immunoglobulin-negative cells are normally unable to leave the bone marrow and enter the peripheral, secondary lymphoid organs, it is still not clear how surface-bound immunoglobulin molecules control, in concert with accessory and costimulatory molecules, the maturation of B cells.

Finally, mature B cells may be stimulated by foreign antigen to enter the cell cycle, mature to immunoglobulin-secreting plasma cells or become long-lived memory B cells. T-cell-independent antigen will not induce longevity and memory. With the help of T cells, however, B cells can become long-lived, can switch the expression of the H chain they express and can hypermutate the V regions of their IgH and IgL chain genes. Consequently, antigen can select the better-binding mutant B cells in germinal centers (GCs) and expand them selectively over other mutants that have not improved their specificity for the antigen. Recently, Honjo and his colleagues [1] made the landmark discovery that a GC-specific activator-induced cytidine deaminase is a key factor controlling two GC-specific events — somatic hypermutation and class switch recombination of immunoglobulin genes. Jacobs and Bross suggest in their article (pp 208–218) that this enzyme may function as a catalytic subunit of an RNA-editing complex or as the deoxy-cytidine deaminase in the regions of DNA where hypermutation and class switching occurs. This deamination could induce staggered DNA double-strand breaks, which are repaired by an error-prone nonhomologous repair pathway, thus introducing mutations.

Several reviews focus on the events of T cell development, activation and memory cell formation. MacDonald, Radtke and Wilson (pp 219–224) review the cellular commitment events and signals that accompany early stages of T cell development. Using a clonal assay, precursor cells restricted to T/myeloid or B/myeloid lineages have been recently defined among fetal progenitor populations. Although the clonal assay confirmed the existence of a T/NK-restricted precursor in the fetal thymus, it failed to detect lymphoid-restricted fetal precursors capable of yielding both T and B cells. Adult marrow, in contrast, reportedly contains ‘common lymphoid precursors’ (CLPs), restricted to T, B and NK lineages. Interestingly, a recent study shows that cytokine signaling through an ectopically expressed cytokine receptor can divert lymphoid-restricted CLPs into the myeloid lineage. Other important findings demonstrate that the Notch1 signaling molecule promotes T cell development at the expense of B cell development in the thymus and thus appears to influence the T cell versus B cell fate decision. Conversely, although some models have suggested that the choice between the αβT cell and γδT cell lineages is determined by instructive signaling by the pre-TCR versus the γδ TCR, new evidence suggests that commitment in this case may be at least partly independent of these signals.

The review by Hogquist (pp 225–231) addresses the late stages of αβT cell development. She emphasizes competing models of how signaling through the TCR regulates selection and CD4/CD8 lineage commitment. Interestingly, new

evidence suggests that the duration of critical signals may be more important than their intensity. In fact, peptide–MHC complexes that bind a TCR with low affinity and stimulate positive selection may give a more sustained, albeit less intense, signal than high-affinity complexes. The positive selection signal, however, apparently differs qualitatively from negative selection signals; recent evidence suggests that the TCR must interact with CD3 δ in order to mediate positive but not negative selection. The interaction with CD3 δ requires the α -chain-connecting peptide (α -CPM), a segment of the TCR α chain just outside the membrane. Evidence suggests that low-affinity TCR ligands lead to sustained ERK activation via α -CPM interactions with CD3 δ and that this type of signaling may underlie positive selection. In the case of the CD4/CD8 decision, much new evidence supports the model in which the fate decision is influenced by the extent of Lck signaling. Again, some evidence suggests that the duration of this signal, rather than its intensity, may be critical for fate determination. The role of Notch signaling in CD4/CD8 lineage commitment is also being investigated intensively, based on earlier work showing that activated Notch1 favors the development of CD8 lineage cells. Although a conditional knockout of Notch1 did not affect CD4/CD8 T cell development, Notch2 and Notch3 are also expressed in the thymus and may function in a redundant manner. Although new evidence has emphasized that Notch activation stimulates survival signals in thymocytes, a recent report suggests that enhanced survival cannot fully explain the effects of activated Notch on CD4/CD8 lineage commitment.

Berg and Kang (pp 232–241) summarize recent efforts to define the molecular mediators of T cell development. In the early stages, a role for IL-7 in activating recombination and expression of TCR γ genes has been documented. Furthermore, the transcription factor E2A alters the pattern of V genes rearranged in this locus. The mechanisms involved are not established but studies of TCR δ rearrangement suggest that local histone deacetylation may be a key event in providing accessibility of specific loci to the recombination machinery. Other basic helix–loop–helix transcription factors such as HEB, Id and SCL also play roles in early T cell development. The understanding of factors regulating death of thymocytes will be aided by studies demonstrating reduced survival of thymocytes in mice lacking PI3K γ , ROR γ or E2A proteins. Surprising findings include recent reports that T cell development is not demonstrably impaired in mice completely lacking all JNK1 and JNK2 activity, in mice lacking the glucocorticoid receptor or in mice lacking PKC θ .

After seeding the periphery, CD4 $^+$ T cells can undergo further differentiation into Th1 and Th2 cells, which produce different sets of cytokines. Asnagli and Murphy (pp 242–247) focus their review on factors and receptors that direct the differentiation and maintenance of these differentiated T helper populations. The major pathway of Th2 differentiation requires IL-4 signaling and consequent Stat-6 activation, leading to cMAF and GATA-3 activation. GATA-3 may act by inducing remodeling of the chromatin in the

vicinity of the IL-4 and possibly other Th2 cytokine genes. Interestingly, in the absence of IL-4 signaling or Stat-6, some Th2 differentiation still occurs. It is proposed that non-IL-4 signals may lead to GATA-3 activation and that GATA-3 may positively regulate itself, reinforcing this pathway of Th2 differentiation. In the case of Th1 differentiation, IL-12 signaling and Stat-4 activation play a critical role. Recently, Tbet, a novel transcription factor, has been shown to induce IFN- γ transcription and repression of IL-4 and IL-5 expression in differentiating T cells and may play a central role in Th1 cell development. Finally, recent studies are described that suggest that Th1/Th2 cells may not be as stable a differentiated phenotype as previously thought. Most interesting are studies indicating that a sustained and effective Th1 immune response against pathogens may require a continuous source of IL-12.

Sprent and Surh (pp 248–254) review recent progress in understanding the generation and maintenance of memory T cells. New studies show that memory T cells can arise from cells that had previously produced perforin, IL-2 or granzyme B, arguing that memory T cells are descended from differentiated, antigen-specific effector T cells. Interestingly, naïve T cells expand and acquire memory markers in response to self-antigens in lymphopenic hosts, suggesting that not all cells with memory markers are derived from cells that previously responded to foreign antigens. The factors that control maintenance of memory T cells have also received recent attention. Strong evidence now indicates that memory T cells, unlike naïve T cells, survive and even proliferate in the absence of interactions with MHC molecules. Various studies, including analyses of knockouts of the IL-15 gene or its receptor, show that survival and proliferation of memory CD8 $^+$ T cells require the action of IL-15. IL-2, in contrast, appears to restrain CD8 $^+$ memory T cells. Factors controlling maintenance of CD4 $^+$ memory cells have not been identified.

It is evident that advances in molecular and cellular technologies have accelerated the pace at which firm conclusions can be made in the study of lymphocyte development. Consequently, the earlier simplistic models of these events gain new dimensions and the full complexity of the system is gradually revealed. The field now develops a more sustainable intellectual framework that increasingly borrows information and concepts from other disciplines. In turn, studies of lymphoid development are also making important new contributions to these other disciplines. It is to be expected that these advances will continue to narrow the gap between immunology and traditional molecular and developmental biology, making the immune system a prime playground for functional genomics, proteomics and bioinformatics.

Reference

1. Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinikai Y, Honjo T: **Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme.** *Cell* 2000, **102**:553–563.