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Lymphocyte development. Overview

Klaus Rajewsky and **Harald von Boehmer**

Lymphocyte development has always attracted the interest of researchers looking at it either as an easily accessible model of lineage determination and cellular differentiation in higher organisms or as the basis for an understanding of the immune system as such. The present collection of reviews contains examples of both categories, without of course comprehensively covering this vast area of research.

Merkenschlager and Wilson choose T cell development to exemplify the developmental interplay and regulatory properties of chromatin modifications and the various classes of small, non-coding RNAs, which have emerged as a new layer of biological control over the last years. Clear patterns of histone modifications have been identified that mark actively transcribed or silenced genes, or genes poised to be transcribed in the anticipated response of a cell to developmental or other stimuli. These patterns, which may remain stable in the cells over many generations, are actively maintained, retaining a high level of plasticity. Small, non-coding RNAs, certain classes of which have been shown to be involved in heterochromatic gene silencing in mammalian cells, are likely important players in this control. While a role for microRNAs in mammalian transcriptional control remains elusive, small RNAs of this class play distinct roles in the control of many genes involved in lymphocyte development, by regulating mRNA translation and stability. The genomes of higher organisms encode hundreds of microRNAs, which mostly differ in their target specificity. MicroRNAs are often stage-specifically expressed and may target multiple components of transcriptional or signaling networks. They usually down-regulate target protein expression, but there are examples of a reversal of this functional activity under certain (e.g. stress) conditions. Overall, the impact of small, non-coding RNAs on the physiology and pathology of the immune system is just beginning to be unraveled.

The plasticity of chromatin modification patterns in developing hematopoietic cells is reflected in the developmental plasticity of the hematopoietic system in terms of lineage determination. Cobaleda and Busslinger discuss in their paper the astounding extent to which cytokines produced in the environment or forced expression of certain transcription factors can divert progenitor cells in a given cell lineage into other hematopoietic lineages or lead to the transdifferentiation of cells in a given lineage. In the case of mature B cells, inactivation of the B cell “commitment” factor Pax5 can result in the dedifferentiation of these cells into uncommitted progenitor cells, which can subsequently give rise to mature T cells when exposed to an appropriate environment. There is some evidence that this developmental plasticity of lymphocytes may contribute to switches of gene expression programs observed in certain human malignancies. Cellular reprogramming has become one of the hottest topics in present-day biomedical research, and as in so many other connections, studies in the immune system have played a pioneering role in this development.

With respect to B cell development, the present collection of reviews focuses largely on developmental processes following the expression of a functional, non-autoreactive B cell antigen receptor (BCR) on the developing cells; we trust that the fascinating earlier developmental stages, in which the cells are driven by pre-B cell receptor expression into proliferation and subsequently enter a compartment where BCR "editing" occurs, and whose signaling requirements are presently being uncovered, will be a topic of the next collection of reviews on lymphocyte development. Allman and Pillai discuss the origin, habitat and function of the various subsets of mature B cells, namely follicular and marginal zone (MZ) B cells, as well as the B1a and B1b subsets. Their review covers enormous ground, documenting the substantial progress made in this area over the last years as well as pointing out the many aspects where knowledge remains fragmentary. Major issues addressed in this paper are BCR-related and –unrelated signals driving the cells into the various compartments and controlling their maintenance and functional activity, developmental relationships between the various subsets, the "niches" in which the cells reside (including the recently identified niche for follicular B cells in the bone marrow, where the cytokine Mif rather than BAFF produced by bone marrow-resident dendritic cells may be critical for their survival), the notion that B1b cells represent memory cells originating from follicular B cells and devoted to IgM production, the distinct signals driving cells of the various subsets into responses, and the role of B cells as antigen capturing and transporting cells. Directly relating to the Allman and Pillai review is the paper of Stadanlick and Cancro, who discuss the foundations of the hypothesis that competition of peripheral B cells for BCR- and BAFF:BAFF-receptor-mediated survival signals provides the basis for positive and negative selection of peripheral B cells. In this picture the BCR and BAFF-receptor mediated survival signals are integrated intracellularly by a molecular cross-talk, which may largely rely on the canonical and alternative □F□□ signaling pathways activated by these receptors.

The differentiation of B cells into plasma cells and the maintenance of the latter is the subject of the review by Tarlinton and Radbruch. A complex network of signaling cascades and transcription factors controlling the differentiation of germinal center B cells into plasma cells on the one side and memory B cells on the other has been identified over the last years, so that intriguing models of the control of these differentiation processes have become available. However, much work remains to be done before we fully understand which (combination(s) of) signals drive the cells into and along either pathway under physiological conditions. Most plasma cells arising in T cell dependent responses are short-lived, but some 10 to 20% become long-lived cells, continuing to produce specific antibodies over extended periods of time. The maintenance of these cells requires the migration of their plasmablast progenitors into specialized survival niches, where they terminally differentiate and for which newly arriving and resident cells seem to compete. While many cytokines, chemokines and their receptors, as well as other molecules controlling these processes have been identified, our understanding of the control of plasmablast migration and maturation, and of the signals keeping plasma cells alive in their niches, is again still incomplete.

A special review in the B cell section is devoted to the generation and function of IgA-producing B cells in the gut-associated lymphatic tissue (GALT). Fagarasan discusses evolutionary, developmental and functional features of these cells and the antibodies they secrete. IgA-secreting B cells are found in different locations in the GALT, namely the gut lamina propria, mesenteric lymph nodes and Peyer's Patches and the so-called isolated lymphoid follicles (ILFs). The origin of these cells in terms of B cell subsets and the signals controlling their differentiation and activation differ for the cells in the various locations. Key regulators of IgA responses in the gut are resident dendritic cells, which imprint gut-homing properties into B cells in their vicinity through retinoic acid production, and promote IgA secretion by these cells through the additional production of interleukin 6 and 5. In the presence of TGFβ, the dendritic cells also promote T cell-independent class switching to IgA, by BAFF and APRIL secretion.

The complex migration pathways and modes of activation provide the B cell system in the gut with a unique flexibility in its vital interaction with the complex and ever changing composition of the bacterial gut flora.

Cobaleda and Busslinger as well as Boehm address the dispute concerning the phenotype of hemopoietic cells that migrate to the thymus and most efficiently produce thymocytes. Presently the focus is on precursors not yet committed to lymphoid lineages but the developmental plasticity of various precursors and their dedifferentiation upon exposure to environmental cues introduces a note of caution. Not disputed is the fact that the thymus is an organ where different T sublineages are generated and cells undergo positive and negative selection according to the specificity of their receptor. Apart from thymic lymphocytes the thymus consists of epithelial cells and fibroblasts with epithelial cells playing a key role in organogenesis and lymphocyte selection. Boehm describes how cells from the endoderm of the anterior foregut under the influence of factors from the mesenchyme develop into epithelial cells that express Foxn1. It seems clear that early Foxn1-expressing epithelial cells are at least bipotent giving rise to cortical and medullary epithelial cells. The full maturation of the medulla requires signaling through the lymphotoxin beta receptor while the autoimmune regulator AIRE involved in expression and presentation of peripheral tissue antigens is regulated by RANK signaling via TRAF6 involving lymphoid tissue inducer cells. In adult life thymic epithelial cells exhibit a significant turnover with AIRE expression occurring only in post-mitotic cells. At present it is not clear how Foxn1 is regulated and in which way it contributes to organogenesis. Of interest is that Foxn1 expression in the rudimentary thymus-anlage of Foxn1-deficient adult mice results in the formation of a functioning thymus. Boehm further discusses several experimental tools such as thymic organoids, nude blastocyst complementation as well as fish models, the latter adding feasibility of less expensive forward genetic screens.

Once the thymus anlage is colonized by hemopoietic precursors these cells undergo differentiation and selection as described in many reviews. Cheroutre adds one further example of lineage fate determination by addressing the apparent conversion of $CD4^{+}8^{+}$ DP cells into so-called CD8alfa alfa cells that are assumed to exert regulatory functions in the gut. While such cells have been shown to result from TCR-agonist stimulation, Cheroutre points out that there are already CD8alfa alfa-expressing precursors of these cells prior to any possible TCR-agonist stimulation and that the generation of these cells crucially depends on pre-TCR signaling: Such precursors represent 5–10% of DP thymocytes that do express CD8 alfa alfa homodimers in addition to CD8 alfa beta heterodimers and unlike conventional DP cells do not undergo extensive cell death when confronted with TCR-agonist ligands. The fact that intrathymic injection of such cells results in accumulation of CD8 alfa alfa cells in the gut of injected mice provides evidence independently of previous fate mapping studies for the thymic origin of CD8alfa alfa TCR alfa beta-expressing cells in the gut.

The remainder of the DP cells either die or are subject to positive or negative selection depending on the specificity of their TCR for thymic MHC molecules. Thymic cortical epithelial cells are considered to participate in TCR-ligand presentation that results in positive selection. In this context the finding of Murata and colleagues is of interest that thymic cortical epithelial cells express a unique proteasome beta-type subunit named beta5t which is incorporated into the 20S proteasome instead of beta5 or the INFgamma-induced beta5i subunit. The other two subunits in the thymic proteasome represent the normal INF-gamma inducible beta1i and beta2i rather than beta1 and beta2 subunits. Instead of methionine, the S1 pocket of beta5t contains threonine and was therefore predicted to have weaker chymotrypsin-like activity compared to beta5 or beta5i subunits. In fact, in beta5t-deficient mice the positive selection of CD8 SP cells is severely impaired. Thus positively selecting thymic cortical epithelial cells can exhibit a unique set of peptide-MHC complexes that are not encountered

elsewhere in the body. Apart from this novel and very intriguing aspect of positive selection perhaps mediated by peptides that can be found nowhere else on the body, the unique expression of beta5t in cortical epithelial cells provides an important vehicle to identify and target cortical epithelial cells.

Another aspect of positive selection concerns the TCR- dependent generation of CD4 and CD8 T cells. Several years ago it was proposed that this lineage decision is strongly influenced by Notch signaling since overexpression of intracellular Notch resulted in increased numbers of CD8 SP cells. However, these conclusions could not be confirmed in several mice deficient in Notch-dependent transcription at this point in T cell development. Nevertheless, presenilin-deficient mice, i.e. mice deficient in generating intracellular Notch by the second proteolytic cleavage of the Notch receptor, were found to be deficient in $CD4^+$ SP thymocytes as reported by Fowlkes and colleagues. In a scholarly review, these authors describe the various Notch signaling pathways in addition to the canonical CSL-dependent transcriptional pathway that could contribute to the different conclusions regarding the role of Notch in CD4/8 lineage fate. Furthermore the authors point out that some deficiencies in Notch-dependent transcription become manifest at different stages of thymocyte maturation and thereby may contribute to the complexity of results. The conclusion from the presenilin studies is that Notch synergizes with TCR signaling and for this reason CD4 SP cells are reduced in presenilin-deficient mice that lack activity of all four Notch receptors.

The final review dealing with T cell specificity concerns an important aspect of MHC-restricted antigen recognition by T cells, namely the question whether there is evolutionary selection for germline-encoded TCRs recognizing MHC molecules , as originally proposed by Jerne, or whether the apparent preoccupation of the TCR repertoire with MHC is mostly due to positive selection that requires coligation of TCR- and MHC-binding CD4 and CD8 coreceptors by thymic MHC molecules. Obviously these two ideas are not mutually exclusive but so far there has been very little evidence for conserved contacts between TCR and MHC in germline-encoded CDR1 and CDR2 regions of the TCR. The review by Marrack, Kappler and colleagues points out that this may have been due to a too conservative definition of “conserved contacts”. The authors have looked at contacts of TCRs that have undergone positive and negative selection by a single peptide-MHC complex as opposed to TCRs that have undergone positive selection by a single peptide-MHC complex and negative selection by a large set of peptide-MHC complexes. TCRs selected in the former scenario are highly crossreactive on a variety of MHC complexes and the authors have analyzed how such receptors bind to MHC. The results show that certain Vbetas and certain Valphas “almost always” use the same amino acids in the CDR1 and CDR2 regions to contact MHC, that the contact requires the usual diagonal orientation of the TCR over MHC and that the contacted residues of the MHC are located on the upper surfaces of the MHC molecules. In this way the TCR is allowed easy access and actually may shift to fulfill other contact requirements imposed by the non-germline-encoded CDR3 region. While such apparently “conserved” contacts have been reported for certain Valphas and Vbetas, the prediction is that similar but not necessarily identical conserved contacts can be found with other variable regions. Of interest here is the observation, recently published in *Immunity*, that mutation of these conserved residues results in diminished T cell activation. It is presently an open question whether the putative evolutionary selection of these apparently conserved contacts had to do with constraints imposed by the MHC-binding CD4 or CD8 coreceptors

Biographies

Harald von Boehmer is Professor of Pathology at Harvard University and Principal Investigator at the Dana-Farber Cancer Institute. He was a permanent member of the Basel Institute for Immunology and Director of Unité INSERM 373 in Paris. He has worked on T cell

development, in particular on structure and function of the pre-TCR as well as on TCR-dependent positive and negative selection including CD4/8 lineage commitment. Other studies have addressed peripheral tolerance, T cell memory and regulatory T cells.

Klaus Rajewsky is a Principle Investigator at the Immune Disease Institute and Fred S. Rosen Professor of Pediatrics and Professor of Pathology at Harvard Medical School. He moved to Boston in 2001, from the Institute for Genetics at the University in Cologne and the Mouse Biology Program of the European Molecular Biology Organization in Monterotondo, Italy. His work has been mainly in B cell biology and mouse genetics.