Thymus Lineage Commitment: A Single Switch

Stephen M. Hedrick1,*

1University of California, San Diego, La Jolla, CA 92093-0377, USA
*Correspondence: shedrick@ucsd.edu

In this issue of Immunity, He et al. (2008) establish the logic and circuitry that determine CD4-CD8 lineage specification. It all comes down to an eighty base pair silencer switch.

Developmental contingencies are endlessly fascinating. There’s something about a circuit that measures small differences and accurately amplifies them into an error-free outcome. Contingencies represent the opposite of chaos and the epitome of robustness. In developmental immunology, none has received more attention than the CD4 versus CD8 T cell lineage commitment, perhaps because it is intertwined with the storied concepts of MHC restriction and thymic selection.

The principles of lineage commitment consist of a basis for the decision, the conceptual underlying logic, and the analog circuitry used the compute an all-or-none result. We understand the first, I propose that an understanding of the second is close at hand, and with recent breakthroughs we have the tools to understand the third. The first is easy; the specificity of the receptor (TCR) determines the outcome of the differentiation pathway—but what is the logic? Do class II- and class I-specific TCRs transmit quantifiably different signals (instruction), or does the developing thymocyte venture...
a guess and survive or not depending on its accuracy (stochastic selection)? These two models were initially proposed, each was tested successfully, and each had its proponents. As we might have predicted from similar debates in science, the truth has elements of each.

A clue came from an analysis of MHC class II-deficient mice. Flow cytometry showed the lesion correlated with an absence of mature CD4+ T cells but with one curious finding. Although there were no developing thymocytes that displayed the CD4+CD8+ phenotype, there was a stubbornly persistent CD4+CD80 population. The inference was that this population had abortively attempted the wrong pathway, and this was said to be evidence for selection. The observation was highly influential in that it gave rise to a field devoted entirely to ever-more-detailed analyses of the iconic CD4 versus CD8 bivariant FACS plot. Thymocyte subsets, based on graded CD4 and CD8 expression, were finely dissected into intermediate steps with vectored trajectories, and there arose a new generation of models describing asymmetric signaling requirements and default pathways. Still, none was rigorously tested.

A breakthrough came in the form of the kinetic signaling model, which is a deceptively simple but conceptually distinct variation of all previous notions of CD4–CD8 lineage commitment (Singer, 2002). The idea is that recognition of a ligand by CD4+CD8+ thymocytes results in positive selection. This, in turn, causes a reduction in the intensity of CD8 expression that can be mapped, in part, to the E8III enhancer in the intensity of CD8 expression that can selection. This, in turn, causes a reduction for CD8+ lineage development and directly affect the silencer activity of the Cd4 gene (Taniuchi et al., 2002). The Runx molecules are surely part of the lineage-specification network.

The spectacular discovery of a single transcription factor that is necessary and sufficient for CD4 lineage commitment now forms the basis for a detailed description of the circuitry. One group found, by chance (but with enlightened insight), a mouse mutation, termed HD (T helper deficient), lacking CD4+CD8+ thymocytes and T cells. Through genetics and analysis by transgenesis, they mapped the mutation to a single base in the DNA binding domain of a transcription factor designated as ThPOK, but known alternatively as c-Krox, Zfp67, and the official symbol, Zbtb7b (zinc finger and BTB domain containing 7B) (He et al., 2005). Another group independently identified this lineage-commitment factor from a differential gene expression analysis of CD4+ and CD8+ T cells (Sun et al., 2005). Zbtb7b is first seen in the CD4+CD80 subset of thymocytes, and in HD mice (predicted to be a Zbtb7b loss of function), MHC class II-specific thymocytes incorrectly differentiate into the CD8+ T cell lineage. Moreover, enforced expression of Zbtb7b in thymocytes causes MHC class I-specific cells to incorrectly differentiate into the CD4+ T cell lineage. Zbtb7b is thus necessary and sufficient for CD4 lineage specification, and from this arises the principle that positive selection is separate from lineage commitment. Work is now centered on identifying the downstream targets of Zbtb7b, and also characterizing its own cis-acting transcriptional regulation—the bottoms-up approach taken to another stage (He drick, 2002). Along with another recently published article (Setoguchi et al., 2008), work by He et al. (2008) in this issue of *Immunity* at once reinforces the kinetic signaling model as a principle of CD4–CD8 lineage specification and focuses attention on a silencer-enhancer governing Zbtb7b expression.

The report by He et al. (2008) is really two related studies. In the first, they sought to establish a basis for their previous observation that Zbtb7b is first expressed at the proposed lineage-commitment step—CD4+CD80. The prediction was that strong or extended signaling is the origin this difference, and they showed this by treatment of MHC class II-deficient mice with antibody specific for TCRβ. The result was an induction of Zbtb7b in CD4+CD80 cells and the appearance of CD4+CD8+ thymocytes. This result is an affirmation that continued signaling at this all-important stage, regardless of its origin, is necessary for CD4 lineage commitment. One could say that MHC class II-mediated signaling is stronger at this juncture and therefore instructive, but that misses the essence of the logic. This experiment is also telling in that positive selection requires MHC recognition because Lck is entirely bound by CD4 and CD8 (Van Laethem et al., 2007). Given that anti-TCRβ is unlikely to coordinate the inclusion of CD4 (and its attendant Lck), I infer that signaling leading to lineage commitment is different from that required for positive selection.

To distinguish between the requirement for strong or extended signaling at the lineage-commitment stage, He et al. (2008) produced HD;B2m-/ mice and analyzed the CD4+CD80 population. Placing these cells into culture presumably interrupts TCR-CD4 signaling, and the result was that Zbtb7b disappeared and the cells expressed CD8. Furthermore, Zbtb7b expression was rescued by the addition of antibody specific for CD3. With caveats concerning thymocyte development in culture, this is consistent with the requirement for continued TCR-mediated signaling at this decision stage in order to maintain CD4 lineage commitment. The class of MHC recognition is unimportant; rather, it is only the extension of signaling to maintain Zbtb7b expression (Figure 1).

In the second part, He et al. (2008) analyzed the cis-acting regulatory sequences needed for Zbtb7b expression. Although expression is mediated by multiple enhancer-like sequences (Figure 1), the most interesting facet of the work was the identification of a 5’ distal sequence that has characteristics of a silencer and enhancer. Deletion of this distal response
element (DRE) caused reporter expression in both CD4 and CD8 lineages, yet, a sequence or sequences in this region also functioned as an enhancer largely restricted to the CD4*CD8lo stage. This would appear to be further evidence that the CD4*CD8lo stage is a truly distinct subset along the T cell developmental pathway.

A region of 300 bp near the DRE was found to be conserved in mammals and marsupials and contains motifs predicted to bind Gata, Runx, NFκB, and E-box factors. After deletion mutants were generated and then tested with their DRE-deleted reporter construct, the Gata, Runx, and E-box sites appeared to be irrelevant, and they defined an 80 bp region important for silencing. Analysis of multiple founders was consistent with weak silencing outside the 80 bp region, and thus a contribution by Runx or Gata was not entirely eliminated. Through this elegant, extensive, in vivo analysis, He et al. (2008) appear to have defined a silencer element that is controlled by one or more transcription factors or factors that embody CD4-CD8 lineage specification. Yet, there is one complication.

In a recent report, Setoguchi et al. (2008) described their continued analysis of Runx as a repressor important for CD4-CD8 lineage specification. A number of transcriptional repressors, including the Runt-Runx family, act by way of a corepressor known as Groucho. In addition to conditional mutations in Runx1 and Runx3 previously analyzed, these investigators produced a Runx1 mutant lacking the conserved VWRPY Groucho-interaction motif at the carboxyl terminus. Analyzing mice with various combinations of Runx genes deleted or mutated, they found that Runx3-deficient mice with a Runx1-VWRPY mutation completely lacked CD8+ T cells—even in the absence of MHC class II genes.

The investigators used ChIP-on-chip to find sequences in the Zbtb7b locus that bind to the Runx binding partner, Cbf/2. Two were found and one corresponded with the DRE. Setoguchi et al. (2008) similarly found the deletion of this gene element derepressed the Zbtb7b locus in CD8 T cells, but differing from the above results, the Runx sites were necessary for efficient silencer activity. On the other hand, these Runx complexes were shown to bind even in cells expressing Zbtb7b, and therefore, Runx binding does not itself confer the specificity of silencing.

Although they differ in a role for Runx-Groucho-mediated repression, the two studies establish very similar and important principles underlying CD4-CD8 lineage specification. Zbtb7b is regulated by an enhancer-silencer element in the distal-most region of the upstream regulatory region, and it acts at the critical CD4*CD8lo stage of development. The element includes Runx sites, but importantly, there is a critical factor yet to be identified. The tools are now at hand to understand CD4-CD8 lineage specification to a degree previously only achieved with saturating mutagenesis screens in simple metazoans. As referred to above, there are probably principles yet to be discovered, or as Groucho himself said, "Those are my principles ... if you don’t like them I’ve got others."

**REFERENCES**


