

huis, F.M., van Ojik, H.H., Sedlik, C., da Silveira, S.A., Gerber, J., de Jong, Y.F., et al. (2002). *Immunity* 16, 391–402.

Ji, H., Ohmura, K., Mahmood, U., Lee, D.M., Hofhuis, F.M., Boackle, S.A., Takahashi, K., Holers, V.M., Walport, M., Gerard, C., et al. (2002). *Immunity* 16, 157–168.

Meyer, D., Schiller, C., Westermann, J., Izui, S., Hazenbos, W.L.,

Verbeek, J.S., Schmidt, R.E., and Gessner, J.E. (1998). *Blood* 92, 3997–4002.

Nimmerjahn, F., Bruhns, P., Horiuchi, K., and Ravetch, J.V. (2005). *Immunity* 23, this issue, 41–51.

Ravetch, J.V., and Bolland, S. (2001). *Annu. Rev. Immunol.* 19, 275–290.

Immunity, Vol. 23, July, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.immuni.2005.07.003

Selection and Lineage Specification in the Thymus: Commitment 4-Stalled

How CD4⁺CD8⁺ thymocytes commit to CD4 helper versus CD8 cytotoxic lineages is a central unresolved question in developmental immunology. In this issue, Sarafova et al. (2005) show that engineering CD4 for shutoff immediately after positive selection misdirects cells to the cytotoxic lineage. The result highlights the distinction between positive selection and lineage commitment and provides new impetus for re-examining lineage models.

Differentiation of double positive (DP, CD4⁺CD8⁺) thymocytes into distinct T cell lineages with helper versus cytotoxic functions serves as a paradigm for binary decisions in vertebrate development. However, this lineage decision can only be understood in the context of the requirement for DP cells to undergo positive selection following appropriate interactions with MHC-peptide complexes. Following positive selection, DP cells, which express $\alpha\beta$ T cell antigen receptors (TCR), differentiate into either MHC class II-restricted CD4⁺ T helper cells or MHC class I-restricted CD8⁺ T cytotoxic cells by way of a CD4⁺8^{lo} intermediate stage. The mechanism by which such lineage commitment occurs has been subject to long-standing and intense scrutiny, and multiple models have been put forth. Although early results were interpreted as supporting a stochastic/selective mechanism of lineage choice, recent work has been most consistent with instructive models, in which the CD4 and CD8 coreceptors transmit qualitatively or quantitatively different signals (Germain, 2002). For example, differential recruitment of Lck by CD4 and CD8 would result in strong or weak TCR signaling and in commitment to CD4 versus CD8 lineages (Hernandez-Hoyos et al., 2000). Support for such a mechanism in bipotential decisions comes from recent evidence that quantitative signals contribute to the $\gamma\delta$ vs. $\alpha\beta$ T cell developmental checkpoint (Robey, 2005).

A distinct model, based on temporal regulation of signaling through the TCR:coreceptor complex, has been advanced by Singer and his colleagues (Singer, 2002). This “kinetic signaling” model postulates that lineage commitment is determined by whether the TCR:MHC interaction is sustained or truncated when, following posi-

tive selection, cells progress to the CD4⁺8^{lo} stage. Down-regulation of CD8 would cause disruption of the TCR:MHC I interaction, resulting in a shorter signal and commitment to the CD8 lineage. Conversely, continuous signaling due to the sustained CD4-dependent TCR:MHC II interaction would commit cells to the CD4 lineage. This model is the only one to explicitly suggest that a postpositive selection signal is responsible for lineage commitment, although it is possible that signals for selection and commitment are coupled. It is clear, from analysis of the *hd/hd* mutant mouse, in which all class II-specific thymocytes are misdirected to the CD8/cytotoxic lineage, that positive selection and lineage commitment are distinct and separable processes (Keefe et al., 1999). Models for lineage commitment have largely ignored the implications of this fact, but a new study by Sarafova et al. (2005) in this issue of *Immunity* highlights the importance of this distinction and provides new insights into the role of signaling following positive selection.

Sarafova and her colleagues (Sarafova et al. 2005) have engineered a mouse in which they could examine the contribution of CD4 expression following CD4-dependent positive selection of MHC class II-specific cells. They expressed CD4 under the regulation of a CD8 enhancer, E8_{III}, previously shown to regulate reporter gene expression only in DP thymocytes and to shut off following positive selection (Ellmeier et al., 1998). When this transgene was introduced into mice lacking endogenous CD4, expression of CD4 was limited to DP cells. In these mice, MHCII-directed positive selection remained intact, and CD4 was downregulated in parallel with CD8. Remarkably, when these mice were also rendered deficient for $\beta 2m$, such that all positive selection was directed by MHCII, most of the selected thymocytes were redirected to the CD8 lineage (Figure 1). When such mice additionally expressed a transgenic MHCII-specific TCR, that normally directs cells towards the CD4/helper T cell lineage, all mature thymocytes bearing this receptor expressed CD8. In $\beta 2m$ -deficient mice, CD8⁺ T cells are usually absent, and only class II-specific CD4 lineage cells are present. The MHCII-restricted unconventional CD8⁺ T cells were reduced significantly in the periphery of the engineered mice, presumably due to impaired transduction of TCR-mediated homeostatic survival signals. Nevertheless, despite being MHCII-restricted, the unconventional CD8⁺ T cells had no CD40L expression, were able to mediate a strong cytotoxic response, and expressed levels of

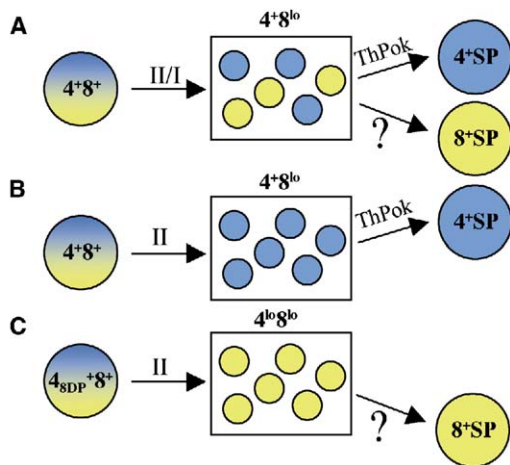


Figure 1. Postpositive Selection CD4 Expression Is Required for Commitment to the CD4 Lineage

(A) Positive selection of CD4⁺CD8⁺ thymocytes by MHCII or MHCI leads to MHCII-restricted CD4⁺ or MHCI-restricted CD8⁺ T cells through a CD4⁺8^{lo} intermediate.

(B) In $\beta 2m$ -deficient mice, positive selection occurs only on MHCII, and all thymocytes become MHCII-restricted CD4⁺ T cells.

(C) Sarafova and colleagues (Sarafova et al. 2005) find that when positive selection is allowed to occur through CD4/TCR:MHCII interactions followed by CD4 downregulation, all thymocytes become MHCII-restricted CD8⁺ T cells.

perforin and cathepsin W comparable to those in wild-type CD8⁺ cells, suggesting that they had differentiated into functional cytotoxic T cells. Thus, loss of expression of CD4 from transitional thymocytes immediately postselection resulted in cells adopting the CD8/cytotoxic cell fate. These results clearly demonstrate that CD4 expression is required after positive selection for differentiation of the helper T cell lineage.

Sarafova et al. (2005) argue that their results are most consistent with a kinetic signaling model in which lineage commitment is determined solely by whether signaling from the TCR persists or ceases. Despite the elegant simplicity of this model, it remains possible that qualitatively different signals occurring at different stages of development result in positive selection versus lineage commitment. The transcription factor defective in the *hd* mice, cKrox (also named Th-POK), is required for commitment to the CD4 lineage, but not for positive selection (He et al., 2005; Sun et al., 2005). Its expression in CD4⁺8^{lo} intermediate cells destined to differentiate towards the CD4 lineage may be a result of either prolonged TCR signaling or of a distinct signal that could be mediated in part through the CD4 coreceptor. Sarafova et al. (2005) point out that in mice lacking CD4 there is differentiation of MHCII-restricted CD4 “wannabe” T helper cells and conclude that CD4, per se, is not required for commitment to the helper lineage. This result is consistent with the kinetic signaling model in that there would be sustained coreceptor-independent signaling spanning positive selection and the subsequent intermediate CD8^{lo} stage, mediated by high affinity TCR:MHCII interactions. However, the converse example is not consistent with the model. Thus, when positive selection of CD8-deficient thymocytes

was rescued by using higher affinity altered peptide ligands, the resulting MHC class I restricted cells were CD8 “wannabe” thymocytes of the cytotoxic lineage, not CD4⁺ helper cells (Goldrath et al., 1997). In addition, a corollary of the result presented by Sarafova and colleagues is that sustained CD8 signaling after positive selection should misdirect MHC class I-restricted T cells to the CD4/helper lineage. Such an outcome has not been clearly demonstrated. It therefore remains possible that qualitative or quantitative differences in TCR and coreceptor signals contribute to the lineage choice.

Significant advances have been made in identifying transcription factors that contribute to lineage decisions during thymocyte differentiation. Understanding the mechanisms by which these molecules are regulated will undoubtedly help to unravel how the CD4/CD8 lineage choice is achieved. The present paper firmly establishes that CD4-dependent signaling after positive selection is required for differentiation of MHC class II restricted thymocytes to the CD4 lineage. Together with the recent studies on the role of cKrox in CD4/helper cell differentiation, this new work raises interesting questions that should now be testable. It will be of particular interest to learn whether, following positive selection, CD8 lineage differentiation is a default pathway that can be diverted to the helper lineage by TCR/CD4-mediated signaling, or whether it is itself induced by a distinct signal. A related question is whether it will be possible to mimic a signal such as that involved in CD4 lineage commitment to divert MHC class I selected thymocytes to the helper cell program. The current studies emphasize that a better understanding of signaling pathways in the postselection CD4⁺8^{lo} thymocytes will be required to at long last resolve the controversy around the mechanism of lineage specification.

Amélie Collins and Dan R. Littman

Howard Hughes Medical Institute
Molecular Pathogenesis Program
Skirball Institute of Biomolecular Medicine
New York University School of Medicine
540 First Avenue
New York, New York 10016

Selected Reading

Ellmeier, W., Sunshine, M.J., Losos, K., and Littman, D.R. (1998). *Immunity* 9, 485–496.
 Germain, R.N. (2002). *Nat. Rev. Immunol.* 2, 309–322.
 Goldrath, A.W., Hogquist, K.A., and Bevan, M.J. (1997). *Immunity* 6, 633–642.
 He, X., He, X., Dave, V.P., Zhang, Y., Hua, X., Nicolas, E., Xu, W., Roe, B.A., and Kappes, D.J. (2005). *Nature* 433, 826–833.
 Hernandez-Hoyos, G., Sohn, S.J., Rothenberg, E.V., and Alberola-Lla, J. (2000). *Immunity* 12, 313–322.
 Keefe, R., Dave, V., Allman, D., Wiest, D., and Kappes, D.J. (1999). *Science* 286, 1149–1153.
 Robey, E. (2005). *Immunity* 22, 533–534.
 Sarafova, S.D., Erman, B., Yu, Q., van Laethem, F., Guintert, T., Sharrow, S.O., Feigenbaum, L., Wildt, K.F., Ellmeier, W., and Singer, A. (2005). *Immunity* 23, this issue, 75–87.
 Singer, A. (2002). *Curr. Opin. Immunol.* 14, 207–215.
 Sun, G., Liu, X., Mercado, P., Rhiannon Jenkinson, S., Kyriotou, M., Feigenbaum, L., Galera, P., and Bosselut, R. (2005). *Nat. Immunol.* 6, 373–381.