Commentary

CD4/CD8 Lineage Commitment: Back to Instruction?

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Mechanisms of lineage commitment are of considerable interest in biology, and lineage commitment in the immune system represents no exception to this rule. In the thymus, the TCR α/β lineage diverges into the $CD4^+8^-$ and $CD4^-8^+$ lineages, which exhibit distinct functional programs after antigenic stimulation. To a large extent, these programs are fixed before antigenic stimulation (1–5) (Fig. 1).

Work with transgenic α/β TCRs has shown that the specificity of the TCR for either class I or class II thymic MHC molecules ultimately decides whether a developing T cell becomes a long-lived, mature $CD4^-8^+$ or $CD4^+8^-$ lymphocyte, respectively (6–9). Before positive selection of immature $CD4^+8^+$ thymocytes was discovered, it was postulated that CD4/CD8 lineage commitment occurred by an instructive mechanisms such that coengagement of the α/β TCR and the CD8 or CD4 coreceptor by either class I or class II MHC molecules would result in different signals that would direct the differentiation into the $CD4^-8^+$ and $CD4^+8^-$ lineages, respectively (10).

While initial results in TCR transgenic mice were consistent with this notion (6–9), subsequent thinking (11) as well as analysis of various mutant mice revealed that there was room for other, noninstructive explanations (1, 3, 12–15). In particular, the discovery of so-called intermediate $CD4^+8^{lo}$ and $CD4^{lo}8^+$ subsets (12, 15) in various MHC-deficient mice led to the hypothesis that early CD4/CD8 commitment was of a stochastic nature. This assumed that $CD4^+8^{lo}$ and $CD4^{lo}8^+$ cells were on their way to becoming $CD4^+8^-$ and $CD4^-8^+$ mature and long-lived T cells, a view that appeared to be supported by the fact that $CD4^+8^-$ cells with class I–restricted TCRs and $CD4^-8^+$ cells with class II–restricted TCRs could be rescued by CD8 and CD4 transgenes, respectively, which were expressed in all α/β T cells (1, 3, 4, 13, 14). Collectively, these data were interpreted to indicate that CD4/CD8 lineage commitment and positive selection could be divided into two steps: in the first step, coengagement on $CD4^+8^+$ cells of the TCR and coreceptor by thymic MHC molecules in $CD4^+8^+$ cells would lead to a stochastic lineage commitment accompanied by either CD4 or CD8 coreceptor downregulation, whereas in the second step, coengagement of TCRs and coreceptors by either class I or class II MHC molecules would result in rescue from programmed death of cells with matched receptor molecules only. This view became known as the stochastic/selective model of CD4/CD8 lineage commitment (Fig. 1).

There were, however, concerns with this model too: direct evidence that $CD4^+8^{lo}$ and $CD4^{lo}8^+$ cells were indeed on their way to becoming $CD4^+8^-$ and $CD4^-8^+$ mature and long-lived cells was lacking. Also, rescue of "unorthodox" subsets with mismatched TCRs and coreceptors by coreceptor transgenes was not as significant as could have been expected if early CD4/CD8 lineage commitment were a stochastic event. Significantly, analysis of $CD4^+8^{lo}$ subsets in vivo (16) and in vitro (17) suggested that these cells contained precursors not only for $CD4^+8^-$ but also for $CD4^-8^+$ T cells. This finding and the notion that, at least in vitro, $CD4^+8^-$ cells could develop from $CD4^+8^{low}$ cells even when derived from mice devoid of classical MHC molecules (17) led authors to postulate a default/instructive model in which $CD4^+8^-$ commitment occurs regularly, even in the absence of classical MHC molecules, whereas commitment to the $CD4^-8^+$ lineage requires an instructive signal delivered when TCR and CD8 coreceptor bind to class I MHC molecules. These experiments can obviously only be performed after cell separation, i.e., after binding of CD4 and CD8 antibodies to the cells in question, which may or may not have consequences for further phenotypic changes. Also, there is at present no general consensus that $CD4^+8^{low}$ cells, committed to the $CD4^+8^-$ lineage, exist in MHC-negative mice (12, 17). Finally, the proponents of the default/instructive model do not want to rigorously rule out any class II MHC ligation of the TCR as an initial step in $CD4^+8^-$ commitment (17). The default/instructive model is illustrated in Fig. 1.

To complete the circle of CD4/CD8 lineage commitment models and experiments that are consistent with them, Itano et al. (18) report in this issue novel experiments that are consistent with an instructive/selective model. As explained above, an instructive model requires different signals that are generated after coengagement of TCR and coreceptor by either class I or class II MHC molecules. Since CD4 and CD8 coreceptors have different cytoplasmic tails, these tails could have a role in generating special signals; in fact, the CD4 tail associates much more strongly with $p56^{lck}$ than does the CD8 tail (19). Therefore, attempts were made to see whether a swap of coreceptor tails would result in changes in CD4/CD8 lineage commitment. This was done previously (20) and in a different way again in the work by Itano et al. described in this issue (18). The authors report that a CD8α/CD4 chimeric transgene, in which the CD4 cytoplasmic tail has been hooked into the CD8α extracellular and transmembrane region (CD884), when

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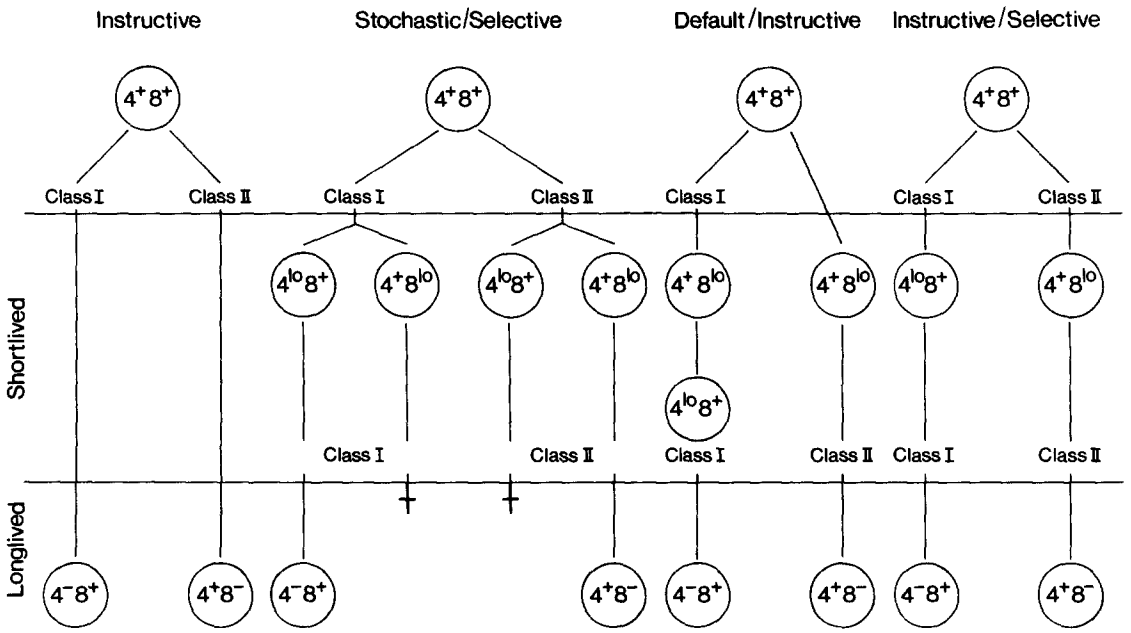

Figure 1. Various hypotheses of CD4/CD8 lineage commitment and positive selection that have been proposed in recent years.

expressed together with a CD8β transgene, engages significantly more $p56^{lck}$ than a CD8α transgene. The same transgene causes an increase in $CD4^+8^-$ cells that express a class I MHC–restricted TCR. Although this increase could be attributed to a more efficient rescue of stochastically generated $CD4^+8^-$ cells with a class I MHC–restricted TCR, the authors argue that this cannot serve as the sole explanation, because there is a concomitant decrease in $CD4^-8^+$ cells, even when competition for putative selection niches appears to be absent. The authors offer adequate, possible explanations as to why such changes were not seen in an earlier attempt of a similar kind (20). The novel model that emerges from these studies is a modified instructive scheme: quantitative differences in signaling induced by coengagement of TCR and coreceptors by either class I or class II MHC molecules produce a bias in lineage commitment such that the stronger signal favors $CD4^+CD8^-$ and the weaker signal favors $CD4^-8^+$ commitment. After receptor downregulation, a "confirmatory" step due to coligation of TCRs and coreceptors ensures survival of those cells with matched TCR and coreceptor expression, while others die. This can be named the instructive/selective model as shown in Fig. 1.

Obviously, by now we are (painfully) aware of the fact that we have at least four distinct CD4/CD8 lineage commitment models, each with a set of consistent experiments. Clearly, what we need are experiments that refute any of these hypotheses. However, these recent experiments have also produced some facts that are agreed upon: it appears no longer reasonable to assume that a short-lasting TCR–MHC ligation results in lineage commitment and positive selection. Rather, these events require consecutive if not continual (21, 22) receptor engagement.

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References

1. Davis, C.B., N. Killeen, M.E. Crooks, D. Raulet, and D.R. Littman. 1993. Evidence for a stochastic mechanism in the differentiation of mature subsets of T lymphocytes. *Cell.* 73: 237–247.
2. Kirberg, J., A. Baron, S. Jakob, A. Rolink, K. Karjalainen, and H. von Boehmer. 1994. Thymic selection of $CD8^+$ single positive cells with a class II MHC–restricted receptor. *J. Exp. Med.* 180:25–34.
3. Baron, A., K. Hafen, and H. von Boehmer. 1994. A human CD4 transgene rescues $CD4^-8^+$ cells in β2-microglobulin deficient mice. *Eur. J. Immunol.* 24:1933.
4. Chan, H.C., C. Waltzinger, A. Baron, C. Benoist, and D. Mathis. 1994. Role of coreceptors in positive selection and lineage commitment. *EMBO (Eur. Mol. Biol. Organ.) J.* 13:

4482–4489.

5. Corbella, P., D. Moskophidis, E. Spanopoulou, C. Mamalaki, M. Tolaini, A. Itano, D. Lans, D. Baltimore, E. Robey, and D. Kioussis. 1994. Functional commitment to Calper T cell linage precedes positive selection and is independent of TCR MHC specificity. *Immunity*. 1:269–278.
6. Teh, H.S., P. Kisielow, B. Scott, H. Kishi, Y. Uematsu, H. Blüthmann, and H. von Boehmer. 1988. Thymic major histocompatibility complex antigens and the αβ T-cell receptor determine the CD4/CD8 phenotype of T cells. *Nature (Lond.)*. 335:229–233.
7. Sha, W.C., C.A. Nelson, R.D. Newberry, D.M. Kranz, J.H. Russell, and D.Y. Loh. 1988. Selective expression of an antigen receptor on CD8-bearing T lymphocytes in transgenic mice. *Nature (Lond.)*. 335:271–274.
8. Berg, L.J., A.M. Pullen, d.S.G.B. Fazekas, D. Mathis, C. Benoist, and M.M. Davis. 1989. Antigen/MHC-specific T cells are preferentially exported from the thymus in the presence of their MHC ligand. *Cell*. 58:1035–1046.
9. Kaye, J., M.L. Hsu, M.E. Sauron, S.C. Jameson, N.R. Gascoigne, and S.M. Hedrick. 1989. Selective development of $CD4^+$ T cells in transgenic mice expressing a class II MHC–restricted antigen receptor. *Nature (Lond.)*. 341:746–749.
10. von Boehmer, H. 1986. The selection of the α, β heterodimeric T cell receptor for antigen. *Immunol. Today*. 7:333–336.
11. Robey, E.A., B.J. Fowlkes, J.W. Gordon, D. Kioussis, H. von Boehmer, F. Ramsdell, and R. Axel. 1991. Thymic selection in CD8 transgenic mice supports an instructive model for commitment to a CD4 or CD8 lineage. *Cell*. 64:99–107.
12. Chan, H.C., D. Cosgrove, C. Waltzinger, C. Benoist, and D. Mathis. 1993. Another view of the selective model of thymocyte selection. *Cell*. 73:225–236.
13. Itano, A., D. Kioussis, and E. Robey. 1994. Stochastic component to development of class I major histocompatibility complex–specific T cells. *Proc. Natl. Acad. Sci. USA*. 91:220–224.
14. Robey, E., A. Itano, W.C. Fanslow, and B.J. Fowlkes. 1994. Constitutive CD8 expression allows inefficient maturation of $CD4^+$ helper T cells in class II major histocompatibility complex mutant mice. *J. Exp. Med*. 179:1997–2004.
15. van Meerwijk, J.P.M., E.M. O'Connell, and R.N. Germain. 1995. *J. Immunol*. 154:6314–6323.
16. Lundberg, K., W. Heath, F. Kontgen, F. Carbone, and K. Shortman. 1995. Intermediate steps in positive selection: differentiation of $CD4^+CD8^{intermediate}TCR^{intermediate}$ thymocytes into $CD4^-CD8^+TCR^{high}$ thymocytes. *J. Exp. Med*. 181: 1643–1651.
17. Suzuki, H., J.H. Print, L.G. Genler, and A. Singer. 1995. Asymmetric signaling requirements for thymocyte commitment to the $CD4^+$ versus $CD8^+$ T cell lineages: a new perspective of thymic commitment and selection. *Immunity*. 2: 413–425.
18. Itano, A., P. Salmon, D. Kioussis, M. Tolaini, P. Corbella, and E. Robey. 1996. In press. The cytoplasmic domain of CD4 promotes the development of CD4 lineage T cells. *J. Exp. Med*. 183:731–741.
19. Veillette, A., M.A. Bookman, E.M. Horak, and J.B. Bolen. 1988. The CD4 and CD8 T cell surface antigens are associated with the internal membrane tyrosine–protein kinase $p56^{lck}$. *Cell*. 55:301.
20. Seong, R.H., J.W. Chamberlain, and J.R. Parnes. 1992. Signal for T-cell differentiation to a CD4 cell lineage is delivered by CD4 transmembrane region and/or cytoplasmic tail. *Nature (Lond.)*. 356:718–720.
21. Kisielow, P., and A. Miazek. 1995. Positive selection of T cells: rescue from programmed cell death and differentiation require continual engagement of the T cell receptor. *J. Exp. Med*. 181:1975–1984.
22. Pircher, H., P. Ohashi, R.L. Boyd, H. Hengartner, and K. Brduscha. 1994. *Eur. J. Immunol*. 24:1982–1987.