The Journal of Immunology

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J Immunol 2016; 196:1983-1984; ;
doi: 10.4049/jimmunol.1600032
http://www.jimmunol.org/content/196/5/1983

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Pillars of Immunology

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One of the central principles of adaptive immunity is the subdivision of T lymphocytes into functionally distinct subsets. Although multiple T cell subsets were shown in recent years to emerge from the thymus, most mature T cells consist of MHC class II (MHC II)–restricted $CD4^+$ T helper cells and class I–restricted $CD8^+$ cytotoxic T cells that arise from bipotential $CD4^+8^+$ (double-positive [DP]) thymocyte progenitors. Elucidation of the mechanism by which these two lineages are specified has been one of the major quests in developmental immunology during the past three decades. As advances were made in understanding TCR signaling, it was not possible to demonstrate qualitatively distinct signals transduced by TCRs associated with CD4 (which interacts with MHC II molecules) versus CD8 (which binds to MHC class I [MHC I]). Several models for this lineage choice were proposed during the 1990s, but it was the publication highlighted here in the *Pillars of Immunology* series (1) that set the current course of investigation in this area of developmental immunology.

Diverse experimental strategies led to an initial proposal that the lineage choice is stochastic, with shut-off of either CD4 or CD8 coreceptor following productive interactions of the TCR with peptide–MHC, such that only cells with appropriate pairing of TCR and coreceptor would be selected for further maturation (2, 3). This model was supplanted by a deterministic model, in which alternative fates were proposed to be instructed by distinct signals through CD4- and CD8-associated kinases (4). Both the stochastic/selective and the instructive models suffered from a variety of inconsistencies, and they were revealed to be overly simplistic when the groups of Shortman and Singer identified a post–positive selection intermediate stage ($CD4^+8^{lo}$) that gave rise to both $CD4^+$ and $CD8^+$ mature single-positive (SP) thymocytes (5, 6). On the other hand, $CD4^{lo}8^+$ cells gave rise exclusively to $CD8^+$ SP T cells, indicating that the lineage choice proceeds in an asymmetric manner. The presence of $CD4^+8^{lo}$ thymocytes specific for MHC I had been previously interpreted as evidence for a stochastic/selective mechanism (2), but the new findings suggested that these cells represented a key intermediate stage worthy of further investigation, motivating the experiments described in the *Pillars of Immunology* article by Alfred Singer and colleagues (1) that is reprinted in this issue of *The Journal of Immunology*. This study, which marked a turning point in how we view the T helper/cytotoxic lineage choice, highlighted two novel findings that have guided subsequent investigation in this field. The first, emphasized in the article's title, was the demonstration that all DP thymocytes selected by interaction with either MHC I or MHC II initially shut off expression of CD8, but only MHC I–selected cells then reactivate CD8 expression while silencing CD4 expression. The second finding was that IL-7, independently of TCR signaling, stimulates coreceptor reversal in the intermediate $CD4^+8^{lo}$ cells, in which CD8 expression is absent. This was demonstrated by stripping the coreceptors with pronase and evaluating their re-expression. In fact, strong TCR signaling was shown to prevent IL-7–induced coreceptor reversal in MHC I–specific thymocytes.

The results led Brugnera et al. (1) to propose a "kinetic signaling" model, in which those T cells selected by interaction with MHC II retain strong signaling through the $CD4^+8^{lo}$ intermediate stage, thus allowing further differentiation into CD4 SP cells, and cells selected by MHC I have attenuated signaling upon downregulation of CD8, allowing them to respond to IL-7 and undergo coreceptor reversal and acquisition of the characteristics of cytotoxic cells.

The kinetic signaling model has dominated the field during the past decade, in large part because of continued efforts by Singer and colleagues to buttress it with additional data. Taking advantage of *cis*-regulatory elements that permit selective expression of coreceptors at distinct stages of development, they generated a variety of transgenic mouse models to test their hypothesis. One prediction of the model is that interruption of TCR signaling following positive selection of MHC II–specific cells would result in their becoming sensitive to IL-7 and adopting the cytotoxic T cell fate. Indeed, when CD4 expression was regulated by an enhancer restricted to the DP stage, its shut-off following positive selection resulted in diversion of MHC II–specific T cells to the CD8/cytotoxic lineage (7). When CD4 was under the regulation of an enhancer that comes on only after positive selection, expression of CD4 was insufficient to rescue class II–specific T cells into the CD8/cytotoxic lineage, arguing against the stochastic/selective model (8). Other studies indicated that further upregulation of CD4 expression following positive selection of MHC II–restricted cells contributes to the CD4/helper lineage choice and suggested that CD4 transgene-mediated redirection of such cells into the CD8/cytotoxic lineage (3) may have been due to insufficient TCR signaling rather than rescue following stochastic downregulation of CD4 expression (9).

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Abbreviations used in this article: DP, double-positive; MHC I, MHC class I; MHC II, MHC class II; SP, single-positive.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1600032

The studies on thymocyte lineage choice carried out by Singer's group have occurred in parallel with investigation by multiple laboratories of transcriptional regulation in T cell development. The transcription factors ThPOK and Runx3 were found to be critical for specification of CD4 and CD8 SP cells, respectively, and to mutually repress each other's expression (10). Whereas Runx3 inhibits ThPOK through direct activity on its gene silencer (11), it is not yet known whether ThPOK binds to the *runx3* locus to inhibit its expression. Runx3 was shown to be upregulated even in the absence of TCR signaling upon ligation of the IL-7R (12), but this was prevented by ThPOK through its induction of inhibitors of cytokine signaling, including suppressor of cytokine signaling 1, whose inactivation was previously shown to predispose cells to develop toward the CD8 lineage (13, 14). ThPOK may thus act indirectly to prevent expression of Runx3. However, in another publication, conditional inactivation of IL-7R resulted in partial reduction in CD8 SP cells, but no reduction in Runx3 expression (15). It therefore remains unclear if regulation of Runx3 fully conforms to the predictions of the kinetic signaling model.

Differentiation of CD4 SP cells is additionally dependent on the transcription factor GATA-3, in the absence of which there is no upregulation of ThPOK in MHC II–selected thymocytes (16). A recent study showed that GATA-3 also represses expression of Runx3 before ThPOK is upregulated in the $CD4^+8^{lo}$ intermediate thymocytes (17). Because upregulation of ThPOK in intermediate thymocytes fated for the CD4 lineage precedes expression of Runx3 in the $CD4^+8^{lo}$ cells directed to the CD8/cytotoxic lineage (18), GATA-3 expression may have a key role in specification of the two lineages. A key question is whether GATA-3 function in this process is somehow instructed by TCR/CD4 interaction with MHC II during positive selection or whether it is acquired as a consequence of prolonged signaling, as proposed by the kinetic signaling model. One of the predictions of the model is that retention of TCR signaling by class I–selected T cells after positive selection, through continued expression of the CD8 heterodimer, should route these cells to the CD4/helper lineage, owing to inability of the cells to respond to IL-7. This has not been demonstrated, but Singer's group has shown that the amount of CD4 expressed following positive selection is critical in ensuring error-free lineage choice (9). It may, therefore, be critical to achieve just the right level of CD8 expression to redirect class I–selected T cells toward the CD4/helper lineage. The possibility remains, however, that specific instructive signals are coupled to positive selection of MHC II–restricted T cells, directing them to the CD4 SP lineage. Regardless of whether kinetic signaling explains the mechanism by which both helper and cytotoxic lineages are specified, the paper by Brugnera et al. remains the pivotal publication guiding how those in the field of T cell development currently formulate experiments to elucidate the mechanism of T cell lineage bifurcation.

Disclosures

The author has no financial conflicts of interest.

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