

Positive Selection in the Thymus: An Enigma Wrapped in a Mystery

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One of the many mysteries that loomed over the field of immunology in its dark ages (that period prior to characterization of the TCR and peptide binding by MHC molecules) was the process of T cell development and selection of the receptor repertoire in the thymus. The work of Hogquist and colleagues was the culmination of theoretical and experimental work starting decades earlier (1, 2), and it established a clear vision of the enigmatic process known as “positive selection” (3). The first and most famous observation, which drove the field, was that T cells did not recognize Ags as such, but rather as some combination of Ag and allelic variations of MHC class II molecules (4, 5) or MHC class I Ags (6–8). “MHC restriction” was magic enough, but there soon emerged an even more perplexing concept that appeared to separate the recognition of Ag from that of MHC molecules.

Experiments showed that the repertoire of mature T cells was biased by the allelic variants of MHC molecules expressed by the radioresistant epithelial cells of the thymus (9–12). In the basic experiment, hematopoietic stem cells from an MHC heterozygous strain were used to reconstitute a lethally irradiated homozygous parental strain. In the vernacular of the time, this was referred to as an F1 → parent radiation chimera. A number of weeks after reconstitution, the APCs were derived from the hematopoietic stem cell donor (expressing both parental MHC haplotypes), whereas the T cells were “educated” in the thymus of the parent strain. Immunization of these mice showed that the specificity of the T cell response was dominated by recognition of Ag in association with the parental MHC molecules; that is, even after Ag-specific selection and expansion, the repertoire was biased by thymic education. This concept was understood as “positive selection,” and the proposal was that developing T cells required a TCR-transduced, MHC-specific signal from the radioresistant thymic epithelial cells to survive and complete maturation. As described below, this influential experiment is still difficult to understand. To borrow from theoretical physics, it might have been wrong, but wrong in an interesting way.

In rapid succession we learned that the TCR used a simple Ig-like combining site (13, 14), and the target of TCR recognition was the complex of a short peptide bound to MHC

molecules (15–18). Furthermore, the process of positive selection was dramatically affirmed. In TCR transgenic mice, CD8⁺ or CD4⁺ T cells would mature only if the appropriate self MHC class I or class II molecules were present (19, 20).

The question was, how is the bias in selection for thymic MHC molecules impressed on the specificity of Ag-induced effector T cells? Could the selection process be peptide independent, do special thymic peptides exist, or are there sufficient self-peptides to cross-react in some form with the universe of foreign Ags? The answer was approachable only if the peptides presented during thymic development could be controlled, and two key advances paved the way. One was the ability to observe T cell development in fetal thymic organ cultures (21), and the second was the development of mutant mouse strains unable to assemble peptide–MHC complexes on the cell surface (22, 23). Reports combining these tools showed that the maturation of CD8⁺ T cells in organ cultures from either of two mutant strains of mice depended upon the addition of β_2 -microglobulin (β_2m) and some source of octamer peptides. Furthermore, the number of maturing CD8⁺ T cells was dependent on the complexity of the added peptides (24, 25). The conclusion was that peptides had a role beyond simply stabilizing MHC class I molecules, and presumably this meant that the TCR on developing thymocytes must have specificity for thymic peptide–MHC complexes. Yet, this interaction must be substantially weaker than that which gives rise to negative selection or mature T cell activation, and the essential question was, what is the nature of the TCR-peptide interaction that promotes positive selection?

Contemporaneously, studies were carried out on the properties of antigenic peptides that would activate cloned, Ag-specific T cells. Stimulatory peptide Ags were shown to consist of MHC-binding amino acids (vernacular: anchor residues) and, separately, amino acid side chains that were directly recognized by the TCR (epitope residues). Peptides harboring single amino acid substitutions at the latter positions were often diminished in their activity, but in addition, they were shown to inhibit T cell activation by concurrently added stimulatory peptides. Such inhibitory peptides could act as antagonists (26, 27), or they could induce a biologically unresponsive (anergic) state (28). These altered peptide ligands were assumed to have a weaker TCR interaction—either a slower on-rate or a faster off-rate, or both.

Everything was in place for Hogquist and her colleagues to address at last the essential question concerning the process of positive selection; i.e., what is the nature of peptide–MHC recognition that promotes survival and differentiation of developing thymocytes (3)? OT-I $B_2m^{-/-}$ (T cells specific for

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Abbreviation used in this article: β_2m , β_2 -microglobulin.

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a peptide of OVA, SIINFEKL, bound to H2K^b) fetal thymic organ cultures produced CD4⁺CD8⁺ thymocytes and a smattering of mature CD4⁺CD8⁻ and CD4⁻CD8⁺ thymocytes by the end of the culture period. The addition of the OVA octamer peptide, SIINFEKL, and β 2m caused an apparent deletion of maturing thymocytes such that only CD4⁻CD8⁻ thymocytes remained. Clearly, as shown in other less physiological experiments, the nominal peptide able to activate mature T cells was also capable of deleting developing thymocytes (29).

Positively selecting peptides were predicted to engage the TCR without leading to T cell activation, and this predicted quality was reminiscent of altered peptide ligands. As such, Hogquist and coworkers tested a variety of peptides that were able to bind H2K^b with equal affinity, including single amino acid SIINFEKL variants. The results showed that peptides that stabilized MHC K^b on the cell surface but were unrelated to SIINFEKL did not result in CD8⁺ T cell maturation. These peptides included a "null" peptide with K^b anchor amino acids and serines at the epitope positions. Such a peptide was designed to bind K^b but neither hinder nor contribute to TCR-MHC binding. The SIINFEKL analogs were prescreened using an in vitro deletion assay, and only those altered peptide ligands that caused little to no deletion were tested for positive selection. The results showed that four of the peptides promoted the maturation of CD8⁺ T cells with all of the characteristics predicted for an Ag-reactive mature T cell: an HSA^{lo} phenotype, enhanced Bcl-2 expression, and proliferation in response to SIINFEKL. Further analysis showed that all four peptides displayed antagonism of SIINFEKL-specific target lysis: two were strictly antagonists, meaning they did not stimulate T cells at any concentration tested, whereas two were antagonists at low concentrations and agonists at higher concentrations. Those two that were agonists would also induce a measure of deletion in β 2m^{+/-} fetal thymic organ cultures.

The major implication of these results is that positive selection results from a highly specific TCR-mediated interaction that depends upon signals distinct from those needed for productive T cell activation and proliferation. A nuance is that peptides might serve to promote positive selection, negative selection, or T cell activation, depending on their abundance or context of presentation. On the basis of this consideration alone, a self peptide that was scarce in the thymus but abundant elsewhere in the body might be capable of eliciting an autoimmune reaction; however, clearly, other factors influence this outcome, including expression of the transcription factor AIRE in the thymus (30) and layers of tolerance mechanisms operative in secondary lymphoid organs (31). Given the results in F1 \rightarrow parent chimeras cited above, the repertoire of TCRs selected in this manner must show a strong preference for the universe of foreign peptides bound to the allelic variations of MHC molecules. Yet, to date, such allelic specificity does not appear to be manifest in TCR-MHC-peptide structures (32, 33). Thus, despite the clarity revealed by Hogquist et al., the process of thymic selection is still not without its mysteries.

Disclosures

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