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Proving Negative Selection in the Thymus

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Today, the idea that many self-reactive T cells are destroyed during ontogeny in the thymus seems almost self-evident. Yet proving this concept of "central tolerance," or "negative selection," was surprisingly difficult and depended upon a remarkable piece of detective work by the Kappler/Marrack group. Their definitive proof for negative selection was published quite recently, in 1987, and provided the last piece in a puzzle posed 40 years earlier by the classic experiments of Owen.

In studying binovular twin cattle sharing a common placenta, Owen (1) observed long-lasting chimerism of erythroid cells in the twins, implying induction of a state of mutual tolerance. This fundamental discovery led Burnet and Fenner (2) to predict that contact with Ag in very early life would induce specific tolerance, which was verified by the crucial transplantation studies of Billingham et al. in the early 1950s, first in twin cattle and then in neonatal mice (reviewed in Ref. 3). These findings together with para-biosis studies of Hasek (4) were highly influential at the time and led a few years later to formulation of the clonal selection theory by Burnet (5). Then came another vital piece of information, namely the discovery of Miller in 1961 that transplantation reactions and other forms of cellular immunity are controlled by the thymus (reviewed in Ref. 6). These experiments ushered in the age of T cells and led rapidly to the current view that self tolerance is due largely to deletion of immature T cells in the thymus during early ontogeny. Thymus-grafting experiments provided strong evidence that tolerance is induced within the thymus itself (6). But then came T suppressor cells and a decade of chaos (reviewed in Ref. 6).

This is not the place to discuss T suppressor cells and how many of the amazing claims about these cells have now simply melted away into obscurity. The point to emphasize is that, during the heyday of these cells in the 1970s and early 1980s, many immunologists turned away from the concept of central tolerance by deletion. Instead, it was argued that self tolerance reflected anergy rather than cell death and that T cells differentiating in an allogeneic thymus were deleted of thymic MHC-specific CTL precursors (reviewed in Ref. 7). However, there was still the objection that such functional clonal deletion reflected anergy rather than cell death and also that either deletion or anergy induction could be under the control of T suppressor cells. The key problem was that there was no way to distinguish between deletion and anergy. What was needed was a method for detecting individual Ag-specific T cells.

This problem was solved by the development of TCR-specific mAbs in the early 1980s (reviewed in Ref. 8). The Marrack/Kappler group was a major contributor to this field, and, with others, it soon developed a series of mAbs specific for the \( \alpha \beta \) TCR. For their work on thymic tolerance, the breakthrough came from studies with a VB-specific mAb prepared from BALB/c mice immunized with SWR T cells. From the immunized mice, Kappler et al. (9) isolated a B hybridoma, termed KJ23a, which had specificity for a new VB polymorphism, VB17a. This polymorphism was expressed in SWR and a few other rare mouse strains but was missing from BALB/c, C57BL/10, and other common strains. With KJ23a mAb, the authors prepared a panel of VB17a+ T hybridoma lines. These lines were then tested for H-2 alloreactivity, i.e., by determining whether the hybridomas were able to synthesize IL-2 when exposed to H-2-different spleen cells. The highly surprising finding was that nearly all of the VB17a+ hybridomas displayed specific H-2 alloreactivity for I-E molecules (the donor SWR strain being I-E\(^+\)); by contrast, I-E alloreactivity was almost undetectable for VB17a- hybridomas.

In a companion paper, the authors used FACS analysis with KJ23a mAb to define VB17a expression on mature T cells (10). As expected, VB17a+ cells accounted for a sizeable proportion (4–14%) of T cells from four strains, SWR, SJL, SJAg, and C57L, that carried the VB17a allele (established by Southern blot analysis). Staining of BALB/c and other common strains was negative, which correlated with these strains being VB17b rather than VB17a. The crucial finding was that mature VB17a+ T cells were virtually undetectable in the C57BR strain, even though this strain was known to carry the VB17a allele. This must have been a eureka moment for the authors because they knew that, of the five VB17a strains tested, only C57BR expresses an I-E molecule (the other strains have a mutation in the \( \text{E}^\alpha \) gene and consequently cannot express an I-E molecule, an E\(^\alpha\)-E\(^\beta\) heterodimer, on the cell surface). Hence it seemed highly likely that the absence of VB17a T cells in C57BR mice reflected deletion of these cells through contact with I-E molecules during their development in the thymus. In line with this interpretation, crossing SWR mice with various I-E\(^+\) strains led to a marked reduction in numbers of VB17a T cells. This reduction applied to mature T cells in the thymus as well as to peripheral T cells. However, the decisive finding was that I-E expression did not reduce VB17a expression at the level of immature T cells, i.e., CD4\(^+\)8\(^-\) (then termed L3T4\(^+\)/Lyt2\(^-\)) thymocytes. The conclusion therefore was that VB17a T cells did develop initially in an I-E\(^+\) thymus but were then eliminated during the transition of CD4\(^+\)8\(^-\) cells into mature...
CD4$^+$ and CD4$^-$ cells. The data thus provided unequivocal evidence for tolerance via clonal deletion.

At the time, the authors presumed that negative selection was directed to I-E molecules per se. However, it later became clear that the selecting ligands were actually endogenous superantigens (Mtv-8, -9) derived from mouse mammary tumor viruses (11). Like other superantigens, these ligands associate preferentially with I-E molecules and are recognized by particular TCR V$\beta$ segments, notably V$\beta$17a as well as V$\beta$5 and V$\beta$11. Negative selection in the thymus was rapidly confirmed by other groups and shown to apply to conventional Ags as well as to superantigens and to reflect cell death in situ (reviewed in Ref. 12).

Although clonal deletion in the thymus is now an established fact, it is clear that full induction of tolerance requires peripheral mechanisms, especially inhibition by T regulatory cells (the expurgated successors of T suppressor cells) (13). Thus, during the last decade, it has become apparent that ablating T regulatory cells or interfering with other forms of peripheral tolerance can lead to severe autoimmune disease. The implication therefore is that negative selection is “leaky.” But why? The most obvious possibility is that tissue-specific Ags, the main targets for autoimmune disease, are not expressed in the thymus and thus cannot induce intrathymic tolerance. However, recent work has shown that many Ags thought to be tissue-specific are actually synthesized in small amounts in the thymic medulla (14). In light of this finding, virtually all self-reactive T cells may be subject to at least some level of negative selection in the thymus. Nevertheless, because medullary expression of tissue-specific Ags is generally very low, one can envisage that negative selection to these Ags is limited to high-affinity T cells, leaving low-affinity T cells to escape to the periphery. These latter cells may be the “real” targets of T regulatory cells.

References