Commentary

The Molecular Jury: Deciding Whether Immature Thymocytes Should Live or Die

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The TCR repertoire, initially generated by random rearrangement of TCR α and β loci, is heavily shaped by the thymic selection processes. $CD4^+CD8^+$ thymocytes expressing TCRs that will in the peripheral lymphoid tissue recognize foreign antigens presented by self MHC are delivered survival and possibly differentiation signals by self peptide/self MHC complexes in the thymus (1). This process, known as positive selection, is also associated with a commitment of $CD4^+CD8^+$ thymocytes to the $CD4^+CD8^-$ or $CD4^-CD8^+$ lineage. Remaining thymocytes expressing TCRs not reactive with self peptide/MHC complexes are thought to die by default. In both cases of cell death in the thymus (peptide/MHC-induced or by default), thymocytes die by apoptosis (2). In addition, thymocytes expressing TCRs with potentially harmful reactivity with self peptide/MHC must be silenced or eliminated. This process of negative selection can be achieved by physical deletion (3–5) or by a variety of non-deletional mechanisms, including down-modulating the levels of coreceptor molecules (4, 6), rendering cells anergic (7, 8), or raising the activation thresholds in T cells (9).

One of the approaches to understand the apparent paradox of how the interaction of the same TCR with the self peptide/MHC complexes can result in two profoundly different outcomes (positive or negative selection) was to identify the cells in the thymus responsible for inducing the selection processes. Evidence for the major role of thymic epithelium in promoting positive selection is overwhelming. Early bone marrow chimera and thymus transplantation experiments have established that the MHC restriction pattern of antigen recognition in the periphery was efficiently imprinted by thymic epithelial cells (10–14). Subsequently, transgenic mice with limited tissue expression of the MHC class II have established the thymic cortex as the selection site (15). Thymus reaggregation cultures demonstrated convincingly that cortical epithelial cells were exclusively required for the conversion of $CD4^+CD8^+$ to $CD4^-CD8^+$ or $CD4^+CD8^-$ thymocytes (16, 17). Finally, long-term thymic epithelial cell lines were shown to imprint new MHC restriction patterns of antigen recognition after intrathymic injection (18, 19).

Recently, evidence demonstrating the ability of other cell types to induce positive selection has accumulated. Fibroblast cell lines injected intrathymicly were shown to induce positive selection (20, 21). In nude mice cells of unknown origin, but apparently non-thymic residents, imprinted restriction of T cells to the host MHC haplotype. This MHC restriction pattern could only be detected after transfer of a mixture of bone marrow and spleen cells to MHC mismatched SCID recipients to allow full maturation of T cells (22). Finally, wild-type bone marrow-derived cells can relatively inefficiently impose an MHC restriction pattern on themselves in MHC class I–deficient mice (23). Thus, under certain experimental conditions cells of non-epithelial origin may induce positive selection, but epithelial cells seem the most efficient, and in many cases the only, promoters of positive selection.

Early experiments assessing the capacity to induce tolerance suggested that bone marrow-derived, but not thymic epithelial cells can induce tolerance to MHC (24, 25). This was supported by findings that thymic epithelial cells could not induce deletion of particular TCR $V\beta$ expressing thymocytes that were readily deleted by the bone marrow derived cells (26). However, the ability of thymic epithelium to induce tolerance remained controversial, as reports accumulated suggesting that tolerance to defined peptide antigens or minor histocompatibility complex antigens (27, 28), as well as partial tolerance to major histocompatibility antigens (8, 29, 30) could be induced by thymic epithelium. Recent studies have demonstrated tolerance induced by thymic epithelium is complete with respect to the thymic stromal tissue itself, i.e., tolerized mice accept thymus grafts, but reject other tissues (31). These findings suggest that the lack of tolerance observed in some cases was due to differences in the peptides expressed by tolerazing thymic epithelial cells and skin grafts or bone marrow-derived cells used as stimulators in in vitro assays of tolerance. In addition, lack of expression of superantigens (sAg), revealed either by the inability of thymic epithelial cells to stimulate sAg-reactive T cell hybridomas (32), or by the lack of specific mRNA expression (33), might explain the inability of thymic epithelial cells to induce deletion of sAg-reactive $V\beta$ TCRs (26, 32, 34). In an in vitro deletion assay, a variety of cell types induced deletion of $CD4^+CD8^+$ thymocytes (35), including the thymic epithelial cell lines that also induced positive selection (36, 37). It can be therefore concluded that the ability to induce tolerance is not unique to any particular cell type in or outside the thymus. Still, bone marrow-derived cells may be more efficient since they induce deletion of $CD4^+CD8^+$ thymocytes whereas thymic epithelial cells induce non-deletional tolerance (38, 39).

In this issue Kishimoto et al. (40) describe experiments that may offer a molecular basis for distinct roles of thymic epithelial and bone marrow-derived cells in thymic selec-

tion events. The authors determined survival and death rates in suspension cultures of CD4$^+$CD8$^+$ thymocytes expressing the transgenic 2C TCR, specific for α-ketoglutarate dehydrogenase-derived octamer (p2Ca) or nonamer (QL9) peptides presented by H-2L^d (41). The affinity of the 2C TCR for the H-2L^d complexed to p2Ca is relatively high ($K_M = 2 \times 10^6$ M^{-1}) whereas the affinity for the H-2L^d complexed to QL9 is even higher ($K_M = 1$–2×10^7 M^{-1}) (42, 43). 2C CD4$^+$CD8$^+$ thymocytes were stimulated by either of the peptides presented by Drosophila cells expressing H-2L^d alone, or coexpressing adhesion/costimulatory molecules ICAM-1, B7.1, or both. In the absence of either adhesion molecule on Drosophila cells, only QL9 could induce apoptosis. The expression of B7.1 molecule enhanced the apoptosis induced by QL9. In addition, p2Ca induced apoptosis as well in the presence of B7.1. The expression of both B7.1 and ICAM-1 increased further the death rate induced by both peptides. Surprisingly, expression of the ICAM-1 in the absence of B7.1 inhibited the low levels of apoptosis induced by QL9, despite the fact that CD4$^+$CD8$^+$ cells were significantly activated as determined by the induction of CD69 expression. The results suggest a direct relationship between the strength of extracellular stimulation of CD4$^+$CD8$^+$ cells (defined as a sum of avidities of different intercellular interactions), and the induction of apoptosis. If the TCR recognizes peptide with a relatively higher binding affinity, apoptosis can be induced without costimulation. Additional adhesion/costimulatory interactions further enhance the apoptotic response. However, the protective effect of ICAM-1 against apopto-

sis induced by QL9 peptide suggests that the strength of extracellular stimulation is not the sole factor determining the fate of CD4$^+$CD8$^+$ cells, and that the quality of the signal (likely related to the use of specific intracellular activation pathways) is also important.

How do these findings from in vitro deletion assay apply to selection in the thymus? The patterns of the ICAM-1 and B7.1 molecule expression in the thymus are very suggestive (44, 45). Cortical epithelial cells express ICAM-1, but not B7.1. By contrast, both B7.1 and ICAM-1 are expressed in the medulla by bone marrow-derived cells as well as medullary epithelium. Despite tissue specific expression of some peptides (31), most MHC-bound peptides seem to be shared by positively selecting epithelium and bone marrow-derived cells (46, 47). If the in vitro studies apply to the in vivo situation, then one can envisage several outcomes of thymic selection with the involvement of same peptides expressed by different cell types (Fig. 1). As mentioned earlier, positive selection probably reflects more than just a survival signal (1) and it is not clear at this point whether survival of cells in the in vitro assay performed by Kishimoto et al. represents positive selection, non-deletional tolerance, or both. Since virtually all cells in the thymus express ICAM-1 (44), stimulation with peptide/MHC complex alone does not occur in vivo. Therefore, there may be a positive correlation between the signal strength and the fate of CD4$^+$CD8$^+$ cells, as illustrated in Fig. 1.

Which molecular interactions contribute to the signal received by CD4$^+$CD8$^+$ thymocytes? Different presenting cells in the thymus may directly affect the signal delivered
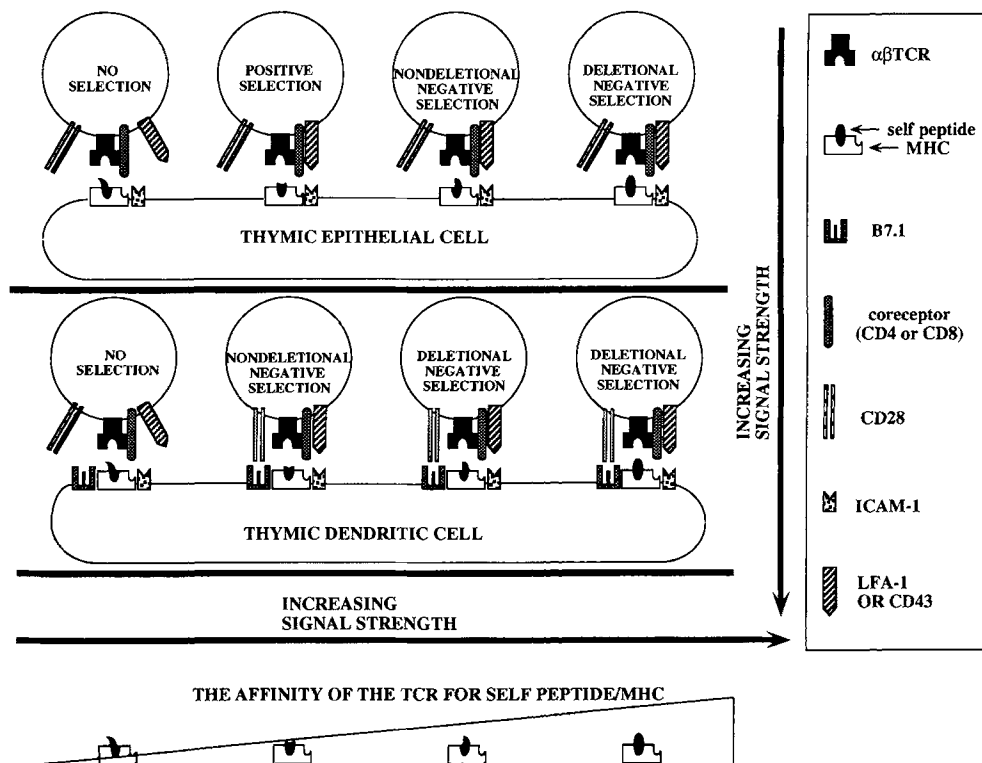
Figure 1. Thymocyte fate in the function of the signal strength received from different stromal cells. Note that the effect of the number of individual receptor-ligand interactions has not been illustrated. Adhesion/costimulatory molecule interactions in addition to those depicted here probably exist.

to immature thymocytes through (a) MHC-associated self peptides interacting with the TCR; (b) MHC molecules interacting with coreceptor molecules; and (c) adhesion/costimulatory molecules. TCR engagement seems to be the crucial event. Both the quality (defined as affinity and/or efficacy) and quantity of TCR-peptide/MHC interaction can determine the survival or death of thymocytes (6, 48–54). However, in some of these models survival was the sole criteria for positive selection, and the responsiveness to antigen has not been determined (51, 52). Although coreceptor molecules have to engage the same MHC molecule as the TCR (55), overexpression of the coreceptor molecules may enhance negative selection (56, 57), suggesting that the local concentration of the coreceptor molecule at the interface between the immature thymocytes and antigen presenting cells may be lower than that of the TCR. Thus, the number of coreceptor molecules engaged in antigen recognition events may be limiting under normal circumstances. Finally, stimulation of thymocytes with anti-CD28 antibody increases the anti-CD3 induced cell death of $CD4^+CD8^+$ thymocytes (58), and interaction with ICAM-1 may be necessary for in vitro deletion by dendritic cells (59).

If ICAM-1 expression by Drosophila cells can convert apoptosis induced by the high affinity ligand for TCR into survival, the following question arises: can cortical epithelial cells, that express ICAM-1, but not B7.1, induce tolerance? Cortical epithelial cells likely express additional adhesion/costimulatory molecules that may synergize with ICAM-1 to produce signal strength sufficient to induce apoptosis. Under the physiological conditions, therefore, it could be expected that the avidity of the TCR and coreceptor interaction with the cortical epithelial peptide/MHC complex plays a critical role in determining the outcome of interaction- no interaction results in the death by default; weak interaction results in positive selection; somewhat stronger interaction will lead to nondeletional tolerance; and finally, very strong interaction leads to cell death. It remains to be determined whether very high strength of the signal is ever achieved in the case of cortical epithelial cells. Bone marrow-derived cells, best represented by dendritic cells, offer so many adhesion/costimulatory ligands for $CD4^+CD8^+$ thymocytes, that even the very low signal strength achieved by the TCR and coreceptor interaction with the peptide/MHC complex is given sufficient amplification to induce tolerance rather than positive selection. Medullary epithelium probably provides the range of signal strengths either equal to, or somewhat lower than those provided by dendritic cells, but higher than those provided by cortical epithelium.

There are many aspects of the signal strength model that can be tested. One prediction, for example, would be that bone marrow-derived cells from B7-deficient mice should be more efficient at promoting positive selection than their wild-type counterparts. Conversely, B7.1 expressed by the thymic cortical epithelium should reduce the potency of these cells to induce positive selection. B7.1-transfected thymic epithelial cell line was less efficient than its mock-transfected counterpart in promoting the appearance of $CD4^-CD8^+$ thymocytes after intrathymic injection into $\beta2$-microglobulin–deficient mice (60) offering an indication that the latter prediction may be valid. Some questions still remain open, such as whether survival in the deletion assay reflects positive selection or non-deletional tolerance and whether signals imprinting MHC restriction in T cells or differentiation into mature $CD4^-CD8^+$ or $CD4^+CD8^-$ thymocytes are delivered by same cells in the thymus. The findings of Kishimoto et al. (40) offer a powerful model to study the nature of intracellular signals that mediate survival or death of immature $CD4^+CD8^+$ thymocytes.

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