A differential-avidity model for T-cell selection

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The processes of positive and negative selection during thymic development shape the repertoires of antigen specificities displayed by T cells. This rids the animal of potentially autoreactive T cells and, at the same time, ensures that they are capable of major histocompatibility complex (MHC)-restricted recognition of antigen. Paradoxically, both processes involve the engagement of the T-cell receptor (TCR) on immature thymocytes with peptide/MHC complexes expressed on thymic stromal cells. Here, Philip Ashton-Rickardt and Susumu Tonegawa suggest that the critical parameter determining the outcome of this interaction is the number of TCRs occupied by peptide/MHC complexes and that this, in turn, is determined by the avidity of the TCR–MHC interaction: low avidity resulting in positive selection and high avidity resulting in negative selection.

The molecular recognition of antigen is fundamental to the adaptive immune system in allowing the host animal not only to respond to potentially pathogenic invaders, but also to distinguish these invaders from benign self components. Unlike the antigen receptor of B cells, which can bind free antigen, T-cell receptors (TCRs) recognize complexes composed of antigen-derived peptides and self major histocompatibility complex (MHC) molecules. Thus, the TCR exhibits dual specificities, one for the antigen and the other for the MHC molecule, the latter being referred to as MHC-restriction specificity[1,2]. X-ray crystallographic studies have revealed that the peptide-binding site of the MHC comprises a groove between the two antiparallel α helices of the N-terminal domains of either the MHC class I molecule[3,4] or the MHC class II molecule[5]. Most of the MHC class I-binding peptides are generated in the cytosol and are transported into the endoplasmic reticulum by an ATP-dependent peptide transporter encoded by the *TAP-1* and *TAP-2* genes[6], where they associate with newly synthesized MHC class I molecules[7]. Peptide binding is essential for the efficient assembly and intracellular transport of MHC class I molecules, as well as for the stable expression of these molecules on the cell surface[7]. Peptide/MHC class I complexes are recognized by $CD8^+$ cytotoxic T cells (CTLs) (Ref. 8), while peptide/MHC class II complexes are recognized by $CD4^+$ T helper cells[7].

Thymic development shapes the repertoire of T-cell antigen specificities

The genes encoding the α and β chains of the TCR undergo random rearrangement in immature $CD4^-CD8^-$ (double negative, DN) or $CD4^+CD8^+$ (double positive, DP) thymocytes, generating TCRs with great structural diversity[9]. Although the repertoire of TCRs expressed by thymocytes leaving the thymus is large, it is substantially less than that of immature thymocytes. This intrathymic reduction in the diversity of the TCR repertoire occurs through two types of cellular selection process[9]. To ensure that T cells leaving the thymus will be capable of MHC-restricted antigen recognition, only those immature DP thymocytes that express TCRs with sufficient binding affinity for peptide/self-MHC complexes expressed on thymic stromal cells are given the signal to differentiate any further[10–12]. This process is known as positive selection[13,14]. The majority of DP thymocytes do not undergo positive selection and are eliminated by programed cell death (PCD). Engagement of TCRs with MHC class I molecules results in differentiation into $CD4^-CD8^+$ T cells, whereas engagement of TCRs with MHC class II molecules leads to differentiation into $CD4^+CD8^-$ cells[15]. Subsequently, negative selection eliminates, through clonal deletion, those T cells that are potentially autoreactive[16,17]. This process is also thought to be triggered by engagement of TCRs on immature thymocytes with peptide/self-MHC complexes expressed on thymic stromal cells. However, one of the central questions that has remained unsolved, and has sometimes been referred to as the 'thymic paradox', is how the similar interactions that take place in the thymus between TCRs and peptide/self-MHC complexes trigger two completely different cell fates during positive and negative selection.

The ligands recognized during thymic selection

Experiments with TCR-transgenic mice have demonstrated that negative selection depends on the recognition of peptide/self-MHC complexes. Typically, the addition of the nominal antigen to *in vitro*[18,19] or *in vivo*[20,21] TCR-transgenic systems results in clonal deletion of the antigen-specific clone. Recently, the

ligand recognized in positive selection has been identified as a peptide/self-MHC complex. This was demonstrated using fetal thymus organ culture (FTOC) systems based on two different MHC class I-deficient mice, namely β_2-microglobulin (β_2-m) (Ref. 22) and TAP-1-mutant mice[23], which both exhibit impaired positive selection of $CD8^+$ T cells. In TAP-1^- mice, the cell-surface expression of class I molecules is severely impaired and the addition of exogenous MHC class I-binding peptide restores stable expression of peptide/MHC class I expression on the surface of cells[23]. Indeed, the addition of several MHC class I-binding peptides to FTOC of TAP-1^- mice rescued MHC class I expression on thymic stromal cells, although only some of these peptides promoted the positive selection of $CD8^+$ T cells[24]. Furthermore, as observed in FTOC of β_2-m^- mice, complex mixtures of MHC class I-binding peptides were more efficient than single peptides in promoting the positive selection of $CD8^+$ T cells[25]. Therefore, it is apparent that both in positive and negative selection, not only is self MHC recognized by the TCR, but also many other self peptides are recognized. It is this that determines the repertoire of mature T cells.

Differential-avidity model of thymic selection

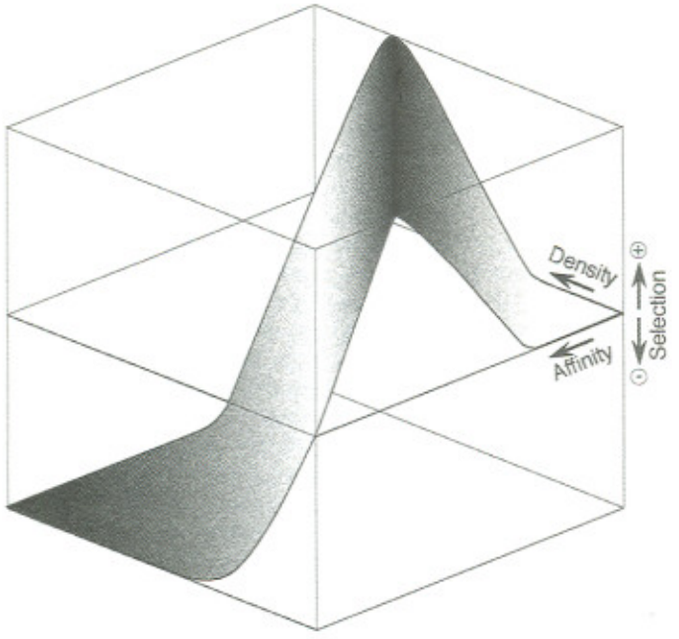
The positive and negative selection of a lymphocytic choriomeningitis virus (LCMV) peptide-specific H-2D^b-restricted T-cell clone (P14) was investigated using TAP-1^- and TAP-1^+ mice transgenic for P14 TCR α and β genes[26]. It was found that positive selection of transgenic $CD8^+$ P14 cells was impaired in TAP-1^- mice[27]. Analysis of FTOC from TAP-1^- and TAP-1^+ P14-transgenic mice, which were supplemented by various H-2D^b-binding peptides, revealed that both the positive and negative selection of the precursors of $CD8^+$ P14 cells were peptide specific[27]. Thus, the nominal antigen peptide of LCMV (residues 33–41) could positively select $CD8^+$ P14 cells when its expression on thymic stromal cells was kept at a relatively low density. However, when the same peptide was expressed at a higher density in TAP-1^- FTOC, negative selection occurred. In TAP-1^+ FTOC, lower input concentrations of antigen peptide than that required for negative selection in TAP-1^- FTOC were sufficient to achieve negative selection. Another H-2D^b-binding peptide [the influenza nuclear protein peptide IF-NP 1934 (366–374)], which must be assumed to have a very low affinity for the P14 TCR, could positively select $CD8^+$ P14 cells on TAP-1^- FTOC when expressed at an artificially high density on thymic stromal cells. A variant LCMV peptide, LCMV-8.1, which is 1000-fold less potent in the activation of P14 spleen cells than the LCMV (33–41) peptide, but has a similar binding affinity for H-2D^b, required a higher input concentration than the LCMV peptide for optimal positive selection of $CD8^+$ P14 cells. Although this variant peptide positively selects $CD8^+$ P14 cells in TAP-1^- FTOC, it negatively selects these cells in TAP-1^+ FTOC when used at the same input concentration (P.G. Ashton-Rickardt and S. Tonegawa, unpublished).

Recently, Sebzda *et al.*[28] also found that the P14 clone can be positively selected by a low concentration of the LCMV peptide, and negatively selected by a higher concentration of the same peptide, in FTOCs derived from P14-transgenic mice with a disruption in the β_2-m gene. While the conclusion drawn from these studies is consistent with the one discussed above, there is a serious discrepancy with respect to the concentrations of the peptide at which positive or negative selection occurs. Thus, in our FTOC system, positive selection was observed within a range of 10^{-5}–10^{-4} M input concentration of the LCMV peptide, whereas Sebzda *et al.* reported positive selection at 10^{-12} M. Likewise, negative selection takes place at 10^{-4} M or higher in our system, whereas Sebzda *et al.* report that it takes place at 10^{-6} M. It is difficult to define the exact cause(s) of these differences when the two studies were carried out in different laboratories using different knockout mice. However, it is likely that one major cause stems from the well-known fact that the β_2-m-knockout mouse is leaky with respect to blocking the surface expression of H-2D^b (Ref. 22).

Based on the results described above, it may be postulated that the critical parameter determining whether a given T-cell clone is positively or negatively selected is the avidity of the interaction between the TCRs of the thymocyte and the peptide/self-MHC complexes of the thymic stromal cells[29]. Avidity is defined as the number of engaged TCRs per thymocyte and is the product of several factors, including: the intrinsic affinity of the TCR for peptide/MHC; the density of the TCR; and the density of the peptide/MHC complexes. The co-receptors CD4 and CD8 are included in this functional definition of the TCR. When the avidity is lower than a minimal threshold, no signal is delivered and the thymocyte is not rescued from PCD. When the avidity is above this threshold, and within a certain range, the signal for positive selection is delivered. When the avidity is higher than this range, then the signal for negative selection is delivered. Figure 1 models the effects of variations in peptide/MHC density and intrinsic affinity for the TCR on the developmental fate of a given T-cell clone.

Such a differential-avidity model explains why $CD8^+$ P14 cells are more susceptible to negative selection in TAP-1^+ compared with TAP-1^- FTOC. In TAP-1^+ thymi, the basal level of avidity necessary for positive selection has already been reached by the interaction between the TCRs and peptide/self-MHC complexes. By contrast, in TAP-1^- thymi, in which surface MHC class I expression is severely impaired, the basal level of avidity is too low to drive positive selection. Therefore, the increase in avidity resulting from the addition of the same concentration of LCMV (or LCMV-8.1) peptide results in negative selection in TAP-1^+ FTOC and positive selection in TAP-1^- FTOC.

The avidity necessary for positive selection is lower than that required for negative selection which, in turn, is lower than that required for activation of mature T cells. It is almost certain that the repertoire of self peptides utilized in the thymus for positive selection is substantially smaller than the repertoire of foreign peptides that need to be covered by mature T cells. This hierarchy in relative avidities makes immunological sense, since lower avidity means higher crossreactivity. Thus, the interaction between the TCR and self-peptide/MHC during positive selection should be more crossreactive than the interaction that occurs between



g. 1. *Relationship between the developmental fate of a T-cell receptor (TCR) on a thymocyte clone with the density and affinity of major histocompatibility complex (MHC)/peptide recognized on the thymic stromal cell. In this scheme, there are three developmental outcomes from the engagement of the TCR with a peptide/MHC complex of a given affinity at a certain density. These are: no selection (colorless area, selection = 0); positive selection (red area, selection = 0 − positive values); and negative selection (blue area, selection = 0 − negative values).*

the TCR and foreign-peptide/MHC during activation of mature T cells, thereby allowing for the selection of a wide range of TCR specificities. Since negative selection apparently requires less avidity than activation, and hence is more crossreactive than peripheral recognition, there is a margin for safety in the self-tolerance achieved by negative selection. The difference in avidity requirements between positive and negative selection means that T-cell clones with overtly self-reactive TCRs are eliminated while, at the same time, those capable of recognizing self-peptide/MHC complexes are allowed to differentiate.

The differential-avidity model and other models of thymic selection

The differential-avidity model, in its minimal form, does not depend on dedicated thymic stromal cells specializing in positive (e.g. cortical epithelium) and negative (e.g. medullary bone-marrow-derived cells) selection, as was proposed in an earlier model of thymic selection known as the 'altered-ligand' hypothesis[30]. This model sought to explain the thymic paradox by proposing that, while self peptides bound to MHC on negatively selecting bone-marrow-derived cells are representative of those found in the periphery of the animal, positively selecting epithelial cells generate a unique set of self peptides. Since these epithelial-specific peptides would be found only in the thymic cortex, there would be no risk of mature T cells being activated by encountering self-peptide/MHC complexes in the periphery. However, direct analysis of peptides eluted from thymic MHC molecules has not supported the idea that cortical epithelium presents a unique set of peptides[17]. In addition, the observation that a single peptide can induce either positive or negative selection depending on the concentration applied to cultured thymi argues against the notion that different sets of self peptides act as ligands for positive and negative selection[27]. Furthermore, recent studies showing that a variety of cell types can induce positive and negative selection further challenge the altered-ligand hypothesis[17,30].

Other models proposed to explain thymic selection have postulated that TCR engagement resulting in negative selection arises from high-affinity interactions with self-peptide/MHC, and that positive selection arises from low-affinity interactions of TCR with self MHC (Ref. 31). In the differential-affinity model, the critical parameter determining the developmental fate of a thymocyte is the intrinsic affinity of the TCR for a given self-peptide/ MHC complex. However, it has been shown that the density of peptide/MHC complexes on thymic stromal cells also determines the developmental fate of a thymocyte clone. For example, a high-affinity peptide (the P14 nominal antigen) can both negatively and positively select depending on the level of expression of the peptide on thymic stromal cells[27].

It has been demonstrated that closely related variants of antigenic peptides can still stimulate T-cell clones *in vitro* (so-called peptide agonists) and/or dominantly inhibit the stimulation of a T-cell clone by the antigenic peptide (so-called peptide antagonists)[27,32,33]. In a recent paper, Hogquist *et al.*[34] reported that all the peptides that could induce the positive selection of H-2K^b-restricted ovalbumin (OVA)-specific CD8$^+$ T cells in FTOC from β_2-m$^-$ mice possessed antagonistic properties. These data are consistent with the notion that positive selection is mediated by a dedicated set of variant peptides that bind TCRs but cannot deliver an activation signal to mature T cells or a deletion signal to immature T cells, either because of their low intrinsic affinity or their inability to induce a conformational change in the engaged TCR. However, these authors also found that one of these antagonistic peptides, E1, can deliver a negative selection signal. Furthermore, the possibility exists that doses of OVA peptide appropriate for positive selection may not have been tested. Alternatively, it is possible that there is an upper limit in the intrinsic affinity of the TCR for the peptide/MHC complex beyond which the signal for positive selection would not be delivered (as discussed in Ref. 35).

There are other findings that support the notion that, except for extreme cases, affinity is not the sole parameter that determines the selection of a T-cell clone. Thus, the natural LCMV peptide variant LCMV-8.1, which has agonistic activity, can positively select CD8$^+$ P14 cells in TAP-1$^-$ FTOC (Ref. 27) and can negatively select the same cells in TAP-1$^+$ FTOC (P.G. Ashton-Rickardt and S. Tonegawa, unpublished) or TAP-1$^+$ mice[36]. Furthermore, Page *et al.*[37] have recently reported the negative selection of an MHC class II-restricted anti-cytochrome T-cell clone by several antagonist peptides in suspension cultures.

Signaling in thymic selection

The hypothesis presented here states that a given MHC-binding peptide has the capacity to induce both positive and negative selection of a T-cell clone as long as its intrinsic TCR-binding affinity is above the minimal threshold that permits induction of a physiochemical alteration in the engaged TCR, this being required for signal delivery. Whether this results in positive or negative selection depends on the number of engaged TCRs on the thymocyte; when this number is below a certain threshold, positive selection occurs; when this number is above the threshold, negative selection takes place.

How the difference in the number of engaged receptors leads to differential signals during positive *versus* negative selection is at present a matter of speculation. One possibility is that thymocytes undergoing negative selection receive more of the same signals than do thymocytes undergoing positive selection. In this case, the differential effects are based on a quantitative difference in the amount of a common signal (Fig. 2). Alternatively, qualitatively different signals may be generated depending on the number of engaged receptors. For instance, it is quite feasible that as the density of the engaged receptors increases many of them may aggregate into higher-order structures. This may lead to the generation of a novel signal that is different to that generated by engaged TCRs of lower densities, which would remain either as monomers or lower-order aggregates (Fig. 2). Several recent experiments suggest possible mediators of differential signals for positive and negative selection. Thus, the protein tyrosine kinase $p56^{lck}$, which associates with the TCR co-receptors CD4 and CD8, is activated in thymocytes undergoing positive but not negative selection[38]. Mice deficient in the CD45 phosphatase, encoded by a version of the gene utilizing exon 6, show a defect in positive selection, whereas all other aspects of thymic development including negative selection seem to be normal[39].

Natural self peptides for thymic selection

The studies carried out to date using TAP-1- or β_2-m^- mice have examined whether, and under what conditions, a given MHC class I-binding peptide can promote positive or negative selection of $CD8^+$ T-cell clones. They do not directly address the issues involving the structure or composition of thymic peptides that are actually used during the selection processes. For instance, the antagonist or agonist peptides that induce positive selection when added to FTOC may or may not be utilized *in vivo* in the thymus as ligands in positive selection. An obvious next task is to identify the natural self peptides that are involved in the selection of a known T-cell clone and characterize them with respect to the sequence and cell-surface density. The use of transgenic and knockout mice, combined with state-of-the-art peptide technology, may permit resolution of at least some of these issues in the near future.

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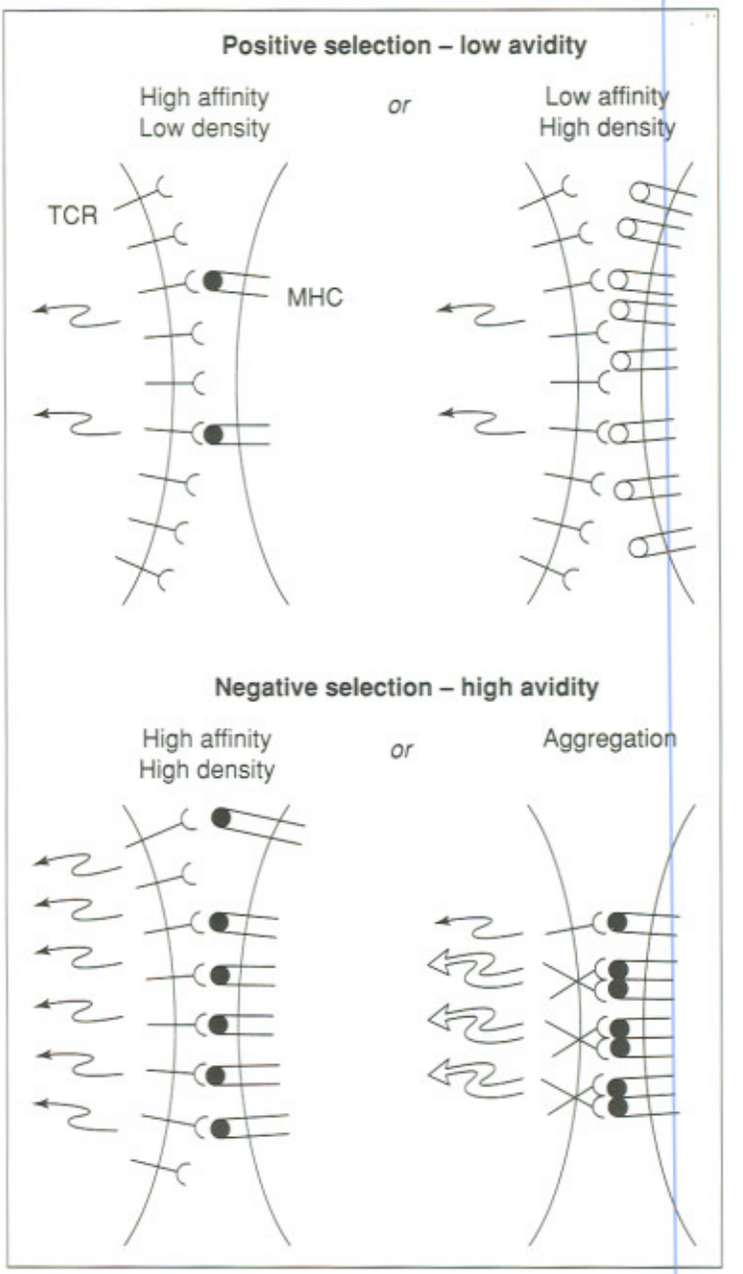

Fig. 2. *Model describing how transduction of signals by the T-cell receptor (TCR) leads to positive and negative selection. Low-avidity interactions between the TCR and the major histocompatibility complex (MHC)/peptide leads to positive selection, whereas high avidity leads to negative selection. Negative selection may occur by an increase in the frequency of a signal leading to positive selection or in response to novel signals generated uniquely by high-avidity interactions. Filled circles denote high-affinity peptide, open circles denote low-affinity peptide.*

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