European Journal of Immunology

# The effects of thymic selection on the range of T cell cross-reactivity

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Based on the results of a computational model of thymic selection, we propose a mechanism that produces the observed wide range of T cell cross-reactivity. The model suggests that the cross-reactivity of a T cell that survives thymic selection is correlated with its affinity for self peptides. In order to survive thymic selection, a T cell with low affinity for all self peptides expressed in the thymus must have high affinity for major histocompatibility complex (MHC), which makes it highly cross-reactive. A T cell with high affinity for any self peptide must have low MHC affinity to survive selection, which makes it highly specific for its cognate peptide. Our model predicts that (1) positive selection reduces by only 17% the number of T cells that can detect any given foreign peptide, even though it eliminates over 95% of pre-selection cells; (2) negative selection decreases the average cross-reactivity of the pre-selection repertoire by fivefold; and (3) T cells responding to foreign peptides similar to self peptides will have a lower average cross-reactivity than cells responding to epitopes dissimilar to self.

Received 1/6/05
Revised 26/8/05
Accepted 29/9/05

[DOI 10.1002/eji.200535098]

**Key words:** Computational modeling · Cross-reactivity · T cells · TCR · Thymic selection

## Introduction

The ability of an individual T cell to recognize related antigenic peptides is an essential part of the body's defense against mutating pathogens. T cells detect intracellular pathogens by binding to foreign peptides presented by major histocompatibility complex (MHC)-encoded proteins on the surfaces of infected cells or on specialized antigen-presenting cells [1–3]. Although T cells are specific to their cognate peptides, they cross-react with many others [4]. It has been estimated that a T cell can detect an average of $10^6$ different peptides [5]. However, not all T cells are equally cross-reactive; it has been observed that the number of peptides to which a single T cell can respond varies widely [6].

T cell recognition of antigen is determined by binding interactions of T cell receptors (TCR) with peptide-MHC complexes (pMHC). Each T cell expresses thousands of copies of identical TCR that bind with high affinity to their cognate peptide presented by MHC. T cells detect the presence of pathogens when their receptors have sufficiently high affinity for the pMHC on a target cell and remain in contact with pMHC for a time sufficient to generate a stimulatory signal. Because the pMHC presents a single binding target for the TCR [7], both the peptide and the structure of the presenting MHC molecule play a role in determining affinity.

TCR are initially generated by V(D)J recombination, with specificities to a wide range of peptides, including

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**Abbreviation:** **pMHC:** peptide-MHC complex

self peptides generated from normal proteins produced by healthy cells [8, 9]. Most self-reactive T cells are screened out early in their maturation process in the thymus, where they are exposed to a large array of the body's peptides presented on MHC molecules [10]. During positive selection, T cells that have an extremely low avidity to self peptides bound to MHC die by neglect [11–13]. It is believed that this process eliminates T cells that have such poor avidity to MHC that they would not bind to any pMHC. Negative selection eliminates those that bind too tightly to MHC-self peptide complexes, ensuring that potentially self-reactive T cells are eliminated [8]. About 1–3% of pre-selection T cells pass both these "tests" and leave the thymus to become active T cells [14].

We created a computational model to test the hypothesis that thymic selection acts upon a random T cell repertoire to generate a T cell repertoire with a wide range of cross-reactivities. The model uses a few assumptions about TCR-pMHC interactions and thymic selection to generate a repertoire of T cells specific for a large number of simulated peptides and with a wide range of cross-reactivities. We determine the consequences of thymic selection by analyzing the repertoires before and after selection; then we discuss the implications of the results on the function of positive selection and antigenic escape.

## Results

We studied the effects of thymic selection on the T cell repertoire using a computational model. This model, described in detail in the Materials and methods section and in [15], generates a set of 30 000 random peptides to represent "self" peptides expressed in the thymus of an organism and a set of T cell clones, each with a single randomly generated TCR, to represent the pre-selection T cell repertoire. Each peptide is presented by one of three different MHC types to form pMHC. For all T cell-pMHC combinations, the model computes the antigenic distances between the T cell and peptide and between the T cell and the presenting MHC. Antigenic distance represents how well the T cell binds to the peptide or MHC; the smaller the distance, the tighter the binding. Antigenic distance is reported in arbitrary units defined by the model's implementation. The affinity of the T cell for a particular pMHC is inversely related to the sum of its antigenic distances to the peptide and the MHC. The model's thymic selection process eliminates T cell clones with a low affinity for all self pMHC (positive selection) and those with high affinity for any self pMHC (negative selection). We assume that a mature T cell can detect and respond to an MHC-foreign peptide complex if it has an affinity for the pMHC that is greater than or equal to that required to induce negative selection in the thymus. Fig. 1 depicts the relationship between a T cell's affinity for self peptide and for MHC during different stages of thymic selection.

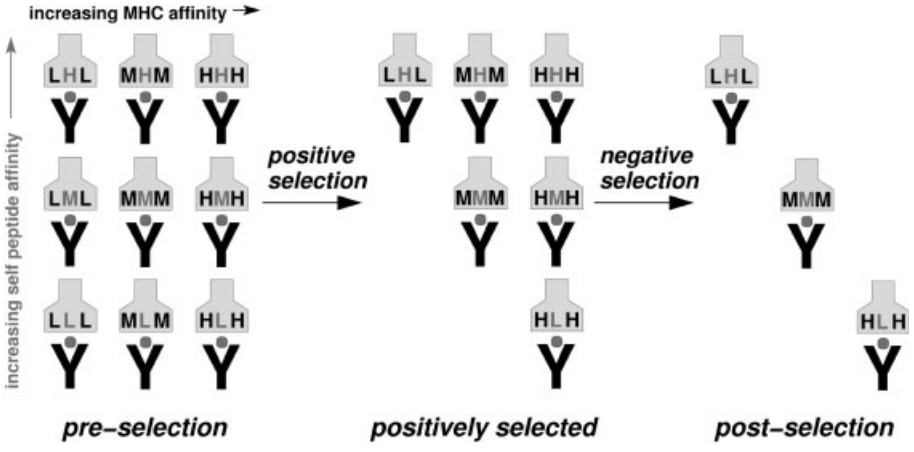


**Figure 1.** Thymic selection introduces a relationship between MHC and self peptide affinity in T cells. The figure depicts T cell affinity for self pMHC during three stages of maturation. The first, on the left, is the pre-selection repertoire, which has all possible combinations of affinities that TCR can have for their selecting pMHC in the thymus. The second, in the middle, is the positively selected repertoire in which only the cells with sufficient affinity for self pMHC survive. The final stage, on the right, is the mature repertoire, after negative selection, which eliminates T cells with high affinity for self pMHC. TCR are shown as gray blocks, the self peptides to which they have the highest affinity as small filled circles, and the presenting MHC as Y-shaped objects. In the model, the total affinity of a T cell for pMHC is the sum of its affinity for the MHC and the peptide portions of the complex. Therefore, the affinity in the figure is represented as three letters in the figure; the middle letter represents the affinity for self peptide, which can be high (H), medium (M) and low (L), and the outer letters the affinity for the presenting MHC. Thus, "HLH" represents a TCR that has high affinity for MHC and low affinity for self peptide.

 

The effects of thymic selection in the model are determined by comparing the T cell repertoire before and after selection, using two measures: the peptide coverage of T cells and their antigenic distances to a pMHC. We define peptide coverage to be the fraction of random peptides that a T cell can detect when the peptide is presented on the MHC type associated with its selecting pMHC, which is the self pMHC to which the T cell has the highest affinity during thymic selection. The larger the coverage, the greater is a T cell's cross-reactivity.

## The impact of positive selection on responses to foreign peptides

We quantified the effects of thymic selection on the T cells that could respond to random foreign peptides. For each of 1000 randomly generated foreign peptides presented by MHC, an average of $231 \pm 15$ (mean $\pm$ standard deviation) pre-selection T cell clones out of $2.5 \times 10^8$ could respond to it, *i.e.* approximately 1 in $10^6$. Of these, $23 \pm 5.5$ (about 10%) survived thymic selection, with $40 \pm 14$ (17%) eliminated during positive selection and $168 \pm 19$ (73%) by negative selection. These figures are somewhat surprising because the number of pre-selection cells that can respond to a particular epitope in our model is reduced by only 17% by positive selection, even though positive selection eliminates over 95% of pre-selection T cells [16]. We repeated the experiment using alternative parameter choices, described in the Materials and methods section, in order to determine the effects of allowing a greater fraction of T cells to survive positive selection and of increasing the role of MHC in TCR-pMHC binding. The impact of positive selection on the number of cells responding to a foreign pMHC was smaller (reducing the number of responding cells by 5%) when the model was adjusted to positively select 3% of pre-selection cells rather than the 0.8% used in the base model. Its impact was also reduced (reducing the number of responding cells by 0.5%) when the contribution of MHC to TCR-pMHC binding was increased from 40% to 58% of total binding to incorporate contributions from both conserved and polymorphic residues (see Materials and methods for details).

There is a tradeoff between the cost of maintaining T cells and the potential benefit of having them during an immune response. By preferentially eliminating the majority of pre-selection T cells that are unlikely to respond to antigen because of their low affinity for MHC, the immune system reduces the cost of maintaining the T cell repertoire without incurring a large cost at the time of antigenic challenge. Our quantitative estimates could be verified in the laboratory by comparing the number of pre-selection clones that respond to a particular pMHC before and after thymic selection. We predict that although most T cells do not survive positive selection, its impact on the number of T cells that respond to any particular foreign peptide will be small.

## Negative selection increases peptide binding specificity

To determine the effects of negative selection, the model's T cell repertoire was examined before and after negative selection. We generated 1000 random T cell repertoires, and for each of these one random foreign peptide was generated and associated with one of three MHC types. The peptide coverages and the distances to the foreign pMHC of all T cells in the repertoires were measured for the positively selected repertoire before and after negative selection. Negative selection reduced the average T cell's peptide coverage, *i.e.* the fraction of pMHC recognized, from $4.7 \times 10^{-4}$ to $9.8 \times 10^{-5}$, nearly a fivefold difference. In Fig. 2a, this can be observed by the decrease in T cells with high coverage after negative selection. The change in average cross-reactivity was greater when the model parameters were altered either to allow a larger fraction of pre-selection cells to be positively selected (the average cross-reactivity was reduced by over 12-fold) or to increase the contribution of MHC to TCR-pMHC binding (over 500-fold). T cells with high MHC affinity are likely to be eliminated because they can survive only if they have low affinity for all self peptides, as illustrated in Fig. 1. Because average cross-reactivity (*i.e.* coverage) decreases after negative selection, specificity to foreign peptide increases. In Fig. 2b, we show that the average antigenic distance between T cells and foreign peptides is decreased after negative selection. This finding agrees with the observation that negative selection increases the specificity that TCR have to foreign peptides [17–19].

## T cell cross-reactivity is lower for epitopes similar to self

To compare the T cells responding to foreign peptides similar and dissimilar to self, we measured the cross-reactivities of the simulated T cells responding to 10 000 different foreign pMHC at various antigenic distances from self pMHC. Fig. 3 plots the average peptide coverage of T cells that could respond to the foreign pMHC against the distance from the pMHC to the nearest self pMHC. The average coverage of responding T cells is correlated with the foreign pMHC's distance to the nearest self peptide. For pMHC distant from all self pMHC, the average T cell coverage is about $1.5 \times 10^{-4}$.

 

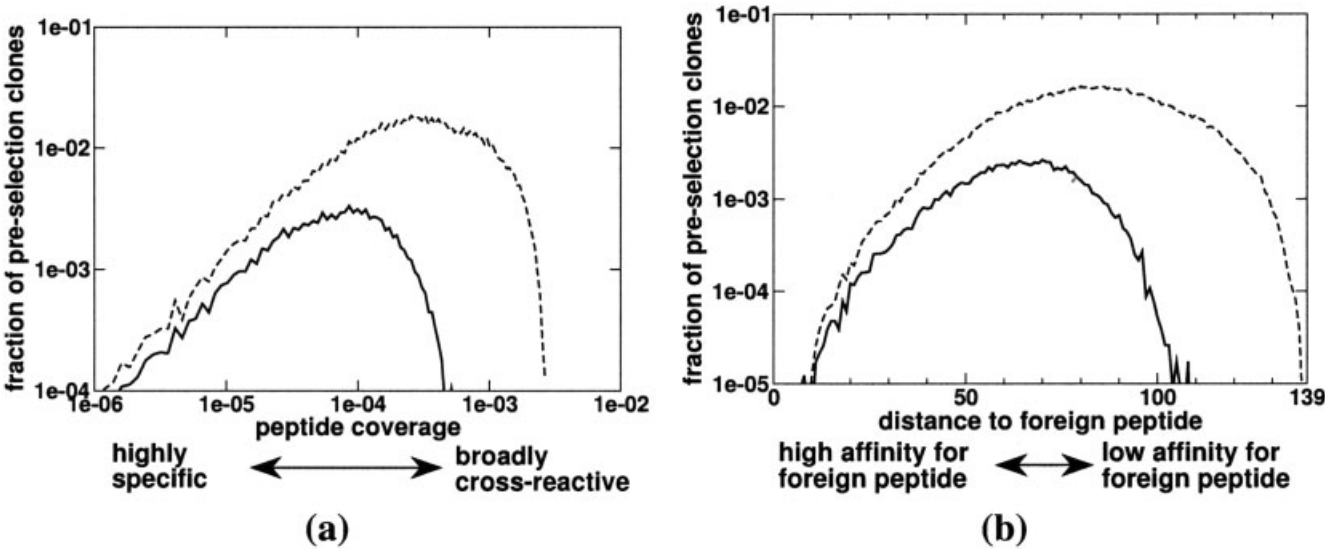


**Figure 2.** Effects of negative selection on the T cell repertoire. A T cell repertoire was generated for a single MHC-foreign peptide complex. The pre-selection repertoire of T cells with affinity for the foreign pMHC is subjected to positive, then negative selection. In (a), the peptide coverage distributions of the repertoire as a fraction of the pre-selection T cells are indicated by the dashed line for the positively selected repertoire, and the solid line for the post-negative selection repertoire. Peptide coverage is a measure of T cell cross-reactivity. In (b), the fraction of clones whose peptide-binding regions are at each distance from the foreign pMHC are indicated by the dashed line for the positively selected repertoire and the solid line for the post-negative selection repertoire. Antigenic distance has an inverse relationship with affinity. The results shown are the averages from 1000 trials.

For epitopes close to self, the average coverage of T cells that recognize them is about $0.5 \times 10^{-4}$. Epitopes that are too similar to self are detected by fewer T cells because negative selection creates "holes" in the TCR repertoire by eliminating self-reactive T cells.

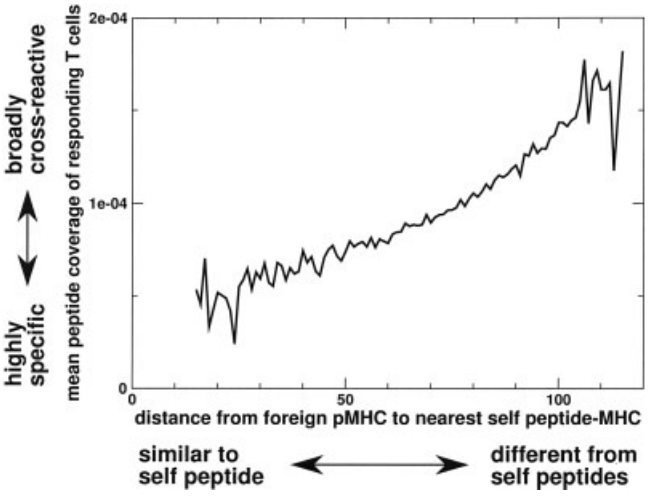


**Figure 3.** T cell coverage *vs.* similarity between an epitope and self. Random epitopes (10 000) were generated, and the distances between these epitopes and their nearest self peptides were measured. A new thymically selected T cell repertoire was created for each of these epitopes, and the average peptide coverage of these cells is plotted against the distance between the epitope and the nearest (most similar) MHC-self peptide complex.

## Discussion

In our computational model, thymic selection eliminates T cells solely on the basis of their affinities for MHC-self peptide complexes. TCR are randomly generated, so a pre-selection TCR's affinity for MHC is independent of its affinity for self peptide. However, thymic selection imposes a dependence between a surviving T cell's affinity for MHC and its affinity for peptide. After selection, a T cell's affinity for MHC is inversely related to its affinity for the nearest self peptide (Fig. 1). Because the model's implementation of thymic selection allows only T cells with a very narrow range of affinities for their closest MHC-self peptide complexes to survive, if a T cell has a certain affinity for its selecting peptide, then its affinity for the MHC that presents that peptide must fall within a very narrow range for it to survive selection. Although these results are derived from an affinity-based model of thymic selection [20], the model could be extended to accommodate avidity-based assumptions [21, 22].

In our model, a T cell's affinity for MHC determines its cross-reactivity. T cells having high affinity for MHC require little binding energy from the peptide, and thus can bind to a much larger set of peptides (*i.e.* have a higher cross-reactivity) than those that bind poorly to MHC. Because a T cell's affinity for MHC depends on its affinity for self peptides, its cross-reactivity is also determined by its affinity for self peptides. In [6], there is tantalizing evidence to support this idea. In that study,

 

**Table 1.** Affinity for MHC affects T cell fate[a)]

| | TCR affinity for MHC | | | | |
|---|---|---|---|---|---|
| | very high | high | medium | low | very low |
| self peptides that mediate positive selection | all | H/M/L | H/M/ | H/ | none |
| affinity for self peptides to avoid negative selection | none | /L | /M/L | H/M/L | all |
| peptides that activate mature T cells in the periphery | – | H/M/L | H/M/ | H/ | – |
| cross-reactivity | – | broad | medium | low | – |

[a)] The table summarizes the properties of T cell clones based on their affinities for MHC (very high, high, medium, low, and very low). The first two rows list the affinities these T cell groups must have for self peptides for them to survive positive and negative selection in the thymus, which can be high (H), medium (M), and low (L). The third row lists their affinities for peptides that will activate them as mature T cells. The final row lists the cross-reactivities of these T cells.

the cross-reactivities of two T cells positively selected on a single MHC-peptide complex were characterized. One T cell was highly specific for a peptide similar to the selecting peptide. The other T cell was specific to a peptide that was unrelated to the selecting peptide, and it had a high peptide binding degeneracy. We postulate that the first T cell had a high affinity for its selecting peptide and a low affinity for MHC, and the second had low affinity for the peptide and high affinity for MHC. More studies are required to quantify the relationship between the cross-reactivity of a T cell and its affinity for self peptides.

T cells generated in the absence of thymic selection can reveal the cross-reactive range of the pre-selection repertoire. In [23], TCR were selected *in vitro* to have high affinity for a particular set of MHC-foreign peptide complexes. It was found that cells expressing these TCR tended to react to self peptides on the MHC that presented the foreign peptide [23]. One would expect these T cells to have high affinity for both the foreign peptide and its presenting MHC. The consequence of having high affinity for MHC would be highly degenerate peptide binding, allowing T cells to react to self peptides [24]. Presumably, such cells would normally be eliminated *in vivo* by negative selection because of their high affinity for MHC, not because of high affinity for a self peptide.

It is widely believed that the purpose of positive selection is to eliminate T cells with such low affinity for MHC that they would not be likely to bind to foreign peptides presented by MHC. It has even been suggested that self peptides are just "stand-ins" for foreign peptides during positive selection [7, 10]. We believe that self peptides play an essential but overlooked role in positive selection. Although positive selection in the model tends to eliminate T cells with low MHC affinity, some of these T cells can be "rescued" by having high affinity for a self peptide. Conversely, T cells with higher affinity for MHC can be eliminated by having low affinity for all self peptides (Fig. 1, Table 1). Therefore, positive selection does not simply purge the repertoire of T cells with low affinity for MHC; it removes T cells that have "sub-optimal" MHC affinity given the T cell's affinity for its selecting peptide.

The degree of T cell cross-reactivity is a compromise between the conflicting needs of coverage and specificity. High cross-reactivity allows for greater coverage of foreign peptides, which could in turn lead to greater numbers of cells responding to infection [5]. Low cross-reactivity allows the body to finely discriminate between self and non-self peptides. Although one can estimate a single optimal T cell cross-reactivity based on these constraints [5, 25, 26], using a wide range of cross-reactivities would provide both good coverage and fine discrimination. In our model, the number of peptides that a single post-selection T cell could detect varied by over two orders of magnitude (Fig. 2a). Our model's thymic selection process allowed only T cells that are almost, but not quite, able to detect MHC-self peptide complexes to survive to maturity. T cells that are specific to peptides that are not similar to any self peptide must have high MHC affinity and hence be highly cross-reactive, while those specific to peptides that are similar to a self peptide must have low MHC affinity and cross-reactivity (Table 1). This strategy is an efficient way for the body to detect foreign peptides with a limited number of T cells. Other mechanisms, such as "tunable activation thresholds", could adjust the cross-reactivities of individual T cells [27–30] and make the repertoire generation process even more efficient.

Our finding that T cells responding to epitopes similar to self peptides are less cross-reactive than those responding to dissimilar epitopes (Fig. 3) impacts on the ability of the immune system to eliminate a mutating pathogen. Pathogens can escape the immune system's response when their epitopes mutate and reduce or abrogate recognition by T cells responding to the original epitope [31–34]. A highly cross-reactive T cell

 

might recognize both an epitope and its variants, enabling it to eliminate new mutants (as discussed in [35]), while a T cell that is highly specific would be easier to escape through mutation. If T cells responding to an epitope that is similar to a self peptide are highly specific, then variant epitopes would more easily escape the immune response. This implies that mutants evade immune system detection more easily as an epitope becomes more similar to self peptides, which in turn suggests that the immune system could drive pathogens to express epitopes that mimic self. These "molecular mimics" could either trigger autoimmune responses by activating T cells that cross-react to a self peptide [36, 37] or evade immune surveillance.

## Materials and methods

We used a computational model of T cell binding and thymic selection that was previously described in [15]. An expanded description of the model's implementation of peptide binding and thymic selection is presented below.

### TCR ligand binding model

Strings of digits were used to represent the binding surfaces of receptors and ligands. We adopted the digit string representation defined in [38]. For all strings used in the model, digits take values between 0 and 127 inclusive. The model in [38] uses a wider range of 0 to 255, but we reduced the range to increase computational efficiency without significantly affecting the model's behavior. A random six-digit string is generated to represent each self peptide in the simulation. It has been suggested that $10^3$–$10^5$ self peptides are involved in thymic selection [39–41], so the model creates 10 000 random "self peptide" strings for each of the three MHC alleles in the model. Three alleles are sufficient to represent the MHC diversity within an MHC homozygous organism. A model with only one MHC allele might produce qualitatively similar behavior, but the number of alleles affects the outcome of thymic selection quantitatively [42]. Because we assume that each distinct peptide in the body is presented by a single MHC allele, each peptide string in the model is associated with exactly one of the three MHC alleles. Each of the alleles is associated with a random four-digit string to represent the portion of MHC visible to the TCR. A peptide string is then concatenated with its associated MHC's string to form a single ten-digit pMHC string that interacts with TCR (Fig. 4). The string lengths of four and six were chosen to be proportional to the contributions of the polymorphic parts of the MHC and peptide to the binding interaction with TCR [43], and not to represent the actual number of amino acids involved. The more conserved regions of MHC, which are away from the peptide-binding groove, tend to interact with the complementarity-determining region (CDR)1 and CDR2 of the TCR, *i.e.* the less diverse parts of the TCR in contact with MHC. Although the conserved portions of MHC may contribute substantially to the binding energy with TCR to the extent that they add a constant contribution to TCR-pMHC binding in the model, they would not affect thymic selection or cross-reactivity. Nonetheless, we also tested the model using strings of lengths seven and five to represent the MHC and peptide contributions, respectively, and 12-digit strings to represent the TCR. Here, the longer MHC string is used to represent both the conserved and polymorphic parts of the molecule in contact with the TCR [44], and the model assigns all 12 digits of the TCR strings randomly because it assumes that the portions of the TCR in contact with the conserved parts of MHC are as variable as those in contact with the polymorphic parts. A more detailed theoretical analysis of the roles of the conserved and polymorphic parts of the MHC in T cell selection will be presented elsewhere. The binding surface of each TCR is represented by a randomly generated string of the same length as the pMHC, *i.e.* a 10- or 12-digit string. The similarity between a TCR string and a pMHC string determines the affinity that the T cell has for a cell expressing that peptide. Each T cell is assumed to express many copies of the same TCR, so a single TCR string is sufficient to represent a T cell's specificity for pMHC.

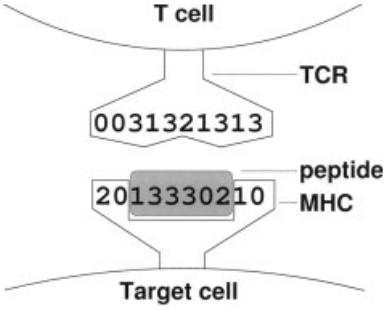


**Figure 4.** The digit string representation of TCR binding. Each T cell receptor, peptide, and MHC type is represented by a digit string. The digits shown in the figure take values between 0 and 3, while the digits in the model are larger, having values from 0 to 127. Peptide strings are concatenated with a string associated with one of the MHC types to form a single pMHC string. Affinity is proportional to the similarity of the TCR string to the pMHC string.

Antigenic distance, which has an inverse relationship with affinity, is a measure of how well a T cell receptor "matches" a pMHC. To compute the antigenic distance between a TCR and a pMHC string, the strings are aligned so each digit in the TCR is opposite a digit in the pMHC (see Fig. 4). The distance between each opposing pair of digits is then computed by the "XOR rule" described in [38]. For strings that use only two digits, 0 and 1, the XOR rule would assign a distance 0 when the opposing TCR and pMHC digits were the same (*i.e.* both 0 or both 1) and assign a distance 1 when the opposing digits were a 0 and a 1. Our model uses digits that take values between 0 and 127 and the XOR rule assigns a value between 0 (a perfect match) and 127 (an extreme mismatch) to each pair of opposing digits. The total antigenic distance between a TCR and pMHC is then the sum of the distances at each of the ten positions. Thus, the total antigenic distance between a TCR and a pMHC ranges from 0 (a perfect match at all bit positions) to 1270 (a perfect mismatch at all bit positions). The affinity of the interaction is inversely proportional to the antigenic distance, so the smaller the distance or better the match, the higher the affinity.

 

### Thymic selection model

The model subjects random pre-selection TCR strings to a process that mimics thymic selection. Random strings are generated to represent the TCR of the pre-selection T cell repertoire. The distance between each of these pre-selection TCR strings and all of the self pMHC strings is computed. A positive selection process eliminates T cells with TCR that are too far from (dissimilar to) all self pMHC, and a negative selection process eliminates those with TCR that are too close (similar) to any self pMHC. Only T cells with TCR strings that are at an intermediate distance from self pMHC survive thymic selection (Fig. 5).

For each TCR, we designate the nearest (most similar) MHC-self peptide complex as its "selecting" pMHC. The distance between a T cell's TCR string and its selecting peptide determines whether or not the cell survives thymic selection. If the selecting pMHC is too close, then the T cell is eliminated by negative selection; if it is too far, then it is eliminated by positive selection. All other self pMHC, which are farther from the TCR than the selecting peptide, do not affect its chance of surviving selection.

The positive and negative selection thresholds are found using the distribution of expected distances between a random TCR string and its selecting pMHC. The distribution was computed using the algorithm described in [38]. The T cells that are between these two thresholds are in the "window" of distances that survive thymic selection in the model (Fig. 5).

Various combinations of positive and negative selection thresholds were tested to find a combination that satisfies constraints derived from mouse data. In mice, 1–3% of pre-selection T cells survive thymic selection [14], and about one half to two thirds of cells that survive positive selection are eliminated during negative selection [24, 45–49]. We found that a thymic selection window consisting of strings at distances between 140 and 149 inclusive from the selecting pMHC roughly satisfies the constraints. This window allows 0.81% of the pre-selection T cells to survive both positive and negative selection, positively selecting 2.1% of pre-selection T cells and then eliminating 61% of these during negative selection. We also ran the model with parameters that allowed 3% of pre-selection T cells to survive thymic selection by positively selecting about 8% of pre-selection cells and then eliminating about 63% of them during negative selection. When the model used TCR strings of length 12 to increase the contribution of MHC in the TCR-pMHC interaction, 2.1% of pre-selection T cells were positively selected and 63% of the survivors were eliminated during negative selection. We assume that a mature T cell initiates an immune response when exposed to a pMHC that is at a distance from its TCR less than the negative selection threshold of 140.

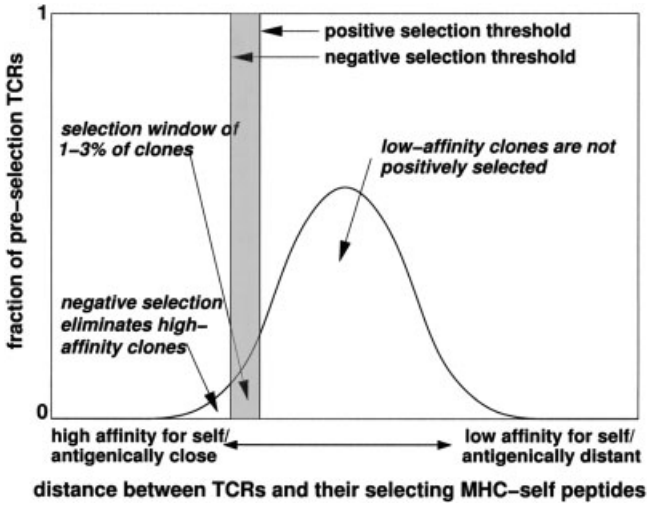


**Figure 5.** The thymic selection window computation. The expected distribution of distances between a random pre-selection TCR and the nearest MHC-self peptide complex is plotted. The T cells with TCR that are to the "right" of the positive selection threshold die by neglect during thymic selection, while negative selection eliminates those to the "left" of the negative selection threshold. Those that are between the two thresholds survive selection. The figure is not drawn to scale.

**Acknowledgements:** We thank Vincent Detours and José Borghans for helpful discussions. This work is supported by the National Institutes of Health grant T32 CA80416 to D.L.C.; the James S. McDonnell Foundation (21st Century Research Awards/Studying Complex Systems), the National Health and Medical Research Council, and the Sylvia and Charles Viertel Charitable Foundation to M.P.D.; NIH grant P20 GM066283, the Defense Advanced Research Projects Agency grant F30602–02-1-0146, and National Science Foundation grants ANIR-9986555, CCR-0331580, and CCR-0311686 to S.F.; NIH grant RR-1P20RR18754 to S.F. and A.S.P.; and NIH grants R37 AI28433 and R01 RR06555 to A.S.P. Portions of this work were performed under the auspices of the United States Department of Energy under contract W-7405-ENG-36.

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