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Activation-threshold tuning in an affinity model for the T-cell repertoire

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Naive T cells respond to peptides from foreign proteins and remain tolerant to self peptides from endogenous proteins. It has been suggested that self tolerance comes about by a 'tuning' mechanism, i.e. by increasing the T-cell activation threshold upon interaction with self peptides. Here, we explore how such an adaptive mechanism of T-cell tolerance would influence the reactivity of the T-cell repertoire to foreign peptides. We develop a computer simulation model in which T cells are tolerized by increasing their activation-threshold dependent on the affinity with which they see self peptides presented in the thymus. Thus, different T cells acquire different activation thresholds (i.e. different cross-reactivities). In previous mathematical models, T-cell tolerance was deletional and based on a fixed cross-reactivity parameter, which was assumed to have evolved to an optimal value. Comparing these two different tolerance-induction mechanisms, we found that the tuning model performs somewhat better than an optimized deletion model in terms of the reactivity to foreign antigens. Thus, evolutionary optimization of clonal cross-reactivity is not required. A straightforward extension of the tuning model is to delete T-cell clones that obtain a too high activation threshold, and to replace these by new clones. The reactivity of the immune repertoires of such a replacement model is enchanced compared with the basic tuning model. These results demonstrate that activation-threshold tuning is a functional mechanism for self tolerance induction.

Keywords: activation-threshold tuning; tolerance induction; T-cell repertoire; mathematical models; computer simulation models

1. INTRODUCTION

T cells in the vertebrate immune system are stimulated by short peptides presented in the groove of molecules from the major histocompatibility complex (MHC). In normal uninfected cells MHC molecules are loaded with 'self peptides' of autologous origin. Although naive T cells require interaction with self peptides presented on MHC molecules to obtain survival signals (Ernst *et al.* 1999), these interactions fail to trigger full activation and clonal expansion of naive T cells. Conversely, foreign peptides presented on activated professional antigen-presenting cells will activate those naive T cells recognizing the molecular complex of the peptide bonded in the groove of the MHC molecule, and will trigger clonal expansion and differentiation into memory/effector phenotypes. The T-cell repertoire is enormously diverse and consists of more than 10^8 clones expressing different T-cell receptors (TCRs; Arstila *et al.* 2000; Kesmir *et al.* 2000). Naive T cells are highly specific (Borghans *et al.* 1999; Borghans & De Boer 2002): for example, about 1 in 10^5 naive T cells will become stimulated by a viral peptide (Blattman *et al.* 2002).

Whether or not naive T cells become fully activated by the peptides presented by MHC molecules depends on the signals delivered by the cell presenting these peptides, and on the avidity of the TCR for the MHC–peptide complex. This functional avidity is influenced by the intrinsic affinity between the TCR and the MHC–peptide complex, the density of the TCR and MHC molecules on the respective surfaces, and by a host of co-receptors and co-stimulatory molecules associated with T-cell activation (Anderton & Wraith 2002). Thus, the lack of naive T-cell activation upon presentation of self peptides, and the full-blown stimulation triggered by foreign peptides, is determined by the same variety of factors. One crucial element is tolerance induction in the thymus, which selects T cells with an intermediate avidity for the MHC–self peptide complexes into the functional repertoire (Ashton-Rickardt & Tonegawa 1994).

To explain the low functional avidity of peripheral naive T cells for MHC–self peptide complexes, Grossman & Paul (2000) introduced the concept of 'tuning' the T-cell activation thresholds. They proposed that the interactions between TCR and MHC–peptide complexes induce biochemical changes in the T-cell signalling and activation machinery that alter the sensitivity of T cells to subsequent stimulation. Ample evidence identifying various molecules that can tune T-cell reactivity now supports this hypothesis (Anderton & Wraith 2002). Mathematical models for optimizing signal-to-noise ratios in T-cell activation models have led to similar notions of down-tuning T-cell reactivity such that the normal spectrum of MHC–self peptide complexes, i.e. the noise, fails to trigger T-cell activation (Noest 2000). Self tolerance induction in the thymus may also involve down-tuning of the reactivity of those

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thymocytes recognizing MHC–self peptide complexes. This would 'save' the cells from deletional tolerance, and yield an optimal reactivity of the peripheral naive T-cell repertoire without risking autoimmunity (Grossman & Paul 1992, 2000; Grossman & Singer 1996; Noest 2000).

In an extensive review, Anderton & Wraith (2002) identify many candidate molecules capable of tuning T-cell sensitivity. In several studies, transgenic (Tg) T-cell populations were cultivated under varying antigenic environments to compare the various dose–response curves for antigenic stimulation. In one approach, T cells were cultivated with antigen-presenting cells (APCs) that expressed variable numbers of different peptides on their MHCs (Dubois *et al.* 1998; Wong *et al.* 2001). In another, Tg T cells were cultivated with their specific ligand, or with altered versions of that ligand that were either hyper- or hypostimulating for these T cells (Nicholson *et al.* 2000). The common result was that increased antigenic stimulation leads to decreased sensitivity of the T-cell population. Similarly, several studies report that T-cell sensitivity decreases during T-cell development in the thymus (Chidgey & Boyd 1997, 1998; Davey *et al.* 1998; Lucas *et al.* 1999). Finally, a recent study by Huseby *et al.* (2003) showed that the cross-reactivity of epitope-specific T-cell populations to similar or unrelated peptides decreased during negative selection, which might be attributable to tuning of the activation threshold. Unfortunately, it remains difficult to distinguish whether the observed changes in sensitivity and/or cross-reactivity are due to the adaptation of the activation thresholds of a 'pure' population of Tg T cells, all carrying the same TCR, or to the selective outgrowth of a subset of T cells from a population of T cells that is heterogeneous in terms of receptor specificity (Bouneaud *et al.* 2000; Anderton *et al.* 2001).

Although the data suggest that tuning of the activation threshold is an existing phenomenon, it remains unclear whether, and to what extent, tuning contributes to self tolerance. Here, we investigate to what extent activation-threshold tuning generates T-cell repertoires that are both tolerant to self and sufficiently reactive to non-self. We have developed a computer simulation model for induction of self tolerance by activation-threshold tuning, and compare the results of the novel model to those of previous models that were based on deletional tolerance induction. In those models, clones obtained a pre-defined and evolutionary optimized clonal cross-reactivity and were purged from the functional T-cell repertoire if they cross-reacted with any of the self peptides present (De Boer & Perelson 1993; Borghans *et al.* 1999; Borghans & De Boer 2002).

2. MODELS FOR T-CELL TOLERANCE INDUCTION

(a) *Deletion model: probabilistic inactivation of auto-reactive clones*

The impact of tolerance induction on the diversity of the functional T-cell repertoire has been studied before in probabilistic modelling studies addressing the diversity of the T-cell repertoire. De Boer & Perelson (1993) and Borghans *et al.* (1999) defined a clonal cross-reactivity p, which is the chance for a randomly selected clone to react to a random epitope (i.e. antigenic determinant).

Table 1. Observed clonal cross-reactivity p under different tolerance-induction scenarios.
(Θ is the T-cell activation threshold. Simulations were all run with $S = 7143$, s.d. = standard deviation over three runs of each 10^7 matches (10^3 foreign antigens and 10^4 T-cell clones).)

	value $\times 10^{-4}$	s.d. $\times 10^{-4}$
deletion model, $\rightarrow$ all $\Theta = 16$	1.41	0.08
tuning model $\rightarrow$ variable Θ values	0.94	0.04
tuning model with clonal replacement $\rightarrow$ all $\Theta \leqslant 16$	1.97	0.02

Equation (2.1) states that the chance to mount an immune response against a random foreign antigen, P_i, is the chance that at least one of the clonotypes present in the functional repertoire R reacts to the foreign epitope. The functional repertoire R consists of all clones of the initial repertoire R_0 that are not reactive to any of the S self epitopes. Those clones that do recognize a self epitope (with chance p) are functionally deleted from the initial repertoire, i.e.

$$P_i = 1 - (1 - p)^R = 1 - (1 - p)^{R_0(1-p)^S}. \quad (2.1)$$

The *optimal* cross-reactivity $\hat{p}$ of this immune system can be found by maximizing P_i by solving $\mathrm{d}P_i/\mathrm{d}p = 0$, which yields $\hat{p} \approx 1/S$ (De Boer & Perelson 1993). Substituting $p = \hat{p}$ in equation (2.1), we can approximate the repertoire size after tolerance induction to S self epitopes using $((1 - 1/S)^S \approx 1/\mathrm{e}$ (since $S \gg 1$), i.e. $R \approx R_0/\mathrm{e}$. Thus, a fraction 1/e of the initial repertoire survives tolerance induction, i.e. 63% of the initial clonotypes (R_0) is functionally deleted, which is not an unrealistic number (Van Meerwijk *et al.* 1997). Substituting $p = \hat{p}$ and $R = R_0/\mathrm{e}$ in equation (2.1), the chance to mount an immune response against an antigenic challenge is approximated by

$$P_i \approx 1 - \mathrm{e}^{-R_0/\mathrm{e}S}. \quad (2.2)$$

The reactivity of an 'optimal' immune repertoire is thus solely dependent on the ratio of the repertoire size R_0 over the number of self epitopes S. For example, for P_i to be higher than 0.95, R_0/S should be higher than *ca*. 10. The main intent of this paper is to investigate how activation-threshold tuning can improve on this deletion model.

(b) *Simulation model: activation-threshold tuning*

To quantify the effect of tuning of the activation threshold on the T-cell repertoire reactivity we develop a 'tuning model'. We consider T cells that have already passed the criteria for positive selection, and focus on the effects of negative selection by ligand-induced adaptation of the activation threshold of individual T-cell clones.

In our model, each T-cell clone is specified by its TCR, which for the sake of simplicity is represented by a bit string (i.e. a sequence of ones and zeros) of length 32. This allows for a potential T-cell repertoire of $2^{32} \approx 4.3 \times 10^9$ clones. We have simulated the tuning model with double sized bit strings as well, but this does not affect our main results (see also § 3c and table 1). T-cell ligands (MHC loaded with self or foreign peptides) are also represented by random bit strings of length 32.

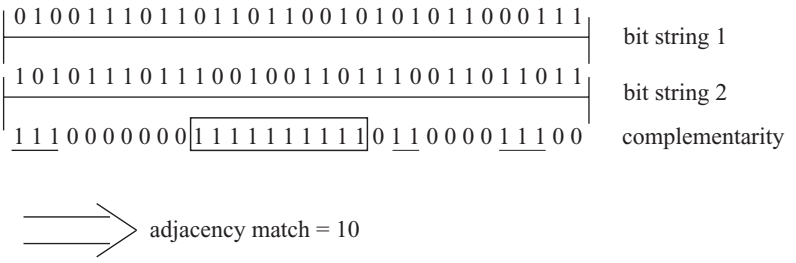

Figure 1. The adjacency match algorithm with all complementary bits underscored. The box denotes the longest sequence of adjacent complementary bits, i.e. the adjacency match of the bit strings depicted. One possible interpretation of the bits is that they represent amino acids with different physical properties, e.g. hydrophobic and hydrophilic or positively and negatively charged amino acids.

As we have chosen not to incorporate MHC in our modelling approach explicitly, we consider the subset of T cells restricted by one particular MHC molecule. Thus, organisms expressing n different MHC alleles would have T-cell repertoires that are n-fold larger than those presented here. For models that incorporate MHC explicitly, see Detours & Perelson (1999, 2000) and Detours *et al.* (1999, 2000).

The match between a T-cell clone and a self or foreign epitope is determined by scoring the complementarity between the bit strings representing the T-cell clone and the epitope, respectively. For simplicity, we assume that the complementary matching is possible in one orientation only. No frame shifts between the strings are allowed for (figure 1).

The bit-string model was not chosen for being realistic, but because it is a convenient and simple shape space model. For the matching algorithm we used both the hamming and adjacency matches, and found similar results. The 'hamming match' between two bit strings equals the sum of all complementary bits, and the 'adjacency match' equals the longest block of adjacent complementary bits (figure 1; De Boer & Perelson 1991; Detours *et al.* 1996). Here, we only present the results obtained with the adjacency match, because it embodies the nonlinear nature of the T-cell MHC–peptide interaction better than the hamming match does. For example, one foreign epitope can trigger clones that have quite different TCRs (Douek *et al.* 2002). Additionally, one point mutation in the epitope often suffices to induce viral escape from T-cell recognition (Borrow *et al.* 1997). These properties of T-cell specificity are poorly captured in the hamming match algorithm.

Note that by using the bit-string representation we neglect any influence that the surface density of TCRs and MHC–peptide complexes might have on the outcome of the encounter between a T cell with an APC. In our model, only the affinity of the interaction between a TCR and an antigen plays a role in the processes of activation and tuning of the T cells. Furthermore, using one bit string to represent a T-cell clone implies that clone size is not important for the chance to react to an antigen. This is justified as we only study primary responses, in which precursor frequencies are typically low.

In our model, a T-cell clone enters thymic selection with an activation threshold of zero. During negative selection this clone goes through subsequent interactions with self epitopes, that increase its activation threshold, and hence decrease its cross-reactivity. Recent experimental evidence supports that T-cell cross-reactivity decreases during negative selection (Huseby *et al.* 2003). After having scanned all self epitopes, the self epitope for which the T cell has the highest affinity will have tuned the T cell most strongly, and therefore defines its activation threshold (Θ). Thus, a T cell will not react to any self antigen but will remain able to respond to those foreign peptides for which it has an affinity higher than that for the best matching self peptide, i.e. those peptides that breach its activation threshold. Summarizing, a T cell with a match of L to its best matching self antigen will be given an activation threshold $\Theta = L$, and can hence respond to any epitope with a match larger than L.

(c) *Visualizing tolerance induction in phase space*

One can visualize tolerance-induction mechanisms in a geometric manner using the concept of shape space (Perelson & Oster 1979; Segel & Perelson 1987; Smith *et al.* 1997, 1999; Lapedes & Farber 2001). T cells and MHC–peptide complexes can be seen as points seeded randomly in a multi-dimensional space (i.e. in the case of a bit string of length 32, a 32-dimensional space). For the sake of simplicity we will project this multi-dimensional space on a two-dimensional plane in a Venn diagram-like manner (figure 2). The dimensions of this space represent the physicochemical properties that determine the binding affinity of receptors and ligands.

We can depict the repertoire shaped by a deletional mechanism, as modelled with the deletion model, as a collection of equally sized T-cell clonotype recognition circles (T) in shape space (figure 2*a*). The size of these recognition circles is assumed to be optimal in terms of rendering the highest possible repertoire reactivity to non-self while remaining tolerant to all self peptides (see equations (2.1) and (2.2)). A clone is deleted if it recognizes one of the self epitopes (S), i.e. if a self peptide lies within its recognition circle. In this model the functional repertoire size is therefore always smaller than the initial repertoire size (i.e. $R \approx R_0/\mathrm{e}$, see § 2a).

Tolerance induction through tuning of the T-cell activation threshold implies cellular adaptation to the current self environment. Thus, the recognition circles (i.e. the cross-reactivities) of lymphocyte clones are formed adaptively to the self ligands (figure 2*b*). The tuning model yields a better coverage because:

(i) all clones that are (functionally) deleted in the deletion model survive in the tuning model, albeit

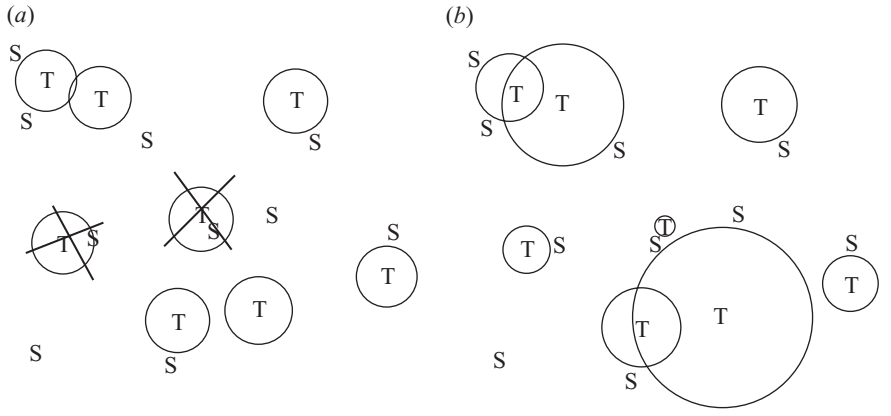

Figure 2. An illustration of the different tolerance-induction mechanisms. The affinity between a T cell (T) and an MHC–peptide complex is represented by the distance between them in shape space (Perelson & Oster 1979; Segel & Perelson 1987; Smith *et al.* 1997, 1999; Lapedes & Farber 2001). (*a*) A model of tolerance induction by deletion of clones that recognize a self peptide ('deletion' model) and (*b*) a model of tolerance induction by tuning of the T-cell activation threshold ('tuning' model). The sizes of the circles depict the clonal cross-reactivities p of T-cell clones.

with smaller recognition circles than those of the fixed clonal cross-reactivity, p; and

(ii) the space covered by clones that survive negative selection in the deletional model will either be as large or even be larger in the tuning model. The more space is covered by the T-cell repertoire, the higher the chance to react to a random foreign antigen.

3. RESULTS

Having described the basic aspects of both the deletion model and the tuning model we ask how these two different tolerance-induction mechanisms influence immune reactivity. To do this we will compare both models analytically and numerically in terms of their reactivity to random foreign antigens.

(a) *Analytical comparison*

In § 2 we introduced a deletion model (equation (2.1)) that describes the reactivity of an immune system given a certain initial repertoire size, a certain number of self peptides and a certain optimal clonal cross-reactivity. Activation-threshold tuning, alternatively, depends solely on the initial repertoire size and the number of self peptides, i.e. thymocytes are not assumed to have any prior knowledge of what levels of stimulation should be interpreted as self and what levels as non-self.

Although the clones in the tuning model will have different activation thresholds, and hence also different cross-reactivities, there is a straightforward 'mean-field'-like approximation of the model that allows for an analytical comparison to the deletion model. An average p, henceforth denoted by $\bar{p}$, can be derived as follows. During tolerance induction, a lymphocyte clone is matched to all S MHC–self peptides. The chance that, after being tolerized to S complexes, a clone will have a better match to a subsequent foreign antigenic challenge than to all S MHC–self complexes, equals $1/(S+1)$. For large S, this can be approximated by $\bar{p} = 1/S$.

Note that this formula is correct only if bit strings are infinitely long. Only then, every T-cell/self peptide match would have a unique value, and the chance that the last match is the largest match can truly be approximated by $1/S$. For bit strings of finite length the average clonal cross-reactivity is expected to be lower than $1/S$. Keeping this in mind, we use this estimate of $\bar{p}$ and calculate the chance that an antigenic challenge evokes an immune response in the mean-field tuning model (see also equation (2.1)):

$$\bar{P}_i = 1 - (1 - \bar{p})^{R_0} = 1 - \left(1 - \frac{1}{S}\right)^{R_0} \approx 1 - \mathrm{e}^{-R_0/S}. \qquad (3.1)$$

Equations (2.2) and (3.1) are very similar in the sense that P_i depends on the ratio of the pre-selection repertoire R_0 and the number of self antigens. The main difference is that the R_0/S-ratio needs to be e-fold larger in the deletion model to obtain the same immune reactivity as in the tuning model. For P_i to be higher than 0.95, R_0/S should be higher than 3 in the tuning model. Because the approximated average clonal cross-reactivity in the tuning model is equal to the optimal clonal cross-reactivity in the deletion model, this e-fold improvement is solely due to the fact that clones are not deleted in the tuning model. Although the clonal cross-reactivity in the deletion model and in the tuning model are based on very different biological assumptions, namely evolutionary optimization versus adaptation to local antigenic environment, respectively, they are both *ca.* $1/S$.

(b) *Numerical comparison*

The estimate for $\bar{p}$ that leads to the e-fold difference between tuning and deletion models is only valid for situations in which the interaction between the TCR and the MHC–peptide complex was of infinite dimensions. In reality, dimensions are finite. To study how good an approximation the analytical model is for the tuning model, we performed numerical comparisons.

To compare the deletion model with the tuning model numerically, we simulated the former by giving all clones the same fixed activation threshold (cross-reactivity). A clone will be deleted whenever one of the self peptides breaches the fixed activation threshold.

As we wish to study the model around an optimal cross-reactivity (i.e. $\hat{p} \approx 1/S$) we have to find the right Θ, S combination (where Θ is the T-cell activation threshold). The

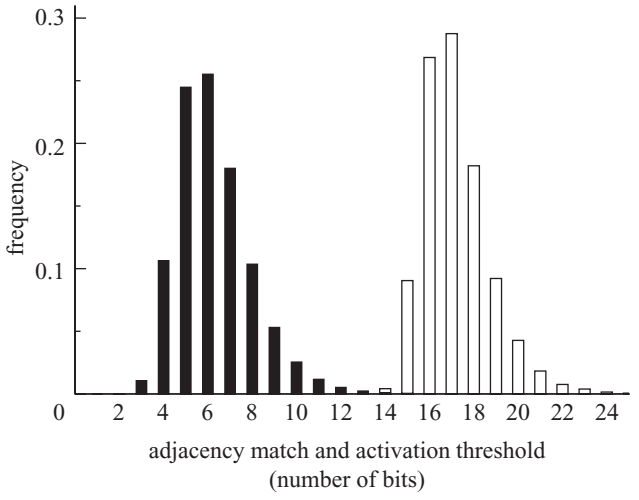

Figure 3. Adjacency match distribution (filled bars) and activation-threshold distribution (open bars). The adjacency match distribution shows the probabilities for adjacency matches of certain lengths given a one-to-one bit-string match. The activation-threshold distribution represents the probabilities for certain T-cell activation thresholds (Θ) after tolerance induction to $S = 7143$ self peptides. To simulate the deletion model, which assumes a fixed, optimal clonal cross-reactivity ($p \approx 1/10^4$), we had to find the Θ, S combination corresponding to this cross-reactivity. To do this, we summed the chances for matches of length 32 to length L. The L for which this cumulative probability amounted to *ca*. 10^{-4} was used as the fixed activation threshold of all T-cell clones in the simulation of the deletion model. The number of T-cell clones used to generate the distributions was 10^5. Note that, for all $\Theta > 15$, the chance to obtain a Θ of a particular value can be easily estimated. For instance, the chance for $\Theta = 20$ is the chance of getting a block of adjacent complementary bits of length 20 (2^{-20}) times the number of possible positions this adjacency match could be found in, times the number of trials (S) *minus* the chance for an adjacency match longer than 20. For Θ values less than 15, the calculation becomes more complicated because one has to take account of multiple simultaneous matches in the bit string.

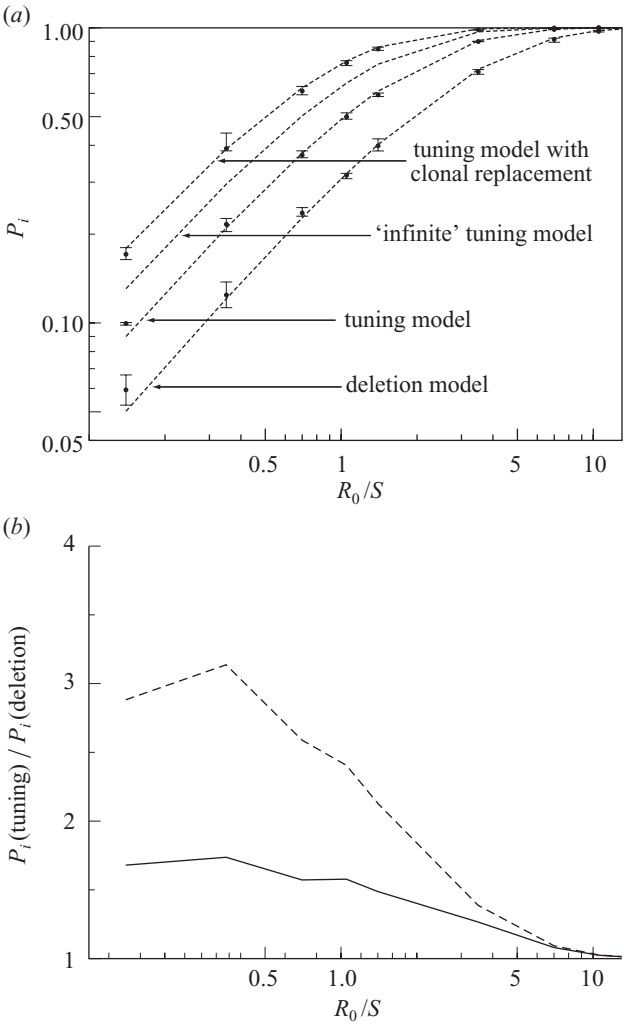

Figure 4. (*a*) Repertoire reactivities for the different tolerance-induction models. The chance to make an immune response is higher for the simulations of the tuning model than for the deletion model. However, the expectation of the reactivity of the 'infinite' tuning model based on equation (3.1) was even higher. Deletion and replacement of T-cell clones with a high activation threshold ($\Theta = 16$) increases the repertoire reactivity even more. Dashed lines represent expectations based on equations (2.1) and (3.1). The simulation results are shown by error bars (average and range out of three simulations). Models were all simulated with the same number of self epitopes. The simulations of the deletion model were run with a fixed activation threshold of 16 bits, which approximates to a clonal cross-reactivity of 1.4×10^{-4}. To operate close to the optimum we therefore simulated with $1/1.4 \times 10^{-4} = 7143$ self ligands. The *observed* cross-reactivity (see § 3c) in the tuning model for this parameter setting was $0.94 \times 10^{-4} \pm 0.042 \times 10^{-4}$. The number of foreign antigens in the simulations was 10^3 and the number of T-cell clones varied between 10^3 and 10^5. (*b*) The repertoire reactivity that is gained by tolerance induction by activation-threshold tuning versus deletional tolerance induction decreases with increasing repertoire sizes. Plotted are P_i (tuning)/P_i (deletion) for the basic tuning model (solid line) and the tuning model with clonal replacement (dashed line). In organisms with large repertoire sizes, the quantity of T-cell clones can compensate for the somewhat inferior quality of clones generated by the deletion model.

number of self peptides presented per MHC molecule was estimated to be 10^4 (see Appendix A). We therefore aim to use a p close to $1/10^{-4}$. From the distribution of random adjacency matches (see figure 3) we observed a p of 1.4×10^{-4} at an activation threshold of 16 bits (for more details see legends of figures 3 and 4*a*). We therefore set $\Theta = 16$ and $S = 1/1.4 \times 10^{-4} = 7143$. In the tuning model, Θ is free and S is also set to 7143.

(c) *Clonal cross-reactivity*

The observed clonal cross-reactivity in the deletion model is set by the Θ, S combination used in the simulations. To see how good an approximation $\bar{p} = 1/S$ is for the cross-reactivity in the tuning model we measure the cross-reactivities directly from simulations in which we test the immune response to 10^3 random (foreign) antigens (table 1).

Due to the finite length of the bit strings, the observed clonal cross-reactivity in the tuning model is lower than $1/S$. Simulations of the tuning model with double-sized bit strings confirmed that the difference between observed

and expected clonal cross-reactivity diminishes with increasing bit-string length (data not shown).

The expected repertoire reactivities generated by the analytical models (dashed lines) and the repertoire reactivities obtained from the simulations (error bars) are displayed over a range of repertoire sizes in figure 4*a*. Note that because the number of self peptides is kept constant ($S = 7143$), increasing R_0/S is equivalent to increasing the repertoire size. The data generated by simulating the deletion model with a fixed Θ fit well with the expectation calculated with equation (2.2). The repertoire reactivity P_i of the tuning model is indeed lower than expected based on equation (3.1), but it remains higher than that of the deletion model. Summarizing, figure 4*a* shows that tolerance induction through tuning of the activation threshold is a functional alternative to deletional tolerance induction based on an optimized, and hence fixed, clonal cross-reactivity. Organisms with many more clones than self peptides (e.g. mice and men) recognize (nearly) all random foreign epitopes. Organisms with more self peptides than T-cell clones have a low probability of mounting an immune response to a foreign epitope in all of the models. In § 3d we discuss how repertoire reactivities could be further increased.

(d) *Clonal deletion and replacement*

In the tuning model, lymphocyte clones with a high affinity for any of the self epitopes presented by their restricting MHC molecule will obtain a high activation threshold. Such T-cell clones will contribute little to the host immunity against pathogens. Deletion of such functionally anergic clones would thus seem an important complement to a tolerance mechanism by activation-threshold tuning.

If one were to apply clonal replacement in the deletion model, the functional repertoire size would not be reduced by tolerance induction, i.e. $R = R_0$ and $P_i = 1 - (1 - p)^{R_0}$. The optimal p for this expression has a trivial solution, $p = 1$, and hence clonal replacement does not make sense in the deletion model.

The tuning model allows for a more mechanistic implementation of clonal replacement because one could argue that only clones with too high an activation threshold are replaced. Thus, we define a deletion threshold Δ and replace all clones with an activation threshold Θ larger than Δ by novel clones. Because this procedure decreases the average activation threshold of the T-cell population, it increases its average clonal cross-reactivity (table 1) and the observed repertoire reactivity P_i (figure 4*a*). For organisms with small T-cell repertoire sizes, e.g. take $R_0/S = 1$, tolerance induction through tuning and clonal replacement yields a T-cell repertoire that will respond to 80% of foreign antigens. Given that pathogens are usually represented by more than one epitope, this seems to render sufficient protection. In the deletion model, alternatively, the T-cell repertoire will respond to less than 35% of all foreign antigens for the same R_0/S (figure 4*a*).

Substituting the observed clonal cross-reactivity of tuning with clonal replacement into equation (3.1) we find that this expected repertoire reactivity also fits well with the simulation data. Thus, our mean field approximation describes the simulation results well.

Figure 4*b* shows how much better the tuning models perform than the deletion model. The graphs depict the ratio of P_i of the tuning models (with and without clonal replacement) versus that of the deletion model for a wide range of repertoire sizes. For large repertoire sizes, the differences in clonal cross-reactivities associated with the various models become less important because low cross-reactivity of the individual T-cell clone can be compensated by high numbers of clones. Thus, the ratio of the repertoire reactivity obtained by different tolerance-induction mechanisms goes to one for large repertoire sizes.

Thus, for mice and men ($R_0/S \gg 10$), there would be little adaptive benefit in a tuning versus a deletional tolerance induction mechanism. Notably, the assumption underlying the deletion model used here is that T cells have evolved an *optimal* clonal cross-reactivity. Our results show that this optimized deletion model even performs marginally *less* well than a tuning mechanism that does not require optimization of the activation threshold. Summarizing, our results show that the more parsimonious tolerance mechanism built upon tuning of T-cell activation thresholds works at least as well as a deletion model with an optimized cross-reactivity.

4. DISCUSSION

The conceptual framework of activation-threshold tuning was developed by Grossman and colleagues (Grossman & Singer 1996; Grossman & Paul 2000). In their model, T-cell adaptation is governed by the balance between excitation and de-excitation factors. Because the dynamics of de-excitation factors were assumed to be intrinsically slow, the outcome of a stimulus by foreign or self antigens depends mainly on the excitation factors. Foreign antigens will cause very fast increases in excitation factors whereas, for example, tissue-specific self ligands or temporary upregulation of some self ligand will induce much slower increases in excitation factors. As long as the de-excitation factors can keep up with the excitation factors, tolerance is maintained. Activation-threshold tuning was proposed to be a mechanism active in both central and peripheral tolerance induction.

More recently, the need for activation-threshold tuning was shown to arise from the first principles of signal detection theory (Noest 2000). A T cell receives many statistically independent noisy signals and will have to compare these signals with some background distribution of signals (self ligands) to decide whether the stimulation should result in activation. A T cell will accept a certain rate of false alarms, which is the probability that any signal in the signal distribution when *no* intruder is present exceeds the activation threshold. As a T-cell clone does not in advance know the set of signals it can 'see', it is clear that the activation threshold will have to be adapted so as to obtain an acceptable rate of false alarms.

Another model concerning T-cell tolerance, which used the concept of an activation threshold, was developed by Van Den Berg *et al.* (2001). In their stochastic model, T cells perceive a total signal from the interactions with ligands that are presented in different concentrations in the antigen presentation profile. In this model, however, thresholds are not tunable and self/non-self discrimination

relies on self peptides being presented in lower concentrations than foreign peptides.

All models mentioned here take account of the concentration at which ligands are presented. Here, we have chosen to focus on the implications of activation-threshold tuning of individual cells on the reactivity of the T-cell repertoire, and have omitted the concentrations of ligands. Although it remains an open question whether activation threshold tuning is operating during self tolerance induction, we have demonstrated that activation-threshold tuning would be a functional alternative to tolerance induction by deletion. Activation-threshold tuning generates T-cell repertoires that are somewhat more reactive to foreign antigens than repertoires resulting from (functional) deletion of self reactive T-cell clones. Tuning generates clones of low cross-reactivity in areas that would otherwise not be covered (owing to deletion) and tuning covers the space near self peptides much better. Tuning works without evolutionary optimization of the clonal cross-reactivity and is hence a very flexible tolerance induction mechanism, that would allow thymocytes, and maybe also adult T cells (see below), to adapt to the particular antigenic environment.

Additionally, activation-threshold tuning is flexible in terms of a modelling framework as it enables us to implement assumptions about tolerance-induction mechanisms in an intuitive and mechanistic way (e.g. clonal deletion and replacement). Tuning of the activation threshold provided sufficient immunity in organisms with large repertoires, but organisms with small repertoires performed poorly (figure 4*a*). For example, amphibians are thought to have a much smaller T-cell repertoire than mammals (Langman & Cohn 1987; Du Pasquier *et al.* 2003). One could therefore ask how such a small repertoire can provide sufficient protection. Clonal deletion and replacement could enhance the repertoire reactivity in such cases. Though this seems promising, one should keep in mind the potential danger posed by increasing clonal cross-reactivities; a highly cross-reactive T cell is much more prone to induce autoimmunity.

Some data suggest that the sensitivity of mature peripheral T cells can also be tuned. For instance, naive $CD4^+$ T cells responded to contact with MHC–peptide complexes by increasing the expression level of the accessory molecule CD5, which dampens TCR signalling (Smith *et al.* 2001). In experimental data it is the consensus view that the transition from primary to memory response results in increased ligand sensitivity (Busch *et al.* 1998; Busch & Pamer 1999; Fasso *et al.* 2000; Slifka & Whitton 2001; Kedl *et al.* 2002). In our tuning model framework, the T-cell activation threshold and T-cell sensitivity are inversely linked. Thus, an increase of the T-cell sensitivity (as observed comparing naive and memory T cells) would imply a decrease in the activation threshold, which in its turn could imply breaking self tolerance unless sufficient safety margins between tolerance and the activation threshold are included. Such safety margins would, however, considerably reduce the repertoire reactivity. Unless sensitivity and cross-reactivity are two independent properties of T lymphocytes, it is hard to imagine how T-cell sensitivity to foreign ligands could be increased while, at the same time, tolerance to self peptides would be maintained.

Summarizing, by using the activation-threshold tuning model, we have improved upon the classical deletion model for T-cell tolerance induction both qualitatively and quantitatively. Qualitatively by having a mechanism that somatically sets the cross-reactivity of T cells, and quantitatively by increasing the reactivity of the mature T-cell repertoire. However, it remains unlikely that self tolerance is mediated solely by tuning mechanisms. In the thymus, more than 50% of the positively selected T cells are deleted during negative selection (Van Meerwijk *et al.* 1997). We have argued that deletional tolerance could be operating on top of a tuning mechanism to replace those clones that have obtained too high activation thresholds by the tuning mechanism. Finally, one should keep in mind that self tolerance induction is just one of the constraints on T-cell specificity (Borghans *et al.* 1999; Borghans & De Boer 2002). Preventing inappropriate cross-reactions between different types of pathogen would select for T cells that are 'as specific as possible' (Borghans *et al.* 1999; Borghans & De Boer 2002). It remains to be established whether and how peripheral tuning mechanisms could help to prevent such inappropriate cross-reactivities.

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APPENDIX A

Equations (2.2) and (3.1) show that the ratio of the functional repertoire diversity (R_0/e and R_0 in the deletion and tuning models, respectively) over the number of self epitopes (S) determines the reactivity of the modelled immune system. The diversity of the human T-cell repertoire has been estimated to lie between 10^7 and 10^{11} clones (Arstila *et al.* 2000; Kesmir *et al.* 2000). The number of different self peptides presented per given MHC allele has been identified to be *ca.* 10^3–10^4 (Hunt *et al.* 1992).

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