Thymic selection does not limit the individual MHC diversity

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The number of different major histocompatibility (MHC) molecules expressed per individual is widely believed to represent a trade-off between maximizing the detection of foreign antigens, and minimizing the loss of T cell clones due to self-tolerance induction. Using a mathematical model we here show that this argument fails to explain why individuals typically express of the order of 10–20 different MHC molecules. Expression of extra MHC types decreases the number of clones surviving negative selection, but increases the number of positively selected clones. Based on experimental parameter estimates, we show that the number of clones in the functional T cell repertoire would in fact increase if the MHC diversity within an individual were to exceed its normal value, until more than one hundred different MHC molecules would be expressed. Since additional MHC types also increase the number of presented pathogen peptides, resistance against pathogens only decreases at unrealistically high MHC diversities exceeding 1,500 different MHC molecules per individual.

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1 Introduction

Textbook immunology holds that the number of different MHC molecules expressed per individual is limited due to self-tolerance induction in the thymus [1–10]. During negative selection, clonotypes that recognize thymic MHC–peptide complexes with too high an avidity are deleted, or rendered tolerant by other mechanisms [11]. Excessive expression of MHC types could thus lead to depletion of the T cell repertoire. In the absence of negative selection, one would expect a large individual MHC diversity to be evolutionarily favorable, because it facilitates the detection of pathogens. The number of different MHC molecules per individual is therefore widely thought to represent a trade-off between pathogen presentation and negative selection in the thymus. In order to enter the functional T cell repertoire, however, lymphocytes also need to be positively selected, *i.e.* recognize MHC–self peptide complexes with sufficient avidity [12, 13]. A large MHC diversity increases the fraction of lymphocytes that is positively selected. Although the net effect of positive and negative selection on the T cell repertoire is hard to predict intuitively, the consensus is that increasing the MHC diversity decreases the repertoire by negative selection.

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Here, we present a simple mathematical model to test this widely used explanation for the limited individual MHC diversity. Since our aim is to study if thymic selection *per se* can limit an individual's MHC diversity, the only components of our model are positive and negative thymic selection and MHC-peptide presentation. Other factors that may limit an individual's MHC diversity, such as MHC–peptide densities at the surface of cells, are omitted in the model as they would only confound our analysis. The role of such alternative factors is addressed in the discussion.

2 Results

2.1 Functional repertoire size

Since experimental estimates for positive and negative selection have become available, a simple mathematical model suffices to study the effect of MHC diversity and thymic selection on the functional T cell repertoire. Consider an individual with M different MHC molecules and an initial T lymphocyte repertoire consisting of R_0 different clones. In order to enter the functional T cell repertoire, clones need to be positively selected by *at least one* of the MHC molecules, but need to avoid negative selection by *all* of the MHC molecules. Let p and n denote the (unconditional) chances that a clone is positively selected by a single MHC type, because its avidity is higher than a threshold T_1, or negatively selected

because its avidity exceeds a higher threshold T_2, respectively (see Fig. 1). Thymocytes can thus only be negatively selected by MHC molecules by which they are also positively selected [14], *i.e.* $n<p$. The number of clones in the functional repertoire R can then be expressed as

$$R=R_0[(1-n)^M-(1-p)^M], \quad (1)$$

i.e. the functional repertoire R contains all T cell clones that fail to be negatively selected, except the ones that also fail to be positively selected by any of the M different MHC molecules of the host.

In mice, approximately 5% of the T cells produced in the thymus end up in the mature repertoire [15–18], and at least 50% of all *positively* selected T cells have been shown to undergo negative selection in the thymus [14]. Thus, approximately 90% of all thymic T cells fail to be positively selected by any of the MHC molecules in the host [14]. To obtain estimates for the chances of positive selection p and negative selection n by a single MHC type, one needs to take into account how many different MHC molecules shaped the T cell repertoires in these mice. In inbred CBA CaH WEHI mice, the percentage of T cells produced in the thymus that enter the peripheral repertoire has been estimated at 3% [16]. Since these mice were MHC homozygous, and hence had 6 different MHC molecules (3 of MHC class I, and 3 of class II), the values of p and n can be solved from $(1-p)^M=1-2R/R_0=0.94$ and $(1-n)^M=1-R/R_0=0.97$ with $M=6$, yielding $p=0.01$ and $n=0.005$. Similarly, in (BALB/c × C57BL/6)F1 mice, having 16 different MHC molecules (6 of class I and 10 of class II), a thymic efflux of 5% was observed [15], which by solving $(1-p)^M=0.9$ and $(1-n)^M=0.95$ with $M=16$ yields $p=0.007$ and $n=0.003$. Both class I and class II MHC molecules are incorporated in these calculations, because positive selection and at least part of negative selection take place at the double positive (DP) stage [19, 20], when thymocytes express both CD4 and CD8 coreceptors. Together, these experiments thus give reasonably consistent estimates for the chances of negative and positive selection, yielding $n\approx0.005$ and $p\approx0.01$ per MHC molecule.

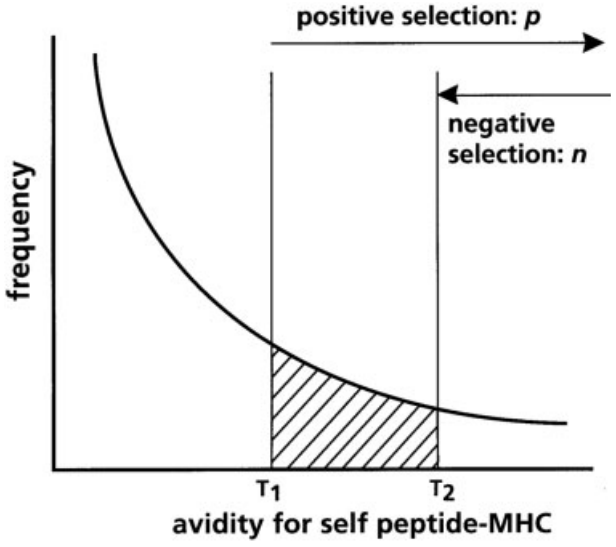

Fig. 1. Positive and negative selection. The curve depicts the distribution of thymocyte avidities for self peptide–MHC complexes (see also [42]). In our model, the chance p to be positively selected by a single MHC type is the chance that the thymocyte avidity exceeds threshold T_1. Thymocytes with avidities exceeding the upper threshold T_2 are negatively selected (with chance n per MHC type).

Applying these experimental estimates to Equation (1) reveals that the functional T cell repertoire size R increases until an individual diversity of about $M=140$ different MHC molecules (see the solid curve in Fig. 2a). Even if we allow for a two- to threefold variation in the selection chances p and n, the optimal MHC diversity per individual is at least $M=57$ MHC molecules (see the dashed curve in Fig. 2a). Thus, the functional repertoire actually increases when the MHC diversity M exceeds its normal value of six different MHC molecules in inbred mice, or eighteen in fully heterozygous humans. This result can be understood as follows: The first 10–20 MHC molecules positively select parts of the T cell repertoire that are almost non-overlapping (Fig. 3a). Thus, for each extra MHC molecule, the functional repertoire R gains all T cell clones that are positively but not negatively selected by that MHC molecule. At higher MHC diversities, some clones will be positively selected by more than one MHC type (Fig. 3b). The number of new clones added to the functional T cell repertoire by an additional MHC molecule therefore decreases. Additionally, clones that are positively selected by multiple MHC types run a higher risk of being negatively selected by at least one of those MHC molecules. The net effect of positive and negative selection remains positive until the MHC diversity exceeds $M=140$. Beyond that limit, the functional repertoire reduces by negative selection (Fig. 2a), since almost all lymphocyte clones have already been positively selected by at least one MHC type (Fig. 3c).

One can calculate from Equation (1) how strong positive and negative selection would need to be to find an evolutionary optimum at low individual MHC diversities. Even for an optimum at 18 MHC molecules per individual, which is still likely to be higher than the average individual MHC diversity, numerical analysis reveals that the

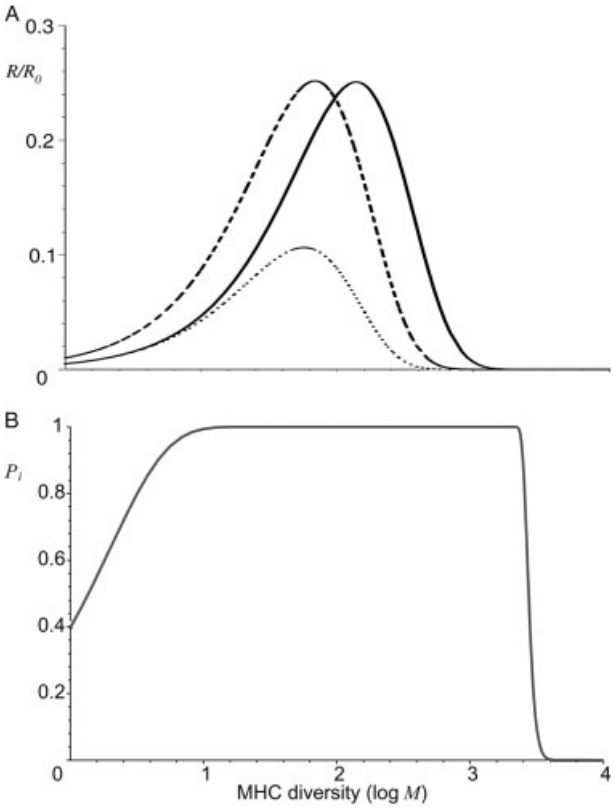

Fig. 2. How MHC diversity affects the size of the functional T cell repertoire, and the chance to mount immune responses. (a) The number of clones in the functional repertoire R is plotted as a fraction of the total initial lymphocyte repertoire R_0. The solid curve gives the functional repertoire for p=0.01, and n=0.005, while for the dashed curve p=0.02 and n=0.01 (50% of positively selected cells lost due to negative selection), and for the dotted curve p=0.02 and n=0.015 (5% thymic output). Note that at M=6 indeed 3% of the initial T cell repertoire ends up in the functional lymphocyte repertoire [16]. (b) The chance P_i to mount an immune response against a pathogen is plotted as a function of the MHC diversity (M). Resistance against infections can be maximal even if the size of the functional repertoire is far from its maximum. Parameters are: q=.02 [27], r=10^{-5} [28], R_0=10^{10} [4], e=25, p=0.01, and n=0.005.

positive selection chance p would need to be 6%, and the corresponding chance of negative selection n=5.2% (to keep 5% thymic output). This would imply that the percentage of positively selected thymocytes that are lost due to negative selection in a heterozygous individual with eighteen different MHC molecules would be as high as 93%, which is far from the 50% estimate by van Meerwijk et al. [14]. Additionally, with these parameters the majority of cells (62%) would be lost due to negative selection, which is at odds with the finding that most thymic T cell deletion is due to lack of positive selection [12–14, 21].

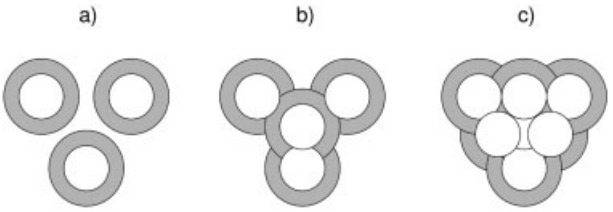

Fig. 3. Cartoon of the effect of MHC diversity on the functional T cell repertoire. (a) At a low MHC diversity, all MHC types select nearly non-overlapping parts of the T cell repertoire (see the gray circles). Additional MHC types therefore increase the number of T cell clones that enter the functional repertoire. Clones that are deleted by new MHC types (see the white circles) were not functional anyway in the absence of those MHC molecules. (b) At a somewhat larger MHC diversity, MHC molecules start deleting parts of the repertoire that were positively selected by other MHC molecules. Yet, the net contribution of additional MHC types to the functional repertoire is positive. (c) At even larger MHC diversities almost all lymphocytes have been positively selected by at least one MHC type of the host, so that additional MHC molecules delete functional lymphocytes and hence reduce the size of the functional repertoire.

In the above equations, all thymocyte loss occurs during the selection process. We cannot exclude the possibility, however, that part of thymic cell loss is due to a lack of expression of functional T cell receptors (TCR). In that case, the parameter R_0 in our equations would have to be rewritten as fR_0, where f is the fraction of clones that "audition for selection" [18]. The selection parameters can then be solved from $f(1–p)^M$=$1–2R/R_0$ and $f(1–n)^M$=$1–R/R_0$. If failure to participate in the selection process would form a significant part of thymocyte loss, *i.e.* if f would be small, the estimates of the parameters p and n in our model would increase, and the optimal MHC diversity per individual would consequently decrease. Failure to express a functional TCR may be due to unsuccessful rearrangement of the TCRβ chain at the double negative (DN) stage, or of the TCRα chain at the DP stage. Since DN cells represent only about 2% of the total number of thymocytes [22], unsuccessful β chain rearrangements do not contribute significantly to thymocyte loss. After one round of α rearrangement at the DP stage, only 5/9 of the thymocytes is expected to express a functional TCRα chain. Thymocytes can go through multiple rounds of α rearrangements, however [23–26]. As a consequence, the great majority (*i.e.* 80–90%) of cell loss in the thymus is thought to be due to elimination during the selection process and not to failure to participate in the selection process [18, 24]. Even in the extreme case that 50% of thymocytes are lost before selection by MHC–peptide complexes, however, the selection parameters p and n would remain within the range analyzed in Fig. 2a, yielding an optimal MHC

diversity that is far beyond the individual MHC diversity found in nature.

2.2 Probability of an immune response

Although this calculation shows that the consensus explanation for the limited individual MHC diversity is untenable, one could argue that evolution does not operate on the size of the functional T cell repertoire. MHC diversity also increases the number of MHC–peptide complexes by which a pathogen is presented to the immune system, and thus increases the chance that at least one clone in the T cell repertoire induces an immune response. To study which individual MHC diversity renders the highest protection against infections, the model of Equation (1) needs to be extended with antigen presentation. Let q be the chance that a particular MHC molecule presents a random peptide, and r the probability that the resulting peptide–MHC complex is recognized by one of the R^* clonotypes in the functional repertoire that were positively selected by that particular MHC molecule. If pathogens are typically represented by e different peptides that could possibly bind the MHC, then P_i, the chance of an individual to mount an immune response against a random pathogen, is given by

$$P_i = 1-[1-q+q(1-r)^{R^*}]^{eM}. \quad (2)$$

In words, no immune response is induced (with chance $1-P_i$) if on all MHC types, all epitopes are either not presented (with chance $1-q$), or presented but not recognized by any of the R^* clonotypes [with chance $q(1-r)^{R^*}$]. The number R^* of clonotypes in the functional repertoire that are expected to be positively selected by a particular MHC type of the individual, can be calculated from

$$R^* = (p-n)R_0\,(1-n)^{M-1}. \quad (3)$$

Thus, R^* contains all lymphocyte clones that (i) survive both positive and negative selection by the particular MHC molecule, and (ii) are not negatively selected by any of the other (M–1) MHC molecules.

The chance of peptide–MHC binding is estimated to be q=0.02 [27], while we take a conservative estimate of e=25 suitably processed peptides per pathogen. Estimating the chance that a T cell clone recognizes a peptide presented by an MHC molecule by which it was positively selected at $r=10^{-5}$ [28], we have calculated the immune response probability P_i as a function of the MHC diversity M (Fig. 2b). The response chance initially increases with the MHC diversity, because (i) more pathogen peptides are presented, and (ii) positive selection increases the functional T cell repertoire, as shown above. At an MHC diversity of about $10<M<20$, the response chance saturates, simply because $P_i \approx 1$. Although the number of clones in the functional lymphocyte repertoire is still augmented by increasing the MHC diversity, recognition of pathogens is already ensured thanks to the high number of MHC–peptide complexes by which each pathogen is presented. Importantly, the chance to respond (P_i) starts to decrease only at MHC diversities exceeding 1,500 different MHC molecules per individual. Only then is there such a vast reduction of the functional repertoire by negative selection that the response chance collapses despite the elaborate presentation of pathogen peptides. Even if the system is triggered by many pathogens (*e.g.* 10^5), we still find a very wide range of MHC diversities yielding good protection against infections (*i.e.* $P_i \approx 1$, not shown). If pathogens are represented by much more than 25 epitopes (*e.g.* e=1,000), the recognition chance increases at low MHC diversities, and collapses at even higher MHC diversities (not shown).

3 Discussion

Summarizing, our calculations show that thymic selection fails to explain why heterozygous individuals express maximally eighteen different classical MHC molecules. Increasing the MHC diversity beyond the normal level would in fact increase the functional T cell repertoire (see also [29]), until more than a hundred different MHC molecules are expressed. Even beyond that limit, immune protection can be near-certain because of the large number of different MHC–peptide complexes by which pathogens are presented.

Remarkably, some previous studies did report a low optimal number of MHC types due to negative selection in the thymus [4–6, 9]. All of these models are not consistent, however, with the experimental finding that most thymic T cell deletion is due to lack of interaction with self MHC molecules [12–14, 21, 30]. One of the models [5] did not at all account for positive selection; all clonal deletion occurring in the thymus was due to negative selection. In that case, the current model also predicts depletion of the T cell repertoire even at low MHC diversities. Two other models [4, 6] did incorporate both positive and negative selection of T cells by MHC molecules in the thymus, but involved too stringent negative selection. T cells that failed to be positively selected by a particular MHC molecule could nevertheless be negatively selected by the same MHC molecule. According to Nowak et al. [4], the functional repertoire is given by

$$R = R_0\,[1-(1-p)^M\,](1-n^*)^M, \quad (4)$$

where n^* is the *conditional* probability that a positively selected clone is negatively selected by a random MHC molecule, *i.e.* $n^*=n/p$. Since negative selection was still evaluated on all M MHC molecules, T cells with avidities too low to be positively selected by a particular MHC molecule could nevertheless be negatively selected by that same MHC molecule. At the optimum of 40 different MHC molecules [4], the chance to respond to a single epitope was only 0.2%. For the novel experimentally based parameters used in the current study, *i.e.* substituting p=0.01 and n^*=0.5, Equation (4) yields an optimum at 1–2 different MHC molecules per individual, in which as much as 99.5% of the total T cell repertoire is lost during thymic selection, reconfirming that the model by Nowak et al. [4]involved too stringent negative selection. The discrepancy between these previous model results and our finding that there is no optimum at low individual MHC diversities is due to the fact that positive selection forms a strong bottleneck during thymic selection that was previously not fully taken into account. We cannot exclude the possibility, however, that in species where most thymocytes *are* lost due to negative selection, an optimum at a low MHC diversity may occur.

Having rejected the consensus explanation that the MHC diversity per individual is limited to avoid repertoire depletion [1–10], we are left with the question what *can* explain the limited number of different MHC molecules per individual. The current model provides an alternative explanation. Thanks to the degenerate binding of peptides to MHC molecules [27, 31, 32], a limited individual MHC diversity may simply be sufficient to have a good chance to present and respond to pathogens. Hence, the selection pressure for more MHC diversity per individual vanishes. Another possibility is that the number of different MHC molecules per individual is limited to avoid the induction of inappropriate, cross-reactive immune responses. For example, self-specific clonotypes that are not fully tolerized by their self epitopes can be triggered by a cross-reacting foreign antigen and subsequently induce an autoimmune disease [33–36]. Once the individual MHC diversity is sufficient to ensure presentation and recognition of at least one epitope per pathogen, having a greater diversity of MHC molecules may be detrimental, because it would increase the risk to induce such inappropriate cross-reactive responses (see also [36, 37]). Alternatively, it has been proposed that the limited MHC diversity per individual may help to reach a critical concentration of MHC–peptide ligands at the surface of antigen-presenting cells, required to induce an effective immune response by focussing the T cell repertoire at a few epitopes only [38].

Interestingly, in polyploid species of *Xenopus* with up to 72 chromosomes (instead of 20 chromosomes in a normal diploid), it has been observed that only one MHC locus is expressed, while the MHC genes on all other chromosomes are silenced [39, 40]. This suggests that a low MHC diversity per individual is advantageous, and not merely sufficient for protection against infections. Mate choice experiments in sticklebacks also suggest the presence of a low optimal MHC diversity within individuals. While female sticklebacks with fewer MHC alleles than the population average tend to select males with more MHC alleles, females with many MHC alleles select males with relatively few alleles [41]. Having shown that the advantage of a low MHC diversity per individual does not lie in T cell repertoire diversity, the current study provides the incentive to identify and discriminate between alternative explanations.

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