Diversity in the Immune System

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1 INTRODUCTION

Diversity is one of the key characteristics of the vertebrate immune system. Lymphocyte repertoires of at least 3×10^7 different clonotypes [2] protect humans against infections, while avoiding unwanted immune responses against self-peptides and innocuous antigens. It is this lymphocyte diversity that forms the main difference between the immune systems of invertebrate and vertebrate species. Invertebrates are protected from pathogenic invasions by broad-spectrum pathogen-associated recognition molecules, recognizing conserved pathogenic structures [29, 33]. On top of these innate responses, which have been preserved in vertebrate species, vertebrates evolved an adaptive immune system, which has the capacity to respond to a virtually infinite variety of antigens. Adaptive immunity evolved when gene rearrangements were employed to generate highly diverse lymphocyte repertoires [1, 20, 34].

Another important source of diversity in the immune system is due to the genes coding for major histocompatibility (MHC) molecules. For a cellular immune response to be induced, the proteins of a pathogen need to be degraded into peptides, which are subsequently bound to MHC molecules on the surface of antigen-presenting cells. The resulting MHC-peptide complexes can be recognized by T-cell receptors. In humans, each individual expresses

Design Principles for Immune System & Other Distributed Autonomous Systems. L. A. Segel and I. R. Cohen, eds. Oxford Univ. Press, 2000.

three classical MHC class I genes (HLA A, B, and C), and three MHC class II gene pairs (coding for the α and β chains of HLA DP, DQ, and DR) [28]. The population diversity of histocompatibility molecules is extremely large, and predates the evolution of vertebrates. For some MHC loci, more than one hundred different alleles have been identified [49, 70]. Due to the high population diversity of MHC molecules, different individuals typically mount immune responses against different subsets of peptides of any pathogen. Pathogens that escape from presentation by the MHC molecules of one particular host, may thus not be able to escape from presentation in another host with different MHC molecules.

The mechanisms underlying the diversity of the adaptive immune system and the MHC complex are very different. The diversity of lymphocyte receptors is due to the evolution of somatic diversification mechanisms [1]. Genes coding for the V, D, and J segments of lymphocyte receptors are somatically rearranged, and imprecise joining of the gene segments, addition of nucleotides, and somatic hypermutation subsequently add to the diversity of lymphocytes [28]. The result is an extremely diverse, semirandomly generated, repertoire of lymphocytes that bind their ligands with great specificity. The diversity of MHC molecules, in contrast, is not due to any special diversification processes. The mutation rate of MHC molecules is similar to that of most other genes [47, 57]. Studies of nucleotide substitutions at MHC loci have revealed that there is Darwinian selection for diversity at the peptide-binding regions of MHC molecules [25, 26, 47, 48]. Contrary to lymphocyte receptors, MHC molecules bind their ligands with great degeneracy [23, 31, 36].

This chapter gives a review of our research on the evolutionary selection pressures underlying the diversity of lymphocytes and MHC molecules. We hypothesize that the adaptive immune system stores the appropriate effector mechanisms against the antigens it encounters (see also Swain et al. [64]). For example, lymphocytes specific for food- and self-antigens switch to a tolerant mode, while lymphocytes recognizing pathogens switch to a particular responsive mode. Once lymphocytes have been instructed as to which type of immune response to mount, they recall their appropriate effector mechanism whenever they recognize their specific epitope [53, 64]. The immune system thereby learns to associate antigens with the appropriate type of immune response against them. Recall responses may be harmful, however, if different antigens requiring different modes of response trigger the same clonotype. The likelihood of such inappropriate responses increases with the degree of cross-reactivity of lymphocytes and with the number of peptides per antigen that are presented to the immune system.

In section 2 we show that a somatically learning adaptive immune system requires a high degree of diversity. Repertoire diversity allows the immune system to reconcile specificity (which is required to avoid inappropriate, crossreactive immune reactions) with reactivity to many antigens (see also Borghans and De Boer [9, 10] and Borghans et al. [11]). Interestingly Cohen discusses in this book how the immune system may achieve a high degree of specificity

using degenerate receptors. In section 3 we investigate why the number of different MHC molecules expressed per individual is so limited as compared to the large population diversity of MHC molecules. We demonstrate that it is unlikely that the individual MHC diversity is limited due to T-cell repertoire depletion during negative selection, as has been proposed [17, 28, 47, 69] and modeled [18, 44, 66] before. We demonstrate that the selection pressure for more individual MHC diversity vanishes once of the order of ten different MHC molecules per individual have been expressed. Excessive individual MHC diversity has the added disadvantage that it increases the chance to mount inappropriate immune responses, such as autoimmune responses by clones that have escaped tolerance induction. The limited number of MHC molecules per individual may thus reflect a compromise between recognition of many antigens and avoidance of self-reactivity. In section 4, we demonstrate that despite the limited expression of MHC molecules per individual, host-pathogen coevolution can account for a very large *population* diversity of MHC molecules. Using a genetic algorithm, we show that MHC diversity is to be expected in host populations adapting to pathogens with short generation times (see also Beltman [5]).

2 DIVERSITY OF LYMPHOCYTES

During a primary immune reaction, the immune system has to decide which type of immune response is most appropriate [64]. No immune response should be induced against self-antigens and innocuous antigens, while pathogens are eliminated by qualitatively different immune responses, varying from cellular to humoral responses, and varying in, for example, immunoglobulin isotype and cytokine expression [28, 64, 76]. The decision as to which type of immune response to mount is based upon many factors, such as signals from the innate immune system [6, 15, 16, 21, 29, 30, 38, 39, 40, 56], the local tissue environment [77], tissue damage [37], and success-driven feedback mechanisms (see Segel [58, 59]). These signals collectively form the "context" of an antigen.

We hypothesize that, apart from dealing with antigens, one of the main functions of the adaptive immune system is to store the appropriate modes of response against different antigens in differentiated lymphocytes [10, 11]. If effector or memory clones recognize a subset of the epitopes that are expressed by an antigen, they contribute to the antigen context, and provide information on the type of immune response that is to be induced. Being fairly independent of costimulatory signals, such instructed lymphocytes help to eliminate pathogens upon re-encounter even before any tissue damage has been done, and help to induce appropriate immune responses against new antigens that correlate with previously encountered antigens. Instructed lymphocytes can also direct the differentiation of new naïve lymphocytes. Tolerant T cells have been shown to be able to transfer their nonresponsive phenotype to other, naïve cells [51, 72], even if those naïve cells have a different specificity [65].

Analogously, memory lymphocytes of a certain responsive mode may direct the differentiation of new naïve clonotypes [10, 32], for example, *via* cytokine secretion or interactions with dendritic cells [13, 54, 55].

If instructed lymphocytes are too crossreactive, however, they may induce inappropriate responses [3, 45, 46, 75]. Here we show the results of a simulation model that we have developed to study under which circumstances storage of appropriate effector mechanisms can help the induction of new, appropriate immune responses, while avoiding inappropriate, crossreactive responses. We find that lymphocyte specificity is required to avoid inappropriate immune responses, and that repertoire diversity does not hamper the role of instructed lymphocytes in the induction of immune responses to new antigens.

2.1 STORAGE OF APPROPRIATE IMMUNE RESPONSES

We simulate the storage of effector mechanisms against different antigens in an immune system with R_0 different clonotypes. The immune system is sequentially challenged with different antigens, each requiring a certain type of immune response, and each consisting of e different (immunodominant) epitopes. Both the appropriate type of response to an antigen, and the clonotypes recognizing its epitopes, are selected randomly. Each clonotype has a certain mode. Clonotypes specific for the S different tolerance-inducing self-epitopes are initialized in the tolerant mode; all other clonotypes are initially naïve. Due to recognition of an antigen, naïve clonotypes may switch to a particular responsive mode (such as Th1, Th2, IgA, IgE, etc.). Different modes are represented by integer numbers $0, 1, 2, \ldots, m$, where 0 means naïve, 1 means tolerant, and $2, 3, \ldots, m$ identify particular responsive modes. In our simulations, every epitope that the immune system encounters is recognized by precisely one clonotype, which is selected randomly. Repertoire diversity is thus inversely related to the crossreactivity of clonotypes. Depending on the degree of lymphocyte crossreactivity, one clonotype may recognize multiple epitopes.

Whenever epitopes of antigens in our simulations are recognized by previous memory clones, these memory clones determine what type of immune response is induced. The modes of response suggested by different memory clonotypes might not be identical, however. Any conflicts are resolved by treating each signal as a "vote" in the decision-making process. The ultimate decision is the mode for which there is a majority count. In case there is a tie, the decision is chosen randomly from the largest votes. In the absence of cross-reacting memory lymphocytes, we assume that the combination of the innate immune response, the context of the antigen, and possibly feedback mechanisms, ultimately leads to the appropriate type of immune response. This might not be unreasonable, because the innate immune system has learned about different kinds of pathogens and antigenic contexts over evolutionary time.

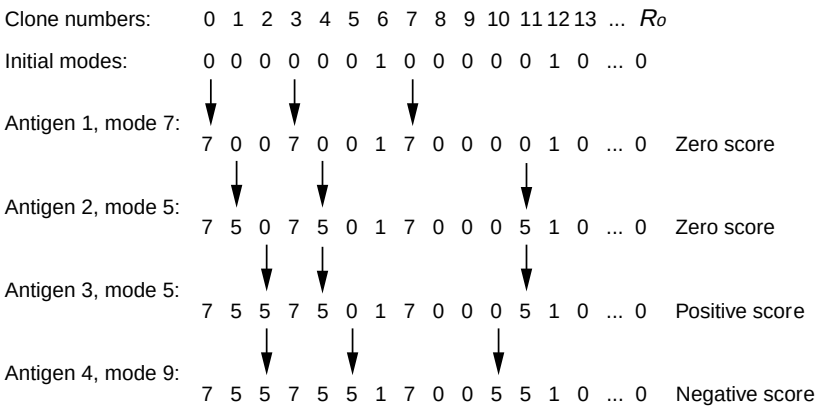

FIGURE 1 A simple example of a simulation with $e = 3$ different epitopes per antigen. After self-tolerance induction most clonotypes are naïve (i.e., mode 0), except clonotypes 6 and 12 which have been initialized in the tolerant mode (i.e., mode 1). The first antigen has to be rejected by an immune response of mode 7, and triggers clonotypes 0, 3, and 7. Since these three clonotypes are naïve in the primary response, the decision as to which type of immune response to mount is made by the innate immune system. Thus, clonotypes 0, 3, and 7 become memory clones of mode 7, antigen 1 is rejected, and no score is obtained. Similarly, antigen 2 triggers three naïve clonotypes, which subsequently switch to memory mode 5. Antigen 3 triggers two memory clones that overlap with antigen 2 (i.e., clones 4 and 11), and triggers the naïve clone 2. Because of the memory votes by clones 4 and 11, an immune response of mode 5 is triggered. This yields a positive score. Clone 2 correctly switches to mode 5. Antigen 4, requiring mode 9, coincidentally triggers a memory clone (2) which is in mode 5. Thus, an inappropriate immune response is induced, yielding a negative score. naïve clonotypes 5 and 10 incorrectly switch to mode 5.

In our simulations, once a decision has been made, all naïve clonotypes involved in a primary immune response switch to the corresponding memory mode. Even if an inappropriate response is triggered, naïve lymphocytes switch (to the incorrect) mode. In accordance with experimental data, memory clonotypes do not switch mode [41, 52]. If previous memory clones have the majority vote and thereby establish the correct mode of response, a positive score is given. All cases in which previous memory clones establish an incorrect mode of response yield a negative score. In the default situation, in which naïve lymphocytes adopt the mode of the innate immune system, no score is added. Figure 1 provides an example of a small simulation.

Obviously, the adaptive immune system will only give a positive contribution to the decision-making process if there are groups of structurally related antigens that require a similar type of immune reaction. To account for such groups of antigens, a fraction P_m of all antigens in our simulations is a mutant of another antigen. Mutant and wild-type antigens always require

identical modes of response and share half of their epitopes; the other epitopes are chosen randomly.

2.2 SOMATIC LEARNING REQUIRES LYMPHOCYTE SPECIFICITY

Figure 2 illustrates how the performance of an immune system that has been challenged with one thousand different antigens depends on the diversity of the lymphocyte repertoire. All antigens have been presented to the immune system only once; i.e., we study a "worst case" scenario, ignoring the conventional benefits of immunity obtained when the same antigen rechallenges the immune system. The two panels show the fraction of challenges yielding a positive score, and a negative score, respectively. The different curves in figure 2 depict different levels of correlation between the pathogens, i.e., $P_m = 0$ (solid), $P_m = 0.1$ (dotted), and $P_m = 0.2$ (dashed).

Figure 2(a) shows that memory clones help to make correct decisions whenever (i) there is some correlation between the antigens *and* (ii) the lymphocyte repertoire is sufficiently specific. At a very low repertoire diversity, hardly any positive score is obtained because most lymphocytes have been tolerized by self-epitopes (see also De Boer and Perelson [18]). At an intermediate repertoire diversity, the repertoire is no longer depleted during tolerance induction but the positive scores that are obtained are largely coincidental. Even if there is no correlation between the antigens (see the solid curve), these positive scores occur because of random crossreactions. Above a diversity of $R_0 = 10^5$ clonotypes, this randomness disappears and the positive scores hardly depend on the diversity of the immune system. Whatever the diversity of the system, a recurring epitope always triggers the same clonotype. Increasing the repertoire size R_0, and hence the specificity of the system, therefore does not impair the positive contribution of memory lymphocytes to the decision making during immune reactions.

Figure 2(b) demonstrates that lymphocyte systems of low diversity are prone to make mistakes due to crossreactivity. At a low diversity, previous memory clones specific for epitopes of unrelated antigens tend to induce wrong types of immune responses; on the other hand, clones that have previously been tolerized by self-epitopes hinder the induction of immune responses to subsequent pathogens. Figure 2(b) shows that such mistakes (i) disappear at a large repertoire diversity, and (ii) hardly depend on the correlation between the antigens.

Summarizing, these simulations demonstrate that in immune systems that store the appropriate modes of responsiveness against many different antigens, the avoidance of harmful, inappropriate responses requires a highly specific immune repertoire. High specificity counteracts the demand that all antigens should be recognized, however [35]. Although the current simulation model does not allow for nonrecognition, we know from previous modeling [9, 11] that responsiveness against many antigens can be reconciled with specificity by selecting for a sufficiently diverse immune repertoire.

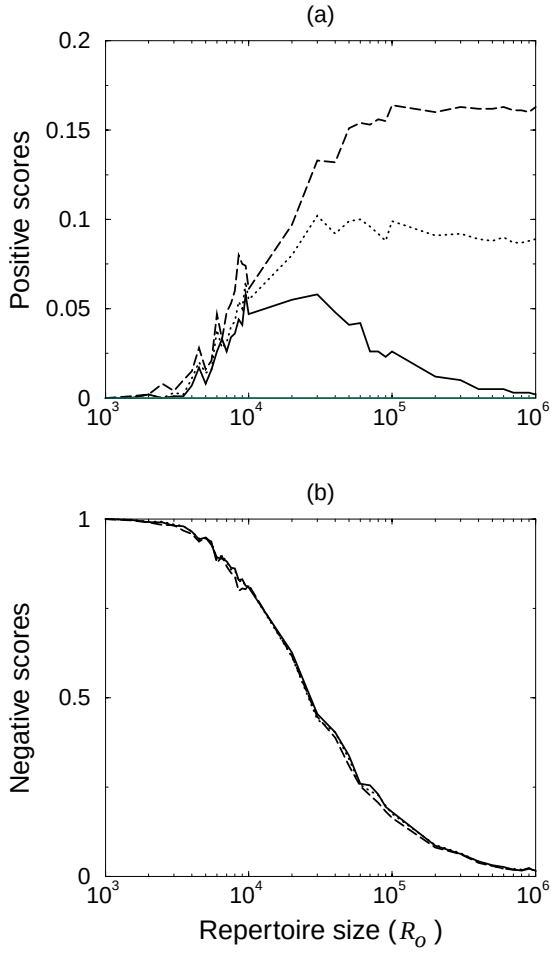

FIGURE 2 The performance of lymphocyte systems of different diversities (R_0) challenged with one thousand different antigens. (a) The fraction of challenges that yield a positive score thanks to previous memory clones making correct decisions. (b) The fraction of challenges yielding a negative score due to inappropriate immune responses induced by previous memory clones or lack of responsiveness due to cross-reactive tolerant clones. The different curves denote different degrees of correlation between the antigens that are encountered: $P_m = 0$ (uncorrelated antigens, solid curves), $P_m = 0.1$ (dotted curves), and $P_m = 0.2$ (dashed curves). Related antigens share 50% of their epitopes. There are $e = 6$ different epitopes per antigen, $S = 10^3$ self-antigens, and ten different modes ($m = 9$).

3 WHAT LIMITS THE INDIVIDUAL MHC DIVERSITY?

In sharp contrast with the highly specific binding between epitopes and lymphocytes, peptide binding to MHC molecules is very degenerate [23, 36]. The chance that a random peptide binds a random human MHC molecule is 0.1% to 10% [31]. Degenerate MHC-peptide binding allows the immune system to present a great variety of peptides and, hence, to mount immune responses against many pathogens. It is generally thought that this selective advantage also explains why individuals tend to be MHC heterozygous (see, e.g., Doherty and Zinkernagel [19], Hughes and Nei [25, 26, 27], and Takahata and Nei [67], and section 4 of this chapter). Indeed, in a study of patients infected with HIV-1, it was shown that the degree of heterozygosity of MHC class I loci correlated positively with a delayed onset of AIDS [14].

Since immunity against pathogens requires the presentation of pathogen peptides on host MHC molecules, the number of MHC genes expressed in vertebrates is, in fact, surprisingly small. Just like favoring MHC heterozygosity, one would expect evolution to favor the expression of many MHC genes per individual. In reality, however, the MHC diversity per individual (i.e., of the order of ten different MHC molecules [28]) pales into insignificance in comparison to the huge diversity of MHC alleles in populations (i.e., up to hundreds of alleles per locus [49, 70]). Using a probabilistic model, we here study which mechanisms may underlie the limited expression of different MHC molecules per individual. In the next section we will investigate the large population diversity of MHC molecules.

It is often quite loosely argued that the number of different MHC molecules per individual is limited due to self-tolerance induction [17, 28, 47, 69]. During negative selection in the thymus, clonotypes that recognize thymic MHC-peptide complexes with too high an affinity are tolerized [43]. Excessive expression of MHC molecules might thus lead to depletion of the T-cell repertoire. Nowak et al. [44] translated this verbal argument into a mathematical model and concluded that self-tolerance induction can indeed account for a realistically low individual MHC diversity. We have criticized this model by a different calculation, leading to the opposite conclusion that negative selection fails to explain the limited MHC diversity observed in nature [12].

Consider an individual with M different MHC molecules and a total lymphocyte repertoire consisting of R_0 different clones. Expression of many different MHC molecules reduces the functional T-cell repertoire due to negative selection. On the other hand, it enlarges the functional repertoire due to positive selection: only T cells that bind thymic MHC-peptide complexes with sufficient affinity enter the functional T-cell repertoire [22, 71]. If a peptide is presented by one of the MHC molecules of a host, the number of clones that can possibly recognize the peptide-MHC complex is the number of clones R_M that is positively selected by the particular MHC molecule, and not negatively

selected by any of the M MHC molecules of the host, i.e.,

$$R_M = hR_0(1-t)^M \ . \tag{1}$$

Here, t is the fraction of thymocytes that is deleted by negative selection per MHC molecule and h is the chance that a T lymphocyte surviving negative selection is positively selected on the particular MHC molecule. Equation (1) reflects the negative effect of expression of many different MHC molecules on the number of lymphocytes surviving negative selection. It has been estimated that approximately 90% of all thymic T cells fail to be positively selected on any of the MHC molecules of a host [68]. At least 50% of all *positively selected* T cells have been shown to undergo negative selection in the thymus [68]. The remaining 5% of all thymic T cells end up in the mature repertoire [61, 71]. Since an individual has typically of the order of ten different MHC molecules, these experimental estimates translate into $h = 0.005$ and $t = 0.005$ per MHC molecule.

If the individual is exposed to an antigen consisting of e different (immunodominant) epitopes, the chance P_i to make an immune response is:

$$P_i = 1 - (1 - q + q(1-p)^{R_M})^{eM} \ . \tag{2}$$

Here, q is the chance that an MHC molecule presents a randomly chosen peptide and p is the chance that a clonotype that has been positively selected by the MHC molecule in question recognizes a random peptide presented by that MHC molecule. No immune response is induced if, on all MHC molecules, all epitopes are either not presented (with chance $1-q$), or presented but not recognized by any of the R_M clonotypes (with chance $q(1-p)^{R_M}$). Equation (2) reflects the positive effect of expression of many different MHC molecules on both the presentation of antigens and the positive selection of lymphocytes.

The solid curve in figure 3 shows that good protection against pathogens is achieved (i.e., $P_i \simeq 1$) for an individual MHC diversity between 10 and 2000 different molecules. This result thus contradicts the conclusion drawn by Nowak et al. [44] that the individual MHC diversity is limited to avoid repertoire depletion during tolerance induction. Instead we find that repertoire depletion occurs only at an unrealistically high individual MHC diversity. Since different MHC molecules select basically nonoverlapping sets of T-cell clones [7, 22], addition of extra MHC molecules tends to *enlarge* the functional repertoire. The essential difference between the model by Nowak et al. [44] and the current model is that in the previous model, T cells that fail to be positively selected on a particular MHC molecule can nevertheless be negatively selected on that MHC molecule [12]. Thus, the realistically low MHC diversity claimed by Nowak et al. [44] hinges upon an unrealistically stringent negative selection.

Having disputed the commonly accepted argumentation that negative selection limits the individual MHC diversity [17, 18, 28, 44, 47, 66, 69], the question remains which other mechanism can explain the limited number of

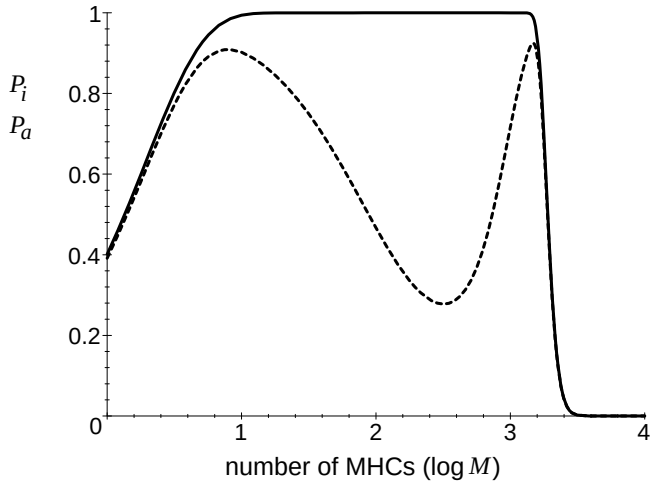

FIGURE 3 The solid curve denotes the chance P_i to mount an immune response as a function of the number of different MHC molecules per individual M. The curve shows that T-cell repertoire depletion occurs only at an unrealistically high individual MHC diversity. The dashed curve denotes the chance P_a to mount an *appropriate* immune response. Once an individual expresses of the order of ten different MHC molecules, additional MHC diversity increases the chance that autoimmune responses are induced. Parameters are: $q = 0.05$ [31], $p = 10^{-8}$, $R_0 = 10^9$, $e = 10$ [18], $h = 0.005$, $t = 0.005$, and $S_i = 2 \times 10^4$.

MHC molecules per individual. The solid curve in figure 3 suggests one possibility. The flat top of the curve demonstrates that the selection pressure for a higher MHC diversity vanishes once about ten different MHC molecules per individual have been expressed. This rather limited individual MHC diversity may thus simply be sufficient to have a good chance to present and respond to antigens.

3.1 AVOIDING INAPPROPRIATE RESPONSES

Elaborating on the theme of section 2, we investigate an alternative explanation for the limited individual MHC diversity, namely the need to avoid inappropriate immune responses. As discussed above, inappropriate responses occur when different antigens requiring different modes of responsiveness trigger the same clonotype. An example of an inappropriate response is when a self-specific clonotype that is ignorant of its self-epitope is triggered by a crossreacting foreign epitope and subsequently induces an autoimmune disease [3, 45, 46, 75]. The likelihood of such inappropriate immune responses increases with the number of epitopes that are presented to the immune system.

Once there are sufficient MHC molecules to ensure presentation of antigens, having a greater diversity of MHC molecules may thus be detrimental.

To study this hypothesis, we extend the above-described model with the chance P_t to stay tolerant to all self-peptides. This is expressed as the chance that during an immune response, on all of the M MHC molecules of a host, foreign epitopes are either not presented (with probability $1-q$), or presented but not recognized by any of the responding, ignorant self-specific clonotypes (with probability $q(1-pa)^{R_M}$):

$$P_t = (1 - q + q(1-pa)^{R_M})^{eM} \ . \tag{3}$$

The probability a that a clone from the functional repertoire is ignorant and self-specific[1] is given by:

$$a = 1 - (1-p)^{qS_iM^*} \ , \tag{4}$$

where S_i denotes the number of self-epitopes that fail to induce self-tolerance, and M^* denotes the expected number of MHC molecules that positively select one particular clone from the functional repertoire:

$$M^* = \frac{Mh}{(1-(1-h)^M)} \ . \tag{5}$$

Note that the decrease in P_t with increasing M is due to (i) the increasing presentation of foreign epitopes, and (ii) the increasing fraction of ignorant, self-specific lymphocytes a, due to the increasing number of peptide-MHC complexes formed by self-antigens that fail to induce tolerance. The chance P_a to mount an appropriate response to an antigen is the chance P_t to stay tolerant minus the probability that all clones fail to respond:

$$P_a = P_t - (1 - P_i) \ , \tag{6}$$

where P_i is given by eq. (2).

The dashed curve in figure 3 shows that involving the chance to mount an autoimmune response yields a sharply defined, low optimal MHC number, i.e., eight MHC molecules per individual (left-hand top). Yet, the chance P_a to make an appropriate immune response in that optimum remains close to one. Apparently, the system can reconcile the need to respond to many antigens with the need to avoid crossreactive, autoimmune responses, by selecting for a relatively low MHC diversity. At the left-hand top of the P_a curve, adding MHC molecules hardly increases the chance P_i to mount an immune response against an antigen (see the solid curve), while it significantly decreases the chance P_t to stay self-tolerant (see the dashed curve).

[1]Note that eq. (3) may give an underestimation of P_t if clones recognize multiple peptide-MHC complexes coming from one antigen. In our parameter setting, this chance is negligible for $M < 10^5$ since the probability that a particular clone recognizes an MHC-peptide complex during challenge with one antigen is $M^*t < 5 \times 10^{-4}$.

Interestingly, the dashed curve in figure 3 has a second peak at a very high number of different MHC molecules per individual. At the second peak, both self- and foreign epitopes are presented as many different MHC-peptide complexes. The immune system then finds a balance between prevention of autoimmunity due to a severely depleted repertoire, and immunity against foreign antigens thanks to the formation of many different peptide-MHC complexes per epitope.[2] This scenario is extremely wasteful, since at the top only 0.06% of the total lymphocyte repertoire survives thymic selection. If autoimmunity is less of a problem, the P_a curve looses the two sharply defined peaks. For example, if lymphocytes are highly specific (e.g., $p = 10^{-9}$), the risk of autoimmunity by crossreactions becomes negligible, and the P_a curve and the P_i curve become almost indistinguishable. Nevertheless, the dashed curve in figure 3 shows that an increase in autoimmunity due to crossreactions is a possible side-effect of expression of a large individual MHC diversity.

Summarizing, our model suggests that the evolution of a limited number of MHC genes per individual does not result from repertoire depletion during self-tolerance induction in the thymus. Instead, it may either reflect a low requirement of MHC diversity due to degenerate peptide-MHC binding (solid curve, fig. 3), or reflect the need to avoid inappropriate, crossreactive immune responses (dashed curve, fig. 3).

4 POPULATION DIVERSITY OF MHC MOLECULES

Despite the limited expression of different MHC molecules per individual, the MHC diversity of populations is extremely large. A commonly held view is that MHC polymorphism is due to selection favoring MHC heterozygosity. Since MHC molecules are codominantly expressed, and different MHC molecules bind different peptides, MHC heterozygous hosts can defend themselves against a larger variety of pathogens compared to MHC homozygous individuals. This hypothesis is known as the theory of "overdominance" or "heterozygote advantage" [19, 25, 26, 27, 67]. Alternatively, it has been proposed that the large polymorphism of MHC molecules is due to the high speed at which pathogens adapt to their hosts, due to their relatively short generation times. Since evolution will favor pathogens that avoid presentation by the most common MHC molecules in the host population, there will be a permanent selection force favoring hosts that carry rare—e.g., new—MHC molecules. Since hosts with rare MHC alleles have a relatively high fitness, the frequency of rare MHC alleles will increase and common MHC alleles will become less frequent. The result of this "frequency-dependent selection" is a dynamic equilibrium, maintaining a polymorphic population [4, 8, 62, 63].

The mechanisms behind the selection for MHC polymorphism have been debated for over three decades. It has been argued that selection for het-

[2]The position and height of the second peak should be taken with care since our equations may become imprecise at very high values of M.

erozygosity alone cannot explain the high MHC diversity observed in nature [48, 73]. Several models have been developed to study the effects of selection for heterozygosity and frequency-dependent selection on the polymorphism of MHC molecules (see, e.g., Takahata and Nei [67], Wills [73], and Wills and Green [74]). To our knowledge, however, a direct comparison of both hypotheses in one model has never been made. In order to make such a comparison, we have simulated the coevolution of hosts and pathogens using a genetic algorithm [24].

4.1 A SIMULATION MODEL OF MHC DIVERSITY

An extensive description of our model has been published previously [5]. We here confine ourselves to a very brief summary of the model structure. In our model, hosts are diploid and consist of bit strings representing their MHC alleles; pathogens are haploid, and their peptides are also represented by bit strings. Peptide presentation by an MHC molecule may occur at different positions on the MHC molecule, and is modeled by complementary bit matching. If the number of complementary bits at the best matching position on an MHC molecule exceeds a predefined threshold, a peptide is considered to be presented by the particular MHC molecule. In the simulations presented here, the chance that a random MHC molecule presents a randomly chosen peptide is 7.3%. Hosts carrying different MHC molecules will therefore typically present different peptides of pathogens.

At each generation, every host in our simulations interacts with every pathogen. The fitness of a host is proportional to the fraction of pathogens that it can present; the fitness of a pathogen is proportional to the fraction of hosts that it can infect without being presented by the host's MHC molecules. All individuals are replaced by fitness-proportional reproduction at the end of each generation. During reproduction, point mutations can occur. One cycle of fitness determination, reproduction, and mutation defines a generation. To account for the shorter generation time of pathogens, we let pathogens go through several generations per host generation.

4.2 MHC DIVERSITY BY HOST-PATHOGEN COEVOLUTION

To study the origin and maintenance of the MHC polymorphism, all hosts in our simulations initially express one and the same MHC molecule, while the pathogens are initialized randomly. The average fitnesses of the pathogens and the hosts are initially close to 0.5 (see fig. 4). Thanks to their relatively short generation times, the pathogens in our simulations evolve to evade presentation by the MHC molecules of the hosts. Since there is no initial MHC diversity, the average fitness of the pathogens immediately increases (see fig. 4(a)). Any pathogen that is able to infect one host is able to infect all hosts and, hence, rapidly takes over the pathogen population. As a consequence, the average fitness of the hosts initially drops (see fig. 4(b)). Under this selection

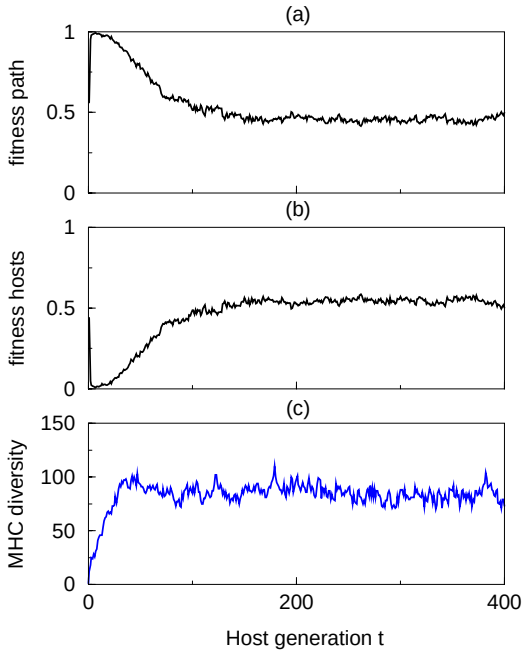

FIGURE 4 The average fitnesses of pathogens (a) and hosts (b), and the average number of different MHC molecules in the population (c), in a coevolutionary simulation in which the pathogens evolve one hundred times faster than the hosts, plotted against the host generation t. The coevolution is initialized with MHC-identical hosts and random pathogens. Results come from a simulation with 200 hosts, each carrying 1 MHC gene with 2 alleles, and 50 different pathogen species, each consisting of maximally 10 different pathogen genotypes, which carry 20 different epitopes each. The epitopes are 12 bits long, while the MHC molecules are 35 bits long. A peptide is presented by an MHC molecule if at least 11 out of 12 bits bind. The probability of mutation, i.e., a bit flip, is $\mu = 0.001$ per bit per generation.

pressure caused by the pathogens, the hosts develop an MHC polymorphism: the number of different MHC molecules in the host population rapidly increases to reach a high equilibrium diversity (see fig. 4(c)). Figure 5 demonstrates that the eventual MHC population diversity that is attained depends on the relative generation time of the pathogens. The faster the pathogens evolve, the larger the resulting MHC polymorphism.

In order to study to what extent the arising MHC diversity is caused by selection for heterozygosity and to what extent by frequency-dependent selection, we performed simulations in which the pathogens do not evolve. Instead, the hosts are exposed to a new, randomly chosen pathogen popula-

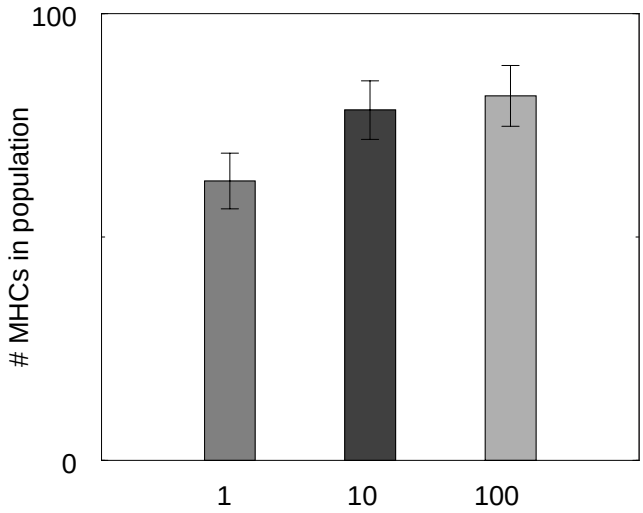

FIGURE 5 MHC molecules become polymorphic. The average number of different MHC molecules arising in the host population increases with the speed at which the pathogens coevolve. Results are shown for three different simulation types: 1: pathogens evolving as fast as the hosts, 10: pathogens evolving ten times faster than the hosts, 100: pathogens evolving one hundred times faster than the hosts. The averages were taken over one hundred generations, between $t = 900$ and $t = 1000$. The error bars denote the standard deviations of the average host and pathogen fitnesses in time. For parameters, see the legend of figure 4.

tion at every host generation (denoted by R). Due to the absence of pathogen evolution, these simulations reflect the MHC diversity that develops under selection for heterozygosity only. Figure 6(a) shows that mere selection for heterozygosity gives rise to an MHC polymorphism that is almost twice as small as the polymorphism arising when hosts and pathogens coevolve. To check if the MHC molecules arising in the host population are really different from each other, and do not differ at a few mutations only, we have also plotted the average Hamming distance between all different MHC molecules in the host population (fig. 6(b)). We find that host-pathogen coevolution increases the genetic distance between MHC molecules. We therefore conclude that rapidly coevolving pathogens provide a considerably larger selection pressure for a functionally diverse set of MHC molecules than mere selection for heterozygosity.

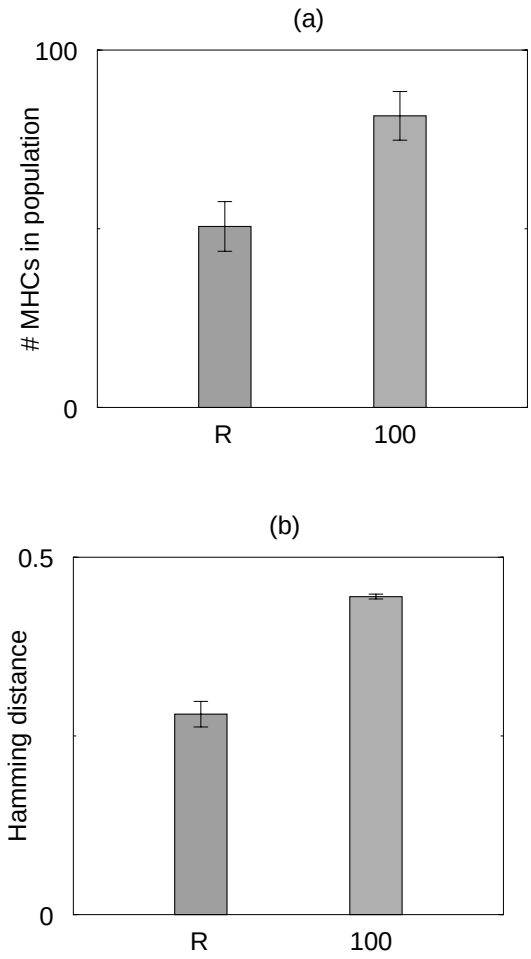

FIGURE 6 Selection for heterozygosity *versus* frequency-dependent selection. (a) The average number of different MHC molecules in the host population, and (b) the average Hamming distance between the different MHC molecules. We have plotted a coevolutionary simulation in which the pathogens evolve one hundred times faster than the hosts (100), and a simulation in which the pathogens do not evolve, but are instead chosen randomly at every host generation (R). The coevolutionary simulation (100) represents the MHC diversity that evolves in the presence of both frequency-dependent selection and selection for heterozygosity, while the simulation with random pathogens (R) represents the MHC diversity that evolves under selection for heterozygosity only. The diversity of pathogens in the R simulation was adjusted to the typical pathogen diversity evolving in the coevolutionary simulations.

5 DISCUSSION

In this chapter, we have studied several sources of diversity in the vertebrate immune system. In particular, we have studied the diversity employed by lymphocytes, which are responsible for the *recognition* of antigens, and the diversity of major histocompatibility (MHC) molecules, which are responsible for the *presentation* of antigens to the immune system. In principle, lymphocytes and MHC molecules are involved in the same task, i.e., to allow immune responses to many foreign antigens, while avoiding inappropriate responses such as autoimmunity. Given the diversity of foreign and self-molecules, it is perhaps not surprising that both MHC molecules and lymphocytes have a high degree of diversity. Nevertheless, they differ fundamentally in the level at which their diversity is expressed. While any vertebrate individual expresses a huge diversity of B and T lymphocytes, the diversity of MHC molecules is mainly evident at the population level. This suggests that MHC and lymphocyte diversity play quite distinct functional roles.

We have proposed that the existence of a diverse lymphocyte system reflects an adaptation of vertebrate hosts to a quickly changing pathogenic world. By storing the appropriate modes of response against different antigens, the vertebrate immune system is able to learn on a somatic time scale. This allows the immune system (i) to respond more promptly and appropriately upon re-encounter of an antigen, even if some of its epitopes have mutated, and (ii) to respond appropriately to whole classes of correlated antigens, even if the immune system has been exposed to only one of their members [60]. We have shown that such a somatically learning system requires sufficient specificity and diversity. If lymphocytes are too crossreactive, inappropriate responses may be induced when unrelated antigens trigger one and the same clone. Immune diversity is required to reconcile reactivity to many antigens with a very specific storage of the appropriate immune responses against them [9, 10, 11].

In sharp contrast with the specificity of lymphocytes, MHC molecules bind their ligands with great degeneracy [23, 31, 36]. This degeneracy, combined with the large degree of heterozygosity of MHC loci, allows the presentation of a large variety of T-cell epitopes to the immune system. Regarding the role of MHC molecules in antigen presentation, it is surprising that the number of different MHC molecules expressed per individual is so limited compared to the large population diversity of MHC molecules. A commonly used argument is that a large individual MHC diversity would impair the T-cell repertoire during self-tolerance induction [17, 18, 28, 44, 47, 66, 69]. As we have shown, however, extra MHC molecules mainly deplete lymphocytes that were not positively selected anyway in the absence of those MHC molecules. As a result, a very wide range of individual MHC diversities—varying from 10 to 2000 different MHC molecules per individual—yields excellent immunity against antigens. The selection pressure for a larger MHC diversity within an individual, however, fades away once there are of the order of ten different MHC

molecules per individual. This suggests that the limited individual MHC diversity found in nature reflects a lack of selection for more MHC diversity than what is needed for sufficient presentation of antigens. This is in agreement with the fact that only little correlation has been found between MHC haplotypes and resistance against particular infectious diseases [50, 74].

In contrast to the lack of correlations between MHC molecules and resistance against infectious diseases, strong correlations have been found between certain MHC haplotypes and susceptibility to autoimmune diseases [42, 74]. Such correlations are to be expected if autoimmunity is due to mimicry between foreign-peptide-MHC complexes and self-peptide-MHC complexes. We have extended our model with autoimmunity, by including ignorant self-specific clonotypes that can be triggered by foreign antigens. Our analysis demonstrates that avoidance of crossreactive, autoimmune responses yields a selection pressure for a limited individual MHC diversity.

Despite the fact that different selection pressures may limit an individual's MHC diversity, our genetic algorithm shows that there is selection for a large diversity of MHC molecules at the population level. A large population diversity of MHC molecules allows different individuals to respond differently to identical antigens, thereby giving protection against coevolving pathogens. Just like the individual diversity of lymphocytes, the population diversity of MHC molecules may thus reflect an adaptation of slowly evolving hosts in a rapidly changing world of pathogens.

ACKNOWLEDGMENTS

We are grateful to André Noest and Joost Beltman for their contributions to sections 3 and 4, respectively, and thank Can Keşmir for discussing the concepts and equations of this chapter.

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