Adaptive immunity as a specific storage system
of immunological decisions

José A.M. Borghans & Rob J. de Boer

*Theoretical Biology,*
*Utrecht University*
*Utrecht, The Netherlands.*

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Abstract

During primary encounter of an antigen, the immune system has to decide which type of immune response is most appropriate. For instance, noncytopathic viral infections typically require a cellular response while elimination of most bacteria typically requires a humoral response. Such immunological decisions are based upon many factors such as signals from the innate immune system, and/or the local tissue environment. We hypothesise that the choice of the most appropriate type of immune response against each antigen is stored by the immune system in the form of differentiated clonotypes. Clonotypes that are triggered (or tolerized) by an antigen switch from a naive phenotype to a new, stable mode of responsiveness (or unresponsiveness), allowing the appropriate type of immune reaction to be regenerated upon restimulation of the clone. The adaptive immune system may contribute to the decision as to which type of immune response to mount, when novel antigens carry epitopes that the system has seen previously. This may go wrong, however, if differentiated cells coincidentally respond to new antigens. We develop a simulation model and a probabilistic model to investigate under which circumstances storing appropriate responses helps the immune system to rapidly make correct decisions. We find that lymphocytes need to be specific in order to avoid inappropriate, cross-reactive responses. Lymphocyte diversity is required to reconcile specificity with reactivity against many antigens. Increasing the diversity of the immune system does not hamper the positive contribution of memory lymphocytes in subsequent responses.

Appropriate responses are stored by memory cells

Specific immunological memory is one of the most striking features of the vertebrate immune system. By inducing a cellular or humoral response to invading pathogens, the immune system is able to remove pathogens and to remember them specifically. Thus, vertebrates are able to respond faster and more efficiently upon reinfection. Adaptive immunity, and the accompanying ability of specific immunological memory, evolved at the transition from invertebrates to vertebrates, when gene rearrangements were employed to generate highly diverse lymphocyte repertoires [4, 69, 129]. Because lymphocyte receptors are at least partially randomly generated, the adaptive immune system requires self tolerance processes to avoid autoimmunity and mechanisms that allow naive lymphocytes to develop appropriate effector functions.

During primary antigenic encounter, there is a whole array of signals informing the immune system on the nature of the antigen that is being recognized [23, 75, 104, 105, 140–142, 182]. For example, the localization of the antigen [234], the presence of conserved bacterial peptides [104, 140, 141, 149], the type of tissue damage [135], and the cytokines and chemokines that are locally expressed [1, 158, 168], all influence the type of immune response that is induced. Based on these signals, which collectively form the
“context” of the antigen [52, 53], a complex decision is made as to whether to respond or not, and if so which effector mechanism to use. For efficient elimination, different pathogens require qualitatively different immune responses, varying from cellular to humoral responses, and varying in e.g. immunoglobulin isotype and cytokine expression [106, 209]. For example, antigen encounter in the gut will tend to induce IgA responses, gram-negative bacterial infections expressing LPS and causing tissue damage will generally trigger B cell responses, and recognition of viral RNA will typically induce a cytotoxic T cell response [106].

Over evolutionary time, certain antigenic contexts may have become correlated with the corresponding appropriate types of immune response. Alternatively, it has been proposed that the choice of immune response is determined somatically, by (success-driven) feedback mechanisms [190]. By whatever route the decision is made, we hypothesise that the adaptive immune system stores the appropriate modes of response against different antigens in differentiated lymphocytes. The immune system thereby somatically learns to associate the epitopes it has encountered with the appropriate modes of response against them.

Memory cells influence subsequent responses

Once lymphocytes have been instructed as to which type of immune response to mount, they recall their appropriate mode of response when restimulated in subsequent infections [177, 209]. It has been shown that differentiated T helper cells recall their cytokine expression even in the presence of adverse costimulation (see [177] and references therein). Cytokine production is somatically imprinted in differentiated lymphocytes by chromatin remodelling and DNA demethylation. Instructed lymphocytes can thus epigenetically transfer their appropriate mode of response to their daughter cells [25, 26, 176].

Memory lymphocytes can greatly influence immune responses against subsequent infections. The ease with which they are triggered, even at very low antigen concentrations [5, 6, 40, 49, 170, 181, 204, 217], may explain why immune responses tend to be dominated by memory lymphocytes from previous infections, a phenomenon termed “original antigenic sin” [73, 114, 139]. It has for example been shown that the CD8+ T cell response against influenza is dominated by memory cells that cross-react with previous influenza infections [92, 197]. Even for unrelated viruses such a bias to stimulation of previous memory clones has been observed [193].

Instructed lymphocytes can also direct the differentiation of other, naive lymphocytes [147]. CD4+ T cells from transplantation-tolerant mice, for example, have been shown to render naive cells tolerant upon adoptive transfer. Since the so induced tolerant cells can in turn tolerate other naive lymphocytes this process was called “infectious transplantation tolerance” [98, 172, 225]. Later, it was demonstrated that infectious suppress-
sion can also take place between lymphocytes of different specificities. Anergic T cells, rendered anergic via T–T cell presentation of their antigen, appeared to actively suppress other T cell clonotypes, provided that both the anergic cells and the responder cells were confined to the same antigen-presenting cell [210]. Analogously, memory lymphocytes of a certain responsive mode may direct the differentiation of new, naive clonotypes, for example via cytokine secretion [168]. It has been proposed that T cells affect each other’s differentiation via interactions with dendritic cells, which in turn promote the differentiation of responding T cells to different cytokine profiles [37, 178, 180, 208]. Cytokine-mediated T helper cell differentiation is characterized by a positive feedback: many cytokines promote their own expression by affecting the differentiation of particular T cell subsets [180]. Such a positive feedback has for example been observed for IL-2, IL-4, IFN-γ, IL-10 [90], and TGF-β [187]. Spreading of a responsive memory phenotype from one (self) epitope to another has also frequently been observed in autoimmune diseases [119, 218].

If cross-reacting memory clones happen to have the correct phenotype for a subsequent antigenic challenge, they are obviously advantageous to their host. Since memory cells are not confined to the lymphoid tissue and freely enter the solid tissue [44, 148, 234], they can respond anywhere and any time their specific epitope is encountered. Additionally, responses due to memory cells are typically more prompt than primary immune responses [5, 6, 40, 49, 77, 170, 181, 204, 217], because memory cells are more sensitive to low antigen doses, have less stringent requirements for costimulation, and have already been instructed for the appropriate mode of response. Thus tissue damage by pathogens upon reinfection and upon pathogen dissemination to other organs can be prevented. Moreover, if pathogens mutate their antigenic structure, previous memory lymphocytes recognizing epitopes that have remained unaltered may direct the differentiation of new clonotypes recognizing altered epitopes of the pathogen.

Memory lymphocytes may also cause immunopathology, however. Being fairly independent of signals from the innate immune system and the local tissue environment, they run the risk of mounting inappropriate responses. Different antigens may possess overlapping epitopes and thereby trigger memory lymphocytes with inappropriate phenotypes. Moreover, self-reactive clonotypes that have escaped self tolerance induction may cause autoimmunity upon stimulation by external antigens [12, 159, 160, 232].

We have hypothesised in Chapter 2 that the immune system should be specific to minimize the risk of mounting inappropriate immune responses by cross-reactivity [30, 34]. Here a simulation model is developed to study under which circumstances immunological memory can help the induction of new, appropriate immune responses, while avoiding inappropriate cross-reactive responses. We find that both requirements are met whenever the lymphocyte system is sufficiently specific and diverse. Although this result may seem to be at odds with the high cross-reactivity of T cells proposed by Mason [130], a calculation in the Discussion shows that both views are perfectly compatible.
A simulation model

To illustrate the basic principles of our hypothesis, we consider an immune system consisting of $R_0$ clonotypes. Each clonotype has a certain mode, being either naive, tolerant, or responsive in a particular type of response. The modes are represented by integer numbers $0, 1, 2, \ldots, m$, where 0 means naive, 1 means tolerant, and $2, \ldots, m$ identify the different types of responsive modes (such as Th1, Th2, IgA, IgE, etcetera). In the simulations presented here, there are ten different modes. When the immune system is challenged with an antigen we allow every epitope to be recognized by precisely one clonotype, which is selected randomly. Depending on the cross-reactivity of the system (here inversely related to its diversity), each clone may recognize multiple epitopes. Clonotypes specific for tolerance-inducing self epitopes are initialized in the tolerant mode; all other clonotypes are initially naive. At birth the system therefore consists of clonotypes with mode zero or one. Self tolerance induction need not be complete. In our simulations, a fraction $f$ of the self-specific clonotypes is initialized in the tolerant mode; the other self-specific clonotypes remain ignorant of their respective self epitopes [50, 169, 185]. Such ignorant clonotypes may induce autoimmunity when they become triggered by pathogens [12, 159, 160, 232]. After birth the system is challenged with different antigens, each represented by $e$ different (immuno-dominant) epitopes, and each requiring a certain mode of response. Both the appropriate mode of response to an antigen and the clonotypes recognizing its epitopes are selected randomly beforehand. Pathogens never kill their hosts, i.e. the simulations are continued even if an inappropriate response is induced.

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 need 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 need not be unreasonable, because the innate immune system has learned about different kinds of pathogens and antigenic contexts over evolutionary time.

In our simulations, once the system has decided which type of response to make to a particular antigen, all naive clonotypes recognizing that antigen switch to the corresponding memory mode. Even if an inappropriate mode of response is triggered, naive lymphocytes switch (to the incorrect) mode. In accordance with experimental data [151, 176], memory clonotypes involved in a response to an antigen do not switch mode.

The performance of the model immune system is recorded by counting scores. In the default situation, in which a decision is made by the innate system and the clonotypes recognizing an antigen simply adopt the mode of the innate system, no score is given. All
Chapter 3

Figure 1. A simple example of a simulation with $e = 3$ different epitopes per antigen. After self tolerance induction most clonotypes are naive (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 naive 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 naive 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 naive 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. Naive clonotypes 5 and 10 incorrectly switch to mode 5.

cases in which previous memory clones establish the correct mode of response against an antigen (without being responsive to any self antigens) yield a positive score. The cases in which previous memory clones establish an incorrect mode of response against an antigen yield a negative score. This includes the cases in which the majority of the memory clonotypes involved is in the tolerant mode, and the adaptive system thus refrains from responding. We also score the number of autoimmune responses, which are induced when naive clonotypes that are ignorant of their self epitopes are triggered into one of the responsive modes, by pathogens cross-reacting with those self epitopes [12, 159, 160, 232]. An example of a small simulation is given in Figure 1.

Obviously, the adaptive immune system will only give a positive contribution to the decision making process if there are groups of structurally related antigens, e.g. coming from the same pathogen family or species, that require similar types of immune reactions. 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. All antigens are presented only once, i.e. we study a “worst case” sce-
nario, ignoring the conventional benefits of immunity obtained when the same antigen rechallenges the immune system.

**Somatic learning requires specificity**

In Figure 2, the performance of immune systems that have been challenged with one thousand different antigens is plotted as a function of the immune system diversity $R_0$, and hence as a function of the specificity of clonotypes. Panels $a$, $b$, and $c$ give the fraction of challenges that yield a positive score, a negative score, and an autoimmunity score, respectively. The different lines in the panels depict different degrees of correlation between the antigens, 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 [62]). 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 cross-reactions. Above a diversity of $R_0 = 10^9$ 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 primary immune reactions.

At a low diversity, unrelated antigens expressing different epitopes and requiring different modes of immune response will tend to trigger the same clonotypes. Figure 2$b$ illustrates that the adaptive immune system hence makes many mistakes. Previous memory clones recognizing epitopes of unrelated antigens tend to induce wrong types of immune response; clones that have previously been tolerized by self epitopes hinder the induction of immune responses to subsequent antigens. Figure 2$b$ shows that such mistakes (i) disappear at a large repertoire diversity, and (ii) hardly depend on the correlation between the antigens.

Figure 2$c$ demonstrates that at a very low diversity, autoimmunity hardly occurs. This is due to the large fraction of clonotypes that have been tolerized by self epitopes. At a somewhat higher diversity, many autoimmune responses are induced due to cross-reactions between foreign antigens and ignored self peptides. Such coincidental cross-reactions disappear if the immune repertoire is very diverse. Summarizing, Figure 2 illustrates that in immune systems that store the appropriate modes of response in differentiated lymphocytes, the benefits of immune memory outweigh the accompanying disadvantages whenever the immune repertoire is sufficiently specific.
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 due to lack of responsiveness caused by cross-reactive tolerant clones. (c) The fraction of challenges leading to autoimmunity caused by ignorant, self-specific clones that are triggered by cross-reacting pathogens. 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, a fraction $f = 0.5$ of all $S = 10^3$ self antigens induces tolerance, and there are ten different modes ($m = 9$).
A probabilistic model

In the simulation model described above, each epitope always triggers precisely one clonotype. Thus, the model’s immune repertoire never fails to recognize an antigen. It is more realistic to let clonotypes respond to epitopes with a certain probability $p$, so that any epitope triggers on average a fraction $p$ of all clonotypes. In that case, specificity required to avoid inappropriate cross-reactive immune responses needs to be reconciled with sufficient cross-reactivity to allow immune responses against many different antigens. We employ a probabilistic model to show that this conflict can be solved if lymphocyte repertoires are sufficiently diverse (see also [30, 34]).

Let us consider an extreme scenario, by assuming no correlation between the antigens that are encountered and considering an infinitely large variety of types of immune responses. Thus there is no positive contribution of memory lymphocytes. Instead we focus on preventing inappropriate immune responses. Any situation in which an antigen triggers a clonotype recognizing a self epitope is considered to be wrong. We no longer distinguish between autoimmunity due to stimulation of naive self-specific lymphocytes, and absence of response due to tolerant lymphocytes instructing naive lymphocytes to adopt the tolerant phenotype. Just as in the simulation model, there are $S$ different self epitopes and $R_0$ clonotypes in the repertoire.

First consider the probability of mounting an appropriate immune response to an antigen that expresses $e$ different epitopes. Having an infinite number of modes and no correlation between the antigens that are encountered, any responding memory clonotype is considered to cause an inappropriate immune response. Thus, an immune response will only be appropriate if all clonotypes responding to an antigen are neither self specific nor of the memory phenotype. In an animal with $S$ different self epitopes that has previously encountered $M$ different foreign epitopes, the probability $v$ that a responding clonotype is naive (i.e. not responsive against any of the $M$ previously encountered epitopes) and not specific for any self epitope is:

$$v \simeq (1-p)^{S+M},$$

where we write “approximately equal” because of possible overlaps between $S$ and $M$. The probability that an antigen does not trigger any inappropriate immune response is the chance that each clonotype in the repertoire either fails to respond (with probability $1-p$), or responds and is naive and not specific for any self epitope (with probability $pv$). This should hold for all of the $e$ different epitopes of the antigen. Subtracting the probability that all clones fail to respond to the antigen gives the probability $P_a$ of mounting an appropriate immune response:

$$P_a = (1-p + pv)^{eR_0} - (1-p)^{eR_0}.$$
Repertoire diversity reconciles specificity with reactivity

In Figure 3 the probability of mounting an appropriate immune response is plotted as a function of the cross-reactivity parameter $p$, for immune systems that need to avoid cross-reactivity with $S + M = 10^5$ different epitopes. A low value of the recognition probability $p$ corresponds to a highly specific immune system; a high $p$ value to a very cross-reactive immune system. From left to right, the four curves represent immune repertoires with $R_0 = 10^{11}$, $R_0 = 10^9$, $R_0 = 10^7$, and $R_0 = 10^5$ clonotypes, respectively. Whatever the diversity of the immune system, there is an optimal level of cross-reactivity, above which many mistakes are made, and below which the immune repertoire frequently fails to recognize antigens [30, 34, 62].

The three left-hand curves all have a cross-reactivity region in which the induction of an appropriate immune response to an antigen is very likely, i.e. $P_a \simeq 1$. The width and height of this region increase with the diversity of the immune system $R_0$. At a low repertoire diversity ($R_0 = 10^5$) many inappropriate immune responses are expected. Such inappropriate responses can be prevented in large immune repertoires by being

![Figure 3. The probability $P_a$ of mounting an appropriate immune response against an antigen with $e = 10$ different epitopes, as a function of the cross-reactivity $p$ of lymphocytes. Cross-reactivity with $S + M = 10^5$ self epitopes and previously encountered epitopes needs to be avoided. If lymphocytes are very specific the immune system frequently fails to mount an immune response against an antigen; if lymphocytes are very cross-reactive many inappropriate immune responses are induced. Large immune repertoires can afford to be very specific, and thereby attain a larger maximum value of $P_a$, than small immune repertoires.

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sufficiently specific. Thus, high repertoire diversity reconciles specificity (to avoid inappropriate immune responses) with reactivity against many antigens.

**Inappropriate responses to novel antigens increase with age**

![Graph](image_url)

Figure 4. The probability $P_a$ of mounting an appropriate immune response against a new antigen as a function of the (logarithm of the) number of epitopes $S + M$ with which cross-reactivity should be avoided. Since any immune system will encounter more and more different epitopes with age, the horizontal axis also reflects the age of the immune system. Parameters are $R_0 = 10^7$, $e = 10$, and $p = 10^{-7}$.

Since the adaptive immune system learns on a somatic time scale, adults tend to be better protected against infections than naive individuals. In the probabilistic model we have chosen to disregard any advantages conferred by memory lymphocytes. Although this choice is artificial, it reveals an interesting insight. Figure 4 shows that as the memory repertoire builds up with age, the chance to induce appropriate immune responses to novel antigens decreases. On the horizontal axis we have plotted the number of epitopes $S + M$ with which cross-reactivity should be avoided. Since this number can only increase with age, it also reflects the age of the immune system. As the memory repertoire becomes more diverse, the chance increases that previous memory clones cross-react with new antigens and induce inappropriate immune responses. This is in good agreement with observations that childhood diseases such as measles and chickenpox typically cause more severe problems in adults than in children [28]. Although the naive repertoires of adults should still be sufficiently diverse to recognize any new antigen [10], we postulate that adult immune responses may be hampered by inappropriate effector mechanisms induced by previous memory clones.
We have studied the hypothesis that the adaptive immune system stores the immunological decisions made during primary immune responses in specific lymphocytes. Lymphocytes that have been instructed as to which type of immune response to mount recall this instruction whenever they recognize their specific epitope. This allows the immune system (i) to respond appropriately and promptly 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. We conjecture that the qualitative property of a memory clone, i.e. its mode of response, is essential in immunological memory [181, 209], on top of the conventional increase in precursor frequency. An experimental comparison of naive and memory lymphocytes supports this idea: when equal numbers of naive and memory lymphocytes were transferred to Rag\(^{-/-}\) mice, memory cells proliferated and performed their effector function much faster than their naive counterparts [84].

Conventionally, the immune system is thought to be diverse to guarantee an effective immune response to many different antigens (see for example [130]). In contrast, we argue that the high diversity of the adaptive immune system reflects the need to store appropriate modes of immunity against many different antigens in a very specific manner. If lymphocytes were to be degenerate, inappropriate cross-reactive immune responses would tend to be induced. According to our calculations, lymphocytes should be as specific as possible, within the constraints imposed by the size of the immune repertoire (see also [34]). Evolution would thus select for diverse, specific immune repertoires, with avoidance of inappropriate responses as the dominant selection pressure.

The diversity of the adaptive immune system has recently been estimated by Arstila et al. [10]. It was shown that the human naive T cell repertoire consists of at least \(2.4 \times 10^7\) different T cell specificities. The upper bound of the human naive T cell diversity was estimated to be \(10^8\) different clonotypes. Such a repertoire diversity would be perfectly functional according to our model. We have argued however, that the upper bound estimated by Arstila et al. [10] is probably several orders of magnitude too low [111].

Throughout this chapter we have adopted the premise that the innate immune system is capable of judging the infectivity of antigens [104]. Although not central to our argument, some problems remain. As pointed out by Bretscher [39], various non-pathogenic antigens, such as xenogeneic red blood cells and rhesus factor, induce strong immune responses even when administered without any adjuvant. Apparently, in the absence of innate signals, naive clones that become triggered nevertheless switch to some type of responsiveness, probably influenced by a context consisting of the local tissue environment only.

In our simulation model, we have allowed for an instructive role of memory clonotypes in the differentiation of other, naive clones. Upon encounter of their antigen, memory cells indeed recreate (at least part of) the cytokine context in which they themselves
were originally stimulated [180]. There is some evidence, however, that spreading of a memory phenotype from one clonotype to another may not always take place. The decreasing efficacy of repeated influenza vaccinations, for example, has been attributed to old memory clones preventing proper stimulation of naive clonotypes specific for novel epitopes in the vaccine [197]. Although we think the spreading of appropriate modes of responsiveness plays an important role in adaptive immunity, our results do not depend on this assumption. The fact that memory clones themselves have to respond appropriately is sufficient to explain why the adaptive immune system needs to be specific (simulation results not shown).

It has been proposed previously that clonotypes that have switched to a regulator phenotype due to self tolerance induction in the thymus may educate a “second wave” of clonotypes recognizing tissue-specific epitopes [147]. By analogy, in our model, tolerant clones may be helpful in preventing immune responses to antigens correlating with tolerance-inducing self molecules. Such an instructive role of tolerant clones could, however, be abused by pathogens. Pathogens could evade immune responses by the mere expression of proteins cross-reacting with self proteins of their hosts. Spreading of the tolerant phenotype to other clones recognizing truly foreign epitopes of the pathogen would hinder the induction of a protective response. It remains unclear whether tolerant clones specific for self epitopes can indeed obstruct the induction of an immune response against a pathogen. Induction of immune responsiveness by innate signals may overrule absence of responsiveness taught by tolerant clones. Additionally, the large population diversity of MHC molecules [21] may thwart immune evasive strategies based on self mimicry.

Since the theoretical number of different epitopes by far exceeds the size of any immune repertoire, clonotypes need to recognize multiple epitopes in order to ensure an immune response against any pathogen [130]. Mason [130] has estimated the total number of different immunogenic epitopes to be of the order of $6 \times 10^{12}$, and calculated that each clonotype in a repertoire of $R_0 = 10^8$ clones should recognize at least $3 \times 10^8$ different epitopes. Thus, it was concluded that T cells have to be “highly cross-reactive.” Our conclusion that lymphocytes should be highly specific (see also [30, 34]) seems to flatly oppose the conclusion drawn by Mason [130]. Both are fully consistent, however. In the same repertoire with $R_0 = 10^8$ clonotypes and our “optimal” specificity of $p = 10^{-7}$ (equation (2) with $e = 1$), any epitope would trigger only ten clones, but any clone would recognize $10^{-7} \times 6 \times 10^{12} = 6 \times 10^5$ different epitopes.

Summarizing, our models illustrate that specificity is a prerequisite of the adaptive immune system. Reliable storage of immunological decisions to many antigens in differentiated clones requires a highly diverse immune repertoire.