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How Specific Should Immunological Memory Be?[1]

José A. M. Borghans,[2] André J. Noest, and Rob J. De Boer

Protection against infection hinges on a close interplay between the innate immune system and the adaptive immune system. Depending on the type and context of a pathogen, the innate system instructs the adaptive immune system to induce an appropriate immune response. Here, we hypothesize that the adaptive immune system stores these instructions by changing from a naive to an appropriate memory phenotype. In a secondary immune reaction, memory lymphocytes adhere to their instructed phenotype. Because cross-reactions with unrelated Ags can be detrimental, such a qualitative form of memory requires a sufficient degree of specificity of the adaptive immune system. For example, lymphocytes instructed to clear a particular pathogen may cause autoimmunity when cross-reacting with ignored self molecules. Alternatively, memory cells may induce an immune response of the wrong mode when cross-reacting with subsequent pathogens. To maximize the likelihood of responding to a wide variety of pathogens, it is also required that the immune system be sufficiently cross-reactive. By means of a probabilistic model, we show that these conflicting requirements are met optimally by a highly specific memory lymphocyte repertoire. This explains why the lymphocyte system that was built on a preserved functional innate immune system has such a high degree of specificity. Our analysis suggests that 1) memory lymphocytes should be more specific than naive lymphocytes and 2) species with small lymphocyte repertoires should be more vulnerable to both infection and autoimmune diseases. ***The Journal of Immunology*****, 1999, 163: 569–575.**

There is increasing evidence that the vertebrate innate immune system is a homologue of the invertebrate nonclonal immune system and that its evolution preceded the development of the adaptive immune system (1–7). Interestingly, the innate immune system was preserved when the adaptive immune system evolved. Innate immunity forms an essential part of the vertebrate immune system by providing signals for the activation of the adaptive immune system (3–5, 8, 9). A hallmark of immune responses is the "second signal" (10) delivered to the adaptive immune system by innate APC that express the membrane proteins B7.1 and B7.2. In the absence of such costimulatory signals from the innate system, T cells fail to become fully activated and instead become anergic (11). The adaptive immune system is thus dependent on evolutionarily conserved signals. We adopt the view that the innate system imposes its evolutionary knowledge on the lymphocyte system instructing it to mount the appropriate response (1, 5, 8, 9).

This dependence raises an evolutionary problem. It is often argued that the adaptive immune system evolved to cope with rapidly coevolving pathogens. The clonal distribution of randomly rearranged lymphocyte receptors renders a high flexibility, enabling the adaptive immune system to adapt more quickly to coevolving pathogens than the innate immune system can. However, if an adaptive immune response depends strictly on the innate immune system, then pathogenic evasion of an innate response implies evasion of an adaptive immune response (see also Refs. 2 and 3). Viruses have indeed been shown to interfere with the innate immune system by producing proteins, e.g., soluble cytokine receptors or proteins that regulate Ag presentation (12–17), that put the immune system on the wrong track. Rapidly coevolving pathogens thus cannot explain why the adaptive immune system has evolved its diversity. Here, we hypothesize that the specificity of the adaptive immune system is used to specifically store the instructions given by the innate immune system. Using a probabilistic model, we demonstrate that this task is best performed if memory lymphocytes are highly specific.

Building a "world view"

We adopt the view that the innate immune system provides signals about the context of antigenic epitopes (1, 3, 5, 8, 9, 18–22). Depending on 1) the organ where the epitope is detected (23), 2) the presence of conserved pathogen-associated molecular patterns (1, 9), and perhaps 3) tissue damage (24), the innate system signals whether the Ag should be attacked and if so, by which immune effector mechanisms. We conjecture that the evolutionary information provided by the innate system is stored in specific lymphocytes by their switch from their naive phenotype to a particular responsive mode or to a nonresponsive mode. Lymphocytes can thus use their specificity to build up a world view, to learn which epitopes are dangerous, which are harmless, and which immune response is most appropriate (25). They should switch to a tolerant mode, e.g., to anergy, whenever the innate system provides a harmless context, so that lymphocytes specific for self peptides, food Ags, and the intestinal flora can be rendered tolerant (26). Conversely, in a harmful context, lymphocytes should be instructed to mount an appropriate immune response and to enter the solid tissue (23, 26, 27). All instructed lymphocytes, i.e., not only conventional memory cells but also, e.g., anergic cells, thus carry information about the appropriate response for the epitopes they recognize. In our view, immunological memory should thus also

Theoretical Biology, Utrecht University, Utrecht, The Netherlands

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[2] Address correspondence and reprint requests José Borghans, Theoretical Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands. E-mail address: jbo@binf.bio.uu.nl

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be regarded as a qualitative memory of the type of immune response to be made. On top of this comes the conventional quantitative form of memory in terms of increased precursor frequencies.

There is good evidence that during a secondary encounter of the same epitope, lymphocytes recall their appropriate response (28–30) and no longer wait for instructions from the innate system. An example, that a qualitative memory may enable lymphocytes to skip over the innate instructions, is the memory for responsiveness vs nonresponsiveness in mice transgenic for a lymphocytic choriomeningitis viral (LCMV)[3] protein (28). In mice expressing the LCMV protein on their pancreatic β-cells, LCMV-specific T cells were neither tolerized nor activated by the LCMV protein. On infection with LCMV, however, the cells became stimulated and caused T-cell-mediated diabetes. Apparently, once the LCMV-specific lymphocytes had seen LCMV in an infectious context, they were instructed to an aggressive response, which was subsequently remembered such that the LCMV protein on the pancreas was regarded as a harmful Ag. Such an LCMV-specific response could not be induced by LCMV infection in LCMV-transgenic mice that had been tolerized with LCMV peptides (30). Thus, nonresponsiveness vs responsiveness is qualitatively remembered by the immune system.

Another example supporting the concept of a qualitative form of immunological memory is the immunity against vaccinia virus (VV). VV is one of many viruses that express proteins interfering with the innate immune system. It prevents its own presentation on MHC molecules of infected cells, blocks the complement cascade and several cytokines, and neutralizes chemokines in the local environment (17). Tackling the immune system at its innate base, the virus typically prevents the induction of an immune response and thus manages to escape, yet vaccination against poxviruses has been extremely successful (17). Apparently, once an adaptive immune response has been triggered, the host is insensitive to the viral immune evasive strategies. Our interpretation is that a qualitative memory identifies the VV epitopes as harmful, thereby circumventing the need for further innate instructions and enabling the host to prevent secondary VV infections.

An immune system with qualitative memory has obvious advantages. The complex decision whether and how to react to specific epitopes need must be made only once. Memory lymphocytes can thus prevent tissue damage by pathogens on reinfection and on pathogen dissemination to other organs. There is, however, a drawback. Instructed lymphocytes, that are fairly independent of further innate instructions run the risk of mounting inappropriate cross-reactive immune responses. For example, self-reactive lymphocytes that have escaped self tolerance induction may become stimulated by a pathogen and subsequently become aggressive towards self (31, 32). Additionally, memory lymphocytes may cross-react in response to subsequent pathogens (33–35) and induce a memory response of the wrong mode, e.g., Th1 instead of Th2. The immune system should therefore be specific enough to avoid such cross-reactivity mistakes. On the other hand, the immune system should be sufficiently cross-reactive to ensure an immune response against any pathogen. Here we develop a model to calculate the optimal degree of specificity of lymphocytes to fulfill both requirements.

Specificity of memory

To calculate the optimal specificity of lymphocytes, we will define the probability P_s of surviving infection by any specific pathogen and calculate for which degree of lymphocyte cross-reactivity this probability is maximal. Let the degree of cross-reactivity of lymphocytes be called p, i.e., each clonotype has a chance p to respond to a randomly selected epitope. In a naive animal, p corresponds to a conventional precursor frequency. Species having evolved highly specific clonotypes have a low p value, whereas those with cross-reactive clonotypes have a high p value. For simplicity, the affinity of clonotypes is not taken into account. A clonotype either responds to an epitope, if its affinity is higher than a certain threshold affinity, or fails to respond.

Avoiding autoimmunity

To avoid autoimmunity, clonotypes responding to self epitopes should be rendered tolerant, i.e., removed from the functional naive repertoire. Consider an animal with R_0 different lymphocyte clones, and let f be the fraction of all self epitopes S that induce self tolerance. The functional repertoire after tolerance induction R consists of all clonotypes that do not respond to any of the fS tolerizing self epitopes. Suppose the animal is infected by a pathogen, which for simplicity is represented by a single antigenic epitope. The chance of mounting an immune response P_i is the chance that at least one clone in the functional repertoire R will be stimulated by the pathogen, i.e.,

$$P_i = 1-(1-p)^R \quad (1)$$

where the expected functional repertoire size

$$R = R_0(1-p)^{fS} \quad (2)$$

(see Refs. 36 and 37 for similar derivations).

Complete self tolerance induction

First consider the simple case that all of the animal's self epitopes induce tolerance; i.e., consider $f = 1$. In Fig. 1*a*, the probability P_i of making an immune response is plotted against the cross-reactivity parameter p. If the immune system is very specific, there is a large chance that none of the clones will recognize the pathogen. On the other hand, if lymphocytes are very cross-reactive, self tolerance induction impairs the immune system by reducing the functional naive repertoire. The maximum value of P_i (Fig. 1*a*, arrow) is attained for $p \approx 1/(fS) = 1/S$. The optimal specificity to mount immune responses to foreign Ags thus reflects the number of self epitopes that induce self tolerance. This result is identical with the conclusion drawn from previous models (36, 38, 39), namely, that immune systems are diverse primarily because animals have large numbers of self Ags.

Ignored self

Healthy animals, however, harbor potentially autoreactive lymphocytes that seem to be ignorant of their specific self ligands (40, 41) and may cause autoimmunity after stimulation (28, 29, 31, 32). After infection by a pathogen, self tolerance is assured only if none of the ignorant clonotypes is stimulated by cross-reactivities with this pathogen. Let α denote the fraction of potentially autoreactive clones in the functional repertoire, i.e., α is the fraction of clonotypes recognizing at least one ignored self epitope. Since only a fraction p of this subset of clones will be stimulated by the pathogen, the fraction of truly autoaggressive clones in the functional repertoire responding to a particular pathogenic epitope is $p\alpha$. The chance P_t of remaining self tolerant is the chance that none of the clonotypes in the functional naive repertoire falls in this autoaggressive category. We are interested in the probability P_s that the animal will survive the pathogenic attack, i.e., in the probability that the animal will make an immune response and will remain tolerant to the ignored self, i.e.,

[3] Abbreviations used in this paper: LCMV, lymphocytic choriomeningitis virus; VV, vaccinia virus.

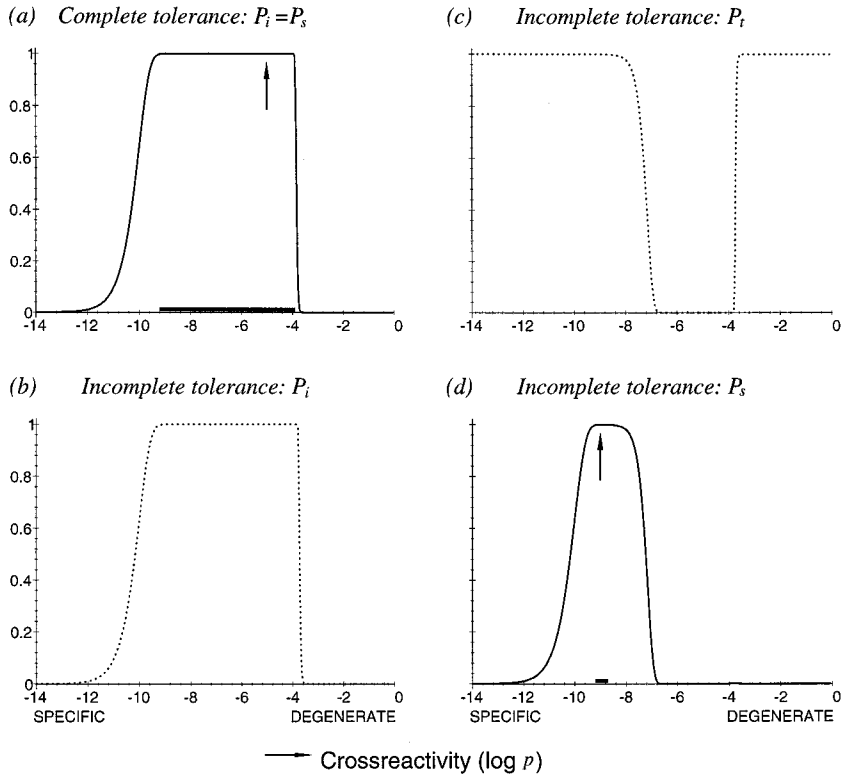

FIGURE 1. Avoiding autoimmunity. The chance of mounting an immune response P_i (*a* and *b*), the chance of remaining self tolerant P_t (*c*), and the chance of surviving a pathogenic attack P_s (*d*), defined by Equations 1–5, plotted against the cross-reactivity p of lymphocytes. Specificity is simply the inverse of cross-reactivity. Arrows show that the optimal cross-reactivity in the case of complete self tolerance induction (*a*) is much larger than the optimal cross-reactivity when some self epitopes fail to induce tolerance (*d*). The black bars in *a* and *d* denote the specificity ranges for which the corresponding survival chances are close to the optimum, i.e., for which $P_s^k > 0.9989$, with $k = 100$ different pathogens infecting a host (see text for further explanation). Parameters are $S = 10^5$, $R_0 = 10^{10}$, and $f = 1$ (*a*) or $f = 0.8$ (*b–d*).

$$P_s = P_t P_{(i|t)} = P_t - (1-p)^R \quad (3)$$

where $P_{(i|t)}$ denotes the conditional probability of making an immune response given that the animal remains tolerant, and

$$P_t = (1-p\alpha)^R \quad (4)$$

and

$$\alpha = 1-(1-p)^{(1-f)S} \quad (5)$$

Note that the intuitive interpretation of Equation 3 is that the survival chance P_s is equal to the overall chance to stay tolerant minus the chance to stay tolerant by making no immune response at all.

The fraction of self epitopes that is ignored is unknown, but taking 20% as an example, the dashed line in Fig. 1*c* depicts the probability P_t that the system will remain tolerant to all ignored self epitopes when stimulated by a pathogen. This probability of tolerance P_t appears to be roughly inversely related to the probability of immunity P_i (Fig. 1*b*, dashed line). This is because lymphocyte specificities that help epitope recognition, including self epitopes, will thwart self tolerance. The survival chance P_s is depicted by the curve in Fig. 1*d*. The arrow in Fig. 1*d* shows that the optimal lymphocyte specificity is much higher now than in the case of complete self tolerance induction. Prevention of autoimmunity to the ignored self apparently requires a high specificity (see also Ref. 37).

If self tolerance induction is incomplete, the most important parameter determining the optimal specificity is the number of lymphocyte clones in the total repertoire R_0: the more lymphocytes are available, the more specific these lymphocytes should be (see Fig. 2*a*). Highly specific lymphocytes reduce the chance of mounting autoimmune responses and thus increase the survival chance of the animal. Surprisingly, the number of self epitopes S, which largely determines the optimal specificity under complete tolerance induction, hardly affects the optimal specificity if self tolerance induction is incomplete. Neither does the fraction of ignored self epitopes $(1 - f)$, in that all curves for which $f < 0.8$ are very similar to the $f = 0.8$ curve.

In practice, selection for the optimal specificity might be hard to accomplish. Once a specificity has been selected for that gives sufficient protection against the typical total number of different pathogens infecting a host (k), the driving force to evolve to an even better specificity vanishes. It might therefore be more informative to consider the range of specificities for which P_s^k is sufficiently large, say larger than 0.9. If an individual is exposed to ~100 different pathogens on average, this range $P_s^k > 0.9$ contains all specificities for which $P, > 0.9989$ (denoted by the black bars in Fig. 1, *a* and *d*, and the "error bars" in Fig. 2). The specificity range for which $P_s^k > 0.9$ in the case of~complete self tolerance induction overlaps with that of incomplete self tolerance and is much wider. If self tolerance induction is complete, the optimal specificity level is thus not defined as sharply as it is when some epitopes fail to induce self tolerance, and more particularly it is not as sharply defined as the $1/S$ value suggested previously (36). Summarizing, repertoires that run the risk of mounting autoimmune responses to ignored self epitopes should be orders of magnitude more specific than repertoires that need only to respond to many pathogens (cf. the recent paper by Mason (42)).

Avoiding responses of an inappropriate mode

A second problem of cross-reactivity is that memory lymphocytes that have acquired a certain mode of immunity during a primary immune reaction may respond to subsequent pathogens (33–35) that require a different mode of response. Besides the widely accepted Th1 vs Th2 modes, many other modes of immunity may exist, varying in the type of lymphocytes, effector mechanisms, and cytokines involved (43, 44). It has been demonstrated experimentally that the cytokine profile of a T cell response is determined by the cytokines present during lymphocyte activation (reviewed in Refs. 22 and 43) and is epigenetically transmitted from mother to daughter lymphocyte (45, 46). Thus, by secreting cyto-

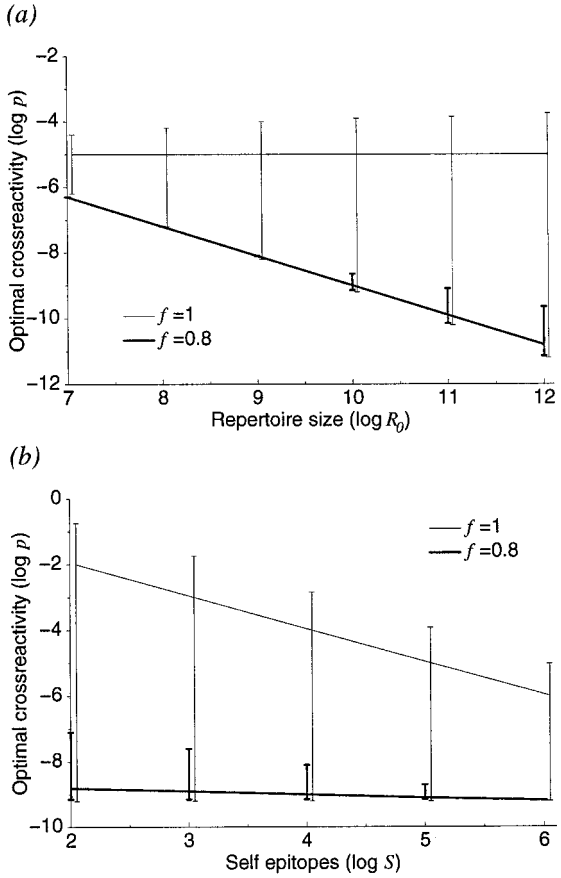

FIGURE 2. What determines the optimal specificity? The optimal cross-reactivity plotted against the size of the total lymphocyte repertoire R_0 (*a*) or against the number of self epitopes S (*b*). If self tolerance induction is complete ($f = 1$), the optimal cross-reactivity decreases as the number of self epitopes increases (*b*). The curves for which $f = 0.8$ are typical for all cases of incomplete self tolerance induction ($f < 1$). The optimal specificity in the case of incomplete tolerance induction is thus hardly dependent on the fraction of self epitopes that induces tolerance (*f*). Results indicate that if self tolerance induction is incomplete, the optimal cross-reactivity depends mainly on the size of the lymphocyte repertoire (*a*) and is hardly dependent on the number of self epitopes (*b*).

kines, cross-reactive memory cells may provide a wrong context for a primary immune response to be induced and can as a consequence impair immunity to subsequent pathogens.

The avoidance of such wrong mode responses is another driving force for the specificity of the adaptive immune system. Consider again an animal with a functional lymphocyte repertoire of R clonotypes (to exclude any effect of self-tolerance induction, Equation 2 is not yet substituted). The chance $P_s(i)$ of surviving infection by the ith pathogen, i.e., the chance of making an immune response without triggering any cross-reactive memory clonotypes, is now dependent on the fraction of memory clones in the repertoire m, and consequently on the number of previous infections ($i - 1$). Only a fraction p of all memory lymphocytes will recognize the ith pathogen, so that the fraction of clonotypes cross-reacting with the present and one previous infection is pm. The chance P_s to survive k different pathogens is the product of all survival chances from the first until the kth pathogen, i.e.,

$$P_s = \prod_{i=1}^{k} P_s(i) \tag{6}$$

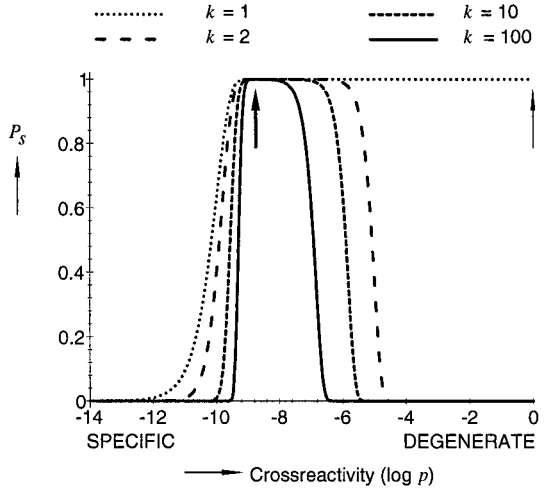

FIGURE 3. Avoiding responses of an inappropriate mode. The chance of surviving a single or multiple different pathogenic attacks, defined by Equations 6–8, plotted against the cross-reactivity (p) of lymphocytes. The curves denote the chances to survive infection by 1, 2, 10 and 100 pathogens, respectively. The optima of the latter three curves nearly coincide. Thick arrow, optimum in the case of infection by a hundred pathogens. Results indicate that if an animal is exposed to multiple different pathogens, and thus runs the risk of mounting cross-reactive immune responses, clonotypes should be much more specific (thick arrow) than they should be if immunity against a single pathogen were the only demand (thin arrow). In the latter case, clonotypes should be maximally cross-reactive ($p = 1$). Parameters are $R = 10^{10}$, $k = 1$ (·····), $k = 2$ (– – – –), $k = 10$ (- - - - -), and $k = 100$ (——).

where, in analogy to Equations 3 and 4,

$$P_s(i) = (1 - pm)^R - (1 - p)^R \tag{7}$$

and

$$m = p(i - 1) \tag{8}$$

Remember that any memory clone of an animal that has survived infection by ($i - 1$) different pathogens can, by our definition, be responsive to a single previous pathogen only.

In Fig. 3, the survival chance P_s is plotted for serial infection by various numbers of pathogens k. Fig. 3 shows that the optimal specificity changes drastically from $p = 1$ (i.e., 100% cross-reactivity), if the animal is exposed to only one pathogen, to a highly specific optimum, in the case of more pathogens. Immunological memory, and the accompanying risk of inducing inappropriate responses by cross-reactivity, thus forces the immune system to be specific. Again, it is the repertoire size R, and not the number of different pathogens k, that largely determines the optimal specificity (Fig. 3).

Of mice and men

Because the optimal specificity to avoid cross-reactive immune responses is largely dependent on the size of the lymphocyte repertoire, our model predicts that the human and the mouse lymphocyte systems may be quite different. To illustrate the predicted differences, the two models of the previous section are combined. The chance $P_s(i)$ to survive infection by the ith pathogen is now the chance that none of the responding clonotypes is either a memory clone or a clone specific for an ignored self epitope, minus the chance that no immune response is made at all, i.e.,

$$P_s(i) = (1 - p(m + \alpha))^R - (1 - p)^R \tag{9}$$

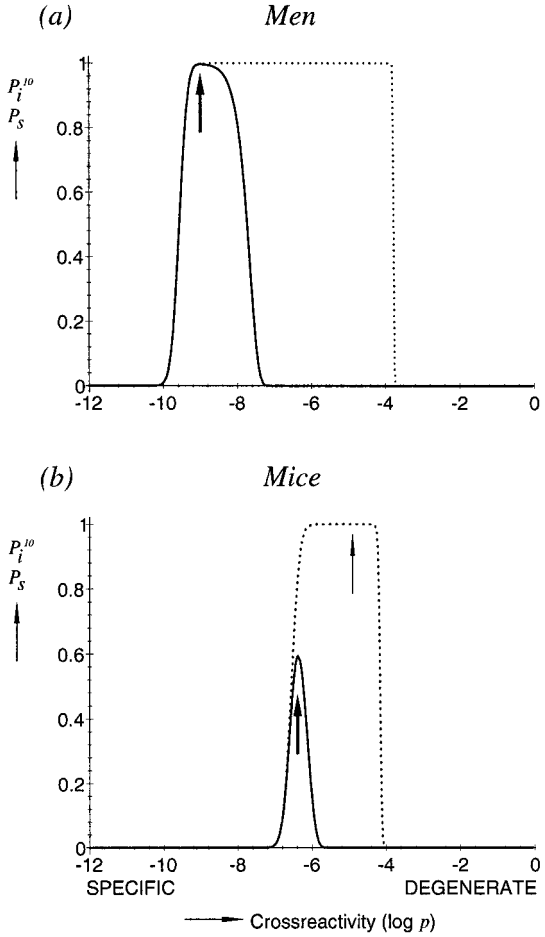

FIGURE 4. Of mice and men. Comparison of the optimal clonotype specificity for humans (*a*) and mice (*b*) if both types of inappropriate cross-reactive immune responses, i.e., autoimmunity towards the ignored self and mode selection failure caused by cross-reactive, old memories, can occur. The chance of surviving after infection by ten different pathogens (P_s, defined by Equations 2, 5–6 and 8–9; ——), and the chance of mounting immune responses against those 10 different pathogens (P_i^{10}, defined by Equations 1 and 2; . . . .), are plotted against the cross-reactivity p of lymphocytes. Thick arrows, optimal cross-reactivity of mouse and human lymphocytes. Human lymphocytes should be orders of magnitude more specific than mouse lymphocytes. Thin arrow, optimal specificity of mice clonotypes if resistance against many of pathogens were the only demand. Parameters are $S = 10^5$, $f = 0.8$, $k = 10$, and $R_0 = 10^{10}$ (for humans (*a*)) and $R_0 = 10^7$ (for mice (*b*)).

where R is given by Equation 2, α by Equation 5, and m by Equation 8. The chance to survive k pathogens is still given by Equation 6. In Fig. 4, the chance of mounting 10 immune responses (P_i^{10}, dashed curves), and the chance of surviving (P_s, solid curves) after serial exposure to 10 different pathogens ($k = 10$) are plotted. The total human lymphocyte repertoire is estimated to consist of 10^{11}–10^{12} T/B lymphocytes, whereas the mouse repertoire consists of $\sim 10^8$ lymphocytes (47, 48). Taking an average clone size of 10 lymphocytes/clone, we estimate the number of clonotypes in humans and mice to be 10^{10} and 10^7, respectively, i.e., a difference of 3 orders of magnitude. Fig. 4 shows that at the optimum of the survival curve, human lymphocytes are orders of magnitude more specific than mouse lymphocytes. This is a new prediction. Previous models (36, 38, 39) have predicted that lymphocytes in mice and humans should be equally specific, i.e., $p \approx 1/S$ (provided that mice and humans have similar numbers of self epitopes).

The need to avoid cross-reactivity with ignored self molecules and the avoidance of inappropriate cross-reactive memory responses are two independent driving forces for the specificity of lymphocytes. For the current parameter setting, the optimal lymphocyte specificity is mainly determined by the need to avoid autoimmune responses. For other parameter settings, e.g., for a lower number of self Ags S and a higher number of pathogens k with which an animal is typically infected, it may be the avoidance of inappropriate memory responses that determines the optimum of the survival curve.

In the optimum, the number of different clones responding to a pathogen is approximately the same for mice and humans. Thanks to the high specificity of human clones, humans should run a lower risk of mounting autoimmune responses than mice. The mouse immune system must make a concession: whereas its protection against infections could be just as good as that of humans (Fig. 4*b*, thin arrow), the need to avoid inappropriate cross-reactive responses forces the mouse immune system to be more specific (Fig. 4*b*, thick arrow). Thus, its resistance against infections is somewhat reduced. Summarizing, mice are predicted to have a smaller survival chance than humans because they suffer more from infections and from autoimmunity.

Discussion

We have argued that the adaptive immune system specifically stores the instructions given by the innate immune system and that the specificity of lymphocytes is used largely for avoidance of inappropriate cross-reactive immune responses (see also Ref. 49). It has been suggested previously that the diversity of the immune system reflects the number of self epitopes that induce tolerance (36, 38, 39). Here, we have shown that if there is any risk of inducing inappropriate cross-reactive immune responses, the immune system needs to be much more specific than had been derived from these previous models (36, 38, 39). In particular, memory lymphocytes should not be triggered by cross-reactive stimulation by food or self Ags (50).

Intuitively, it is hard to see how responsiveness to foreign Ags and avoidance of inappropriate immune responses can be reconciled merely by selecting for a certain degree of lymphocyte specificity (42). In our framework, however, there is an asymmetry between naive and memory clonotypes that allows this conflict to be solved. Inappropriate immune responses come from memory clonotypes only. In our model, naive clones do not run the risk of inducing an inappropriate immune response, because they either remain naive or are properly instructed to switch to the required phenotype. It is this asymmetry that allows for a high optimum of the survival curve at a high degree of lymphocyte specificity.

By considering the risk of cross-reactive autoimmune responses, we have implicitly calculated the optimal specificity of memory lymphocytes. Because naive lymphocytes do not run the risk of inducing inappropriate responses, it might be beneficial to have naive cells that are more cross-reactive than the memory cells. Interestingly, naive B cells indeed appeared to react to a broader range of Ags than did memory B cells (51) (see also Ref. 52 and references therein). Because B cell hypermutation and affinity maturation occur largely after the primary immune response (53, 54), it is tempting to suggest that the function of B cell hypermutation is to induce highly specific memory B cells, on top of inducing a high affinity secondary response (see also Refs. 52 and 55, in which a more general form of specificity maturation was suggested). This idea is supported by the observation that beyond a certain avidity threshold there is no correlation between Ab avidity and protection against infection (56, 57). Recent x-crystallographic

studies uncovered a possible mechanism for specificity maturation: affinity-matured Abs are more specific because they have a more rigid configuration than germline Abs (58). Selection for a high affinity thus seems to imply selection for a high specificity. It has been demonstrated that lymphocytes specific for self Ags are routinely generated during B cell somatic mutations (59). In combination with the strong selective pressure on recognition of the original foreign Ag (60), specificity maturation may reduce the chance of releasing lymphocytes with cross-reactivity for self Ags into the periphery.

Throughout the calculations, the assumption was made that stimulation of a single clone is sufficient for a functional immune response. Obviously, this is a strong simplification. It is very likely that protection against infection and induction of autoimmunity require activation of multiple clones. We have chosen for maximal simplicity, however, because the qualitative results of the model do not depend on such complications. In their protecton theory, Cohn and Langman (61) proposed that lymphocytes act in a concentration-dependent manner; to compensate for their larger lymph volume, large animals would require more lymphocytes of the same Ag specificity than small animals do. We can account for this argument in our model by considering the expected repertoire size per unit volume. All calculations would remain the same, and our claim that immunological memory should be as specific as possible (per unit volume) remains true. It is only the predicted difference between large and small animals that disappears in the protecton version of our model. The protecton model need not be correct, however. Because of lymphocyte recirculation and homing to the sites of infections, large animals may indeed profit from their large lymphocyte repertoire. Even if this is only partly the case, our model correctly predicts a specificity and survival difference between mice and humans.

The high optimal specificities that we calculate seem to be at odds with recent measurements of precursor frequencies performed with MHC/peptide tetramers (62, 63) and with other estimates of lymphocyte cross-reactivity (42). It should be stressed, however, that the optimal cross-reactivities calculated here reflect precursor frequencies in naive animals, which experimentally remain "soft numbers" (63, 64). Naive precursor frequencies may be orders of magnitude lower than the precursor frequencies reported in MHC/peptide tetramer studies after immunization (62, 63). Moreover, the precise quantitative results of our model depend on the specific choice of parameters and simplifications made (see also Ref. 37). For example, we disregarded any safeguards that prevent cross-reactive cells from causing inappropriate immune responses (23, 59). Additionally, there is no affinity in our model, whereas experimental estimates of precursor frequencies depend on the affinity cutoff of the specific assay that is used. Despite these quantitative complications, however, our results show that the need to avoid inappropriate immune responses imposes a strong selection pressure for the specificity of lymphocytes. Importantly, our model shows that the specificity constraints on lymphocytes are even stronger than was concluded previously (36, 38, 39).

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