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Memorizing innate instructions requires a sufficiently specific adaptive immune system

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Abstract

During its primary encounter with a pathogen, the immune system has to decide which type of immune response is most appropriate. Based on signals from the innate immune system and the immunological context in which the pathogen is presented, responding lymphocytes will adopt a particular phenotype, e.g. secrete a particular profile of cytokines. Once stimulated, lymphocytes store the appropriate type of response by differentiating from a naive to a memory phenotype. This allows the appropriate type of immune reaction to be regenerated upon re-stimulation of those memory clones. We developed a computer simulation model in which cross-reacting effector/memory clones contribute to the immunological context of pathogens. If a pathogen is recognized by both naive clones and pre-existing effector/memory clones, the naive lymphocytes adopt the effector mechanism of the memory clone. The adaptive immune system thereby stores immunological decisions and somatically learns to induce the right type of immune response to pathogens sharing epitopes. The influence of effector/memory lymphocytes may be detrimental when they cross-react to new pathogens that require a different kind of immune response. Here, we show that the immune system needs to be sufficiently specific to avoid such mistakes and to profit from the information that is stored in effector/memory lymphocytes. Repertoire diversity is required to reconcile this specificity with reactivity against many pathogens.

Introduction

The efficient elimination of different types of pathogens requires qualitatively different immune responses, varying from cellular to humoral responses, and varying in, for example, immunoglobulin isotype and cytokine expression (1,2). During primary pathogen encounter, the innate immune system plays a key role in determining the nature of the immune response (3). The type of response that is induced is determined by the 'immunological context' of the pathogen, including its localization (4), the presence of conserved bacterial peptides (5–8), and the cytokines and chemokines that are locally expressed (9–11). Based on these signals, lymphocytes differentiate to a memory phenotype and attain a certain effector mechanism. These effector mechanisms are recalled whenever effector/memory lymphocytes are re-stimulated by their specific epitope (2,12). When lymphocytes differentiate, their cytokine production is somatically imprinted by chromatin remodeling and DNA demethylation. Differentiated lymphocytes thereby epigenetically transfer their mode of response to their daughter cells (13–15). The immune system thus learns to associate the antigens it encounters with the appropriate types of response against them.

Memory lymphocytes can greatly influence immune responses against subsequent infections. The ease with which they are triggered (16–24), even at very low antigen concentrations, may explain why immune responses tend to be dominated by memory lymphocytes from previous infections, a phenomenon termed 'original antigenic sin' (25–27). For example, it has been shown that the $CD8^+$ T cell response against influenza is dominated by memory cells that cross-react with previous influenza infections (28,29). Such a bias to stimulation of previous memory clones has even been observed for unrelated viruses (30).

Lymphocytes with a differentiated phenotype can also direct the differentiation of other, naive lymphocytes. $CD4^+$ T cells from transplantation-tolerant mice, for example, have

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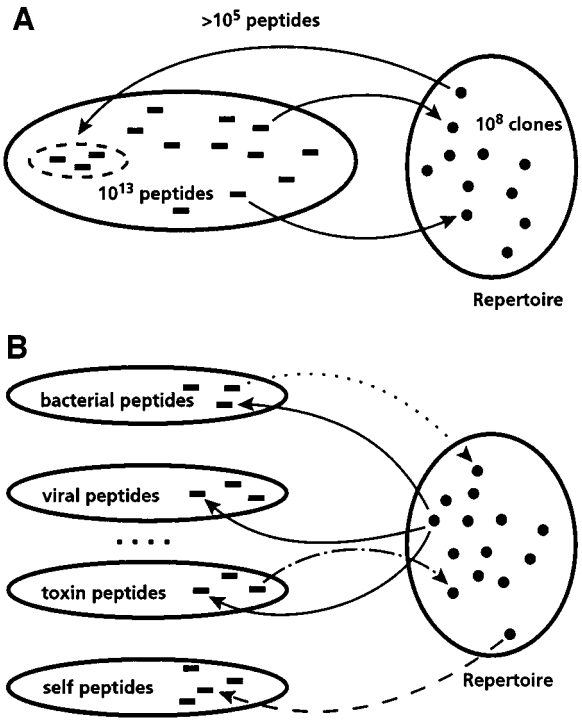

Fig. 1. The lymphocyte repertoire versus the world of antigenic epitopes. (A) Since the theoretical number of epitopes ($\sim 10^{13}$) is much larger than the number of clones in any lymphocyte repertoire (here 10^8), each clonotype needs to recognize multiple epitopes (here $>10^5$) (63). Ideally, however, every epitope triggers only a very small fraction of the lymphocyte repertoire, such that unrelated epitopes are seldomly recognized by the same lymphocyte clone. (B) In our simulations we consider pathogens that come from different structurally related groups. Pathogens from a group all need to be eliminated by one particular type of immune response and have an increased chance of sharing epitopes. If clonotypes recognize self epitopes they become tolerant (with chance f) or remain ignorant (with chance $1 - f$). Clonotypes are not specific for any pathogen group; they may recognize epitopes from different pathogen groups. As long as each epitope triggers only a small fraction of the lymphocyte repertoire, however, the chance that one clone is stimulated by epitopes from multiple pathogen groups remains small.

been shown to render naive cells tolerant upon adoptive transfer. Since the so-induced tolerant cells can in turn tolerize other naive lymphocytes, this process was called 'infectious transplantation tolerance' (31–33). Infectious suppression can also take place between lymphocytes of different specificities, provided that both the suppressive cells and the responder cells are confined to the same antigen-presenting cell (34,35). Analogously, memory lymphocytes of a certain responsive mode may direct the differentiation of new, naive clonotypes, e.g. via cytokine secretion (9,36,37). 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 (38–41). Spreading of an effector/memory phenotype from one (self) epitope to another has also frequently been observed in autoimmune diseases (36,42,43).

Although the innate immune system generally manages to induce the appropriate type of adaptive immune response against pathogens, pre-existing memory lymphocytes can significantly increase the chance to survive infections. Childhood vaccinations and influenza are classical examples. Human adults are typically protected from lethal influenza infection by cross-reactivity to influenza epitopes from previous infections. Novel strains, carrying novel epitopes for which there is no pre-existing memory, can cause incredible mortality, as was the case in the 1918 epidemic (44).

The advantages of memory lymphocytes are several. As they are not confined to the lymphoid tissue and freely enter the solid tissue (4,45,46), they can respond anywhere and any time their specific epitope is encountered. Responses due to memory cells are typically more prompt than primary immune responses (16–24,47,48), because memory cells are more sensitive to low antigen doses, have less stringent requirements for co-stimulation and have already been instructed for the appropriate type of response. Tissue damage by pathogens upon re-infection and upon pathogen dissemination to other organs can thus be prevented. Additionally, if pathogens mutate their antigenic structure, like influenza does over the years and HIV within a single host, memory lymphocytes recognizing epitopes that have remained unaltered may direct the differentiation of new clonotypes recognizing altered epitopes of the pathogen.

Immunological memory is not always beneficial, however. Effector/memory lymphocytes may cause immunopathology when they *coincidentally* cross-react to unrelated pathogens. Being fairly independent of signals from the innate immune system and the local tissue environment, they may induce an inappropriate response and thereby hinder the efficient elimination of a pathogen. Additionally, pathogens may stimulate clonotypes that cross-react with self molecules that fail to induce tolerance. If such clonotypes attain an effector/memory phenotype they may cause autoimmunity when recognizing their specific self epitopes (49–52).

Omitting any protective effects of memory cells, we have previously shown that the immune system should be specific to minimize the number of inappropriate immune responses induced by memory lymphocytes (53,54). Here, we devise a new model that allows us to incorporate (i) a protective role of cross-reacting memory lymphocytes in subsequent related infections and (ii) an instructive role of memory lymphocytes in the differentiation of naive lymphocytes. We find that the immune system profits most from cross-reactions if effector/memory lymphocytes have a low degree of cross-reactivity. The immune system is indeed known to be highly specific: although every clone should recognize many different peptides, only a very small fraction of the lymphocyte repertoire responds to any particular epitope (55) (see Fig. 1a). Our analysis emphasizes that cross-reactivity *per se* should not be considered beneficial. It is the combination of specificity and diversity that enables the immune system to respond reliably to many antigens, and to profit rather than suffer from pre-existing memories.

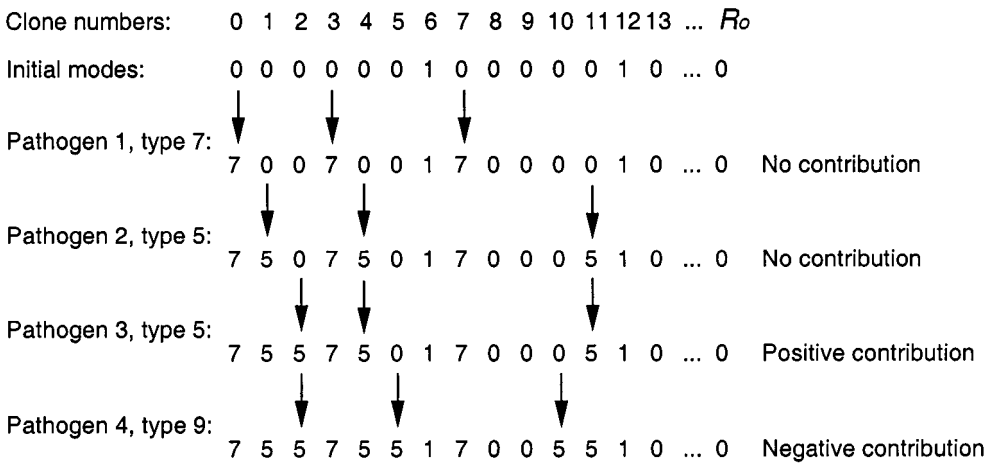

Fig. 2. A simple example of a simulation. After self-tolerance induction most clonotypes are naive (i.e. mode 0), except clonotypes 6 and 12 which have been initialized in the tolerant mode (i.e. mode 1). Each pathogen consists of $e = 3$ different epitopes. The first pathogen has to be rejected by an immune response of mode 7, and triggers clonotypes 0, 3 and 7. Since these three clonotypes are naive in the primary response, the type of immune response is determined by the innate immune system and the immunological context of the pathogen. Clonotypes 0, 3 and 7 differentiate to mode 7, and pathogen 1 is rejected. Similarly, pathogen 2 triggers three naive clonotypes, which subsequently differentiate to mode 5. Pathogen 3 triggers two effector/memory clones that had been triggered by pathogen 2, i.e. clones 4 and 11, and triggers the naive clone 2. Because the type of immune response that is induced is dictated by clones 4 and 11, a response of type 5 results. The presence of the two cross-reacting effector/memory clones was beneficial and clone 2 correctly becomes an effector/memory clone with mode 5. Pathogen 4, requiring an immune response of mode 9, coincidentally triggers an effector/memory clone (2) with mode 5. This cross-reacting effector/memory clone causes the induction of an inappropriate immune response. Naive clonotypes 5 and 10 incorrectly differentiate to mode 5. In total, the presence of effector/memory cells in this simulation was beneficial in 25% of the infections and detrimental in another 25%.

The simulation model

Using a computer model, we simulate the storage of immunological decisions in immune systems consisting of R_0 different lymphocyte clones. Each clonotype is naive, or tolerant, or has a certain effector/memory phenotype. The different states that lymphocytes can attain are represented by integer numbers: naive cells are denoted by 0, tolerant cells by 1, and 2, ..., m identify the different types of effector mechanisms (e.g. T_h1, T_h2, IgA, IgE, etc.). 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 0 or 1. Self-tolerance induction need not be complete. In our simulations, a fraction f of all S self epitopes induces self tolerance. The other self epitopes fail to induce tolerance (56–58) and may cause autoimmunity when the ignorant lymphocytes that recognize them are coincidentally triggered by a pathogen (49–52). After birth the model immune system is challenged with different pathogens, each represented by e different (immunodominant) epitopes. Each naive clonotype has the same chance p to respond to any epitope. Thus p is a cross-reactivity parameter, corresponding to a conventional naive precursor frequency.

Obviously, the information stored in effector/memory lymphocytes can only be useful in response to new pathogens if there are groups of structurally related pathogens. Pathogens could, for instance, come from the same species or family, and therefore share epitopes and require similar types of immune responses (see Fig. 1b). In our simulations, pathogens come from m different groups; each group is defined by a collection of N different epitopes, from which pathogens are assembled by randomly picking e epitopes. The smaller N, the larger the structural correlation between pathogens in a group. Pathogens that come from the same group always need to be eliminated by the same type of immune reaction. Thus, once the immune system has responded to several pathogens from, for example, the group of cytopathic viruses, it may respond more efficiently using its memory clones when a new cytopathic virus sharing an earlier epitope is encountered. In our simulations, all pathogens are encountered only once, i.e. we study a 'worst case' scenario, ignoring the conventional benefits of immunity obtained when the same pathogen re-infects the body.

If pre-existing memory clones recognize a subsequent pathogen, they dictate the type of immune response against that pathogen. The nature of the response that is induced is determined by the effector mechanism of the majority of memory clones recognizing the pathogen. (If there is no unique majority, the type of response that is induced is chosen randomly from the different effector mechanisms that occur most frequently.) In the absence of cross-reacting effector/memory lymphocytes, we assume that the combination of the innate immune response, the context of the antigen and possibly feedback mechanisms (59,60), ultimately leads to the appropriate type of immune response. This need not be unreasonable, because the innate immune system has learned how to respond to different kinds of pathogens and antigenic contexts over evolutionary time. Once the system has decided which type of response to make to a particular pathogen, all naive clonotypes that recognize the pathogen in our simulations differentiate to become effector/memory cells

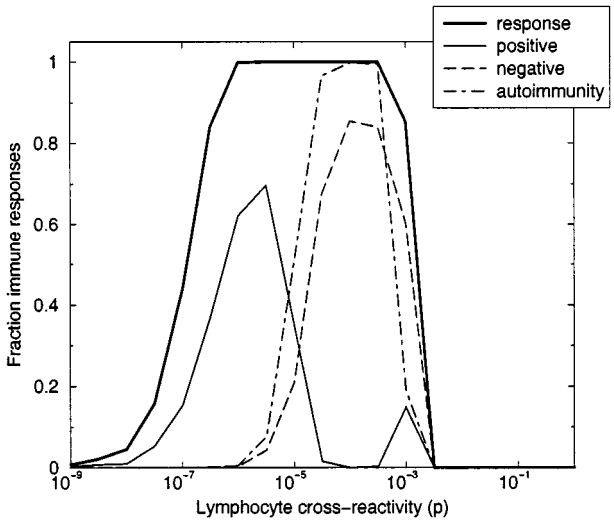

Fig. 3. The performance of a diverse immune system. The fractions of different immune responses have been plotted for different degrees of cross-reactivity (p), after challenge with 10^3 different pathogens. The thick curve denotes the fraction of challenges that induce any immune response at all. The fraction of challenges in which pre-existing effector/memory clones induce the correct type of immune response is denoted by the thin curve. The fraction of challenges in which pre-existing effector/memory clones induce an inappropriate immune response is denoted by the dashed curve. The fraction of challenges leading to autoimmunity, caused by ignorant self-specific clones that are triggered by foreign antigens, is denoted by the dash-dotted curve. This repertoire is most functional at a cross-reactivity of $p \sim 10^{-6}$, because the chance of immunity is then close to 1, and the net contribution of pre-existing effector/memory cells is high. There are $e = 6$ different epitopes per pathogen, pathogens come from $m - 1 = 8$ different groups, each consisting of $N = 10^3$ different epitopes, a fraction $f = 0.8$ of all $S = 10^4$ self epitopes induces tolerance and there are $R_0 = 10^6$ clonotypes.

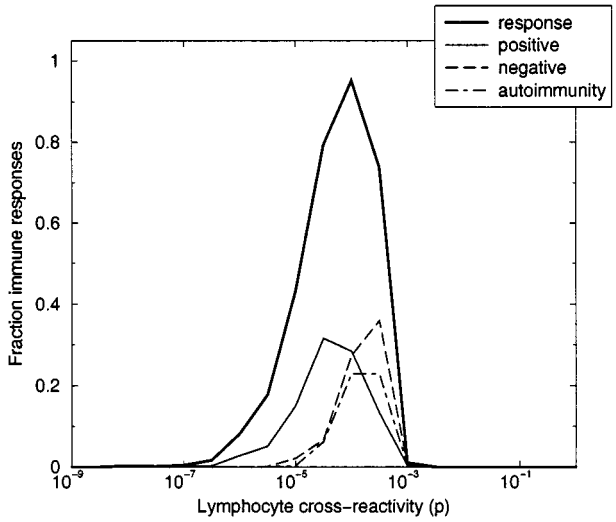

Fig. 4. The performance of a less diverse immune system. The repertoire consists of $R_0 = 10^4$ clonotypes; for other parameters and the interpretation of the different curves, see the legend of Fig. 3. This repertoire has the largest chance of making an immune response at $p \sim 10^{-4}$. With such a high cross-reactivity, however, many mistakes are made.

with the corresponding effector mechanism. Even if an inappropriate type of response is induced, naive lymphocytes acquire the corresponding (incorrect) effector mechanism. Memory clonotypes involved in a response to an antigen do not switch effector type (15,61). Pathogens never kill their hosts, i.e. the simulations are continued even if an inappropriate response is induced.

The performance of the model immune system is followed by counting the fractions of infections in which the presence of effector/memory clones helps or hinders the induction of an appropriate immune response. In the default situation, the pathogen is only recognized by naive clonotypes, and the effector type of the responding clonotypes is determined by the innate immune system and the immunological context of the pathogen. All cases in which pre-existing effector/memory clones establish the correct type of response against a pathogen (without being responsive to any self antigens) contribute to the protection of the individual. The cases in which effector/memory clones establish an incorrect type of response against a pathogen are detrimental. We also count 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 (49–52). An example of a small simulation is given in Fig. 2. The above gives a full description of our model simulations. The C-code of our program is available upon request.

Results

Somatic learning requires specificity

We have studied how the performance of an immune system that is challenged with 10^3 different pathogens depends on the cross-reactivity p of its lymphocytes (Fig. 3). The probability of immunity (i.e. the probability that all 10^3 pathogens are recognized by at least one non-tolerant clone, see the thick line in Fig. 3) is close to 1 in a wide range of intermediate cross-reactivities. A too cross-reactive immune repertoire fails to respond to foreign antigens because the majority of lymphocytes has been rendered non-functional during self-tolerance induction (53,54,62). If lymphocytes are too specific, on the other hand, the immune repertoire frequently fails to recognize a foreign antigen. Thus, the simulations confirm that sufficient cross-reactivity is required to ensure an immune response against any pathogen (63).

Within the range of cross-reactivities yielding a high chance of immunity, effector/memory clones only tend to make correct decisions if they are sufficiently specific (see the thin line in Fig. 3). At a relatively high cross-reactivity, i.e. $p \sim 10^{-3}$, the positive contribution of effector/memory clones is largely coincidental. Even if there is no structural relationship between the pathogens, such a protective effect is observed because of random cross-reactions (not shown). Since there are eight different responsive modes in our simulations, the probability with which effector/memory clones coincidentally induce the right type of immune response is 1/8. If lymphocytes are sufficiently specific, this randomness disappears and the fraction of immune responses in which the presence of pre-

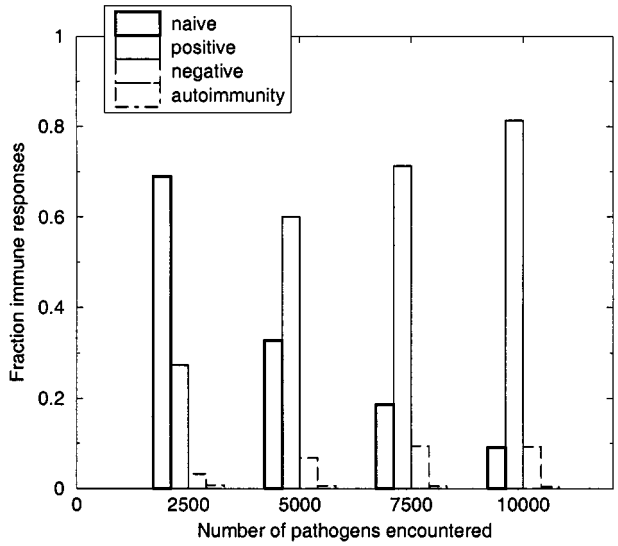

Fig. 5. Immune responses in the elderly. Since the total number of antigens to which an individual has been exposed increases with age, the horizontal axis reflects the age of the individual. The fraction of infections that lead to a naive immune response (thick bars) decreases with age. With age, the fraction of infections in which cross-reacting effector/memory cells are involved increases. Hence the number of efficient immune responses increases (thin bars), but also the numbers of inappropriate cross-reactive immune responses (dashed bars) and autoimmune responses (dash-dotted bars) increase. Parameters are $R_0 = 10^6$, $N = 10^4$, $e = 6$, $S = 10^4$, $f = 0.8$, $m - 1 = 8$ and $p = 10^{-6}$.

existing effector/memory cells is beneficial becomes much higher (see the thin line in Fig. 3).

If lymphocytes are relatively cross-reactive, i.e. $10^{-5} < p < 10^{-3}$, unrelated pathogens (expressing different epitopes and requiring different types of immune responses) will often trigger the same clonotypes. Effector/memory clones that remain after challenge with one pathogen can thus lead to a wrong type of immune response against a subsequent, unrelated pathogen. The dashed line in Fig. 3 illustrates that the adaptive immune system hence makes many mistakes. At a relatively high cross-reactivity, the immune system also makes many autoimmune responses, due to cross-reactions between foreign antigens and ignored self peptides (see the dash-dotted line in Fig. 3). Both types of cross-reactions disappear if the immune repertoire is sufficiently specific.

Summarizing, our simulations illustrate the importance of specificity in the immune system. In immune systems that store the appropriate types of immune response against different antigens in effector/memory lymphocytes, the benefits of immune memory outweigh the accompanying disadvantages only if the immune repertoire is sufficiently specific.

Repertoire diversity reconciles specificity with reactivity

Despite the need for lymphocyte specificity, a certain minimal level of cross-reactivity is required to ensure the recognition of any pathogen infecting the body (63) (see the thick line in Fig. 3). The cross-reactivity that is minimally required reduces as the size of the lymphocyte repertoire increases (54). In highly diverse lymphocyte repertoires, reactivity to many foreign antigens can therefore be reconciled with specificity. Indeed, if the model repertoire consists of $R_0 = 10^6$ clones with a recognition probability $p \sim 10^{-6}$, the chance to make an immune response is close to 1, there is a positive contribution of cross-reacting effector/memory lymphocytes in the majority of the challenges, and inappropriate cross-reactions hardly occur (see Fig. 3). If the immune system is less diverse (see Fig. 4), however, the cross-reactivity that is required to ensure immunity against many pathogens is so high (i.e. $p \sim 10^{-4}$) that the net contribution of cross-reacting effector/memory lymphocytes is no longer positive. A highly diverse and specific immune repertoire is thus superior to a cross-reactive repertoire, because the disadvantages of immunological memory can be avoided, while the protective role of effector/memory lymphocytes in subsequent immune responses is maintained. Evolution is therefore expected to select for highly diverse and specific immune repertoires.

Inappropriate responses to novel pathogens increase with age

The diversity of the memory repertoire increases with age as the adaptive immune system learns on a somatic time scale. One would therefore expect adults to be better protected against infections than naive individuals. The chance that an immune response to a novel pathogen develops from scratch, i.e. in the absence of any effector/memory lymphocytes, indeed decreases with age (see the thick bars in Fig. 5), and the chance that a pre-existing effector/memory clone helps the induction of an effective immune response is considerably higher in adults compared to naive individuals (see the thin bars in Fig. 5). For unrelated novel pathogens, however, the chance that a previous memory clone cross-reacts, and thereby induces an inappropriate immune response, also increases with age (see the dashed bars in Fig. 5). Although the naive repertoire of adults should still be sufficiently diverse to recognize any new antigen (64), adult immune responses may therefore be hampered by inappropriate effector mechanisms induced by previous memory clones. This could explain why childhood diseases such as measles and chickenpox typically cause more severe problems in adults than in children (65).

Discussion

We have studied how the immune system can use the information that is stored in effector/memory lymphocytes during subsequent immune responses. We propose that, by being part of the immunological context, cross-reacting effector/memory lymphocytes influence the choice of immune response against subsequent pathogens and the differentiation of naive lymphocytes. Since effector/memory lymphocytes recall their mode of response whenever they recognize their specific epitope, they allow the immune system to respond effectively to pathogens that share structural relationship with previously encountered pathogens. Structural similarity may occur within pathogen families, but is even more prominent for pathogens that alter their genetic make-up, such as influenza and HIV. Paradoxically, we find that the immune system profits most from cross-reactions if effector/memory lymphocytes have a low degree of cross-reactivity. Too much

cross-reactivity is detrimental because memory lymphocytes will cross-react with unrelated antigens that require a different type of response [see also (54)]. The chance of a pre-existing effector/memory clone helping the induction of a subsequent immune response also increases at a high degree of lymphocyte specificity. Evolution is thus expected to select for a high lymphocyte specificity, thereby ensuring the reliability of cross-reactions.

The 'education' of naive lymphocytes by cross-reacting effector/memory lymphocytes in our model is reminiscent of the education of ignorant self-specific lymphocytes by tolerant clones that was proposed by Modigliani *et al.* (66). There is growing evidence that anergic cells are not inert and can actively regulate responses induced by other lymphocytes (67,68). The anergic cells can be $CD25^+$ (68) and could hence belong to the well-studied class of $CD25^+$ regulatory T cells (69,70). This regulatory mechanism may play an important role in maintenance of tolerance to self antigens. As we do not study self-tolerance induction here and only challenge our model immune system with pathogens, we have not included any instructive role of tolerant clones. Despite their regulatory function in self tolerance, there is no evidence that tolerant clones would prevent primary immune responses to pathogens in an inflammatory context.

The high demands on lymphocyte specificity that result from our study seem to conflict with the current trend to stress the cross-reactivity of lymphocytes (63,71–73). Both points of view are in fact complementary, however. First of all, we think the tendency to stress lymphocyte cross-reactivity is partially due to the fact that considerable cross-reactivity has been observed during positive selection (74,75) and peripheral maintenance of lymphocytes (76–78). In contrast, our analysis deals with lymphocyte specificity during immune responses. Since the molecular mechanisms involved in lymphocyte selection, maintenance and stimulation are very different, so may be the demands on specificity. Secondly, our analysis confirms that for a given repertoire diversity a minimal degree of cross-reactivity is required to allow immunity against many pathogens (63) (see Figs 1, 3 and 4). Our main result is that cross-reactivity *per se* should not be considered beneficial, however. A higher cross-reactivity than is minimally required hampers the use of effector/memory cells in subsequent responses and increases the chance of inducing inappropriate immune responses by coincidental cross-reactions. Our model demonstrates that in order to give good protection against infections, having a diverse and specific lymphocyte repertoire is a much better alternative than having cross-reactive lymphocytes. It is thus not surprising that the adaptive immune system evolved to be highly diverse and specific.

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References

1 Janeway, C. A. and Travers, P. 1997. *Immunobiology. The Immune System in Health and Disease.* Garland, New York.
2 Swain, S. L., Bradley, L. M., Croft, M., Tonkonogy, S., Atkins, G., Weinberg, A. D., Duncan, D. D., Hedrick, S. M., Dutton, R. W. and Huston, G. 1991. Helper T-cell subsets: phenotype, function and the role of lymphokines in regulating their development. *Immunol. Rev.* 123:115.
3 Fearon, D. T. and Locksley, R. M. 1996. The instructive role of innate immunity in the acquired immune response. *Science* 272:50.
4 Zinkernagel, R. M., Ehl, S., Aichele, P., Oehen, S., Kundig, T. and Hengartner, H. 1997. Antigen localisation regulates immune responses in a dose- and time-dependent fashion: a geographical view of immune reactivity. *Immunol. Rev.* 156:199.
5 Janeway, C. A. 1989. Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harbor Symp. Quant. Biol.* 54:1.
6 Medzhitov, R. and Janeway, C. A. 1996. On the semantics of immune recognition. *Res. Immunol.* 147:208.
7 Medzhitov, R. and Janeway, C. A. 1997. Innate immunity: impact on the adaptive immune response. *Curr. Opin. Immunol.* 9:4.
8 Muraille, E. and Leo, O. 1998. Revisiting the T_h1/T_h2 paradigm. *Scand. J. Immunol.* 47:1.
9 Paul, W. E. and Seder, R. A. 1994. Lymphocyte responses and cytokines. *Cell* 76:241.
10 O'Garra, A. 1998. Cytokines induce the development of functionally heterogeneous T helper cell subsets. *Immunity* 8:275.
11 Abbas, A. K., Murphy, K. M. and Sher, A. 1996. Functional diversity of helper T lymphocytes. *Nature* 383:787.
12 Richter, A., Lohning, M. and Radbruch, A. 1999. Instruction for cytokine expression in T helper lymphocytes in relation to proliferation and cell cycle progression. *J. Exp. Med.* 190:1439.
13 Bix, M. and Locksley, R. M. 1998. Independent and epigenetic regulation of the interleukin-4 alleles in $CD4^+$ T cells. *Science* 281:1352.
14 Bird, J. J., Brown, D. R., Mullen, A. C., Moskowitz, N. H., Mahowald, M. A., Sider, J. R., Gajewski, T. F., Wang, C. R. and Reiner, S. L. 1998. Helper T cell differentiation is controlled by the cell cycle. *Immunity* 9:229.
15 Reiner, S. L. and Seder, R. A. 1999. Dealing from the evolutionary pawnshop: how lymphocytes make decisions. *Immunity* 11:1.
16 Veiga-Fernandes, H., Walter, U., Bourgeois, C., McLean, A. and Rocha, B. 2000. Response of naive and memory $CD8^+$ T cells to antigen stimulation *in vivo*. *Nat. Immunol.* 1:47.
17 Rogers, P. R., Dubey, C. and Swain, S. L. 2000. Qualitative changes accompany memory T cell generation: faster, more effective responses at lower doses of antigen. *J. Immunol.* 164:2338.
18 Cho, B. K., Wang, C., Sugawa, S., Eisen, H. N. and Chen, J. 1999. Functional differences between memory and naive CD8 T cells. *Proc. Natl Acad. Sci. USA* 96:2976.
19 Bruno, L., Kirberg, J. and Von Boehmer, H. 1995. On the cellular basis of immunological T cell memory. *Immunity* 2:37.
20 Sprent, J. and Tough, D. F. 1994. Lymphocyte life-span and memory. *Science* 265:1395.
21 Ahmed, R. and Gray, D. 1996. Immunological memory and protective immunity: understanding their relation. *Science* 272:54.
22 Ahmed, R. 1996. Tickling memory T cells. *Science* 272:1904.
23 Tough, D. F., Borrow, P. and Sprent, J. 1996. Induction of bystander T cell proliferation by viruses and type I interferon *in vivo*. *Science* 272:1947.
24 Pihlgren, M., Dubois, P. M., Tomkowiak, M., Sjogren, T. and Marvel, J. 1996. Resting memory $CD8^+$ T cells are hyperreactive to antigenic challenge *in vitro*. *J. Exp. Med.* 184:2141.
25 Fazekas de St Groth, S. and Webster, R. G. 1966. Disquisitions on original antigenic sin: I. Evidence in man. *J. Exp. Med.* 124:331.
26 McMichael, A. J. 1998. The original sin of killer T cells. *Nature* 394:421.
27 Klenerman, P. and Zinkernagel, R. M. 1998. Original antigenic sin impairs cytotoxic T lymphocyte responses to viruses bearing variant epitopes. *Nature* 394:482.
28 Haanen, J. B., Wolkers, M. C., Kruisbeek, A. M. and Schumacher, T. N. 1999. Selective expansion of cross-reactive $CD8^+$ memory T cells by viral variants. *J. Exp. Med.* 190:1319.
29 Smith, D. J., Forrest, S., Ackley, D. H. and Perelson, A. S. 1999.

Variable efficacy of repeated annual influenza vaccination. *Proc. Natl Acad. Sci. USA* 96:14001.
30 Selin, L. K., Nahill, S. R. and Welsh, R. M. 1994. Cross-reactivities in memory cytotoxic T lymphocyte recognition of heterologous viruses. *J. Exp. Med.* 179:1933.
31 Waldmann, H., Qin, S. and Cobbold, S. 1992. Monoclonal antibodies as agents to reinduce tolerance in autoimmunity. *J. Autoimmun.* 5:93.
32 Qin, S., Cobbold, S. P., Pope, H., Elliott, J., Kioussis, D., Davies, J. and Waldmann, H. 1993. 'Infectious' transplantation tolerance. *Science* 259:974.
33 Honey, K., Cobbold, S. P. and Waldmann, H. 1999. CD40 ligand blockade induces $CD4^+$ T cell tolerance and linked suppression. *J. Immunol.* 163:4805.
34 Taams, L. S., Van Rensen, A. J. M. L., Poelen, M. C. P., Van Els, C. A. C. M., Besseling, A. C., Wagenaar, J. P. A., Van Eden, W. and Wauben, M. H. M. 1998. Anergic T cells actively suppress T cell responses via the antigen-presenting cell. *Eur. J. Immunol.* 28:2902.
35 Taams, L. S., Boot, E. P., Van Eden, W. and Wauben, M. H. 2000. 'Anergic' T cells modulate the T-cell activating capacity of antigen-presenting cells. *J. Autoimmun.* 14:335.
36 Lehmann, P. V., Targoni, O. S. and Forsthuber, T. G. 1998. Shifting T-cell activation thresholds in autoimmunity and determinant spreading. *Immunol. Rev.* 164:53.
37 Harris, D. P., Haynes, L., Sayles, P. C., Duso, D. K., Eaton, S. M., Lepak, N. M., Johnson, L. L., Swain, S. L. and Lund, F. E. 2000. Reciprocal regulation of polarized cytokine production by effector B and T cells. *Nat. Immunol.* 1:475.
38 Stumbles, P. A., Thomas, J. A., Pimm, C. L., Lee, P. T., Venaille, T. J., Proksch, S. and Holt, P. G. 1998. Resting respiratory tract dendritic cells preferentially stimulate T helper cell type 2 (T_h2) responses and require obligatory cytokine signals for induction of T_h1 immunity. *J. Exp. Med.* 188:2019.
39 Ridge, J. P., Di Rosa, F. and Matzinger, P. 1998. A conditioned dendritic cell can be a temporal bridge between a $CD4^+$ T-helper and a T-killer cell. *Nature* 393:474.
40 Bottomly, K. 1999. T cells and dendritic cells get intimate. *Science* 283:1124.
41 Rissoan, M. C., Soumelis, V., Kadowaki, N., Grouard, G., Briere, F., De Waal Malefyt, R. and Liu, Y. J. 1999. Reciprocal control of T helper cell and dendritic cell differentiation. *Science* 283:1183.
42 Lehmann, P. V., Forsthuber, T., Miller, A. and Sercarz, E. E. 1992. Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen. *Nature* 358:155.
43 Tuohy, V. K., Yu, M., Yin, L., Kawczak, J. A. and Kinkel, R. P. 1999. Spontaneous regression of primary autoreactivity during chronic progression of experimental autoimmune encephalomyelitis and multiple sclerosis. *J. Exp. Med.* 189:1033.
44 Gibbs, M. J., Armstrong, J. S. and Gibbs, A. J. 2001. Recombination in the hemagglutinin gene of the 1918 'Spanish flu'. *Science* 293:1842.
45 Mondino, A., Khoruts, A. and Jenkins, M. K. 1996. The anatomy of T-cell activation and tolerance. *Proc. Natl Acad. Sci. USA* 93:2245.
46 Butcher, E. C. and Picker, L. J. 1996. Lymphocyte homing and homeostasis. *Science* 272:60.
47 Flynn, K. and Mullbacher, A. 1996. Memory alloreactive cytotoxic T cells do not require costimulation for activation *in vitro*. *Immunol. Cell Biol.* 74:413.
48 Croft, M., Bradley, L. M. and Swain, S. L. 1994. Naive versus memory CD4 T cell response to antigen. Memory cells are less dependent on accessory cell costimulation and can respond to many antigen-presenting cell types including resting B cells. *J. Immunol.* 152:2675.
49 Ohashi, P. S., Oehen, S., Buerki, K., Pircher, H., Ohashi, C. T., Odermatt, B., Malissen, B., Zinkernagel, R. M. and Hengartner, H. 1991. Ablation of 'tolerance' and induction of diabetes by virus infection in viral antigen transgenic mice. *Cell* 65:305.
50 Oldstone, M. B., Nerenberg, M., Southern, P., Price, J. and Lewicki, H. 1991. Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. *Cell* 65:319.
51 Zhao, Z. S., Granucci, F., Yeh, L., Schaffer, P. A. and Cantor, H. 1998. Molecular mimicry by herpes simplex virus-type 1: autoimmune disease after viral infection. *Science* 279:1344.
52 Bachmaier, K., Neu, N., De la Maza, L. M., Pal, S., Hessel, A. and Penninger, J. M. 1999. Chlamydia infections and heart disease linked through antigenic mimicry. *Science* 283:1335.
53 Borghans, J. A. M. and De Boer, R. J. 1998. Crossreactivity of the T-cell receptor. *Immunol. Today* 19:428.
54 Borghans, J. A. M., Noest, A. J. and De Boer, R. J. 1999. How specific should immunological memory be? *J. Immunol.* 163:569.
55 Murali-Krishna, K., Altman, J. D., Suresh, M., Sourdive, D. J., Zajac, A. J., Miller, J. D., Slansky, J. and Ahmed, R. 1998. Counting antigen-specific CD8 T cells: a re-evaluation of bystander activation during viral infection. *Immunity* 8:177.
56 Schluesener, H. J. and Wekerle, H. 1985. Autoaggressive T lymphocyte lines recognizing the encephalitogenic region of myelin basic protein: *in vitro* selection from unprimed rat T lymphocyte populations. *J. Immunol.* 135:3128.
57 Cibotti, R., Kanellopoulos, J. M., Cabaniols, J. P., Halle-Panenko, O., Kosmatopoulos, K., Sercarz, E. and Kourilsky, P. 1992. Tolerance to a self-protein involves its immuno-dominant but does not involve its subdominant determinants. *Proc. Natl Acad. Sci. USA* 89:416.
58 Peterson, D. A., DiPaolo, R. J., Kanagawa, O. and Unanue, E. R. 1999. Quantitative analysis of the T cell repertoire that escapes negative selection. *Immunity* 11:453.
59 Segel, L. A. and Bar-Or, R. L. 1999. On the role of feedback in promoting conflicting goals of the adaptive immune system. *J. Immunol.* 163:1342.
60 Bergmann, C., van Hemmen, J. L. and Segel, L. A. 2001. T_h1 or T_h2: how an appropriate T helper response can be made. *Bull. Math. Biol.* 63:405.
61 Murphy, E., Shibuya, K., Hosken, N., Openshaw, P., Maino, V., Davis, K., Murphy, K. and O'Garra, A. 1996. Reversibility of T helper 1 and 2 populations is lost after long-term stimulation. *J. Exp. Med.* 183:901.
62 De Boer, R. J. and Perelson, A. S. 1993. How diverse should the immune system be? *Proc. R. Soc. Lond. B Biol. Sci.* 252:171.
63 Mason, D. 1998. A very high level of crossreactivity is an essential feature of the T-cell receptor. *Immunol. Today* 19:395.
64 Arstila, T. P., Casrouge, A., Baron, V., Even, J., Kanellopoulos, J. and Kourilsky, P. 1999. A direct estimate of the human $\alpha\beta$ T cell receptor diversity. *Science* 286:958.
65 Boodley, C. A. and Jaquis, J. L. 1989. Measles, mumps, rubella and chickenpox in the adult population. *Nurse Pract.* 14:12.
66 Modigliani, Y., Bandeira, A. and Coutinho, A. 1996. A model for developmentally acquired thymus-dependent tolerance to central and peripheral antigens. *Immunol. Rev.* 149:155.
67 Jooss, K., Gjata, B., Danos, O., Von Boehmer, H. and Sarukhan, A. 2001. Regulatory function of *in vivo* anergized $CD4^+$ T cells. *Proc. Natl Acad. Sci. USA* 98:8738.
68 Taams, L. S. and Wauben, M. H. 2000. Anergic T cells as active regulators of the immune response. *Hum. Immunol.* 61:633.
69 Taams, L. S., Smith, J., Rustin, M. H., Salmon, M., Poulter, L. W. and Akbar, A. N. 2001. Human anergic/suppressive $CD4^+CD25^+$ T cells: a highly differentiated and apoptosis-prone population. *Eur. J. Immunol.* 31:1122.
70 Stephens, L. A., Mottet, C., Mason, D. and Powrie, F. 2001. Human $CD4^+CD25^+$ thymocytes and peripheral T cells have immune suppressive activity *in vitro*. *Eur. J. Immunol.* 31:1247.
71 Hiemstra, H. S., Van Veelen, P. A., Willemen, S. J., Benckhuijsen, W. E., Geluk, A., De Vries, R. R., Roep, B. O. and Drijfhout, J. W. 1999. Quantitative determination of TCR cross-reactivity using peptide libraries and protein databases. *Eur. J. Immunol.* 29:2385.
72 Wilson, D. B., Pinilla, C., Wilson, D. H., Schroder, K., Boggiano, C., Judkowski, V., Kaye, J., Hemmer, B., Martin, R. and Houghten, R. A. 1999. Immunogenicity. I. Use of peptide libraries to identify epitopes that activate clonotypic $CD4^+$ T cells and induce T cell responses to native peptide ligands. *J. Immunol.* 163:6424.
73 Hemmer, B., Kondo, T., Gran, B., Pinilla, C., Cortese, I., Pascal, J., Tzou, A., McFarland, H. F., Houghten, R. and Martin, R. 2000. Minimal peptide length requirements for $CD4^+$ T cell clones—

implications for molecular mimicry and T cell survival. *Int. Immunol.* 12:375.
74 Ignatowicz, L., Kappler, J. and Marrack, P. 1996. The repertoire of T cells shaped by a single MHC/peptide ligand. *Cell* 84:521.
75 Lee, D. S., Ahn, C., Ernst, B., Sprent, J. and Surh, C. D. 1999. Thymic selection by a single MHC/peptide ligand: autoreactive T cells are low-affinity cells. *Immunity* 10:83.
76 Beverley, P. C. 1990. Is T-cell memory maintained by crossreactive stimulation? *Immunol. Today* 11:203.
77 Matzinger, P. 1994. Immunology. Memories are made of this? *Nature* 369:605.
78 Tanchot, C., Lemonnier, F. A., Perarnau, B., Freitas, A. A. and Rocha, B. 1997. Differential requirements for survival and proliferation of CD8 naive or memory T cells. *Science* 276:2057.