The paradox of alloreactivity and self MHC restriction: Quantitative analysis and statistics

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Although 1–24% of T cells are alloreactive, i.e., respond to MHC molecules encoded by a foreign haplotype, it is generally believed that T cells cannot recognize foreign peptides binding foreign MHC molecules. We show using a quantitative model that, if T cell selection and activation are affinity-driven, then an alloreactivity of 1–24% is incompatible with the textbook notion that self MHC restriction is absolute. If an average of 1% of clones are alloreactive, then according to our model, at most 20-fold more clones should, on average, be activated by antigens presented on self MHC than by antigens presented on foreign MHC. This ratio is at best 5 if alloreactivity is 5%. These results describe average properties of the murine immune system, but not the outcome of individual experiments. Using supercomputer technology, we simulated 100,000 MHC restriction experiments. Although the average restriction ratio was 7.1, restriction was absolute in 10% of the simulated experiments, greater than 100, although not absolute, in 29%, and below 6 in 24%. This extreme variability agrees with experimental estimates. Our analysis suggests that alloreactivity and average self MHC restriction both cannot be high, but that a low average restriction level is compatible with high levels in a significant number of experiments.

On the one hand, a wealth of experimental data suggest that T cells are self restricted: they recognize pathogens presented by self MHC molecules, but they ignore them if presented by foreign MHC (1–5). On the other hand, 1–24% of T cells are alloreactive: they respond to MHC molecules from a foreign MHC haplotype (6, 7). Thus, a significant number of T cell receptors (TCRs) bind foreign MHC molecules, i.e., they are not self MHC restricted. We analyze this paradox quantitatively by estimating the level of restriction compatible with observed alloreactivity frequencies.

Most experimental evidence for self restriction comes from measuring the reactivity of the repertoire against a few particular antigens, and from the investigation of a limited number of self/foreign haplotypes combinations (reviewed in ref. 5). Both low and nearly absolute self restriction levels have been measured in these systems (5, 8–11). The small number of antigens, self/foreign MHC haplotypes pairs, and TCR specificities studied, and the discrepancies among the data produced suggest that these experiments should be repeated on a large number of systems to obtain reliable averages of the restriction level in the murine immune system. Unfortunately, doing so is technically difficult. To circumvent this problem, and because the issue is quantitative, we adopt a computational approach.

We previously proposed a mathematical model of T cell selection (12–14) relating quantitatively the parameter driving T cell repertoire generation to properties of the mature repertoire, including alloreactivity and self MHC restriction levels. Its main underlying assumption is that affinities between TCRs and MHC–peptide complexes drive T cell maturation and selection in the thymus, and activation in the periphery. Here, we use this model to analyze how alloreactivity and self restriction are quantitatively related, and we simulate the outcome of self restriction experiments for 100,000 different systems to get an accurate statistical picture of the degree of self MHC restriction.

Quantitative and Statistical Analyses

Overview of the Model. Our analysis relies on a mathematical model of affinity-driven selection of the T cell repertoire. The model involves a minimal representation of MHC molecules, peptides, and TCRs that support the notions of affinity, ligand diversity, and ligand size. Positive and negative selection affinity thresholds are inferred from experimental estimates of the stringencies of the overall selection process and of negative selection.

Protein Shapes and Binding Affinities. The binding of two proteins can be described with a relatively small number of parameters, such as their geometric shape, charges, and hydrophobicity. All of these parameters combine to form the protein's "generalized shape" as defined in ref. 15. As in previous simulation studies (reviewed in ref. 16), we model the generalized shape of a protein as a string of digits. The strength of binding of two proteins is then defined as the degree of complementarity between the digits representing their generalized shapes (Fig. 1). Only the interface between TCRs and MHC–peptide complexes (framed region in upper diagram in Fig. 1) is represented in the model. We define the affinity, K, between two digit-string proteins, as the sum of their individual digit interactions.

Our model describes residues at the interface between TCRs and MHC–peptide complexes, not the full structure of these molecules. MHC and peptide are random strings, l_m and l_p digits long, respectively. Affinities are always computed on aligned strings, so the central digits of TCRs always contact a peptide, and digits at the extremities, a MHC. This modeling choice follows from studies according to which TCRs bind MHC–peptide complexes with a common orientation (17). The model is independent of the linear arrangement of digits. In particular, it is equivalent to a model with digits arranged in a two-dimensional array mimicking the solvent accessible surface of proteins (as proposed in refs. 18 and 19).

TCRs, MHCs, and Peptides. In our model, it is assumed that l_m and l_p are the same for all MHCs and all peptides. This assumption is reasonable because we restrict our analysis to class I MHC, which present peptides of fixed length. The number of MHC alleles expressed in an individual is n_m. A given MHC allele can present a panel of n_p distinct self peptides. We also assume that because of allele-specific binding motifs, MHC molecules of different haplotypes present different subsets of self peptides (20), which is mathematically equivalent to presenting the same peptides in different conformations (13). A TCR is selected by a self environment composed of $n_m \times n_p$ MHC–peptide complexes.

Our goal is to measure self restriction and alloreactivity, which depend, by definition, on MHC polymorphism, and on the specificity of TCRs. Therefore, MHC polymorphism-independent effects do not need to be part of the model. Hence we make the following legitimate simplifications. The effect of

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Abbreviation: TCR, T cell receptor.

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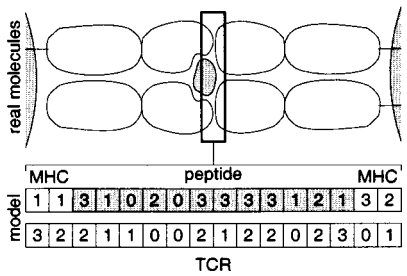

Fig. 1. Digit-string representation of MHC–peptide and TCR interaction. MHC–peptide complexes are constructed by inserting a peptide string of length l_p digits in an MHC string of length l_m digits. TCRs are sequences of l_m+l_p digits chosen randomly. The interaction strength between two facing digits in the two aligned strings is a measure of their complementarity. The method for computing this strength is given in ref. 12. The affinity, K, is the sum of interaction strengths of contacting digits in the two aligned strings.

T cell coreceptors is omitted. Conserved MHC residues are not represented, i.e., the n_m MHC segments are interpreted as the polymorphic parts of MHC molecules accessible to TCRs. To our knowledge, there is no evidence for a germline encoded bias of TCRs toward recognition of some particular peptides, and bias toward recognition of MHC most likely results from interaction with MHC conserved residues (21–23), which are not taken into account here. Thus, assuming that preselection TCRs are random is justified in the context of the model (see ref. 12 for a more extensive discussion of this issue). The peptide does not influence the MHC in the model. Thus, we exclude from our scope of investigation the altered self hypothesis (5, 24) according to which the TCR senses peptide-induced structural features of the MHC rather than the peptide itself.

Positive and Negative Selection. Selection is implemented by introducing affinity thresholds for positive and negative selection, K_{pos} and K_{neg} ($K_{pos} < K_{neg}$). Clones binding at least one self MHC–peptide complex with affinity K larger than K_{pos} survive positive selection. Negative selection deletes clones binding one or more self MHC–peptide complexes with affinity K larger than K_{neg}. The values of K_{pos} and K_{neg} are derived from experimental data by considering the fractions of clones surviving the different stages of selection (13).

A clone will become part of the peripheral repertoire if its affinity K falls between K_{pos} and K_{neg}. The fraction f of clones allowed to reach the periphery is

$$f = f_{pos} \cdot f_{neg}, \qquad [1]$$

where f_{pos} is the fraction of clones surviving positive selection (a similar parameter is used in ref. 25), and f_{neg} is the fraction of positively selected clones that survive negative selection (25–28). The values of f and f_{neg} can be inferred from recent experimental data (see below).

T Cell Activation and Self Tolerance. A criterion for activation of selected T cells has to be defined in our model to study alloreactivity and antigen response frequency. A clone is considered activated by a set of MHC–peptide complexes if the affinity of binding between its TCR and at least one MHC–peptide complex in this set is greater than K_{neg}. The repertoire in the model is self tolerant by construction, because no clone having an affinity larger than K_{neg} to a self MHC–peptide complex can survive negative selection.

Quantitative Hypotheses Investigated. The quantitative parameters driving affinity-based selection are not constrained by definitive experimental evidence (14), and they may vary between different individuals. Instead of focusing our analysis on a particular parameter set, we investigate the model over parameter ranges encompassing a very wide array of biologically reasonable quantitative hypotheses. These ranges (Table 1) were inferred from experimental data (14). It has been shown that they include all of the parameter sets leading to a T cell repertoire compatible with experimentally observed levels of self MHC restriction, alloreactivity, and foreign antigen response frequency (14).

Average Properties vs. Individual Experiments. To measure self MHC restriction, one has to select a particular pair of self/foreign haplotypes to be compared, to choose particular antigens against which immune responses are measured, and to perform an experiment using the T cells of a particular animal that has a unique repertoire. This set of choices will be referred to as a "restriction experiment." Similarly, an "alloreactivity experiment" requires the choice of a particular self/foreign MHC haplotype pair, and a particular repertoire from which the T cells to be challenged are taken.

We developed elsewhere (13) mathematical formulas that give the average responses for all restriction and alloreactivity experiments possible in the context of the model. These formula are used here to investigate the trade-off between alloreactivity and self restriction. However, they do not give information about the variability among experiments.

Computer simulations were run to get such information. They consist in the explicit construction of a T cell repertoire followed by measurements of self restriction (see Fig. 3). These computationally intensive simulations (13) were run on a parallel supercomputer developed at Los Alamos National Laboratory (see ref. 29, or http://cnls.lanl.gov/avalon/).

Estimating Self MHC Restriction and Alloreactivity. The extent of self MHC restriction has typically been estimated by comparing the effector activity against foreign peptides presented by self MHC with the activity against foreign peptides presented by foreign MHC (3, 5, 30–34). In our model, rather than examining response intensity, we measure the number of responding clones. We call R the fraction of clones in the selected repertoire that respond to a foreign peptide presented on self MHC in a particular experiment, and $\langle R \rangle$ its average over many experiments. Thus, R is a response frequency. Similarly, R_a is the fraction of clones in the selected repertoire that respond to a foreign peptide presented on foreign MHC, and $\langle R_a \rangle$ denotes its averaged counterpart. Thus, R_a is the response frequency in the context of foreign MHC. We define the restriction ratio for a particular experiment as R/R_a. The average restriction ratio can be defined in two ways: either as the ratio of average response frequencies $\langle R \rangle / \langle R_a \rangle$, or as the average of the ratio of response frequencies $\langle R/R_a \rangle$. Both alternatives are investigated.

Situations in which the immunizing antigen cannot be presented by either self or foreign MHC because of Ir genes defects are not taken into account in our measurement of self MHC restriction. An experimental system in which the antigen can be presented by self, but not by foreign, MHC would appear absolutely restricted to self MHC. Because this result depends only on the antigenic peptide and foreign MHC, and not on the T cell repertoire, this effect is different from the restriction acquired during positive selection, as revealed by bone marrow chimera experiments (35) and transgenic systems (36). Our analysis focuses on the acquired self restriction revealed by these experiments.

Alloreactivity is the fraction of T cells responding to products of a foreign MHC haplotype. Again we distinguish between alloreactivity, a, for a particular experiment, and average alloreactivity, $\langle a \rangle$. As suggested by experimental data (reviewed in ref. 20), we assume that molecules encoded by different MHC

Table 1. Parameters driving affinity-based T cell repertoire selection

Name	Definition	Trade-off analysis	Simulations
n_m	No. of MHC class I loci	3*	3
n_p	No. of self peptides presented by a given MHC allele	$\{10^2, 10^3, \ldots, 10^8\}$	10^3
n_t	No. of T cell clones submitted to selection	†	6.6×10^8
l_m	No. of MHC polymorphic residues accessible to TCR‡	$\{2, 3, \ldots, 8\}$	4
l_p	No. of peptide residues accessible to TCR‡	$\{4, 5, \ldots, 11\}$	6
f	Fraction of selected clones	{0.19, 0.37, 0.75, 1.5, 3}	1.5%§
f_{neg}	Fraction of positively selected clones that survive negative selection	{20, 37, 95}	37%
d_{max}	Discreteness of affinity distribution	255¶	255

Two kinds of analysis are presented. First, the trade-off between self MHC restriction and alloreactivity is investigated by calculating (using probability theory, see ref. 13) these two quantities for all the 5,880 combinations of parameters that can be generated from the sets of values given in the column "Trade-off analysis" (i.e., $1 \times 7 \times 7 \times 8 \times 5 \times 3 \times 1 = 5{,}880$ parameter sets). A biological justification for these parameter ranges is given in ref. 14. Second, computer simulations were run using the parameter values given in the "Simulations" column.

*The number of MHC class I loci in the mouse is known with absolute certitude, thus there is no need to investigate different hypotheses regarding this parameter.

†The mathematical equations upon which the trade-off analysis relies gives averages over all TCRs that can be produced in the context of the model. The underlying model makes unnecessary any assumption about TCR repertoire size (13). However, such an assumption is necessary in the simulations because they require the explicit construction of TCR repertoires (13).

‡Alternative interpretations of l_m and l_p are possible (see ref. 13).

§The size of mature repertoires in the simulations is $n_t \times f = 0.015 \times 6.6 \times 10^8 \approx 10^7$ clones.

¶This parameter controls how well computer generated affinity distributions approximate their continuous real world counterparts (53). It has no effect on the model if chosen large enough (13). Thus, there is no need to investigate more than one value for this parameter.

alleles present different subsets of self peptides, or present the same self peptides in different conformations [these two hypotheses are equivalent in the context of the model (13)]. This assumption leads to lower bound estimates of alloreactivity (12).

Results

Average Self MHC Restriction and Average Alloreactivity Are Not Independent; They Are Inversely Correlated. Self MHC restriction of the repertoire implies that TCRs recognize peptides presented on self MHC but not those on foreign MHC. This notion seems at odds with the observed high alloreactivity frequency, which implies a massive recognition of foreign MHC products (11). A quantitative assessment of this issue follows.

Given that at least $x\%$ of the repertoire is alloreactive, what is the average restriction ratio, $\langle R\rangle/\langle R_a\rangle$, at best? We focus on $\langle R\rangle/\langle R_a\rangle$ rather than $\langle R/R_a\rangle$ because of statistical uncertainty with regard to the latter quantity (see below). The answer, according to our model, is shown in Fig. 2*a* for alloreactivities x ranging from 0.01% to 5%. Calculations of $\langle a\rangle$, $\langle R\rangle$, and $\langle R_a\rangle$ were performed by using the formulas developed in ref. 13, for all of the parameter values given Table 1. For each value of x investigated, we plotted the maximum restriction among the parameter sets giving both $\langle a\rangle \geq x$, and $\langle R\rangle$ in the experimental range 10^{-6}–10^{-4} (37, 38). As shown Fig. 2*a*, alloreactivity correlates inversely with self restriction. Thus, there is a trade-off between these two quantities. If $\langle a\rangle \geq 1\%$, then the average restriction ratio $\langle R\rangle/\langle R_a\rangle$ is at best 19.8. Alternatively, if the alloreactivity $\langle a\rangle \geq 5\%$, then the average restriction ratio is at best 5.3.

One may also ask: given that $\langle R\rangle$ is in the experimental range 10^{-6}–10^{-4} and that the restriction ratio $\langle R\rangle/\langle R_a\rangle$ is greater than y, what is the maximum compatible alloreactivity level $\langle a\rangle$? As shown Fig. 2*b*, the answer to this question confirms the existence of a trade-off between self restriction and alloreactivity. Average alloreactivity is below 12% for any restriction level. Assuming, as suggested by Stockinger *et al.* (37), that at least 6 times more clones recognize foreign peptides presented on self MHC than peptides presented on foreign MHC gives $\langle a\rangle \leq 3.1\%$. A restriction level ≥ 100 implies $\langle a\rangle \leq 0.04\%$, an unrealistically low value.

Self MHC Restriction Is Not Absolute on Average, Although Absolute Restriction Is Observed in Particular Experiments. None of the 5,880 quantitative hypotheses we analyzed (Table 1) is compatible with absolute average restriction in the sense that $\langle R_a\rangle = 0$ and $\langle R\rangle > 0$. Although restriction ratios as large as $\langle R\rangle/\langle R_a\rangle = 328$ can be derived from some parameters sets, restricting the analysis to sets giving $\langle R\rangle$ in the experimentally measured response frequency range 10^{-6}–10^{-4} implies that if $\langle a\rangle \geq 1\%$, then $\langle R\rangle/\langle R_a\rangle \leq 19.8$ (Fig. 2). These average results however do not preclude the possibility of absolute restriction in particular experiments. Simulations performed using the standard parameter set (Table 1) indicate that as many as 9.8% of all experimental systems could be absolutely restricted to self MHC, i.e., give $R_a = 0$.

Self MHC Restriction Is Subject to Extreme Statistical Variations Among Experiments. The prediction that restriction is absolute in a significant number of experiments, but relatively low on average, raises concerns about the statistical properties of this phenomena, and on the ability of the few experimental systems studied so far to depict it accurately. A distribution of this quantity (Fig. 3) was obtained by simulating the generation of 10 T cell repertoires and measuring the restriction level of each of them for 10,000 distinct combinations of foreign haplotypes and immunizing peptides, i.e., 100,000 distinct computer experiments were performed. The restriction ratio, R/R_a, is above 100 for 29% of experiments but below 6 for 24% of them. These data are in agreement with the discrepancies observed among experimental measurements of self restriction (see *Discussion*). Because some experiments give absolute restriction (i.e., R/R_a is infinite), both the mean and the standard deviation of R/R_a are infinite. Ignoring these experiments when averaging still gives $\langle R/R_a\rangle = 100 \pm 208$, confirming the extreme spread of the distribution. Using the same parameters and the alternative definition of the average ratio leads to $\langle R\rangle/\langle R_a\rangle = 7.1 \pm 0.5$. Comparing the standard deviations resulting from the two definitions leads

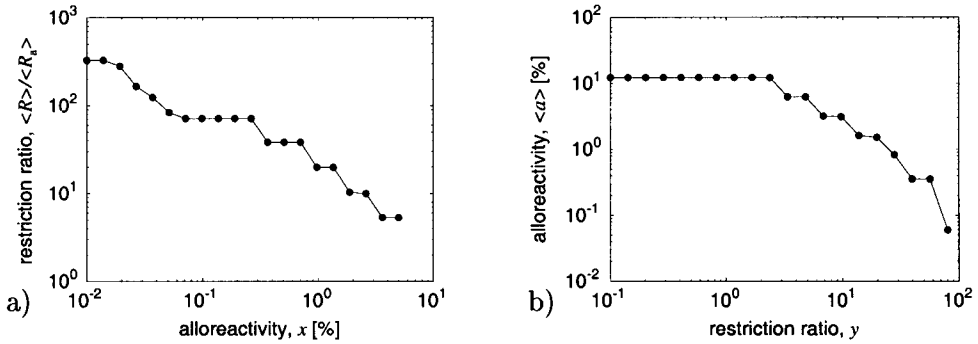

Fig. 2. Trade-off between self MHC restriction and alloreactivity. See text for explanations.

to the conclusion that $\langle R\rangle/\langle R_a\rangle$ is statistically acceptable, while $\langle R/R_a\rangle$ is misleading because of the large standard deviation in this quantity.

The extreme spread of the distribution suggests that the small number of experimental systems investigated so far (reviewed in ref. 5) is unlikely to give an accurate picture of the phenomena of self MHC restriction as assessed by the quantity R/R_a.

Discussion

Research on self MHC restriction has been particularly intricate, and rich in controversies (5), partly because the notions of alloreactivity and self MHC restriction are tightly entangled.

The requirement for haplotype compatibility in T–B cell cooperation was first exhibited in 1973 (1). This result was soon extended to macrophage-induced T cell proliferation (2), cytolytic response (3, 39), and delayed-type hypersensitivity (4, 31). Early restriction experiments however, were received with skepticism by many who suspected that "allogenic effects" were interfering with the measured responses (5). The cytotoxic T lymphocyte assays of Zinkernagel and Doherty (3), and Shearer *et al.* (39) provided strong support of the concept of self restriction, because they were known to be free of allogenic effects (5).

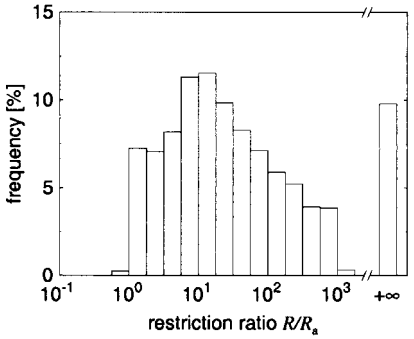

Fig. 3. Distribution of the restriction ratio, R/R_a, over 100,000 experiments. Simulations were run as follows (see ref. 13 for more details). Ten sets of n_p peptide sequences and n_m MHC alleles were generated and used to select 10 repertoires of 10^7 TCRs each. A set of 100 peptides was then generated for each TCR repertoire, and R was measured for all 10×100 combinations of TCR repertoires and peptides. The peptides were presented in association with the MHC used to drive selection. Next, for each of the 10 TCR repertoires, a set of 100 MHC haplotypes (each made of n_m MHC molecules) was generated. Each of these haplotypes was associated with 100 peptides, and R_a was measured for each of the 100×100 haplotype/peptide combinations. These peptides were assumed to be extracted from the same "pathogens" as the 100 peptides (per repertoire) used to measure R. Finally, R/R_a was calculated for the $10 \times 100 \times 100 = 100,000$ combination of TCR repertoires, "pathogens," and foreign haplotypes.

Much subsequent research was aimed at understanding the origin of self MHC restriction. The theory of adaptive differentiation (40), according to which restriction is acquired during T cell development, received experimental confirmation in cytotoxic T lymphocyte (35) and helper (32) systems. The role of the thymus in this process was established shortly afterward (41). These developments continued to be complicated by allogenic effects. Many authors observed restriction only in T cell populations depleted of alloreactive cells (5). For example, Bennick and Doherty (5, 42) filtered H-2^k-specific T cells through (H-2^k $\times$ H-2^b)F_1 mice to tolerize them to H-2^b. The resulting T cells could generate cytotoxic responses against H-2^k virus infected cells but not against infected cells expressing H-2^b. However, depletion of alloreactive cells did not guarantee self restriction in all systems. Schwartz (5) compiled the results of 18 studies relying on this technique and involving either cytotoxic T lymphocytes or helper cells, and found that significant allogenic responses occurred in 11 of them. Similar discrepancies were observed in bone marrow chimera experiments in which $(A \times B)F_1$ T cells developed in irradiated parents of A or B haplotypes and were then challenged with stimulators bearing A or B. For example, in the pioneering experiment of Bevan (35), the levels of ^{51}Cr released by MHC-compatible and MHC-incompatible stimulators differed by factors ranging from 3 to 30, i.e., both weak and strong restriction levels were observed. Blanden and Andrew (43) examined 53 such chimeras and reported absolute restriction in 10 of them. Allorecognition was significant in the other 43. Although MHC-compatible targets were consistently lysed more efficiently, in some experiments, the quantities of ^{51}Cr released by MHC-compatible and MHC-incompatible targets differed only by a factor 2. These discrepancies suggest that self restriction is a very variable phenomena and raise questions about its average level.

We addressed these issues quantitatively by estimating the average level of self restriction that is compatible with known alloreactivity frequencies, and by estimating the spread of individual restriction measurements around this average level.

The mathematical model underlying our analysis assumes that the affinity between TCRs and MHC–peptide complexes drives T cell selection and activation. The surface density of MHC–peptide complexes on antigen presenting cells is thus ignored in our model. Avidity effects may be significant *in vivo* and *in vitro*, because positive selection could occur for T cells having TCRs for which low binding affinity—i.e., lack of restriction—is compensated for by high ligand density (44). Our calculations ignore such situations, and may therefore overestimate self MHC restriction. We cannot determine the amount of overestimation, because the frequency of T cells selected by low affinity/high density ligands is unknown.

The compatibility between self MHC restriction and alloreactivity was investigated by calculating the highest self MHC

restriction ratio possible for a given alloreactivity frequency. We found that there is a trade-off between restriction and alloreactivity: both cannot be high. If, on average, 1% of clones respond to MHC molecules from a foreign haplotype, then, on average, the restriction ratio is at most 20, i.e., at most 20-fold more clones are activated by foreign antigens presented on self MHC than by foreign peptide presented on foreign MHC. The restriction ratio is at most 5 if the alloreactivity of the repertoire is 5%. These results contrast with the view presented in many textbooks that self MHC restriction is absolute (45–52).

These estimates concern the average properties of the murine immune system, not the outcome of individual experiments. Computer simulations of 100,000 MHC restriction experiments were run to determine how representative are the few experimental systems in which self restriction has been examined. We found that the outcome of individual measurements is extremely variable. In our simulations, the restriction ratio is greater than 100 in 29% of the experiments but lower that 6 in 24% of them. Absolute restriction is observed in 10% of the cases. Thus, the model provides a statistical explanation for the discrepancies among the experiments mentioned earlier, and it suggests that low average self restriction is compatible with very high self restriction in a significant number of experiments.

The solution to the paradox suggested by these results is that there is no need to reconcile high self restriction and high alloreactivity, because self restriction is not high on average.

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