A Solution to the Rheumatoid Factor Paradox

Pathologic Rheumatoid Factors Can Be Tolerized by Competition with Natural Rheumatoid Factors[1]

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Rheumatoid factors (RF) associated with arthritic joint erosion are only seen transiently, if at all, in nondiseased individuals. Therefore, a tolerance mechanism must exist that prevents pathologic RF B cells from expressing Abs. Surprisingly, it has been shown that pathologic RF B cells are not tolerized by any previously established tolerance mechanism such as deletion, receptor editing, anergy, or prevention of memory establishment. How are pathologic RF cells tolerized? By simulating the RF response with a cellular automaton model immune system, we demonstrate that pathologic RFs can be tolerized by the novel mechanism of "competitive tolerance" with natural, nonpathologic RFs. We then demonstrate that competitive tolerance can be broken when a sequestered pool of expanding B cells are inappropriately subjected to chronic stimulation (as appears to occur in MRL/*lpr* mice and in patients with rheumatoid arthritis). ***The Journal of Immunology,*** **1997, 159: 1728–1738.**

There are two general classes of rheumatoid factor (RF)[4]: natural and pathologic. While both natural and pathologic RFs bind IgG Fc, the two types of RF differ by several key features (summarized in Table I), the most important being that pathologic RFs are associated with rheumatoid arthritis (RA) while natural RFs are not.

The natural RF response borders on the bizarre. Primary immunization induces little or no natural RF response, while secondary immunization induces a natural RF response that is an order of magnitude higher than the Ag-specific Ab response (1, 2). This natural RF response is T cell dependent. The T cell help does not come from autoreactive T cells but rather from normal, foreign Ag-specific T cells. Natural RF cells can acquire intermolecular help from these normal Th cells during the secondary response to foreign Ag, since IgG will bind the foreign Ag to which the T cells are reacting (see Fig. 1) (3, 4).

Surprisingly, the RF cells participating in this secondary response usually lack the hallmarks of secondary or memory-type B cells: they are polyclonal, polyspecific, mostly IgM, and undermutated. Even in response to tertiary and further stimulation, the natural RF response fails to mature; that is, natural RF cells seem to be unable to progress beyond a response that qualitatively resembles a typical primary response (albeit a massive one) (5–10).

A large fraction of B cells (up to 10%) produce natural RF in normal mice (1, 11, 12). This observation has been explained on an ontologic but not etiologic basis. The molecular details of how this high frequency of natural RF cells arises are now clear: analyses of light chain V_L gene usage in RF reveal that 5 to 17% of V_L genes contain the framework region FR3 critical for IgG binding (10, 13, 14). No correlation between natural RF activity and V_H sequence was observed. This was interpreted to mean that natural RF activity resides primarily in V_L. Why the preimmune repertoire should contain so many natural RF cells, however, is puzzling. The high precursor fraction suggests that natural RFs perform important functions, some of which have been established (15, 16).

High titers of pathologic RFs are found in the sera and the synovial fluid of patients with RA and are strongly correlated with the severity of the disease (17). A wide survey of mammals and avians also demonstrates a correlation between RF levels and joint erosion (18). Inbred mouse strains that appear to have a general breakdown of peripheral tolerance (e.g., MRL/*lpr*) also produce high titers of pathologic RF (19, 20), and when these pathologic RFs were introduced into normal mice, these otherwise normal mice developed disease (21). These observations are consistent with pathologic RF being an active participant in joint degradation as well as vasculitis.

In contrast to natural RF cells, pathologic RF cells undergo clonal expansion and memory establishment as evidenced by their class switch, Fc isotype restriction, oligoclonality, and high R:S ratios in CDRs (see Table I) (10, 19–24). As many as one-third of all B cells isolated from an MRL/*lpr* mouse spleen have been shown to be clonally-related pathologic RF producers (25). Intuitively, one might suppose that pathologic RFs are tolerized through one of the previously established tolerance mechanisms:

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[4] Abbreviations used in this paper: RF, rheumatoid factor; RA, rheumatoid arthritis; V_L, variable light chain; V_H, variable heavy chain; R:S, replacement to silent; CDR, complementarity-determining region; PALS, periarteriolar lymphatic sheath; EGC, ectopic germinal center; IMMSIM, computer simulation of immune system cells and their interactions.

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deletion (26–29), receptor editing (30, 31), anergy (32–34), or blockage of the memory response (35–38). Surprisingly, however, it has been shown that none of these tolerance mechanisms actively function in normal mice to tolerize pathologic RF. When pathologic RFs that have developed in an MRL/*lpr* mouse are presented to a normal mouse immune system as a transgene-encoded Ab, these otherwise normal mice fail to delete the pathologic RF cells, do not anergize the pathologic RF B cells, and appear not to block the pathologic RF cells from entering the memory response (39, 40).

The above observations raise the following interrelated questions: The natural RF response—a T-dependent response—appears highly stimulated but does not mature; what prevents the maturation of natural RF in normal mice? Why do normal mice not tolerize transgenic high affinity RF cells? And what mechanisms can account for pathologic RF Ag-driven RF maturation in disease conditions?

Invaluable to the understanding of RFs' paradoxic behavior is an immune system that is controllable down to the level of a single B cell. Since no current animal model allows this level of fine control, we have explored these questions with the IMMSIM cellular automaton model immune system. This model has been successfully used to simulate primary immune responses, secondary immune responses, affinity maturation, and hypermutation (41–43). Here we will show as a first step in resolving the RF paradox that the IMMSIM model can properly simulate the natural RF response. We then use the model to derive a novel solution to the RF paradox and provide insight into RF-associated disease.

Materials and Methods

A qualitative description of IMMSIM

IMMSIM is a computer simulation of the cells of the immune system and their interactions. IMMSIM simulates B cells, T cells, and APCs. Simulated B and T cells (but not APCs) each possess a receptor represented by a binary string of 12 bits with a fixed directional reading frame. B cells and APCs (but not T cells) possess 12-bit MHCs. B cells and APCs interact directly with Ag, but T cells interact only with antigenic peptides presented on IMMSIM's MHC receptors.

Ags are defined by a number of 12-bit segments representing epitopes and peptides. Abs are also defined by bit strings. IMMSIM Abs have a paratope that is identical to the receptor of the B cell that secreted it. Abs also contain a constant Fc region: a binary string that is identical for all Abs. The Fc is thus available to be recognized by any B cell or Ab whose receptor is complementary to it; such Abs are analogous to RFs. RF cells thus have the ability to clear IgG/Ag immune complexes. APCs also have constitutively-expressed FcRs to allow APCs to clear immune complexes.

Three types of specific (sequence dependent) interactions are allowed between the simulated cells: 1) B cell receptors may bind Ag, 2) Abs may bind Ag, and 3) TCRs may bind peptide-complexed MHC molecules (on B cells or APCs). In all three cases interactions are dependent on the complementarity between the two elements. All specific interactions in the model are defined by interaction strengths. If an epitope matches the receptor of a B cell, for instance, the Ag is allowed to bind that B cell with some probability. In this context, "binding" is synonymous with effective binding. Ineffective binding (e.g., B cells that bind but do not present Ag) is not explicitly modeled (but is implicitly modeled by binding efficiency). If APCs bind Ag, they present that Ag on their MHCs; if T cells bind the MHC/peptide of the APCs, those T cells are activated; if activated T cells bind the MHC/peptides of B cells, the bound B cells are activated. Once interactions occur, they are irreversible.

The probability of binding is determined by the complementarity between the bit strings representing the Ag and receptor: zeros in the Ag match ones in the receptor and vice versa. The binding probability is a monotonic function of the number of matching bits. In our model, zero- and one-bit mismatch Abs bind at 100% efficiency; two-bit mismatches bind at 51.2% efficiency, and three-bit mismatches bind at 12.8% efficiency. We arrived at the relative binding of two- and three-bit mismatch Abs through a parameter search: significantly lower binding efficiencies of two- and three-bit mismatch preclude appropriate natural RF secondary responses (data not shown). While higher two- and three-bit mismatch binding efficiencies can also simulate appropriate natural RF responses (data not shown), if we choose these higher values, the repertoire has little room to mature in binding efficiency.

Once the Ag is "endocytosed" by an interacting IMMSIM B cell, the Ag is broken down into peptides. These peptides are then presented on the MHC molecule of the cell as an MHC/peptide complex. The leftmost six bits of the MHC molecule form the histotope. The processed peptide binds with its agretope half to the rightmost six bits of the MHC molecule (which represent the desetope or binding groove). The automaton's T cells will then see the leftmost six bits and the nonagretope portion of the peptide as one continuous bit string.

A final critical feature of the IMMSIM automaton is a thymus. In the IMMSIM thymus, as in a real one, T cells develop tolerance through negative selection. Initially, all 2^{12} T cell types are created. Any T cell containing a TCR that can bind bare MHC molecules or MHC molecules that are presenting self peptides will be deleted. In our model, we defined the Fc as a self peptide so that RF cells are denied RF-specific T cell help (i.e., T cells that can bind MHC/Fc are deleted). Positive selection is also accounted for in the IMMSIM thymus. T cells that can bind MHC molecules at low affinity are selected and leave the thymus. In our case, roughly 7.8% of T cells will bind foreign Ag/MHC. Only after the T cells are negatively and positively selected in the thymus are they allowed to enter the IMMSIM grid.

The IMMSIM grid

The IMMSIM grid, a 32×32 triangular grid of compartments, is an abstract space in which all IMMSIM interactions occur. The IMMSIM grid may be associated with the germinal center or other lymphoid tissue but only if the limitations of the IMMSIM grid are appreciated. The IMMSIM grid is two dimensional and has no boundary conditions (i.e., the grid is topologically a torus in which the top compartments are adjacent to the bottom one and the left compartments abut the right ones). In the modeling presented in this paper, compartments of the IMMSIM grid are probabilistically uniform for initial cell and Ag entry, so there is no delineated T cell zone, for example. In the future, we plan to enhance the IMMSIM grid to include spatial considerations so that we may model more precisely the germinal center and other lymphoid tissues.

Time steps—what happens in IMMSIM

A full account of all IMMSIM logic has been published previously (41). The following is a brief account of IMMSIM logic tailored to our experiments.

During each time step, all interaction are limited to cells, Abs, and Ags in the same compartment of the IMMSIM grid. We stress that all interactions within each compartment are probabilistic for each individual cell, Ag, and Ab (with interaction probabilities based on interaction strengths). This is in contrast to clonal models in which all cells of a particular type move in lockstep. So, for example, many B cells that present foreign Ag will not be activated by T cell help during any one time step or even during an entire simulated immune response.

As the first action of every time step, activated T cells are allowed the probablistic opportunity to stimulate APCs and B cells (appropriately, if the APCs and B cells are not presenting foreign Ag, the probability of activation is zero). Then overage cells die ($t_{1/2}$ = 10 time steps for virgin and plasma cells; $t_{1/2}$ = 50 for memory B and activated T cells). Next, all generalized Ag/Ab-type interactions occur in random order: Ab, B cells, and APCs bind Ag (foreign or self depending on specificity). Then old Abs are deleted ($t_{1/2}$ = 10 time steps). Next, stimulated B and T cells multiply (four divisions for excited B cells, three divisions for excited T cells). New cells are born. Multiplied B cells differentiate into plasma and memory cells, and plasma cells release Abs. Finally, all entities are allowed to diffuse to adjacent compartments.

Repertoire size

The total number of specificities for all the variable elements in the system is 2^{12} or 4096. We allowed Ag to bind to Abs with zero-, one-, two-, or three-bit mismatches. A maximum of 299 of the 4096 Ab types are thus competent to interact with any antigenic type. We found that by setting the initial B cell population at 4000 we could keep computation time at a reasonably low 2 to 5 h per run and operate within 128 MB of memory.

Antigen

Two Ag injections were scheduled for each simulation: one at time step zero and a second at time step 200. It takes about 100 time steps for an immune response to develop, so with this injection schedule primary and secondary responses to the Ag can be qualitatively distinguished. The bit string for the Ag epitope we injected was 111111110101 (thus, our Ag is perfectly bound by Ab 000000001010). A self Ag was provided for most

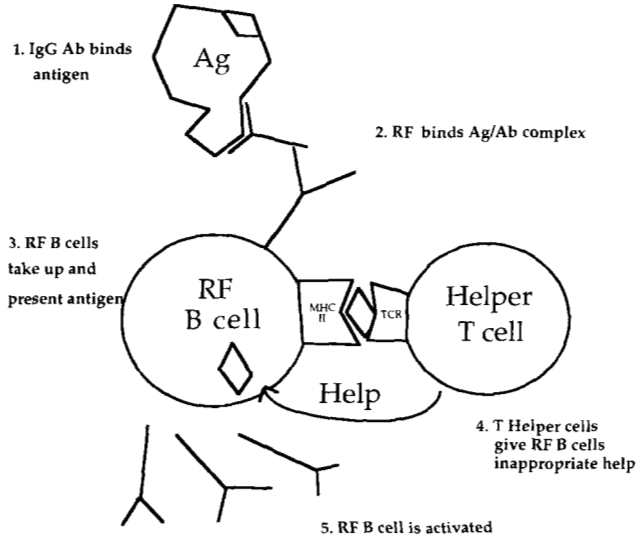

FIGURE 1. An illustration of RF cells acquiring intermolecular help from secondary antigenic challenge.

of these simulations by defining an Fc portion for the Abs (we assigned Fc the value 000000001010), which does not, in itself, bind foreign Ag. The values selected for the Fc and the Ag prohibit cross-reactive Abs because the Ag and Fc are 12-bit mismatches to each other; a minimum of 6-bit-mismatch binding would have to be allowed if a particular Ab were to bind both Ag and Fc.

Of course, real Ags often have more than one epitope. The single foreign epitope we model can be thought of as a representation of all strictly foreign epitopes for purposes of this paper. In other words, from the RF point of view, the specificity of any one IgG is irrelevant.

Pathologic vs natural RF

Since pathologic RFs exhibit the hallmarks of Ag-driven affinity maturation (Table I), it is a reasonable assumption that pathologic RFs have higher binding efficiencies for IgG Fc than do natural RFs. Although direct affinity comparisons between natural and pathologic RFs are not available, it has been demonstrated that highly mutated RFs indeed have higher affinities for IgG Fc than do their clonal progenitors (44, 45). For the purpose of these experiments, we defined natural RFs as two- and three-bit mismatch RFs and defined pathologic RFs as zero- and one-bit mismatch RFs. These parameters are conservative because if they are improper (i.e., if pathologic RFs have lower affinities for IgG Fc than do natural RFs), then the competitive tolerance mechanism provided by natural RF (and, hence, the conclusions of this article) will be significantly strengthened rather than weakened.

Precursor frequencies

We modified the IMMSIM automaton to allow us to specify the probability with which B cells carrying zero- to three-bit mismatches to Ag or IgG Fc would leave the bone marrow. We set the precursor frequency for the Ag-specific B cells to a previously predicted value of 0.1% (i.e., 1 out of 1000 B cells will bind Ag to some degree) (46). To obtain the 0.1% frequency, we gave zero-bit mismatch B cells a 0.00033% chance of leaving the bone marrow; a 0.0040% chance to one-bit mismatch B cells; a 0.022% chance to two-bit mismatch B cells; and a 0.074% chance to three-bit mismatch B cells (0.00033% + 0.0040% + 0.022% + 0.074% = 0.1%). The relative frequencies were chosen from the combinatorics of 12-bit systems: for every perfect (zero-bit mismatch) Ab type there are 12 one-bit mismatch Ab types, 66 two-bit mismatch Ab types, and 220 three-bit mismatch Ab types.

To set the precursor frequency of natural RF cells, we chose a midrange value of 7% from the experimental (3–10%) and theoretical (4–15%) values (1, 10–13). When modeling the natural RF response, pathologic RF (0- and 1-bit mismatch) B cells were disallowed from the initial repertoire; 2-bit mismatch RF cells had a 1.6% chance of leaving the bone marrow; and 3-bit mismatch RF cells had a 5.4% chance of leaving the bone marrow (0 + 0 + 1.6% + 5.4% = 7.0%). Again, the relative frequencies of 2- and 3-bit mismatch Abs were dictated by the combinatorics of the 12-bit system.

Table I. *Key features of the two types of RF*

Natural RF	Pathologic RF
Polyclonal: produced by a high frequency of preimmune B cells	Oligoclonal: derived from a small number of preimmune B cells
Does not class switch from IgM	Class switched to IgG or IgA
Can bind a wide range of IgG Fcs	Often isotype specific
Undermutated	Hypermutated
Low CDR R:S	High CDR R:S
Naive	Enters memory
No pathology	Causes inflammation and subsequent joint vascularization

Class switch

The Ag-specific Ab response class switches in vivo from mainly IgM in the primary to mainly IgG in the secondary. We model this in IMMSIM by introducing a RF-bindable (i.e., IgG) Fc only following the second Ag injection (time step 200). Additionally, at the onset of the second injection, we discard all previous Ag/Ab complexes (which would inappropriately "class switch" if we allowed them to remain). This simulated class switch allows for an appropriately high secondary RF response while preventing any inappropriate primary RF response. If we do not initiate this class switch, a significant natural RF response is generated against the primary Abs that is contrary to experimental RF data (data not shown). We currently are working on the dynamics of class switch in the IMMSIM system and plan in the future to provide IMMSIM with a less synthetic class switch.

Results and Discussion

Ag injections generate primary and secondary responses

Our initial simulations include a characteristically small percentage (0.1%) of Ag-specific B cells in the initial repertoire as predicted by the Cohn protecton theory (46). This 0.1% precursor frequency allows a high RF cell/Ag-specific B cell ratio in the context of the IMMSIM grid. Here we confirm that the IMMSIM system successfully generates distinct primary and secondary responses to Ag with this precursor fraction of Ag-specific B cells: the generation of memory B and T cells elicits a narrower and higher secondary than primary Ab response (Fig. 2*A*), and Ag is cleared faster during the secondary than during the primary response (Fig. 2*B*).

Modeling natural RF

It is crucial to establish that IMMSIM can accurately simulate the eccentricities of the natural RF response. The results are presented in Figure 3*A*. With a natural RF precursor frequency of 7% (the mean of observed and theoretic ranges), IMMSIM recapitulated the major features of the natural RF response. As in vivo, there was no natural RF response to the primary Ag injection. Also as in vivo, the natural RF response to secondary Ag injection was 10 times higher than the Ag-specific Ab response. The absence of a primary but presence of secondary natural RF response is due to the simulated class switch of Ag-specific Abs from IgM to IgG. We attribute the extent of the RF response to the high precursor frequency of low affinity natural RF. Significantly, a lower fraction of preimmune natural RF cells failed to recapitulate the natural RF response (Fig. 3*B*).

Low levels of pathologic RF cells will expand in the absence of tolerance

Since pathologic RF cells seem not to be tolerized by deletion, anergy, receptor editing, or memory blockage (39, 40), it is reasonable to consider that a small number of pathologic RF cells might be present in normal B cell pools. Indeed, measurable titers

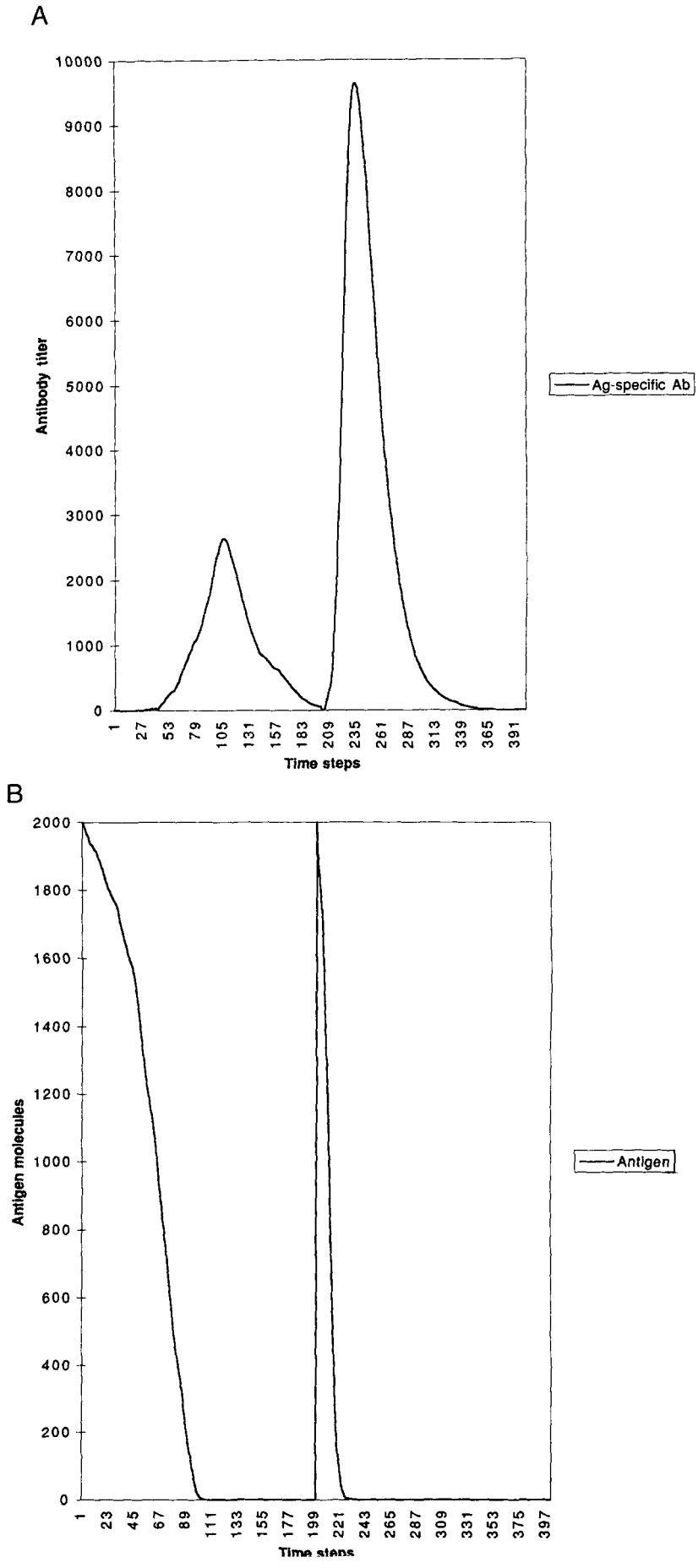

FIGURE 2. IMMSIM utilizing the Cohn protecton theory-specified 0.1% Ag-specific B cell precursor fraction recapitulates the hallmarks of primary and secondary immune responses. *A*, Ag-specific Abs exhibit a wide, low magnitude primary and a narrow, high magnitude secondary response. This is a control experiment for the Ag-specific B cell precursor fraction parameter. Ag-specific B cells were set at a total precursor frequency of 0.1%. For exact base-mismatch breakdowns see *Precursor frequencies* under *Materials and Methods*. No RFs are present in this run. In "wet" biology, measured Ab titers are dependent on the binding affinity of the Abs. A higher affinity Ab in a mixed pool of Abs will manifest as a higher titer than will a lower affinity Ab even if both Abs are in fact at the same concentration. To reflect this, we have weighted the Abs in this figure according to their binding affinities. Zero- and one-base mismatch Abs are given 100% weight, two-base mismatch Abs are given 51.2% weight, and three-base mismatch Abs are given 12.8% relative weight (see *A qualitative description of* IMMSIM under *Materials and Methods*). *B*, Ag is cleared more rapidly during the secondary then the primary response. Plotted are Ag units. A total of 2000 units of Ag were added at time steps zero and 200.

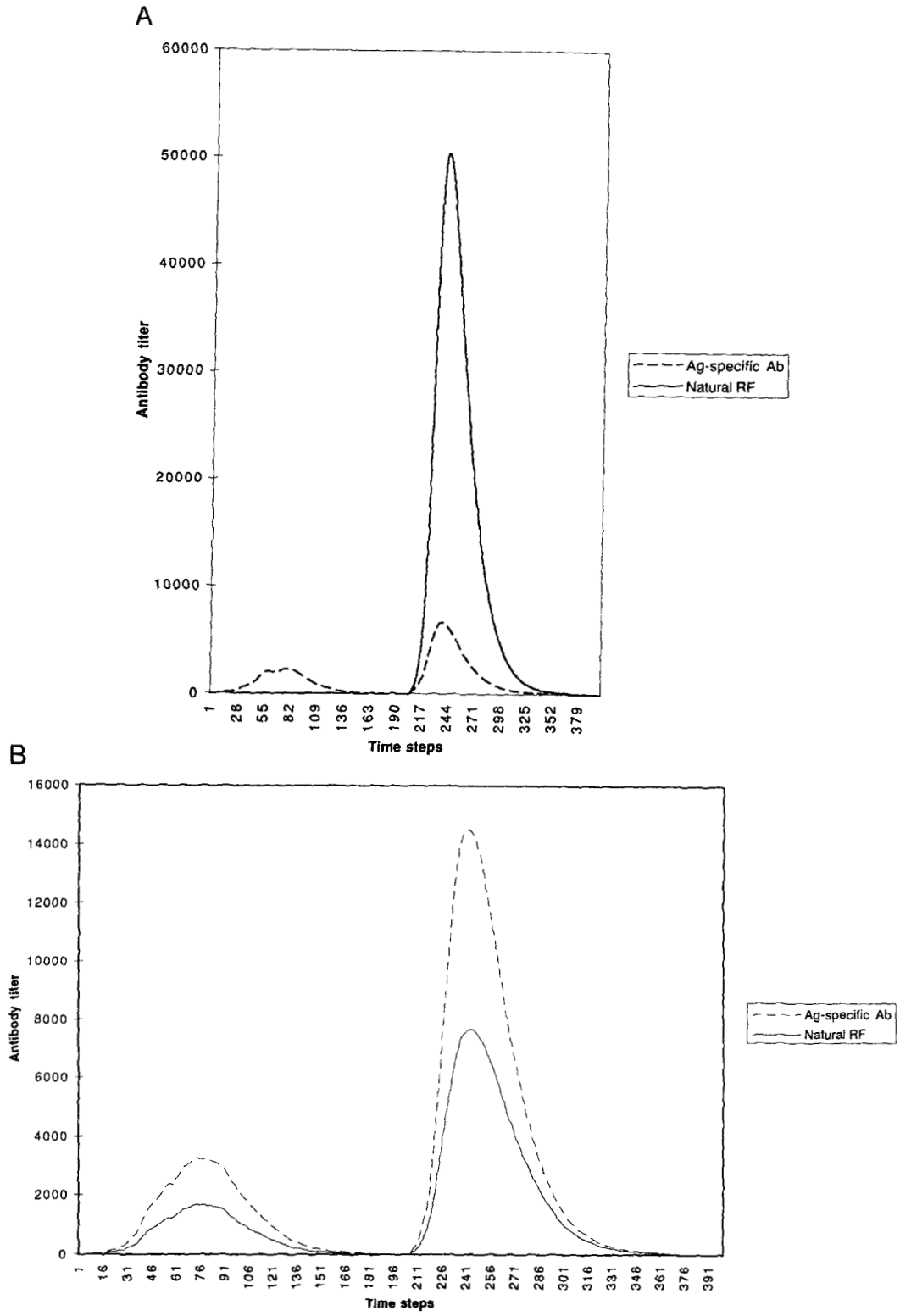

FIGURE 3. Seven percent natural RF cell precursor frequency recapitulates the natural RF response. *A*, The natural RF cell precursor fraction was set at 7%. The precursor frequency of Ag-specific Ab is 0.1% as in Figure 2. The secondary natural RF response is about 10 times the Ag-specific Ab response, which is consistent with in vivo data. The data presented are weighed titers (see Fig. 2*A* legend). The absence of RF in response to primary Ag injection is a result of the Ag-specific Abs' class switch from IgM to IgG (see *Class switch* under *Materials and Methods*). *B*, A 1/10 (0.7%) natural RF cell precursor frequency is unable to recapitulate the natural RF response.

of pathologic RFs are sometimes found in nondiseased individuals (47). One might conjecture that a low frequency of pathologic RF cells might produce insignificantly low pathologic RF titers during a secondary response (and thus, that a low frequency of pathologic RF cells, in itself, obviates tolerance mechanisms). We tested this conjecture. We specified a pathologic RF cell frequency of 0.1%

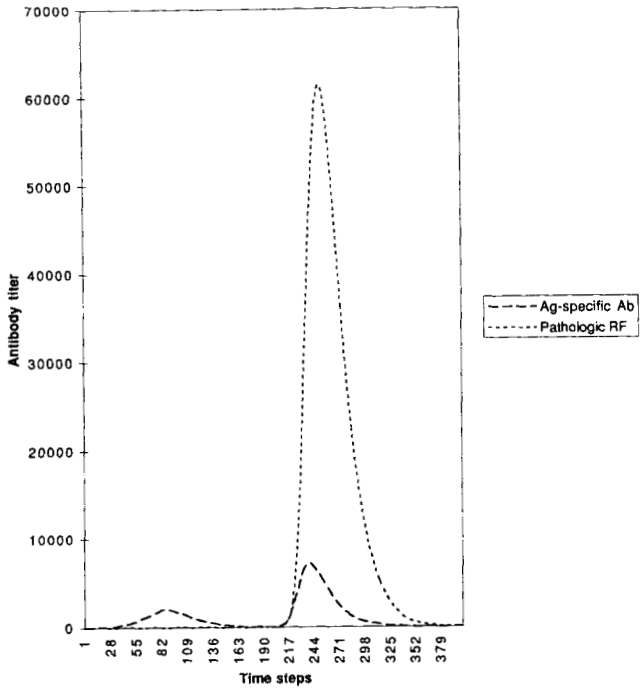

FIGURE 4. One-tenth percent pathologic RF cell precursor frequency generates high RF response. The precursor frequencies of Ag-specific Ab and pathologic RF cells are both 0.1% as in Figure 2. The secondary pathologic RF response is ~10 times the Ag-specific Ab response even though both types of B cells are initially present in equal amounts. The data presented are weighed titers (see Fig. 2*A* legend). The absence of RF in response to primary Ag injection is a result of the Ag-specific Abs' class switch from IgM to IgG (see *Class switch* under *Materials and Methods*).

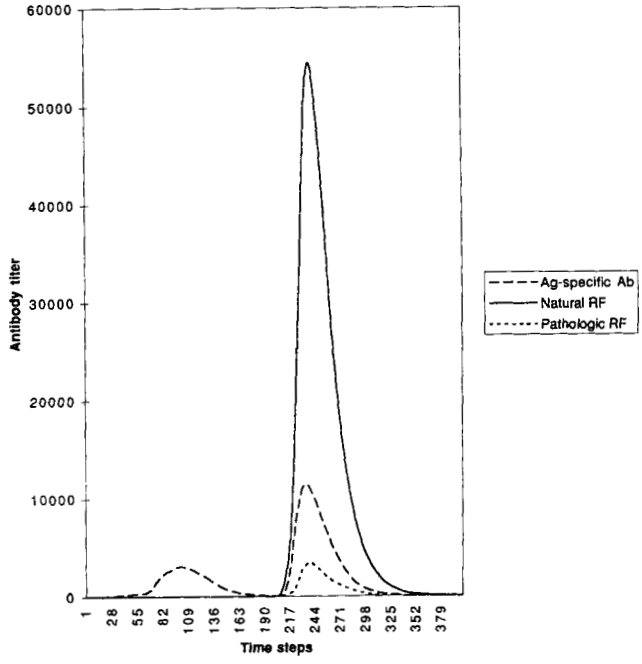

FIGURE 5. Seven percent precursor fraction of natural RF cells can dramatically lower the stimulation received by pathologic RF cells. Natural RF cells formed 7% of the preimmune repertoire along with 0.1% pathologic RF cells. High concentration, low affinity natural RF effectively competes with low concentration, high affinity pathologic RF for Ag (i.e., for IgG Fc-binding sites). The precursor frequency of Ag-specific Ab is 0.1% as in Figure 2. The data presented are weighed titers (see Fig. 2*A* legend). The absence of RF in response to primary Ag injection is a result of the Ag-specific Abs' class switch from IgM to IgG (see *Class switch* under *Materials and Methods*).

(i.e., as much as any other Ag-specific B cell type) and subjected this B cell pool to the same Ag injections as before (Fig. 4). As seen, a 0.1% precursor frequency of pathologic RF cells produced a pathologic response of roughly the same massive titer as that produced by 7% natural RF (Fig. 3). This outcome is a clearly a departure from what is observed in the nondiseased state.

We considered that 0.1% was too high a frequency for pathologic RF cells in the nondiseased state. Even supposing that the pathologic RF cell frequency is too high, surely one pathologic RF cell will sometimes be found in a germinal center. No tolerance method is perfect. Accordingly, we modeled the addition of a single RF cell to the B cell pool. In 37 out of 100 runs (data not shown), that one pathologic RF cell acquired massive stimulation (i.e., it and its daughter cells produced a higher Ab titer than did the Ag-specific B cells).

Therefore, our model would seem to indicate that if even a single pathologic RF cell escapes tolerance, that one cell will often expand and produce high titers of pathologic RF. This suggests that a low frequency of pathologic RF cannot, by mere virtue of its low frequency, relax the need for a strong tolerance mechanism.

Modeling coexistence of pathologic and natural RF

Thus far, in Figure 3 we modeled natural RF in the complete absence of pathologic RF, and in Figure 4 we modeled pathologic RF in the absence of natural RF. In vivo, neither of these situations is particularly plausible. Natural RFs are still present in diseased individuals, and pathologic RFs are transiently observed in nondiseased individuals and in principle could mature from the bone marrow (2, 47). To obtain a more realistic model of the RF response, we reconstituted the B cell pool to contain both the 7% natural RF from Figure 3 and the 0.1% pathologic RF from Figure 4. Although the two types of RF produce equivalent titers when separate, would they do so when combined? Would pathologic and natural RF each produce half of the total RF response, or would either high affinity pathologic RF or high population natural RF dominate? Representative results of this experiment are presented in Figure 5. As seen in the figure, low levels of high affinity pathologic RF were almost entirely outcompeted by high levels of low affinity natural RF. Pathologic RF received only a fraction of the stimulation it previously received when no natural RF was present (Fig. 4).

More significantly, perhaps, when we seeded the B cell repertoire with a single pathologic RF cell in the presence of 7% natural RF, the single pathologic RF appears never to have acquired high levels of stimulation (the pathologic RF titer exceeded the Ag-specific Ab titer in 0 of 100 runs) (not shown). Indeed, the single pathologic RF cell failed in all 100 runs to produce an Ab titer higher than 500 when natural RF was present (compared with a single pathologic RF cell without natural RF which produced an Ab titer >500 in 80 of 100 trials) (not shown).

We conclude that a high frequency of natural RFs normally can outcompete a small number of pathologic RFs for IgG Fc stimulation.

Competitive tolerance: a solution to the RF paradox

Even though pathologic RFs are likely to arise as a result of fortuitous mutations in non-IgG-binding B cells, high titers of Ag-driven, mature RFs are only observed in diseased individuals. One

might conclude from this that pathologic RF cells are tolerized. Nonetheless, experimental evidence shows that the canonical tolerance methods—deletion, receptor editing, anergy, and blockage of memory establishment—seem not to operate on pathologic RF cells (39, 40). When these observations are coupled with the data presented in this paper, we conclude that the large precursor frequency of natural RF may tolerize pathologic RF. This "competitive tolerance" mechanism may operate by preventing pathologic RF cells from receiving stimulation. The immune system may maintain a high precursor frequency of a particular autoantibody B cell type so that no one B cell can receive sufficient stimulation to drive it to higher affinity and so that pathologic B cell "tolerance escapees" will not receive further stimulation. There is no a priori reason to assume that competitive tolerance might not play a role in tolerizing against other pan-antibodies. We propose that any autoantibody B cell specificity that is found to comprise an unusually high precursor fraction of the preimmune repertoire will be subject to competitive tolerance.

The competitive tolerance response to excessive antigenic stimulation

Can competitive tolerance be maintained against chronic antigenic stimulation? In order to explore this question, we tested the ability of competitive tolerance to withstand multiple injections of Ag. We started a single pathologic RF cell in the IMMSIM grid along with a 7% pool of natural RF. We then subjected that IMMSIM grid to 2000-U Ag injections every 200 time steps for 4000 time steps. As is illustrated in Figure 6*A*, chronic antigenic stimulation eventually broke competitive tolerance. This simulation may explain the finding of Nemazee and Sato that Ag injections given in quick succession lead to a transient pathologic RF response (2) (i.e., the same set of B cells may have been subjected to multiple antigenic stimulations). Likewise, the same cells that are present in our IMMSIM grid were not allowed to leave (except by a relatively slow death rate).

In light of our previous finding, we thought it likely, even obvious, that a single burst of antigenic stimulation would break competitive tolerance by providing Ag and thus IgG Fc in high amounts. In this scenario, pathologic RF cells would receive stimulation before natural RF could bind the excess Ag. To test this idea, we carried out such an experiment; the results are presented in Figure 6*B*. In this experiment, pathologic RF was allowed to form 0.1% of the B cell repertoire in the context of 7% natural RF cells. Instead of injecting 2000 Ag units, we increased that amount by a factor of five. As seen in Figure 6*B*, raising the Ag level by a factor of five raised the Ag-specific Ab by a factor of four and the natural RF by a factor of two (which is as one might have predicted). The pathologic RF, however, did not significantly change with increased antigenic stimulation. Not only was competitive tolerance not broken by this increased dose of Ag, competitive tolerance appears not even to have been strained by it. A comparison of T cell data from this experiment with data from the experiment outlined in Figure 5 reveals that T cells are not the limiting factor (data not shown). While a large amount of Ag may lead to the breaking of competitive tolerance, it may be imperative that pathologic RF cells be submitted to that antigenic stimulation multiple times before competitive tolerance is likely to be broken.

*MRL/*lpr *mice and pathologic RF: an escape from competitive tolerance*

The MRL/*lpr* mouse strain—a model organism of systemic autoimmune disease—produces high titers of pathologic RF along with a distinct subset of other autoantibodies (e.g., anti-ssDNA, anti-dsDNA, antihistones) (19, 20) linked to a defective *fas* apoptosis gene (48–51). Despite several proposals for roles of *fas* in the thymus or germinal centers, there is not yet any simple explanation for why many autoantibodies arise in MRL/*lpr* mice. One might have supposed that, simply, autoreactive B cells are no longer centrally deleted from the repertoire of mice lacking functional *fas*; however, this is demonstrably not true for some autoantibodies (52). In any case, such a simplistic explanation cannot explain the high levels of pathologic RF in MRL/*lpr* mice because pathologic RF cells are not deleted in normal mice (39, 40). What insight can predictions from the IMMSIM model provide to pathologic RF production in MRL/*lpr* mice?

As we have demonstrated, in the IMMSIM model, competitive tolerance can be broken if B cells sequestered in a germinal center are subjected to chronic antigenic stimulation. Such sequestered pools have been associated with RF production in diseased MRL/*lpr* mice—that is, high RF production is primarily associated with a dense accumulation of IgG2a plasma cells in the central T cell zone of periarteriolar lymphatic sheaths (PALSs) (53). Neither this dense accumulation of IgG2a plasma cells nor subsequent high RF production is observed in normal mice or even in nondiseased MRL/*lpr* mice. This sequestration model is consistent with the *fas* defect. Presumably, clusters of plasma cells sequestered to the inner PALS are normally disrupted by *fas*-mediated apoptosis.

B cells sequestration may break competitive tolerance in RA

The mechanisms by which pathologic RF break competitive tolerance in RA are not so constrained as they are in the MRL/*lpr* mouse model. RA mechanisms are not necessarily linked to an apoptosis or even to a general tolerance defect. We can, however, limit the scope of probable models because there are no obvious defects in the B cell repertoire of RA patients; neither a low preimmune fraction of natural RF cells nor a high initial fraction of pathologic RF cells is likely to be a cause of RA. The most promising model that can explain how RA patients have broken competitive tolerance is the "sequestration" model.

As in the MRL/*lpr* mouse model, disease-associated foci in which competitive tolerance might be broken have been observed and reported. Another high correlate to RA is the ectopic germinal center (EGC). These EGCs—clusters of B cells in nonlymphoid tissues—have been observed in the synovial tissue of RA (but not nondiseased) patients (54, 55). In these EGCs high numbers of Ag-driven somatic mutations and clonal expansion have been observed. Given the high titers of pathologic RF found in the RA synovium, it seems reasonable that the EGCs are producing pathologic RF, although this has yet to be demonstrated directly. It has also been postulated that these EGCs may arise in reactive arthritis patients as a result of chronic infection in the synovium's local environment. These EGCs may provide just the type of sequestered system that would allow for the breaking of competitive tolerance through chronic stimulation.

A clinical role for natural RF in the treatment of RA?

If competitive tolerance is about to be broken but not completely broken—that is, if the levels of IgG are high but not overwhelming—then increased levels of natural RF may still be able to compete with pathologic RF and thus reestablish competitive tolerance. We turn back to the IMMSIM model to illustrate and test this point. Again, we allow the same normalized numbers of pathologic B cells to arise in the initial repertoire, but we increase the natural RF to the level found at the peak of a 7% natural RF response to secondary Ag stimulation. Can this increased level of natural RF increase the effectiveness of competitive tolerance? From the results of this experiment (Fig. 7), it is obvious that increased levels of natural RF can indeed prevent pathologic RF from receiving

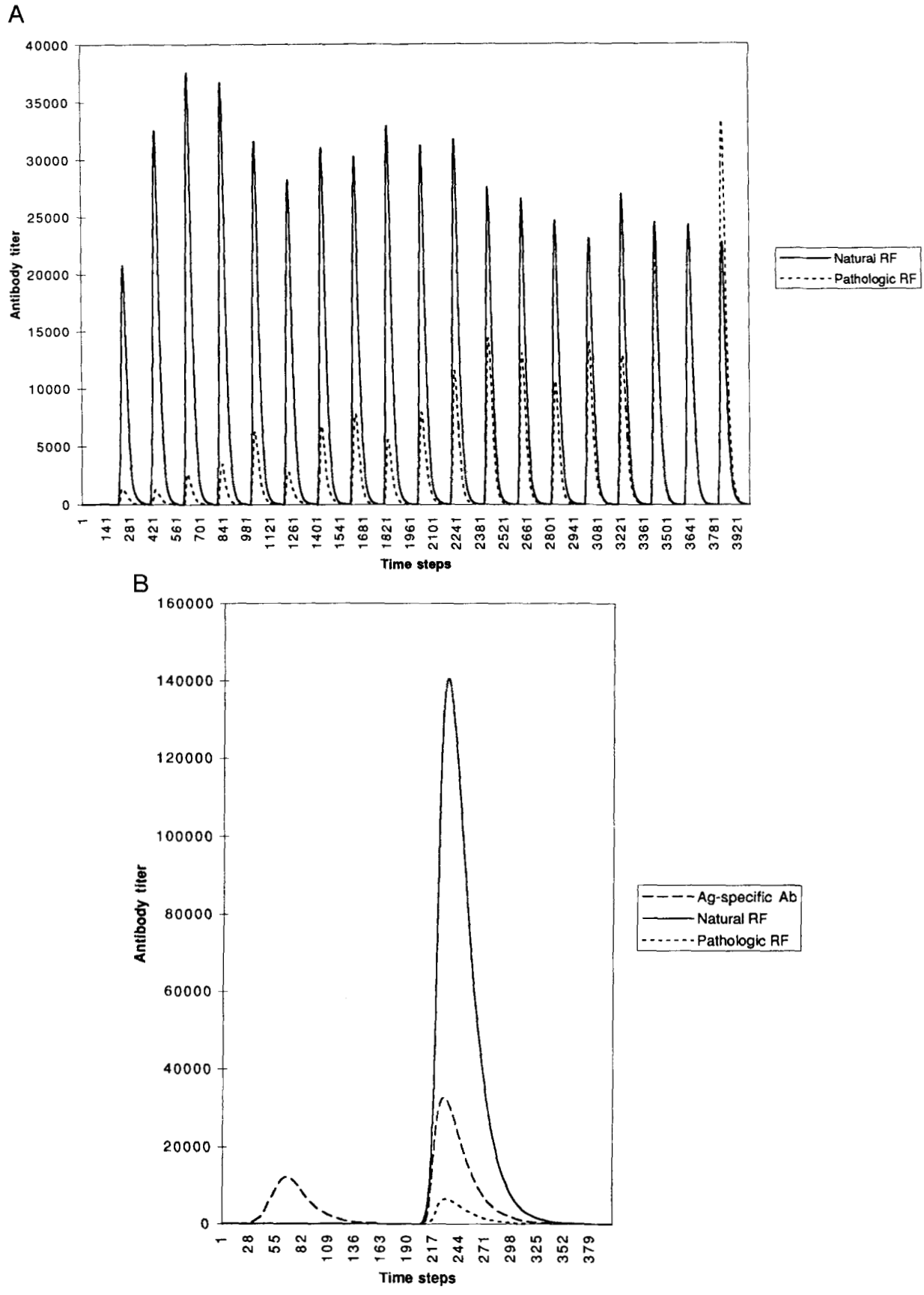

FIGURE 6. The competitive tolerance response to excessive antigenic stimulation. *A*, A single pathologic RF cell was placed in the repertoire along with 7% natural RF. The germinal center was then subjected to Ag stimulations (2000 U) every 200 time steps. *B*, The parameters in this run were identical to those used in Figure 5 with one exception: 10,000 Ag units were injected at time steps 0 and 200. While Ag-specific Ab and natural RF both rise in response to increased Ag levels, pathologic RF remains stable (i.e., at the same level as in Fig. 5.)

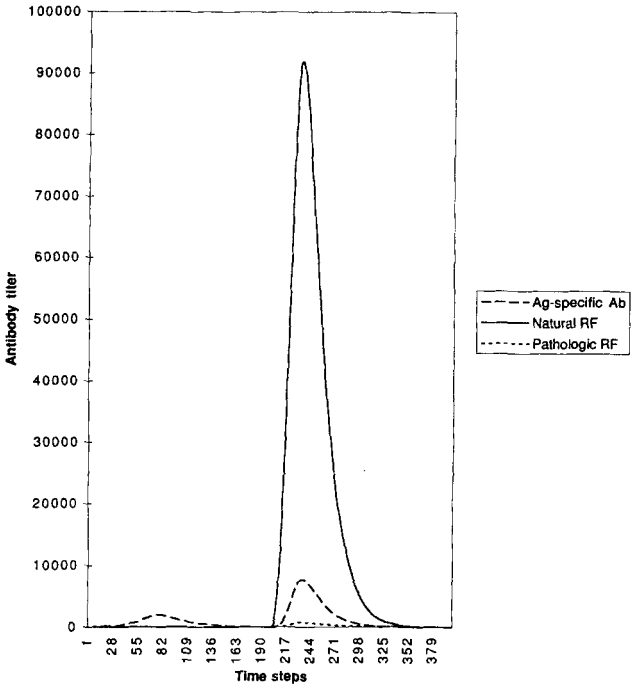

FIGURE 7. Treatment of RA by secondary Ag injection: timely secondary Ag injection can re-establish competitive tolerance. We added the same number of pathologic RF cells to the repertoire that were added in Figures 4, 5, and 6*B*. However, we boosted the numbers of natural RF cells to equal those found in at the peak of a secondary immune response. At a peak response, natural RF cells number ~11,000 in the Figure 3 run (data not shown). We changed the absolute numbers of B cells to 12,000 for this one run to accommodate 11,000 natural RF cells. To add in the same number of pathologic B cells as we had in Figures 4, 5, and 6*B*, we therefore had to change the pathologic RF cell precursor frequency to 0.033%. Similarly, we retained the same absolute numbers of Ag-specific B cells by specifying 0.033% of the repertoire as Ag specific. The Ag-specific B cell binding type breakdown is: zero-base mismatch Abs form 0.00011% of the preimmune repertoire; one-base mismatch Abs form 0.0013% of the preimmune repertoire; two-base mismatch Abs form 0.0074% of the preimmune repertoire; and three-base mismatch Abs form 0.025% of the preimmune repertoire. Pathologic RF fails to receive significant stimulation when the natural RF preimmune repertoire is increased by prior secondary stimulation. All other variables were set as in Figures 3 to 5.

practically any stimulation. Is this observation useful? Can we in fact raise natural RF levels in vivo? The basis for this suggestion is the commonly reported symptom by RA patients of flare anticipation. If flare anticipation is experienced in response to developing EGCs or to prior events, pathologic RFs may not yet have risen to high titers. Any secondary antigenic stimulation (e.g., tetanus toxoid) (56) could then raise natural RF levels before pathologic RFs are stimulated. Competitive tolerance could possibly be reestablished and may provide a protective barrier against pathologic RF.

Summary and conclusions

RFs provide a fascinating paradox: how are pathologic RFs tolerized in the apparent absence of classical tolerance mechanisms? Competitive tolerance can solve this paradox. By the competitive tolerance model, pathologic RF cells are unable to accumulate sufficient foreign Ag to acquire intermolecular T cell help.

Along with the fundamental paradox, RFs provide other significant puzzles for which a common solution can be found under the aegis of competitive tolerance. For instance, why are so many natural RF cells found in nondiseased mammals? Our data indicate that competitive tolerance relies on a high precursor fraction of natural RF cells in order to prevent pathologic RF cells from accumulating Ag and thus intermolecular help.

What defects lead to pathologic RF production in disease situations? In IMMSIM, competitive tolerance may be broken if B cells are subjected to chronic antigenic stimulation. Such stimulation can occur in vivo when B cells are inappropriately sequestered. This may explain the significance of plasma cells in PALSs in *fas*- and *fas* ligand-deficient mice and EGCs in RA patients. In this model, pathologic RF cells break competitive tolerance by accumulating sufficient antigenic stimulation to acquire intermolecular T cell help.

Why does the natural RF response fail to mature? We believe that the same competitive tolerance that prevents pathologic B cells from accumulating Ag and subsequent T cell stimulation will also prevent any one natural RF cell from being driven to pathogenicity (we are currently modeling this directly). Implicit to this model is that the signal for an RF cell to become a plasma cell cannot be the same as the signal that drives the cell to memory. This view is supported by natural and pathologic RF data in that it is clear from the natural RF response that natural RF cells can become plasma cells without appearing to class switch or hypermutate. Our interpretation of these data is that a lower level of stimulation is necessary to initiate a plasma response than is necessary to lead to a classic memory response (including class switching and hypermutation). This may be applicable to B cells in general. Unlike other models that rely on a lack of T cell help to prevent RF maturation (57), this model can withstand foreign-specific, intermolecular T cell help. Of course, mechanisms that rely on natural RF cell deletion following IgG Fc exposure in the absence of T cell help may work in conjunction with competitive tolerance to prevent natural RF maturation.

It is a current limitation of the IMMSIM model that we only assess binding probabilities as a measure of relative B cell "fitness." To simulate a reasonable natural RF response, the binding probability of natural RF was left high relative to pathologic RF. Thus, our current modeling of competitive tolerance has been limited to considering those pathologic RFs that are of only moderately higher affinity (about two to eight times higher) than natural RFs. We cannot yet comment on the entire range of pathologic RF/natural RF fitness strengths accommodated by competitive tolerance. It is interesting to note, however, that extremely avid pathologic RFs (ones that bind IgG Fc several orders of magnitude better than those analyzed here) are subject to deletion (M. Shlomchik, manuscript in preparation). Thus, for pathologic RF, competitive tolerance appears to bridge the gap between clonal ignorance and central tolerance.

There are, of course, many ways in which competitive tolerance might be strengthened. For instance, FcRs on B and other cells may mask IgG Fc. By including FcRs only on APCs and RF cells, we have modeled a worst-case scenario. Any lowering of IgG Fc binding by pathologic RF cells will decrease the need for competitive tolerance by preventing pathologic RF cells from binding immune complexes and thus from acquiring intermolecular T cell help. Similarly, if pathologic RFs in fact have lower binding affinities for IgG Fc than do natural RFs, then natural RFs will compete for IgG Fc better than those we have modeled and thus will provide even stronger competitive tolerance than we demonstrate.

It is increasingly appreciated that tolerance through strict self/non-self recognition mechanism is inherently leaky. Otherwise

normal individuals do, in fact, generate several autoantibodies in spite of deletion, anergy, and editing. With the advent of "danger" models of immune response, significant subsets of the immunology field have undergone a definite shift from strict self/non-self determination paradigms (58). As a consequence, the avenues of thought about tolerance mechanisms have been significantly broadened to the point at which it is reasonable to consider a subset of autoantibodies that may be resistant to many classical tolerance mechanisms—so-called "clonal ignorance" (39, 59–62). These apparently tolerance-resistant autoantibodies would include Abs that can indirectly receive T cell help through "bridging" mechanisms (26). Examples of bridging Abs may include anti-DNA (which can form anti-DNA/DNA/viral protein complexes, e.g.,), antihistones (antihistone/histone/DNA/Ag), and of course anti-IgG (anti-IgG/Ag-specific Ab/Ag). Recent evidence for anti-DNA competitive tolerance has been reported in autoimmune NZW/NZB mice: DNA injection lowers anti-DNA nephritis even while increasing anti-DNA titers (63). We interpret this surprising result to indicate that natural anti-DNA Abs were expanded and so provided competitive tolerance against pathologic anti-DNA stimulation. If tolerance is to be maintained in the face of bridging mechanisms, one may invoke canonical tolerance mechanisms, but these mechanisms, while helpful, cannot be perfect. Competitive tolerance is a reasonable solution to this general problem.

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