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Poor repertoire selection in symmetric idiotypic network models

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1. Summary

The selection of B and T cell repertoires is known to be influenced by idiotypic interactions during early ontogeny. Early B cell clones are multispecific, have numerous idiotypic interactions, produce IgM antibodies, and may constitute a separate cell lineage (characterised by the Ly1 or CD5 marker). Furthermore, because early B cells are self-reactive, self antigens should play a crucial role in the repertoire selection.

Previously we developed a theoretical model of idiotypic B cell interactions. The model is based on a (symmetric) bell-shaped interaction function. The symmetry and the shape are a consequence of the process of receptor crosslinking. Assuming that early (i.e. IgM) B cell interactions are independent of helper T cell activation, we now apply this model to the problem of idiotypic repertoire selection. In the model the molecular structures of B cell receptors (i.e. of the idiotypes) and of self antigens are represented by random (bit-)patterns. Interactions are based on complementary matches between these patterns. Therefore, the repertoire selection is brought about by stimulatory networks based upon complementary matches.

The results show that the presence of a self antigen specifically shapes the B cell repertoire. The selection depends on the nature of the antigenic signal; we incorporate either stimulatory or inhibitory (i.e. tolerizing) self antigens. Clones stimulated by the self antigen tend to become agressive in the network; tolerized self-reactive clones tend to become suppressed. Thus, idiotypic interactions play a facilitating role in self/non-self discrimination. However, since the idiotypic selection is only a tendency, the immune system should not rely on it.

We next incorporate two classes of B cells. We call them the early, or "IgM", and the late, or "IgG", B cells (IgG B cells appear after the development of the IgM repertoire). IgG B cells may have only a few idiotypic interactions. We investigate whether the early IgM repertoire, which is (partly) selected by self antigen, influences the selection of the late IgG repertoire. It turns out that this influence can only be non-specific. Either we find a similar tendency in the IgG repertoire, or we find that the IgG repertoire influences the IgM repertoire non-specifically.

Thus, despite the fact that this theoretical work readily confirms empirical results showing that the manipulation of early idiotypic interactions can have specific and long-lasting effects, the presupposed physiological role of these interactions, in terms of self-reactivity or repertoire selection, fails to develop in our models.

Key words: Idiotype; mathematical model.

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2. Introduction

The potential repertoire of (naive) antibody molecules is larger than 10^{10} different receptors. The actual repertoire, however, is estimated to remain below 10^8 different antibody receptors [1]. Thus, the formation of the actual antibody repertoire must involve (several) selection mechanisms. One of them seems to be the idiotypic network. The establishment of the adult B cell repertoire was demonstrated to depend on idiotypic interactions during early ontogeny [2–6]. T cell repertoires, in turn, are

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mainly tained and selected by the B cell repertoire [7]; T cells recursively influence the B cell repertoire [8].

During early ontogeny, idiotypic interactions are very common [9, 10]. These early interactions are found among the so-called "natural" antibodies, which are of the IgM type, and which preferentially use specific V gene families [11]. Furthermore, a special class of B cells, which is identified by the Ly1 or CD5 marker, might be responsible for these networks of natural antibodies. These Ly1 B cells appear during early ontogeny, are long-lived, are multispecific, are probably independent of helper T cells, and play a role in autoimmunity [12, 13]. It is thus possible that the numerous idiotypic interactions which occur during early life, and which seem to play a role in adult repertoire selection, are a special property of the Ly1 B cells [6, 14].

Additionally, these natural antibodies are often self-reactive, i.e., have numerous interactions with self antigens. Network interactions with the self environment are believed to play a functional role in "self-assertion" and repertoire selection [7, 10, 5, 16]. These authors argue that 10% of the lymphocytes form a formal network of self-reactive specificities. The majority (90%), however, are not connected to the network and form a compartment of immunocompetent but resting lymphocytes. Immune responses to external antigens originate in the latter compartment.

The most detailed data on idiotypic repertoire selection are obtained with a four-layered (i.e. Ab_1-Ab_4) "paper network" of several perinatal monoclonal IgM antibodies that are known to play a role in the immune response to phosphorylcholine and dextran [4–6]. The data show that *in vivo* manipulation of the developing mouse immune system with members of this network influences the adult B cell response to these antigens. Administration of Ab_2's, Ab_3's, or Ab_4 enhances or suppresses the adult anti-PC and anti-DEX responses. Administration of Ab_2 or Ab_3's restores the suppressive effect of an early dose of Ab_4. Manipulation with these anti-idiotypic antibodies requires strict timing. For each antibody there appears to be a specific time window (of a few days) within which administration has an enhancing or a suppressing effect.

We here investigate whether these highly connected idiotypic networks can indeed account for a *sensible* selection of millions of clones simultaneously. We think that it remains to be established whether a huge IgM network, with all its multispecific and degenerate interactions, is able to perform such a complex task. Our theoretical approach to this (informatic) question is to construct large networks that are based on complementary matches between many lymphocyte clones. These theoretical networks are confronted with a stimulatory or inhibitory self antigen. We investigate to what extent these networks are able to accomplish a selection of repertoires that is interpretable in terms of self-reactivity. If self-tolerance and repertoire selection are distributed properties of network interactions, similar properties are expected to arise in these model networks.

Our previous (theoretical) work concentrated on stability, and on the generation of idiotypic memory and proliferation control in (adult) networks [17–20]. Symmetric idiotypic networks were shown to be poor at generating proliferation regulation. Idiotypic memory was shown to imply endless activation of all clones of the network. Subsequently [21] we proved that the behaviour of B cell network depends stringently on the incorporation of helper T cells. MHC-restricted helper T cells, for instance, may altogether prevent B–B idiotypic interactions from becoming functional. We had to conclude that idiotypic networks provide poor adult immune systems [22].

These results, however, do not apply to the abovementioned early networks of IgM antibodies. Since these networks develop before T cells appear, early idiotypic interactions seem to be helper-independent [7, 23]. Additionally, the endless activation that we have described for our model networks may well correspond to the autonomous network behaviour that is described for developing immune networks [15]. Indeed, whilst adult B cells are described as resting cells, early B cells are generally in an active state [9, 10, 16]. Our previous results showed that the occurrence of such autonomous behaviour depends on the connectivity of the network [17, 20]. Once the connectivity exceeds a certain threshold, idiotypic interactions in combination are sufficient to activate individual clones. The network thus loses its virgin (i.e., resting) state.

Here we apply these previous insights to the prob-

lem of idiotypic repertoire selection. We deliberately set the connectivity of the network to values at which autonomous activation occurs. In the absence of antigenic stimulation, all clones proliferate and settle into a stable state in which they are either aggressive or suppressed. The distribution of aggressive and suppressed clones is in fact the actual repertoire. The environment in which the idiotypic network is embedded is determined by the presence of a single self antigen (which either induces tolerance or activates the complementary clones). We thus investigate the interplay between the influence that this single self antigen and the multitude of idiotypic interactions exert on the establishment of the antibody repertoire.

3. The model

In the model it is assumed that all B-lymphocyte clones are regulated by three processes: (1) influx of newborn cells from the bone marrow, (2) normal turnover (decay) of cells, and (3) proliferation. Idiotypic interactions influence the rate of cell proliferation. This means that we focus on stimulatory idiotypic interactions. We incorporate only B cells in this paper (i.e., we assume that these idiotypic B cell interactions are T cell-independent). We lump each clone of B cells and the antibody it produces into one population (X_i). The network consists of N clones, each of which is characterised by a unique random receptor shape. We consider one self antigen (X_0) which is also represented by a random shape. Any clone (X_i) recognises another clone or antigen (X_j) if (part of) their respective shapes can be matched complementarily. The accuracy of this match specifies the affinity (A_{ij}) of the interaction between clone X_i and clone X_j ($0 \leq A_{ij} \leq 1$).

3.1. *Influx/efflux*

In the absence of idiotypic interactions the clone size is determined solely by the balance between the source (S_i) of cells from bone marrow and the death (D) of cells in the periphery. This suffices for a stable virgin state at a clone size of S_i/D. Whenever we incorporate tolerance induction by the self antigen (X_0), the source of self-reactive cells is reduced in proportion to the affinity and the concentration of the self antigen. Self-reactive cells are then assumed to be deleted before they appear in the peripheral circulation. For peripheral cells the self antigen remains stimulatory.

3.2. *Symmetry*

If idiotypic recognition is based on complementary matching and receptor crosslinking, idiotypic interactions seem necessarily symmetric. If idiotype "i" matches "j", "j" should also match "i"; if "i" antibodies crosslink "j" receptors, "j" antibodies can do the same. Hoffmann [24, 25] first proposed this simple and attractive symmetry theory. Note that complementary matching of parts of the idiotype implies that idiotypes can be matched onto themselves. We omit such autoantibodies [26]: we set all A_{ii} to zero. The symmetry assumption will be relaxed at the end of this paper because the idiotypic interaction between an early IgM and a late IgG B cell is not necessarily symmetric.

3.3. *Crosslinking function*

Because B cells are most probably activated by the crosslinking of the antigen receptors [27], it is to be expected that the rate of cell activation will increase if the concentration of the crosslinking agent (here antigen or antibody) increases. Whenever these concentrations become too high, however, the efficacy of cell activation by receptor crosslinking decreases. Receptor crosslinking thus follows a bell-shaped curve [28]. The present model is based on this curve. Stimulatory idiotypic interactions (which lead to proliferation) develop if antibody concentrations are intermediate. Inhibitory interactions are incorporated as overstimulation, i.e., as a reduction of the rate of proliferation whenever the concentrations of anti-idiotypic antibody become too high.

We thus consider N clones of B lymphocytes (X_i) with an influx of S_i cells per day from the bone marrow and a rate D of cell turnover. Clones proliferate in response to the total of complementary shapes (i.e. αX_i), which consist of the anti-idiotypic antibodies X_j ($1 \leq j \leq N$) and one self antigen (i.e. X_0). The strength of the complementary interaction is determined by the symmetric affinity matrix A ($A_{ij} = A_{ji}$). The rate of cell proliferation is governed by a growth function $G(X_i, \alpha X_i)$. The function G( ) is maximally one; proliferation per cell

then proceeds at a rate P per day. G( ) generates a (log) bell-shaped curve [20]. We thus propose the following equations:

$$\alpha X_i = \sum_{j=0}^{N} A_{ij} \cdot X_j \quad (1),$$

and for the bell-shaped growth function of each clone ($1 \leq i \leq N$):

$$G(X_i, \alpha X_i) = \frac{\alpha X_i}{P_1 + F \cdot X_i + \alpha X_i} \cdot \frac{P_2}{P_2 + \alpha X_i} \quad (2),$$

with the differential equation ($1 \leq i \leq N$):

$$X_i' = S_i - D \cdot X_i + P \cdot X_i \cdot G(X_i, \alpha X_i) \quad (3).$$

But, if the self antigen (X_0) induces tolerance and $A_{i0} > 0$:

$$X_i' = \frac{S_i}{T \cdot A_{i0} \cdot X_0} - D \cdot X_i + P \cdot X_i \cdot G(X_i, \alpha X_i) \quad (3').$$

Antigen (X_0) is incorporated as a constant.

3.4. *Parameters*

The parameter setting is: $S \approx 100$ cells d^{-1}, $D = 1$ d^{-1}, $P = 1.5$ d^{-1}, $P_1 = 500$, $P_2 = 10^6$, $F = 0.01$, $T = 10^5$. Buffering ensures that large X_i populations (i.e. $F \cdot X_i \approx P_1$) cannot be stimulated by small antigen and/or anti-idiotype concentrations [20]. The virgin population density equals $S/D \approx 100$ cells. The influx is slightly different for each clone (to prevent settlement into unstable equilibria): S has a mean of 100 cells per day with a 10% standard deviation. Maximum proliferation proceeds at a rate $P - D = 0.5$ cells per cell per day (this corresponds to a doubling time of about 16 h).

3.5. *Complementary matching*

Each population X_i ($0 \leq i \leq N$, i.e. antigen or clone) is characterised by a random shape: here by a bit-pattern. Populations interact with one another if their shapes can be matched complementarily. Interaction requires such a match over only part of the shape: this part is the mask. Patterns can be shifted along each other in order to find the location of the best possible match. For instance, the pattern 0000*101100* (i.e. of length 10) matches perfectly to the pattern *1101001*100 over a mask of length 8 if the patterns are shifted two positions (as is indicated by the italics). This rule is similar to procedures described previously [20, 29]. A perfect match yields an affinity of one (i.e. $A_{ij} = 1$). The affinity is reduced in proportion to the number of mismatches within the mask for the best possible alignment. The affinity is set to zero if the number of mismatches exceeds a certain threshold. We thus generate symmetric affinity matrices. The connectivity of such a matrix is defined as the average number of interactions per clone (i.e. the number of non-zero elements per row of the A matrix). For each matrix the interactions with antigen (i.e. the A_{i0} elements) allow for an ordering of the clones into the conventional Ab_1, Ab_2, Ab_3, to Ab_n levels. We will concentrate on the selection of the Ab_1 repertoire (i.e. of the clones that see the self antigen).

4. Results

We reported previously that each clone attains one of three stable states: virgin, aggressive (or immune) or suppressed [19, 20]. The virgin state is the balance between influx and efflux (i.e., $X_i \approx S_i/D$); this state involves hardly any idiotypic stimulation (i.e. $G(X_i, \alpha X_i) \approx 0$). In the aggressive state a clone is enlarged (due to proliferation); aggressive clones proliferate further whenever they are further stimulated (i.e., whenever αX_i increases). In the suppressed state a clone is also enlarged. A suppressed clone is overstimulated: it is smaller than an aggressive clone and cannot proliferate any further (i.e., whenever αX_i increases, proliferation decreases). The aggressive and suppressed states are maintained by *reciprocal stimulation* between the complementary clones. An aggressive and a suppressed clone proliferate at an equal rate. However, they occupy different positions on the crosslinking curve G( ). An aggressive clone is located to the left of the maximum of the bell-shaped curve, a suppressed clone to the right of it.

These three states are the immediate consequence of the simple idiotypic interactions of our model. For instance, Fig. 1 depicts the 0-isoclines of two complementary clones (X_1 and X_2 for $A_{12} = 1$). At the stable intersects we have indicated the respective states for X_1 (i.e. V: virgin, A: aggressive, and S: suppressed). We showed previously that antigenic stimulation of X_1 leads to a switch from the virgin

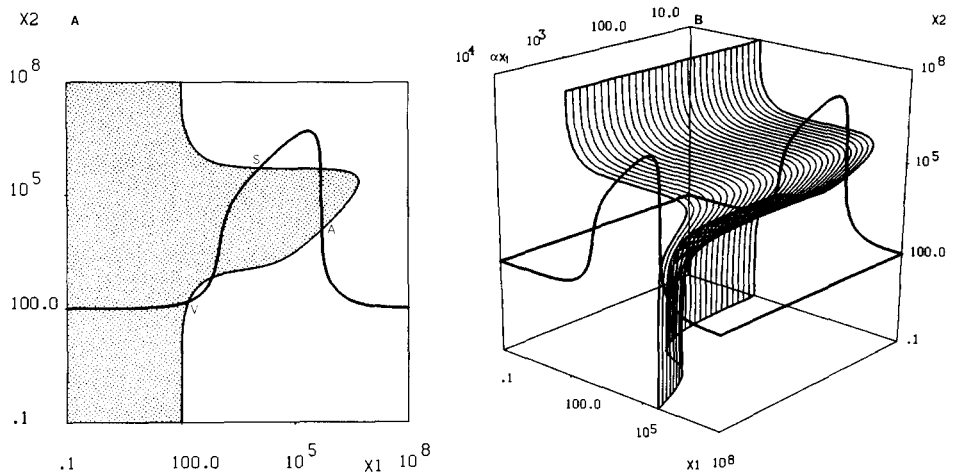

Fig. 1. The equilibrium states of a 2-D model. The figure depicts the state of the system as a function of various concentrations of two complementary clones (X_1 and X_2). This state space is divided into regions of qualitatively different behaviour (by the 0-isoclines for X_1 and X_2). X_1, for instance, increases in the shaded region. At a 0-isocline the derivative equals zero (see Segel [36] for further explanation). At intersections of 0-isoclines one thus finds equilibria (because all derivatives equal zero). (A) The $X_1' = 0$ and $X_2' = 0$-isoclines. At the stable intersects we have indicated the stable states for X_1: V (virgin), A (aggressive), and S (suppressed). (B) The same isoclines as a function of further anti-idiotypic (i.e. αX_1) stimulation of X_1. The virgin state disappears whenever X_1 sees too many complementary clones (i.e. whenever αX_1 is too high). (Isoclines are drawn by means of GRIND [37].)

to the aggressive state. We concluded that the (2-D) network is able to remember such an exposure to antigen specifically [19, 20].

In Fig. 1b the same isoclines are drawn as a function of further idiotypic stimulation of X_1 (i.e., αX_1 increases along the additional axis). The figure shows that the virgin state disappears whenever X_1 is stimulated by too many complementary clones (i.e., whenever αX_1 is sufficiently large). Hence, whenever the connectivity of the network exceeds a certain threshold, clones always proliferate and can only be aggressive or suppressed (see also [20]). Thus, such networks react autonomously, i.e., without exposure to antigen (cf. ref. [15]). We have facilitated this autonomous behaviour in the current parameter setting; we have deliberately enlarged the parameter determining the virgin state (i.e. S_i 10-fold) and lowered the parameter determining the onset of proliferation (i.e. P_1 2-fold) in comparison with our previous model [20]. In the subsequent high-D networks the connectivity will always be such that the networks respond autonomously. This requires only a few (<5) connections per clone.

Fig. 2a depicts an example of a 100-D network with an average connectivity of seven connections per clone. The network is based on complementary matches of idiotypes of length 30 with a mask of length 20. The (6) clones of the Ab_1 level are stimulated by a self antigen. Therefore, the Ab_1 clones are the first to proliferate. As a consequence the Ab_2 clones respond and the whole network is activated (Fig. 2a). This network settles into a steady state with 35% of the clones in the aggressive state and 65% in the suppressed state. We consider this distribution of aggressive and suppressed clones to be the actual repertoire. Due to the autonomous activation, this network behaviour remains very similar in the absence of the self antigen (see e.g. ref. [20]).

4.1. *Repertoire specificity*

In Fig. 2b we analyse the specificity of the repertoire as it is induced by self antigen. Two networks (i.e. that of Fig. 2a and a randomly chosen other one) are stimulated with self antigen or with slight variants of this original self antigen. The variants are made by changing a few bits in the original antigen bit-pattern. These variations are plotted along the *X*-axis (zero changed bits define the original self antigen). The *Y*-axis indicates the difference between the repertoire induced by each variant and that induced by the original self antigen (i.e. we plot the

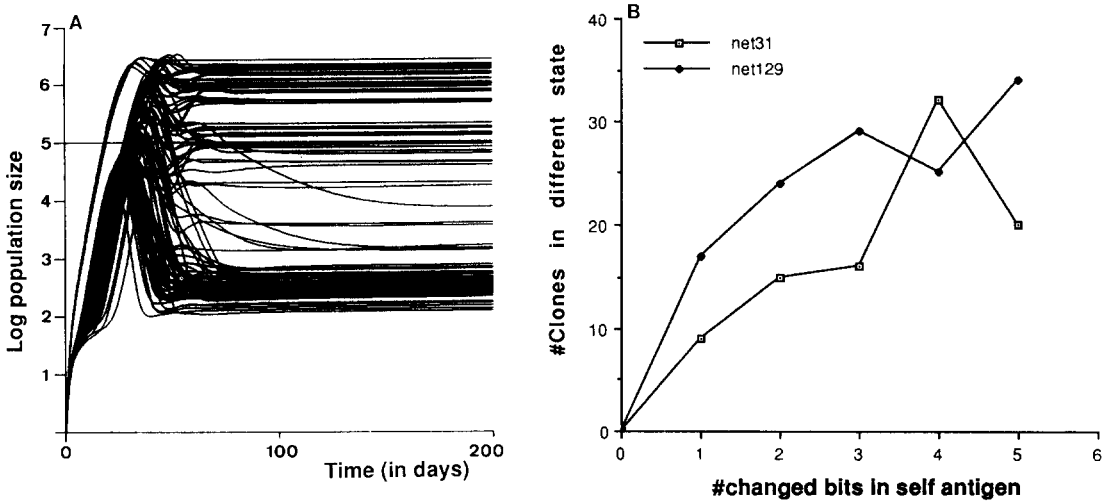

Fig. 2. Examples of 100-D networks with stimulatory self antigen. (A) The behaviour of one network, showing the respective clone sizes, plotted in time. The first clones to proliferate are the self-reactive clones. This in turn activates the Ab_2, the Ab_3, and the Ab_4 clones. Within ~100 days the network settles into an equilibrium, i.e. into the actual repertoire, in which all clones are aggressive or suppressed. (B) The specificity of the Ab_1 repertoire. Two networks (i.e. ◆ and ⧈) are stimulated with slight variants of the self antigen. Variants are made by changing a few bits in the antigen bit-pattern. We score the number of clones that settle into a state different from the state into which they settle with the original self antigen. The figure shows that the repertoire depends specifically on the shape of the antigen. (Numerical integration by means of a variable time-step Runge-Kutta method [38].)

number of clones that settle into a different state). The figure shows that repertoire induction is very specific. A variation in the original bit-pattern at only one position, makes about 10% difference in the selected repertoires.

Early manipulations with a single dose antibody (here X_i) also influence repertoire development in this model. Introduction of 1000 units of a randomly chosen idiotype around day 10 or 20 suffices for a settlement into a different stable equilibrium, i.e., into a different adult repertoire (not shown). These results confirm the experimental observations [3–6] that early manipulations (with antigen or idiotype) specifically influence repertoire development.

4.2. *Repertoire selection*

Repertoire selection thus turns out to be specific, but is it also determined by the nature of the antigenic signal? Fig. 3 depicts the Ab_1 repertoires of 10 randomly different networks. Each network is confronted with 3 different circumstances. The self antigen either (1) induces tolerance (i.e. the left bars in Fig. 3a), or (2) is absent (i.e., a control situation; the middle bars), or (3) activates complementary clones (i.e., the right bars). Due to the random differences in network structure, the total number of clones in the Ab_1 level varies between the networks. This variation is plotted along the *X*-axis. The *Y*-axis depicts the number of (Ab_1) clones that finally end up in the aggressive state. The average connectivity is (again) ~7 connections per clone. The presence and the nature of the self antigen clearly influence the selection of the self-reactive repertoire. The bars on the right are generally larger than the (middle) control bars, whereas the bars on the left are generally smaller. Thus clones activated by the self antigen tend to become aggressive, and clones tolerized by the self antigen tend to become suppressed. These results are summarised in Fig. 3b, which depicts the total number of clones in the aggressive and suppressed state for each situation.

The fact that tolerized clones tend to become suppressed is interesting. If the network interactions were non-existent, all tolerized clones would eventually respond vigorously to the self antigen. Since the antigen is stimulatory in the periphery, and because tolerance induction in newborn cells is incomplete (i.e. $S_i/(T \cdot A_{i0} \cdot X_0) \neq 0$), each cell that escapes from the tolerance induction will proliferate (and give rise

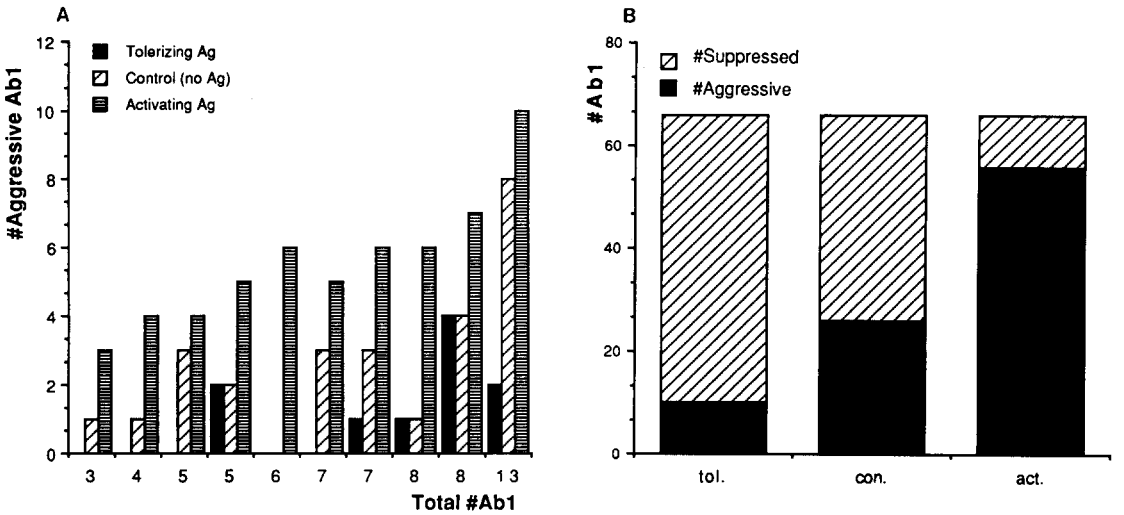

Fig. 3. The self-reactive repertoire in 10 randomly different 100-D networks. The networks are based on idiotypes of length 30 sites with a mask of 20 sites. This generates a connectivity of ~7 interactions per clone. (A) The number of aggressive self-reactive clones. The self antigen is either tolerizing (left bars), absent (middle bars), or stimulatory (right bars). Due to random variation the number of self-reactive clones varies from 3 to 13 (see the *X*-axis). The *Y*-axis plots the number of these self-reactive clones that become aggressive (the remaining clones become suppressed). (B) The total number of aggressive and suppressed clones in these 10 networks. The black bars depict the total number of aggressive clones, the striped bars the suppressed clones. The nature of the self antigen clearly influences repertoire selection.

to a large clone) once it arrives in the periphery. A clonal deletion process needs a fail-safe mechanism. Apparently, idiotypic network interactions tend to provide this. The time delay with which the tolerized clones appear in the periphery turns out to be sufficient for the network to suppress these clones. (The rate at which tolerized cells appear in the periphery is determined by our T parameter, which must be large.) This leads to the important conclusion that network interactions may play a facilitating role in the process of self non-self discrimination. In conclusion, network interactions do shape the repertoire.

However, this selection is only facilitating, and is insufficient for self/non-self discrimination. In the absence of the tolerance process (see the right bars), the self-reactive clones tend to become aggressive (i.e., autoaggressive). Moreover, even in the presence of the tolerance process, self/non-self discrimination is only a tendency, and hence is not a very reliable process. In Fig. 3b, 10 out of 66 tolerized clones end up in the aggressive (i.e., autoaggressive) state. Note that in the buffering parameter (F) in the model ensures that autoaggressive clones do not explode. If the buffering were to be omitted the network behaviour would be disrupted by the autoaggression.

4.3. *IgG versus IgM B cells*

The general idea of idiotypic repertoire selection involves two classes of B cells. It is argued that B cells appearing early in ontogeny form a network which plays a role in the selection of the clones appearing later. A special lineage of B cells might be responsible for these early networks [6, 12–14]. For convenience, we here refer to these two classes as the *IgM* and the *IgG* B cells. The networks are doubled in size and split into one half of early IgM clones, and one half of late IgG clones. The new class of IgG clones only starts to appear after (an arbitrary period of) 200 days; this is after the repertoire of the early IgM clones has settled (see Fig. 2a). The networks are again based on idiotypes of length 30 with a mask of 20 sites. Thus during the first 200 days the IgM network again has a connectivity of about seven IgM connections per clone. However, once the size of the network doubles (due to the appearance of the IgG clones), this connectivity also doubles. After day 200 the connectivity is about 13 connections for each clone (of both classes). We investigate the same three

situations: the self antigen is either (1) tolerizing, (2) absent, or (3) stimulatory.

It is unclear how the IgM network will select the IgG repertoire. The IgM network is shaped by the self antigen: activated self-reactive IgM clones (i.e. Ab_1's) *tend* to become aggressive. Hence the Ab_2 level of the IgM network should *tend* to become suppressed. Self-reactive IgG clones (i.e. Ab_1's) are thus expected to be stimulated by antigen *and* the IgM Ab_2's. Moreover, the Ab_2's of the new IgG clones are expected to be suppressed by the self-reactive (i.e. Ab_1) IgM clones. Thus, one might expect self-reactive IgG clones also be become aggressive. Note that this should lead to autoimmunity. Tolerized IgM clones, on the other hand, *tend* to become suppressed. Suppression is maintained by the complementary (i.e. Ab_2) IgM clones. Thus, self-reactive IgG clones, being delayed by the tolerance process and being suppressed by the Ab_2 IgM clones, are expected to become suppressed. In conclusion, the additional selection pressures of the IgM network might given rise to a more pronounced selection in the IgG repertoire.

Fig. 4 again depicts the total number (from 5 different networks) of aggressive and suppressed self-reactive clones. The left 3 bars depict the IgM repertoire at day 200 (i.e., before the IgG clones emerge). The 6 bars on the right depict the late repertoire (at day 500) of the IgM and the IgG clones. The early IgM repertoire is similar to that depicted in Fig. 3; this is obvious since it is the same situation. However, the late IgM repertoire is also identical to the early IgM repertoire (i.e. bars 1–3 are equal to bars 4–6). Thus the extension of the network with IgG clones has no effect on the established early repertoire. The repertoire of the self-reactive IgG clones (bar 7–9) resembles the IgM repertoire qualitatively. Like the IgM clones, tolerized IgG clones tend to become suppressed, and activated IgG clones tend to become aggressive. This tendency is, however, of the same magnitude as that of the IgM repertoire. Apparently, the additional selection pressures of the IgM network have very few specific effects.

A difference between the late IgM and IgG repertoires is the high percentage of suppressed clones in the latter repertoire. This is a general influence of the IgM network on the IgG network, which is due to the increase in network connectivity that occurs

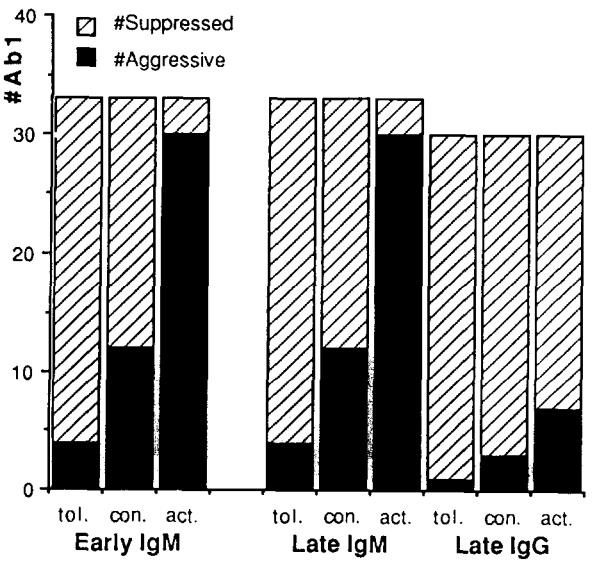

Fig. 4. The summed early and late IgM and IgG self-reactive repertoires of five different 200-D networks with tolerizing, absent, or stimulatory self antigens (see Fig. 3). The three bars on the left depict the actual self-reactive IgM repertoire at day 200 (i.e. just before the IgG clones appear). The six bars on the right depict the late IgM (left) and IgG (right) repertoires. The lengths and masks of the idiotypes are the same as those in Fig. 3; the connectivity is now ~ 13 interactions per clone (due to the doubled dimension). The IgM network influences the IgG repertoire non-specifically.

when the IgG clones appear. We showed previously that an increase in network connectivity increases the proportion of suppressed clones [17]. We conclude that, in our model networks, the early IgM network can only shape the late IgG repertoire in a very non-specific way (i.e. by increasing the percentage of suppressed clones).

4.4. *Robustness*

This poor repertoire selection by the IgM network might of course be a consequence of the drastic simplifications that we had to make in order to develop a reasonable model. However, since the assumptions of our model (i.e. the general crosslinking function and the source and decay terms) seem well-established facts, we consider our results to be general, and not typical only of the networks developed here. However, in order to test the robustness of our results we now investigate two variations of the assumptions of the model.

4.5. *Asymmetric interactions*

It is generally argued that the early ($CD5^+$) IgM clones are involved in numerous idiotypic interactions, whereas the late IgG B cell clones have few (or no) idiotypic interactions [10]. The early IgM clones preferentially use specific V regions for assembling the antigen receptor [11]. Hence the high idiotypic connectivity of these early IgM clones could be due to different molecular properties of these V regions. A problem that remains, however, is the nature of the idiotypic interaction between an early IgM clone and a late IgG clone. The IgM B cells might be able to interact with the IgG cells, but the IgG clones may fail to recognise the IgM clones. Although such an asymmetric molecular interaction seems strange, we can easily incorporate it in our model. This is achieved by lengthening the mask for the IgG clones, i.e., by assuming that IgG clones require a stronger match. For reasons of equivalence, the binding of IgG to the antigen is kept similar to that of IgM.

Fig. 5 summarises the results of 5 such asymmetric networks. The IgM clones again have a mask of 20 sites, and the IgG clones one of 22 sites. The connectivity of the IgM clones is hence the same as above (i.e. ~7 and ~13 connections per clone). The connectivity of the IgG clones is about ~3 connections per clone (we have omitted a few clones that were not connected to the network). The early IgM repertoire is identical to the one described in Fig. 4 (we used the same five networks). However, the late IgM repertoire now deviates from the early IgM repertoire. Interestingly, the late IgG repertoire also differs from that in Fig. 4. Stimulated IgG clones still tend to become aggressive, but tolerized IgG clones no longer tend to become suppressed. The tolerized repertoire resembles that of the control situation. Due to the lower connectivity, idiotypic interactions no longer facilitate the process of self/non-self discrimination.

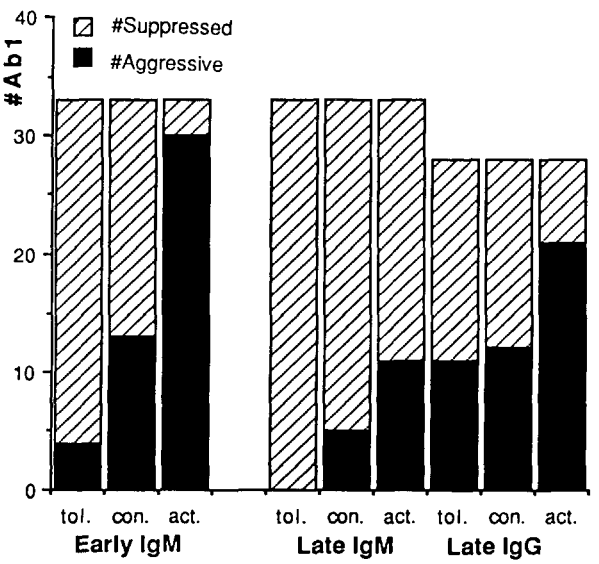

Fig. 5. As Fig. 4, but with a lower connectivity for the late IgG clones. The connectivity of the IgM clones is the same, that of the IgG clones is based on idiotypes with a length of 30 sites and a mask of 22 sites. This generates a connectivity for the IgG clones of ~3 interactions per clone. The IgG network influences the IgM repertoire non-specifically.

The late IgM repertoire has changed, in that it now contains a higher percentage of suppressed clones. This general effect is caused by the increase in network connectivity due to the appearance of IgG clones (as was explained above). The fact that it is now the IgG clones that (non-specifically) influence the IgM repertoire, and not vice versa, is a consequence of the low connectivity of the IgG clones. Because IgG clones are only stimulated by a few complementary clones (i.e. for IgG clones αX_i is small), such clones tend to become aggressive. Due to the asymmetry, however, the IgM clones are overstimulated (i.e. for IgM clones αX_i is large). We conclude that the established IgM repertoire altogether fails to select the IgG repertoire. The selection that takes place is again non-specific and is now mediated by the IgG repertoire.

Although the IgG repertoire seems to select the IgM repertoire, both repertoires are specifically determined by the self antigen. Changing single bits in the self antigens generates similar changes in all three repertoires (i.e. early and late IgM, and IgG), such as those described for the early IgM repertoire in Fig. 2b).

4.6. *Network structure*

The specification of the lengths and masks of our idiotypes is based on the idea that several determinants (i.e. idiotopes) are present on each idiotype. As a consequence of this idea, paths through the network diverge widely into unrelated branches. The structure of biotic (and our theoretical) networks is

thus expected to be degenerate. Because it might be argued that the present failure of repertoire selection is due to the degenerate topologies of our networks, we here allow for more structure in the networks.

Fig. 6 summarises results of networks that are based on idiotypes of which both length and mask are 8 sites. Thus complementary matching no longer involves any shifting of idiotypes. Idiotypes are only considered to match complementarily if the match is perfect (yielding $A_{ij} = 1$), or if there is one mismatch (yielding $A_{ij} = 0.5$). The resulting connectivity (for the *entire* 200-D network) is ~7 connections per clone. The results depicted in Fig. 6 are very similar to those described in Fig. 4. Due to the lower connectivity, however, the overall percentage of suppressed IgG clones is somewhat lower. We conclude that our results do not depend on the degeneracy of the networks that we have analysed. The failure of specific repertoire selection seems to be a robust property of these networks.

5. Discussion

These results show that whenever large idiotypic networks mature in the presence of an additional complementary shape (here self antigen), this shape specifically influences repertoire selection. Repertoire selection can therefore easily be manipulated by the introduction of specific shapes at certain time intervals. However, although the repertoire selected by each shape is highly specific, the shape of the repertoire can only *tend* to reflect the nature of the antigenic signal. If it is assumed that a second repertoire of late B cells is selected by an earlier established network, results become even worse. The early and late network do not seem to be able to influence each other specifically.

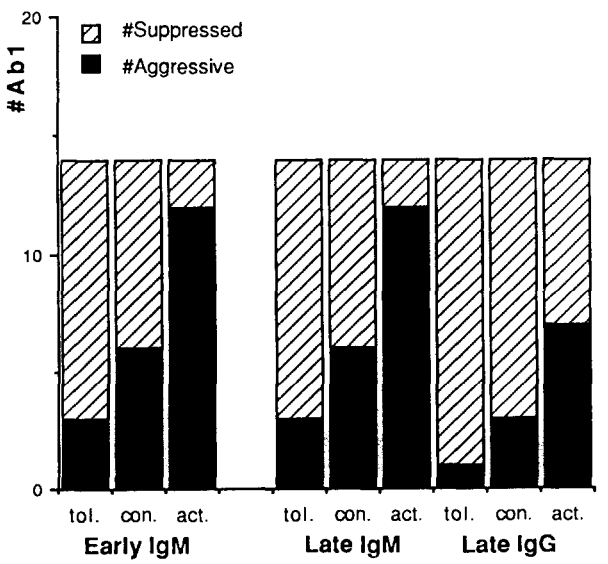

Fig. 6. As Fig. 4, but for idiotypes of a length of 8 sites and a mask of 8 sites. This allows for more structure in these networks. The connectivity for the entire 200-D networks is ~7 connections per clone. The results remain comparable to those of Fig. 4.

The networks were shown to play a facilitating role in self/non-self discrimination. Cells that escape from tolerance *tend* to become suppressed by the network. We also concluded however that, since suppression is only a tendency, this is not a very reliable process. Empirical evidence, nevertheless, supports this idea. In the case of T cell tolerance, Zöller [30] concludes that "remaining autoreactivity is controlled by the establishment of an equilibrium between idiotypic and anti-idiotypic elements". Other authors consider self/non-self discrimination to be a distributed network property resulting from self-reactive activity [7, 31, 32]. However, whether or not self/non-self discrimination is distributed, our networks, which are based on complementary matching rules, fail to complete this (informatically very complex) task of controlling a very large number of autoreactive clones.

The formulation of mathematical models necessarily involves drastic simplifications. Nevertheless, we think that the current model grasps the essence of the processes by which idiotypic networks could select the antibody repertoire. One simplification is the continuous influx of Ly1 or IgM B cell clones in our model. It is known that Ly1 B cells are only produced during perinatal life [12, 13]. This was ignored in order to make our model more general, i.e., our IgM clones need not be Ly1 clones. Moreover, because all IgM clones proliferate in our model, they are relatively independent of the influx of newborn cells. Thus, if the reduction in influx were incorporated it should not make any difference.

Another omission is the fact that we have ignored T cells. The early IgM repertoire might be independent of helper T cells (as we have assumed), but the late IgG B cells are certainly not. Moreover, some authors have suggested that Ly1 B cells select the helper T cell repertoire which, in turn, determines

the late (here IgG) B cell repertoire [23]. Additionally, several authors [7, 33] have demonstrated that the development of T and B cell repertoires depend stringently upon one another. Huetz et al. [33] show that the selection of the "natural" B repertoire can only take place in the presence of activated T cells. Unfortunately, the role of T cells in idiotypic networks is unpredictable unless one knows whether anti-idiotypic T cells are MHC-restricted, and whether they form B_1-T_2-B_3 or T_1-T_2-T_3 networks, or just assist in B_1-B_2-B_3 networks [21]. Thus it remains unclear how the incorporation of T cells would affect our results. However, since repertoire selection would still be based on complementary matching rules, it is at least possible that the present model still grasps the essence of these ideas.

Several empirical reports have established that, under normal physiological circumstances, idiotypic interactions prevail during early life. From these data it is concluded that idiotypic interactions are *functional*. Indeed, the findings with the paper networks [4–6] (which were discussed in Section 2) clearly demonstrate that early idiotypic interactions influence repertoire development. A recent study [34] provides evidence for complex network dynamics among natural antibodies in normal mice. Treatment of adult mice with low doses of one of two complementary (IgM) idiotypes reduces the *normal* fluctuations in the serum concentration of both idiotypes for periods up to three months. Hence, immune network dynamics could be directly observed. Note however, that other studies [14] fail to find any influence of transferred Ly1 B cells on the repertoire selection in recipient mice.

Our results suggest, however, that whenever these ongoing network interactions are interpreted in terms of their specific self environment, they fail to account for the desired (distributed) properties. In our formal network early idiotypic interactions are also prevalent and functional (in terms of manipulations); nevertheless they can only form noisy background (cf. ref. [35]). In order to test whether our conclusions can indeed be generalized to other formal networks, we are currently extending our model with: (1) an equation for free antibodies, (2) sequential appearance of connected idiotypes, (3) replacement of unsuccessful idiotypes by novel ones, and (4) with several self antigens.

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