

MEMORY BUT NO SUPPRESSION IN LOW-DIMENSIONAL SYMMETRIC IDIOTYPIC NETWORKS

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We present a new symmetric model of the idiotypic immune network. The model specifies clones of B-lymphocytes and incorporates: (1) influx and decay of cells; (2) symmetric stimulatory and inhibitory idiotypic interactions; (3) an explicit affinity parameter (matrix); (4) external (i.e. non-idiotypic) antigens. Suppression is the dominant interaction, i.e. strong idiotypic interactions are always suppressive. This precludes reciprocal stimulation of large clones and thus infinite proliferation. Idiotypic interactions first evoke proliferation, this enlarges the clones, and may in turn evoke suppression. We investigate the effect of idiotypic interactions on normal proliferative immune responses to antigens (e.g. viruses).

A 2-D, i.e. two clone, network has a maximum of three stable equilibria: the virgin state and two asymmetric immune states. The immune states only exist if the affinity of the idiotypic interaction is high enough. Stimulation with antigen leads to a switch from the virgin state to the corresponding immune state. The network therefore remembers antigens, i.e. it accounts for immunity/memory by switching between multiple stable states. 3-D systems have, depending on the affinities, 9 qualitatively different states. Most of these also account for memory by state switching.

Our idiotypic network however fails to account for the control of proliferation, e.g. suppression of excessive proliferation. In symmetric networks, the proliferating clones suppress their anti-idiotypic suppressors long before the latter can suppress the former. The absence of proliferation control violates the general assumption that idiotypic interactions play an important role in immune regulation. We therefore test the robustness of these results by abandoning our assumption that proliferation occurs before suppression. We thus define an "escape from suppression" model, i.e. in the "virgin" state idiotypic interactions are now suppressive. This system erratically accounts for memory and never for suppression. We conclude that our "absence of suppression from idiotypic interactions" does not hinge upon our "proliferation before suppression" assumption.

1. Introduction. The immune system consists of a large number ($> 10^7$) of different clones of lymphocytes. The lymphocytes comprising such a clone all share identical antigen receptors. The variable (V) regions of these receptors are generated by random processes such as somatic recombination and somatic mutation (Early *et al.*, 1980; Berek *et al.*, 1985). The primary repertoire can therefore be visualized as a large (random) array of receptor molecules. Out of the total array of (random) antigen receptors, an antigen "selects" those structures (those paratopes) that provide a mirror image of the antigenic structure (the epitope, Jerne, 1974). This is clonal selection theory (Burnet,

1959). Only the clones with a receptor (paratope) that fits to one of the antigen epitopes become activated and proliferate. Following a period of proliferation, the B-cells acquire an effector function: they secrete their antigen receptors as antibody molecules which in turn eliminate the antigen. In the present paper we ignore the important facts that most B-cell responses require help from helper T cells.

Jerne proposed his network hypothesis in 1974. Network theory is an implication of the axiom that the (random) primary repertoire is complete (Jerne, 1984). Jerne argued that the V-regions of lymphocyte receptors should also be recognized by other receptor molecules (paratopes). The antigenic determinants provided by a receptor molecule are called idiotypes (Jerne, 1974). The interaction between two receptor molecules (i.e. a paratope seeing an idiope) is an idiotypic interaction. The set of such interactions defines the immune network. Via their idiotypic anti-idiotypic interactions, different clones should thus be able to interact in either a stimulatory or a suppressive manner. The existence of network interactions, i.e. of idiotypic interactions, has since been proved with numerous experimental data (Jerne, 1984). Moreover the possibility that the functioning of immune systems is regulated by network interactions seems intriguing. Several authors have suggested that immune networks may account for: (1) the maintenance of steady states (Jerne, 1974; Cooper *et al.*, 1984); (2) the control of proliferation (Jerne, 1974; Cooper *et al.*, 1984); (3) the development of immunological memory (i.e. immunity, see Jerne, 1974); (4) low zone tolerance (Jerne, 1974); (5) self-non-self discrimination (Hoffmann, 1975; Jerne, 1984; Holmberg *et al.*, 1986). Similarly, idiotypic networks have aroused considerable theoretical interest (Richter, 1975; 1978; Hoffmann, 1975; 1979; 1980; 1982; Hiernaux, 1977; Seghers, 1979; Gunther and Hoffmann, 1982; Fey *et al.*, 1984; Fey and Eichmann, 1985; Kaufman *et al.*, 1985; Kaufman and Thomas, 1987; Farmer *et al.*, 1986; De Boer, 1988; Segel and Perelson, 1988; Perelson, 1988).

Jerne (1974) visualized the immune response as the "escape from suppression": in the absence of antigen (i.e. in the virgin state) network interactions are suppressive. According to Jerne (1974) this would guarantee the existence of a stable virgin state; see however De Boer and Hogeweg (in press). Antigens, e.g. viruses, perturb the suppressed state of the network, which results in proliferation and antibody production. For T cells similar "escape from suppression" models were proposed (based on the analyses of limiting dilution curves) (Cooper *et al.*, 1984; Fey *et al.*, 1984; Fey and Eichmann, 1985).

Hoffmann (1975; 1978; 1980) developed a 2-D model (the "plus-minus" model) of the idiotypic network. This model incorporates the very important assumption that idiotypic interactions are symmetric, i.e. if clone X_i recognizes X_j , X_j should also recognize X_i . If recognition corresponds to complementary

matching, an idiotypic interaction should indeed be symmetric. Moreover, cells are activated by receptor crosslinking (Hoffmann, 1980), which is a process that cannot discriminate between paratope and idiotype. Additionally a considerable amount of experimental evidence favours the idea that idiotypic interactions are symmetric (Hoffmann, 1980; Jerne, 1984). However, although Hoffmann's (1975) original suggestion that idiotypic interactions are symmetric is very attractive, it has been followed by only few authors (Fey *et al.*, 1984; Fey and Eichmann, 1985; Jerne, 1984). Note that symmetric network theory disposes of the distinction between paratope and idiotype; instead it regards V-regions as "sticky ends" (Hoffmann, 1980).

Here we adopt the symmetric network theory. However we abandon the "escape from suppression" hypothesis: in our model we assume that the virgin state is devoid of (suppressive) network interactions. Such a model accounts for a (stable) virgin state maintained solely by the influx and turnover of newborn cells. Furthermore, we think that the most important role that suppression could play in regulating immune reactions is the control of excessive proliferation. This requires strong suppressive interactions when lymphocyte populations are large, and not, as "escape from suppression" implies, in the virgin state. We therefore assume that it is the large anti-idiotypic populations that are suppressive.

Here we investigate the effect that idiotypic interactions can have on the (normal) proliferative immune response to external (i.e. non-idiotypic) antigens. The effect of idiotypic interactions is studied as a function of (1) the affinity of the idiotypic interaction and (2) the number of idiotypic interactions (i.e. the size and connectance of the network). We thus require a model that incorporates: (1) influx and efflux of newborn cells; (2) symmetric stimulatory and suppressive idiotypic interactions; (3) affinity; (4) interaction with external antigens. None of the previous models described in the literature adequately incorporates these requirements; we therefore develop a new model.

2. Previous Models. Hoffmann (1982; 1987) reviewing the previous idiotypic network models concluded that most of the models were inadequate because they: (1) were asymmetric; (2) lacked influx or efflux; (3) ignore clonal selection; (4) could not fulfil the Unpredictability Axiom. The Unpredictability Axiom states that the strength (i.e. affinity) of the idiotypic interactions must vary considerably, and that results obtained with network models should be robust to small variations in the interaction strength. In this review, Hoffmann however does not engage in a critical discussion of the symmetric network models put forward by him (1975; 1979; 1980) and by Gunther and Hoffmann (1982). These models nevertheless have a number of inadequacies.

The Hoffmann models consists of two complementary clones, X_+ and X_- ; idiotypic interactions induce proliferation (k_1) and suppression by IgM (k_2)

and by IgG (k_3). The cells have a turnover rate k_4 and appear at a rate S from thymus or bone marrow:

$$dX_+/dt = X_+ \cdot (k_1 \cdot X_- \cdot E_1 - k_2 \cdot X_- \cdot E_2 - k_3 \cdot X_-^2 \cdot E_3 - k_4) + S.$$

The "effectivity" (E_i) of the idiotypic interactions is determined by the concentration of antigen specific (T cell) factors of either the "+" and the "-" specificity:

$$E_i = 1/(1 + (X_+ \cdot X_-/C_i)^{N_i}), \quad i = 1, 2, 3,$$

for which (Hoffmann, 1979):

$$N_1 = 1, \quad N_2 = 2, \quad N_3 = 2.$$

The saturation or effectivity terms of these models incorporate the product of the idiotypic and anti-idiotypic ($X_+ \cdot X_-$) population as a "whole". This product is large, i.e. the terms saturize, if (1) both populations are large, and (2) one of the two populations (X_+ or X_-) is sufficiently large. If this product is large, the X_+ population proliferates at a rate $k_1 \cdot C_1$ whatever the actual size of the X_+ population. Similarly, IgM suppression approximates zero in all circumstances in which this product is large. Only IgG suppression depends on the X_+/X_- ratio. Per individual cell, these terms thus allow for infinite proliferation or suppression rates. By contrast, the saturation or efficiency of an idiotypic interaction between populations should remain interpretable at the level of the individual cells that comprise these populations. Indeed, the physiological constraints of individual cells (e.g. a minimum time span required for cell division) suffice for defining more realistic saturation effects. Moreover, the "global" effectivity terms of the Hoffmann models severely hamper the extension of these models with external antigen and/or additional lymphocyte clones.

The Hoffmann models omit affinity and external antigen. Incorporation of affinity is relatively easy and enables us to investigate whether these models fulfil the Unpredictability Axiom. It appears that the existence of four stable states, as described by Hoffmann (1979) and Gunther and Hoffmann (1982), requires a high affinity interaction. If affinity is lowered the Hoffmann (1979) model loses the two immune states, whereas the model of Gunther and Hoffmann (1982) loses the suppressed state and has only one immune state. The extension of the Hoffmann models with antigen is, by contrast, very complex because of the global nature of the efficiency terms. Stimulation with antigen should increase the production of T cell factors, and should therefore block the idiotypic interaction between the plus and minus clone.

We have tested a variety of implementations of external antigens in the Hoffmann (1979) model. These antigens grow exponentially and are eliminated

by the IgM and IgG terms. This however failed to generate the classical picture of an immune response: proliferation followed by antigen regression, and finally settlement in an immune state. Depending on its initial size and/or its growth rate, antigen is either immediately eliminated or expands infinitely. Importantly, in our experiments with these models, the system never ended up in the suppressed state if we started with "physiological" initial conditions (i.e. in the virgin or immune state with or without antigen). Thus Hoffmann's suppressed state is "unattainable". We conclude that although the Hoffmann models define "escape from suppression" systems that account for switching to immune states and from IgM to IgG, the models are inadequate for investigating the role of idiotypic interactions in proliferative immune reactions to antigens.

3. *A New Network Model.* In our model we make two important choices: (1) idiotypic interactions are symmetric; (2) all populations are identical except for the antigen receptor (i.e. the idiootype). We choose for symmetry because it seems the most minimal (and, in our opinion, most likely) implementation of a recognition interaction based on complementary matching and receptor crosslinking. Secondly, we only consider one type of lymphocyte, i.e. we do not differentiate between "helper" and "suppressor" interactions, because: (1) idiotypic network theory was originally (Jerne, 1974) defined for identical clones of B-lymphocytes; (2) symmetric network theory (Hoffmann, 1979; 1980; Gunther and Hoffmann, 1982) has demonstrated that identical populations adopt "helper" or "suppressor" functions (i.e. phenotypes) depending on the idiotypic circumstances.

Although these two choices enable us to define a simple and attractive model of the (possibly) very complex idiotypic network, they necessitate another (third) choice: the distribution of stimulatory and inhibitory interactions. Idiotypic interactions are empirically known to be both positive and negative. During an immune reaction the responding idiootype can evoke the production of anti-idiotypic antibodies (Cosenza, 1976; Cerny, 1982). This is a positive interaction similar to a proliferative response to antigen. Administration of anti-idiotypic antibodies can both enhance (Eichmann and Rajewsky, 1975; Vakil and Kearny, 1986; Bernabe *et al.*, 1981) and suppress (Hardt *et al.*, 1972; Eichmann, 1974; Vakil and Kearny, 1986) the idiotypic immune response. Because all populations are identical in our model, and because idiotypic are symmetric, we have no *a priori* basis to discriminate between stimulatory and inhibitory interactions. The effect of an idiotypic interaction can therefore only depend on the (temporal) idiotypic circumstances, i.e. on the concentration of idiootype and anti-idiootype, and on the affinity of the interaction.

In contrast to what "escape from suppression" (Jerne, 1974) implies, we assume that idiotypic interactions are suppressive when the concentrations of

anti-idiotypic are large. In accordance with our previous work on proliferative immune reactions (De Boer and Hogeweg, 1986; 1987a,b) we thus assume that populations have to enlarge (by proliferation) before they can have a significant (here suppressive) effect. Idiotypic interactions are therefore absent or low if the anti-idiotypic populations are small, e.g. in the virgin state. In between, i.e. for intermediate-sized anti-idiotypic populations, interactions are stimulatory. In fact, this "proliferation before suppression" assumption corresponds to the widely accepted idea that the immune response is most vigorous at intermediate antigen concentrations. We, nevertheless, test the robustness of our results for our "proliferation before suppression" assumption by analysing an "escape from suppression" version of our model.

Clones have a constant influx (S_i) of cells from the bone marrow. Cells have a constant turnover rate (D). Differences between long-lived memory cells and short-lived effectors are thus deliberately ignored. Affinity is a parameter ranging between zero (no interaction) and one (maximum interaction). We ignore the possibility of an affinity threshold, i.e. a minimum affinity required for possible interaction. A clone interacting with a number of clones, interacts with the sum of the anti-idiotypic interactions (αId = anti-Idiotype). One specific immune network can now be defined completely by an affinity matrix A . A_{ij} specifies the affinity between X_i and X_j . Symmetry in our model simply means $A_{ij} = A_{ji}$. Clones never recognize themselves (all $A_{ii} = 0$).

For external antigens we consider viruses (Vr_i) that grow exponentially. We assume, for simplicity, that virus elimination is identical to anti-idiotypic suppression. The interactions among idiotypes are thus equivalent to the interactions with external antigens, which is the basic assumption of idiotypic network theory. The interaction with antigen is however asymmetric because a virus usually activates lymphocytes but cannot suppress them (the AIDS virus is an exception to this rule). For reasons of simplicity we assume that each clone can see only one virus, and that viruses are seen by only one clone; viruses are always seen with maximum affinity. A virus is numbered according to the number of the clone that recognizes it. (If viruses are seen by more clones similar results can be obtained.)

We propose the following model:

$$\alpha Id_i = \sum_{j=1}^n A_{ij} \cdot X_j, \quad (1)$$

$$F(X_i, Vr_i, \alpha Id_i) = \frac{B \cdot X_i \cdot (Vr_i + \alpha Id_i)^M}{K_B^M + (F \cdot X_i)^M + (Vr_i + \alpha Id_i)^M} - \frac{C \cdot X_i \cdot \alpha Id_i^M}{K_C^M + (F \cdot X_i)^M + \alpha Id_i^M} \quad (2)$$

$$\frac{dX_i}{dt} = S_i - D \cdot X_i + X_i \cdot F(X_i, Vr_i, \alpha Id_i), \quad (3)$$

and for the antigens,

$$\frac{dVr_i}{dt} = R \cdot Vr_i - \frac{C \cdot Vr_i \cdot X_i^M}{K_C^M + (F \cdot Vr_i)^M + X_i^M} \quad (4)$$

The interaction function $F(X_i, Vr_i, \alpha Id_i)$ seems rather baroque. However, for $F=0$ and $M=1$ it simply specifies the difference between two conventional saturation functions (one for proliferation and one for suppression). Because we choose $K_B \ll K_C$ the proliferation term (B) outweighs the suppression term (C) at low population (X_j) densities. By choosing $C \gg B$ (i.e. the maximum suppression rate \gg maximum proliferation rate), we assume that the suppression term over-rides the proliferation term at high population densities. Suppression is thus dominant (cf. Richter, 1978). For minimalization reasons it is important to know that most of the results described in this paper can also be accounted for by simpler models, i.e. $F=0$ and/or $M=1$ (see Fig. 1).

The model incorporates a "buffering" term (F), see e.g. Richter (1978), in order to avoid that the interaction with X_i cells can become independent of the size of X_i . This would lead to unrealistic model behaviour (especially in high dimensional networks). By choosing $F \ll 1$ we make these functions follow conventional saturation kinetics whenever X_i populations are small. At the level of individual cells $F < 1$ means that one X_j cell can stimulate or suppress several X_i cells.

Parameters. We exclude long-lived memory cells from our model: all cells live about 5 days ($D=0.2 \text{ days}^{-1}$). If life-long memory occurs it can thus only be caused by a state switch of the network. An influx of about $S_i \approx 20$ cells per day per clone thus generates a virgin clone size of $S_i/D = 100$ cells. This is a reasonable estimate, De Boer and Hogeweg (1987a). A normal proliferation cycle takes about 16 hr: a continuous increase of about 0.5 cells per cell fits this estimate. The maximum per cell increase is $B-D$; B should therefore equal 0.7 days^{-1} . Suppression is dominant (i.e. $C \gg B$); we take $C = 25 \text{ days}^{-1}$. The suppression threshold is higher than the proliferation threshold (i.e. $K_B \gg K_C$): $K_B = 10^3$ and $K_C = 10^6$. At low population densities, the effect of buffering should be negligible: $F=0.01$. The virus has a doubling time similar to the lymphocyte clones: $R=0.5 \text{ days}^{-1}$. Unless explicitly stated otherwise, we use $M=2$ throughout.

In Fig. 1 we analyse the interaction between X_i ($i=1$) and X_j ($j=2$) for various interaction functions ($F(X_i, Vr_i, \alpha Id_i)$), i.e. for $M=1$, $F=0$; for $F=0.01$ and/or $M=2$). The shaded regions depict the respective $X'_1 > 0$ zones, i.e. the regions in which proliferation dominates suppression. The line at the boundary of such a region is the O -isocline. The figure also shows several other X_1 -isoclines (i.e. 10, 100, ..., 10^6). Figure 1a (i.e. $F=0$, $M=1$) has an

extremely high ($> 10^6$) proliferation region: about $10^8 X_1$ cells proliferate in response to only $10^4 X_2$ cells (i.e. 10^{-4} stimulator cells per X_1). This is unrealistic. If we include buffering ($F=0.01$, Fig. 1b) this artefact is eliminated: the O -isocline closes. The maximum (net) proliferation rate is now about 10^4

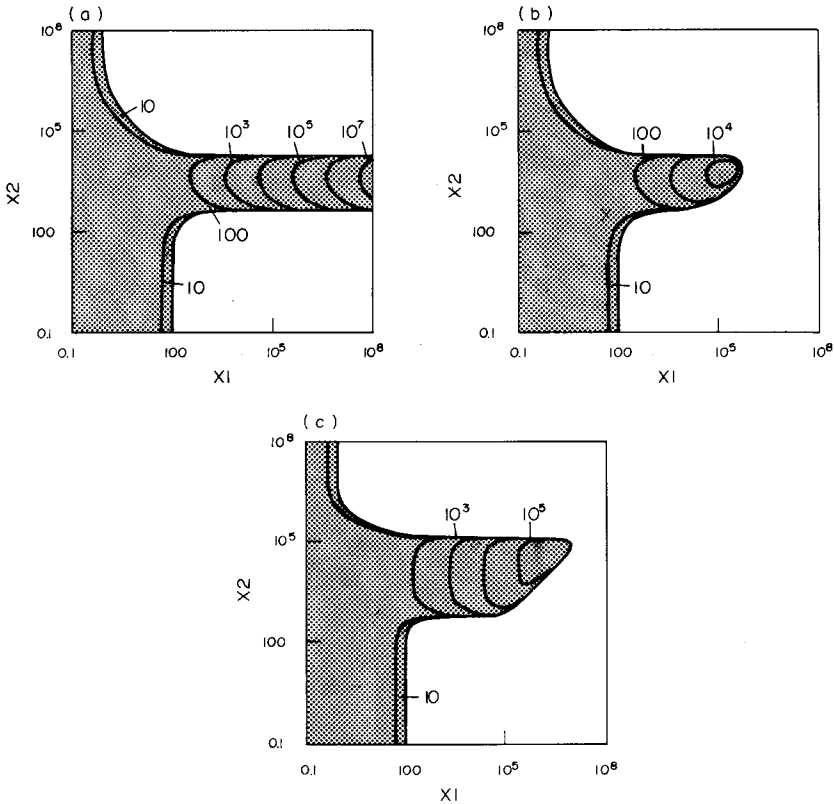


Figure 1. The 0, 10, . . . , 10^7 isoclines of the X_i ($i=1$) clone in an X_1 - X_2 state space, for three different versions of equation (2). (a) $F=0$ and $M=1$ (no buffering); (b) $F=0.01$ and $M=1$ (buffering); (c) $F=0.01$ and $M=2$ (buffering and a sigmoid dose response curve). The regions where proliferation dominates suppression ($X_1 > 0$) are shaded.

cells per day (for an X_1 population of about 10^5 cells). If $M > 1$ the saturation functions in equation (2) become S-shaped (Hill functions). The isoclines for $M=2$, are shown in Fig. 1c: the shape of the O -isocline is somewhat different from that of Fig. 1b ($M=1$). The proliferation region is wider: proliferation thus occurs for a larger range of X_2 values. In addition the maximum proliferation rate is higher. The switch between proliferation and suppression is thus more pronounced. The O -isoclines for even larger M values (e.g. $M=5$) are very similar to those of Fig. 1c. The maximum proliferation rate is however

ten times higher. We take $M=2$ and $F=0.01$ (Fig. 1c), because we do not want our results to depend on too extreme a saturation function.

The models are analysed by means of GRIND (De Boer, 1983); GRIND numerically searches for isoclines and performs numerical integration by means of ROW4A (Gottwald and Wanner, 1981).

4. Results

A 2-D network. Addition of the $X'_2=0$ isocline to Fig. 1c yields Fig. 2a. The O -isoclines of the two interacting idiotypes ($A_{12}=1$) intersect in five equilibria. The phase plot in Fig. 2b shows that three of these are stable (the stable states are encircled in Fig. 2a). The steady-states are (1) V : the virgin state, both clones are at their respective S/D values, a value too low for initiating an idiotypic interaction; (2) I_1 and I_2 : the immune states for X_1 and X_2 respectively. The immune states are asymmetric: in I_1 , X_1 is immune, thus suppressing X_2 , and in I_2 the situation is reversed. X_2 is suppressed in the I_1 immune state: it is far smaller than X_1 and cannot respond to its antigen (Vr_2) any further (see below). X_2 is nevertheless enlarged in the suppressed state; it is this enlargement of X_2 that sustains the immune state of X_1 . Note that the enlargement of X_2 means that X_2 is still stimulated by X_1 (i.e. the interaction is not negative): we can however call X_2 suppressed because it can never proliferate again (e.g. to Vr_2).

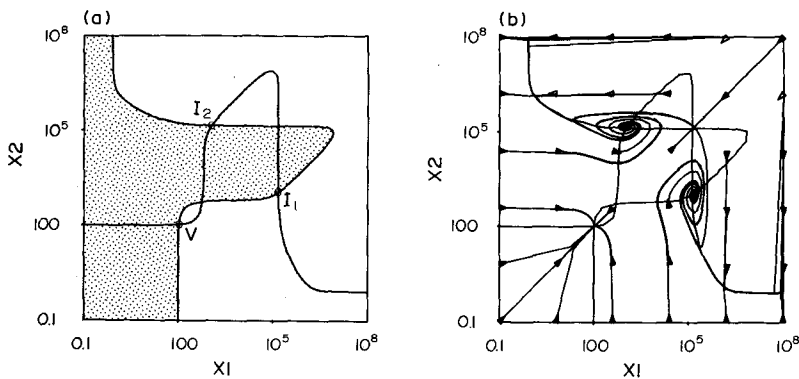


Figure 2. The X_1 and $X'_2=0$ isoclines of a 2-D system ($A_{12}=1$). (a) The $X'_1 > 0$ region is shaded, and the stable equilibria are encircled. (b) The same O -isoclines in combination with trajectories starting at various points in the state space. The picture shows the oscillatory behaviour around the immune states.

Our V , I_1 and I_2 are equivalent to virgin, immune and anti-immune states of the Hoffmann (1979; 1982) models. The present states are however generated with far simpler, and in our opinion better, saturation functions (note that even $F=0$, $M=1$ (equation (2)) yields these three states). The present model however lacks the stable suppressed states of the Hoffmann models. In the same

region (i.e. both X_1 and X_2 large) however, the clones do suppress each other. In this model, concomitant suppression is not a stable situation. Other differences are the absence of interactions in our virgin state (Hoffmann virgin states are maintained by suppression) and the fact that our immune states are situated above the source/decay (S/D , i.e. no interaction) equilibrium, whereas those of the Hoffmann models are situated below it. In the Hoffmann models idiotypic interactions can only decrease population levels.

Affinity. The form of the O -isoclines and hence the existence of the steady states depend on the affinity of the X_1 - X_2 idiotypic interaction (A_{12}). This is analysed in Fig. 3, which includes an affinity axis. The front of this cube is identical to Fig. 2 ($A_{12} = 1$); the V , I_1 and I_2 state are again indicated. At the back ($A_{12} = 10^{-3}$) the system only has a virgin state. Note that the position of the virgin state is hardly affected by the affinity. In the Hoffmann (1979) model, by contrast, the X_1 and X_2 population size in the virgin state decreases if the affinity increases (not shown).

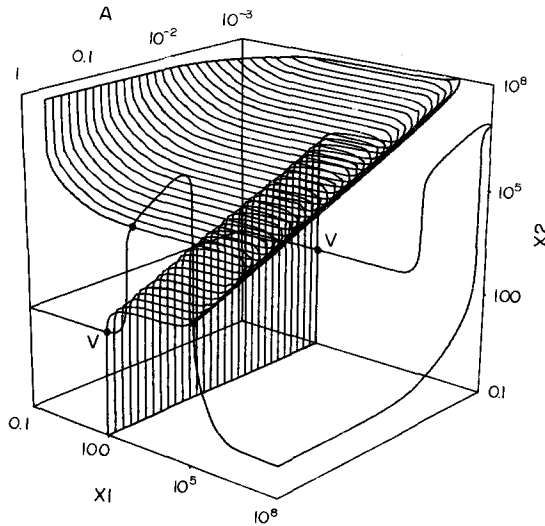


Figure 3. The X_1 and $X_2 = 0$ isoclines as a function of the affinity (A_{12}) of the idiotypic interaction. The front plane of this 3-D space is identical to Fig. 2. The $X_1 = 0$ isocline plane is shaded, and the stable equilibria for $A_{12} = 1$ and $A_{12} = 10^{-3}$ are encircled.

We conclude from Fig. 3 that for immune states to exist the affinity must be sufficiently large. Clones with a low affinity interaction (e.g. 10^{-3}), can never switch to an immune state, whatever their respective population densities. Thus although we omitted an affinity threshold from our model, i.e. a minimum affinity below which idiotypic interaction is impossible, the model nevertheless generates one. This emergent threshold is caused by the double

saturation terms (i.e. by buffering): if $F=0$ (Fig. 1a) the O -isoclines run straight into infinity and hence always intersect. Dynamically, i.e. by numerical integration, these systems ($F=0$) however never reach such high immune states.

Any specific 2-D network corresponds to a slice through the cube of Fig. 3. We concluded above that, depending on this affinity, the model yields different results: (1) if affinity is low ($A_{12} \leq 0.006$) the 2-D system only has a virgin state (i.e. it cannot switch); (2) if affinity is sufficiently high ($A_{12} > 0.006$) the system has virgin and immune states (i.e. it might be capable of switching). For low affinities, results are again qualitatively different. For instance, for $A_{12} = 0.01$, the system has one virgin and one immune state.

Antigen. First consider the interaction of one clone (X_1) with its virus (Vr_1). This is the no network situation: all A -matrix elements equal zero. The O -isoclines of this (1-D) system are shown in Fig. 4a. The $Vr'_1 = 0$ isocline is straight: the virus decreases whenever "its" lymphocyte clone exceeds a size of about 10^5 cells. The $X'_1 = 0$ isocline is more complex: at low Vr_1 densities X_1 remains in its virgin state (S/D). Higher Vr_1 densities induce X_1 proliferation: the O -isocline bends to the right. At high X_1 densities, however, proliferation requires higher Vr_1 densities (due to buffering): the O -isocline bends upwards. Every virus infection (trajectory) should start at the virgin state of X_1 : we start at $X_1 = 100$ and $Vr_1 = 10$. The virus expands to about 10^6 infected cells (Fig. 4), and meanwhile induces X_1 proliferation. As a result of this immune reaction, the virus regresses (i.e. dips to about 0.01 infected cells). Thus X_1 is no longer stimulated and declines: as a consequence the virus regrows. After a number of oscillations the system settles in a stable equilibrium with about 10^3 infected cells (Vr_1) and 10^5 immune cells (X_1). We call this "virus dormancy": the virus remains present at a (harmless) low density, kept under control by a stimulated (enlarged) clone. This is a simple form of immunity: reintroduction of the same virus never leads to virus expansion because the system quickly returns to the dormancy equilibrium with hardly any clone proliferation. Note that many biotic viruses remain present in a similar dormant state (Bellanti, 1985). We conclude that virus dormancy can account for the "memory" phenomenon. Such an immune state does not require either long-lived memory cells or idiotypic interactions.

Note however that at various stages of the immune response the virus density was less than one infected cell. Although in a stochastic model this might correspond to a low but finite probability, we prefer to eliminate such (improbable) circumstances. Virus densities of less than one cell are therefore set to zero: this is virus elimination. If we do this in Fig. 4, the virus is rejected in the first cycle and the clone returns to its virgin state due to a lack of immune stimulation. Thus dormancy, and hence immunity, is lost.

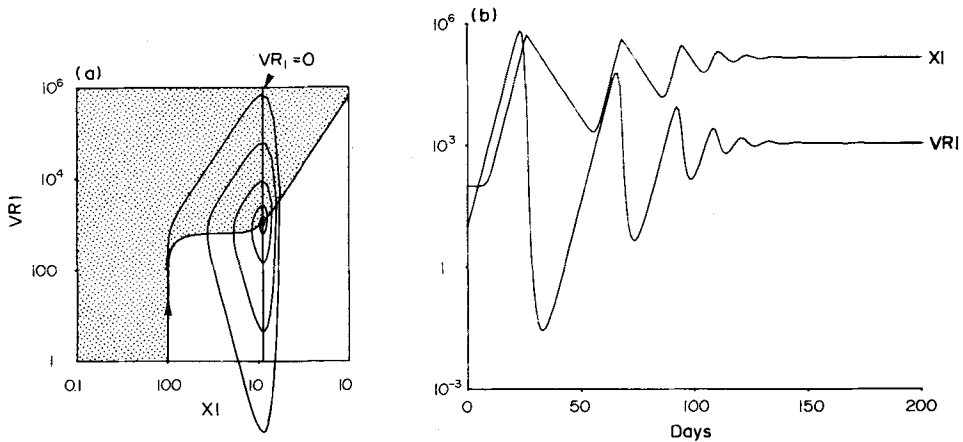


Figure 4. The interaction with X_1 and Vr_1 without any network interaction. (a) Shows the $X_1' = 0$ and the $Vr_1' = 0$ isocline (the $X_1' > 0$ region is again shaded). The trajectory depicts the immune response following the introduction of $Vr_1 = 10$ in the virgin state of X_1 ($X_1 = 100$); the latter is marked by the circle. The response is highly oscillatory and settles in the stable intersect between the two isoclines. (b) The time plot of the same reaction.

Switching. We next study this virus in a 2-D network, e.g. that of Fig. 2 ($A_{12} = 1$). Following the immune reaction to Vr_1 this 2-D system switches from virgin to immune (i.e. to I_1 , Fig. 5a). This is a “correct” switch: X_1 attains an immune state following an X_1 primary response to Vr_1 ; X_2 becomes suppressed. Experimental data also describe stable state switches that are based on differences in population sizes (Bernabe *et al.*, 1981). Figure 5b shows the time plot of this reaction: during the normal immune response the responding idiotypic stimulates the production of the anti-idiotypic. This is also described experimentally (Cosenza, 1976; Cerny, 1982; Möller and Fernandez, 1986). Around day 25, the virus is rejected and X_1 remains immune. Reintroduction of the same virus immediately leads to virus regression. Introduction of Vr_2 in the V state would of course lead to the I_2 state: the system is symmetric. Introduction of Vr_2 in the I_1 state, i.e. after Vr_1 rejection, leads to infinite growth of Vr_2 in the absence of X_2 proliferation. X_2 really is suppressed.

This type of switching behaviour only occurs if $A_{12} \geq 0.02$ (analysed dynamically by introducing viruses for a variety of different affinity values). Thus if affinities are distributed uniformly between one and zero, we expect 98% of all 2-D systems to switch to immunity after a primary immune response.

Static analysis. We analyse the X_1, X_2, Vr_1 system statically in Fig. 6. At the back ($Vr_1 = 1$) Fig. 2a ($A_{12} = 1$), and at the bottom ($X_2 = 0.1$) Fig. 4a can be recognized. In the cube, the $Vr_1' = 0$ isocline is straight: it depends on X_1 only.

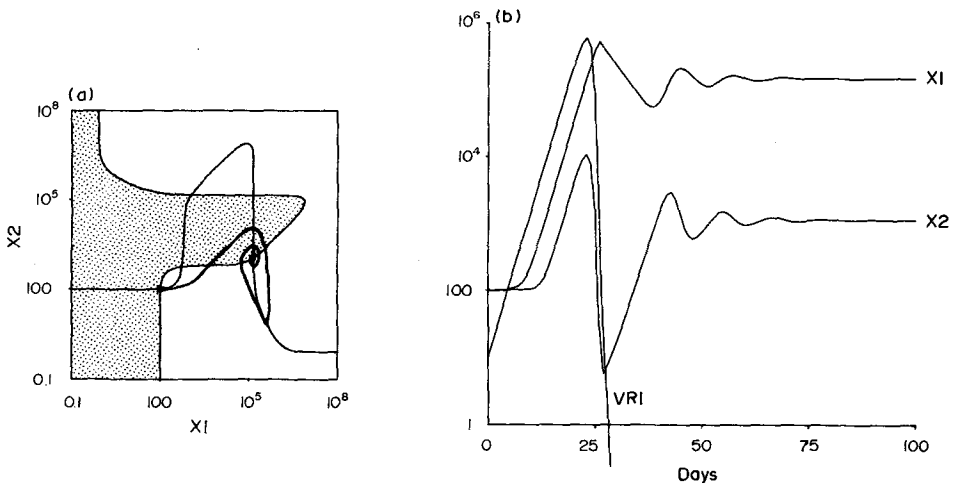


Figure 5. The interactions between X_1 , X_2 and Vr_1 , i.e. a 2-D network with antigen. We again depict the 2-D state of Fig. 2a ($A_{12} = 1$), but we additionally project the immune responses to Vr_1 in the figure (the fat line). This settles in the immunity equilibrium. The immune response is also shown in a time plot (b).

The $X_2' = 0$ isocline is independent of Vr_1 , i.e. it remains identical to that in Fig. 2. The upper part of the X_1 isocline is also independent of Vr_1 ; the lower part, by contrast, bends to higher X_1 values at high Vr_1 values. X_1 can thus be further stimulated by Vr_1 . At the upper part of the isocline X_1 is suppressed: an increase of Vr_1 cannot influence the position of the X_1 steady-state. By contrast in the immune state (I_1), X_1 is enlarged and it is still capable of further expansion. Infinite Vr_1 values allow for infinite X_1 populations. An X_1 population in the I_2 state however remains constant whatever the Vr_1 density. Moreover the X_1 population in the I_2 state is too small to cause Vr_1 regression: it is situated left of the $Vr_1' = 0$ isocline. We conclude that X_1 is "immune" in the I_1 state and "suppressed" in the I_2 state.

From the results presented hitherto we conclude that in 98% of all the cases (if affinity is distributed uniformly) a 2-D network accounts for immunity/memory due to a "correct state switch" following a primary response. Moreover, the switching behaviour of this model is robust: even simpler models (i.e. those based on equation (2) for $F=0$ and/or $M=1$) perform the same switches (albeit for a somewhat smaller range of affinity values). The present results are thus not the mere consequence of the choice of the form of the saturation function.

Suppression. Data like that in Fig. 5b, i.e. oscillations in the network response to Vr_1 , are usually interpreted in the form of idiotypic suppression (Cosenza, 1976; Bona, 1982). It indeed seems as if the enlargement of the anti-idiotypic clone (X_2) is responsible for the decline of the idiotypic (X_1)

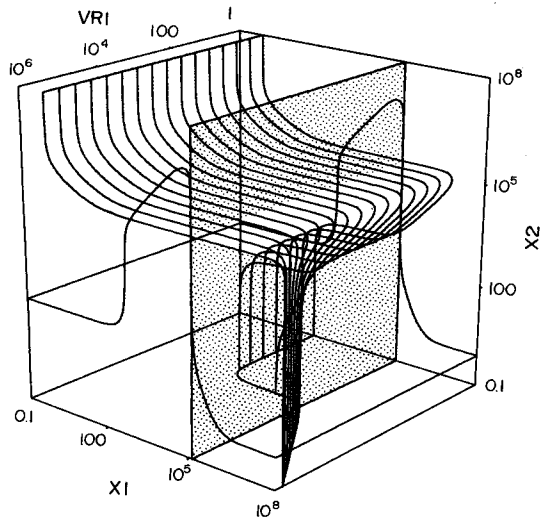


Figure 6. A static representation of the interaction between X_1 , X_2 and Vr_1 . The $X_1' = 0$, $X_2' = 0$ and $Vr_1' = 0$ isoclines in a X_1 , X_2 , Vr_1 state space. The $X_1' = 0$ isocline plane is shaded. The $Vr_1' = 0$ isocline is straight and situated at about $X_1 = 10^5$; the $X_2' = 0$ isocline is identical to that of Fig. 2 ($A_{12} = 1$). The proliferation region of X_1 expands if Vr_1 increases.

population. In the model however this is not the case: X_1 declines because the virus regresses, X_2 declines because X_1 declines. Experimentally, it was also shown that the decline of the responding idiotype (X_1) is not caused by the anti-idiotypic clone produced during the response (Möller and Fernandez, 1986). In the model an anti-idiotypic clone (here X_2) can never suppress the idiotypic clone (X_1) that responds to antigen because X_2 is necessarily smaller than X_1 . X_1 is always ahead because it responds first (to antigen). Thus it is never the anti-idiotypic that (down) regulates the proliferation of the idiotypic clone (X_1) but it is the proliferating idiotypic that suppresses its anti-idiotypic "suppressors". Proliferating clones "free" themselves of idiotypic interactions.

Conversely, suppression (proliferation regulation) does arise in these models if the system is (experimentally) manipulated by the introduction of anti-idiotypic (X_2) prior to the introduction of the antigen (Vr_1). Such a procedure gives the anti-idiotypic an advantage over the idiotypic, and the system moves toward the I_2 state (in which X_1 is suppressed). In the absence of such experimental manipulations, i.e. in normal networks, this cannot happen.

Suppression, i.e. down regulation of the proliferating idiotypic, is also absent from the models based on simpler interaction functions (i.e. $F=0$ and/or $M=1$). The absence of proliferation regulation is therefore a robust property of symmetric networks. Moreover, without experimental manipulation, the suppressed state of the (symmetric) Hoffmann models is also "unattainable".

Furthermore, asymmetric versions of our model can (sometimes) account for suppression (i.e. if $A_{21} \gg A_{12}$). We conclude that symmetric networks fail to account for the control of proliferation (i.e. for suppression). This is a counterintuitive result because: (1) the model incorporates strong negative (suppressive) interactions ($C \gg B$); (2) suppression is most intense at high population densities (i.e. after proliferation).

Escape from suppression. In order to test whether the absence of suppression hinges upon our “proliferation before suppression” assumption, we define an “escape from suppression” model (Jerne, 1974). According to this model idiotypic interactions are basically suppressive; antigen perturbs this suppressed state, thus allowing the clone to proliferate. This means that we have to replace equation (2). In equation (2') anti-idiotypic (αId_1) no longer induces proliferation (i.e. αId_1 is absent from the proliferation term); anti-idiotypic suppression, by contrast, has remained identical. Antigen induces proliferation (as it does in equation (2)), and, additionally, reduces the rate of anti-idiotypic suppression (if $Vr_1 \approx K_C$):

$$F(X_i, Vr_i, \alpha Id_i) = \frac{B \cdot X_i \cdot Vr_i^M}{K_B^M + (F \cdot X_i)^M + Vr_i^M} - \frac{C \cdot X_i \cdot \alpha Id_i^M}{K_C^M + (F \cdot X_i)^M + Vr_i^M + \alpha Id_i^M} \quad (2')$$

In the “virgin” state ($X_i \approx 100$) idiotypic interactions have to be suppressive; we therefore set $K_C = 100$ in equation (2'). Virus elimination (equation (4)) is however kept identical between the two models (i.e. for internal equivalence reasons (Irvine and Savageau, 1985); K_C in equation (4) is kept at 10^6). All other parameters can remain the same. Note that in this escape from suppression model $K_C \ll K_B$; we thus first have suppression and then proliferation.

The $X_1 - X_2$ state space of this model (for $A_{12} = 1$) has three equilibria (Fig. 7a); two of them (V_1 and V_2) are stable. Both correspond to some sort of virgin state, i.e. they are reached in the absence of antigen. Which of the two is reached depends on the respective influx parameters (S_i). We thus have to consider two different initial states for the introduction of antigen. For a wide range of affinity values (Fig. 7b) the system either has one virgin state, with hardly any idiotypic interactions, or the two virgin states described above. Immune states (situated at elevated population sizes) are absent.

Introduction of a virus (here Vr_1) in the two virgin states indeed leads to escape from suppression (Fig. 8). If Vr_1 is introduced in the V_1 state (Fig. 8a), X_1 starts to proliferate when the virus is sufficiently large, i.e. after about 10 days. This increase of the idio type further suppresses the anti-idiotypic (X_2 further decreases). Thus, again, the proliferating idio type suppresses its anti-idiotypic suppressors and “escapes” from further regulation. Following virus

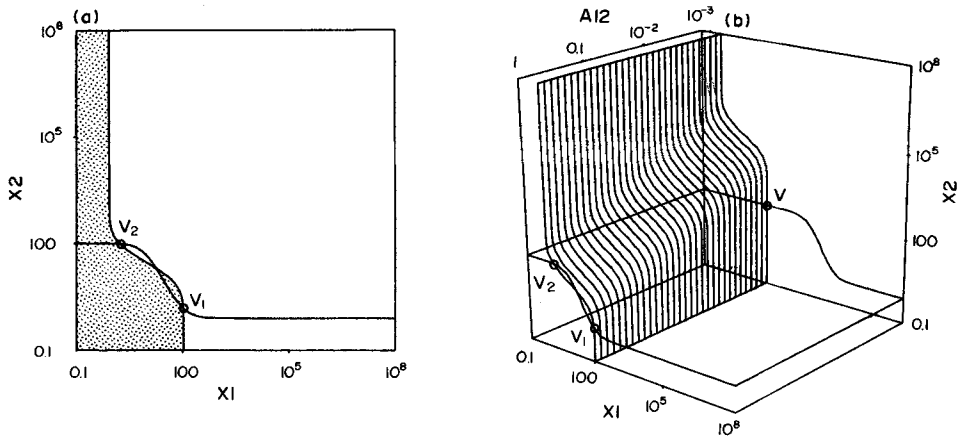


Figure 7. An “escape from suppression” model. The $X_1' = 0$ and $X_2' = 0$ isoclines are displayed in the X_1, X_2 state space for the affinity $A_{12} = 1$ (a), and as a function of affinity (b). Stable equilibria are encircled; the $X_1' > 0$ region is shaded. The system has one or two virgin states but no immune states.

rejection the system returns to the V_1 state. We conclude that both immunity and proliferation regulation are absent from this system. Similar behaviour is obtained when Vr_1 is introduced in the V_2 state (Fig. 8b). Because the idiotypic starts at a smaller population level the immune reaction is somewhat (but minimally) delayed. Following idiotypic (X_1) proliferation the anti-idiotypic again becomes (further) suppressed. Thus again proliferation regulation by the anti-idiotypic is impossible. Following virus rejection the system does however switch from V_2 to V_1 ; this gives the idiotypic a small advantage (compare Fig. 8a with 8b).

This switch from V_2 to V_1 is a form of immunological memory. However, it is rather peculiar because: (1) it only develops erratically, i.e. if $S_1 < S_2$; (2) if X_1 is called immune in the V_1 state, X_2 should be called immune in the (virgin!) V_2 state. Hence, immunity occurs in escape from suppression models, but only erratically and/or in the absence of the corresponding antigen. Moreover, note that this escape from suppression network fails to account for the production of anti-idiotypes during an immune response (Cosenza, 1976; Cerny, 1982; Möller and Fernandez, 1986). During the immune reaction the idiotypic escapes but the anti-idiotypic becomes further suppressed. The anti-idiotypic is thus eliminated and the system no longer behaves as a network.

We conclude that in a symmetric system, escape from suppression has two meanings: (1) stimulation by antigen cancels the suppressive anti-idiotypic interaction; (2) the anti-idiotypic clones are suppressed to a level near elimination. Secondly, we conclude that our “proliferation before suppression” model behaves in a superior way, and, most importantly, that the absence of

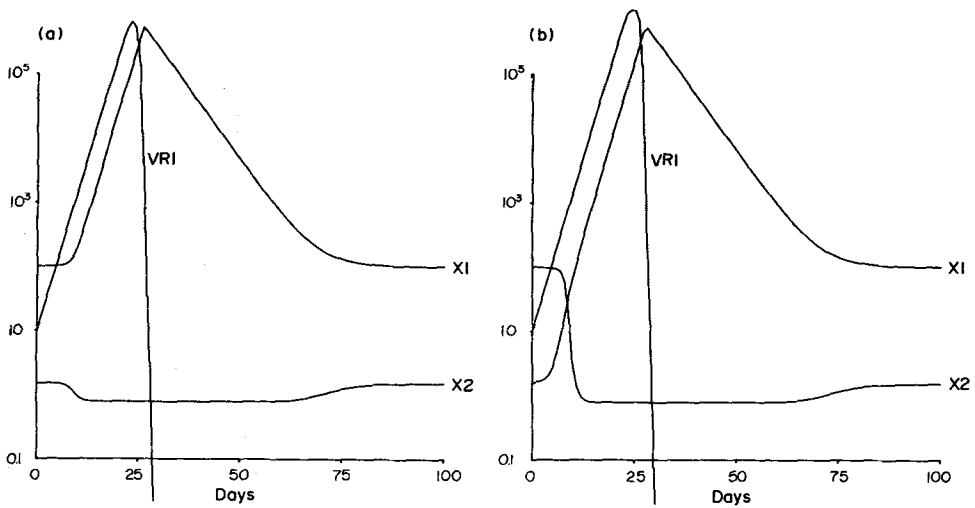


Figure 8. Immune responses in the escape of suppression model. In each of the two virgin states ((a) V_1 ; (b) V_2) Vr_1 is introduced in a dose of 10 infected cells. In both cases the anti-idiotypic clone (X_2) becomes further suppressed. The system always returns to V_1 .

suppression in such a model does not hinge upon the order of proliferation and suppression.

3-D system. We therefore proceed with the analysis of our original [equations (1)–(4), $K_B < K_C$] model. Expanding that 2-D model into three dimensions is technically easy. The analysis of a 3-D model is however much more complex because we now have to consider a number of affinity parameters, each of which is expected to vary widely. Consider the conventional scheme of the immune networks (i.e. X_1 sees antigen, X_2 sees X_1 , X_3 sees X_2 , and so on). Thus in our symmetric A -matrix: $A_{12} = A_{21} > 0$ and $A_{23} = A_{32} > 0$, and all other A elements equal zero. Qualitatively we thus have to consider two affinity parameters. Additionally however, a 3-D network allows two qualitatively different antigens: (1) Vr_1 an antigen seen by a clone with one connection to the network; (2) Vr_2 an antigen seen by X_2 with two connections (i.e. to X_1 and X_3). Vr_3 is qualitatively identical to Vr_1 : the system is symmetric in X_2 .

We have analysed this 3-D system by the introduction of these two antigens for all different combinations of the two affinity parameters (A_{12} and A_{23}). We thus attained nine qualitatively different stable states. In the absence of antigen the systems always remains in the 3-D virgin state. Antigenic stimulation yields 2-D immune states (as described above) if one of the affinities is sufficiently low. The weakly connected clone remains virgin, the other two switch to immune or suppressed. If both affinities are sufficiently high, the system switches to states

where either two clones are immune and one is suppressed, or to states where two clones are suppressed by one immune clone.

Most of these switches correspond to "correct" immunity phenomena. In fact, depending on the circumstances, the additional idiotypic interaction in the 3-D system can both facilitate or inhibit the correct switching behaviour. Inhibition is more common however. If X_1 is stimulated by antigen, and the interaction between X_2 and X_3 is much stronger than that between X_1 and X_2 , the correct switching behaviour is disrupted; X_1 then generally returns to virgin. Proliferation regulation does not occur in 3-D systems: even clones with two idiotypic connections suppress both anti-idiotypic clones before these can become suppressive. We conclude that the results obtained with the 2-D network can largely be repeated with 3-D systems.

5-D network. The most straightforward extension of the above 3-D network is one in which X_1 is seen by two clones of level 2, which each in turn are seen by one clone of level 3. Thus X_1 is doubly connected (as X_2 was) to 2-D systems identical to those analysed above. The affinity matrix now has 4 symmetric connections, i.e. 8 non-zero elements. We distribute these values uniformly between one and zero (by a pseudo random number generator). We want to find out whether an idiotypic interaction between X_1 and X_2 (which accounts for correct switching in 98% of the cases), can be disturbed by the three additional idiotypic interactions of a 5-D network. We have tested 40 such systems. Because all randomly drawn A_{12} values exceeded 0.01, we expect all 40 networks to switch to an I_1 state after the introduction of Vr_1 . Such a switch was indeed found for all 40 networks. The immune states of X_1 are somewhat lower because X_1 can now be suppressed by two clones of level 2; X_1 immunity is thus less vigorous (i.e. may lead to virus dormancy instead of virus rejection).

However, if we connect this system randomly instead of according to the above scheme, results become different. We assign to A_{12} a random value between zero and one; additionally such values are assigned to three other randomly chosen connections. We again analysed 40 networks; again a switch to immunity of X_1 was expected for each of them. However we find only 32 such switches: in 8 cases (20%) X_1 returns to its virgin state. Thus the general occurrence of correct switching behaviour (i.e. immunity/memory) seems to require a specific structure of the network. It is not at all clear whether biotic immune networks fulfil these conditions. Switching can for instance be disturbed if X_1 is only connected to X_2 , and if X_2 is suppressed due to other network interactions. Such "disturbance" already occurred in 3-D systems. We conclude that the expansion of the present model to a random high- D network may reduce the model's capacity to switch, i.e. to generate immunity. We are currently working on this question.

5. Discussion.

Thresholds. The most important decision that has to be made during the development of an idiotypic network model is how to distribute the positive and negative interactions (i.e. Jerne's, 1974, third dualism). In asymmetric models the (asymmetric) affinity matrix may define non-overlapping sets of positive and negative interactions (i.e. it defines a different distribution of affinities on each side of the diagonal). Thus each individual idiotypic anti-idiotypic interaction has either a positive or negative sign. Farmer *et al.* (1986) represent paratopes and idiotypes by bit-patterns which yields such an asymmetric affinity matrix. The Richter (1978), the Hoffmann models (1979; 1982), and the present model instead define a switch in sign depending on the size of the anti-idiotypic clone.

Jerne's (1974) "escape from suppression" idea was followed by Richter (1978): his suppression threshold is lower than the proliferation threshold (his $B > D$). In the Richter "virgin state" however, network interactions are absent, i.e. the population densities are far below the suppression threshold (Hiernaux, 1977). In the Hoffmann models (1979; 1982) the virgin state is maintained by suppression; the suppression terms switch off at high population densities (even in the "suppressed state"). Thus, the Hoffmann models are "escape from suppression" systems.

In this paper we chose the opposite (our $K_B < K_C$), i.e. idiotypic interactions are absent in the virgin state and only large clones evoke suppression. We think this makes most sense if the stimulation and suppression processes are considered at the level of individual cells. Stimulation, and hence proliferation, of a cell requires crosslinking of its receptors by anti-idiotypic antibody. An increase in anti-idiotypic antibody is thus expected to increase proliferation. Suppression can occur by various mechanisms: (1) the crosslinking process may become less efficient if anti-idiotypic antibody concentrations become too high (Goldstein, 1988); (2) massive release of antibodies by idiotypic plasma cells will neutralize anti-idiotypic antibodies by complex formation; (3) coating of cells with anti-idiotypic antibodies may initiate the complement cascade, and lead to cell lysis (Eichmann, 1974; Hoffmann, 1979). All these mechanisms probably require higher antibody concentrations than does the initial crosslinking process that leads to proliferation.

Parameter constraints. We choose the proliferation saturation constant (K_B) larger ($10 \times$) than the virgin population size in order to keep network interactions low in the virgin state. The choice of $M=2$, i.e. an S-shaped dose response curve, also facilitates the preservation of the virgin state: if $M=1$ the 2-D virgin state only exists if $A_{12} \leq 0.88$. Thus in a model with linear ($M=1$) saturation function the switching range is somewhat smaller ($0.04 \leq A_{12} \leq 0.88$). Because the virus grows relatively fast ($R=0.5$), the immune reaction has

to be quite vigorous (i.e. to bring about much proliferation). This facilitates the establishment of network interactions because clones easily grow above the threshold required for inducing the proliferation of an anti-idiotypic clone (K_B). In addition, virus rejection is more severe following a vigorous immune reaction; this prevents dormancy development (De Boer and Hogeweg, 1986). High lymphocyte turnover rates (D) increase the instability (i.e. the oscillations) and lead to increased virus dormancy development. The abundant switching behaviour of this model occurs however for various parameter settings (not shown).

Immunological memory. The fact that an immune network can account for immunological memory does not mean that the memory phenomena displayed by biotic immune systems are indeed generated by network state switches. The existence of long-lived memory cells (Jerne, 1984; Levy and Coutinho, 1987) provides a very simple alternative explanation. An alternative memory cell that would account for immunity is a cell which by proliferation and differentiation becomes insensitive to network suppression (Cooper *et al.*, 1984). Thus by adding this assumption, one can account for memory in an "escape from suppression" model. Immunity generated by memory cells is not stable; although these cells are long-lived, they decline slowly. The main difference between network immunity and the two forms of memory cell immunity is that the former is (actively) maintained by proliferation whereas the latter corresponds to a resting state. Thus experimental data describing the existence of small, resting, long-lived cells (Jerne, 1984; Levy and Coutinho, 1987) support the memory cell alternative. Moreover the dynamics of (long-lived) memory cells can account for self-non-self discrimination (De Boer and Hogeweg, 1987a, 1987b). Nevertheless, immunity generated by state switching remains an intriguing possibility; the present results prove that if proliferation precedes suppression it is a feasible possibility.

Suppression. The fact that our symmetric model fails to account for the suppression of proliferating clones violates the most important role of the immune networks. If anti-idiotypic interactions were indeed able to control excessive responses this would be very important (e.g. in self-non-self discrimination). In our model however this is impossible. Note that suppression can be generated by "experimental" manipulation, e.g. by the introduction of large anti-idiotypic clones, but never evolves during a "physiological" immune reaction. Experimental data that thus demonstrate the existence of suppression therefore provide no evidence for a role for suppression in the regulation of immune reactions. We think that suppression will be absent from most, if not all, symmetric networks because a proliferating clone is always ahead and hence should always be able to suppress its anti-

idiotypic suppressors long before these can become suppressive. Our present parameter setting, i.e. proliferation before suppression ($K_B < K_C$) and dominant suppression ($C \gg B$), should facilitate the suppression of large proliferating clones because the suppression interaction is most intense in this region. Moreover, if we make our model asymmetric (by defining an asymmetric affinity matrix) suppression sometimes occurs. We thus conclude that symmetric network theory seriously violates the role of anti-idiotypic suppression in the control of (excessive) proliferation. In our extensions of the Hoffmann models we reach the same conclusion because proliferating clones moving in the direction of the immune state (thus suppressing the anti-idiotypic partners) never end up in the suppressed state. The absence of suppression in our model suggests that the model can be further simplified by the removal of the negative interactions ($C > B$). Indeed, an alternative model would be one in which suppression only reduces stimulation. This can easily be incorporated by setting $C = B$: such a (2-D) model generates a very similar result (submitted).

Network dimensions. The results obtained with our 2-D network also occurred in our 3-D networks and in 5-D networks of a specific structure. Randomly connected 5-D networks however showed different results. This suggests that the present (low-D) results may depend strictly on the form and dimension of the network. Recent work (submitted) confirms this: the low-D results described here can hardly be repeated in high-D networks.

The Hoffmann models (1975; 1979; 1980; 1982) assume that the 10^7 -D immune network comprises separate pairs of interacting clones (plus minus clones). Wikler *et al.* (1979) showed that antibodies of the third level (Ab3) are idiotypically related to the Ab1 level; Ab4 in turn is related to Ab2. These data suggest that the network is "flat" instead of "open-ended". Similarly, Jerne (1984) concludes that "one characteristic of the idiotypic network is the occurrence of pairs of antibodies, of preferred partners". If these interpretations are correct, the idiotypic network is a (1-D) stack of independent (2-D) systems. We think that such a flat system should not be called a network (at least all comparisons with e.g. neural networks break down). In addition, as Jerne (1984) also argues, it implies that anti-idiotypic interactions have to be totally different from immune reactions to non-network antigens (epitopes) because the latter involve several hundreds, instead of one, antibody molecules. Moreover, idiotypes can have as many as 40 antigenic determinants (idiotopes) (Novotny *et al.*, 1987), and, secondly, anti-idiotypic clones obtained from immature immune systems do show a high connectance to other clones (Holmberg *et al.* 1984; 1986; Vakil and Kearny, 1986).

If the idiotypic network would indeed be nothing more than a collection of independent pair-wise complementary interactions, the conclusions of the

present paper should be valid for the complete 10^7 - D immune network. However, we recently (submitted) found that if the high- D idiotypic network is open-ended, i.e. is not organized in such monogamous pair-wise interactions, the system behaviour becomes non-sensical because of extensive percolation of idiotypic signals. Therefore, it is an interesting question to analyse which cellular interactions in large (possibly randomly connected) idiotypic networks might cause the functional reduction to the pair-wise idiotypic interactions described above.

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