International Journal of Bifurcation and Chaos, Vol. 6, No. 9 (1996) 1691–1702

NATURAL TOLERANCE AS A FUNCTION OF NETWORK CONNECTIVITY

V. CALENBUHR* and F. J. VARELA
CREA, Ecole Polytechnique, 1, rue Descartes, F-75005 Paris, France
E-mail: fv@ccr.jussieu.fr vcalenbu@ulb.ac.be

H. BERSINI
IRIDIA, Université Libre de Bruxelles,
C.P. 194/6, 50, Ave. F. Roosevelt, B-1050 Bruxelles, Belgique,
E-mail: bersini@ulb.ac.be

Received October 23, 1995; Revised November 9, 1995

This article investigates the following basic question: in the relatively stable molecular environment of a vertebrate body, can a dynamic idiotypic immune network develop a natural tolerance to endogenous components? Our approach is based on stability analysis and computer simulation using a model that takes into account the dynamics of two agents of the immune system, namely, B-lymphocytes and antibodies. We investigate the behavior of simple immune networks in interaction with an Ag whose concentration is being held constant as a function of the connectivity matrix of the network. The latter is characterized by the total number of clones, N, and the number of clones, C, with which each clone interacts. The idiotypic network models typically become unstable in the presence of this type of Ag. We show that idiotypic networks that can be found in particular connected regions of NC-space show tolerance towards auto-Ag without the need for ad hoc mechanisms that prevent an immune response. These tolerant network structures provide dynamical regimes in which the clone which interacts with the auto-Ag is suppressed instead of being excited such that an unbounded immune response does not occur. Possible implications for the future treatment of auto-immune disease such as IvIg-treatment are discussed in the light of these results. Moreover, we propose an experimental approach to verify the results of the present theoretical study.

1. Introduction

This paper is concerned with the following fundamental question: can a dynamic idiotypic immune network which develops in the relatively stable somatic molecular environment provide a natural tolerance to endogenous components? And if so, what are the mechanisms for this tolerance? We have studied these problems for the case of the possibly simplest immune networks and have proposed a solution [Calenbuhr *et al.*, in press; hereafter referred to as CBSV]. Here we extend this study and focus on larger immune networks.

Natural tolerance to endogenous components is central to an organism-centered view of the immune system, that is, where the immune system is seen as being centrally responsible for a somatic identity,

*Present address: Institut für Medizinische Informatik, Statistik und Epidemiologie, Universität Leipzig, Liebigstr. 27, D-04103 Leipzig, Germany, Calenbuhr@imise.uni-leipzig.de

and only secondarily to a defensive role [Varela & Coutinho, 1991, 1993; Tauber, 1994]. Yet, also in the classical clonal selection view of the immune system natural tolerance is a contended issue. In recent years the interest in the understanding of idiotypic networks has produced a number of models and significant insights [Varela & Coutinho, 1991; De Boer & Perelson, 1990]. In these models the dynamics (of soluble and cellular fractions) and metadynamics (turnover of clones and new recruitment) of the network have been emphasized. Until recently, the network's interaction with an external antigen (Ag) has been studied with several degrees of sophistication, always considering the Ag as an infectious agent being controlled by the network dynamics [De Boer & Hogeweg, 1989; Neumann & Weisbuch, 1992a,b; Weisbuch *et al.*, 1993]. However, the case of continuously present Ag (such as those found on tissues and in the somatic environment), a situation corresponding to the one found in auto-Ag and possibly in auto-immune disease, has received much less attention. This problem has been treated so far mainly with simplified cellular automata [Stewart & Varela, 1991] and has been dealt with in full dynamical detail for the first time by Detours *et al.* [1994] and CBSV.

In general, it is believed that sophisticated and perhaps redundant control mechanisms avoid that the immune system attacks molecules of the host organism. It is well known that large interaction networks have often coexisting dynamical regimes with sometimes largely differing dynamical properties. For example, a system can have several coexisting chaotic attractors or chaotic attractors coexisting with oscillatory ones. A question of primary importance then is whether tolerance towards auto-Ag can be achieved by the diversity of dynamical configurations or regimes (the so-called dynamical repertoire[1]) that the model can display when some stable auto-Ag[2] are considered. That is, can bifurcations of the system give rise to branches of different stability properties to attain bounded response of a perturbed clone?

In fact, in a recent study (CBSV) it was shown that in a simple three clone immune network, tolerance can indeed be found for certain dynamical regimes. The different types of dynamics are a function of the connectivity of the idiotypes. There are only two possible connectivity configurations in the simple three clone network. One of them leads to a chaotic regime which is characterized by the dynamical equivalence of the clones. It was shown that the interaction of the system in this regime with a continuously present and constant Ag causes a degeneration of the chaotic attractor into an attractor in which the clones are no longer equivalent, namely a periodic one. Instead of launching an unbounded immune response, the system is reciprocally stabilized with the constant Ag. The other dynamical regime, a limit cycle becomes unstable when coupled to an auto-Ag.

These results suggest to consider the problem of natural tolerance not in the framework of a theory that relies on detailed cellular and molecular mechanisms to prevent auto-immunity, but instead to focus primarily on network connectivities and the corresponding dynamical repertoire. CBSV studied only a very simple prototypic network. In this paper we extend the analysis to larger networks that allow for a multitude of different dynamical regimes. We examine their behavior in the presence of a continuously present Ag with respect to their stability properties.

Biological networks and networks of oscillators have received much attention in recent years, see for example [Tsang *et al.*, 1991] and references therein. Researchers were mainly interested in the behavior of coupled oscillators as a function of system parameters such as coupling strength and distribution of natural frequencies [Matthews *et al.*, 1991]. The present study differs in respect to these works: the immune network studied here is not made up of individual oscillators. Only when two basic building units are coupled together, the system starts to oscillate. Moreover, here we are interested in the impact of structural changes of the connectivity matrix on the system behavior and not that of parameter values.

The paper is organized as follows. In the second section we present a brief description of our model and discuss its behavior as a function of network connectivity. In the third section we briefly describe the behavior of the basic three clone system in the presence of auto-Ag and extend this discussion to larger networks.

[1]We use the term "dynamical repertoire" to refer to all dynamical regimes that the system can have. Hence, dynamical repertoire must not be confounded with the standard use of repertoire, repertoire size etc., commonly used in immunology.

[2]By a stable auto-Ag we understand an Ag that can stimulate the IS and whose concentration is always being held constant.

2. The Model

2.1. *Basic model without interactions*

The model was originally proposed by Varela *et al.* [1988] and discussed in [Varela & Stewart, 1990; Stewart & Varela, 1990]. We have since used a slightly modified version of this model, by the use of differently shaped activation functions. Despite its simplicity the model shows a rich dynamical behavior, notably the occurrence of oscillations and chaos [Bersini, 1992; Calenbuhr *et al.*, 1993; Bersini & Calenbuhr, 1995; Calenbuhr & Bersini, 1993, also in preparation]. Similar models have been intensively studied by de Boer *et al.* [1993a,b].

Our model describes the interactions between a soluble and a cellular compartment of variable V-regions, whose behaviors are described by the following differential equations:

$$\frac{df_i}{dt} = -k_1\sigma_i f_i - k_2 f_i + k_3\ \mathrm{mat}(\sigma_i)b_i \qquad (1)$$

$$\frac{db_i}{dt} = -k_4 b_i + k_5\ \mathrm{prol}(\sigma_i)b_i + k_6 \quad i = 1,\ldots,n; \qquad (2)$$

where f_i denotes the concentration of the ith type (clone) of antibody, and b_i the population of the ith type (clone) of B-lymphocytes. The first term in (1) describes the kinetics of the formation of antibody-antibody complexes, the second term accounts for the rate of inactivation of Ab's and the third term describes the production of Ab's by B-cells (B-cell maturation). The first term in (2) accounts for the death-rate of B-cells, the second term for the proliferation of B-cells and the third term represents the production of B-cells in the bone marrow.

The antibody-antibody and antibody-B-lymphocyte interactions are specified by the connectivity matrix **M**, whose entries $m_{i,j}$ determine whether (antibody- or lymphocyte-) species i interacts with species j and define the function, σ_i, which is called the field:

$$\sigma_i = \sum_{j=1}^{j=n} m_{i,j} f_j \qquad (3)$$

We will restrict the analysis here to Boolean affinities, such as the ones obtained empirically by Eliza measurements [Stewart & Varela, 1989]: an entry "1" in the connectivity matrix (CM) indicates a threshold affinity between clones f_i and f_j, while a "0" indicates the absence of affinity. For example, a situation where all members react only with their nearest neighbors results in a CM in which all elements are zero except for the direct neighbor elements of the diagonal. We will refer to that case as the open chain. Adding non-zero corner elements to this matrix is equivalent to closing the chain of interaction case (see Fig. 1 for the 3-clone case):

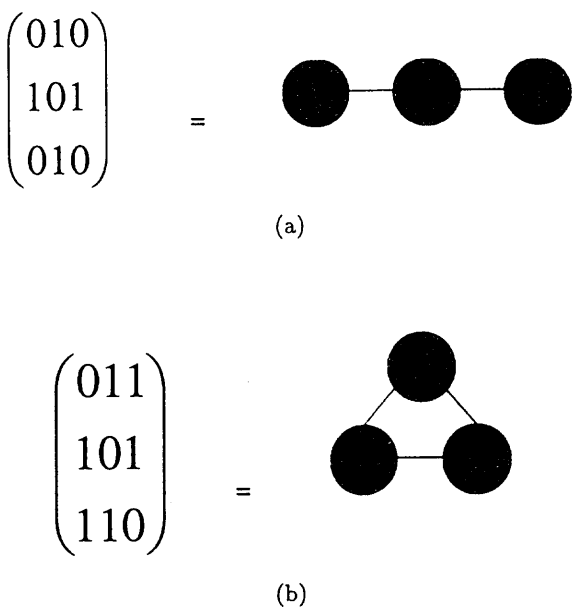
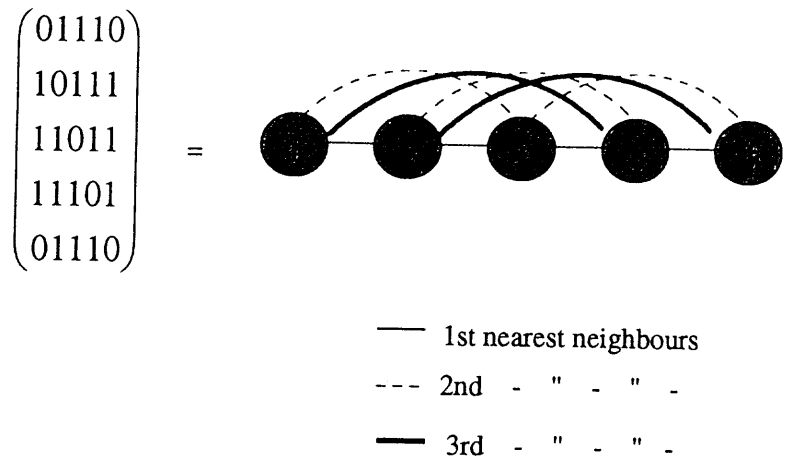


Fig. 1. Connectivity matrices and the corresponding interaction scheme for the 3-clone open chain case (above), and the 3-clone closed chain case (below).

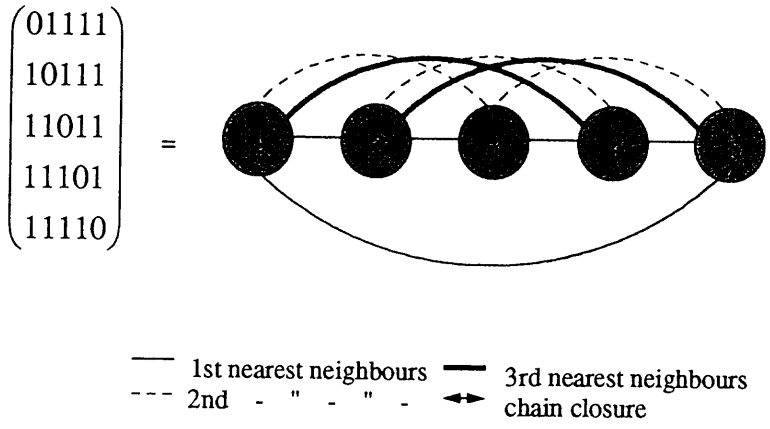
In the simple 3-clone case only first nearest neighbor interactions are possible. For a larger number of interacting clones, however, it is also possible to have long-range interactions. Note that short-range or long-range interaction in our case does not refer to any chemical or physical property of the interacting Ab's. First, second, third etc. nearest neighbor interactions are all of the same strength in our case. The chosen terminology serves only to label the clones and their interaction symmetry.

There are three parameters that characterize the isotropic connectivity matrices used in our case. First, the number of clones, second the maximal number of nearest neighbor interactions in a network and third, open or closed chain constellation, e.g., 5/3o would designate a network of five clones with up to three nearest neighbor interactions and an open chain constellation (see Fig. 2).

The distinction between open- and closed-chain cases may appear artificial and superfluous. However, we have several reasons to stick to this nomenclature. In many cases, the behavior of larger networks can be related to properties of the 3-clone



(a)



(b)

Fig. 2. Connectivity matrices and the corresponding interaction scheme for the open chain case (above) and closed chain case (below) of a 5/3 network.

cases. This is particularly true for the phenomenon of network fragmentation in which a large network shows the behavior of several smaller and independent non-interacting networks, which will be briefly touched upon in the next paragraph (unpublished results).

The functions Mat and Prol determine how B-cells mature and proliferate upon activation:

$$\mathrm{mat}(\sigma_i) = \exp - \left\{ \frac{\ln(\sigma_i/\mu_m)}{s_m} \right\}^2 \qquad (4)$$

$$\mathrm{prol}(\sigma_i) = \exp - \left\{ \frac{\ln(\sigma_i/\mu_p)}{s_p} \right\}^2 \qquad (5)$$

The parameter values for the results presented here are as follows: $k_1 = 0.0016[\mathrm{conc}^{-1}d^{-1}]$; $k_2 = 0.02[d^{-1}]$; $k_3 = 2.0[d^{-1}]$; $k_4 = 0.1[d^{-1}]$; $k_5 = 0.2[d^{-1}]$; $k_6 = 0.1[d^{-1}]$; $\mu_m = 80[\mathrm{conc}^2]$; $s_m = 0.5$; $\mu_p = 120[\mathrm{conc}^2]$; $s_p = 0.5$.

The discussion in this paper is based on a system dynamics in which the number of clones and their connectivity remains constant as a function of time, i.e. we study systems without meta-dynamics. The basic behavior of the system including meta-dynamics has been discussed in Stewart & Varela [1991] and Detours *et al.* [1994].

2.2. *Coupling with an auto-Ag*

The coupling of an Ag whose concentration remains constant is the simplest case to represent an autologous antigen. From the mathematical point of view

it introduces the least modification at the level of the equations. For this case (3) is replaced by

$$\sigma_i = \sum_{k=1}^{k=n'} l_{i,k} \mathrm{Ag}_k + \sum_{j=1}^{j=n} m_{i,j} f_j \qquad (6)$$

whereby Ag_k denotes an auto-Ag coupled to the network via the interaction matrix l. In the following discussion we will drop all indices with the understanding that there is always only one auto-Ag present.

2.3. *Computational aspects*

The system's equations were integrated using a fourth order Runge-Kutta method with adaptive stepsize. The solutions with auto-Ag are different from the solutions without auto-Ag as the coupling of an auto-Ag to the network leads to a new dynamical regime. We have tried to find as many attractors as possible for a specific connectivity matrix by performing computer simulations using random starting values.

As will be discussed below, there is a critical range of auto-Ag that can lead to an unbounded immune response. This range is in general defined by $\mu_m < [\mathrm{Ag}^i] < \mu_p$. Moreover, in many cases stability and instability depend on which particular clone is perturbed. It was therefore necessary to run simulations for various auto-Ag concentrations and different perturbed clones. We are not interested in calculating bifurcation diagrams, but instead stability diagrams that indicate the stability of the system as a function of the connectivity matrix in the critical auto-Ag concentration range. We have to distinguish three different types of stability. If the system remains stable irrespective of which clone is perturbed, then the corresponding connectivity matrix is called safe. If the system is unstable irrespective of which clone is perturbed, its connectivity matrix is called unsafe. If stability or instability depend on which clone is coupled to the auto-Ag, the corresponding connectivity matrix is called dangerous. The reason for this will become clear in Sec. 3. Often a network of a particular connectivity has several dynamical regimes. Some of which are stable, some are unstable. If at least 50% of all tested starting conditions lead to stable solutions, we classify the behavior as stable and vice versa. In reality, what we have found indicates that one half stable and one half unstable situations do not occur. We have always found at least 75% of stable or unstable cases.

This categorization omits a lot of useful and important information. However, it also reveals some interesting patterns, which would otherwise not have been detected.

3. Results

3.1. *Behavior of the system without Ag-interaction*

The behavior of the networks defined above cover the whole spectrum from fixed points over limit cycles to chaos. Often several co-existing regimes are found. A detailed discussion would go beyond the scope of this paper and is therefore deferred to other publications [Detours *et al.*, in preparation; Calenbuhr *et al.*, in preparation]. We therefore briefly indicate some general results and tendencies:

1-, 2- and 3-clone systems

In the 1-clone case the system has one fixed point. In the 2-clone case the clones oscillate out of phase and have the same amplitude. Various types of behavior are found for the 3-clone case, namely oscillations (open chain) and chaos (closed chain). In the open chain case clones 1 and 3 always oscillate in phase and have the same concentration, while clone 2 oscillates in phase opposition. The amplitude of clone 2 is twice as large as that of clones 1 or 3.

The chaotic regime is characterized by the formation of pairs of clones that synchronize (as is the case in the 3-clone open chain case). However, as soon as a new pair is forming it breaks up and a new attempt is made to form a pair with the other clone. The clones participating in the pair formation are selected randomly and the attempts to form a pair occur irregularly. From a dynamical point of view, all the clones are equivalent or interchangeable. The phenomenon just described is reminiscent of the frustration phenomena found in neural networks [Marcus *et al.*, 1991; Atiya & Baldi, 1989] and spin glasses [Toulouse, 1977] and was therefore named "frustration induced chaos". A complete description will appear elsewhere [Bersini & Calenbuhr, 1995; Calenbuhr & Bersini, 1993, also in preparation].

Open versus closed chain

Up to 17 clones and considering only first nearest neighbors, systems with an uneven number of clones

are always characterized by oscillations in the open chain case and chaos in the closed chain case (exception: 7 clones open chain, which also displays chaos) [Calenbuhr *et al.*, 1993]. Even numbered systems always show oscillations up to this size. For larger systems, this clear distinction remains no longer valid. Also, if longer range interactions are present this simple classification scheme breaks down and more complex rules have to be applied.

Network fragmentation

From about 25 clones onwards, our networks show in many cases a very interesting behavior; namely, the large network behaves as if it was made up of several smaller and independent non-interacting networks. This phenomenon is called network fragmentation [Detours *et al.*, 1994; also Calenbuhr *et al.*, in preparation]

Chaos versus oscillations

Chaos and oscillations are the most prominent behavior, although fixed points can also be found. In many cases co-existing regimes are found. Chaotic regimes become rarer with increasing network size.

3.2. *Interaction with auto-Ag*

1-, 2- and 3-clone systems

A fixed auto-Ag corresponds to a situation of a molecule that is always immediately being replenished. Although mathematically simple, it is to be noted that for the system a constant Ag is the hardest possible perturbation, and one most likely to lead to an unbounded immune response. With the parameter values employed here, there is a critical case for the 1-clone system when the auto-Ag concentration is in the range $\mu_m < 80 = [\mathrm{Ag}] = 180 < [\mathrm{Ag}_c]$, where there is an unbounded immune response.

For the 2-clone system the critical range is smaller, since the perturbed clone receives additional stimulation from the unperturbed clones raising its mean field faster into a region where the activation functions decrease. Before and after the critical region the perturbed clone oscillates at a high level, while the unperturbed ones oscillate at a low level. This concentration pattern is important, as it is representative for other cases.

The range of the critical region depends on the parameter values and the number of clones and their interaction scheme. In general, the critical zone leading to instability lies, roughly speaking in the range $\mu_m < [\mathrm{Ag}^i] < \mu_p$.

The 3-clone case is interesting because it represents the simplest network with more than one interaction possibility. We begin with the 3-clones closed chain case (3-ccc case), as illustrated in Fig. 3. In the absence of auto-Ag the system displays aperiodic behavior. The introduction of an auto-Ag leads to several changes in the behavior of the system of which we shall mention only those results

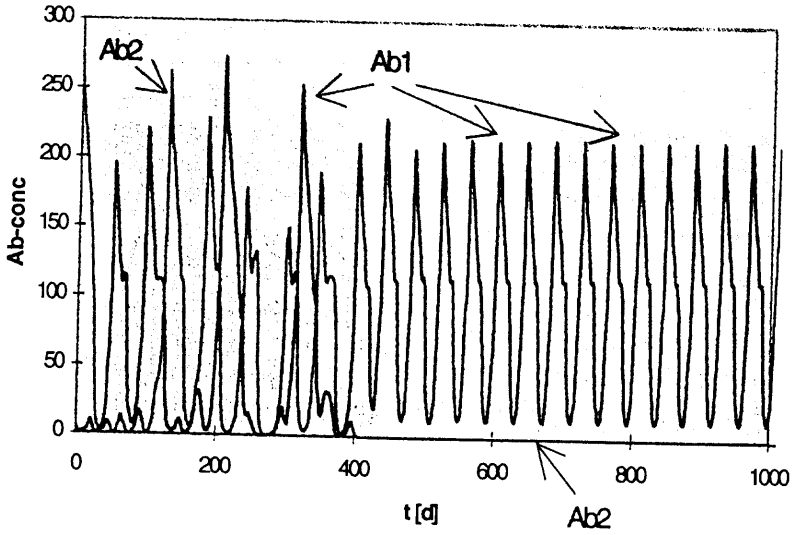


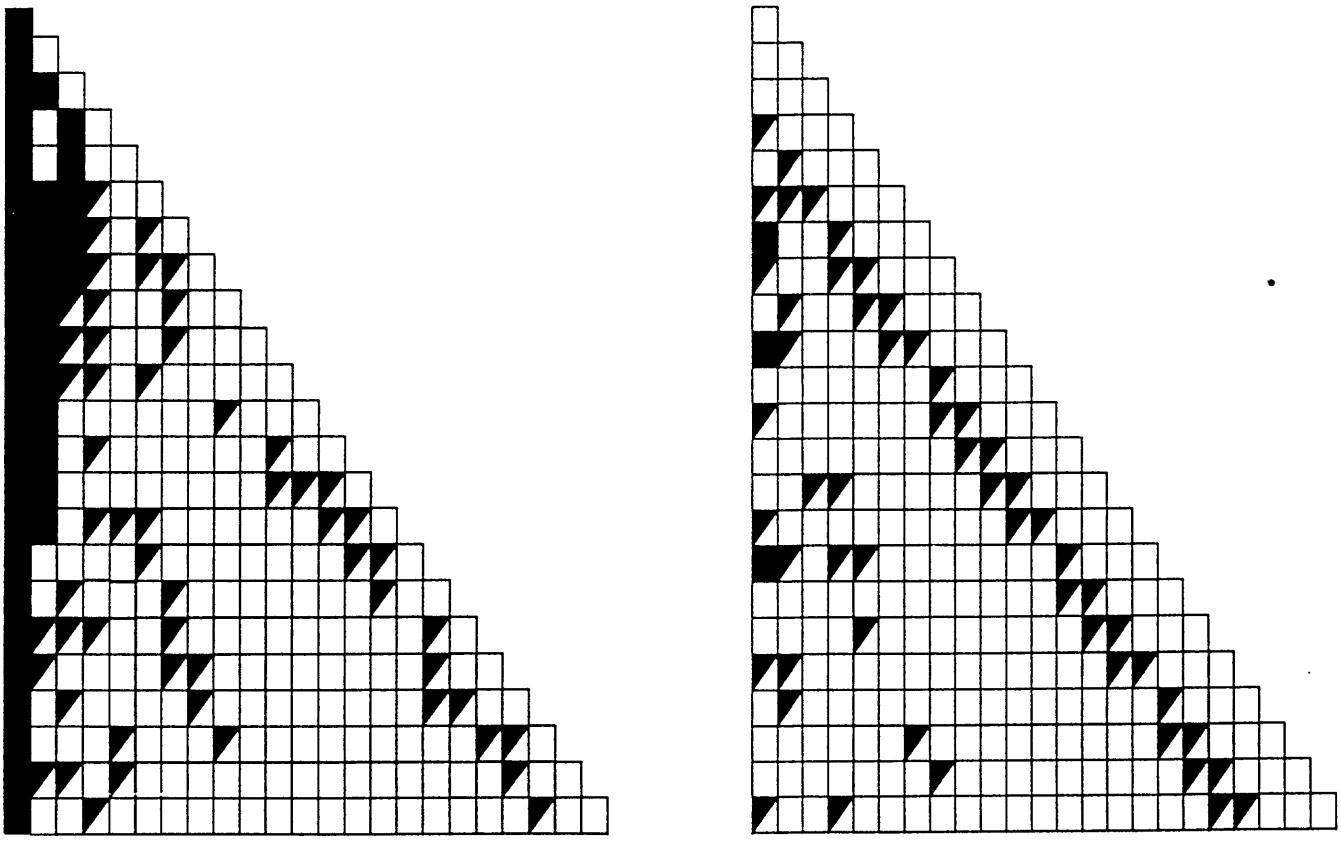
Fig. 3. Time series of the 3-clone closed chain system with and without auto-Ag interaction. For $0 > t > 400$ d there is no interaction with auto-Ag and one finds a chaotic regime. Upon introduction of a fixed Ag at $t = 400$ d, the chaotic attractor degenerates into a periodic one with the perturbed clone (2 in this case) oscillating in the low concentration range, while the two unperturbed clones oscillate in the high concentration range. $[\mathrm{Ag}] = 100$.

that are important for the following discussion. A deeper analysis can be found in CBSV. The interaction with the auto-Ag causes the chaotic attractor to degenerate into one of several possible periodic attractors. These are characterized by two different concentration patterns. In one case the perturbed clone oscillates with low amplitude, while the two others oscillate with a high amplitude. The two non-perturbed clones always have the same concentration and oscillate out of phase with the perturbed one (Type I behavior, in accordance with the terminology in CBSV). In the other case the perturbed clone oscillates at a high level and out of phase with the other two that oscillate at a low level (Type III).

Roughly speaking for two values, $[\mathrm{Ag}^i] < \mu_m$ and $[\mathrm{Ag}^i] > \mu_p$ the system is always stable. That means that the presence of Ag neither leads to an unbounded increase of one of the clones nor is any of them totally suppressed. For $\mu_m < [\mathrm{Ag}^i] < \mu_p$ (the critical region) the system has one stable regime (bounded response), corresponding to Type I concentration patterns, and one unstable regime (unbounded response), corresponding to Type III concentration patterns.

The interaction of an auto-Ag with the 3-clone open chain system leads to several oscillatory regimes which are all characterized by high level oscillations of the perturbed clone and low level oscillations of the unperturbed one. In the critical range these regimes become all unstable and lead to an unbounded immune response.

There are three basic results that are interesting as far as concerns the extrapolation from smaller systems to larger ones; namely:



(a) (b)

Fig. 4. Stability diagrams for (a) the open chain case and (b) the closed chain case. Along the left edge of the triangle, the number of clones in the network increases from three (top of the triangle) to 25 (left, bottom corner). From left to right, the number of nearest neighbors is plotted. For example, the left bottom corner corresponds to a system consisting of 25 clones, with only first nearest neighbor interactions. The right bottom corner of the triangle corresponds to a 25 clone system with 1st, 2nd,... up to 24th nearest neighbor interactions. Dark squares correspond to unstable (shore) regions, half filled squares to dangerous (reef) zones and white squares to safe (deep sea) areas. See also explanations in the text.

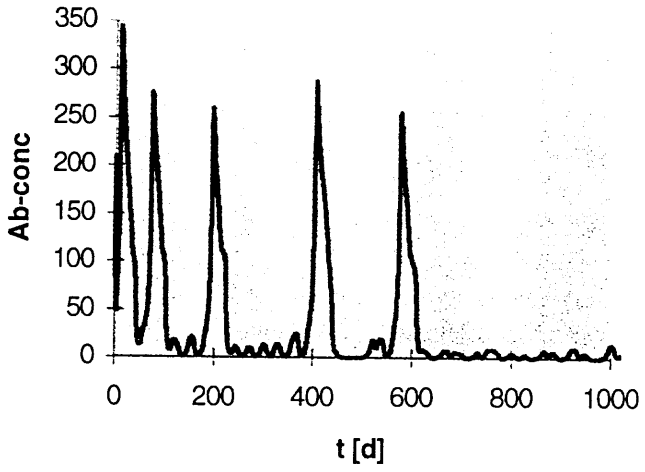
(a) stability as a function of the system dynamics, i.e. chaotic versus oscillatory dynamics,
(b) stability as a function of the connectivity matrix, i.e. closed versus open chain configurations,
(c) stability as a function of the concentration level of the perturbed clone.

Larger networks

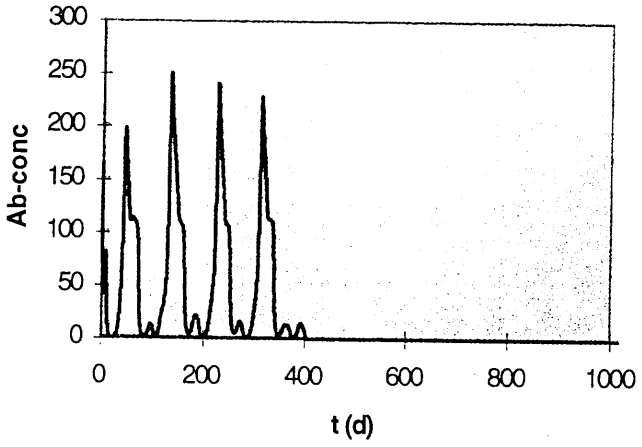
In what follows we shall describe the behavior of networks of varying connectivity and up to 25 clones. The reason for this is that above this limit network fragmentation starts to play an important role in the determination of the system behavior. Such a discussion would go beyond the scope of the present paper. We shall concentrate exclusively on interactions of the IS and auto-Ag in the critical range.

Open versus closed chain

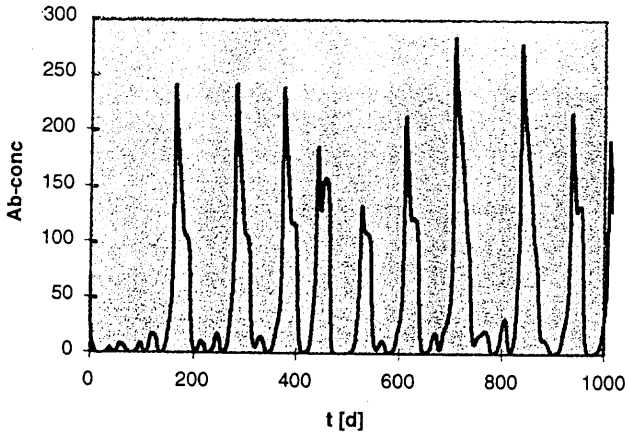
The NC-diagram (N = number of clones, C = number of connections) in Fig. 4 depicts the stability of the system as a function of its connectivity matrix. On the x-axis, the number of nearest neighbor connections are indicated, while on the y-axis the total number of clones can be found. For networks with only first nearest neighbor interactions (i.e. chains) there is always instability for the open chain case, while both, stable and unstable solutions are found in the closed chain cases. The simple



(a)



(b)



(c)

Fig. 5. Time series of the first (a), second (b) and third clone (c) of the 19/9-cl system without auto-Ag interaction for $t < 400\ d$ and with auto-Ag interaction for $t > 400\ d$. Upon introduction of the Ag, the chaotic regime persists, but the perturbed clone (2) is forced into the low concentration range, while the two unperturbed clones remain in the high concentration range. [Ag] = 100.

pattern of closed chain connectivity corresponding to stability, and open chain connectivity corresponding to instability such as found in the three clone case no longer remains true. However, the diagram, shows that closed chain connectivities lead more often to tolerance than open chain connectivities.

Chaos versus oscillations

In general, oscillatory regimes tend to be unstable, while chaotic regimes tend to be stable. Chaotic regimes can therefore not always be classified as safer or more advantageous than oscillatory ones, as was suggested by the analysis of the 3 clone case.

General tendencies

In the open chain case the safe, dangerous and unsafe regions, respectively, are connected. To use an analogy, the unsafe region in the low connected part of Fig. 4 will be referred to as shore region, while the large safe region in the medium- to high-connected areas can be compared to deep sea. At the rims of the deep sea area one finds reefs. This is also a useful terminology, as the reefs reach into the safe region of the deep sea.

In the closed chain case we identify some of the characteristics of the open chain case, namely a connected deep sea area with some irregular reefs with low and medium connected connectivity matrices. Also, the large connected reef forming a diagonal in the region of near maximally connected connectivity matrices is found in the closed chain case. The major difference between open and closed chain case is the absence of the shelf region in the closed chain situation.

A direct extrapolation of what was found in the 3-clone case has not been possible. The extrapolation of the behavior of the small system to larger ones allows us only to identify tendencies. There is, however, an important exception. The concentration pattern of the tolerant regime are always characterized by high level concentrations of the unperturbed and low level concentrations of the perturbed clone. In the unstable case, the perturbed clone can always be found in the high concentration range, see Fig. 5. It is important to note that the stable regime does not necessarily have to be oscillatory as in the case of the 3-clone closed chain situation. For larger networks, the system can live with the auto-Ag and can have either oscillatory, chaotic or fixed point dynamics.

An interesting structure is the distinct diagonal made of reefs in the closed chain case. This structure can also be found in the open chain case, however less marked. The mean number of connections per clone can be defined as degree of connectivity. In this sense, lines of iso-connectivity can be found along vertical lines in the diagram. Note that the diagonal does not correspond to lines of iso-connectivity, but that the diagonal of the closed chain systems is shifted to the left compared to the open chain case. On average, a clone in a closed chain system has more connections than a clone in an open chain system. If we suppose that a certain connection density leads to the unsafe regions on the diagonal, then this density is reached earlier for a closed chain system. It can be easily verified that the mean number of connections per clone in a particular N/C-closed network lies between the values obtained for the corresponding $N/(C-1)$-open and $N/(C+1)$-open networks.

4. Discussion

4.1. *Connectivity, stability and scaling*

The idiotypic network models studied to date do not have explicit mechanisms that stop short an immune response in the presence of a constant Ag. The fundamental question that we addressed here is different, namely, whether it is possible to have a coherent co-existence between the network and a constant somatic auto-Ag solely on the basis of the dynamic repertoire of the system. We have systematically studied the behavior of immune networks of different size and with diverse types of connectivity matrices. It was indeed found that for large connected areas in the NC-space the dynamical repertoire provides stable dynamical regimes. These allow the system to live with an auto-Ag. Moreover, there are areas in the NC-space in which instability prevails. However, there are also so-called dangerous zones interspersed irregularly as well as regularly in the stable regions.

An important issue in every study of network behavior is the determination of scaling laws. The behavior just described, however, is one of the reasons that make it quite unlikely that the system under study has scaling laws at the level of the stability properties as a function of the connectivity matrix. Another reason is the appearance of network fragmentation, which — roughly speaking — plays a role in systems of more than 25 clones.

We therefore have only studied networks of up to this size. As the phenomenon of network fragmentation is not yet fully understood, it appeared to be more appropriate to study only a selected and limited corner of the NC-space triangle. Nevertheless, some tendencies can be uncovered. In general, higher connected immune networks tend to provide tolerant regimes, while unstable, i.e. auto-immune ones as well as dangerous zones tend to concentrate in regions of smaller connectivity. The exception in both, open and closed chain cases is a narrow connected dangerous area along a line of iso-connectivity in the high connectivity region.

It is important to note that the characterization as unstable (shore), dangerous (reef) and stable (deep sea) zones does not stem from the exclusive presence of one of the behaviors in a particular zone but from its strong dominance (at least 75%) over the other behaviors, i.e., when moving horizontally along the connectivity-depth-axis in the NC-diagram, we do not encounter phase transition like behavior that corresponds to one particular type of behavior, i.e. stability or unstability, but transitions in the relative frequency of occurrence of these phenomena.

In CBSV it was suggested that closed chain connectivities and/or the resulting chaotic dynamics provide tolerant modes. This result from the simple 3-clone case cannot be extended without modifications to larger systems and more complex connectivity matrices. However, in general one finds that oscillatory modes tend to be unstable. Moreover, closed chain connectivities lead only rarely to instability. In general, several regimes can coexist. The principal result that can be generalized is the following. In the three clone case it was found that stable regimes are always characterized by the following concentration pattern: oscillations of the perturbed clone in the low concentration region, while the unperturbed clones oscillate in the high concentration range. This result can be generalized. Whenever, the perturbed clone is in the low concentration range, while the unperturbed clones are in the high concentration range, then — whatever the dynamics, i.e. whether it be chaotic, oscillatory or fixed points — the system will be stable, i.e. tolerant.

4.2. *Tolerance and autoimmunity*

We have seen that connectivity space can be divided into several zones characterized by a particular type of behavior. Do these results provide any useful insight with respect to the etiologies auto-immune disease and their treatment? In the framework of our network interpretation, auto-immune disease could be due to defects in the network structure [Varela & Coutinho, 1991]. In particular, intravenous injection of pooled Ig (IvIg) is a successful clinical practice which could be explained by a camouflage- or fill out-effect of the network defects [Kaveri *et al.*, 1991]. Further, in a case study of a patient suffering from Hashimoto's thyroiditis we have shown the changes in network dynamics before and after IvIg treatment [Dietrich *et al.*, 1993]. Often auto-immune patients display remission of symptoms after IvIg treatment, but the symptoms recur after a couple of months.

CBSV suggested that a large perturbation of the immune system by injection of Ig could indeed induce a shift from an unstable to a stable regime in the case of coexisting attractors. Furthermore, in the course of several months one would expect the system to shift back to the old attractor, as the effect of the perturbation dies out. In the case of larger networks with more complex connectivity structures, this still remains a valid possibility, as we almost always find unstable regimes co-existing with stable ones. This scenario also implies that, given a certain connectivity, stability or unstability, i.e. tolerance or auto-immunity would result from the attractor that is selected. Which, in turn, depends on starting conditions. In the course of the life span of an individual, these are determined by the term k_6 in Eq. (2). Hence, tolerance and auto-immunity would depend on this factor.

The present results also suggest another alternative scenario. IvIg injection could not only lead to a change in concentration of several clones involved and thereby inducing a shift from one regime to another. An injection could also introduce new clones into the system and thereby change the actual connectivity matrix of the system. This in turn could bring the system into a region of NC-space of different stability properties. Moreover, one expects that also in this case the perturbation would die out, such that the system shifts back to the original regime.

A definitive answer as to whether these scenarios are realistic can only be obtained by experiment. Such experiments would require knowledge of connected clones. The smaller the number of clones involved, the better. If a pure change in concentration of the clones can cause disappearance of

auto-immune symptoms in the organisms, then the former scenario would be a good candidate for a possible explanation for the mechanisms involved in relation to IvIg-treatment. If, however, changes at the level of the connectivity matrix play a role, then only detailed knowledge of the injected clones can help us further. As such a detailed information can probably not be obtained, it is perhaps more adequate to manipulate the connectivity matrix of the selected network in such a way as to suppress one of the clones and investigate the effect of IvIg-treatment with and without such a modification.

4.3. *Future studies*

We conclude by indicating some of the directions to take in future work. First, we have to test the robustness of our results when relaxing the constraint of Boolean type affinities. Secondly, would a system with meta-dynamics select those interaction schemes favoring the stable regimes in the presence of constant Ag? Thirdly, how do fragmented networks behave in the presence of auto-Ag?

Acknowledgments

VC gratefully acknowledges financial support from the European Community. The authors would like to thank the members of the Paris-Brussels group in theoretical immunology for many discussions, in particular A. Coutinho and J. Stewart.

References

Atiya, A. & Baldi, P. [1989] "Oscillations and synchronizations in neural networks: An exploration of the labeling hypothesis," *Int. J. Neural Systems* **1**, 103–124.

Bersini, H. [1992] "The interplay between the dynamics and metadynamics of the immune network," in *Artificial Life III*, ed. Langton, C. (Addison-Wesley, New Jersey).

Bersini, H. & Calenbuhr, V. [1995] "Frustration induced chaos in a system of coupled ODE's," *Chaos, Solitons and Fractals* 5(8), 1533–1549.

Calenbuhr, V., Bersini, H., Varela, F. J. & Stewart, J. [1993] "The impact of the structure of the connectivity matrix on the dynamics of simple model for the immune network," in *Proc. of the 1st Copenhagen Symp. on Computer Simulation in Biology, Ecology and Medicine*, ed. Mosekilde, E. (Soc. Comp. Sim. Int., Ghent).

Calenbuhr, V. & Bersini, H. [1993] "Frustration induced chaos and intermittency," IRIDIA Technical Report, Université Libre de Bruxelles; also in preparation for publication.

Calenbuhr, V., Bersini, H., Stewart, J. & Varela, F. J. [1995] "Natural tolerance in a simple immune network," *J. Theor. Biol.* **177**, 199–213.

De Boer, R. & Hogeweg, P. [1989] "Unreasonable implications of reasonable idiotypic network assumption," *Bull. Math. Biol.* **51**, 381–408.

De Boer, R. & Perelson, P. [1990] "Size and connectivity as emergent properties of a developing immune network," *J. Theor. Biol.* **149**, 381–424.

De Boer, R., Kevrekidis, I. G. & Perelson, A. S. [1993a] "Immune network behavior I: From stationary states to limit cycle oscillations," *Bull. Math. Biol.* **55**, 745–780.

De Boer, R., Kevrekidis, I. G. & Perelson, A. S. [1993b] "Immune network behavior I: From oscillations to chaos and stationary states," *Bull. Math. Biol.* **55**, 781–816.

Detours, V., Bersini, H., Stewart, J. & Varela, F. J. [1994] "Development of an idiotypic network in shape space," *J. Theor. Biol.* **170**, 401–414.

Detours, V., Calenbuhr, V. & Bersini, H. [1995] "Clustering phenomena in idiotypic networks," IRIDIA Technical Report, Université Libre de Bruxelles; also in preparation for publication.

Dietrich, G., Varela, F. & Kazatchkhine, M. [1993] "Manipulating the human immune network with IgG," *Europ. J. Immunol.* **23**, 2945–2950.

Kaveri, S.-V., Dietrich, G., Hurez, V. & Kazatchkine, M. D. [1991] "Intravenous immunoglobulins (IVIg) in the treatment of autoimmune diseases," *Clin. Exp. Immunol.* **86**, 192–198.

Matthews, P. C., Mirollo, R. E. & Strogatz, S. H. [1991] "Dynamics of a large system of coupled nonlinear oscillators," *Physica D*, 293–331.

Neumann, A. U. & Weisbuch, G. [1992a] "Window automata analysis of population dynamics in the immune system," *Bull. Math. Biol.* 54(1), 21–44.

Neumann, A. U. & Weisbuch, G. [1992b] "Dynamics and topology of idiotypic networks," *Bull. Math. Biol.* 54(5), 699–726.

Stewart, J. & Varela, F. [1989] "Exploring the connectivity of the immune network," *Immunol. Rev.* **110**, 37–61.

Stewart, J. & Varela, F. [1990] "Dynamics of a class of immune networks. II. Oscillations of the humoral and cellular compartments," *J. Theor. Biol.* **144**, 103–115.

Stewart, J. & Varela, F. [1991] "Morphogenesis in shape space: Elementary meta-dynamics of immune networks," *J. Theor. Biol.* **153**, 477–498.

Tauber, F. [1994] *The Immune Self. Theory or Metaphor. A Philosophical Inquiry* (Cambridge University Press).

Toulouse, G. [1977] *Commun. Phys.* **2**, 115.

Tsang, K. Y., Mirollo, R. E., Strogatz, S. H. & Wiesenfeld, K. [1991] "Dynamics of globally coupled oscillator array," *Physica D*, 102–112.

Varela, F., Coutinho, A., Dupire, B. & Vaz, N. [1988a] "Cognitive networks: Immune, neural, and otherwise," in *Theoretical Immunology, Part II (SFI Series on the Science of Complexity)*, ed. Perelson, A. S. (Addison Wesley, New Jersey), pp. 359–375.

Varela, F. & Stewart, J. [1990] "Dynamics of a class of immune networks. I. Global stability of idiotype interactions," *J. theor. Biol.* **144**, 93–101.

Varela, F. & Coutinho, A. [1991] "Second generation immune networks," *Immunol. Today* **12**, 159–167.

Varela, F. J. Stewart & A. Coutinho [1993] "What is the immune system for?," in *Thinking about Biology, SFI Series*, eds. Stein, W. & Varela, F. J. (Addison Wesley, New Jersey).

Weisbuch, G., Zorzenon Dos Santos, R. M. & Neumann, A. U. [1993] "Tolerance to hormones in an idiotypic network model," *J. Theor. Biol.* **163**, 237–253.