A Graph Model for the Evolution of Specificity in Immune Systems

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Abstract

The immune system protects the body against health-threatening entities, known as antigens, through very complex interactions involving antigens and the system's own entities. One remarkable feature resulting from such interactions is the immune system's ability to improve its capability to fight antigens commonly found in the individual's environment. This adaptation process is called the evolution of specificity. In this paper, we introduce a new mathematical model for the evolution of specificity in immune systems, based on Jerne's Functional Network Theory. The evolution of specificity is modeled as the dynamic updating of connection weights in a graph whose nodes are related to the network's idiotypes and their concentrations. At the core of this weight-updating mechanism are the increase in specificity caused by clonal selection and the decrease in specificity due to the insertion of uncorrelated idiotypes by the bone marrow. As we demonstrate through numerous computer experiments, for appropriate choices of parameters the new model correctly reproduces, in qualitative terms, all immune functions, including the immune response, the immune memory, and the distinction between self and non-self entities.

Keywords: Immune-system specificity, immune response, immune memory, self non-self distinction in immune systems, models of the immune system, clonal selection, functional network.

1 Introduction

The immune system is responsible for protecting the body against potentially dangerous agents. A deep knowledge of the mechanisms that underlie this complex system is essential in the fight against several illnesses, such as AIDS, cancer, and various auto-immune diseases, and is also of interest to computer scientists, who employ immune-related notions, such as the immune memory, to develop tools capable of solving computational problems. Immune-inspired computational tools are called artificial immune systems, and can also benefit from new discoveries in immunology to improve their performance.

We introduce a new model of the immune system, based on Jerne's Functional Network Theory. The network in our model is represented by a weighted directed graph whose weights correspond to the degrees of affinity among network idiotypes. The model's main contribution is a new concept in immune networks, namely the evolution of specificity by dynamically updating network weights. This process mimics the increase in network specificity, biologically due to

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clonal selection, as well as the decrease in network specificity, biologically due to the insertion of new idiotypes by the bone marrow.

Our model was designed to qualitatively reproduce immune-system functions. In order to verify that this is indeed achieved, and also to highlight the performance of the immune network, computer experiments were carried out. The first phase of this experimentation process comprised the determination of adequate values for each parameter of the model. This was not a simple task, as there is a relatively large number of parameters and also it is difficult to establish direct relationships between actual biological quantities and suitable parameter values. The essence of this first phase was then to investigate a wide range of possibilities in the search for parameter values.

Once a suitable set of parameter values was identified, the remainder of the experimentation process yielded results that are very successful in demonstrating that the model correctly reproduces immune-system functions in qualitative terms, and also very clearly indicate a tremendous influence of specificity evolution on the network's overall performance. Several more detailed conclusions can be drawn from the results, essentially concerning the model's structure and the influence of parameter values on performance.

The remainder of the paper is organized as follows. Section 2 is a summary of immune-related concepts, especially those more closely related to our discussion in this paper. Our model is presented in Section 3, which is followed by the results of our computer experiments in Section 4. Conclusions follow in Section 5.

2 The Immune System

The immune system is in charge of protecting the body against foreign and potentially harmful agents that come into it. It is also responsible for destroying body cells whose behavior is abnormal or dangerous, as in the case of cancerous or virus-infected cells. The immune system is a very complex system, comprising a very large number of elements that interact with the dangerous agents and among themselves also. What results from such interactions are the so-called immune functions, among which are the immune response, the immune memory, and the capability of discriminating between self and non-self entities.

The potentially harmful entities, both self and non-self, are made of and also produce chemical substances that can be recognized by the immune system. These substances are called antigens and the corresponding reaction of the immune system is the immune response. When the immune system develops an immune response against a certain antigen, it becomes better prepared to fight it in future occasions, showing a remarkable memory, which is the basis of vaccine immunization and is known as the immune memory.

The immune system is capable of recognizing virtually any antigen occurring in nature, including antigens from the very body it must protect. However, in healthy individuals this does not happen because the immune system has a way to prune those entities that might react against self-antigens. Auto-immune diseases occur when this mechanism stops working properly.

2.1 Main Components

The immune system's main components include molecules, cells, and organs. Molecules are responsible for maintaining inter-cellular communication (cytokines), neutralizing and removing antigens (antibodies and blood complement), and controlling and maintaining cellular functions (cell-building molecules).

Lymphocytes are the immune system's most important cells. There are two main types of lymphocyte, known as B cells and T cells. There are also other cells with important functions in the immune system, as for example macrophages and dendritic cells.

B cells are precursors of cells of yet another type, known as plasma cells, whose function is to produce antibodies. These molecules bind to antigens to help macrophages and the blood

complement recognize antigens that should be destroyed and removed from the body. A binding takes place when there is high affinity between receptors of the immune-system entities and the antigens. The affinity between the receptors and antigens is based on the complementarity of properties, as for example geometric shape and chemical affinity. Although it is often assumed in theoretical studies that each B cell has receptors that can recognize and bind to only one type of antigen, real B cells, notwithstanding their high specificity, can recognize more than one type of antigen; in fact, they recognize a group of antigens very similar to one another. When a B cell recognizes an antigen, it becomes stimulated and starts producing plasma cells and other B cells with receptors similar to its own.

There are two types of T cell, helper T cells and killer T cells. Helper T cells participate in the activation process of the other types of lymphocyte (B cells and killer T cells), while killer T cells are in charge of destroying intruders or body cells producing abnormal substances. T cells, like B cells, can recognize antigens with high specificity, but they are capable of recognizing only antigens bound to MHCII (major histocompatibility complex type II) molecules, which act in essence like a repository for sample substances and antigens. Most body cells have this type of molecule on their surfaces and attach samples of the substances they are producing to those molecules so T cells are capable of discriminating whether those substances are normal and well known or abnormal and probably harmful. In the latter case, killer T cells are activated and contaminate the cell's interior with their cytotoxic substances.

Lymphocyte receptors are known as paratopes, while the antigen regions that can be recognized by the immune system are called epitopes [8]. The immune response is the result of cell activation due to the affinity between paratopes and epitopes. The immune system repertoire of paratopes is limited, so in order for its recognition capabilities to be all-encompassing, it has been argued that some conditions need to be satisfied [10]. These are that each paratope must recognize a small group of slightly different epitopes; that the repertoire of paratopes must be in the order of 10^6, and that paratopes must be randomly distributed along the possible range of different paratopes.

The body organs most closely related to the immune system are the bone marrow, the lymph nodes and the thymus. The bone marrow produces immune-system cells as it produces the other blood cells. The lymph nodes are spread along the entire body and their function is to facilitate the contact between helper T cells and antigens bound to the other immune-system cells. The thymus is a dense organ where T cells mature. It is in the thymus that the aforementioned pruning of self-reactive helper T cells happens.

2.2 Immune Functions

The immune response is the set of actions taken by the immune system to face the attack of potentially harmful agents. It starts with the agent being recognized and generally ends with its total removal from the body. There are two types of immune response, the innate immunity and the acquired immunity. The former appears during fetal development and is the immune mechanism the individual is born with. This type of immunity is only effective on some types of antigen, and it does not show any memory or specificity. The acquired immunity, by contrast, is developed during the individual's life. It goes through an improvement process that leads the immune system to efficiently protect the individual against antigens commonly found in the individual's environment. This type of immunity is highly specialized and shows strong memory and specificity. There are two types of acquired immunity, the humoral and the cellular.

The humoral immunity is thus called because its main effectors, the antibodies, are found in the body's fluids, also called humors. When a B cell recognizes an antigen complementary to its paratopes, it becomes stimulated. It also takes the antigen into its cytoplasm for division into the peptides that eventually get bound to the MHCII molecules on its surface. Helper T cells recognize the MHCII-peptide ensemble and become activated, thereby liberating the cytokines that signal the previously stimulated B cells to start producing plasma cells and other B cells. This proliferation occurs with high mutation rates (process known as hypermutation), which is why the paratopes of the resulting B cells are not exact copies of the originals [9]. The antibodies

produced bind to the antigens, thus signaling macrophages and the blood complement to destroy any antigen with attached antibodies.

The cytokines liberated by the activated helper T cells also signal the killer T cells to destroy all cells on whose surface the MHCII-peptide ensemble is to be found. This is the essence of cellular immunity.

The first time the immune system finds a certain antigen, it develops an immune response, called the primary immune response, to fight it. If in the future the system finds the same or a similar antigen, it develops a new response called the secondary immune response [5]. The secondary response is normally more efficient and much faster than the primary response, because the immune system seems to already know the mechanisms needed to destroy the antigen. In fact, each time the immune system fights an antigen it becomes better prepared to face the same antigen when it next enters the body. This memory is known as the immune memory and is the basis of vaccine immunization. Immune memory is generally not permanent; in most cases it eventually ends, which seems to indicate that the information needed to face the attack of an antigen is kept for a limited period of time only.

The same properties that endow the immune system with the power to fight any foreign agent also confer to it the capability of reacting against body entities. This does not happen to healthy individuals because of a mechanism that prunes helper T cells that recognize body tissues. This pruning process takes place basically in the thymus and is based on two properties helper T cells show in the presence of high antigen concentrations. These properties are known as tolerance (or anergy) and suppression. In the presence of very high concentrations of antigens, helper T cells either simply ignore their presence (tolerance) or die (suppression). As the thymus is a dense organ containing high concentrations of all self-antigens, it induces tolerance and suppression on self-reactive helper T cells during their maturation. As these lymphocytes are essential to develop both humoral and cellular immunity, reactions against self-antigens become automatically prevented.

2.3 Immune System Theories

There are two main theories that try to explain the macroscopic behavior of the immune system. They are the Clonal Selection Theory and the Functional Network Theory.

The Clonal Selection Theory was originally proposed by Macfarlane Burnet in 1959 [2]. The idea underlying the theory is that only those cells that recognize antigens proliferate, being in this way selected from all others [10]. A clone is a group of cells with similar paratopes. This theory basically describes the immune response as it was explained in Section 2.2. According to it, the immune memory is due to the presence of lymphocytes with longer lifetime. These cells are called memory cells and are the product of the proliferation of activated lymphocytes. The discrimination between self and non-self antigens is attributed to the pruning of self-reactive lymphocytes during their maturation process.

Jerne proposed the Functional Network Theory in 1974 [8]. According to this theory, the immune-system components do not act as isolated entities but as an auto-regulated network in which they recognize other components and can also be recognized by them.

Such interactions are based on the fact that those components have not only paratopes to recognize entities but also epitopes that can be recognized by other entities. In fact, antibody and B-cell receptors all have the same structure. In both cases one finds light and heavy chains, in each chain constant and variable regions. Variable regions can bind to antigens while constant regions determine the mechanism to be used in order to destroy the antigen. Experiments have shown that variable regions contain sub-regions that can be recognized by other entities of the immune system; that is, antibodies and B-cell receptors have epitopes. The group of epitopes belonging to an antibody or B-cell receptor is called an idiotype and each idiotypic epitope is known as an idiotope [8]. According to Jerne, these idiotypes have a functional role in the network.

When the immune system develops an immune response against an antigen, antibodies are produced. Other cells of the immune system recognize the idiotypes of the antibodies and new

antibodies are produced. If there were no mechanism of control, antibody production would never stop and cause an antibody demographic explosion. However, this does not happen because, according to Jerne, there is a mechanism that regulates system activity. This mechanism is the functional network.

Each entity of the immune system (cell or antibody) has paratopes and idiotypes so it can recognize and also be recognized by other system entities. These entities, called clones in an attempt to generalize the homonymous notion in the Clonal Selection Theory, are characterized by paratope-idiotype couples, denoted generically by p_x-i_x and illustrated in Figure 1. Using this figure as example, the functioning of the network can then be described as follows. Before an antigen comes into the body the network remains in population equilibrium. Clone p_1-i_1 stimulates clone p_3-i_3, which in turn inhibits clone p_1-i_1. Meanwhile, clone p_2-i_2 stimulates p_1-i_1 and p_1-i_1 inhibits p_2-i_2. This stimulation-inhibition interplay maintains the clonal population's balance in the network. When antigens are introduced in the system, they react with the elements of clone p_1-i_1, which causes the population of this clone to decrease, and takes the network out of balance. The excitatory action of p_1-i_1 over p_3-i_3 decreases, leading the population of p_3-i_3 to decrease, too. As a result, the inhibition of p_3-i_3 over p_1-i_1 decreases. While the population of p_1- i_1 remains low, inhibition over p_2-i_2 remains low as well, so p_2-i_2 tends to increase in population, increasing also its stimulatory effect over p_1-i_1. This stimulation makes the population of p_1-i_1 increase, increasing the stimulation of p_1-i_1 over p_3-i_3 as a consequence. As a result, p_3-i_3 has its population increased until its inhibitory effect over p_1-i_1 is strong enough to counterbalance the excitatory effect of p_2-i_2 over p_1-i_1 and to make the population of p_1-i_1 stabilize.

2.4 Immune-System Models

There are two basic formalisms widely used to represent the immune network in mathematical models of the immune system. They are the multidimensional vector space called the shape space and graphs.

As we discussed earlier, the affinity among idiotypes and paratopes is due to the complementarity that exists between molecules in physical or chemical terms. Letting the shape space be an n-dimensional vector space, each complementarity property can be represented by one dimension. A point $(x_1, \ldots, x_n)$ in the shape space represents an idiotypic clone of the network. One possibility to indicate affinity in the shape space is to use geometric complementarity among points, so the clone complementary to $(x_1, \ldots, x_n)$ is $(-x_1, \ldots, -x_n)$. Other rules can also be used to define complementarity among idiotypes in the shape space, one of them being the Hamming distance.

Variations of the shape-space formalism can be used to represent special features of the immune system. For example, it is known that an idiotypic clone in the network can be stimulated by more than one clone (this property is known as cross link [10]). In order to represent this property in the shape space, a sphere of small radius surrounding the point of total complementarity can be used. All points inside the sphere have affinities with the original point but affinity strength decreases with the distance to the center [4]. There are also other versions of the shape space.

Each idiotypic clone of the network can alternatively be represented by a node of a graph, whose edges can then be used to indicate complementarity. Edges may be undirected or directed, respectively in the case of symmetric or asymmetric complementarity relations. In the former case, an edge connecting nodes n_1 and n_2 means that node n_1 is complementary to node n_2 and node n_2 is complementary to node n_1. The latter case, which seems to be more suitable to represent natural clones, allows the paratopes of n_1 to be complementary to the idiotypes of n_2, for example, while the paratopes of n_2 are not complementary to the idiotypes of n_1. Graphs allow greater diversity of network topologies when compared to shape spaces. They also allow irregular network configurations in which each node has a different number of complementary nodes.

One prominent model that employs the shape-space formalism is the B model of De Boer [3]. Here we give a relatively detailed account of the B model because our model, to be introduced in Section 3, can be regarded as extending the B model in several ways. The B model is one of the simplest mathematical representations of the immune system; it only takes into account the evolution of B-cell populations and regards these lymphocytes as being sufficient to develop an immune response. The model uses the shape-space formalism and there is only one system of equations to describe the evolution of the populations of idiotypic clones as a function of time. For clone i, the equation is

$$\frac{dx_i}{dt} = m + [pg(h_i) - d]\, x_i,$$

where

- x_i is the population of idiotypic clone i;
- m is the rate at which new clones are inserted by the bone marrow;
- d is the death rate of clones;
- p is the proliferation rate of clones;
- g is an activation function; and
- h_i is the so-called field of clone i, which depends on the populations of the clones complementary to clone i and on the affinity between each of them and clone i.

The field of clone i is given by

$$h_i = \sum_j J_{ij} x_j,$$

where J_{ij} is the interaction strength (affinity) between clones i and j. J_{ij} is an element of what we henceforth call the stimulation matrix. This matrix is symmetric, which reflects the symmetry of the affinity relation in the B model.

The activation function g models the three possible activation states of a clone: the virgin state, in which the clone is not yet stimulated, the immune state, in which the clone proliferates, and the inhibited or suppressed state, in which proliferation stops. In the original model, a Gaussian function is used as activation function, but in some variations [1] the Gaussian is substituted by a window function, as shown in Figure 2. When the field is lower than l_1, the clone remains virgin; if the field is between l_1 and l_2, the clone is in the immune state; and if the field is grater than l_2, the clone is inhibited.

In the original model, another Gaussian is used to determine the elements of the stimulation matrix. This is achieved by identifying clones with points in a discrete version of the shape space and making the Gaussian's maximum coincide with the point of total complementarity. Radial decay is used for the other points.

The B model has been extensively used in studies of the immune network's dynamics, and several variations thereof have been created to address specific issues on different properties of the network. One important variation is given by Harada and Ikegami [6], used to analyze the evolution of the network's specificity in the presence of antigens.

The specificity of a paratope is related to its ability to recognize idiotypes, so the higher the affinity between a paratope and an idiotype, the higher the specificity of the paratope to recognize that idiotype. The specificity of the network is given by its capability to recognize only a few antigens but with high affinity. Harada and Ikegami use yet another discrete version of the B model to analyze the evolution of specificity in a network with fixed stimulation matrix. They show that only those clones with certain paratopes that show high affinity with antigens increase their populations, while other clones of the same idiotype tend to maintain their populations unaltered.

3 The Model

In this section, we present a detailed description of our model. We start by considering a shape-space variant in which each clone is extremely selective. Each paratope can recognize only one idiotype and each idiotype can only be recognized by one paratope with the highest possible affinity. Biological clones, as discussed earlier, are very selective but can nonetheless recognize more than one idiotype with different affinity levels. Hence biological clones can be represented in this variant of the shape space as small regions of the space, the larger the region the less specific the clone, because it can interact with a greater number of other clones. Henceforth, we refer to these regions of the space as clusters.

Each cluster of the shape space can be represented as a node in a graph, with edges linking nodes representing related clusters. Each edge has a weight that corresponds to the affinity level between clusters. Weights can be positive or negative, depending respectively on whether the influence of one cluster upon the other is excitatory or inhibitory. In Figure 3, a network with three nodes is depicted. Node A stimulates node B, which means that the paratopes of clones in node B recognize the idiotypes of clones in node A, and this happens with an average specificity of 0.1. In accordance with the Clonal Selection Theory, the presence of clones in node A stimulates clones in node B, and as a result the population of clones in node B increases. This increase causes a population reduction in node A, because the paratopes of the clones in node B bind to the idiotypes of the clones in node A, removing such clones from the system. So the excitatory (positive) influence of node A upon node B generates a simultaneous inhibitory (negative) influence of node B upon node A. In the network of Figure 3, node B is stimulated by nodes A and C, while nodes A and C are inhibited by node B. There is no interaction between nodes A and C. This means that the paratopes of clones in node B can recognize, with different specificities, idiotypes of clones in nodes A and C, but paratopes of clones in nodes A and C do not recognize any idiotype in the network. In the sequel, for simplicity we refer to clusters or to nodes interchangeably.

The specificity of a cluster can be estimated by the number of edges incoming to it from other clusters and by the weights of those edges. A highly specific cluster has few such edges with high weights. The specificity of the immune system grows in the presence of antigens: when a particular antigen is recognized by a group of lymphocytes they start proliferating, and due to hypermutation the idiotypes of the new cells differ a little from those of the original cells. Mutants with higher levels of affinity with the antigen keep proliferating, while others eventually die. This process, repeated several times, leads the system to a higher specificity level. An uncontrolled growth of specificity would make it necessary to increase the number of cells beyond reasonable limits in order to guarantee that every possible antigen could still be recognized. In order to avoid this, the bone marrow decreases network specificity concomitantly with the increase promoted by clonal selection: it introduces new lymphocytes based on pre-established genetic patterns that have no correlation whatsoever with antigens. Such uncorrelated idiotypes reduce the network's specificity to recognize currently present antigens.

The network's immune memory depends highly on the growth of specificity, because the system becomes better prepared to fight a certain antigen every time it finds that antigen. However, as remarked earlier the immune memory cannot last indefinitely, because the growth of specificity is reversed in order to maintain the system's cell population below reasonable bounds.

Our model grew out of the B model and is based on two systems of equations. The first system represents the populations of clones in the network clusters, and the other dictates how edge weights are to change in order to represent the evolution of network specificity. For simplicity, we present our model's equations for discrete instants given by the naturals.

For $t \geqslant 0$, the variation in clone population for cluster i is given by

$$x_i(t+1) = x_i(t) + b + \left[pg(h_i^+(t)) - d\right] x_i(t) - uh_i^-(t), \tag{1}$$

where

x_i is the clone population of cluster i;

b is the rate at which new clones are inserted by the bone marrow;

p is the proliferation rate of clones;

h_i^+ is the excitatory field of clone i;

g is the activation function;

d is the death rate of clones;

u is the inhibition rate; and

h_i^- is the inhibitory field of clone i.

The fields h_i^+ and h_i^- depend on the clone populations at neighboring clusters and also on the affinities among the paratopes and idiotypes involved. For a network with N clusters,

$$h_i^+(t) = \sum_{j=1}^{N} w_{ij}^+(t)\left[x_j(t) + a_j(t)\right], \tag{2}$$

and

$$h_i^-(t) = \sum_{j=1}^{N} w_{ij}^-(t) x_j(t). \tag{3}$$

In (2) and (3),

w_{ij}^+ is the affinity with which the idiotypes of clones in cluster j are recognized by the paratopes of clones in cluster i;

a_j is the amount of antigen with epitope similar to the idiotype of clones in cluster j; and

w_{ij}^- is the affinity with which the paratopes of clones in cluster j recognize the idiotypes of clones in cluster i .

Note that w_{ij}^+ represents the excitatory effect of cluster j upon cluster i, thence the reason why h_i^+ is called an excitatory field. Similarly, w_{ij}^- represents the inhibitory effect of cluster j upon cluster i, thence the denomination as an inhibitory field for h_i^-.

Note also that the proliferation and death terms in (1) depend on the number of clones in cluster i. The reason for this is clear: only existing clones can proliferate or die, and do so proportionally to how many clones there are. Inhibition, on the other hand, does not work the same way, as it only depends on the number of clones in clusters that inhibit cluster i. These clones will bind to clones in cluster i to remove them from system, and they may exist even if there are no clones in cluster i, leaving the system ready to react rapidly if a sudden population increase in cluster i takes place.[1] The activation function g is a window function like the one in Figure 2, and models the three possible activation states, the virgin state, the immune state, and the inhibited state.

Our second system of equations indicates how the affinities among network clusters evolve. For $t \geqslant 0$, we have

$$w_{ij}^+(t+1) = f_{ij}(t) w_{ij}^+(t) + r, \tag{4}$$

where

$$f_{ij}(t) = \frac{\delta \eta_{ij}(t)}{\max\limits_{k=1,\ldots,N} \eta_{ik}(t)} + 1 - \frac{\delta}{2} \tag{5}$$

[1] Thus, in order to be completely rigorous, in (1) we must use the maximum of 0 and $b + \left[pg(h_i^+(t)) - d\right] x_i(t) - uh_i^-(t)$ instead of simply the latter quantity. This prevents $x_i(t+1)$ from falling below 0.

with

$$\eta_{ik}(t) = w_{ik}^{+}(t)\left[x_k(t) + a_k(t)\right].$$

In (4) and (5),

f_{ij} is the weight expansion or contraction factor;

r is the noise inserted by the bone marrow; and

δ is the difference between the maximum expansion factor and the maximum contraction factor.

The weight w_{ij}^{+} is updated according to the factor f_{ij}, which in turn depends on the affinities among clusters and on cluster populations. The excitatory influence of cluster j on cluster i is represented by the product of the affinity weight with which cluster j stimulates cluster i and the population at cluster j. Clusters exerting higher excitatory influences on cluster i induce the production of mutants at cluster i with higher chances of further proliferation. As a result, cluster i becomes more competent to recognize clusters exerting higher excitatory influences, meaning an increment in specificity. In the model, a linear function is used to determine the weight update factor. This is shown in (5) and plotted in Figure 4. The maximum weight-population product gives the maximum weight expansion factor of $1 + \frac{\delta}{2}$, while all others are scaled proportionally between $1 - \frac{\delta}{2}$ and $1 + \frac{\delta}{2}$. Factors greater than one expand weights, while those less than one contract weights. The δ parameter determines how fast weights, and hence network specificity, are altered.

Weights w_{ij}^{+} can be regarded as the elements of the stimulation matrix w^{+}, and w_{ij}^{-} of an inhibition matrix w^{-}. In the model, we assume $w^{-} = -(w^{+})^{T}$ at all times, where the superscript T indicates matrix transpose, that is, $w_{ij}^{-} = -w_{ji}^{+}$.

Another essential element in (4) is the random-noise term r intended to represent the decrease in specificity caused by the bone marrow. This term has maximum amplitude R. The greater the value of R, the greater the spreading effect due to the insertion of new clones by the bone marrow.

In our model, antigens are introduced in the cluster whose idiotype resembles the antigen's epitopes more closely. Antigens do not have paratopes, so they cannot inhibit other clusters; therefore, their presence only affects the excitatory field. We model the removal of an antigen by a function that depends on the populations of clones complementary to the antigen, because those are the clones that will bind to the antigen and remove it from the system. The equation describing the evolution of antigen population for $t \geqslant 0$ is

$$a_i(t+1) = a_i(t) + \tau \sum_{j=1}^{N} w_{ij}^{-}(t) x_j(t),$$

where

a_i is the antigen population and

τ is the antigen removal rate.

4 Simulation Results

In order to evaluate the model's behavior and also the influence of each parameter on its performance, a number of tests were performed. The main goal in performing such tests has been to demonstrate that, with the right parameters, the model behaves qualitatively like the immune system. Two different plot types are used to show the results. Plots of the first type are three-dimensional, as in Figure 5, for example, and depict the clone populations of all clusters at all times during the simulation. Plots of the second type are two-dimensional, as in Figure 8, for example, and are meant to represent the network's immune response against antigens

inserted in some cluster or clusters. For each cluster where an antigen is present, these plots show the amount of antigen at all simulation times.

Results on two different network sizes are given next. For all tests, the elements of the initial stimulation matrix were sampled uniformly from the interval $[0, 2]$. Likewise, all initial node populations were sampled uniformly from $[0, 1]$.

4.1 A Five-Node Network

Our first network has five nodes. In order to determine the most suitable values for the model's parameters, we first deactivate the evolution of specificity and analyze the network's steady or homeostatic state under these conditions. The parameters involved in this case are the insertion rate by the bone marrow (b), the death rate (d), the proliferation rate (p), and the inhibition rate (u). All the other parameters are kept at values that do not interfere with network behavior ($\tau = 0$, $\delta = 0$, $R = 0$, $l_1 = 0$, and $l_2 = 10^4$). The first simulations were performed using the initial matrices

$$w^+(0) = \begin{pmatrix} 0 & 0.3669 & 0.3703 & 0 & 0 \\ 0 & 0 & 0 & 1.8244 & 0 \\ 0 & 0.8022 & 0 & 0 & 0.4865 \\ 1.1319 & 0 & 0.9573 & 0 & 1.0544 \\ 0.6080 & 0.4870 & 0 & 0 & 0 \end{pmatrix}$$

and

$$w^-(0) = \begin{pmatrix} 0 & 0 & 0 & -1.1319 & -0.6080 \\ -0.3669 & 0 & -0.8022 & 0 & -0.4870 \\ -0.3703 & 0 & 0 & -0.9573 & 0 \\ 0 & -1.8244 & 0 & 0 & 0 \\ 0 & 0 & -0.4865 & -1.0544 & 0 \end{pmatrix}.$$

Two criteria were used to choose the first parameter values. The first criterion was to maintain the transient period within a reasonable span of time, neither so large as to make simulations last too long nor so short as to make the transient period hardly detectable. The second criterion was to keep simulation numbers from overflowing.

Three possible network attractors have been identified. A static attractor (Figure 5), in which cluster populations reach a stable level; a periodic oscillatory attractor (Figure 6), in which cluster populations oscillate with a fixed period; and a state characterized by the unbounded growth of cluster populations (Figure 7).

The insertion rate of clones by the bone marrow (b) and the death rate (d) determine whether the network's node populations will grow within bounds or not. When the effect of d is small compared to that of b, populations grow without bounds. This behavior is certainly not biologically plausible, so this state and hence the parameters leading to it must be discarded.

The other two parameters, the proliferation rate (p) and the inhibition rate (u), determine the oscillatory behavior of the network. When proliferation is too pronounced compared to inhibition, network oscillations are bound to have a large amplitude and can eventually saturate the network. That is the reason why these parameters have to be calibrated so to counterbalance each other. When this happens, the network evolves towards a static state in which no oscillations are present. It so happens, however, that the decision between the two sets of parameters is a delicate one, so we delay it until having analyzed the immune functions for each attractor.

We first tested the static attractor, and for such introduced an antigen in cluster 2 at three different times. The first parameter to adjust at this moment is the antigen removal rate (τ), which determines how fast antigens are removed from the network. Using small values for τ makes the time antigens stay in the network relatively long, in turn forcing simulation times to be even longer in order to clearly observe the immune memory. By contrast, using large values

causes antigens to be eliminated so fast that it is difficult to observe the network's immune memory. Figure 8 shows the effect of a large τ, Figure 9 the effect of a small τ, and Figure 10 shows the effect of an intermediate value for τ.

We are then in position to activate the evolution of specificity, and to analyze the parameters related to it, δ and R. The former parameter has an important influence on the network's immune memory. Using large values for δ improves network memory, but if the value is too large the network may reach saturation, as shown in Figure 11. Large values for δ also make it more difficult to recognize other types of antigen, because the network reaches high levels of specificity to recognize the first antigen very quickly. On the other hand, the immune memory becomes rather ineffective if δ is too small, as shown in Figure 12. A suitable value for δ yields the behavior shown in Figure 10.

The other parameter related to the evolution of specificity is the random-noise amplitude (R). In order to determine the most suitable value for this parameter, it is necessary to insert two types of antigen in the network (one in node 2 and the other in node 3, for example). A small value makes it difficult for the network to recognize the second type of antigen inserted, as Figure 13 shows. On the other hand, a high value makes the network loose its memory too fast, because the noise-spreading effect reverses the increase in specificity (see Figure 14) and can also lead the network to saturation. Suitable values for this parameter make the network retain its immune memory against the first antigen, and at the same time helps the network develop an efficient response against the second antigen. Figure 15 depicts this case.

We turn last to the parameters related to activation and inhibition, namely l_1 and l_2. In order to determine appropriate values for them, it is important to remember that they are to be compared against a weighted sum of cluster populations plus a certain amount of antigen (cf. (2)). So suitable values for them must be compatible with those of such a summation. Let us analyze l_2 first. This parameter regulates the network's tolerance to high amounts of antigen. In general, a high amount of antigen is a large value if compared to a cluster's population. As such populations are in the order of units, a value of 50 may be considered large.

The use of large values for l_2, such as 100 or 1000 (Figures 16 through 19), does not prevent clonal proliferation for an antigen amount of 50. So a smaller value such as 10 must be used. In this case, clonal proliferation is totally blocked for an antigen amount of 50, as Figures 20 and 21 show, but not for the amount of 5 used in the tests we already discussed; this is shown in Figure 22.

Suitable values for l_1 must be small relative to the aforementioned summation so to avoid interference with the immune response to the antigen amounts used in our tests and to block clonal proliferations in response to small amounts of excitatory idiotypes. A large value for l_1 impacts the immune response negatively as shown in Figures 23 and 24, and must therefore not be used. Small values for this parameter all have a similar influence on network performance, as shown in Figures 25 through 28. We choose the value 1 because it does not interfere with the immune response to the antigen amounts used in our experiments while blocking spurious proliferations efficiently.

Having analyzed the immune response of the network with the static attractor, we now look at the oscillatory attractor. In this case, results depend on the time step at which we introduce antigens in the network. As the network never reaches a steady state, at times the immune response and memory are satisfactory, at times they are not. Unlike the case of the static attractor, in this case the plots do not all have the same shape, as Figures 29 through 31 illustrate. Although it can be argued that the immune response in healthy immune systems is not always equally efficient, such pronounced differences seem unreasonable. This suggests that the oscillatory attractor is not suitable for modeling healthy immune networks.

If we now take into account all the previous considerations, we conclude that a suitable set of parameters for the model are $b = 0.08$, $d = 0.03$, $p = 0.001$, $u = 0.002$, $\tau = 0.02$, $\delta = 0.06$, $R = 0.05$, $l_1 = 1$, and $l_2 = 10$. We use these values henceforth unless otherwise noted.

Let us then look at the homeostatic state of the network again. Figures 32 and 33 illustrate the evolution of cluster populations for two different initial weight matrices. In both cases the evolution of specificity was deactivated. Figure 32 corresponds to matrix $w_1^+(0)$, while Figure

33 corresponds to matrix $w_2^+(0)$. As the figures show, surface shapes are not always equal; they depend on the weight matrix. The matrices are

$$w_1^+(0) = \begin{pmatrix} 0 & 0.3669 & 0.3703 & 0 & 0 \\ 0 & 0 & 0 & 1.8244 & 0 \\ 0 & 0.8022 & 0 & 0 & 0.4865 \\ 1.1319 & 0 & 0.9573 & 0 & 1.0544 \\ 0.6080 & 0.4870 & 0 & 0 & 0 \end{pmatrix}$$

and

$$w_2^+(0) = \begin{pmatrix} 0 & 1.0321 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0.5380 & 0.1512 & 0 & 0.0641 & 0.6077 \\ 0.3839 & 0.7162 & 0 & 0 & 0.9536 \\ 0.9411 & 0.3822 & 0 & 0 & 0 \end{pmatrix}.$$

When the evolution of specificity is activated, surface shapes undergo important alterations, as Figures 34 and 35 attest. If, on top of that, random noise is introduced, then not even the surface shapes of consecutive experiments for the same weight matrix remain similar to each other (see Figures 34 and 36). Such shape variations are extremely interesting because they do not interfere with the network's immune response, unlike what happened with the oscillatory network, but rather are coherent with the available evidence of biological oscillations in idiotypic populations. To see this, refer to Figures 37 and 38, which represent a first experiment using the weight matrix $w_1^+(0)$, and to Figures 39 and 40, representing a second experiment. In both cases, the immune response is practically the same.

It must also be noted that the initial weight matrix does not have a strong influence on the network's immune response, because the evolution of specificity has a compensatory effect that maximizes the immune response against the particular antigen inserted in the network. This is shown in Figures 38 and 41. The former corresponds to matrix $w_1^+(0)$, the latter to matrix $w_2^+(0)$.

Note, however, that this is no indication whatsoever that weight updates can be done away with. In fact, if one deactivates the evolution of specificity with weight matrix $w_1^+(0)$, the plots of Figures 42 and 43 result. In these figures, even though the network still shows some immune memory, its performance is far below its own performance with evolving specificity.

In order to assess the extent to which the insertion of random noise is effectual, different types of antigen were injected several times into the network. Antigens at node 3 were injected three times, at node 4 twice, and at node 5 twice also. Figures 44 and 45 illustrate the immune response with noise insertion, and Figures 46 and 47 without noise insertion. Notice that in the absence of noise the network's response is good against the first antigen injections, but it has trouble fighting the others.

Finally, we analyze the network's response against a massive attack by injecting antigens at all nodes. Figures 48 and 49 show the network's response with all its features activated. The network, in this case, is capable of efficiently fighting all antigens. Figures 50 and 51 show its performance without a tolerance threshold. In this case, the network is still capable of fighting all antigens, but a population overflow is certain to occur under longer simulation times. Figures 52 and 53 show the network's response without specificity evolution. In this case, the system fails to fight all antigens.

4.2 A Hundred-Node Network

The second network we analyze is a network with one hundred nodes. The most suitable values for the model's parameters depend on the number of nodes in the network, so it is necessary to obtain a new set of parameters for this network. We do, however, use some of the insight obtained while calibrating the five-node network of the previous section. So, right from the

start, we use a static attractor when the evolution of specificity is deactivated. Also, we use the parameters determined previously as reference.

For the case in which specificity does not evolve, Figure 54 shows the network's evolution under the same parameters used for the five-node network. These parameters lead to an undesirable population growth that almost saturates the network. A little variation in the death rate (d) helps overcome this problem and reproduces the same static attractor observed in the five-node network (see Figure 55).

As in the case of the previous section, determining δ requires the immune response to be analyzed. The first value tested was the one used for the five-node network; Figure 56 shows how this parameter performs. It seems to be too small because antigens remain in the network for too long. Figure 57 shows the immune response with a better value for δ.

Figure 58 illustrates the network's performance with the same random noise amplitude used for the five-node network. As the network performance with this value seems satisfactory, we keep that amplitude. As for the value of l_1, for the five-node network it was chosen based on the population of each node; since now populations are still in the same range, we keep that value of l_1 as well.

The amount of antigen used in this network is greater than the amount used before, so l_2 has to be changed. Figures 59 through 62 show the network's evolution and immune response for different values of l_2 and an amount of antigen of 100. Our choice is to let $l_2 = 250$ because it stops clonal proliferation for an amount of antigen of 100 or grater, without interfering in the proliferation process for the amount of 30 that was used in the tests (Figure 63).

After these considerations, the final set of parameters is $b = 0.08$, $d = 0.05$, $p = 0.001$, $u = 0.002$, $\tau = 0.002$, $\delta = 0.1$, $R = 0.05$, $l_1 = 0.1$, and $l_2 = 250$. Figures 64 and 65 show the network's performance of a new stimulation matrix with specificity evolution deactivated, while Figures 66 and 67 show the same network's performance with specificity evolution activated. All the four figures relate to the same weight matrix. Once again, the performance improvement the network undergoes when specificity evolution is activated is evident.

5 Conclusions

We have presented a model of the immune network that uses dynamically updated edge weights in a graph to represent the evolution of specificity. This model has demonstrated that, in essence, the immune network as postulated by the Functional Network Theory is capable of developing all immune functions, as well as maintaining itself regulated in order to avoid the demographic explosion of immune components. It has also shown that the evolution of specificity has a very important role in the network's immune memory.

According to the Functional Network Theory, the immune network's auto-regulation is based only on the interaction among network elements. There is no involvement of any foreign structure. Such interactions can be of two types, excitatory or inhibitory, one opposing the other. The equilibrium between the two types of interaction maintains the network's idiotypic population stable. As these interactions are not perfectly synchronized, the network's idiotypic populations do not remain static, but rather change until the interaction to be felt last compensates the first one. Such relative delay between interactions causes the network's idiotypic populations to change within a reasonable, bounded range, which characterizes the system's attractor. Our results suggest that the most suitable attractor for networks without the evolution of specificity is the static attractor, although this may seem to contradict the intrinsic nature of a dynamical behavior. When compared to oscillatory networks, static networks present a stronger and time-independent immune response, as well as a more robust immune memory.

When the evolution of specificity is activated, static networks start showing idiotypic population oscillations, but unlike oscillatory networks these oscillations are not periodic, they are chaotic. The activation of specificity evolution also leads the originally uniform idiotypic populations of static networks to turn totally dissimilar, showing peaks and valleys. Peaks correspond to activated nodes while valleys to virgin or suppressed nodes. At any time step, the activated

nodes constitute only a subset of the entire node set, but this subset does not remain the same as time elapses. These changes are the idiotypic oscillations observed by Holmes et al. [7] and correspond to a manifestation of the network's idiotypic dynamics.

Still according to the Functional Network Theory, the immune memory is a consequence of the network's dynamics, which is in contrast with the Clonal Selection Theory, according to which the immune memory is due to the presence of longer lasting cells produced during B-cell proliferation. Our results indicate that the immune network's dynamics is perfectly capable of developing immune memory when combined with the evolution of specificity. This latter mechanism does on the one hand lead the network to fight most concentrated antigens increasingly more efficiently, and on the other hand guarantees the network's capability to recognize any antigen coming into the body. There is a trade-off between the duration of the immune memory and the network's ability to recognize any antigen. The immune network is not capable of retaining a memory indefinitely because the only way to do this would be to evolve to such a state of high specificity that only a few antigens would be recognized; memory has therefore to be gradually sacrificed as new antigens appear. This process is supported by two phenomena, the clonal selection that increases network specificity, and the insertion of new, antigen-uncorrelated clones by the bone marrow, this one causing network specificity to decrease.

Network performance in the presence of an antigen highly depends on the network's topology, especially in the case of networks with few nodes. This means that the network's efficiency to fight an antigen depends on its connections and on the weights of those connections. If there is a node that stimulates only a few or none of the other nodes, then the immune response against antigens inserted in that node is very weak or may even not exist. Evolving the network's specificity solves this problem because it allows connections to adapt in order to improve the network's performance against any antigen.

The tolerance threshold has a very important role in self non-self discrimination. The thymus is the organ where helper T cells mature and is a very dense organ. It is at the thymus that self-antigens are presented in very high quantities to helper T cells, causing self-reactive lymphocytes to develop tolerance and then die in order to prevent auto-immune diseases. Tolerance also helps maintain stability in mathematical models when the network faces the attack of several antigens at the same time.

Our model brings two innovations to the modeling of immune networks. The first one is the update of connection weights to reflect specificity growth. This feature dramatically improves the network's immune memory and establishes a similarity with neural networks. The second innovation is the reduction of specificity by means of the insertion of random noise in the weights, aiming at representing the insertion of new clones by the bone marrow. This feature makes it possible for the network to recognize any antigen without sacrificing immune memory.

Acknowledgments: The authors acknowledge partial support from CNPq, CAPES, the PRONEX initiative of Brazil's MCT under contract 41.96.0857.00, and a FAPERJ BBP grant.

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