Mathematical Approaches in Immunology

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SFI WORKING PAPER: 1992-01-002

SANTA FE INSTITUTE

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ABSTRACT

The immune system is complex system of cells and molecules distributed throughout our bodies that can provide us with a basic defense against pathogenic organisms. Like the nervous system, the immune system performs pattern recognition tasks, learns and retains a memory of the antigens that it has fought. In this chapter, I provide a brief introduction to the biology of the immune system and summarize some of the uses of mathematics in understanding its operation.

1. Introduction

The principle defense cells of the body are a class of white blood cells known as lymphocytes. These cells can leave the blood through capillaries, explore tissues for antigens (foreign cells and molecules), and then return to the blood. There are two main classes of lymphocytes: B cells and T cells. B lymphocytes secrete antibody. T cells interact with other cells and play a regulatory role (helper T cells) or directly kill (cytotoxic T cells) virally infected cells and cells that appear abnormal, such as some tumor cells. Helper T cells are the cells that are predominantly infected by the human immunodeficiency virus and their depletion plays a major role in AIDS. Although outside the scope of this lecture, models for the interaction of HIV with T cells have been developed by Perelson (1989c), Nelson & Perelson (1992), Perelson et al. (1992), McLean & Kirkwood (1990), Nowak & May (1991), Merrill (1989) and models of the kinetics of natural killer cell and T cell killing have been developed by Perelson & Bell (1982), Macken & Perelson (1984), Perelson & Macken (1984), Perelson, et al. (1984), and Macken & Perelson (1985b).

Both B cells and T cells have receptor molecules on their surface that can recognize antigen. These cells thus provide specificity to the immune system and are responsible for its apparent cognitive properties: pattern recognition, learning and memory. When B cells become stimulated they secrete a soluble form of their receptor molecule. This molecule is antibody.

Antibody molecules recognize specific chemical groups called *epitopes*. The immune system is capable of making of order 10^7 different antibodies at any one time. Due to their exquisite specificity the immune system uses antibodies as tags with which to label cells and molecules as foreign. Antigen and antibody can form large

complexes that are taken up and eliminated by various cells. A foreign cell with antibody attached to it is quickly eaten by large phagocytic cells such as macrophages. These phagocytic cells lack specificity and eat anything that has antibody attached. For example, they will phagocytose latex beads if they are antibody coated. Antibody bound to the surface of a cell can also initiate a cascade of reactions among a set of high molecular weight serum proteins known as the complement system. This cascade of reactions causes the complement components to assemble a cylinder that penetrates the cell membrane and leads to the death of the cell by disrupting its osmotic balance. Models dealing with the elimination of antigen via the complement cascade have been developed by Perelson & Wiegel (1979), Perelson, Goldstein & Rocklin (1980), DeLisi & Wiegel (1983), and Dower et al. (1984).

In this chapter I will focus on models that deal solely with B cells. T cells and other accessory cells, such as macrophages, are assumed to be present and to provide necessary growth factors so as not to limit B cell activity.

2. Clonal Selection

The most basic task of the immune system is pattern recognition. It must recognize (and then respond) to all foreign cells (e.g. viruses and bacteria) and molecules (e.g. bacterial toxins). The diversity of receptor types used by the immune system is the basis for pattern recognition. The basic idea, called *clonal selection*, was introduced by Sir MacFarlane Burnett (1959). It is related to Darwin's notion of evolution but applied to the cell populations within an individual rather than to animal or plant populations. Recognition in the immune system is performed by B lymphocytes and T lymphocytes. Each of these cells express receptor molecules of a particular shape on their surface. A rather elaborate genetic machine underlies the construction of these receptors and to a first approximation ensures that the receptors have a random shape. Whether the receptor on any particular cell detects anything is thus a random event. Recognition is the first of many steps that may lead to the cell becoming activated. Upon activation the cells proliferate, and grow into a clone, and respond, e.g. secrete antibody if the cell is a B cell or secrete a growth factor if it is a helper T cell. Because antigen selects which clones grow, the theory is called clonal selection. The analogy with natural selection should be obvious, the fittest clones being the ones that recognize antigen the best. For this algorithm to work the receptor population has to be diverse enough to recognize any foreign shape. Immunologists call the collection of receptor shapes the immune system's *repertoire*. The repertoire is called *complete* if it can recognize any foreign shape.

3. Shape Space

A first example of the use of mathematics in immunology is the evaluation of the completeness of the repertoire. Perelson and Oster (1979) developed a simple quantitative model with which they could ask, given a set of N_{Ab} distinct, randomly made antibodies (or receptor types), what is the probability that a randomly encountered antigen is recognized by at least one of the antibodies? The model is based on the notion of *shape space*. The idea is that the degree of binding between a receptor and a molecule that it binds, which immunologists call a ligand, generally involves short-range non-covalent interactions based on electrostatic charge, hydrogen binding, van der Waals interactions, etc. In order for the molecules to approach each other over an appreciable portion of their surfaces, there must be extensive regions of complementarity. Oster and I called the constellation of features important in determining binding among molecules the *generalized shape* of a molecule. Suppose that one can adequately describe the generalized shape of an antibody combining site by N parameters: the length, width and height or the radius of curvature of any bump or groove in the combining site, its charge, etc. Then a point in an N-dimensional space, "shape space" S, specifies the generalized shape of an antibody binding region. If an animal has a repertoire of size N_{Ab}, then the shape space for than animal would contain N_{Ab} different points. We shall assume that these points lie in some finite volume V of shape space, since there is only a restricted range of widths, lengths, charges, etc. that an antibody combining site can assume. For example, one would never find an antibody with a combining site dimension of one meter!

Antigens are not recognized as whole objects but rather are parsed into small regions known as antigenic determinants or epitopes. Antigenic determinants are also characterized by generalized shapes which should lie within V. For example, a combining site with a length of 1 nm cannot be expected to recognize a determinant 10 nm long. In order to estimate how well an animal with a repertoire of size N_{Ab} can recognize molecular determinants, let us assume that an antibody i and antigenic determinant $\bar{i}$ fit together perfectly. If the antibody and antigen shapes are not quite complementary then the two molecules may still bind but with lower equilibrium binding constant or "affinity". At some low level of affinity, e.g. 10^4 M^{-1}, we say the interaction is not specific and that the molecules are not complementary. To describe this we assume that each antibody interacts strongly enough to generate an immune response with all antigenic determinants that are within a small region in shape space surrounding its exact complement. We call this region a "recognition ball". Let $V_{\epsilon_i(K)}$ be the volume of the recognition ball for antibody i when the threshold affinity for specific recognition is set at K. Let $V_{\epsilon(K)}$ be the average volume of a recognition ball in S. (If one wanted to be more precise and not use thresholds one could assign an explicit affinity to each pair of shapes depending upon their location in shape space. This approach was taken by Segel & Perelson (1988). Because each antibody can recognize all antigenic determinants within a recognition ball a finite number of

antibodies can recognize an infinite number of antigens, i.e. one can put an infinite number of points into the volume $V_{\epsilon(K)}$.

To complete the model, let us assume that antibodies are made with random shapes. Thus the N_{Ab} antibodies lie scattered at random in the shape space. If each antibody has roughly the same recognition volume V_ϵ, then the total volume covered by all of the antibodies in the repertoire is $N_{Ab}V_\epsilon$. If this volume is large compared with the total volume of shape space V, then one would expect that the various antibodies would have recognition regions that overlap and completely cover shape space. In fact, each epitope would on average be recognized by $N_{Ab}V_\epsilon/V$ different antibodies, and the probability, P, that an epitope is *not* recognized by some antibody is (Perelson & Oster, 1979)

$$P = (1 - V_\epsilon/V)^{N_{Ab}} \simeq e^{-N_{Ab}V_\epsilon/V} \quad . \tag{1}$$

The expression on the right is the one that would result from assuming a Poisson distribution of antibodies in shape space, i.e. it is the probability of finding a point (i.e. an epitope) lying in no antibody recognition ball.

We can use Eq. (1) to quantify the completeness of the repertoire. Typically, of order 10^{-5} of the B cells in an animal respond to any epitope (cf. Klinman and Press, 1975). This value is an estimate of $p(K)$, the probability that an antibody recognizes a random antigenic determinant with an affinity above the threshold value K. To interpret $p(K)$ within the context of shape space theory notice that if one randomly places an epitope in shape space, the probability that it lands in the volume V_ϵ surrounding any given antibody is V_ϵ/V, the fraction of the shape space volume covered by a single antibody. (An easy way to see this is to consider throwing darts at a two-dimensional version of shape space. Assume that the darts are thrown at random and all hit the board. The probability that a dart will land in a recognition ball is then the area of the ball divided by the area of the dart board.) Thus, if the readout of immune recognition is B cell stimulation, $p(K) = V_\epsilon/V \simeq 10^{-5}$.

With this rough estimate, Eq. (1) predicts that animals with a repertoire of 10^5 antibodies will have marginal immune systems immune systems immune systems immune systems immune systems immune systems immune systems, i.e. e^{-1} or 37% of epitopes will escape detection. However, if $N_{Ab} = 5 \times 10^5$ then P falls to 6.7×10^{-3} and less than 1% of epitopes escape detection. If $N_{Ab} = 10^6$ then $P = 4.5 \times 10^{-5}$ and essentially all epitopes will be recognized. Thus a repertoire of order 10^6, composed of antibodies with random shapes, will be complete. This is interesting because the smallest known immune system, that of a young tadpole, is estimated to have 10^6 lymphocytes and thus a repertoire of order 10^5 to 10^6 (Du Pasquier, 1973; Du Pasquier & Haimovitch, 1976). Smaller immune systems do not exist and the calculation given above suggests that this is the case because such immune systems would recognize antigen so infrequently that they would provide little, if any, protective advantage.

To summarize, the repertoire will be complete if three hypotheses are satisfied: (i) Each antibody can recognize a set of related epitopes, each of which differs slightly in shape. (ii) The repertoire size is of order 10^6 or greater. (iii) The antibodies in the repertoire have shapes that are randomly distributed throughout shape shape.

Experiment has shown that hypothesis (i) is clearly satisfied. Although binding is highly specific, antibodies can bind more than one epitope. If the epitopes have related chemical structures the antibody is called cross-reactive. The strength of binding may differ for different epitopes and is accounted for by differences in affinity.

Although exact repertoire sizes are not known, the best current estimates for mice and humans (the two best studied systems) place the repertoire size at approximately 10^7. Thus we are reasonably confident that hypothesis (ii) is also satisfied.

Hypothesis (iii) is almost certainly not strictly satisfied. Antibodies are not made by a totally random process but are constructed from genes. The genetic machinery involved in generating antibodies is very diverse and error prone so that most immunologists believe that to a good approximation shape space is covered. However, it is clear that one can bred mice to have "holes" in their repertoire. The basic genetic building blocks out of which antibodies are made biases the ultimate repertoire. This bias may be towards antigenic determinants that are found on common pathogens. Thus during evolutionary time the immune system may have learned that there are regions of shape space that are more important to cover than others. The antibody repertoire may thus be biased towards this type of coverage. Notice, however, a weaker form of hypothesis (3) will suffice. Not all antibody shapes need be made at random. It will suffice if a subset of the repertoire of size 5×10^5 to 10^6 is distributed randomly throughout V; other antibodies could be distributed non-randomly and the repertoire would still appear complete.

4. Learning and Memory

For protection, it is not enough to only recognize antigen. The immune system must also have sufficient resources to mount an effective response against pathogens. As in a typical predator-prey situation the size of the lymphocyte subpopulation specific for the pathogen relative to the size of the pathogen population is crucial in determining the outcome of infection. To be more precise, a mouse has approximately 10^8 lymphocytes. If it has a repertoire of 10^7, then *on average* there are only 10 lymphocytes of any particular specificity. Learning in the immune system involves raising the population size of lymphocytes that have proven themselves to be valuable by having recognized antigen. If one can learn from experience where in shape space antigenic determinants most likely lie, then the immune system can allocate more

lymphocytes to the appropriate complementary regions of shape shape and be better prepared to fight an antigen if it is seen again. Thus learning in the immune system involves biasing the repertoire from random towards a repertoire that more clearly reflects the actual antigenic environment.

Learning can occur on two time-scales. First, as mentioned above, over evolutionary time biases can develop in the antibody variable region gene segments that a species carries. Not much is known about the evolution of antibody genes but there are indications that some genes encode the ability to recognize common bacterial antigens. Modeling of antibody V gene evolution using genetic algorithms is being attempted by Forrest and Perelson (1992) and Smith, Forrest & Perelson (1992). Second, during the life of any individual the detailed dynamics of lymphocyte growth, differentiation and competition between clones ultimately reflects the system's interaction with its environment. After an antigen has been seen once, the immune system responds to subsequent encounters with the same antigen with faster and larger amplitude responses. Such responses are called *secondary* immune responses, and they can be attributed to having larger initial clones sizes. Thus, say rather than starting the response with a clone of 10 cells, the system might start with 10^4 cells specific for the antigen. When these cells secrete antibody they would have a large impact. In a primary response, there is a delay due to the fact that the cell population needs to enlarge before it can secrete substantial amounts of antibody. (The more rapid and vigorous secondary response may also be due to differences between naive cells and cells that have seen antigen. For example, secondary response or memory cells may be easier to trigger than naive cells.) Because the total number of lymphocytes in the immune system is regulated, increases in the sizes of some clones, means other clones must decrease in size. Thus, if the immune learns only by increasing the population sizes of specific lymphocytes it must either forget previously learned antigens or constantly decrease the portion of its repertoire that is generated at random and responsible for responding to novel antigens. Because of the experimental difficulties inherent in studying individual clones *in vivo* it is not yet possible to decide to what degree each of these alternative is followed. To gain insights into these and related questions a number of workers have developed quantitative models of cell growth and differentiation in the immune system under various scenarios of immune regulation. We discuss some of these models below.

5. Regulation of Clonal Growth

A central issue in immunology is the regulation of the immune response. What determines which clones are involved, how big the clones grow, and how is the response turned off once the antigen is eliminated? The simplest idea, based on clonal selection, is that particular subsets of B cells (and T cells) are selected for growth

and differentiation by antigen, and turn off when the antigen concentration falls below some threshold. Antigen specific helper and possibly suppressor T cells regulate the magnitude of the response. In this clonal selection view, antigen specific clones respond more or less independently of one another, and are primarily regulated by antigen. An alternative view, suggested by Jerne (1974), is that the immune system is a regulated network of molecules and cells that recognize one another even in the absence of antigen. Because antibodies are created in part by random genetic mechanisms, they must look like novel molecules to the rest of the immune system and thus should be treated like antigens. Due to the completeness of the repertoire the immune system should recognize novel epitopes (called *idiotopes*) on its own antibodies and make antibodies against them. Jerne suggested that during an immune response antigen would directly elicit the production of a first set of antibodies, Ab_1. These antibodies would then act as antigens and elicit the production of a second set of "anti-idiotypic" (anti-id) antibodies Ab_2, which recognize the idiotopes on Ab_1 antibodies. Similarly, a third set of antibodies, Ab_3 could be elicited that recognized Ab_2 antibodies, and so forth. Anti-id antibodies have been found to occur naturally (Binion & Rodkey, 1982) and when injected into animals profoundly alter the animal's antibody repertoire in later life (Eichmann, 1978; Takemori & Rajewsky, 1984; Vakil et al., 1986; Freitas et al., 1988). Further, by idiotypic interactions one clone or an antibody carrying its idiotope should be able to stimulate its anti-idiotypic partner. Such stimulatory idiotypic interactions have been described (Eichmann & Rajewsky, 1975; Cosenza, 1976). Suppressive idiotypic interactions have also been found (Hardt, et al., 1972; Vakil et al., 1986). Whether idiotypic interactions regulate or play a part in regulating the immune system, especially given other levels of control (e.g. antigen processing, interleukins, helper and suppressor T cell circuits, etc.) still needs to be determined.

6. Mathematical Models

Mathematical modeling in immunology has attempted to describe both the clonal selection hypothesis and idiotype network ideas. A brief and necessarily incomplete survey follows. For a more comprehensive introduction to modeling in immunology see Perelson, Weisbuch & Coutinho (1992), Perelson & Kauffman (1991), Perelson (1988), Bell, Perelson & Pimbley (1978), and DeLisi (1983).

6.1. Clonal Selection

Bell (1970, 1971) developed the first model of clonal selection. It was quite complete for its time, and it included the dynamics of antigen binding to B cell receptors and the various stages of B cell differentiation. Sixty or so clones were simulated, each with different affinity for antigen. The simulations showed how clonal selection could account for much of the data on the increase in the average antibody affinity for antigen seen over the course of an immune response. This phenomena is called the *maturation of the immune response.* More recent models of affinity maturation deal with a process known as somatic hypermutation in which point mutations are introduced in the antibody variable region. Mutants with higher affinity for the immunizing antigen are selected for growth and the average affinity increases with time. Models of this process have been developed by Kauffman et al. (1988), Macken & Perelson (1989, 1991), Macken, Hagan & Perelson (1991) and Weinand (1991).

Perelson, Mirmirani & Oster (1976), using optimal control theory, examined in more detail the choices a stimulated cell has in terms of either proliferating and secreting modest amounts of antibody, or giving up the ability to divide and differentiating into a short-lived but rapid antibody secreting cell called a plasma cell. They also examined the possible consequences of repeated exposures to the same antigen and allowed the possible differentiation of a stimulated B cell into a long-lived cell known as a *memory cell* (Perelson, Mirmirani & Oster, 1978). More recently, Batt & Kompala (1990) reexamined this optimization problem for a replicating antigen using a different optimization criterion.

In order for B cells to be stimulated to grow they need to interact with antigen. Interestingly, numerous experiments have shown that the simple binding of ligands to receptors is not sufficient to trigger clonal growth. Receptors need to be cross-linked. This is a state attained when multiple receptors bind to a single (multivalent) antigen. Cross-linking brings and holds receptors close together within the cell membrane and allows subsequent intracellular biochemical reactions to occur. An extensive modeling effort has pursued the development of dynamic and equilibrium models of receptor cross-linking, and to a lesser extent, the growth and inhibition signals that result (Perelson & DeLisi, 1980; DeLisi, 1980, 1981; Wiegel & Perelson, 1981; Perelson, 1981, 1984; Goldstein & Perelson, 1984; Macken & Perelson, 1985a; Dembo & Goldstein, 1978, 1980).

Although we shall not deal explicitly with the roles of helper and suppressor T cells in these lectures, models of immune regulation involving T helper - T suppressor cell circuits have been developed (cf. Herzenberg et al., 1980; Eisenfeld & DeLisi, 1985; Kaufman, 1988; King, 1988).

6.2. Network Models

Due to the great diversity of the immune system and the fact that on average only 1 cell in 10^5 responds to a particular epitope, it has been extremely difficult to experimentally examine interactions between particular clones as would be required to gain a detailed understanding of idiotypic networks. Clones in immune networks can communicate with other over long distances by broadcasting signals in the form of anti-idiotypic antibodies. These antibodies can react with complementary receptors on the surfaces of B cells and T cells throughout the body. In neural networks, one can in principle trace the connections between neurons and thus learn the topology of a network. In the immune system this is not even true in principle and thus one is forced to rely on models to gain even a rudimentary understanding of the degree of interaction among cells in the immune system.

One of the major stumbling blocks in formulating a mathematical model of an idiotypic network is determining a realistic topology for the network. In a system with a repertoire of say 10^7 elements how can one ever determine all of the possible interactions? A number of approaches have been taken. In the first, no attempt is made to classify all the interactions and one simply assumes some simple relationship among idiotypes and anti-idiotypes. For example, Hoffmann's plus-minus network theory (Hoffmann, 1975, 1979, 1980; Gunther and Hoffmann, 1982) only deals with two specificities, an antigen specific population and its anti-idiotypic partner. Richter (1975, 1978) dealt with a linear network; that is a linear sequence of clones and their anti-clones $[Ab_1, Ab_2, ..Ab_n]$, in which antibodies and/or lymphocytes (a distinction between the two was not made) at idiotypic level i interacts with populations at levels $i-1$ and $i+1$, and antigen is defined to be level 0.

$$Antigen \longrightarrow Ab_1 \longrightarrow Ab_2 \longrightarrow \ldots \longrightarrow Ab_\ell$$

Although ℓ, the number of levels in the network could be very high, in early simulation studies ℓ was generally taken to be rather small, i.e. $\ell < 10$. Hiernaux (1977) noticed that the dynamical behavior of Richter's model depended upon whether the network had an even or odd number of levels. He thus analyzed a model in which the linear chain was converted into a simple cyclic network by identifying Ab_1 with Ab_ℓ. More recent models have analyzed Cayley tree and lattice topologies (Weisbuch, De Boer & Perelson, 1990; Weisbuch, 1990; Neumann & Weisbuch, 1992a,b).

A second approach to determining the interactions between clones, is to set up an "affinity matrix" (De Boer, 1988). The non-zero elements in the matrix represent interactions and the magnitude of the elements represents the strength of the interaction. The affinity matrix is the equivalent to a matrix of synaptic weights in neural network models. If one takes the point of view that the network is so large and

complex that it is impossible to to determine the relationships between the elements, then one can simply assign the elements at random with a specified fraction of non-zero elements (cf. De Boer, 1988; Hoffmann et al., 1988; Parisi, 1990). I believe that this approach begins to confront the complexity that inherently can be in a network. Alternatively, specific terms can be placed in the matrix to represent networks of a given topology (cf. Stewart & Varela, 1989). Some data is available from experiments by Zöller & Achtnich (1991), Kearney et al. (1987) and Holmberg et al. (1984) on the structure of networks in neonatal mice which can guide the construction of realistic forms for the affinity matrix.

A third approach, which is one that I have pioneered, assumes that the interactions in a network are determined by the specific chemical interactions between the various cells and molecules in the immune system. The basis of these interactions is what we previously called generalized shape. Thus if one knew the shape of each molecule one could predict which molecules would react and the affinity of their interaction. Even though we do not know the actual shapes of molecules we can develop simple mathematical representations of antibodies that allow us to compute the degree of complementarity between molecules and even assign an affinity to their interaction. In one approach, first introduced by Farmer et al. (1986), one represents the antibody binding site by a binary string of length n. An antigen containing a single epitope is represented by single binary string, whereas antigens with multiple epitopes are represented by multiple strings (Seiden & Celada, 1992). With this representation shape space is a hypercube of dimension n. If one chooses $n = 32$, then one can represent $2^{32} \approx 4 \times 10^9$ different determinants in this shape space. Thus, with a 32 bit computer, one can represent systems with diversity comparable to that expressed in the mammalian immune system.

Complementarity between molecules represented as strings can be defined by any of a number of rules, and the degree of complementarity can be quantified and used as a measure of the affinity of the antigen-antibody interaction. For example, two n-bit strings are complementary if at least some critical number of their bits are complementary; the number of complementary bits exceeding the threshold can be taken proportional to the affinity of the interaction.

Other rules can also be used for determining complementarity. For example, since the strings represent molecules they need not be aligned when they interact. Thus one can also use a match rule in which the sequences are shifted and then checked for complementarity. Furthermore, molecules generally do not interact over their entire length, but rather interactions tend to be localized. To model this one can use a complementarity rule in which the number of adjacent complementary bits is important (De Boer & Perelson, 1991).

A fourth approach, which emphasizes the effects of cross-reactivity among clones, is that of Segel & Perelson (1988). In this approach one describes the shape of a

molecule by one or more real "shape" variables and constructs a system of partial differential equations for the dynamics. This approach has the advantage of leading to equations that can be analyzed analytically, so that qualitative behavior can be predicted, but it relies on a somewhat simplified description of shape space. For example, in Segel & Perelson (1988) we assumed that a single (positive or negative) number x characterizes the generalized shape of antibody (or antigen). This variable x could be viewed as the height or depth of the antibody combining site. Thus an antibody with $x = -2$ has a binding site that is deeper than an antibody with $x = -1$ and can better bind an antigen with a bump of height $x = +2$. Recent structural determinations of idiotype – antiidiotype complexes indicate that a bump on one antibody fits into a groove of the other as envisioned in this simple model. A potential difficulty of a one-dimensional shape space is that an antibody has only one natural complement and the complement of the complement is the original antibody. To generate richer idiotypic structures requires higher dimensional models. Recent work by De Boer, Segel & Perelson (1992) and De Boer, Hogeweg & Perelson (1992) considers two-dimensional shape spaces. Weinand (1991) considers a three-dimensional shape space.

In order to illustrate the types of mathematics used in modeling, I consider some explicit cases in the sections that follow.

6.3. A simple discrete network model

Weisbuch, De Boer & Perelson (1990), De Boer & Perelson (1991), Perelson & Weisbuch (1992), De Boer (1988) and De Boer & Hogeweg (1989b) all considered variations of the following model for B cell clonal dynamics. Let $b_i(t)$ represent the population size of the i*th* clone at time t, $i = 1, \ldots, N$. Most network models postulate an equation of the following type for $b_i(t)$:

$$db_i/dt = m - db_i + pb_i f(h_i) \quad . \tag{2}$$

Here $b_i(t)$ is the population size of clone i, $i = 1, \ldots, N$, at time t, the constant m is a source term that models the rate of supply of new B cells from the bone marrow. The constant d is the per capita B cell death rate, so that an unstimulated cell has a lifetime of magnitude $1/d$. The *activation function* f denotes the fraction of cells that are proliferating, and p is the rate of proliferation of activated cells. The influence of other clones on clone b_i is quantitated by the *field* h_i, which is assumed to be formed of a linear combination of the B cell concentrations b_j:

$$h_i = \sum_{j=1}^{N} J_{ij} b_j \quad . \tag{3}$$

The most crucial feature of this model is the shape of the activation function $f(h_i)$, which is taken to be the biphasic dose-response function

$$f(h_i) = \frac{h_i}{\theta_1 + h_i}\left(1 - \frac{h_i}{\theta_2 + h_i}\right) = \frac{h_i}{\theta_1 + h_i}\,\frac{\theta_2}{\theta_2 + h_i}\ , \tag{4}$$

with parameters θ_1 and θ_2 chosen such that $\theta_2 \gg \theta_1$. The first factor in f increases from 0 to 1, reaching its half-maximal value at θ_1, the second factor decreases from 1 to 0, reaching its half-maximal value at θ_2. For $\theta_2 \gg \theta_1$, the maximum, $\frac{\theta_2}{(\sqrt{\theta_1} + \sqrt{\theta_2})^2}$, is approximately one. This maximum is attained at $h = \sqrt{\theta_1 \theta_2}$. Because $0 \leq f(h) < 1$, we derive from Eq. (2) that the B cells can maximally grow at a rate $p - d$. Thus, in order to allow for net clonal expansion p must be greater than d. Maximally stimulated cells divide about once every 16 h, so $p = 1\ \mathrm{d}^{-1}$ is a typical rate of proliferation. Cells live a few days, so that $d = 0.5\ \mathrm{d}^{-1}$ is a typical death rate.

Below the maximum of $f(h_i)$, increasing h_i increases $f(h_i)$; we call this the *stimulatory regime*. Above the maximum, increasing h_i decreases $f(h_i)$; we call this the *suppressive regime*. Plotted as a function of $log\, h_i$, the graph of $f(h_i)$ is a bell-shaped curve. An important argument for the use of a log bell-shaped function is that receptor crosslinking is involved in B cell activation. The fraction of cell surface receptors cross-linked by a ligand when plotted against the logarithm of the ligand concentration is called a cross-linking curve. For bivalent ligands the cross-linking curve is bell-shaped and symmetric around its maximum (Dembo & Goldstein, 1978; Perelson & DeLisi, 1980; Perelson, 1984).

The topology of the network is determined by J_{ij}. Here we discuss the simple case in which the J_{ij} are computed by assuming that the network has the topology of a Cayley tree (Weisbuch, De Boer, & Perelson; 1990) with c connections per vertex (see Fig. 1). For reasons of simplicity the J_{ij}'s are chosen to be 0 (no interaction) or 1 (maximum interaction). One clone in the network is assumed to react with an injected antigen. This clone is chosen to be the root of the tree and will be called Ab_1. Under these conditions (3) reduces to

$$h_1 = cb_2\ \ ,\ \ h_i = b_{i-1} + (c-1)b_{i+1}\ \ ,\ \ i \geq 2\ . \tag{5}$$

We consider the problem of classifying the different steady states of the network. First, there is a virgin state, corresponding to the case where no antigen has been encountered and there is no internal stimulation of the network. Here all proliferation functions $f(h_i) \simeq 0$, so that at steady state Eq. (2) implies $b_i \simeq m/d$, for all i. After presentation and elimination of an antigen, memory is said to be obtained if some clones in the network establish a stable level significantly larger than m/d. If such a new steady state is to correspond to immunological memory, we also require that when different antigens are encountered, the network remembers these antigens,

and that memory of one antigen should be robust enough not to be destroyed by the presentation of another antigen. In a network model, such a scheme can work if the response to any given antigen remains localized. In such a case, a patch of clones can be modified by encounter with antigen, leaving the remainder of the network unchanged. As long as the patches of clones activated by different antigens do not overlap, all the encountered antigens can be remembered. Further, if network responses are localized then the mathematical analysis is greatly simplified. Instead of solving N equations corresponding to the N different specificities of the immune repertoire, we only have to solve a small set of equations for the interacting clones in a patch.

To show how this might work, consider the case of an antigen reacting with clone Ab_1 that results in excitation of clones Ab_1 and Ab_2 but which leaves clones $Ab_3, Ab_4, \ldots$ essentially at their virgin level (see Fig. 1, top). If one considers steady state solutions of (2) in which a large fraction of cells are activated, i.e. $f(h_i) > 0$, then the source m can be neglected and one expects steady state solutions when $f(h_i) \sim d/p$. For an immune system to be reactive, $p > d$, so that in general there will be two intersections of the line $y = d/p$ with the curve $y = f(h)$, one on each side of the maximum of the bell shaped curve. (If the source m is small but non-zero, then the steady states will be somewhat below the two intersection points). The fields below and above the maximum of the log bell-shaped activation curve, $f(h)$, are called stimulatory and suppressive, respectively. One stable solution occurs when Ab_1 has a low stimulatory field and Ab_2 has a high suppressive field (Weisbuch, De Boer & Perelson, 1990). This state is called an immune state since B cells that recognize the antigen will be maintained at a high population level.

To obtain approximate values for the steady states, which are sufficient to illustrate that a localized response can occur, we note that in the immune state the field felt by clone Ab_1 is small and less than the field $\sqrt{\theta_1\theta_1}$ at the maximum of the log bell-shaped curve. For $h_1 \ll \theta_2$, we approximate $f(h_1)$ by its ascending part, i.e.

$$f(h_1) \sim \frac{h_1}{h_1 + \theta_1} \ . \tag{6}$$

Hence the steady state condition $d/p = f(h_1)$ reduces to

$$h_1 = \frac{d\theta_1}{p - d} \ . \tag{7}$$

Since $h_1 = cb_2$, we find that at steady state

$$b_2 \sim \frac{d\theta_1}{c(p - d)} \ . \tag{8}$$

In a similar way, one can show

$$b_1 \sim \frac{(p - d)\theta_2}{d} - \frac{(c - 1)m}{d} \ . \tag{9}$$

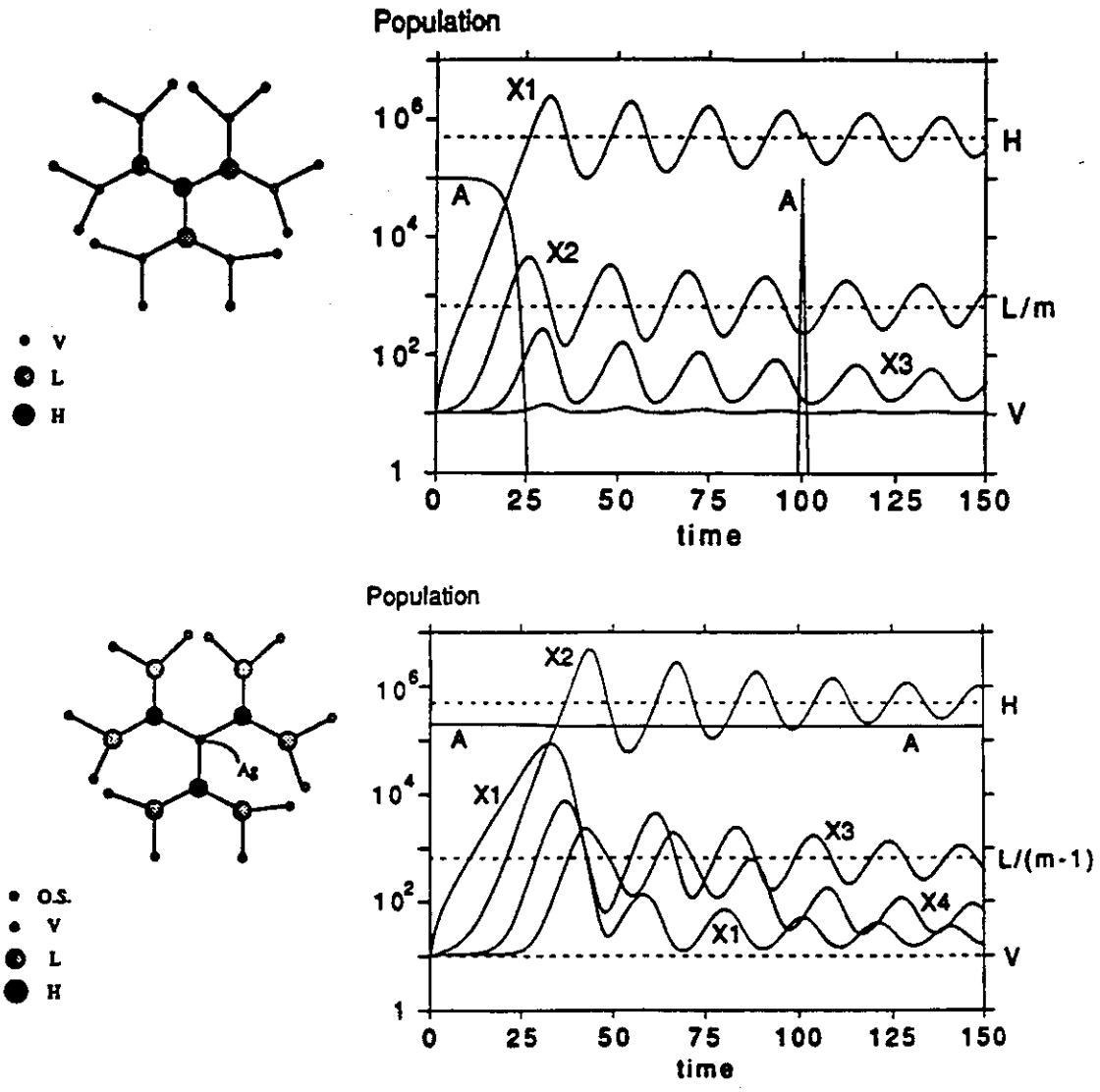

Figure 1. Two localized attractors of the immune network corresponding to immunity (top) and tolerance (bottom). Black circles correspond to large suppressive populations (of the order of θ_2), gray circles to medium excitatory populations (of the order of θ_1), and small white circles to small virgin or oversuppressed populations (of the order of m/d). The two graphs are time plots of the logarithm of the populations (numerical simulation results). A is antigen concentration. That immunization leads to memory is illustrated on the left plot; a second injection of antigen at the same dose as the first injection at time $t = 100$ results in faster elimination than the first injection. Slow decay or persistence of large antigen concentrations results in tolerance. Here a suppressive field acts on b_1 and prevents the clone from expanding so as to eliminate the antigen. GRIND software (De Boer, 1983) was used to generate the figure.

The solution remains localized only if the field h_3 on b_3 is much less than θ_1; otherwise b_3 would also proliferate. The field $h_3 = b_2 + (c-1)m/d$. Thus for localization we require

$$h_3 = \frac{d\theta_1}{c(p-d)} + \frac{(c-1)m}{d} < \theta_1 \ . \tag{10}$$

For typical biological parameter values $d/(p-d) \sim 1$, thus the condition for localization becomes

$$\frac{cm}{d} < \theta_1 \ . \tag{11}$$

This condition is equivalent to requiring that a virgin clone surrounded by c virgin neighbors (at population level m/d) should not be exposed to a field larger than θ_1. If this were not true, the network would self stimulate and the virgin state would not be stable.

A second localized attractor corresponds to a state of tolerance to the antigen (see Fig. 1, bottom). Here a strong suppressive field acts on Ab_1 due to Ab_2's; Ab_2's proliferate due to a low field provided to some extent by Ab_1 but mainly by Ab_3's. The Ab_3's remain low due to the suppressive field generated by Ab_2 and Ab_4's remain nearly virgin. The field equations again allow computation of the steady state population (see Weisbuch, De Boer & Perelson, 1990 for details).

In order to predict which attractor is obtained when antigen is injected into the system one must consider the dynamics of the system in the presence of antigen. Antigen only interacts with Ab_1 in this model, and thus the field $h_1 = cb_2 + \gamma$, where γ is the antigen concentration. We assume Ab_1 leads to the elimination of antigen according to:

$$d\gamma/dt = -k\gamma b_1 \ , \tag{12}$$

where k is a rate constant.

The effects of antigen injection for two different values of k are depicted in Fig. 1. In the first case (top), k is large and the antigen is rapidly eliminated. The dynamics illustrated are typical of a classical immune response. The encounter leads to memory and when antigen is encountered a second time it is eliminated even more rapidly. In the second case (bottom), k is very small so that the antigen concentration remains essentially constant. This is meant to mimic the case of a self-antigen that is constantly being produced by the body. Note that in this case, tolerance rather than immunity results. Neumann & Weisbuch (1992a) have extensively analyzed this model and using various approximations have mapped out the regions of parameter space that given rise to immunity, to tolerance or to non-localized responses.

6.4. The Shape Space Approach

The second approach that I want to illustrate in a little detail deals with partial differential equations rather than systems of ordinary differential equations. Here the shape of a receptor that characterizes a B cell clone is described by a continuous variable x. For concreteness one can think of x as the height of a wedge-shaped region on the receptor, with positive x corresponding to a protuberance and negative x to an indentation. Perfect complementarity, and hence maximum binding affinity, occurs when the protuberance on one molecule and the indentation on another molecule are of the same length. For definiteness, the falling off of binding affinity for less complementary shapes is described by a Gaussian function of the distance between a given shape and its complement. These considerations leads to the following model

$$\partial b/\partial t = m + b(pf(h) - d) \ , \tag{13}$$

where $b(x,t)$ is written as b, and $f(h)$ is again given by the log bell-shaped activation function.

The field $h(x;b)$ felt by B cells of shape x is

$$h(x;b) = \int_{-L}^{L} g(x,\hat{x})b(\hat{x},t)d\hat{x} \ , \tag{14}$$

where $[-L,L]$ is the extent of shape space and

$$g(x,\hat{x}) = G(2\pi\sigma^2)^{-1/2}\, exp[-(x+\hat{x})^2/2\sigma^2] \ . \tag{15}$$

In (15) G and σ are constants determining the amplitude and width of the Gaussian, respectively. If each shape is complementary to only a small fraction of all possible shapes, $\sigma \ll L$. Because we can scale θ_1 and θ_2, we can set $G = 1$ without loss of generality (De Boer & Perelson, 1991). This has the advantage that for $L \gg \sigma$

$$\int_{-L}^{L} g(x,\hat{x})d\hat{x} \approx 1 \ .$$

For a multidimensional shape-space, we replace Eq. (15) with

$$g(x,\hat{x}) = G(2\pi\sigma^2)^{-\ell/2}\, exp[-(x+\hat{x})^2/2\sigma^2] \ ,$$

where ℓ is the dimension of the shape-space and x and $\hat{x}$ are ℓ-dimensional vectors. The use of different variances in different shape-space directions is also possible in this multidimensional model. Sometimes periodic boundary conditions are used. To study the effects of finite shape space size, other boundary conditions are employed wherein clone sizes whose shapes lie outside a certain interval are fixed at zero magnitude.

As might be deduced from our considerations of the discrete network, whenever $m \ll \theta_1 \ll \theta_2$, Eq. (13) formulated on the infinite domain or using periodic boundary conditions has three spatially uniform equilibria $b(x,t) = \bar{b}$ (De Boer, 1988; Weisbuch et al., 1990). We have called these the *virgin*, the *immune*, and the *suppressed* states,

respectively. By Eqs. (14) and (15) the fields of the uniform states satisfy $h(x;\bar{b}) = \bar{b} = constant$, for all x. Each of the three states has its own typical range of values of the field. As in the case of the discrete network one can greatly simplify the analysis by approximating $f(h)$ differently in each of the three states, and easily obtain analytic expressions for the B cell population levels in the three uniform states. The stability of the uniform states can be analyzed by rather standard analytical methods (De Boer, Segel & Perelson, 1992). Many non-uniform steady states also exist, which can be found by numerical methods. Analysis of the nonuniform solutions is thus a type of pattern formation problem, where the patterns form in shape space. Peaks in the shape space pattern correspond to clonal populations that are large, valleys to clonal populations that are small. Learning and memory in this system correspond to obtaining patterns in which clonal populations that are complementary to encountered antigens are high and remain high after subsequent antigen challenges. Patterns can also form in the absence of antigen, which reflect the intrinsic activity of the network. The general analysis of pattern formation in multidimensional shape spaces is a challenging task and remains to be resolved. Some approaches that are currently being explored involve using cellular automata models of approximating systems of equations in which the log bell shaped activation function $f(h)$ is replaced by a piecewise linear function that is 1 when h is between θ_1 and θ_2, and 0 elsewhere (De Boer, Hogeweg, & Perelson, 1992; Stauffer & Weisbuch, 1992)

6.5. Bit-string Models of the Network

The main advantage of the two models discussed so far is that their simplicity allows for mathematical analysis. Thus, in the continuous shape space model one can easily analyze the stability of the three homogeneous steady states of the model to uniform and sinusoidal perturbations (De Boer, Segel & Perelson, 1992). In the discrete network model we were able to find conditions for the existence of localized attractors. However, a major disadvantage is that these models ignore the turnover of clones in the network and have rather simple, idealized topologies.

In the immune system the bone marrow produces novel clones with new receptors at a rate that is sufficient to replace all clones in the network in a few days. Thus, it seems that in the network the addition and deletion of clones takes place on a time scale that is similar to the growth and decay rates of B cells. The addition and deletion of clones was first considered in the simulation model of Farmer, Packard & Perelson (1986). Farmer et al. (1987), as well as Varela et al. (1988), have used the term "meta-dynamics" to denote the change in dynamics of the system owing to the replacement of clones. By adding a form of meta-dynamics to a class of network models, Rob De Boer and I have attempted to account for the effects of clonal insertion and deletion processes in the network (De Boer & Perelson, 1991).

Another feature of the immune system that we have not discussed yet is the fluctuating behavior of individual clones. Recent data published by Lundkvist et al. (1989) and Varela et al. (1991) indicate that antibody concentrations fluctuate on a time scale of about two weeks and that these fluctuations may be chaotic. Network models can exhibit oscillatory or chaotic behavior if we make them more realistic by considering not only the B cell populations but also the antibodies they secrete. Differences in the B cell and antibody life times appear to be responsible for these new forms of behavior (De Boer & Hogeweg, 1989c; De Boer, Kevrekidis & Perelson, 1990; De Boer & Perelson, 1991; De Boer et al., 1992a,b; Perelson & Weisbuch, 1991). Oscillatory behavior has also been found in a model of the immune network developed by Varela and coworkers (Varela et al., 1988; Stewart & Varela, 1989, 1990).

De Boer and I studied the general properties of a network that incorporated metadynamics and contained both B cells and antibodies (De Boer & Perelson, 1991). Because the immune network seems to be most significant during early life (Coutinho, 1989; Holmberg et al., 1989) we also used the model to study the development of the immune network. We were particularly interested in emergent properties like the size and the connectivity of the network.

6.5.1. The Model

The model is composed of a varying number of B cell clones of different specificities that form a network. Each clone is characterized by its specific antibody receptor, which is specified in the model by a bit-string that reflects the "shape" of the antibody (cf. Farmer et al., 1986). A source, intended to model the bone marrow, supplies novel B cell clones that can be incorporated into the network. Two clones can interact via solution phase antibodies whenever their receptor shapes (i.e., bit-strings) are complementary. Cells that become activated, proliferate and differentiate into antibody secreting cells. This maturation process takes a few days. Free antibodies may also react with complementary antibodies to form *complexes*. These complexes, which are analogous to antigen-antibody complexes, are removed from the system.

The field of a clone is defined as in (3). The activation function $f(h_i)$ is the log bell-shaped function introduced in (4).

To model the dynamics of the various B cell clones we assume that cells proliferate at rate p upon idiotypic stimulation, and decay at rate d_B. Thus

$$\frac{dB_i}{dt} = B_i[pf(h_i) - d_B] \; . \tag{16}$$

This differential equation differs from (2) in that it has no source. To model the time-dependent aspects of antibody production we introduced a "gearing up" function,

$G(t)$, that accounts for the time lag associated with the differentiation of stimulated B cells. We use a separate gearing up function for each clone so that $G_i(t)$ can be interpreted as the proportion of mature B cells of type i. Following Segel & Perelson (1989), $G_i(t)$ is given by

$$\frac{dG_i}{dt} = k[f(h_i) - G_i] \ , \tag{17}$$

where k is a constant that determines the characteristic time for gearing up. At $t = 0$, $G_i(0) = 0$ so that there is no initial secretion. After antibodies are secreted they are free in solution. Free antibodies decay at rate d_A and form complexes with complementary antibodies that are eliminated at rate d_C. Thus

$$\frac{dA_i}{dt} = sB_iG_i - d_CA_ih_i - d_AA_i \ , \tag{18}$$

where s is the rate at which a fully mature B cell produces antibody. The formation of an antibody-antibody complex is a reversible reaction, which typically reaches equilibrium rather rapidly. In equilibrium the concentration of A_i–A_j complex is $J_{ij}A_iA_j$. Thus the coefficient d_C multiplies the equilibrium concentration of all possible complexes, A_ih_i.

Summarizing, the model consists of $3n$ differential equations, where n is the number of clones in the system. The size of the network n is determined by the meta-dynamics.

6.5.2. Meta-dynamics

As in Farmer et al. (1986) and Perelson (1988), the shape of each antibody molecule is represented as a bit-string of length L. Antibody molecules are assumed to recognize each other whenever their bit-strings can be matched complementarily. The specific rule that we used was to align the bit-strings and require a complementary match over a stretch of at least T *adjacent* positions. If the strings match over exactly T adjacent positions, we assigned a low affinity, $J_{ij} = 0.1$. If the strings match over more than T adjacent positions, we assigned a high affinity, $J_{ij} = 1$. We set $L = 32$ and varied T in order to vary the a priori matching probability, $P(match)$, of receptors. For the match rule described above, $P(match)$ is the probability of finding a "success run" of length T in a sequence of L Bernoulli trials (e.g., T "heads" in a row in a sequence of L coin tosses). Feller (1968) provides an approximate formula for $P(match)$ that becomes reasonably accurate for $L \geq 2$. Using Feller's result we find for $L = 32$ and $T = 6$, 7, 8, 9, 10, and 11, $P(match) = 0.205$, 0.103, 0.05, 0.024, 0.012, and 0.005, respectively. Experimental data suggest that during early life, clones are connected to 20-25% of all the clones in the network (Holmberg et al., 1984; Kearney et al., 1987). For $L = 32$ such a connectivity, i.e., $P(match) \approx 0.2$, is expected around $T = 6$.

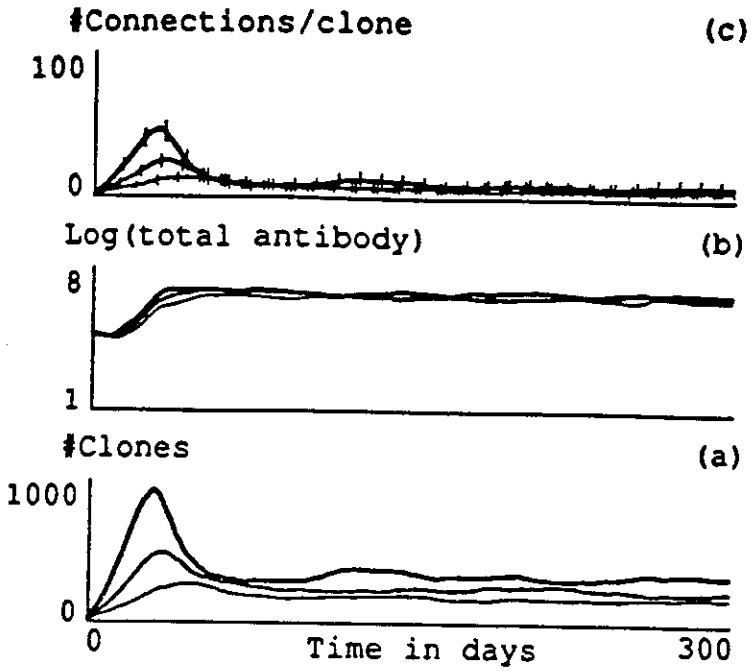

Figure 2. Time dependent characteristics of a sample network. Parameters $p = 1, d_B = 0.5, d_A = 0.05, s = 1, d_C = 10^{-3}, \theta_1 = 10^2, \theta_2 = 10^4, k = 0.2, T = 8, P(match) = 0.05$. We show three values of bone marrow production: $M = 10$, $M = 20$, and $M = 40$. Lines increase in thickness with M. (a) The number of clones in the network, (b) the total antibody on a logarithmic scale (base ten), and (c) the average connectivity. (The bars indicate one standard deviation).

In our simulation M novel clones are produced each day, with each clone containing about 10 cells. Using the string matching algorithm, we compare each newly generated clone with the clones already in the network. A new clone is incorporated in the network only if it recognizes at least one other clone, and if these interactions are sufficiently stimulatory so that $dB_i/dt > 0$. At time intervals of one day, clones are removed if $B_i < 1$ and $A_i < \theta_1/10$, i.e., if the clone consists of less than one cell, and if its antibody population is too small to have any effect. As an initial condition we start with a few randomly generated antibodies, assumed to represent maternal antibodies.

6.5.3. Simulation Results

Figure 2 shows how some global characteristics of the network change in time for several values of the clonal production rate (i.e., $M = 10,\ 20,\ 40$; lines increase in thickness with M). The number of clones in the network n (Fig. 2a), has a large peak

during the first month, whose height increases with M. This early peak sharply declines by the third month, and the network attains an equilibrium size around which it fluctuates. The total amount of antibody produced by the network is shown in Fig. 2b. After a slow decline of the maternal antibodies, the total antibody concentration increases until a steady state level of about 3×10^6 units is attained, where a unit is the amount of antibody secreted by a single activated B cell during one day. This level corresponds to roughly 1 mg/ml, the physiologically observed serum level of IgM. The daily average number of connections per clone, i.e., the connectivity of the network, is shown in Fig. 2c. As in Fig. 2a, Fig. 2c shows an early peak and attains an equilibrium around day 100. This equilibrium is about 6–9 connections per clone.

The fact that equilibrium levels are attained in Fig. 2 shows that the idiotypic network has certain self-structuring properties: interactions within the network determine the network's size and connectivity, and determine the total serum antibody level. The existence of stable "emergent" properties is one of the most interesting features of this network model. As we all know one of the characteristics of living systems is their ability to maintain themselves in the face of constant turnover of their components. Thus, while as a whole the immune system behaves as a coherent system we know that the cells and molecules that make up the system constantly change. Our network model has precisely this property. Stable properties characteristic of the immune system arise even though individual components may only have short lifetime within the system, and may even oscillate in concentration while they are present! Thus, for example, the serum concentration of one particular idiotype may oscillate, consistent with the measurements of Lundkvist et al. (1989) and Varela et al. (1991), whereas the total serum antibody concentration remains quite constant. This is significant because there is no explanation of what physiological processes maintain the serum immunoglobulin concentrations constant. Our model is thus the first quantitative model to address this question.

The emergent properties of the network are further investigated by analyzing a series of networks in which both $P(match)$ and M are varied (Fig. 3). The equilibrium size of the network strongly depends on $P(match)$ (Fig. 3a). Networks comprised of sticky receptors (e.g. $P(match) > 0.1$) remain very small and contain fewer than 200 clones. Conversely, whenever receptors are specific (e.g., $P(match) < 0.01$), the networks become very large. From the shape of the observed curves we conclude that the number of clones is inversely related to $P(match)$. Thus, systems with highly specific receptors will generate very large networks.

The light lines in Fig. 3b depict the network connectivity, i.e., the average number of connections per clone. For low values of $P(match)$ the connectivity increases with $P(match)$ until it saturates at about 10 connections per clone. This saturation is surprising because it means that the connectivity no longer depends on the matching probability. It shows that one cannot deduce $P(match)$ from the connectivity data.

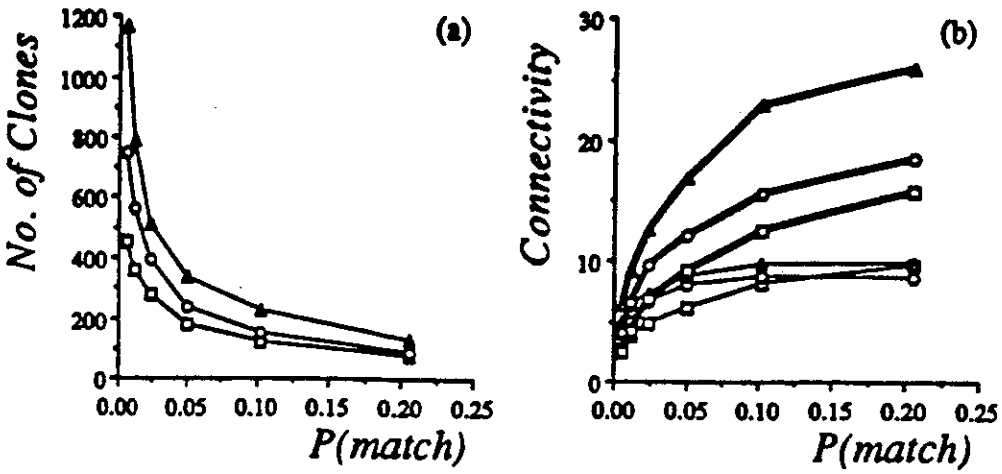

Figure 3. Equilibrium characteristics of a series of networks varying $P(match)$. Parameters as in Fig. 2. The equilibrium values were determined by averaging over the last 100 days of a simulation. We show three values of M: $M = 10$: $\square, M = 20$: $\circ, M = 40$: $\triangle$. (a) The total number of clones (light lines). (b) The network connectivity (light lines), and the expected network connectivity (heavy lines).

The experimental estimate of a connectivity of 20-25% is only a transient in this model, which is attained during the early peak shown in Fig. 3. This is consistent with experiments by Holmberg et al. (1984) and Kearney et al. (1986) in which connectivities of around 20% were observed in newborn mice and then decrease by 10 to 100-fold in adult mice (Holmberg et al., 1986, 1989).

The "expected connectivity" of a network, i.e., the $P(match)$ multiplied by n, the number of clones in the network (as provided in Fig. 3a), is shown by the heavy lines in Fig. 3b. Because the connectivity is always smaller than the expected connectivity, we conclude that the networks in equilibrium select for growth and survival within the network clones with low connectivity. Low connectivity is not an intrinsic property of any particular antibody but rather is determined by the random structure of a clone's receptor and the shapes of the receptors on the other clones that are currently present in the system. A clone that is low connectivity in one simulation may be a high connectivity clone in another simulation – the collection of clones within the system at any given time determines whether a clone will have high or low connectivity. This is a prediction of the model that may be able to be tested in experimental animals.

The fact that the observed equilibrium size of the networks is inversely related to $P(match)$ accounts for a "self-regulatory completeness" of the repertoire: the higher the specificity of the receptors the larger the number of clones becomes in the immune network. Thus over a large range of specificities the size of the system changes so that the repertoire remains complete. This completeness also provides an explanation for

the fact that the networks attain an equilibrium size. The networks grow until every bit-string is expected to be connected to a sufficient number of other clones to remain stimulated. De Boer & Perelson (1991) explain this in more detail.

The incorporation of meta-dynamics in this model was an attempt to account for the rapid turnover of clones in the network. Unfortunately, due to current computational limitations, it is impossible to perform the simulations for realistic values of M. In the immune system the bone marrow produces of the order of $M = 10^5$ to $M = 10^6$ novel clones per day, thus systems with of order of a million differential equations might need to be solved. We have simulated up to $M = 500$ (De Boer & Perelson, 1991). In an attempt to model systems with larger values of M we have simplified the shape space model (Segel & Perelson, 1988, 1990a,b) using the window automaton proposed by Neumann & Weisbuch (1992a) and have implemented this as a cellular automaton (De Boer, Hogeweg & Perelson, 1992). We find that all of the conclusions presented above remain true as M reaches realistic values.

6.6 Conclusions

Mathematical modeling in immunology is a field still in its infancy. The payoffs of developing successful models will be increased understanding of the operation of the immune system, the generation of new ideas, and new experiments to test them, as well as the eventual possibility of conducting immunological experiments *in machina* rather than *in vitro* or *in vivo* (Celada & Seiden, 1992). The models described above already contain insights into how clonal selection works, how immunological networks may be involved in immune memory and how networks may be responsible for controlling the serum level of immunoglobulin. The challenge in immunology, as in brain research, is to understand a system of enormous complexity. One can only hope that the principles and ideas being developed in complex systems, non-linear science, and the tools of computer simulation will help unravel the remaining mysteries.

Acknowledgements

This work was performed under the auspices of the U.S. Department of Energy and supported by NIH Grants RR06555 and AI28433, and the Santa Fe Institute through their Theoretical Immunology Program. Much of the work reported here was done in collaboration with Rob De Boer, Lee Segel, and Gerard Weisbuch.

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