[Frontiers in Bioscience 3, d338-347, March 16, 1998]

MATHEMATICAL MODELING OF IMMUNOLOGICAL REACTIONS

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Received 2/16/98 Accepted 3/9/98

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1. ABSTRACT

The immune system is a highly regulated, complex and integrated system which has evolved to provide the organism with substantial defenses against pathogenic organisms. Over the last several decades there has been an explosion of experimental data in this area, and new techniques in molecular and cellular biology have been crucial in deepening our understanding of immune processes. Most of these new techniques have allowed the isolation of the process or cell under study so that the results can be readily interpretable. At the present time, however, there is an emerging need to understand the system as it functions as a whole and the language of mathematics is the one best suited for this purpose. This review, written from the perspective of an experimental immunologist, describes some of the recent advances in the development of mathematical models of the immune system. Particular emphasis is placed on the rapidly growing field of modeling in HIV infection and T cell activation. Immunology as a whole will benefit from the introduction of the language of mathematics in much the same way as neuroscience has done in the last decade.

2. INTRODUCTION

The immune system functions primarily to protect the organism from invading pathogens. In order to perform this function, the immune system has evolved strategies that allow successful elimination of a wide variety of pathogens, including viruses, bacteria and parasites. These strategies include specific recognition mechanisms that allow the host to distinguish a dangerous pathogen from non-pathogenic organisms or physiological changes in the host. They also allow for the development of killing/inactivation mechanisms tailored to individual pathogens. These responses are highly regulated in order to avoid problems with autoimmunity and to control responses that can, if left unchecked, have grave immunopathological consequences. Thus, the immune response to pathogens is a complex, highly regulated system involving numerous interactions between different cell types. The cells of the immune system communicate with each other by direct cell-cell contact and deliver signals to each other directly, through cell surface molecules, or indirectly, via secreted proteins, known as cytokines. Experimental advances in immunology over the last two decades have been immense and many of the important questions surrounding the issues of pathogen recognition, immune cell development, immune regulation and effector mechanisms are well on the way to being answered.

In order to obtain interpretable data on many of these issues, it has been necessary to devise experimental techniques that isolate the questions of interest. For example, the actions of a particular cytokine can be determined using *in vitro* experiments with individual cell types and purified cytokines. In addition, the *in vivo* relevance of the same cytokine can be determined by generating a mouse lacking this cytokine through the targeted disruption of the cytokine gene. These approaches have been extremely successful in determining the functions of individual cytokines or cell surface molecules. These experiments do not, however, allow a true assessment of the relative importance of each molecule in the naturally occurring immune response, in which all of the cells and cytokines are functioning simultaneously. A good example of this is the case of interleukin (IL-) 2 which, from *in vitro* experiments, was thought to be the most important factor driving T cell proliferation (1, 2). It was a surprise, therefore, that IL-2 knockout mice showed little to no impairment of T cell proliferation, but rather appeared to have defects in T cell death, which lead to the development of inflammatory bowel disease (3-5). Do these results mean that IL-2 is not an important factor for T cell proliferation? Probably not : it is likely that in the normal mouse IL-2 does play a dominant role in T cell proliferation, but that the expansion of pathogen-specific T cells is such an important function of the immune system that other mechanisms can be utilized in the event of IL-2 dysfunction.

These, and other similar experiments, point to the flaws in using pure experimental techniques to address complex issues of immune regulation and development. There is now an increasing need to understand how the immune system functions as a whole in order to be able to better understand the development of diseases such as

autoimmunity, allergy and cancer, and to design effective strategies to treat them. An important concept is that the immune response evolves rapidly in time and its interactions are all highly regulated. The only real way to study the whole integrated system is through the use of mathematical modeling. There has been relatively little interest, on the part of experimental immunologists, in this area, probably due to the complexity of the mathematics necessary for this work and the dearth of mathematicians sufficiently cognizant of the immune system to make such models useful. This situation appears to be changing (6) and is most apparent in the study of human immunodeficiency virus (HIV) infection, where, as discussed in detail below, mathematical models have been useful in challenging existing paradigms of AIDS development and are now an integral part of the HIV research effort.

Mathematical models can serve several distinct purposes. They can be used to analyze experimental results and provide predictions and suggestions for follow-up experiments, or they can attempt to synthesize existing knowledge and provide a theoretical framework for the interpretation of existing paradigms. Both types of model are useful to the experimental immunologist, and the ultimate merit of particular models depends on the specific questions they are designed to answer. The more assumptions that have to be put into the model, the harder it is to be confident about the conclusions. On the other hand, a well designed model can test different assumptions and provide important new insights into questions that cannot be readily answered experimentally.

This review is written from the point of view of an experimental immunologist who has been engaged in close collaboration with mathematicians in the development of mathematical models describing the Th1/Th2 system of immune regulation. As such, this review will focus on aspects of modeling that appear, to this writer, to have contributed significantly to the understanding of the immune system.

3. MODELS OF HIV INFECTION AND OTHER INFECTIOUS DISEASES

An area of intense research in recent years has been that of mathematical models of HIV infection and the progression to AIDS. HIV infects $CD4^+$ cells (T cells and monocytes) through interaction of its envelope glycoprotein, gp120, with CD4 and by interaction with another cell surface protein, the chemokine receptor CXCR4 (7, 8). The infection is characterized by a long latent phase with a low viral load in which $CD4^+$ T cell numbers either remain stable or gradually fall, followed by an increase in viral load associated with a rapid decline of $CD4^+$ T cells and the development of AIDS. This phenomenon has been intriguing to immunologists and virologists alike, and the use of mathematical models has been instrumental in deepening our understanding of this infection. It was realized early on that HIV has a high mutation rate, which would allow the escape of virus mutants from immune detection. This concept formed the basis of one of the first models of HIV infection, which postulated that during the latent phase of the infection the immune system and the virus were engaged in a race for dominance (9). It was postulated that the immune system could successfully remove most of the initial viral inoculum, but that mutant viruses requiring additional responses would continually appear. This process could continue for some time, but because the target for HIV infection was the $CD4^+$ T cell which would be gradually depleted over time, the immune system would eventually be unable to respond to new mutant viruses. This point was termed a “diversity threshold” and, since the immune system would fail to respond to the new mutant virus, AIDS would ensue (9). This model made some important predictions, and much debate and new experiments followed.

A major breakthrough occurred when two groups used mathematical models to calculate the viral turnover and the lifespan of infected $CD4^+$ T cells (10, 11). In these studies it was observed that the use of powerful anti-viral drugs, such as protease inhibitors, resulted in a rapid drop in the viral load and a concomitant increase in the CD4 count. From these results, it was possible to calculate the replication rate of HIV which proved to be much higher than people had expected; in addition, the lifespan of infected cells was calculated to be around 2 days (10, 11). The models were also used to calculate the CD4 lymphocyte turnover and this was found to be of the order of 10^9 $CD4^+$ T cells/day (10, 11). These papers were important in the field because they revealed that HIV infection was a dynamic process with continuing viral replication, even during periods of apparent quiescence and stable CD4 counts. In addition it was shown that the failure of single agent therapy was associated with the emergence of viral mutants, that were resistant to the drug being used (11). These studies changed the way that people thought about HIV infection and made some concrete predictions and suggestions about therapy. Thus, it was argued that therapy should be started as early as possible, with multiple agents, in order to prevent the evolution of drug resistant mutants, which has become the standard mode of therapy.

The mathematical models used in these studies were relatively simple and did not incorporate the immune response to HIV. This has proved to be a point of some debate since some individuals believe that the progression to AIDS is simply a reflection of the virus/host dynamics, and the immune system is only involved because immune cells are targets for infection. Others believe that the immune system plays an important role in the ability of individuals infected with HIV to combat this infection and may determine whether individuals are fast or slow progressors. Many mathematical models have been proposed to address this question, and it is clear that it is possible to reproduce the clinical results using models that do not evoke an immune response to the virus (12-17). One of these models considers that HIV can only productively infect activated $CD4^+$ T cells; this model is capable of reproducing the time course of HIV infection with progression to AIDS, without invoking a diversity threshold (13,14). Another model describes the progression to AIDS as a direct effect of HIV on CD4 depletion coupled with homeostatic mechanisms that lead to holes in the T cell repertoire (16). Whether these models capture the reality of HIV infection or not, they prove that mathematical models can be used to test particular hypotheses.

Recently, models of viral dynamics in the presence of anti-viral drugs have been used to provide

detailed characteristics of viral replication and to suggest the best strategies of drug therapy to maximize HIV control and, potentially, viral elimination (18-23). Wein *et al* proposed a model that assessed the benefit of using a dynamic therapeutic approach in which drug therapy was changed based on the evolution of the infection (23). The results suggested that a dynamic multidrug approach would result in lower viral loads, higher CD4 counts and a delay in the progression to AIDS. Perelson *et al* have used clinical data obtained from patients receiving combination therapies for the first time to obtain accurate assessment of the rate of viral loss and to estimate how long treatment would theoretically have to be continued in order to eliminate the virus (24). These studies predicted that therapy would need to be continued for 2-3 years after the viral load has become undetectable in order to remove all of the virus from the blood and tissue compartments (24). Whether such estimates will be used in clinical practice remains to be seen. Estimates of the lifespan of the infected cell and the viral generation time were also made (25), although these results were challenged by Herz *et al* (22) who suggest that while it is possible to calculate the lifespan of the infected cell it is not possible from the existing data to arrive at an accurate estimate of the intracellular lifespan of HIV.

The issue of the role of the immune response in HIV infection has been directly addressed in several recent models. In these papers, models that either did or did not incorporate the immune response were compared (26-28). In simple models of viral replication and interaction with host cells, it was possible to develop a stable equilibrium of virus and CD4 cells, but this appeared to occur at higher viral loads than are observed clinically. The introduction of the immune (virus-specific CTL response) response into the model resulted in a low viral load (27, 29). The relevance of the CTL response has been questioned since no correlation has been observed between rate of progression and the level of measured specific CTL activity in the blood. However, the model demonstrates a potential explanation for this, by suggesting that the levels of measured CTL response do not necessarily reflect the "strength" of these responses (27). In this case the "strength" or responsiveness of an individual is distinguished from the measured response, and an individual who has an effective CTL response, that maintains the viral load low, may have a low measured CTL response in the blood because there is little viral antigen available to maintain it. Further predictions are made to suggest that the rate of progression is likely to be slow in individuals with strong CTL responses but rapid in those with weak responses. The strength of the CTL response may be reflected in the immunogenetics of individuals such that people with particular MHC alleles might generate particularly effective CTL responses. Thus, the overall conclusion from these studies was that while some aspects of HIV infection can be captured in models of pure viral dynamics, individual variations in disease progression rates may be related to differences in immune responsiveness.

Similar models have been used to address the role of the immune system in other infectious diseases such as hepatitis B (30) and malaria (31). A recent model of the CTL response in the case of malarial infection has used both the concept of host/parasite interactions and that of antagonist peptides in T cell activation (31). In this system, it was observed that two allelic HLA-B35 epitopes were antagonists for each other, such that CTL specific for one epitope would be inhibited by the presence of the other epitope. In addition, the presence of co-infection with malarial strains containing both alleles was observed more frequently than expected (31). A model of these interactions was developed that predicted, among other things, that the increase in observed mixed infections could be attributed to the presence of antagonism both at the level of CTL induction and effector function. In particular, antagonism at the level of CTL induction alone was not sufficient to account for the increase in mixed infections. The complex interrelations between the host immune system and invading pathogens can, in this way, be unraveled with the help of mathematical models.

One of the beauties of mathematical modeling is that it raises questions that may not have been addressed before. The calculation of the $CD4^+$ T cell turnover rate (10^9 T cells/day) during HIV infection was considered by some immunologists to be highly inaccurate and it was proposed that the rate of increase in the CD4 count following anti-viral therapy could be accounted for by the recirculation of $CD4^+$ T cells from lymphoid organs, and there was ample experimental evidence to support this concept (32). As a result of this debate new models of lymphocyte recirculation have been developed, which can be incorporated into existing models of HIV disease (33, 34). However, a recent paper describing $CD4^+$ T cell turnover in uninfected and SIV-infected macaques demonstrated that SIV infection does result in a marked increase in turnover rate for $CD4^+$, $CD8^+$ T cells, NK and B cells (35). The debate on this issue is not over and questions still remain in order to resolve this issue.

Mathematical models of HIV infection, as illustrated above, have provided important new insights into the development of AIDS and pathogenesis of HIV. Even in situations where the models might have been subsequently proved wrong they have provided a forum for debate that has helped researchers, both basic and clinical, deepen their understanding of the disease process and what therapeutic regimens might be most efficacious. The value of a model in this situation, and probably in any situation, is measured by the clarity of the question it aims to answer. By using models to address very specific questions in HIV pathogenesis and treatment, mathematicians have provided important new insights into this devastating disease.

4. MODELS OF T CELL ACTIVATION AND PROLIFERATION

T cells recognize antigen presented as a peptide bound to self MHC class I or class II molecules. The specificity of the TCR:ligand interaction is such that, in general, only foreign peptides are recognized. It has been shown that MHC molecules can bind many different peptides and that at least 2000 different peptides are presented at any one time on a given antigen presenting cell (APC). A particular MHC/peptide complex may only be represented 100 times on an APC and the T cell expresses at least 10^4 T cell receptors (TCR) on its surface. An area of considerable interest, both experimentally and theoretically, has been to determine the requirements for T cell activation such that the T cell is sensitive enough to recognize small numbers of MHC/peptide complexes (36-38) without allowing a significant number of "false

positive" responses that could lead to destructive autoimmune disease. T cell activation is thus both highly specific and extremely sensitive, despite the fact that the measured affinity between TCR and MHC/peptide complex is relatively low (39). Lanzavecchia and colleagues have provided experimental data to support the concept that T cell activation requires, in some cases, serial triggering of the TCR, such that a single MHC/peptide complex can trigger many hundreds of TCR (40). The interaction between individual TCR and MHC/peptide is brief and is determined by the dissociation rate constant; it is postulated that a certain length of association is required in order to trigger the intracellular cascade of events that lead to full T cell activation. They also demonstrated that once the TCR has been triggered it is internalized and degraded (41), whereas the MHC/peptide complex is released to bind more TCR. The experimental evidence for this is based on the observation that the level of TCR expression goes down following T cell activation, and has been elegantly confirmed in studies by Sykulev *et al* who demonstrated that a single MHC/peptide complex could trigger CTL function (36). From these data it has been calculated that up to 8000 TCR/MHC/peptide interactions are required in order to trigger the T cell, in the absence of costimulation (42). Recently Valetutti and Lanzavecchia have developed a computer model of this concept and have been able to demonstrate the function of agonist and antagonist peptides based on varying the time of interaction between TCR and MHC/peptide complex. They suggested that the TCR can be considered a "tunable switch that may transduce different signals depending on the time of ligation" (43).

Several other models have been proposed for this important interaction. One that attracted a lot of attention among immunologists was that of McKeithan, who used the concept of kinetic proofreading as a way to explain the high degree of sensitivity in the presence of high specificity of a low affinity receptor (44). TCR activation was modeled as a series of steps that had to be completed before a full signal to activate was delivered, similar to that described by Hopfield for DNA replication (45). In this scenario the TCR interacts with the MHC/peptide complex leading to a modification of the receptor, which then moves through a series of steps before reaching a state of irreversible activation. Mistakes would be avoided by postulating that each step had a unique dissociation rate constant and, that if dissociation were too rapid, the next step would not be achieved. It was postulated that nonspecific interactions would be avoided since it is unlikely that short-lived interactions would be able to sustain the number of steps required for full T cell activation. This model again predicts that the degree of T cell activation is controlled by the off rate of the TCR/MHC/peptide complex.

A more recent version of the kinetic proofreading model has been proposed and has been termed kinetic discrimination (46). In this case, if a ligand interacts with the TCR to produce a modified receptor, it can either be further modified and eventually lead to T cell activation, or it can dissociate and deliver a negative signal to inhibit activation. This model incorporates the concept of serial engagement and introduces the concept of a suboptimal ligand delivering a negative signal rather than simply failing to activate. This model incorporates, therefore, the experimental observations showing that antagonist peptides can inhibit the simultaneous activation via an agonist peptide. Again, this model is dependent on the dissociation rate constant for the TCR from the MHC/peptide complex.

These models all postulate that variant ligands will result in different signaling events triggered via the TCR, and there has been extensive experimental evidence that this is the case. One of the earliest events triggered via the TCR is an increase in intracellular calcium, which has recently been studied in detail and correlated with the model of kinetic discrimination described above (47). In these experiments Wülfing *et al* identified several types of calcium signal and classified them according to the percentage of cells responding, the quality and timing of the response. The differences in calcium response were correlated with downstream activation events such as cytokine production and proliferation, and the results suggested that the calcium response represented the accumulated signal received by the T cell. Thus, the T cell appeared to have counted the different signals received before the calcium flux is initiated. These results were used to refine the model, and introduced the requirement for the accumulation of a particular amount of an intracellular signaling molecule in order to trigger proliferation, a kind of activation threshold (47). The ability of individual peptides to signal the T cell to accumulate the threshold quantity of signal transduction product could be reproduced in computer simulations of this model. The authors concluded from these results, and the subsequent model, that commitment to T cell activation was not dependent on the rate of MHC/peptide/TCR complex formation or on oligomerization of the TCR, but rather on the accumulation of an intracellular signal transducing molecule (47).

These ideas have been debated for many years and it has been proposed by several groups that oligomerization of TCR, often in the presence of the accessory molecule CD4, was necessary for T cell activation (48, 49). The models of kinetic discrimination and serial engagement do not include any need for oligomerization or complex formation on the T cell surface. Hampl *et al* recently reported that CD4 appeared to bind after the TCR had interacted with MHC/peptide complex, that it was most necessary in short lived (1-2s) interactions, and that it had very little impact on interactions that lasted longer (50). Thus, the presence of CD4 can convert a weak agonist peptide into a strong agonist but it has little effect on already strong agonist peptides. The models described above clearly do not take into account all of the factors known to be important in T cell activation, such as the role of costimulation and CD4 accessory molecules. It has been shown that the presence of CD28 results in a reduction in the threshold number of activated TCR from 8000 to 1500 (42), but these facts have yet to be incorporated into a meaningful model.

T cell activation has also been modeled as a consequence of aggregation of the TCR on the cell surface, and these models differ from those discussed above since they concentrate on the signal transduction pathway initiated following T cell activation (51). In these studies, the investigators develop a model with the aim of understanding antigen-induced unresponsiveness and it is concluded that the activity of phosphorylating enzymes, triggered after activation, does not return immediately to

baseline levels. This could provide a plausible explanation for the phenomenon of T cell unresponsiveness following prior antigenic stimulation, and it is suggested that if suboptimal ligands interact with the TCR they could function by delivering a phosphorylation signal that is insufficient for activation but sufficient to induce unresponsiveness. Thus, this model specified what the negative signal, discussed in the kinetic discrimination model, might be, but used a different paradigm for T cell activation. It will be interesting in the future to see some of these models put together

A recent report by De Boer and Perelson utilizes the Michaelis-Menten type quasi-steady-state assumption which allows for an almost enzymatic amplification of the T cell response (52). This model is the latest in a series by the same group and it represents an improvement over the previous models since it allows for a maximal level of T cell proliferation. This model utilizes an interesting concept of T cell activation but it does not address the fundamental issues of interest to many T cell immunologists, namely the role of antagonist peptides and the high degree specificity and sensitivity in a relatively low affinity receptor.

Another model that has provided some important new insights is a detailed model of T cell activation that incorporates the antigen presentation step. All of the previous models discussed above utilized MHC/peptide complexes as a variable but did not model their generation. A recent model of T cell/APC interaction by Agrawal and Linderman specifically models the antigen processing pathway in antigen-specific B cells and performs a detailed kinetic analysis of this process (53). This model details the path that antigen takes from the surface immunoglobulin of a specific B cell, through intracellular compartments and interaction with newly synthesized MHC molecules. The effects of varying the method of antigen uptake, the affinity of antigen for immunoglobulin, the affinity of peptide for MHC and TCR affinity for MHC/peptide are all examined in detail. This model does not address the fundamental issues of sensitivity and specificity of T cell activation but it elegantly delineates the important parameters of antigen processing and presentation by B cells. It will be interesting to see this kind of model used in conjunction with models that more specifically describe T cell activation.

T cell proliferation is a very late consequence of T cell activation and is thought to be mediated via cytokines such as interleukin (IL-)-2 and IL-4, secreted following activation. The ability of IL-2 and IL-4 to stimulate proliferation is related to the expression of high affinity receptors for IL-2 (IL-2R) and IL-4 (IL-4R) on activated T cells. In our studies of T cell proliferation we have been interested in developing a mathematical model that would take into account two apparently contradictory experimental results. IL-2 and IL-4 have been shown to act in synergy in stimulating T cell proliferation (54, 55). Thus, the presence of IL-4 when only limiting amounts of IL-2 are present will result in an enhanced T cell proliferation that is greater than additive. In contrast, it has been shown that pretreatment of both B and T cells with IL-4 results in a downregulation in the number of high affinity IL-2R on the surface of these cells (56, 57). In addition, whereas both IL-2 and IL-4 can stimulate proliferation of B cells individually, the combined treatment of B cells with IL-2 and IL-4 results in inhibition of proliferation. We developed a mathematical model of these events that was able to provide a plausible explanation for these apparently contradictory results (55). In this model both the synergy effect and the downregulatory effect of IL-4 on IL-2R expression were included. We were able to show that the appearance of synergy or antagonism between IL-2 and IL-4 depended on several variables, including the initial number of IL-2R and IL-4R expressed on the cell and the threshold number of binding events required to stimulate proliferation (55, 58). Thus, if as in the case of B cells, the number of IL-2R is low relative to the number of IL-4R, the presence of IL-4 will result in the downregulation of IL-2R expression to level at which the threshold number of bindings can no longer occur and antagonism will result. In contrast, in T cells that express high levels of IL-2R the synergistic effect of IL-4 on the IL-2 response occurs before the reduction in the number of IL-2R, in the presence of IL-4, falls below the threshold required for proliferation. We have subsequently used this model to estimate the threshold number of binding events required for IL-2-mediated and IL-4-mediated proliferation and have been able to generate synergy terms that allow accurate simulation of experimental results (58).

In this area, apparent paradoxes in experimental results, such as in T cell activation and cytokine effects, can be clarified by the use of mathematical models. In many cases, such as that of IL-2 and IL-4, a mathematical model that incorporates known biological phenomena can be used to simulate apparently contradictory experimental results, demonstrating that the same underlying principles can explain synergy and antagonism in two different systems.

5. SIGNAL TRANSDUCTION MODELS

Activation of the T cell via the TCR, as discussed above, is not thought to require extensive oligomerization/aggregation of the receptor for signals to be transduced. This is still a matter of some debate, but it is clear, on the other hand, that some receptor systems do require a significant degree of aggregation for a signal to be transduced. An example of this is the high affinity Fc receptor for IgE (FcεRI), which is found on mast cells and basophils. When this receptor is triggered degranulation of the mast cell/basophil occurs and mediators important in allergic reactions are released. The kinetics and degree of aggregation required for signaling has been extensively studied by several groups and several models have been developed of this system (59-62). Results that have emerged from these studies have revealed that simple cyclic dimers of bound FcεRI are not sufficient to induce activation (60), and this suggested that complex structures were required in order to trigger internal signaling events. This concept has been investigated further by the identification and detailed kinetic analysis of phosphorylation events occurring following controlled FcεRI aggregation (61, 63). In this way, it could be demonstrated that cyclic dimers of Fc RI only stimulate partial phosphorylation of the receptor (63).

6. IMMUNE NETWORKS

Over 20 years ago Neils Jerne proposed the network theory of immune regulation (64), which postulated that the immune system was regulated by a network of idiotype/anti-idiotype interactions. This

network would be responsible for the control of the immune response and would also allow the development of self tolerance. The concept of networks was very attractive to mathematicians and many models have been developed around this concept (65-68). These models demonstrated that a network of interacting antibody molecules was self-organizing and could generate states of tolerance, immune response and memory. These models were all based on the bell shaped curve of lymphocyte activation, which allowed the inactivation/deletion of clones that were of too high an affinity for the antigen. While this was attractive, the network theory did not become an accepted part of mainstream immunology due to the ability of clonal selection theory to explain immune responsiveness and tolerance through selective clonal expansion or deletion respectively (69). However, it has become apparent that networks of interconnected antibodies do exist involving around 20% of the circulating lymphocytes in the newborn, with this degree of network connection falling significantly with age (70, 71). For some immunologists, clonal selection theory has never adequately explained the maintenance of self tolerance or the development of autoimmunity (69). Recent experiments and models have permitted the refinement of the network theory, and have demonstrated that the presence of a network involving a small percentage (15%) of lymphocytes is sufficient to maintain tolerance, and has unstable properties that could occasionally allow autoimmunity to develop (72-75). In addition, the development of immune memory can be seen to emerge naturally from such a network model, rendering obsolete the requirement for long-lived memory cells or persistent antigen. While the network theory is still, to some extent, on the fringes of mainstream immunology it is a good example of a productive collaboration between immunologists and mathematicians leading to a deeper understanding of immune regulation. In a recent editorial (69), Coutinho elegantly described the evolution of the network theory and postulated that the immune response to foreign antigen is mediated by clonal expansion of lymphocyte clones but that a network involving a small proportion of lymphocytes is necessary for the maintenance of self tolerance. This perspective has resulted in development of a new model that incorporates both B and T cells in a network that is restricted to a small proportion of these cells (76).

7. OTHER IMMUNOLOGICAL MODELS

The development of T cells in the thymus has been a fertile area of research and recently has attracted the attention of modelers (77-79). A recent model by Mehr and colleagues (80, 81) describes the role of mature $CD4^+$ T cells on T cell development in the thymus. It has been observed experimentally that the addition of mature T cells profoundly affects the developing thymocytes, resulting in a reduction in the numbers of double positive (DP) thymocytes and an increase in the numbers of mature $CD4^+$ T cells (81). These effects were markedly increased in older mice. In order to reproduce these results the mathematical model had to introduce two levels of regulation by $CD4^+$ T cells. One was a negative effect on the proliferation and expansion of DP thymocytes and the other was a positive effect on the differentiation of $CD4^+$ T cells.

Several other models of basic immunological phenomena have been reported including models of Th1/Th2 crossregulation (82, 83), the development of germinal centers (84) and B cell homeostasis (85).

8. CELLULAR AUTOMATA IN IMMUNOLOGY

Most of the mathematical models described in the preceding sections involve the use of differential equations; there have been few models using the concept of cellular automata, computer simulations in which the body is depicted as a grid and all of the components of the system are defined. An immune response can be followed in this system by allowing the system to evolve over several discrete time steps and introducing a series of basic rules of interaction among cells, antigen, antibodies etc. The recognition receptors and antigens can be modeled as a series of bit maps and the recognition requirements for activation are specifically defined. The advantage of this type of model is that it allows spatial configuration of the immune system and adds the extra dimension of structure to the model. However, it does not allow extensive mathematical analysis since the results are analyzed after each individual simulation, and only then can the parameters of the automaton be modified. Celada and Seiden have extensively used this modeling system and have produced models of the immune response (86, 87), somatic hypermutation of B cells (88), and the thymus (89). All of these models have been able to reproduce existing experimental data and have suggested new interpretations of the data with further experiments to be performed. Cellular automata can be a useful step for immunologists interested in modeling since all of the elements of the model are defined and it is readily understood. In the complex area of the interacting immune system, when there is potentially insufficient information available to construct a detailed mathematical model, cellular automata can be used to simulate what is known about the interactions and thus to highlight where the gaps in the knowledge are the most problematic.

9. CONCLUSIONS

The explosion of research in immunology has led to the need to develop integrated ways of describing the system. Many of the recent experimental advances in the field have relied on the isolation of the objects of study, using either knockout or transgenic mouse models. These powerful technologies provide important insights into the relative importance of certain cytokines, cell surface molecules and cell types, but do not ultimately tell us how the system functions when all of the components are present and active. It seems, to this immunologist at least, that the language of mathematics is ideally suited to the understanding of the integrated immune system as a whole, and that it will become an increasingly important part of the study of the immune system. Physics was transformed from a study of phenomena into the powerful subject it is today through the introduction of mathematics as its main language. In biological sciences, mathematics have come to play an important role in the study of the nervous system and have contributed greatly the development of quantitative neurobiology. It remains to be seen how profoundly mathematics can influence the study of the

immune system, but in the examples described above it can be seen that its impact is already being felt.

10. ACKNOWLEDGMENTS

This work was supported by NIH grant AI31427. The author wishes to thank Drs. Susan McCarthy, Benoît Morel and Timothy Oriss for critical reading of this manuscript.

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Key words: Immunology, T lymphocytes, HIV infection, mathematical modeling

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