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New Insights into Mathematical Modeling of the Immune System

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Abstract

In order to understand the integrated behavior of the immune system, there is no alternative to mathematical modeling. In addition, the advent of experimental tools such as gene arrays and proteomics poses new challenges to immunologists who are now faced with more information than can be readily incorporated into existing paradigms of immunity. We review here our ongoing efforts to develop mathematical models of immune responses to infectious disease, highlight a new modeling approach that is more accessible to immunologists, and describe new ways to analyze microarray data. These are collaborative studies between experimental immunologists, mathematicians, and computer scientists.

Key Words

Mathematical modeling
Mycobacterium tuberculosis
Microarray immunology

Introduction

The explosion of research in the field of immunology and the increased complexity that it is has revealed have created a need for new methods to assimilate and analyze the data. In addition, the advent of experimental tools such as gene arrays and proteomics poses new challenges to immunologists who are now faced with more information than can be readily incorporated into existing paradigms of immunity. Novel methodologies are required to allow integration of these extensive data sets into our thinking. These data-intensive technologies represent a change from the reductionist approaches that have

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traditionally characterized immunological research. In addition, as we begin to identify more of the components of the immune response, there is a need to model complex interactions in the in vivo setting. Mathematical modeling coupled with computer simulation models provide important new tools that can be used not only to analyze complex datasets but also to model in vivo immune responses *(1).* As described below we have been involved in mathematical modeling of immune responses for several years and have tackled important questions.

In our initial studies, we developed ordinary differential equation (ODE) models to describe the proliferative response of B cells and T cells to interleukin (IL)-2 and IL-4 *(2,3).* In these studies we modeled the apparently contradictory effects of these cytokines on B and T cells, such that IL-2 and IL-4 act synergistically to induce T cell proliferation but antagonize each other in the induction of B cell proliferation *(4).* This could be explained in the model by incorporating two effects: (1) the downregulation of the high-affinity IL-2R by IL-4, through the sequestration of the common γ chain that is shared by both the IL-2 and IL-4 receptors; and (2) a synergistic effect on the signaling induced by IL-2 in the presence of IL-4. The difference in response between B and T cells could thus be modeled by changing the initial conditions of the simulations in which B cells, expressing lower levels of IL-2Rs than T cells, were more susceptible to the downregulating effects of IL-4 on IL-2R expression, whereas the synergy term was dominant in the case of T cells. Although these results were satisfying, the model was very computer intensive and required a supercomputer *(2),* and a large number of parameters had to be estimated. We attempted to broaden this model to a more general analysis of Th1/Th2 crossregulation in vivo but realized that using the ODE approach it was not going to be easy to capture the complexity of the interactions between relevant cell types and the cytokines and mediators that they secrete. On the other hand, as discussed below, we have successfully developed useful ODE-based models of *Mycobacterium tuberculosis* infection that have provided important new insights into this devastating infection. In this review we provide a summary of our efforts in the modeling of *M. tuberculosis* infection in the lung and highlight a new modeling approach that we are developing as well new tools for the analysis of microarray data that we are currently working on.

Modeling *M. tuberculosis* Infection

Many studies in model systems designed to elucidate the immune response to infection are reductionist, highlighting the importance of one or two elements at a time. These fail to account for how the entire system is affected by variations in elements of the system as a whole. Adding mathematical models to the study of infectious diseases to help understand immunity and pathogenesis is powerful. A particularly complex infectious disease is that caused by the bacterium *M. tuberculosis.* Tuberculosis kills 2 million people every year, even though there are drugs available to cure this infection. The bacillus infects via the respiratory route, and causes primary disease in a small fraction of those infected. Although most infected persons are capable of controlling the infection with no signs of disease, the organism resides within the host for years or decades. In approx 10% of infected individuals, reactivation of this clinically latent infection occurs, and active tuberculosis ensues. The immune response to this infection is crucial in preventing both primary and reactivation tuberculosis, and under immunosuppressing

conditions, such as age, malnutrition, and HIV infection, there is an increased risk of active disease (reviewed in ref. *5*). The immune response to *M. tuberculosis* is complex and not well understood because, although we know many of the immune cellular players, we do not understand the basis for establishment or maintenance of latency in terms of the cellular interactions occurring throughout the course of this long infection.

Our previous studies in animal models and those of multiple other groups have provided information about the cell types and cytokines involved in control of *M. tuberculosis* infection (reviewed in ref. *6*). A feature of the immune response to *M. tuberculosis* is the formation of a granuloma, an immune structure composed of T cells, B cells, macrophages, and other cell types, whose function is to provide an immune microenvironment where T cells, cytokines, and macrophages can interact. The bacterium resides within macrophages at the center of the granuloma, and activation of these cells is crucial to the success of the host in controlling the infection.

We have developed several mathematical models to address important questions regarding both the immune response and *M. tuberculosis* infection that have been difficult to approach using traditional experimental methods *(7–12).* In addition we have used these mathematical models to identify mechanisms by which the bacterium subverts the immune response, allowing long-term persistence. An example of the use of ODE models to address difficult experimental questions comes from our work on $CD8^+$ T cells and *M. tuberculosis (11).* This has been a controversial topic in tuberculosis research, because it is difficult to prove that $CD8^+$ T cells are playing an essential role in the control of this infection. This is an important question as rationally designed vaccines are currently being developed, and one must consider whether to target $CD8^+$ T cells in addition to $CD4^+$ T cells. In both mice and humans, $CD8^+$ T cell responses to *M. tuberculosis* are present, but loss of $CD8^+$ T cells in mice gives only a modest phenotype. There is a major difference between human and murine $CD8^+$ T cells: human cells produce a molecule called granulysin, present in the cytotoxic granules of these cells, which directly kills intracellular *M. tuberculosis* following perforin-mediated granule exocytosis *(13).* Murine $CD8^+$ T cells do not have a granulysin homolog, and therefore testing the effects of $CD8^+$ T cells on control of tuberculosis in the murine model is incomplete. We addressed the question of the role of $CD8^+$ T cells in tuberculosis using a mathematical model. The model was built based on known cellular interactions described in the literature together with our experimental results, and has variables that represent T cells, macrophages, specific cytokines, and bacterial populations. The T cells are divided into subsets: TH0 (precursor Th cells), TH1, TH2 for $CD4^+$ T cells and T80 (precursor $CD8^+$ T cells), T8 (those that make IFN-γ) and Tc (cytotoxic cells) for $CD8^+$ T cells. The model was validated by performing various "virtual knockouts" consisting of the depletion of specific cells or cytokines to ensure that published mouse or human data could be recapitulated. Based on changes in the parameter values, the model provides scenarios for primary, latent, and reactivation tuberculosis.

As an example of how we used this model to study the T cell response, we questioned the timing of the development of the two different functional $CD8^+$ T cell subsets. In our mouse model, we found that the $CD8^+$ T cells expressed a cytotoxic phenotype first, and then switched in the chronic phase to producing IFN-γ (and were no longer cytotoxic) *(14).*

Using the ODE mathematical model, we also studied development of disease when $CD8^+$ T cells were cytotoxic first, and then IFN-γ producing; however, we also were able to model the reverse situation. To our surprise, the bacterial loads were lower and latency was "more stable" in the situation where $CD8^+$ T cells produced IFN-γ first, and then were cytotoxic, the opposite of the situation that we observed in our mouse model. We went on to use this model to study the minimum requirement for memory $CD4^+$ and $CD8^+$ T cells that would be protective in a vaccine-challenge model.

The ODE model is currently being expanded further to understand the role of TNF-α in tuberculosis, and how TNF-α neutralizing drugs used to treat chronic inflammatory diseases cause increased reactivation of latent tuberculosis. In addition to modeling the lung, we have also developed a two-compartment model, where the modeling space is the lymph node (for priming of T cell responses) and the lung (for effector T cell and macrophage responses) *(9)*. This model explored the importance of dendritic cells for priming T cells in this model, as well as the kinetics of priming T cells following *M. tuberculosis* infection, which is a much slower process than in many infections.

To understand granuloma formation and function, the ODE model is not the best choice. A granuloma is a three-dimensional spatial object, and is best modeled in a system that allows cellular migration and spatial arrangements. We have used agent-based modeling to provide the framework for addressing important and difficult questions about granuloma formation. Our current goal is to include much more complexity (as in the ODE model) into the agent-based model, and to ultimately link it to the lymph node priming model, to provide a complex and comprehensive approach to the study of immune responses in tuberculosis.

Novel Modeling Approach

The use of ODE models requires detailed knowledge about kinetic parameters that are explicitly included in the equations. Some of these can be measured experimentally whereas others need to be estimated. One concern that arises with this approach is that the more parameters there are, the easier it becomes to introduce inaccuracies in the model that may deviate from the biological reality that the model is trying to capture. We have recently experimented with a new framework for modeling biological processes. The framework includes two main parts. The first is a formal language to express experimental design and data as well as knowledge regarding states and interactions in biological systems. The second is a computer-simulation environment that uses the formal language to define stochastic logical network models and to simulate them. The approach addresses limitations in available measurements, high variability in results, large amounts of data across the field of immunology, and the exceptional complexity of biological systems. The formal language is intuitive and readily understandable by biomedical scientists, facilitating communication across distant disciplines, mathematics and biology.

In developing a general framework for modeling immune functions, we had to deal with several issues. We have developed an approach that (1) is flexible and modular; (2) is understandable by bench scientists without additional training in modeling approaches; (3) addresses the complexity of the system; (4) directly deals with the hierarchical structure (from genes to proteins to cells to organs); (5) addresses the great variability in experimental data (by using logical variables); (6) addresses the insufficient knowledge regarding reaction rates (by using logical variables for reaction rates); and (7) can inter-

act with state-of-the-art experimental techniques, because it can deal with genes and proteins and their interactions.

The rationale for the new framework is based on a few observations. First, the large number of interactions and processing that take place in an immune response, or in other coupled molecular/cellular systems, call for a new paradigm to capture such complex behavior. Second, the variability in experimental results in expression level of receptors, cytokines, microarrays, and others suggests the use of discrete variables with only a few values (e.g., "no expression," "low level," and "high level"). Such abstraction is necessary also for rate constants, because in most cases such rates (on-rate, off-rate) are not known; in the real human condition, variation induced by single nucleotide polymorphisms in genes critical to inflammation and immunity add a further layer of complexity. This is not to say that the model we have developed cannot be made very quantitative: we note that as additional quantitative information becomes available, our modeling platform can become more finely-grained. Third, there is a large body of knowledge regarding molecular interactions in immune response, and this knowledge base is growing rapidly. Thus, it is useful to work in a modeling environment that allows for rapid incorporation of new developments. At the individual cell or molecule level, we use logical variables attaining only a few values; however, at the population level (many cells), our description is in terms of real numbers, thus quantitative at the population level.

Molecules are the fundamental objects in our model and all interactions are formulated in terms of molecules. Cells are treated as more complex objects made up of molecules residing in a few compartments (surface, cytosol, etc.). Finally, organs are objects with a higher level of complexity, consisting of populations of cells. The dynamics in our model are governed by a set of reactions (rules) for molecular interactions that are defined outside the simulation program for maximum flexibility. To build a model using this framework one has to build the following components: (1) the objects in the system, including their molecular content, (2) a set of interactions (rules), (3) specification of desired output.

Objects in the modeling platform are of two types, simple objects and complex objects. The simple objects represent, for example, molecules. These may be genes, proteins, or any other relevant molecule. Such objects are described in terms of their identity, i.e., a name, and abundance that attains one of the discrete values allowed (none, low, high, very high, etc.). A Th1 cell for example can be expressed as Th1:=Obj (IN:={SigTh1X}; SURFACE:= {CD4, CD28, TCR, IL-12R, IL4R, IL-2R}; ObjList:={Mitochondria, Nucleus}), SigTh1X represent a certain signaling typical to a Th1 cell. For example, activation of a Th1 cell via the TCR that stimulates the secretion of IL-2 and IFN-γ.

Objects communicate through surface–surface interactions (via surface molecules), through the secretion of molecules from one object into another, and more. Objects, such as cells, may migrate between compartments that represent organs, the blood, the lymphatic system, etc. This is facilitated using chemokines and their receptors as in reality.

A fundamental component of our framework involves a set of interaction rules, or reactions. This is a set of rules that describe the possible evolution for objects or processes. Owing to the limited knowledge regarding reaction rate constants, we distinguish only a few reaction speeds that in the real system correspond to different orders of magnitudes. We limit our discussion here to three speeds, although the model can easily be extended to have any finite number of speeds as information about these reaction rates

becomes available, and introduce symbols to express them, -> (slow), ->> (moderate), and ->>> (fast). Reactions have a list of reactants (left), a list of products (right), and a speed. Examples include, **IL-12 + IL-12R ->>> IL-12:IL12R,** where IL-12:IL12R denotes the complex formed by IL-12 and its receptor. To deal with secretion of molecules by cells, we have transporter reactions. As an example, consider the transporter reaction **Tr :: IL-2 @ IN ->>> IL-2 @ OUT.** Here **Tr** is a transporter required in order to move **IL-2** from the inside of the cell (**IN**) to the outside (**OUT**), and the rate of transfer is high (**->>>**).

The evolution of a system involves the execution of reactions among objects. Such reactions result in changes in the abundance of both reactants and products. Because we are dealing with a discrete representation of these processes, we need to define addition and subtraction among such numbers. The intuition behind the rules we use is quite obvious if we think of 0 as representing the absence of molecules (or process), 1 representing a low abundance, 2 representing higher abundance, and so on. Our non-traditional arithmetic starts with the rules 0 + x = 0, 1 + 1 = 1 (low + low = low), 1 + 2 = 2 (low + high = high), 2 + 2 = 2 (high + high = high).

A reaction (e.g., A + B -> C) is executed as follows. First, a change in reactants (A and B) and products (C) is calculated, based on abundance and speed mimicking a mass action law, for the log of concentration. This step is followed by the addition of the change in products and subtracting it from reactants, following our non-standard arithmetic. Such changes may be fractional at small time steps, and are interpreted probabilistically. Thus, a change of α is interpreted as a change of 1, but is executed in probability of α.

We have carried out preliminary studies in modeling different cellular systems, during the development of the general framework. This allowed us to examine major challenges in modeling biomedical questions and to build our modeling environment accordingly. In particular, we have initiated modeling of Th1/Th2 differentiation, apoptosis, activation of NF-κB, acute inflammation, the initiation of cancer, and a coarse-level model of the whole immune system (manuscripts in preparation). Preliminary results suggest that this modeling approach is readily usable by immunologists and that it will provide important new insights.

Modeling Immunology in the Age of Microarrays

The immune system is a biological system, unlike physical systems that evolves toward maximal entropy. For example the immune response in general leads to immune memory, which can be considered a "negentropic" process. From the innate response and the detection of a pathogen to the triggering of the adaptive response, there is an intense amount of information processing. As a result modeling the immune response is challenging and stresses the state of the art in modeling.

The advent of microarray data has compounded this challenge. Microarrays provide additional evidence of the degree of complexity and an imperative to meet the challenge. There is more information on a microarray than we able to process at our present state of knowledge. As a result the first instinct has been to use statistical techniques. One popular technique was to divide the genes in clusters of similar activation patterns, on the assumption that genes co-activated or co-inactivated somehow contribute together to the physiological effect observed. This approach, which is in development, has synergized with the theory of networks. Genes acting in concert behave as if they

were part of a network. This approach offers the hope to be able to "understand" the effect of patterns of gene activation. In the parlance of complex system theory, those physiological effects correspond to "emergent properties." Another way to use data from microarrays is to understand the regulation of transcription and its relation with signaling cascades.

Recently, we have used new analysis tools to analyze preexisting microarray data sets. The analysis used data from one of the first microarray experiments *(15).* In these experiments, human peripheral blood monocytes were challenged with *Escherichia coli* and microarray analyses were performed on samples taken at several time points: 0 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 12 h. We concentrated our analysis on the first three time points: 0 h, 0.5 h, and 1 h. The rationale for this choice is that, in the first phase, the transcription factors involved are likely to be those induced from signaling cascades stimulated directly as a result of the LPS binding.

Our analysis was made in three steps:

1. *Selection of the genes:* There is a need to make some kind of selection because more genes are activated than we can analyze. We selected the most highly activated genes, that increased or decreased by a factor of at least 2, on a logarithmic scale (normalized to 0 at time zero). The probability that those genes are spuriously activated is very small, but they represent a subset of the activated genes.
2. *Identification of transcription factors, using a tool called Footer (16).* This tool allows the identification of transcription factors that could have been responsible for the activation of a given gene. Having identified an activated gene, we use the publicly available TRASER (http://genome-www6.stanford.edu/cgi-bin/Traser/traser) to find the 3 kb upstream promoter sequences. Footer analyzes the sequences and returns a list of candidate transcription factors that could have binding sites in the selected sequence and therefore might have been involved in the activation of the gene. We performed this analysis for all the genes of the set of co-activated genes and compared the list of transcription factors. We identified the minimum number of transcription factors that, in combination, could have led to the observed pattern of gene activation. The presence of the same transcription factor or a few transcription factors in the lists of transcription factors for all the genes strongly suggests that these transcription factors could have been responsible for the observed pattern of gene activation. This also suggests that a common signaling cascade may have produced them.
3. *Identification of networks of genes coordinately activated:* Data were further analyzed through the use of Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). Ingenuity is a very large database where the knowledge about co-activation of genes is made directly accessible.

In our early analysis of the data from Boldrick et al. *(15)* we identified 132 genes that were activated after 0.5 h, whereas 192 were activated after 1 h. Of the 132 genes, 94 of which were still activated after 1 h. An intriguing observation is that of the 132 genes activated after 0.5 h, 35 of them build a complete Ingenuity network. These genes are:

ALOX5	BCL7A	CASP10	CCR5	CD151	CD69	CD81
CDC10	CRADD	F3	FOSL1	FTH1	GRO2	HNRPU
ICAM1	IFNGR1	IK	IL1A	IL1B	ITGA6	LYN
MCL1	NFKBIA	NOTCH1	NR4A2	NR4A3	PPP1R15A	RGS1
SCYA3	SCYA3L1	SCYA4	SDF1	SELE	TNF	TNFAIP3

Using Footer, we determined whether the pattern of activation seen after 0.5 h could be attributable to a small set of transcription factors. Footer analyzes the upstream sequences of the desired gene for both *Homo sapiens* and mouse to avoid false positive results. For three of these genes TRASER did not have the corresponding sequence. The seven transcription factors that can bind to the largest number of the remaining 32 genes are:

PPAR-α STAT4 TBP Sp3 STAT3 RXRbeta STAT5A STAT5B

According to Footer, none of these transcription factors bind to all 32 genes. PPAR-α binds to 29 of them (the three left are SELE, ICAM1, and SFD1). STAT4 and TBP bind to 25 of them. PPAR-α and STAT4 together are predicted to bind to all the genes within this network. STAT4 is activated following the binding of IL-12 to its receptors and should not be activated at this early time point. PPAR-α has been shown to inhibit the activation induced by LPS by repressing the function of NK-κB and AP-1 *(17).* Thus, these results have identified two transcription factors that can regulate this network of genes, but, on the basis of these experiments, it is unlikely that these transcription factors would be responsible for the induction of the genes seen at these early time points following LPS stimulation.

This preliminary analysis demonstrates that a combination of approaches can be used when analyzing patterns of activation detected by microarray analysis and these can reveal "networks" of coordinately regulated genes. Learning how to make the culture of networks speak to the physiological effect associated with patterns of gene activation would increase our mathematical toolbox for the modeling the immune system.

Conclusions

We have described some of the new approaches that we are exploring and we anticipate that these will allow us to deepen our understanding of the integrated behavior of the immune system. These studies are continuing under the auspices of a newly created Immune Modeling Center (http://cmpi.cs.pitt.edu/) that will be studying immune responses to three pathogens: *M. tuberculosis,* influenza and *Francisella tularensis.* These are collaborative studies between experimental immunologists, mathematicians and computer scientists and we anticipate that new and accessible tools will be developed for the analysis of large datasets and the modeling of in vivo infections.

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