Modeling Development and Differentiation in the Immune System

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Abstract

In recent years, there has been considerable interest in modeling and simulating the processes of lymphocyte development and differentiation. A recent meeting was aimed at identifying and defining the relevant problems and the various mathematical and computational tools that may be appropriate for dealing with them.

Mathematical modeling of lymphocyte development

At present it is not clear how immune cells integrate the information they receive from their environment (via cytokine and other signals and cell-cell interactions) and how they choose the appropriate course of action in the face of varying challenges. This is specifically true in the case of lymphocyte development and differentiation. Due to the complexity of interactions involved, the use of mathematical modeling and computer simulations to help elucidate immunological processes has been rapidly spreading in recent years [8, 1, 2]. Here we focus on recent results of modeling lymphocyte development, differentiation and selection, as reported in a recent meeting[1].

B cell development and differentiation

The germinal center (GC) reaction is necessary for the generation of high affinity B memory cells. After immunization with thymus-dependent antigens, activated T- and B lymphocytes migrate from the T cell areas of secondary lymphoid tissues (e.g., spleen and lymph nodes) into the B cells zones. Here they proliferate within an antigen-bearing reticulum of follicular dendritic cells (FDCs) to form GCs. Both antigen-specific T- and B cell clones expand in GCs and early GCs are clonally diverse, containing as many as

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ten unique clones. However, late in the primary response the diversity falls and each GC contains only a few distinct clones. This reduction in diversity is largely due to the loss of low affinity B cells. The precise mechanisms by which clonal selection is performed in the GC has not been elucidated, and the frequency of GCs that contain the highest affinity cells has not been addressed in any systematic way. T. Kepler (North Carolina) and A. Perelson (Los Alamos) suggested, based on an optimal control model, that mutation should be "on" in discrete phases. Thus, to generate the largest number of high affinity cells cells should first proliferate without mutation, then mutate and be selected. The selected cells should then repeat this process. The architecture of the GC, in which proliferation occurs in the dark zone (DZ) and selection in the light (DZ), lead to the suggestion that cells cycle through the GC going from DZ to LZ and then back to DZ for another round of proliferation, mutation and selection. G. Kelsoe (Baltimore) has specifically looked for re-entry into the DZ suggested by the Kepler-Perelson model of punctuated selection/proliferation [1]. Dark Zone (DZ) centroblasts move to the Light Zone (LZ) to become Ig^{+} centrocytes. These cells are susceptible to apoptosis by the inappropriate crosslinking of their mIg. Depletion of LZ B cells results in the eventual loss of the GC DZ, consistent with the idea that selected centrocytes return to the DZ and re-initiate proliferation as centroblasts, as suggested by the Kepler-Perelson model.

The group of T. Manser (Philadelphia) has been testing models that propose that the majority of somatic hypermutation and selection events leading to the generation of the memory B cell compartment take place in the microenvironment of the germinal center, and that antigen in the form of immune complexes on FDCs is involved in these selective events. In agreement with GC models for the generation of B cell memory, it was found that the participation of a very high affinity clonotype in the immune response of A/J mice to arsonate does not overtly influence the participation and entry into memory of much lower affinity clonotypes. Immunohistochemical examination of spleen sections from such mice indicates that the two clonotypes undergo primary differentiation in different GCs. Thus, the physical isolation of these clones in individual GCs appears to result in entry into memory being regulated in a "clone autonomous" fashion.

Using phage display technology and in vitro mutagenesis, Manser also reported that the frequency of random mutants of an anti-arsonate V region that have increased affinity for both arsonate and the autoantigen DNA is surprisingly high. The fact that such mutants are never observed in vivo strongly suggests that during mutation-selection an active process of negative selection of autoreactive mutants is operative. Positive selection for increased affinity for arsonate alone could not purge the mutant repertoire of "dual- reactive" B cells that had acquired increased affinity for both arsonate and DNA. Thus, the selective factor limiting entry into the memory B cell compartment does not appear to be affinity per se, but specificity for foreign antigen.

Manser also reported that immune complex deposition on FDCs is dramatically enhanced in mice in which the gene encoding the FcR gamma chain has been "knocked out". Despite this enhanced deposition, somatic mutation and affinity maturation of the B cell response appears unperturbed. These data challenge the current belief that antigen on FDCs plays a direct role in the selection of B cells into the memory compartment.

The process of receptor editing might occur extremely frequently during B-cell development. The fact that most normal B-cells only rearrange on a single κ allele places limits upon the theoretical frequency of receptor editing. A mathematical model was presented (D. Nemazee, Denver) which probed the relationship between antigen receptor crossreactivity and self tolerance. It was predicted that an optimal level of crossreactivity should be selected in evolution which would maximize coverage of foreign antigens after clonal elimination of autoreactive cells. Such an optimal system should be characterized by frequent autoreactivity in the preselected repertoire, requiring massive negative selection. The model predicts that approximately 63% of all newly generated lymphocytes should be autoreactive and eliminated by tolerance [4]. A similar model that reached exactly the same conclusion had been previously published by DeBoer and Perelson [3].

Based on the above results, a theoretical model of light chain allelic exclusion was developed to determine if massive receptor editing was compatible with the observation that about 50% of splenic B-cells bear κ rearrangements only on a single chromosome. In the model autoreactivity was assumed to actively prevent light chain allelic exclusion, as has been proposed by the laboratories of Nemazee and Weigert [5, 6, 7]. Also incorporated in the model was the possibility that light chain proteins are often unable to associate with the B-cell's heavy chain and therefore fail to mediate feedback inhibition of light chain rearrangement. The results suggest that high frequencies of light chain secondary rearrangements as a result autoreactivity and/or light chain non-functionality are possible, but such high frequencies place narrow constraints on the number of rearrangement attempts permitted per cell, which should result in mean rearrangements/cell of approximately 2.5.

M. Weigert (Princeton) and S. Litwin (Fox Chase Cancer Center) have exercised a model of clonal proliferation that allows one to simulate populations of cells that would be sampled after a fixed number of cell divisions. Model cells mutate according to an adjustable schedule, and garner advantage which is conferred by enhanced ability to reproduce. Mutations of several categories, namely: silent framework, replacement framework, silent CDR, neutral CDR, and advantageous CDR mutations are accumulated by each cell in the clone. In particular, the ratio of replacement CDR to silent CDR mutations (R/S) was determined under roughly periodic mutation schedules as well as under continuous ones. Periodic mutation schedules produced only tiny fractions of cells whose R/S ratios are as large as observed values, (e.g. seven) whereas, under some continuous schedules 20% of cells did. It is not clear whether the mechanism responsible for the cyclic mutation is that of centroblast reentry into the GC dark zone, as suggested by this model; there is only indirect evidence for such reentry (G. Kelsoe, Baltimore).

Computer modeling has demonstrated that neither exhaustive editing at the κ light-chain locus nor B-cell death during development (the "crash factor") alone can explain a κ:λ ratio exceeding ten, that is observed experimentally. The BREC model of antibody formation that incorporates both receptor editing and the crash factor – and that can account for κ:λ ratios in accordance with empirical data predicts that the κ chain will usually be exhaustively rearranged before the λ light chain locus is rearranged (Paul Pelavin, Princeton, with M. Weigert and S. Litwin). Preliminary experimental data on exhaustive κ rearrangement in l-expressing B-cells suggests that this does occur. In addition, a computer simulation of the BREC model has attained κ:λ ratios in accordance with empirical data. Since this model predicts that biased expression of κ is dependent on multiple rearrangements, a mouse strain utilizing homologous recombination that will only be able to undergo one rearrangement per κ locus is being developed . It is expected that these mice will have significantly lower κ:λ ratios than wild-type mice.

T cell development, differentiation and selection

Analysis of *in vitro* constructed chimeric thymuses, formed by co-culturing of lymphoid depleted fetal thymus explants with bone marrow cells, or with sorted thymic subpopulations, indicate a bi-lateral interaction of bone marrow cells with the thymic stroma, and feedback effects of mature lymphocytes on cells at earlier steps of development. These observations on the patterns of T cell generation, utilizing the comparison of development of thymocyte progenitors from young and old mice, point at regulatory mechanisms that may be deterministic at the early steps of thymocytopoiesis (A. Globerson, Weizmann Institute, Israel). Feedback effects exerted by mature CD4$^+$ cells on thymocyte development were analyzed in detail using a mathematical model (Ramit Mehr, Los Alamos). It was found that these cells most likely affect thymocyte development through two distinct feedback loops: a negative feedback on total cell population growth, and a positive feedback on cells differentiating to the CD4$^+$ lineage [10]. This analysis utilized a model geared toward analyzing thymocyte differentiation, and the commitment to either the CD8$^+$ or CD4$^+$ T cell lineages. The latter are the primary target of the HIV (AIDS virus), and in this respect the study of their development may shed light on CD4$^+$ cell depletion seen in AIDS patients.

A computer model developed by V. Detours (Los Alamos) for the selection of T cells in the thymus that takes into account the main differentiation stages of thymocytes, as well as the specificity of T cell antigen receptors (TCR), allows researchers to address issues related to the TCR repertoire and the TCR mediated signaling events underlying the selection of thymocytes. Numerical simulations give results in quantitative accordance with the data from normal mice. The model allows a straightforward implementation of transgenic systems in which all T cells express the same receptor, and an assessment of the effect of imperfections in the expression of the transgene (by allowing a few TCRs to be random). Simulations suggest that a large percentage of "leaky" (non-transgenic) TCRs results in subset compositions very similar to that of normal mice, whereas even small "leakiness" results in large temporal fluctuations of the total number of thymocytes. These fluctuations imply that single point measurement on thymuses of transgenic mice may not provide statistically significant results. This model will be utilized to study the mechanisms underlying feedback regulation of T cell development, and CD4/CD8 lineage commitment (Ramit Mehr, Los Alamos).

The thymic medulla has generally been viewed as a quiet compartment, with little proliferation and cell death. Positively selected thymocytes spend an average time of 14 days there before being exported to the periphery, but it is unknown whether these cells leave the medulla on a "first in-first out" basis, or depart randomly during this 14 day period. Using BrdU incorporation, it has been shown that while few medullary single positive (SP) cells are proliferating (2-5% per day), a high proportion (30-35%) of the recent thymic emigrants have taken up BrdU label after only one day of labeling (R. Scollay, Sydney, Australia). The results indicate that many T cells proliferate just prior to emigration from the thymus, and indeed, recently divided cells may be preferentially exported. This suggests that the export of T cells from the thymic medulla may be more complex than previously thought. Given that proliferation of mature SP thymocytes is thought to be TCR dependent, these data raise some important questions. (1) Is the proliferation that precedes thymic emigration antigen driven? Recent data from anti H-Y transgenic mice suggest that it may be so. (2) If so, what is the nature of the antigen (MHC, foreign, thymic or extra-thymic)? (3) If extra-thymic, does this imply that peripheral antigen can recruit antigen specific cells from the thymus on an as needed basis? Computer modeling is presently employed to analyze these thymic processes.

B. Morel (Pittsburgh) reported an on-going interdisciplinary (i.e. in interaction with experimentalists) attempt to model the differentiation of $CD4^+$ T-cells into Th_1 and Th_2 and of their cross-regulation. The Th_1-Th_2 system seems to play a central role in the regulation of the immune system, involving a mix of competition and apparent cooperation. Together they provide a cytokine environment conducive to promote a specific immune response. The immune system can be seen as an adaptive control system, able to address a huge variety of challenges, adjust to their specificity, optimize the response, memorize it and learn from it. This process involves a large number of agents (most of them being effector cells). According to Morel, the Th_1-Th_2 system is part of the "brain" of that system, and he suggests that the apparently irreducible complexity of the immune response could be conceptualized as a form of biological genetic programming, based on a competition between effector functions.

While the immunoglobulin (Ig) structure has been well-characterized by X-ray crystallography, the structure of the T cell antibody receptor (TCR) remains undetermined. Because it is an insoluble membrane-bound protein, the TCR cannot be easily crystallized. However, from models and the recently published β chain structure, it is known that TCR takes on an Ig-like conformation. Computer modeling was used to elucidate the MHC-peptide binding site of the TCR (Connie Lee, Princeton). Using the Shannon information theory, the amino acid diversity at each position in sets of Vα, Vβ and Vκ sequences was analyzed. A canonical repertoire of amino acids, known as framework residues, have thus been found to be crucial for the conserved structure of Ig-variable domains. Within that framework, a striking homology exists between TCR and Ig sequences. This allows the use of the coordinates of the antibody light chain as a template for the TCR. In spite of the similarities, the results show that the TCR framework is markedly more diverse than that of the Ig, suggesting that "framework" residues may be part of the combining site. Hence the TCR may have a larger combining site than antibodies.

The size and composition of the T cell repertoire has also been studied by mathematical modeling (Rob de Boer, Utrecht, the Netherlands; and A. Perelson, Los Alamos). In these models, relevant for adults, it is assumed that the T cell population is maintained by proliferation rather than by thymic generation. However, because T cell stimulation involves interaction with antigen presenting cells (APCs), there are two levels of competition. The first is the presentation of peptides by a limited number of available "sites" on APCs. This form of competition is amongst the peptides and allows for the regulation of total T cell numbers. The second is the competition amongst T cells for access to the APCs. This allows for regulation of the individual T cell clone sizes, and for affinity selection by competitive exclusion. Similar models have been developed by M. Fishman and A. Perelson [15].

Insights from considerations of immune responses

Models of the immune system often need to establish a representation of antigen, antibody, and TCR shapes. To this end, bitstring representations have been used in the literature (e.g., Farmer et. al., 1986; De Boer and Perelson, 1991; Celada and Seiden, 1992). These representations are abstract and it is hard to tell how accurately they reflect the behavior of proteins. To address this question, B. Sulzer (Los Alamos) introduced a simple model of protein folding and binding, called a pseudo-protein model. The pseudo-protein model places a chain of residues in their minimum-energy conformation on a two-dimensional square lattice and evaluates the affinity between two chains as the maximum energy gained, when placing the folded chains next to each other. Each residue carries a charge-like and a hydrophobicity-like attribute which are utilized to determine the energy of conformations. To evaluate the representations, the affinity distribution and the change of affinity upon point mutation were determined. The change of affinity upon point mutation reveals an important qualitative difference between two kinds of bitstring representation: If the affinity between two strings is defined as the total number of complementary bits (global match rule; e.g., Celada and Seiden, 1992), then the affinity changes always by a small amount; if the affinity between two strings is defined as the length of the longest stretch of consecutive complementary bits (local match rule; e.g., De Boer and Perelson, 1991), then the affinity stays the same most of the time, changes a little sometimes and a lot rarely. The behavior of bitstring representations with the local match rule mimics that of the pseudo-protein model. Furthermore, it agrees with the general pattern observed when proteins undergo point mutations. In conclusion, models for processes where point mutations are important (e.g., affinity maturation) are probably more accurate, when they employ local match rules instead of global match rules in conjunction with a bitstring representation.

L. Segel (Weizmann Institute, Israel) discussed the following questions. How might the immune system be set up in order that it performs with decent efficiency the task of ridding the body of pathogens? More precisely, what are possible ways that there could be feedbacks that shift the system into modes of action (e.g. isotypes, specificities) that are more successful in carrying out the above task? One consequence of viewing the immune system as an efficient device for destroying pathogens is that more emphasis should be paid to allergy which is a disease, not because the immune system is misdirected against self, but because it responds inappropriately to nonself. Recent modeling by Segel and colleagues attempts to understand the complexity of immune cell function (each cell type, antibody or cytokine is involved in more than one function, and each function is performed by more than one entity) from principles of effective immune response. The work so far suggests that both humoral and cellular responses could include positive feedbacks based directly on success in destroying pathogens; and that spatial dispersion may be important in success-directed feedback processes.

A model presented by M. Cohn (Salk Institute, California) regards the humoral immune system as constructed of iterated modules of function. These modules are equivalent in their function and are referred to as Protectons [12, 13, 14]. Protecton theory has been implemented in a computer simulation of a primordial synthetic immune system (SIS), designed to be as universal as possible so that the consequences of various theories could be evaluated on the same platform, and based on cellular automata (R. Langman, Salk Institute, California). The behavior of cells in the automaton is defined by entries in a transition table that the user must construct. A modest 30-50 table entries is sufficient to allow the simulation of a recognizable immune system, complete with a somatically selected self-nonself discrimination and a rational means of determining the class of any response as either cell-mediated or humoral. As demonstrated by these simulations, protecton theory ties together all of the single issue models that have been proposed in order to successfully secrete antibody in response to a pathogenic load.

Summary

The approach of mathematical modeling and simulation is a rapidly developing and powerful tool in modern immunology, as demonstrated by several recent workshops[2]. It is being used for a variety of topics where complexity does not enable direct experimental approaches. Modeling approaches vary from exploratory "thought experiments" to tight dialogues with experimental work, often suggesting new directions for experimental studies.

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