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The Multi-scale Immune Response to Pathogens: *M. tuberculosis* as an Example

Denise Kirschner[1]

Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109-0620 kirschne@umich.edu

Summary. The immune response occurs over multiple temporal and spatial scales. Events at the genetic level can influence events at the cellular level and finally manifest at the population scale. Through the example of the human pathogen *Mycobacterim tuberculosis* we explore immune response events over multiple scales and how bridging these scales may ultimately lead to the greatest picture of how this complex system works.

13.1 Introduction

When a pathogen invades a host, the host mounts a response that occurs at several levels of biological organization including genetic, molecular, cellular, tissue and system level. A number of host cells are called into action including antigen presenting cells (APCs) and T cells. At the body's peripheral sites, populations of resident APCs are maintained consisting primarily of macrophage and dendritic cells (DCs). These cells are among the first to encounter pathogens that breach host barriers. Foremost among their responsibilities is the presentation of peptide antigens from pathogens that are taken up at the site of infection in the form of peptide-MHC (pMHC) complexes on their cell surface. Some APCs, namely DCs, migrate to the nearest lymph node (LN) where they activate naïve T cells. Other APCs, namely macrophages, remain at the site of the infection and respond to an influx of activated $CD4^+$ T cells by increasing their presentation and microbicidal activity.

While most of these events occur at the cellular level, they are embedded in the context of multiple biological levels. The initial APC-T cell interaction occurs mainly in the specialized structured environment of the LN. The lymphatic system serves as a conduit for immune cells between tissues, LNs and organs. While the blood supplies

immune cells to the LNs, the lymphatics drain the tissues, acting as the key source of antigens and DCs in most infections. Hence, both tissue- and system-level events play a role in response efficacy. At the same time, APCs may vary in their ability to perform antigen presentation due to events occurring at the molecular and genetic levels. The APC-T cell interaction depends on stable expression of pMHC complexes on the APC surface that in turn depends on pMHC binding affinity. A high degree of variability exists in the peptide-binding region of MHC throughout the human population, resulting in considerable APC heterogeneity, both within a single individual and between individuals. Antigen presentation therefore lies at the crux of the immune response, between the larger scales (tissue- and system-level) that determine its context and the small scales (genetic- and molecular-levels) that determine its constituents. In fact, susceptibility and resistance to some diseases have been linked directly to the basic genetic components underlying antigen presentation.

Certainly there has been a wealth of basic science performed at the molecular and cellular levels attempting to elucidate immunity. However, given its complexity, the multi-scale system is presently impossible to study in an experimental setting. Thus, mathematical and computational models bridging the multiple scales that encompass the immune response are necessary to help uncover mechanisms underlying the dynamics of this complex system.

Mathematical models of the host-pathogen interaction have mainly been restricted to the study of host-viral interactions. Relatively few models have explored bacterial-host interactions [Freter *et al.* 1983, Kirschner & Blaser 1995, Asachenkov 1994, Gordon & Riley 1992, Lipsitch & Levin 1997]. Regardless, most have focused on the single-scale of cellular-level dynamics.

We have made attempts to explore the complex system of immunity by studying the immune response to a specific pathogen. We have studied the interaction of the immune system with the intracellular pathogen *Mycobacterium tuberculosis* at a number of biological and spatial scales. Here we highlight both the biology we are addressing and the mathematical approach taken as a means for beginning to understand the integrated, multi-scale complex system know as the immune response.

13.2 *M. tuberculosis*

Tuberculosis (TB) has been a leading cause of death in the world for centuries. Today it remains the number one cause of death by infectious disease world wide - 2 million deaths per year. TB is not only one of our oldest microbial enemies, but it remains one of the most formidable: An estimated one third of the world population has latent TB—2 billion people. Thus, there is a great need to elucidate the mechanisms of TB disease progression. There are 2 major infection outcomes for TB–latency and active disease; the ability to clear TB has not been demonstrated, although only a subset (~30%) become initially infected upon exposure [Styblo *et al.* 1969] suggesting some (perhaps most) are able to clear upon initial infection.

Reactivation can occur in latent infection, although we do not discuss this here for brevity (see [Singer & Kirschner 2004] for more information). Key issues are to understand immune mechanisms involved in controlling infection leading to latency. To this end, elaborating the primary immune response against the causative agent, *M. tuberculosis* (Mtb), is essential to understanding the functional immune response that leads to latency.

Primary infection usually develops in the alveoli of the lung after inhaling droplets containing Mtb. The bacteria are then ingested by resident alveolar macrophages and begin to multiply [Canetti 1955]. These macrophages are poor at destroying their occupants in part because Mtb can prevent phagosome-lysosome fusion in resting macrophages [Myrvik *et al.* 1984, McDonough *et al.* 1993]. Infected macrophages may burst due to the large number of multiplying bacteria within. Infected dendritic cells or macrophages circulate out through the lymphatic ducts to the draining lymph nodes where the specific immune response is initiated. Here, CD4+ T cells are stimulated to become effector cells, most likely of the Th1 type. These and other effector cells such as CD8+ T cells and monocytes must then be recruited and migrate to the site of infection, interact with cells at the site, where they participate in the formation and function of a unique immunological structure known as a granuloma.

Granuloma formation is dependent on a number of factors, including chemokines, cytokines, cell adhesion molecules and immune effector cells. There exists a large body of literature regarding these individual elements in the immune response in TB; however, little is known about the interaction among these elements that leads to granuloma formation and function. Characterization of the immunologic factors operating during granuloma formation is likely to shed light on our understanding of host defense and pathogenetic mechanisms involved in TB. This is a daunting task as infection with Mtb triggers production of a complex set of immunologic factors, including potent pro- and anti-inflammatory cytokines and chemokines that are capable of interacting with and cross-regulating one another. These analyses are further complicated by the fact that many of the participating members of the tuberculosis immune network possess pleiotropic and often opposing functions. Mathematical models provide a framework for integration of large amounts of data into a complex system that can then be analyzed, and thus is currently the only integrative approach for studying complex biological systems.

13.2.1 Immune cells participating in the immune response to *M. tuberculosis*

Macrophages are the preferred host cell for mycobacteria. These phagocytic cells take up *M. tuberculosis* and are unable to clear it as they normal do most other bacteria. However, if the macrophage receives appropriate cellular and cytokine signals (such as IFN-γ) within an efficient amount of time, then these macrophages can become activated and clear their intracellular load [Nathan *et al.* 1983, Flesch & Kaufmann 1990]. Otherwise, macrophages become chronically infected and will not only

never be able to clear their intracellular bacteria [Armstrong & Hart 1971, Sturgill-Koszycki *et al.* 1994], but will eventually burst due to increasing bacterial numbers or be killed by cytotoxic T cells [Lewinsohn *et al.* 1998, Tan *et al.* 1997].

It is well established that cell-mediated immunity is essential for controlling initial as well as latent Mtb infection both in humans and murine models. CD4+ and CD8+ T cells are believed to be important in this response [Chan & Kaufmann 1995]. Support for the importance of CD4+ T cells comes from the extreme susceptibility of HIV+ subjects to acute and reactive TB. Mice deficient in CD4+ T cells succumb to fatal TB [Leveton *et al.* 1989, Muller *et al.* 1987, Tascon *et al.* 1998, Caruso *et al.* 1999]. CD4+ T cells produce cytokines, such as IFN-γ, and thus activate macrophages to eliminate intracellular Mtb [Caruso *et al.* 1999, Silver *et al.* 1998]. This is partially mediated, in mice and possibly in humans, by the production of reactive nitrogen intermediates, such as nitric oxide, produced by inducible nitric oxide synthase (NOS2) within macrophages [Chan 1993]. Mice deficient in CD8+ T cells are more susceptible to Mtb than are wild type mice [Flynn *et al.* 1992]. CD8+ T cells in the lungs of infected mice can produce cytokines and act as cytotoxic T cells (CTL) for infected macrophages [Dolin *et al.* 1994, Serbina & Flynn 1999, Serbina *et al.* 2000]. Mtb-specific human CD8+ T cells from tuberculosis patients have recently been reported (reviewed in [Flynn & Ernst 2000].

13.2.2 Cytokines Involved in the Response to *M. tuberculosis*

An essential cytokine in control of infection is IFN-γ; mice deficient in this gene are extremely susceptible to acute TB [Flynn *et al.* 1993, Cooper *et al.* 1993]. A consequence of the absence of IFN-γ is the lack of macrophage activation, including NOS2 production [Flynn *et al.* 1993, Cooper *et al.* 1993, Dalton *et al.* 1993]. IL-12 is also required for control of acute TB [Cooper *et al.* 1997b, Cooper *et al.* 1997a]. Human studies have demonstrated that mutations in genes for IFN-γ and IL-12 receptors increase susceptibility to mycobacterial infections [Ottenhoff *et al.* 1998]. TNF is also essential to control of both acute and chronic Mtb infection [Flynn 1995, Adams *et al.* 1995, Mohan *et al.* 2001, Bean *et al.* 1999]. This cytokine has effects on chemokine and adhesion molecule expression and therefore is an apparent key player in granuloma formation [Flynn 1995, Bean *et al.* 1999, Kindler 1989, Mohan *et al.* 2001]. Recently, TNF has shown to be an important cytokine in human studies (with anti-TNF treatment for arthritis), which have induced reactivation of TB [Ehlers 2003, van Deventer 2001, van Deventer 2002], as well as in mouse systems were TNF knock-out mice were highly susceptible to active TB [Mohan *et al.* 2001, Botha & Ryffel 2003].

13.2.3 Chemokines Involved in the Response to *M. tuberculosis*

A successful host inflammatory response to invading microbes requires precise co-ordination of myriad immunologic elements. An important first step is to recruit

intravascular immune cells to the proximity of the extravascular location of infection, preparing them for the process of extravasation. This is controlled by adhesion molecules and chemokines. The field of chemokine research is expanding at a rapid rate. These molecules induce migration of various cells, including monocytes/macrophages, dendritic cells, neutrophils and leukocytes [Baggiolini 1998]. The migration of cells occurs as a result of the integration of various chemokine signals and their receptors [Foxman *et al.* 1997]. There is evidence that cytokines play both direct and indirect roles in modulating this process [Lane *et al.* 1999, Czermak *et al.* 1999, Crippen *et al.* 1998, Koyama *et al.* 1999]. Chemokines in Mtb infection have been investigated to a limited extent [Orme 1999a, Orme & Cooper 1999, Orme 1999b, Orme 1999c]. We begin to elucidate the role of chemokines in our models of the immune response to Mtb.

13.3 *In Silico* Models at Different Biological Scales

Our goal is to illustrate the application of mathematical modeling at different biological scales towards better understanding the immune response to Mtb. To this end, we present 4 distinct models. First, we study the role of antigen presentation at the intracellular level exploring processing and genetic events that are interfered with by *M. tuberculosis* to its favor. Second, we bridge two distinct biological scales: genetic level, immune system events that impact the epidemiology of TB. Next, we explore the immune response to *M. tuberculosis* using a two-pronged approach. We developed a temporal model tracking a spatially homogenous population of cells and cytokines in the lungs. This model was designed with ordinary differential equations. And lastly, we then narrowed the spatial scale to a single granuloma forming and accounted for the heterogeneous spatialization and behavior of cells on an individual level using an agent-based model.

13.3.1 Antigen Presentation and its role in *M. tuberculosis* Infection

Antigen presentation is critical to triggering an appropriate immune response. It is the process whereby peptide fragments of proteins derived from pathogens are presented on an immune cell surface signaling the presence of infection. This process occurs via two pathways. All cells of the body (except red blood cells) have the ability to process and present antigens that are derived from the cytosol. This allows for cells to signal they are infected to the immune response for clearance. This process occurs via the MHC class I presentation pathway. Other cells, termed professional antigen presenting cells, or APCs, present antigen to immune cells for activation via the MHC class II pathway. It is this route of presentation that we focus on here.

Briefly, specialized APCs, dendritic cells and macrophages, take up pathogens or other factors produced by pathogens at the site of infection. Once taken up,

pathogens are sequestered into vacuoles and their proteins are processed into peptides. These peptides are bound by MHC class II (MHC II) molecules, named for the region of the genome in which they are encoded, the major histocompatibility complex. Within this region lie the most polymorphic genes in the human genome, giving rise to MHC molecules with different peptide-binding specificities. Peptide-MHC complexes (pMHC) are displayed on the surface of the APC and are recognized by the T cell receptor on T helper cells that become activated and proliferate in response For a complete treatment of T cell receptors, see Chapter 4 by Lee and Perelson in this book.

13.3.2 A Model for MHC class II Antigen Presentation During Infection with *M. tuberculosis*

While MHC II polymorphism may be the strongest genetic determinant of an antigen presentation outcome due to its effect on pMHC binding, this is by no means the only regulated step. Several critical cellular processes contribute to successful antigen presentation by APCs. These processes occur in the time frame of minutes to hours and can be stated briefly as: (1) uptake of antigen from the extracellular environment and degradation of antigen within endosomal compartments into peptides, (2) synthesis of MHC II molecules, (3) peptide-MHC II binding to form pMHC complexes, and (4) display of pMHC complexes on the APC surface. We review these briefly below, but for a full treatment we refer the reader to a recent review [Bryant & Ploegh 2004].

Exogenous antigens, constituting the bulk source of peptides for MHC II-mediated antigen presentation, generally have three routes of entry to the APC: fluid-phase pinocytosis, receptor-mediated endocytosis, and phagocytosis [Lanzavecchia 1996]. Pinocytosis is a common mode of entry and is our focus. Once taken up, antigens move through a series of increasingly acidified endosomal compartments and are either processed into peptides capable of binding MHC II molecules or degraded. Low pH-activated proteases degrade antigen as it traffics through the endocytic pathway, yielding peptides suitable for binding MHC II [Honey & Rudensky 2003].

MHC II expression is normally low in resident populations of APC that have not been exposed to antigen. However, a number of environmental cues can alter MHC II expression including chemical signals (cytokines) secreted by neighboring cells and direct contact with certain molecules native to pathogens. Such signals trigger a signal transduction cascade in the APC resulting in the up-regulation (or, in a few cases, down-regulation) of MHC II expression. For example, macrophages are often incubated with IFN-γ for *in vitro* studies; in the *in vivo* situation, this would come from T cells or natural killer cells. IFN-γ binds to receptors on the macrophage surface, increasing the expression of class II transactivator (CIITA), a master regulator of MHC II transcription, over a period of hours, leading after a time delay to increased MHC II expression and presumably increased ability to present antigen. Describing the effects of IFN-γ requires consideration of the degradation of IFN-γ in solution and the uptake of IFN-γ by macrophages [Celada & Schreiber

1997]. Shortly after appearing in the endoplasmic reticulum, a nascent MHC II molecule is coupled to invariant chain (Ii) which possesses a cytosolic domain capable of directing the molecule to the endosomal pathway and an extracytosolic domain capable of binding and protecting the MHC II binding groove.

The MHC II molecule arrives in the endosomal pathway with its binding groove still loaded with a remnant of Ii, the class II invariant chain-derived peptide (CLIP). Removal of CLIP occurs in an endosomal compartment, the MIIC, that also contains antigenic peptides and is catalyzed by the MHC-related enzyme HLA-DM [Denzin & Cresswell 195]. Self peptides derived from the body's own proteins are also present within the MIIC and compete with antigenic peptides for binding to MHC II [Adorini *et al.* 1988]. Indeed, in the absence of exogenous antigen self peptides may bind 80% or more of the available MHC II molecules [Chicz *et al.* 1993]. Once a pMHC complex is formed, whether it involves antigenic or self peptide, it is transported to the cell surface where it can be recognized by $CD4^+$ T cells for a period of time until it is either degraded or internalized. These processes appear largely unaffected by IFN-γ in contrast to MHC II expression [Boehm *et al.* 1997].

DCs and macrophages represent two types of so-called professional APCs, i.e. APC that express not only MHC II molecules but also co-stimulatory and adhesion molecules necessary to engage T cells.While DCs take up antigen at the site of infection and migrate to LNs to present antigen, macrophages primarily perform their function as APC at the infection site [Reinhardt *et al.* 2001] Thus, in examining the lung in *M. tuberculosis* infection, we focus our attention on the macrophage.

13.3.3 Many Pathogens Regularly Interfere with the Antigen Presentation Process.

Not surprisingly, since pathogens meet APCs continually as a first line of defense, many have evolved ways in which to inhibit antigen presentation, including both viral and bacterial pathogens. Cytomegalovirus is a viral pathogen that has been shown to inhibit antigen presentation, interrupting the MHC II expression pathway [Miller *et al.* 1998]. An example of one such bacterial pathogen is *M. tuberculosis.* Upon entering the lungs, *M. tuberculosis* is taken up by resident macrophages or DCs, adapts to the intraphagosomal environment, and survives or slowly replicates [Fenton 1998]. To evade immune surveillance, *M. tuberculosis* is known to inhibit antigen presentation via both class I and class II pathways in chronically infected macrophages [Grotzke & Lewinsohn 2005, Brookes 2003, Chang *et al.* 2005]. The mechanisms by which *M. tuberculosis* achieves inhibition of presentation via the class II pathway have not been completely elucidated, though several hypotheses have been proposed [Moreno *et al.* 1998, Hmama *et al.* 1998, Noss *et al.* 2000]. Without a detailed model of the molecular and cellular events of antigen presentation, it is difficult to assess the impact of various mechanisms of inhibition on the display of antigen and ultimately on the immune response. Early models by Linderman *et al* presented a first look at the dynamics of antigen presentation at the cellular level and demonstrated that the rates of endocytosis could be related to the display of antigen

[Singer & Linderman 1990, Singer & Linderman 1991, Petrovsky & Brusic 2004]. However, these models did not account for the more recently understood dynamics of antigen presentation and the role of IFN-γ in increasing MHC II expression. We developed a next-generation model of the molecular and cellular events required for display of antigen on the surface of the APC and describe how it might be used to elucidate the mechanisms pathogens use to interfere with the process [Chang *et al.* 2005]. We use the number of pMHC complexes on the APC surface with respect to time as our output variable and our measure of antigen presentation unless otherwise stated.

Our model uses ordinary differential equations (ODEs) to describe the time-dependent processes essential to antigen processing and presentation [Chang *et al.* 2005]. A previous model of the class I presentation pathway applied a method known as nueral networks [Petrovsky & Brusic 2004]. As detailed earlier, these processes include uptake of protein antigen from the extracellular environment, degradation of antigen within endosomal compartments into peptides, synthesis of MHC II molecules, peptide-MHC II binding to form pMHC complexes, and display of pMHC complexes on the APC surface. ODEs are well suited for modeling dynamical systems when species are well mixed and present in numbers large enough that they can be considered continuous. Both of these conditions are met in the case of MHC II-mediated antigen presentation by macrophages. We represent MHC II molecules using six variables to distinguish between intracellular and surface localizations as well as free, self peptide-bound, and exogenous peptide-bound forms. The portions of our model dealing with exogenous antigen and MHC class II peptide loading will be similar to the simpler model developed by [Singer & Linderman 1990].

Key assumptions made in our model development included the following: (1) Both antigen uptake and processing can be represented as single-step reactions. (2) Events leading up to MHC II expression require long periods of time relative to other events, e.g. peptide-MHC binding, and therefore should be included in our model. Long-lived intermediates of these events, mainly mRNA and protein species, will be represented explicitly, while shorter-lived intermediates such as second messengers will not. (3) Events bridging the appearance of MHC II molecules in the ER and removal of CLIP occur constitutively and therefore can be represented as one event. (4) All forms of MHC class II molecules are capable of being transported to and from the plasma membrane, including peptide-free ("empty") MHC II [Germain & Hendrix 1991, Santambrogio *et al.* 1999]. (5) The reaction scheme MHC + peptide $\Leftrightarrow$ pMHC is sufficiently accurate on the timescales of the experimental conditions we wish to simulate to allow us to forego more complicated models of this process (e.g. in [Beeson & McConnell 1995]). Indeed, our calculations with peptides for which we have pMHC association and dissociation rate constants indicate that we can assume equilibrium binding in the endosome in the presence of the enzyme HLA-DM. (6) Different self peptides bind to MHC II molecules with similar kinetics, despite being derived from various endogenous proteins, and can be represented as a single population. These self peptides will be available for MHC II binding or will be transported to lysosomes and degraded.

Parameters for the model were estimated from published experimental data; many parameters are similar to earlier models [Singer & Linderman 1990, Singer & Linderman 1991, Agrawal & Linderman 1996]. The model was validated under a number of control scenarios. For example, macrophage CIITA, MHC II mRNA, and MHC II protein levels have been reported at various time points by [Pai 2002] and [Cullell-Young *et al.* 2001]; these data were used to verify the MHC expression portions of our model. Other simulations were compared to time courses of antigen presentation in the presence and absence of IFN-γ from the data of [Delvig *et al.* 2002]. In each case we matched both qualitative and quantitatively to the known experimental data (see [Chang *et al.* 2005] for full details of the negative and positive control simulations).

Simulations were run using several ODE solvers to ensure consistency, including the NDSolve feature of Mathematica v4.2 (Wolfram Research, Inc.) and our own solver coded in C and run on Sun UNIX machines. We also performed a detailed sensitivity analysis integrated into the numerical solver.

Using the model described above, we simulated several time courses of antigen presentation. As net pMHC binding affinity was increased in the model (base +/- 25% is shown), the average number of pMHC complexes appearing on the surface over the first six hours of antigen exposure also increased (Figure 13.1). Depending on other conditions in the model, such as extracellular antigen level and level of MHC II expression, this number sometimes dipped below a threshold required to elicit T cell responses, approximately 200 pMHC complexes [Kimachi *et al.* 1997]. These results suggest that some variants of MHC II may hinder the development of adaptive immunity, and that binding affinity is a key parameter a successful immune response.

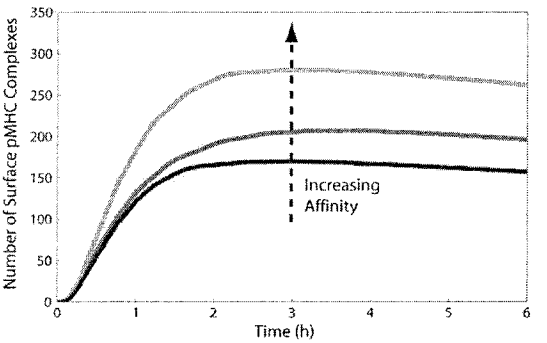


Fig. 13.1. Simulated time courses of surface pMHC levels following exposure to antigen as net pMHC affinity is increased.

13.3.4 *M. tuberculosis* Inhibits Antigen Presentation at Multiple Times using Multiple Mechanisms

Inhibiting antigen presentation at some level is a strategy that many pathogens need to employ to evade immune killing. Because the many processes that constitute antigen presentation are complex and difficult to study individually, many mechanisms have been proposed to explain how pathogens may interrupt one or more of these processes. That *M. tuberculosis* inhibits antigen presentation in macrophages is now well established. Multiple studies have provided a number of hypotheses regarding the mechanism used by *M. tuberculosis* to inhibit antigen presentation, reviewed in [Harding *et al.* 2003], including (H_1) inhibition of antigen processing [Hmama *et al.* 1998, Singer & Linderman 1990] (H_2) of MHC II protein maturation (including delivery of MHC II proteins to the MIIC and Ii processing), (H_3) of MHC II peptide loading [Hmama *et al.* 1998] or (H_4) of transcription of MHC II genes [Noss *et al.* 2000]. Our model addresses why multiple mechanisms have been observed, whether previous experimental protocols favored the detection of some mechanisms over others, and whether alternative mechanisms may exist.

We included into our model of antigen presentation those processes hypothesized to be inhibited by *M. tuberculosis*: antigen processing, MHC class II maturation, MHC class II peptide-loading, and MHC class II transcription. Parameter values were estimated from the literature, mostly *in vitro* studies on mouse cells, and major features of the output, typically surface peptide-MHC levels, were compared to other experimental data. We then used the model to simulate experimental protocols from studies proposing hypotheses and found that some were biased to detecting mechanisms targeting MHC class II expression (Figure 13.2). We also found that mechanisms differed by the timescales on which they were effective (either less than or greater than 10 hours) and therefore might be used in combination by *M. tuberculosis* to ensure continuous inhibition of antigen presentation. Finally, by analyzing the sensitivity of the model to variations in parameter values, we also identified other intracellular processes that may significantly affect antigen presentation (such as self-peptide synthesis) and be targeted by *M. tuberculosis* or other pathogens as a result.

13.4 Genetic Epidemiology of TB- a further look at the impact of antigen presentation in a broader context

One important application of a mathematical modeling approach can be to bridge gaps between biological scales of interest. Clearly, what manifests at the epidemiological level is a result of events that occur at many host-level scales. To illustrate one approach, we explore a link between effects occurring at the level of antigen presentation to effects manifesting at the population level during tuberculosis epidemics.

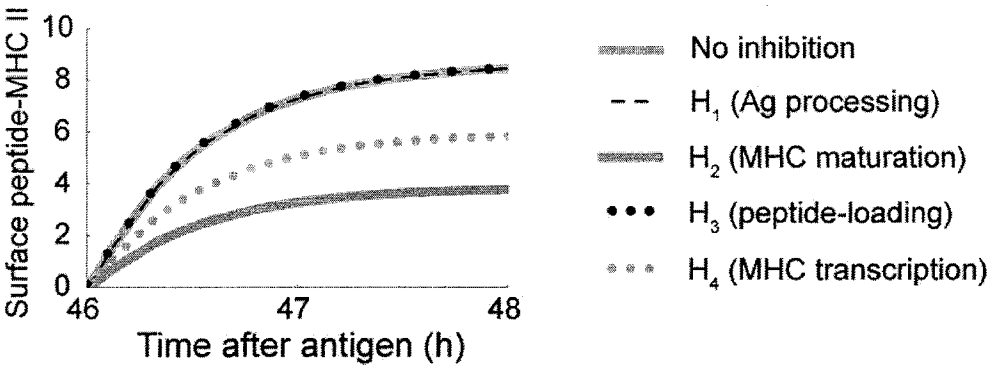


Fig. 13.2. Simulation of one experimental protocol showing that detection of MHC class II expression-targeting mechanisms is favored

Several studies have found that genetic factors influence susceptibility and resistance to *M. tuberculosis* infection [Kramnik *et al.* 2000, Bothamley *et al.* 1993, Goldfeld *et al.* 1998, Selvaraj *et al.* 1998, Bellamy & Hill 1998, Bellamy *et al.* 1998, Wilkinson *et al.* 1999, Hill 1998]. These studies employ a variety of methods including large-scale association-based population case/control studies of candidate genes, family-based linkage analysis, investigation of rare individuals with exceptional mycobacteria susceptibility, and comparison with murine models of disease. Such studies enable identification of particular host genes that influence susceptibility to TB disease.

The major components of susceptibility and resistance to TB appear to be linked directly to the immune response, and in particular to MHC class II molecules. Human MHC molecules are termed human leukocyte antigen (HLA) molecules (but the terms tend to be used interchangeably). Increased susceptibility and resistance to more than 500 diseases has been shown to be associated with various HLA antigens, alleles, or haplotypes (sets of genes that are typically inherited as a unit) [Zachary *et al.* 1996]. In some diseases, HLA expression may influence the balance and strength of the immune response [Pile 1999]. The level and type of immune response to a particular pathogen may vary among populations that have different distributions of HLA molecules.

Many HLA genotypes are implicated in susceptibility to *M. tuberculosis* infection [Bothamley *et al.* 1993, Goldfeld *et al.* 1998, Selvaraj *et al.* 1998, Meyer *et al.* 1998]. Variable binding of mycobacterial antigens to the various HLA molecules may affect the intensity of the adaptive immune response and thus influence susceptibility to TB [Lim 2000, Vordermeier 1995]. Expression of HLA-DR2 is strongly and consistently linked to pulmonary TB and the severe multibacillary form of TB in India [Selvaraj *et al.* 1998, Singh *et al.* 1983, Bothamley *et al.* 1989, Brahmajothi 1991, Rajalingam *et al.* 1996]. HLA-DR2 correlates with increased levels of serum antibody levels [Bothamley *et al.* 1993, Bellamy & Hill 1998, Bothamley *et al.* 1989], indicating an elevated humoral immune response, associated with active disease. The presence of the HLA-DR2 allele may induce tolerance to *M. tuberculosis*, leading to uncontrolled growth of the bacilli [Rajalingam *et al.* 1996]. In addition, HLA-DR2 correlates with

decreased production of key proteins that play crucial roles in granuloma formation and subsequent containment of bacteria [Tracey 1997, Flynn & Chan 2001a, Flynn & Chan 2001b].

13.5 Modeling Epidemic TB

Our goal was to develop a mathematical model of epidemic TB that allowed us to investigate different demographic populations with inherent susceptibility to infection by *M. tuberculosis*. To illustrate our approach, we highlight results related to India where the frequency of the HLA-DR2 allelle is high and prevalence and incidence levels of TB are significantly higher as compared with the rest of the world. We were motivated by previous work from our group which presented a first model of HIV infection within a genetically heterogeneous population, [Sullivan *et al.* 2001].

We have developed a model of epidemic TB using a modified Susceptible-Infected-Removed (SIR) model with mutually-exclusive groups of individuals who are uninfected, latently infected (those infected with *M. tuberculosis* but not infectious), or actively infected with *M. tuberculosis* (those infected AND infectious) [Murphy *et al.* 2002, Murphy *et al.* 2003]. As our goal was to study the effects of a genetically susceptible subpopulation on the dynamics of epidemic TB at the population level, we further subdivide each of these three groups to include individuals carrying a susceptibility allele for MHC II (DR2 in this case), resulting in the six mutually-exclusive populations. Due to extensive diversity in the HLA genetic system, we examine disease relationships based upon the presence of susceptibility with no distinction between homozygotes and heterozygotes. For full details of the model equations and assumptions, [Murphy *et al.* 2002, Murphy *et al.* 2003].

13.5.1 How to include the effects of genetic susceptibility

Two things are important to consider regarding including effects of a susceptibility gene into this model. First, we divided individuals entering uninfected classes into a cohort that was neutral with respect to effects of a gene and a cohort that was susceptible because of the gene. To allow for births into the population, we defined a parameter that represents the fraction of the general population exhibiting a susceptible phenotype. If we consider a specific genotype underlying this phenotype, then this value must be derived from the allelic frequency according to dominance patterns for that allele. In the model implementation, we considered this value to be constant. This could certainly be extended to include a time varying allelic frequency, as we did in [Sullivan *et al.* 2001], to examine selection processes.

Second, based on the observed significant correlations of HLA-DR2 with active TB, we proposed three possible ways that the HLA-DR2 susceptibility allele may affect the susceptible cohort:

1. HLA-DR2+ individuals have an increased probability of direct progression to active TB upon initial infection
2. HLA-DR2+ individuals exhibit an increased reactivation rate from latent to active TB
3. HLA-DR2+ individuals are more likely to transmit and/or receive *M. tuberculosis*.

To account for these potential processes within the modeling framework, we introduced a parameter to describe the possible influence(s) of genetic susceptibility from our 3 hypotheses on baseline (i.e. genetically neutral) parameters. We do not predict specific values for this parameter as none have been identified; rather we use this parameter to indicate where we included influences from hypotheses of genetic susceptibility and studied a wide range of effects. To observe the effects of this variation, we predict 95% confidence intervals on our output measures (prevalence and incidence) based on large variations in this parameter.

Parameter values and initial conditions reflect demographics of India (derived from the WHO and other data [World Health Organization 2001], as this is the population with the highest frequency of the HLA-DR2 allele. For this simple model we also assumed no treatment or therapy, as may be the case for many of the developing countries with the highest burden of TB. Worldwide, the average (baseline) prevalence of TB is approximately 33%, and the average incidence is 135/100K/yr [Bleed *et al.* 2001, Chakraborty 1993]. Figure 13.2 (dashed curves) shows baseline simulations (worldwide) prevalence and incidence simulations together with a 95% confidence interval on the mean derived from an uncertainty and sensitivity analysis, see [Murphy *et al.* 2002, Murphy *et al.* 2003] for all details).

Our goal was to determine what effects to the epidemiological system would likely have to occur to bring prevalence and incidence in line with the significantly higher level known to exist in India (where prevalence of TB is almost 50% and incidence is between 200-400/100K/yr) [World Health Organization 2001]. The model predicted that the scenario when HLA-DR2 affected all 3 hypotheses (listed above) simultaneously yields results most closely in line with current outcomes for India (Figure 13.3, solid curves). The combined effects yield increased values for incidence and prevalence closer to levels that are observed in India where HLA-DR2 is most prevalent. While the combined effects are more representative of current TB burden, they may be too high in some cases. One explanation is that the presence of known resistance alleles may balance these effects.

While the role of genetic susceptibility is not well defined, it is clearly important to understanding the dynamics of infectious diseases. This is a first attempt to show how effects occurring at the immune system scale can impact dynamics in a significant way at the population scale. Further detailed studies along these lines can likely lead to suggested strategies for intervention and control.

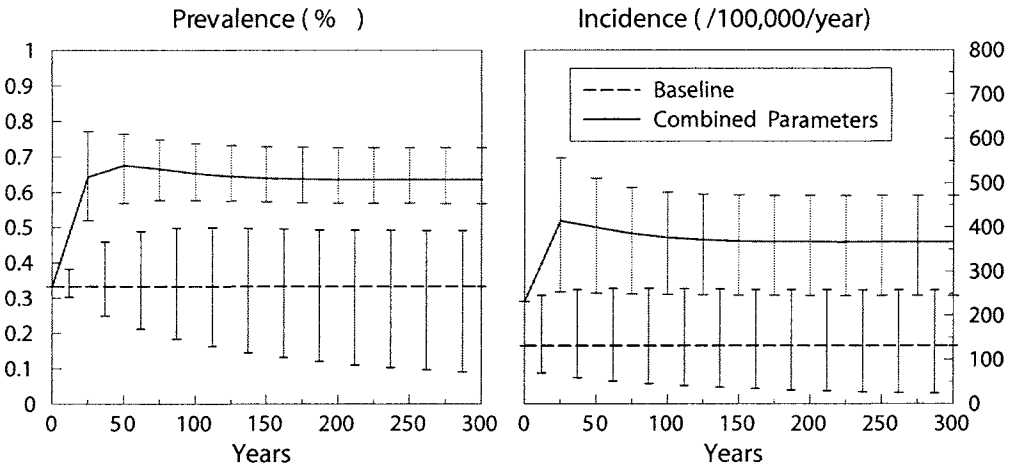


Fig. 13.3. Shown are simulations of the epidemic model for susceptibility to TB over a 300 year period. Panel A indicates the Prevalence, and Panel B shows incidence cases per 100,000/year. The horizontal dashed curves indicate the worldwide (baseline) prevalence and incidence levels with 95% confidence intervals, while the horizontal solid curves indicate the simulated outcomes when all 3 hypotheses are altered indicating the effects of the susceptibility allele (also shown with 95% confidence intervals for variations of parameter values)

13.6 A Temporal Model Tracking the Immune Response to *M. tuberculosis* in the Lung

When a $CD4^+$ T cell encounters an APC, and its T cell receptor (TCR) recognizes the specific pMHC being displayed on the surface of the APC, a series of events follows leading to T cell activation. This interaction between cells bridges to the next biological scale – that of cellular level events. As a first attempt to understand the cellular immune response to infection with *M. tuberculosis*, we have developed a temporal model that qualitatively and quantitatively characterizes the cellular and cytokine control network operational during TB infection in the whole lung [Wigginton & Kirschner 2001]. Using this model we made a first attempt at identifying key regulatory elements in the host response.

This first model was developed to capture infection with *M. tuberculosis* at the site of infection in the lung. Our 'reference space' is the entire lung tissue; however since no data are available in humans, we consider that the simulations take place in bronchoalveolar lavage (BAL) fluid, and we measure all cells and cytokines in units per ml of BAL, as data is available in humans and non-human primates.

While it is likely that the quantitative response differs between the airspace and the interstitium, we relied on the acceptance of BAL as a qualitative predictor of lung environment [Ainslie *et al.* 1992, Moodley *et al.* 2000].

We developed a mathematical system based on the interactions of a number of key cells and cytokines known to be important in TB infection. We tracked both extracellular and intracelluar mycobacteria, the cell populations: Th0, Th1 and Th2 cells, resting, activated and infected macrophages, and four cytokines: IFN-γ, IL-12, IL-10, and IL-4. Our first goal was to develop a model that represents the basic processes of the immune response to Mtb. This model serves as a template on which to add other cells, cytokines, chemokines and interactions as new data warrants to determine how their presence augments or abrogates the system dynamics.

Mathematical expressions were developed representing the interactions between the 8 cell populations and 4 cytokines and parameter values for the rates and rate constants governing each of the interactions were determined (for complete details, see [Wigginton & Kirschner 2001]. Values for most rate parameters were estimated from published experimental data, with weight given to results obtained from humans or human cells and Mtb-specific data over results based on BCG or other mycobacterial species. We outline below how we incorporate these data into the model. Estimates obtained from multiple studies are presented as a range of values. On those parameters for which we have a range, or those for which no experimental data are available, we performed uncertainty and sensitivity analyses to obtain order of magnitude estimates (see the methods outlined above). As an example, we indicate how we estimate the decay rate of IL-10. When IL-10 was administered intravenously to human volunteers, one study estimated its half-life to be 2.3-3.7 hours [Huhn *et al.* 1996]. A similar study estimated this quantity to be 2.7-4.5 hours [Huhn *et al.* 1997]. Therefore, we estimate a range for the half-life from 2.3 to 4.5 hours. The decay rate can be estimated from half-life given by the standard formula $r = ln2/half\text{-}life$. Thus, the decay rate of IL-10 lies in the range [3.69, 7.23] /day. Once the parameters values are estimated, we then simulate the model by solving the differential equations using an appropriate numerical method. Our lab utilizes both packaged software (such as Mathematica and MATLAB) as well as algorithms we coded in C/C^{++} to directly compare results of these different platforms for accuracy.

13.6.1 Simulating Infection Outcomes with *M. tuberculosis*

The negative control, if there are no Mtb present in the system, yields a results with resting macrophages at equilibrium (3 10^5 ml of BAL) and all other populations and cytokines at zero (which agrees with estimates for resting macrophage populations in the lung in healthy individuals). The model also indicates that it is possible to be exposed to an initial bacterial inoculum and then clear infection with no memory of that response (i.e. PPD negative). This outcome is plausible, as it is thought that only 30% of individuals exposed to Mtb become infected (i.e. PPD positive) [Comstock 1982]. The other outcomes for the model are: latency and primary disease. Figure 13.4 presents representative simulations for two given sets

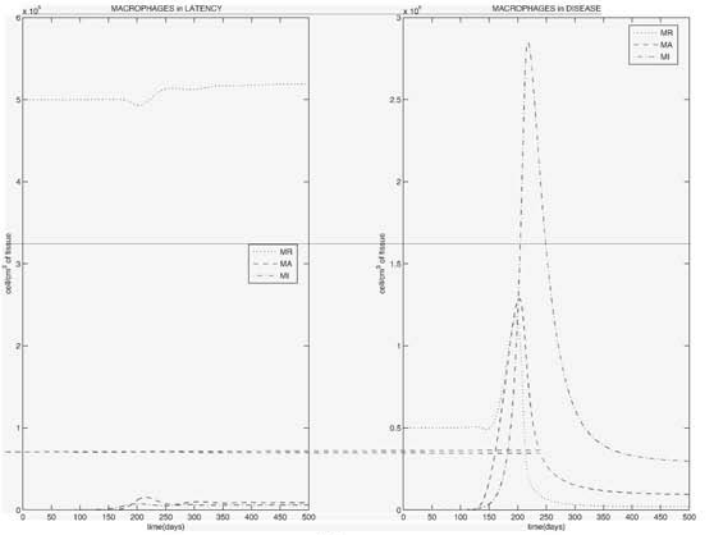
of parameter values - one leading to latency and the other leading to active disease. The different outcomes predicted by the model begs the question: "Which elements of the dynamical system that describes the host response to *M. tuberculosis* govern the different disease outcomes observed?".

Parameter values that govern the rates and behavior of interactions in the model may change from individual to individual and over time within an individual. The virtual experiments reveal that changes in only certain parameters lead to the different disease outcomes – either latency or active disease. Our primary finding is that the rate of T cell killing (via cytotoxic or apoptotic mechanisms) of chronically infected macrophages governs infection outcome. High efficiency of T-cell killing of infected cells, and consequently bacteria, acts to maintain latency, while lower efficiencies lead to active disease. Further, a trade-off exists between the rate of activated macrophage killing of bacteria and T cell cytotoxicity; if macrophage function is compromised, the T cell response must be more potent in order to control infection. However, when the rate of activated macrophage killing of bacteria is considerably increased (beyond values estimated from experimental data), latency is consistently achieved, even for severely compromised T cell function.

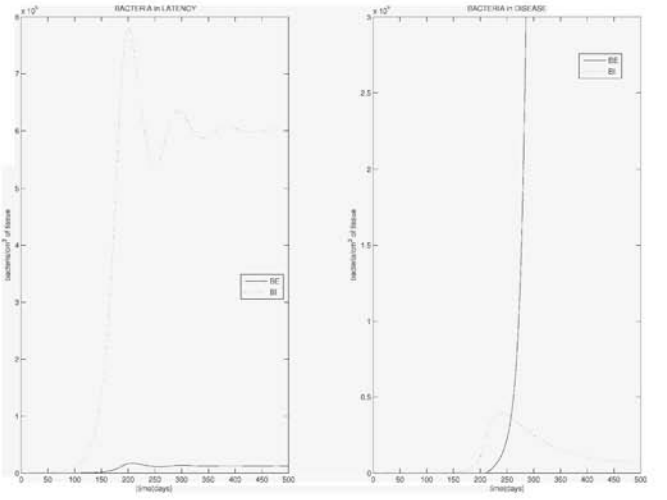
13.6.2 Virtual Deletion and Depletion Experiments

The power of the models we develop is that they can be manipulated in a variety of ways to ask questions about interactions and rates within the system. By doing so, we can explore experimental outcomes on a scale that would be difficult, if not presently impossible, to analyze with other approaches. For example, we can perform both virtual deletion and depletion experiments in this virtual human model for comparison with known experimental results in mice as well as to perform new experiments. Deletion experiments mimic knockout (disruption) experiments whereby we remove an element from the system at day 0, before any infection is imposed into the system. This type of analysis allows us to elaborate which system elements control the establishment of latency. Second, we can simulate depletion experiments by setting the relevant parameters to zero after the system has already achieved latency. These depletion experiments mimic, for example, the addition of antibody that can, to a significant level, neutralize most of a cytokine of one type. This analysis allowed us to determine what elements control maintenance of latency (data not shown- see [Wigginton & Kirschner 2001] for details).

A limitation of this model is that it only tracks temporal dynamics while any spatial aspects are considered homogenous. Moving from a temporal-only model to a spatio-temporal model allows us to elaborate the immune response seen in tissues- that of granuloma formation.



(a)



(b)

Fig. 13.4. Simulations of latency and active disease. The top two panels indicate the bacteria load during latency (left) and disease (right). Shown are the distinct intracellular bacteria (BI) and extracellular (BE) levels over a 500 day time-course. The bottom two panels indicate the macrophage populations over 500 days during latency (left) and disease (right). Shown are resting (MR), infected (MI) and activated (MA) macrophages.

13.7 A Model of Granuloma Formation- the Localized Immune Response to *M. tuberculosis*

The process of granuloma formation leads to a core of dead and infected macrophages together with a centralized necrotic region. These are encircled by activated and resting macrophages as well as CD4+ and CD8+ T cells. Infected macrophages that have not been activated have bacteria growing within them can be killed by activiated CD4+ and CD8+ T cells, which both can act by cytotoxic and apoptotic pathways [Kaleab *et al.* 1990, Kaufmann 1988, Kaufmann 1993, Lewinsohn *et al.* 1998]. Bacteria released are ingested and killed by other activated macrophages. These processes are mediated by a host of elements that must operate in concert to achieve successful granuloma formation. Cells are the key players, but their roles are orchestrated by a number of factors, including chemokines, cytokines, adhesion molecules and their corresponding receptors. Therefore, understanding the dynamic interplay between these immune elements during the time course of granuloma formation and maintenance will provide insight into the mechanisms that control this process. This should distinguish differences between proper functioning granulomas (leading to latency) from those that are unable to contain the bacteria (active disease). A clinical study by [Emile *et al.* 1997] examined granulomas from 14 patients with BCG-induced infection (from receiving the TB vaccine!) . In these cases, it is likely some immune defect (potentially genetically linked) contributed to susceptibility to BCG-induced disease. However, some children suppressed infection while others suffered acute disease. Interestingly, granulomas formed by these two groups of patients were distinct and uniform throughout a given patient. Patients with well-circumscribed, well-differentiated, solid granulomas with activated macrophages and infected macrophages surrounded by lymphocytes containing few bacteria, suppressed infection. Patients with ill-defined, poorly differentiated granulomas with few giant cells and lymphocytes containing a plethora of macrophages filled with bacteria, suffered disseminated disease. Thus, the structure of the granuloma likely determines function which in turn determines whether the host suppresses infection or progresses to active disease. Therefore, understanding granuloma formation will aid in our understanding of the elements that contribute to success or failure of the immune response towards achieving latency in TB.

The importance of the spatial aspect of the immune response to *M. tuberculosis* via granuloma formation has not yet been determined. Likely, the structure plays at least two important roles [Saunders & Cooper 2000]: first is to wall off the bacteria not allowing spread of an infection which cannot be cleared, but second is to facilitate communication between the immune cells affording an optimal, quorum sensing-like interaction [Bonecini-Almeida 1998]. The temporal model developed above is not able to capture this spatial behavior, so new models had to be developed.

To determine the appropriate mathematical tool with which to study the formation and function of granuloma, we developed a series of mathematical models each using a different application, and then performed a formal comparison of each method (see [Gammack *et al.* 2005] for details). Here, we will focus solely on the approach where we used a computational system known as an agent-based model. This allows us to

capture the most discrete and stochastic representation of the forming granuloma. This approach also allows for heterogeneity in space and time.

13.8 The Agent-based Model

We have developed the first model of this type applied in the context of the immune response to a pathogen [Segovia-Juarez *et al.* 2004]. To develop an agent-based model 4 things are necessary: a description on the agents, the rules that govern their behavior, the environment on which they reside and the parameters that govern their interactions. The environment is a key feature of ABMs; important details about modeling environments in general can be found in Chapter 12 by Stepney in this book. The environment is a 2-dimensional lattice representing 2mm x 2mm of lung tissue. The lattice is comprised of grids where the size of each grid can hold the largest cell-type, the macrophage. A single macrophage can reside is a grid with other smaller cell types (such as T cells) and large amounts of effector molecules, such as cytokines and chemokines. The agents are a mix of discrete and continuous entities: immune cells such as macrophages and T cells are discretely tracked, while the bacterial populations and effectors such as cytokine and chemokine are continuously tracked variables. Cells can take on one of several states. A macrophage can be resting, infected or activated, while T cells can take on resting or activated status. There are a complex set of rules that govern the individual behavior of each agent, as well as rules that govern their interactions. These are based on well-documented data. For example, if a macrophage takes up mycobacteria, there is a window of opportunity where a T cell can move into the same grid space occupied by the infected macrophage and activate it via direct cell signaling together with secretion of the cytokine IFN-γ, allowing macrophages to clear the load of intracellular bacteria [Nathan *et al.* 1983, Flesch & Kaufmann 1990, Armstrong & Hart 1971, Sturgill-Koszycki *et al.* 1994]. This is one of the many rules coded into the model (see Figure 13.5).

Many of the parameter values are not known in this setting as they are probabilities and these are difficult to estimate in a wetlab. This makes the use of a detailed uncertainty and sensitivity analyses important in this context. We were the first to apply this analysis to study agent-based models [Segovia-Juarez *et al.* 2004]. For many of the other parameters, we could borrow from what we had estimated previously. For full details please see [Segovia-Juarez *et al.* 2004].

13.8.1 Simulating Granuloma Formation

The behaviors that emerge from this model are complex and of three consistent types. First, a small solid granuloma forms showing containment of bacteria with little to no necrosis forming (Figure 13.6, Panels A, C). Second, we can also generate a larger, more necrotic granuloma that is consistent with dissemination (Figure 13.6,

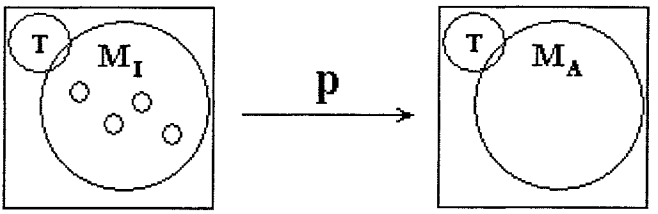


Fig. 13.5. An example of a rule for the agent-based model. If an infected macrophage (M_I) has taken up bacteria (small circles) a T cell can activate it with some probability p, which allows the macrophage to become activated (M_A) and also to clear its intracellular bacterial load.

Panels B, D). Third, we can simulate clearance of all bacteria with no trace of a granuloma (not shown). This last outcome is interesting as it predicts that under certain circumstances the immune response is efficient at clearance. This is suspected as only 30% of individuals exposed to *M. tuberculosis* become infected, however it has not been strictly documented.

The top panels of Figure 13.6 show early time points (2 weeks) in the development of the granuloma under two sets of parameter choices: on the left T cells arrive to the site of infection on day 2 as compared with the right panel where they arrive on day 14. Also, the initial number of macrophages is higher on the right panel than on the left. Within 14 days, it is clear that already the granuloma on the left is more solid and contained than the one on the right which shows more diffusivity. By 6 months (bottom panels) the amount of necrotic tissue (shown in brown) is much greater and the granuloma on the right is much larger as compared with the granuloma forming on the left. Based on the study of [Emile *et al.* 1997] this would indicate that granulomas forming similar to those in the left panels would be able to contain infection, while those on the right would lead to disseminated infection.

The benefit of mathematical modeling here lies in predicting what mechanisms determine these different granuloma outcomes. The sensitivity analysis we employ is based on a partial rank correlation and can identify (with statistical significance) the parameters in the model that when varied correlate to different outcomes. In the simulations shown in Figure 13.6, the timing of effector T cell entry onto the grid (from lymph node homing) is what was determinative. Interestingly, all of the parameters that relate to early numbers of resting macrophages present on the lattice positively correlate with bacteria load. This likely follows since they serve as the primary host for mycobacteria and their presence serves to propagate infection.

Parameter	30 days	60 Days	500 days
Chemokine diffusion rate	0.18	0.13	0.13
Prob. T cell recruitment	-0.36	-0.27	-0.31
Prob. T cell movement	-0.65	-0.54	-0.57
Prob. T cell activates a macrophage	-0.24	-0.16	-0.15
Initial number of macrophages	0.40	0.54	n.s.
Prob. a macrophage is recruited	0.56	0.61	0.75
Speed of activated macrophage	0.31	0.61	n.s.

Table 13.1. Time-dependent partial rank correlations for the 7 parameters in the model (out of 27) that behave as bifurcation parameters driving the system toward containment or dissemination as they are varied. Correlations are shown for total bacteria load as the outcome variable of interest. Similarly, the size of the granuloma or amount of necrosis could be used as outcomes ($p < .001$ in all cases, unless not significant (n.s.)).

Thus, reducing early inflammation (less than 60 days post infection via the influx of too many cells) could be beneficial towards halting infection or tipping the scales in favor of containment. Table 13.8.1 shows all 7 key host parameters with their correlation coefficients over time.

The agent-based approach has its strengths and weaknesses. The strength here is that individual cells can be tracked and at any moment in time all interactions and cell levels can be observed. Weaknesses include an inability for complete mathematical analysis. Regardless, this method uncovers some important features of the host pathogen interaction that we were unable to identify previously with any other approach.

13.9 Discussion

Despite a wealth of information in the biological literature regarding elements of the immune response over genetic, molecular, tissue and system levels, no single representation synthesizing this information into a model of the overall immune response currents exists. In this paper we present approaches for capturing each of these levels to address one specific case: the immune response to *M. tuberculosis*. The next goal is to combine information over the relevant biological and temporal scales to generate a single, integrated multi-scale representation. Such multi-scale models should be developed so that they are sufficiently general that they can be applied to answer a wide range of questions regarding immunity but adaptable enough to answer specific questions regarding, for example, pathogen invasion, tumors, vaccines or auto-immunity. One step towards achieve this goal will be to develop hybrid

models (such as multiple compartment, agent-based models) that include various biological scales. Here we have presented a number of models that each include representations of multiple biological scales, but none are a complete picture of the entire immune response to *M. tuberculosis* and its manifestations at the epidemic level.

Once we can develop multi-scale models, we can apply them towards the generation of hypotheses regarding features of the roles of specific processes in immunity, such as antigen presentation. It is crucial to work under a hypothesis that events occurring at each level (genetic, molecular, cellular, and tissue) of the immune system affect the development of the overall immune response.

For example, the efficacy of vaccines are in part determined by activation of CD4+ T cells. A multi-scale model should enable testing the roles that various factors play in that activation. What is the relationship between antigen dose in the vaccine and the number of mature DCs appearing in a lymph node? Further, what aspects of the antigen presentation process should be targeted to optimize vaccine efficacy? Can our insights help to explain why BCG, the vaccine against TB used for the last 80 years, has failed to control the TB scourge? As theoretical immunologists we are poised to make a strong contribution in this area through hypothesis generation and testing using multi-scale models.