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Technoreview

# Mathematical and computational approaches can complement experimental studies of host–pathogen interactions

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## Summary

**In addition to traditional and novel experimental approaches to study host–pathogen interactions, mathematical and computer modelling have recently been applied to address open questions in this area. These modelling tools not only offer an additional avenue for exploring disease dynamics at multiple biological scales, but also complement and extend knowledge gained via experimental tools. In this review, we outline four examples where modelling has complemented current experimental techniques in a way that can or has already pushed our knowledge of host–pathogen dynamics forward. Two of the modelling approaches presented go hand in hand with articles in this issue exploring fluorescence resonance energy transfer and two-photon intravital microscopy. Two others explore virtual or '*in silico*' deletion and depletion as well as a new method to understand and guide studies in genetic epidemiology. In each of these examples, the complementary nature of modelling and experiment is discussed. We further note that multi-scale modelling may allow us to integrate information across length (molecular, cellular, tissue, organism, population) and time (e.g. seconds to lifetimes). In sum, when combined, these compatible approaches offer new opportunities for understanding host–pathogen interactions.**

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## Introduction

Experimental science has generated tremendous insights into host–pathogen interactions. Mathematical and computational modelling (here, simply 'modelling') offers a powerful and complementary tool for the study of host–pathogen interactions. Like any experimental tool, it can be used in combination with other approaches to offer new insights into biological systems.

Models can be used to describe, simulate, analyse and predict the behaviour of biological systems (Table 1). By turning what is known about the biology into equations and/or rules, you offer a framework by which to *describe* and ultimately understand a system. For example, what are the most important processes occurring, and what may determine the rates of those processes? Answering these questions, which typically involves incorporating detail from many sources, can bring key hypotheses and assumptions to light. Note that we focus here on mechanistic models (as opposed to empirical models, which approximate the shape of a relationship without any mechanistic basis, or statistical models). One can *simulate* the behaviour of a biological system by performing *in silico* experiments, that is, numerically solving the equations/rules that describe the model. Does the model reproduce the known behaviour of molecules, cells or organisms of interest? The results of such *in silico* experiments become the input for further *analysis*, for example, identification of key parameters or mechanisms, interpretation of data, or comparison of the ability of different mechanisms to generate observed data. Finally, a model that successfully describes existing experimental data may be used to *predict* the result of new experiments and generate further hypotheses about a system. For example, novel therapeutics can be tested first via a virtual clinical trial.

## A quick history of modelling in host–pathogen interactions

Modelling has an extensive history in the study of host–pathogen interactions. Models dating as far back as 1760

**Table 1.** Functions of a mathematical/computational model.

| | |
|---|---|
| Describe | |
| | Provide a framework for understanding an experimental system |
| | Clarify hypotheses and assumptions |
| | Consolidate information from many sources |
| Simulate | |
| | Perform *in silico* experiments, including virtual knockouts and deletions |
| | long-time runs (e.g. months to years) |
| Analyse | |
| | Compare different theories or proposed mechanisms |
| | Generate hypotheses |
| | Identify key parameters, molecules, mechanisms or pathways |
| | Study dynamic aspects |
| | Interpret existing experimental data |
| | Identify new biological mechanisms |
| Predict | |
| | Motivate new experiments |
| | Suggest new therapies or vaccine targets |
| | Predict how interventions at one scale would affect another |
| | Offer new explanations for existing data |
| | Virtual interventions (e.g. pharmaceuticals) |

explored the epidemiology of smallpox, malaria and cholera (Farr, 1866; Ross, 1916; Kermack and McKendrick, 1927; Dietz and Heesterbeek, 2000), attempting to clarify the dynamics of epidemics and how disease is spread. More recent epidemiological models explore the dynamics of susceptible, infected and recovered populations of individuals for many diseases and have been instrumental in shaping public health policy (Young and Dye, 2006). Focusing at the level of an individual, models were developed to understand various aspects of the immune system in the absence of infection, for example, generation of antibody diversity, T cell homeostasis and cytokine dynamics (Segel, 1984; Perelson, 1989; Nowak and May, 2000; Segel and Cohen, 2001; Yates *et al*., 2004). The first generation of models to explicitly include a pathogen at the level of the immune system contributed to our understanding of infection with HIV, *Mycobacterium tuberculosis* and *Leukemia cytomegalovirus* (Perelson, 1999; Wigginton and Kirschner, 2001; Ganusov and Antia, 2005). Most recently, detail about signalling pathways is being incorporated into both immune cell and host–pathogen models, with the potential of identifying new drug targets (Goldstein *et al*., 2004; Kinzer-Ursem *et al*., 2006; Kemp *et al*., 2007; Franke *et al*., 2008). Collectively, these models cover spatial scales that range from molecular to cellular to tissue to organism to population and time scales that range from seconds to lifetimes.

In this article, we focus on how modelling can complement experimental tools that are currently being applied to understand host–pathogen interactions. Two other articles in this issue focus on new techniques, intravital multiphoton microscopy (MPM) and four-dimensional (three dimensions in space and one dimension in time) FRET (fluorescence resonance energy transfer), which offer exciting first-time visual data on spatial aspects of host–pathogen interactions in tissues. The first two of our four examples focus on insights gained when modelling is paired with these techniques. The second two examples we offer are additional modelling approaches that complement other techniques in host–pathogen interaction studies.

## Example 1: modelling approaches to complement intravital MPM

Until recently, lymph nodes and other immune sites in the body have been difficult to study *in vivo*. The recent development of imaging techniques such as intravital MPM has allowed novel observations of cellular dynamics. In this issue, Miller and colleagues present details regarding MPM and its role in studying host–pathogen interactions (Konjufca and Miller, 2009).

Multiphoton microscopy has provided useful insights into aspects of immune cell interactions in the lymph node (LN), liver and gut. However, MPM is limited to examination of only a portion of a single tissue and for typically only a few minutes. In the study of LNs, for example, the dynamics of dendritic cell (DC)–T cell interaction have been explored in a ~100 $\mu$m $\times$ 100 $\mu$m window to produce movies that last a few minutes, although DCs and T cells are likely present in LNs for hours to days (Miller *et al*., 2002; 2004a; von Andrian and Mempel, 2003; Catron *et al*., 2004). To obtain statistical significance, many cell paths and behaviours are needed, yet typically MPM experiments are able to track only a handful of cells. In addition, although observations are made of single cell behaviour, the mechanisms driving that behaviour are not directly measurable. Finally, experimental preparations are often modified to increase the opportunity for capturing cellular dynamics. For example, many studies of cells in the LN increase the density of cognate T cells or DCs beyond the physiological range in order to facilitate observation and get statistical significance (Miller *et al*., 2004b; Celli *et al*., 2008).

Models of cell motion have recently been developed. These aim first to replicate observed cell paths in MPM studies and then to predict cell motion over larger areas and longer times and ultimately to understand any implications for immunology [another approach would be to develop algorithms to analyse the large amounts of data generated from MPM studies (Witt *et al*., 2005)]. For example, movement of T cells within a LN was studied (Beltman *et al*., 2007). Using a small-scale (20 $\mu$m) spatio-temporal mathematical model of T cell and DC migration in LN, the sometimes erratic movement of T cells observed with MPM was predicted to be due to the densely packed cellular environment and not some

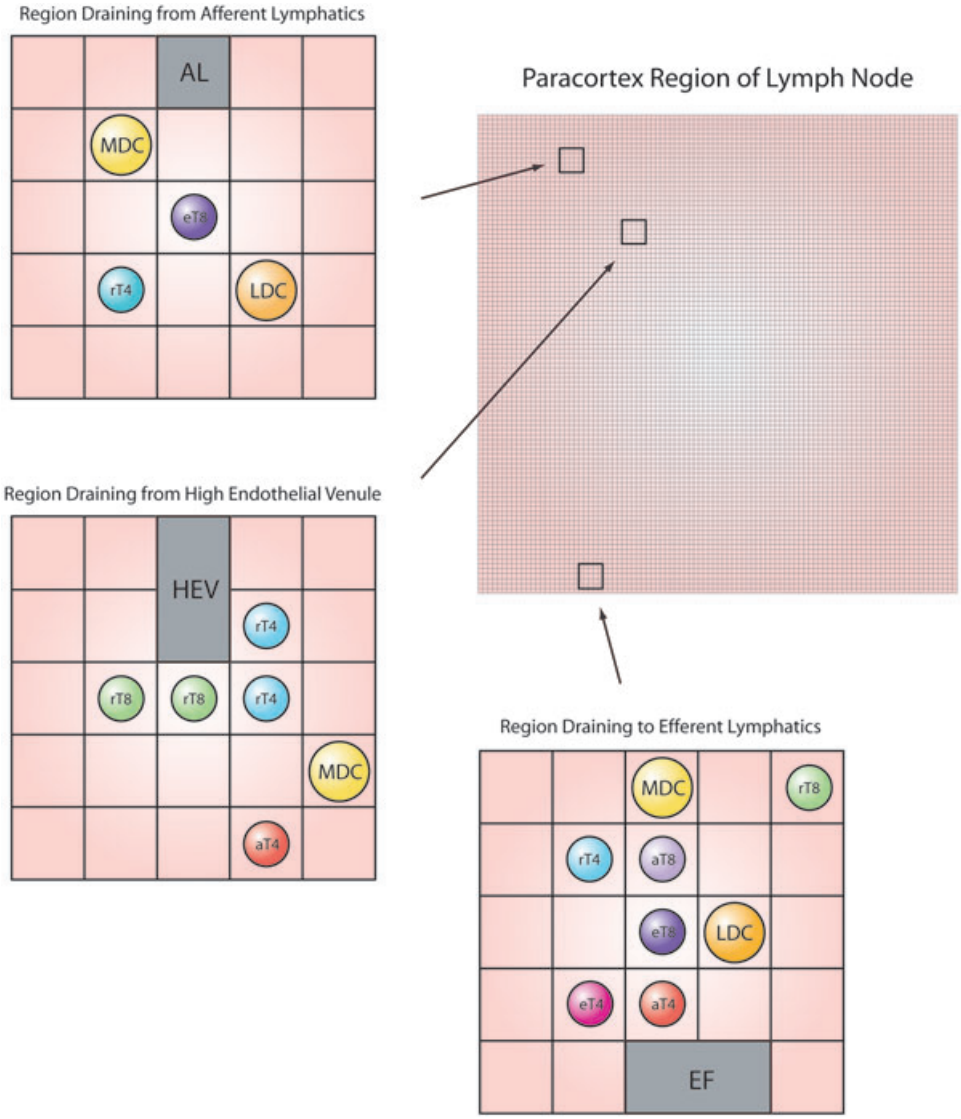


**Fig. 1.** Shown is a grid that represents the paracortoid region of a lymph node (or T cell zone). The grid is subdivided into 20 micron by 20 micron compartments that can hold up to one DC (MDC – mature, LDC-licensed) or up to two T cells (either CD4+ or CD8+ T cells, which are r, resting; a, activated; or e, effector). While DCs exist within one compartment, their influence represents a sweep area that extends to the surrounding compartments around the DC allowing any T cell that comes into those spaces to interact with a DC. T cells enter the LN through the afferent lymphatic ducts (AL) and leave via the efferent lymphatic (EF). DCs enter via high endothelial venules (HEV). Shown are enlargements of regions indicating the existence of these complexities on the grid. A computer model solves the rules described for cells interacting on this grid and the output can be visualized as a time-lapse simulation (see http://malthus.micro.med.umich.edu/lab/movies/LNtzone/).

inherent motility property of T cells. Their experimental colleagues used MPM to confirm these results.

Encounters between T cells and antigen-presenting cells (APCs) can be observed via MPM and lead to T cell activation. How long does it take for an antigen-specific T cell to find its cognate APC 'match' in the LN? Two recent models explore this question using different modelling approaches to describe and make predictions based on MPM data. Preston *et al.* (2006) use asymptotic methods to calculate the time required for a randomly moving T cell to encounter a stationary DC and the threshold level of APCs required for a T cell to have > 50% probability of finding an APC within 24 h. In our work, in contrast, we use an agent-based model (ABM) approach that includes detailed representations of individual CD4+ and CD8+ T cells and DCs in various stages of activation that are present in the LN (Fig. 1). Using this model, we generate individual cell tracks for validation with MPM data from our experimental collaborator Mark Miller and his group (Riggs *et al.*, 2008). A prediction of the ABM model is that

T cell–DC contact begins less than 30 min after cells enter the LN, with an average time for a T cell to find its APC match of several hours.

In addition, we used our ABM to question whether chemotaxis, rather than random motion alone, plays a role in aiding antigen-specific CD4+ T cells in finding their cognate DC within a LN (Riggs *et al.*, 2008). While chemotaxis does increase total number of T cell–DC contacts, it also decreases the number of unique contacts, ultimately leading to the production of fewer activated T cells than a random search strategy. In contrast, other cells in the immune system certainly use chemotaxis: neutrophil chemotaxis is well documented and recent experiments suggest that CD8+ T cells in the LN use a chemotactic mechanism (Castellino *et al.*, 2006).

Most recently, we have explored the relationship between cognate T cell frequency and the density of antigen-bearing DCs present in the LN (T. Riggs, M. Pande, J.J. Linderman, and D.E. Kirschner, in preparation). Understanding how cognate frequency affects T cell activation will be important in interpreting MPM studies that use cognate frequencies elevated above physiological levels. Both T cell frequency and DC density increase the output of primed T cells.

### Example 2: modelling approaches to complement FRET microscopy

Protein–protein interaction networks form the backbone of signalling pathways and constitute a fundamental regulatory mechanism controlling the behaviour of living cells. Until recently, pathway analysis has relied largely on biochemical means (e.g. coimmunoprecipitation, mass-spectroscopy, yeast two-hybrid) to define protein interactions. These measurements provide little dynamic or quantitative information about protein interactions and effectively no information about the subcellular organization of a pathway. As described elsewhere in this issue (Hoppe *et al.*, 2009), microscopy techniques are now being used to investigate the signalling pathways activated during infection. In particular, FRET microscopy can be used to ascertain the binding of specific protein pairs inside live cells by fluorescently labelling them with different variants of fluorescent proteins. The range over which FRET can occur is less than 10 nm and thus the appearance of FRET suggests the association of donor- and acceptor-labelled proteins (Kenworthy, 2001). While *qualitative* information on protein–protein interactions has been routinely inferred from FRET image data, methods for *quantitative* characterization of such interactions from these data have proved more difficult to develop and implement.

As a starting point in the quantitative analysis of FRET images, computational methods for image deconvolution (correcting for optical blurring inherent in any imaging process) and spectral unmixing of image data are required and calibration data are needed (Wu and Pollard, 2005; Hoppe *et al.*, 2008). Next one needs methods to extract quantitative pathway information from these corrected FRET images of protein–protein interactions. In general, information on the location of individual proteins and of various protein–protein complexes as a function of time might be used to extract values for both equilibrium and rate parameters describing protein–protein binding and protein movement. To do this, models could be developed to describe probable events occurring in cells and then, by comparison with image data, to extract or infer corresponding parameter values.

We recently described one such effort to obtain the *in vivo* value of the protein–protein binding equilibrium dissociation constant ($K_d$) in small volume elements of the cell (voxels) (K. Mehta *et al.*, submitted) (Voxels are simply the three-dimensional equivalent of a pixel). Labelled acceptor (A*) and labelled donor (D*) proteins, each tagged with appropriate fluorescent proteins, as assumed to react according to

$$A^* + D^* \underset{K_d}{\longleftrightarrow} A^*D^*$$

where A*D* is the acceptor–donor complex. At the same time, endogenous (unlabelled) forms of A and D also exist and are assumed capable of binding with labelled or unlabelled species. Application of the equations describing this simple model to images allowed us to infer values of $K_d$; one can also infer different values of $K_d$ in different cellular regions (Fig. 2A).

These methods, while still in their infancy, can potentially be applied to study any protein–protein binding interactions. The modelling and analysis may also be extended to analyse image data over time to allow inference of kinetic parameters. Ultimately, obtaining rate and/or equilibrium parameters describing protein–protein interactions may aid not only in signalling pathway reconstruction, including pathways activated during the early stages of infection, but also in understanding how drugs, inhibitors or pathogens might alter those pathways. In addition, we note that terabytes of image data will ultimately be produced as FRET imaging is performed with high three-dimensional resolution over time in living cells, far outstripping human capacity to interpret, digest or analyse. Methods of data reduction and abstraction, including inference of equilibrium and/or kinetic parameters, will be critical to our ability to utilize these data.

### Example 3: modelling approaches to complement epidemiological association studies

In the epidemiological literature, genetic polymorphisms are often observed to be associated with disease

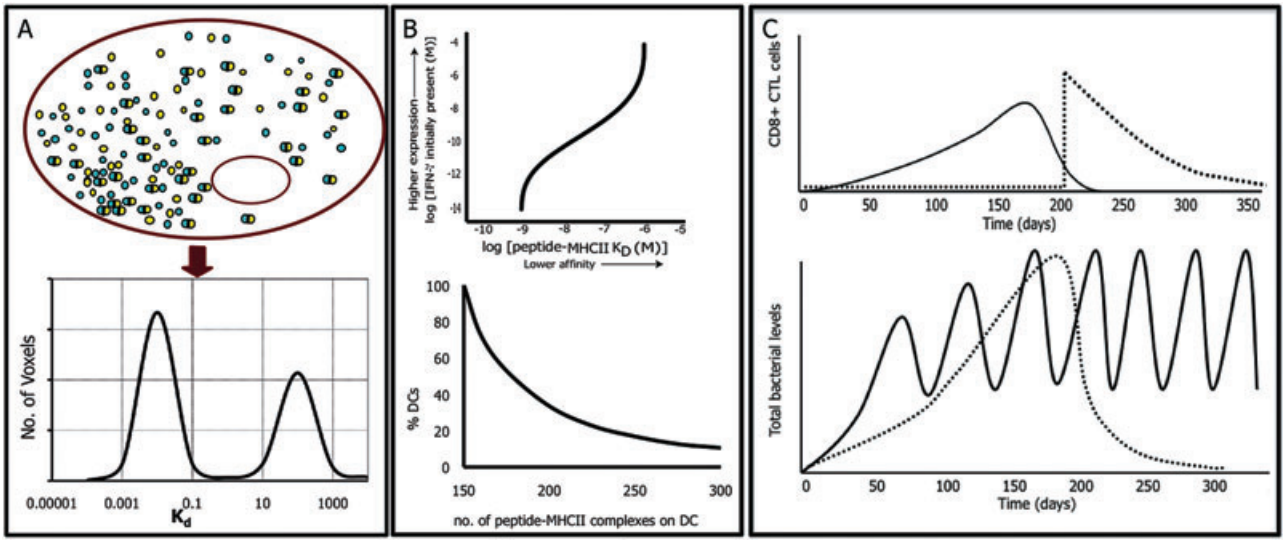


**Fig. 2.** A. Schematic of a cell that has been loaded with acceptor (yellow)- and donor (blue)-labelled proteins to achieve FRET. Analysis of FRET images can be used to obtain values of the equilibrium dissociation constant ($K_d$). Note that one obtains a probability distribution rather than a whole-cell averaged value, potentially allowing identification of multiple distinct values of $K_d$ in different cellular regions.
B. Trade-offs during antigen presentation. In the top plot, we show the values of peptide–MHCII affinity and IFN-γ concentration that give the same response, that is, T cell receptor internalization or T cell cytokine secretion. Within the middle portion of the curve, a deficiency in one parameter can compensate for a change in the other parameter; at the ends of the curve peptide–MHC affinity is dominant. In the lower plot, we show values of the number of peptide–MHCII complexes on a DC and the number of DCs in the LN that give the same number of activated CD4+ T cells leaving the LN. Again, changes in one parameter are observed to compensate for changes in the other.
C. Virtual manipulation of CD8+ T cells during infection with *M. tuberculosis*. Solid curves show the wild-type response similar to that observed in experiments, dashed curves show an alternative simulation. Top panel: CD8+ CTL T numbers grows slowly, peaking after day 150, and then decay (solid curve), while the CD8+ IFN-γ-producing T cells don not appear until day 200 (not shown). In our *in silico* alternative simulation, we switch the order of the differential expression: CD8+ IFN-γ-producing T cells emerge first and slowly decay over time (not shown) while the CD8+ CTL T cells do not appear until day 200 and then slowly decay (dashed curve). Bottom panel, in the wild-type scenario, bacterial load is large, and even oscillating (solid curve), but in the artificial scenario, the bacterial load is cleared (dashed curve).

susceptibility, but inconsistently so. In addition to experimental concerns [e.g. small sample size (Alcais *et al.*, 2001)], there is the very real possibility that multiple polymorphisms simultaneously affect disease susceptibility. For instance, a polymorphism in gene *A*, *A*1, may render the human host more susceptible to disease in the presence of one polymorphism in gene *B*, *B*1, but not in the presence of another polymorphism in *B*, *B*2. Such complications could underlie the difficulty in interpreting results of association studies, which certainly fail to assay all polymorphisms and in fact often assay only a single polymorphism. For example, both particular IFN-γ and major histocompatibility complex class II (MHCII)[1] alleles have each individually been associated with tuberculosis (TB) susceptibility (Teran-Escandon *et al.*, 1999; Lio *et al.*, 2002), but not in all populations. Both genes are relevant to the antigen presentation pathway, a crucial pathway in the development and maintenance of an effective and sustained immune response.

Modelling can play a role in elucidating how multiple factors can simultaneously affect disease outcome and can identify pairs or sets of factors (as represented by parameters in a model) that can compensate for each other. We recently developed an ordinary differential equation model of the events in antigen presentation (Chang *et al.*, 2005; 2008). Our model includes a representation of processes occurring in the APC (peptide–MHCII binding and trafficking and presentation of peptide–MHCII complexes), interaction between an APC and a T cell, and a T cell activation response (cytokine production). This yields a multi-scale model that links molecular and intracellular events to cellular and multicellular outcomes.

One key parameter in the model is the equilibrium binding affinity ($K_d$) of peptide for MHCII. Experimental measurements of $K_d$ are available for some peptides and MHCII (AntiJen database; http://www.jenner.ac.uk/antijen; Blythe *et al.*, 2002) and Immune Epitope Database and Analysis Resource (Peters *et al.*, 2005; http://www.immuneepitope.org/home.do). However, given the plethora of possible peptides (~$10^{11}$) and the large number of MHCII variants (several thousand), experimental measurements for all combinations are unlikely. Algorithms are available and under development that allow for predictions of binding affinities (Chang *et al.*, 2006; Nielsen

[1]We use MHCII for consistency, but in humans this gene is termed HLA.

*et al*., 2008). Other parameters in the model include the level of IFN-γ expression by T cells, and the level of MHCII expression by APCs.

Once validated against experimental data, our model was used to explore whether one factor could compensate for another, which we term a trade-off. Polymorphisms need not affect the same cell or even the same space or time scale to be compensatory. For example, considering that IFN-γ upregulates MHCII expression, could an allele of IFN-γ sufficiently increase the number of MHCII molecules per APC to offset deficiencies exhibited by some MHCII alleles in binding epitopes from *M. tuberculosis*? Within certain ranges of IFN-γ concentration and peptide–MHCII affinity, the answer is yes (Fig. 2B). However, in other ranges, polymorphisms in MHCII may mask the effect of polymorphisms in IFN-γ; that is, the peptide–MHCII binding affinity is dominant. This trade-off could account for inconsistencies in epidemiological studies that attempted to link IFN-γ polymorphisms and TB susceptibility (Moran *et al*., 2007). We also found that MHCII expression and MHCII binding polymorphisms, antigen processing ability and MHCII binding polymorphisms, and peptide–MHCII affinity and affinity of peptide–MHCII complexes for T cell receptors can be compensatory.

In ongoing work, we also find such compensatory relationships in the LN using our ABM based on MPM data discussed under Example 1 (T. Riggs, M. Pande, J.J. Linderman, and D.E. Kirschner, in preparation). For example, an increase in the number of peptide–MHC complexes on a DC surface can in some circumstances compensate for a decrease in the number of antigen-bearing DCs present, trading off to yield the same number of, say, primed T cells (Fig. 2B). This hints at the complexity that may be influenced by pathogens that can alter antigen processing leading to suppressed immunity (Chang *et al*., 2005). In the future, one might use these models to rank polymorphisms by effect, providing a new tool for designing epidemiological association studies.

### Example 4: modelling approaches to complement genetic deletion and molecular depletion studies

The use of genetic knockouts and molecular depletion studies for understanding cells and effector molecules has been of tremendous benefit to immunology. It has allowed the analysis of specific roles played by individual genes and molecules in the pathogenesis of many infectious diseases. For example, depletion of macrophages in a murine model of anthrax helped to underscore their importance in disease progression (Hanna *et al*., 1993). Depletion (or neutralization) of the cytokine IL-17 was shown to be a necessary factor in development of acquired immunity to *Streptococcus pneumoniae* (Lu *et al*., 2008). Humans with the mutation CCR5Δ32 are missing the chemokine receptor CCR5 and are protected from HIV-1 infection (Sullivan *et al*., 2001). As a final example, patients with latent TB who are treated with anti-tumour necrosis factor (TNF) drugs for their rheumatoid arthritis suffer reactive TB, suggesting that TNF plays a major role in containing infection (Wallis *et al*., 2004). In other cases, deletions and depletions provide less informative or even ambiguous results. Still further, there are many deletions and depletions that cannot be performed because of lack of availability of animals with that phenotype or lack of reagents to perform neutralization or depletion studies.

Modelling can be used in both experimentally accessible and inaccessible cases to contribute to our understanding of the roles of particular molecules in immunology. It is possible to perform both virtual (*in silico*) deletion and depletion experiments (cf. Yates *et al*., 2000). Virtual deletion experiments mimic knockouts by removing a molecule or cell type at day 0, before infection is introduced. In virtual depletion experiments the relevant molecule or cell concentrations are set to zero at any time post infection.

For example, we used a model to make predictions on the role of CD8+ T cells in the host–pathogen interaction with *M. tuberculosis* (Sud *et al*., 2006). The immune response to *M. tuberculosis* is cell-mediated and results in the formation of granuloma in the lungs. These are collections of cells and cytokines all aimed at physically containing and immunologically restraining bacteria. Data suggested that there are two subsets of effector CD8+ T cells: those that produce cytokines including IFN-γ and TNF, and those that produce perforin/granzyme and perform CTL activity (Lazarevic *et al*., 2005). These cells were observed to be differentially expressed during disease progression. It is presently impossible to delete or deplete these specific subsets of effector CD8+ T cells. We developed an ordinary differential equation model that represented these CD8+ T cell subtypes in a larger model of the immune response to *M. tuberculosis* and performed virtual deletion and depletion studies (Sud *et al*., 2006). We first either deleted or depleted (done during latency) each of the cell types individually. When the INF-γ-producing CD8+ T cell population was depleted or deleted, the latency state achieved was similar to wild type. When the CTL-CD8+ T cell population was depleted or deleted, latency was still achieved, but with a different phenotype from wild type. Oscillations in bacterial load were observed, a less stable latent state that is more easily perturbed to active disease. Also, the bacterial loads were higher in this latent scenario as compared with wild type. Interestingly, we found that when both subsets of CD8+ T cells were simultaneously deleted, a synergy occurs and the system goes to active disease. These

results suggest that both cell types are required for control of infection, but each plays an independent role in affecting that result.

In a related study, we used our model described above to study the role of differential cell population dynamics in TB disease progression. In mouse studies (and included in the model), CTL-CD8+ T cells appear quickly in the first 20 days following infection and then slowly die off; INF-γ-producing CD8+ T cells appear about day 20 and then increase for more than 6 months (Lazarevic *et al*., 2005). We simulated a case in which the appearance and timing of these cell populations was reversed, and found that the bacterial infection could be cleared (Fig. 2C). This result suggests that one reason individuals may have trouble clearing *M. tuberculosis* infection is because they are producing these different CD8+ T cells subclasses in the wrong order. Treatment and vaccine studies aimed at understanding this could be very powerful in combating infection.

## Conclusions and future directions

We believe that modelling, particularly in combination with experimental studies, is a tool that has much to contribute to the study of host–pathogen interactions. As described above, a multitude of different modelling formalisms are available and appropriate for different situations, similar to experimental techniques.

Most models (and experiments also) focus on a single length (and time) scale. It has become increasingly clear, however, that events at different length and time scales all simultaneously affect the overall outcome of biological processes including host–pathogen interactions. For example, the affinity of particular peptides for MHCII may play a role in the efficacy of antigen presentation, affecting the number of T cells generated which will dictate success or failure of the immune response in a given situation (Kirschner *et al*., 2007). Experiments that simultaneously track molecular, cellular, tissue, organism and population scale events over time are rare and difficult to perform with accuracy. However, multi-scale models offer a distinct advantage in being able to integrate information across scales. Multi-scale modelling requires building and validating models at individual scales and linking them together in a method that is computationally feasible (Alarcon *et al*., 2004; Kirschner *et al*., 2007).

Ultimately, such multi-scale models may allow us to predict how particular interventions at the molecular scale will manifest themselves at higher scales. This understanding may guide the development of effective treatments for an infected host or vaccines for entire populations. Modelling is a tool that will allow these next important steps in the study of host–pathogen interactions.

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