An approach to modelling in immunology

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Abstract

Like most other fields in biology, immunology has been revolutionised by the techniques of molecular biology and the resulting explosion in available experimental data. It is argued that efforts to integrate the data to gain insight into how various subsystems in the immune system interact and function require mathematical modelling and computer simulation in close collaboration with experimentalists. This paper illustrates some of the techniques available for modelling immune systems, and highlights the issues that should be borne in mind by anyone starting down the modelling path.

INTRODUCTION

The human immune system is enormously complex and the battle to understand it has presented problems that pre-date those being faced now by geneticists and molecular biologists dealing with genomic data: abundant information within a theoretical framework that is relatively underdeveloped. Mammalian immune systems show a high degree of evolutionary conservation and through studies of both humans and other species, a great deal of the molecular mechanisms at work and the interactions at the intercellular level that drive immune responses have been uncovered. However, our understanding of the properties of the immune system *as a whole* is still limited, and this in turn constrains therapeutic approaches. This is apparent, for example, in autoimmune diseases or in the case of HIV infection.

In order to put the current challenges into context, we can divide the development of the biological sciences, and immunology in particular, into three stages:

- *Cell biology/physiology era*. This was largely an experiment-driven whole animal or cellular black box approach in which inputs were compared to outputs without much understanding of the genetic or molecular events that linked them. Great insights into system behaviour were gained with relatively simple discriminative experiments.

- *Molecular biology era*. This has been characterised by the recent 'data explosion' from high-throughput molecular biology techniques, the development of advanced experimental techniques and information management, leading to genome sequencing, extensive cataloguing of the structure and function of gene products and the description of metabolic and signalling pathways. However, insight into system behaviour has lagged behind data acquisition, partly due to the reductionist approach that is frequently employed.

- *Post-genomic era*. We are currently faced with the difficult but exciting task of integrating huge experimental data sets with new theoretical frameworks to produce useful models of system behaviour.

It is increasingly being realised that the properties of whole biological systems cannot be deduced by intuition alone, nor deduced directly from the data being provided by the molecular biology

approach. This is due to a number of factors:

- *Combinatorial complexity*. The number of potential interactions between individual elements (eg proteins) in a system grows extremely rapidly with the number of elements. There is increasing evidence that this network of interactions cannot be ignored. The action of every protein will be modulated and affected by the presence of many other proteins. This is not addressed using current experimental technique. One can attempt to determine the function of every gene by knocking them out individually in an animal (a massive job that has been completed for *Drosophila* but not for the mouse). However, to understand the function of a gene it would be necessary to knock out every pair, triplet, etc. of genes. This is clearly not feasible.

- *Feedback* loops appear to be ubiquitous in physiological systems, and these operate at various levels in the immune system. For example, antigen stimulates the formation of the appropriate antibody, which then results in the clearance of the antigen, a classical negative feedback loop. A similar example at the intracellular level is that of NF-$\kappa\beta$ which activates the transcription of I$\kappa\beta$, which binds to NF-$\kappa\beta$ and inactivates it. Positive feedback loops are also seen, for example, when the presence of active TNF-α results in the recruitment of immune cells which then release more TNF-α, as described in the TNF-α model below. Although the effects of a single feedback loop can probably be understood intuitively, this is no longer the case when several feedback loops begin to interact.

- *Delays*. Similarly, most biological effects involve a delay. Thus for instance when a T cell receives a proliferative signal, it first needs to synthesise DNA and undergo the biochemical changes required for cell division. Delays, especially when combined with feedback loops introduce many unforeseen effects into a system, for instance oscillations (eg Haurie *et al.*[1]).

- *Non-linearity*. A system is called non-linear if its response is not always proportional to the stimulus. Non-linearity is all-pervasive in biological systems, and indeed is essential for their functioning. Examples include saturation of signalling pathways or the ability of some signalling molecules to switch on different genes at different concentrations (eg Shimizu and Gurdon[2]). It is well known that even simple non-linear feedback systems can exhibit complex and counter-intuitive behaviour (eg Callard *et al.*[3]).

It is therefore clear that more rapid data acquisition and more sophisticated information management are insufficient by themselves to understand the complexities of the immune system. Our belief is that increasingly mathematical modelling is becoming an essential tool to complement experimental and conventional bioinformatical techniques in immunology. Together, such approaches offer the possibility of gaining new insights into the behaviour of the immune system, of providing new frameworks for organising and storing data and performing statistical analyses, of suggesting new hypotheses and new experiments, and even of offering a 'virtual laboratory' to supplement *in vivo* and *in vitro* work.

However, mathematical modelling in immunology, and in the life sciences more generally, is far from straightforward, and suffers from a number of potential pitfalls:

- *Mathematically sophisticated but biologically useless models*. These can arise because of a lack of biological input, leading to models that are biologically unrealistic,

or address a question of little biological importance. The latter, more insidious, problem often arises because the reasons for constructing the model have not been clearly articulated.

- *Biologically realistic but mathematically intractable models*. The converse problem usually arises because biologists unfamiliar with the limitations of mathematical analysis want to include every known biological effect in the model. Even if it were possible to produce such models they would be of little use since their behaviour would be as complex to investigate as the experimental situation.

Formulating the question

In our experience, the single most important factor in avoiding either of these is to formulate clear *explicit biological goals* before attempting to construct a model. This will ensure that the resulting model is biologically sound, can be experimentally verified and will generate biological insight, or new biological hypotheses. We stress that the aim of a model should not simply be to reproduce the biological data, and indeed often the most useful models are those that exhibit discrepancies from experiment. Such deviations will typically stimulate new experiments or hypotheses. Our ideal is therefore an iterative approach, starting with a biological problem, developing a mathematical model, and then feeding back into the biology (Figure 1). Once established, this *collaborative loop* can be traversed many times, leading to ever-increasing understanding. There are many potential benefits for the biological side of the collaboration. As well as indicating new experimental directions, developing the mathematical formulation of a system may focus thinking and clarify definitions. At the same time it will frequently motivate new mathematical questions and lead to unexpected pay-offs for the mathematical side. It is crucial that both sides benefit in this way and the collaboration is conducted between equals. If either the mathematician is simply seen as providing a service to the biologist, or conversely the mathematician fails to be interested in solving the biological problem (rather than a mathematical one), then it is unlikely that the collaboration will survive, or produce any worthwhile results. It is therefore essential that there is good communication between the disciplines, and this can take a long time

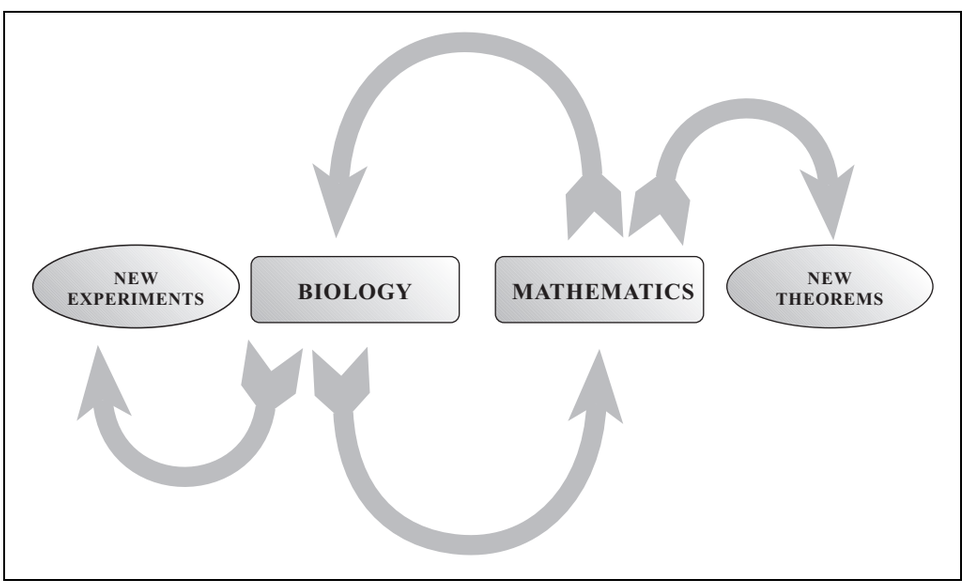

Figure 1: The collaborative loop

Repeated interaction

to establish. Our experience has been that it may be several years for a collaboration to become productive, and often several rounds of discussion before experimentalist and theorist can agree on a common question and methodology. On the other hand we are convinced that such an investment to build an effective collaboration is then repaid many-fold.

It can obviously be difficult for an immunologist unfamiliar with mathematical modelling to know how to start in this area. Our experience has been that mimicking existing role models can be valuable. In this paper, we therefore illustrate a number of modelling issues and approaches using examples developed over the last few years at the Centre for Mathematics and Physics in the Life Sciences and Experimental Biology (CoMPLEX) at University College London.

A BRIEF TOUR OF THE IMMUNE SYSTEM

Adaptive immune responses in vertebrates are generated following exposure to a foreign *antigen*, generally an infectious microorganism such as a virus or bacteria. The infected individual responds rapidly by the production of specific antibodies made by B lymphocytes and by the expansion and differentiation of effector and regulatory T lymphocytes. This response aimed at clearing the infectious agent is coordinated by a network of highly specialised cells that communicate through cell surface molecular interactions and through a complex set of intercellular communication molecules known as *cytokines* and *chemokines*. Following clearance of the infectious agent, the individual is able to respond more rapidly and more vigorously to a second exposure to the same infection (antigen). This is known as immunological *memory*. Induction of immune memory is the basis for long-term immunity to pathogens we have already encountered, either through infection or vaccination. The adaptive immune response is highly specific, and antibodies and T cells generated in response to one pathogen generally fail to respond to antigens from unrelated pathogens. In addition, the immune system is able to discriminate between self and foreign antigens. The processes involved in this tolerance to self-antigens include deletion, anergy and active regulation. Failure of these safety mechanisms can result in autoimmunity, in which the immune response is directed towards the host tissue.

One of the most intriguing features of the immune system is its multi-functionality. Its cellular components are complex, context-sensitive agents that respond in a non-linear fashion to an enormously diverse set of signals from cytokines, chemokines and direct cell–cell interactions. Further, the messenger molecules are typically expressed by several cell types and are themselves multi-functional. Laboratory experiments tend to isolate and study one or two interactions at a time, but this approach alone will not shed light on the interacting whole. Our understanding of the immune system has reached a level such that mathematical modelling is becoming a useful and powerful investigative tool. The abundance of experimental information has led to a situation in which higher levels of description are needed to integrate the data.

The immune system can often be viewed as operating in isolation from other physiological subsystems. This allows us, at least in principle, to describe it from both top-down and bottom-up perspectives. Some authors have prescribed 'goals' to the immune system in order to construct models of its behaviour (see, for example, Segel and Lev Bar-Or[4]). Others have tried to demonstrate how properties may have emerged or evolved by considering optimal solutions to trade-offs (eg selection of T-cell receptor repertoires[5]). Another modelling philosophy is to build dynamical models based on experimental data and attempt to understand the

emergent behaviour of complex interacting systems of cells and/or molecules. The latter approach is the one we have pursued to date. Some of the questions we have started to tackle are the following:

- How can the immune system effectively distinguish between self and foreign antigens? Under what situations does tolerance to self break down?
- How does a coherent immune response emerge from the multiple interactions of the cytokine network?
- How is immune memory maintained in the face of multiple serial infections throughout the life of an individual?

We describe these below. There are obviously many other areas that merit investigation. For further background, the reader is advised to consult one of the many good immunology textbooks, eg Paul[6] and Janeway and Travers.[7] A good mathematical introduction to modelling in biology is Murray,[8] though unfortunately there does not appear to be a comparable text suitable for those with a biological background. Other reviews of the application of modelling to immunology are Perelson and Weisbuch[9] and Morel.[10]

Background reading

MODELLING ISSUES

Level of detail

As indicated above, attempting to incorporate every single known interaction rapidly leads to an unmanageable model. Further, parameter determination in such models can be a frightening experience. Estimates come from diverse experiments, which may be elegantly designed and well executed but can still give rise to widely differing values for parameters. Data can come from both *in vivo* and *in vitro* experiments and results that hold in one medium may not always hold in the other. Further, despite the many similarities between mammalian immune systems, significant differences do exist and so results obtained from experiments using animal and human tissue may not always be consistent. Given the mathematical tools currently available to us and uncertainty in the data, then, any modelling approach is obliged to be coarse-grained to some extent. How does one decide which are the crucial components? Typically the temptation is to include a multitude of factors. In most immunological contexts, many of the interactions, components or parameter values are ill defined, and even for well-characterised systems a thorough exploration of parameter space for exhaustively detailed models is effectively impossible. The experimentalists' insight into which interactions are important is the first resource.

Using experimental data

Robustness

Robustness is another important guiding principle: the cellular components of the immune system operate in noisy media, with significant environmental and genetic variation among individuals, and yet immunity to most pathogens is maintained. Realistic models of biological systems may need to be insensitive to changes in kinetic parameters or concentrations of mediators, eg Barkai and Liebler.[11] This is something we have tried to explore in our models to date. Rather than set out to construct models with these properties, however, robustness can be used as a means of evaluating or comparing models that have been developed purely with the biological data in mind.

Modularity

Despite its complexity, it seems likely that hierarchical or modular descriptions of the immune system are possible. The most obvious example is that of the inter-/intracellular split, in which we consider cells to be 'black boxes' with complicated but nonetheless calculable input/output characteristics. This has certainly been the implicit assumption in many successful models. An excellent review of

modularity in biological systems can be found in Hartwell *et al.*[12]

Another aspect of modularity is the repetition of common motifs or components in molecules involved in cellular interactions or communication. For example, cytokine receptors are typically constructed from several components, some of which may be common to several distinct receptor types. Many cytokine interactions may have arisen from a small set of precursors that duplicated and diversified over time, resulting in repeated units with similar structure but different functions. This modularity can simplify aspects of modelling as it both allows individual parts of the whole system (modules) to be initially considered in isolation and allows the application of common approaches to different aspects of the same system.

Anatomical and spatial considerations

The immune system does not operate in a well-stirred chamber with no structure. The cells move between different anatomical compartments and molecules on the cell surface can be organised into particular spatial patterns that are important for their function. For example, germinal centres are highly organised, temporary structures in lymph nodes within which B cells pass through repeated rounds of antibody mutation, proliferation and selection. This results in the generation of antibodies of high affinity during an immune response, eg Oprea *et al.*[13] Similar spatial compartmentalisation is seen in the thymus, an organ in which newly generated T cells that are both viable and not overly reactive to self are selected and exported to the periphery. At the cellular level, when T cells recognise their antigen there is a reorganisation of molecules on the surface of both the T cell and the antigen-presenting cell (APC) to form the immunological synapse, which has a distinct spatial and temporal organisation.[14]

Stochasticity

One of the major tools in modelling is the use of simple differential equations. When dealing with large, well-mixed populations of cells and relatively long time-scales it is reasonable to use this approach. However, many processes in the immune system are probabilistic or have stochastic elements (see, for example, Borghans *et al.*[5] and van den Berg *et al.*[15]). Examples are the recognition of foreign antigen by T-cell receptors amid a noisy background of self-peptides, the generation of an effective and safe T-cell repertoire, and somatic hypermutation of B cells. We discuss our approach to the first example in the section on 'Cross-talk between T-cell receptors' below.

Many processes, particularly intracellular reactions, involve small numbers of molecules and stochastic effects are likely to be very important. This is an area of research that has received very little attention.

MODELLING EXAMPLES

We now present four examples from our own work illustrating the points developed above. A common theme running through these is the presence of non-linear effects and both negative and positive feedback loops. These are often thought of as simply damping down and amplifying mechanisms respectively. We show that they have a role to play in cellular differentiation, as a rapid response mechanism, as well as in preserving diversity in the memory pool and enhancing the specificity of the immune response. These varied roles of feedback are not intuitively obvious, and become apparent only through modelling.

TNF oscillations

Our first example shows how even extremely simple control systems in immunology can exhibit unexpected behaviour. Motivated by experimental results demonstrating oscillations in the level of the inflammatory cytokine tumour necrosis factor α (TNF-α) in the

Activator inhibitor model

aqueous humour of rabbits receiving corneal allografts,[16] we developed a simple ordinary differential equation model[17] for these oscillations, based on the regulatory interactions between TNF-α and its inhibitors (IL-10, TGF-β, soluble TNF-α receptor). The model is illustrated in Figure 2. Such an intuitive model is then converted into coupled ordinary differential equations, using the simple principle of balancing rates for each cytokine:

rate of change of cytokine concentration
= rate of formation − rate of clearance

This leads to the coupled pair of equations:

$$\frac{dx}{dt} = \nu_1 \frac{(x^n + \varepsilon_1^n)}{(x^n + \alpha^n)} \frac{\beta}{(\gamma + \beta)} - d_1 x \qquad (1)$$

$$\frac{d\gamma}{dt} = k_2 + \nu_2 \frac{(x + \varepsilon_2)}{(x + \gamma)} - d_2 \gamma \qquad (2)$$

where x is the concentration of TNF so that dx/dt represents the rate of change of cytokine concentration. The concentration of the inhibitor is given by γ. The first term on the right of equation (1) models the positive feedback loop shown in Figure 2 (and so is dependent on x, the concentration of TNF). The parameter ν_1 is the maximal rate of TNF production, set by the strength of antigenic stimulus. The parameters ε_1 and α represent, respectively, a baseline level of TNF-α production and the threshold value of the TNF concentration at which positive feedback on its own production becomes apparent. The second term incorporates the negative feedback (dependent on γ, the concentration of the inhibitor), where β sets the threshold inhibitor concentration for negative feedback on TNF production.The final term represents the clearance (or catabolism) of the cytokine, which is considered to be dependent only on the concentration of TNF. The equation describing the rate of change of inhibitor concentration is similar, though the rate of formation of the inhibitor is dependent only on x, as there is no positive feedback loop. In all cases the interactions of x and γ are given by Hill functions, which are a mathematical representation of a standard sigmoid dose response curve.

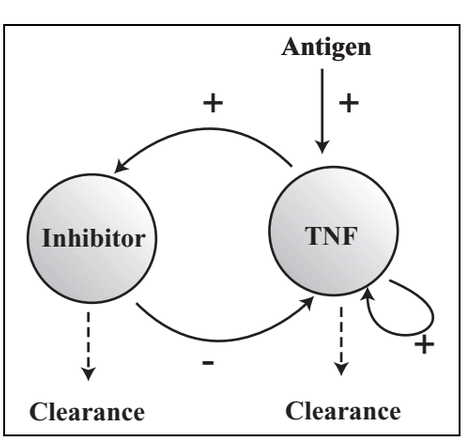

Figure 2: Regulation of TNF-α by both negative and positive feedback. After illustration in Chan *et al.*[17]

For given parameter values, a plot of the time evolution of TNF concentration against inhibitor concentration results in a phase diagram, which reveals the long-term qualitative behaviour of the system. Such plots can be easily generated using general computer algebra software packages (eg Maple™ or Mathematica™) as well as software written for analysis of non-linear dynamical systems (eg DsTool[18]). We can also systematically vary each parameter value and see how this affects the system behaviour. This is known as *bifurcation analysis* and is generally done numerically using dedicated software (eg Auto[19] or Content[20]), though unfortunately such packages are probably not easily used by non-specialists. A list of dynamical systems software is available at Dynamical Systems Software.[21]

In this particular case, bifurcation analysis was used to characterise the qualitatively different solutions of the model. It revealed that even such a simple two-component network could show a rich set of behaviours under quantifiable conditions, including excitability, oscillations (Figure 3), hysteresis,

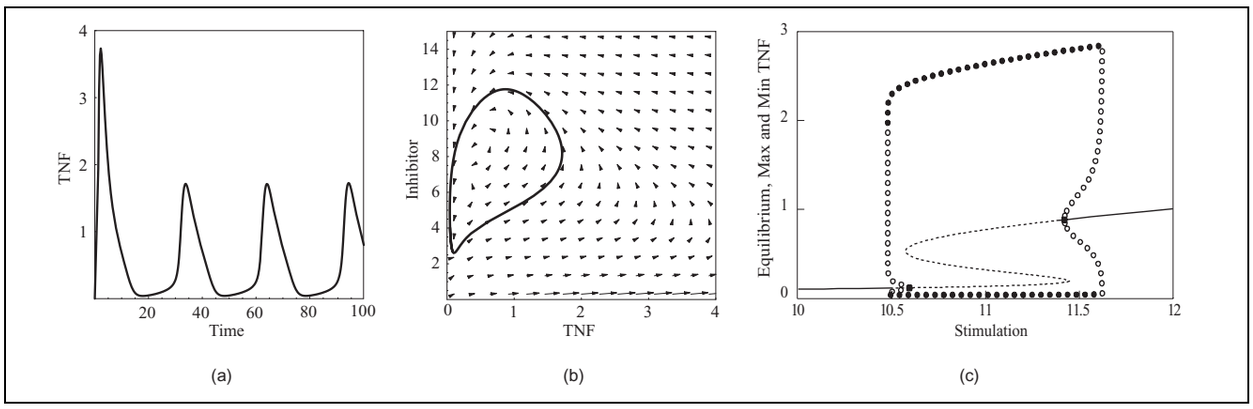

Figure 3: Oscillations arising from feedback interactions between TNF and inhibitors. (a) and (b) The time series and phase diagram representations of the oscillations; (c) bifurcation diagram showing the range of values of the parameter ν_1, the strength of antigenic stimulation, over which oscillations occur. Reproduced from Chan *et al.*,[17] copyright Royal Society of London

threshold behaviour and bistability. One of the interesting predictions of the model is that oscillations exist only for an intermediate degree of antigenic driving, and both increasing and decreasing the antigen load can abolish these oscillations. The possibility of such counter-intuitive behaviour should obviously be borne in mind by those attempting to perturb 'real' cytokine networks for therapeutic purposes, for example, in the use of anti-TNF monoclonal antibodies to treat rheumatoid arthritis.[22]

Th1/2 differentiation

Reducing complexity

Although real cytokine networks are highly complex, it is sometimes possible to reduce the complexity drastically by separating time-scales so that a lower dimensional system is obtained. Then the tools mentioned above (eg phase plane analysis) can be used to analyse this reduced system. Such an approach is described next.

Complex networks of cytokine interactions pervade the immune system but there are a few subsystems that are sufficiently isolated and well studied to be amenable to modelling. One such area is T-helper cell differentiation. After encountering foreign antigen in the periphery, APCs migrate to lymph nodes and display antigen fragments to T cells. APC–T cell encounters of sufficient specificity lead to activation, proliferation and differentiation of T cells into clones with effector functions. An important subset of T cells, CD4+ T helper cells, can be further subdivided into Th1 cells that are involved in cellular immunity and inflammation, and Th2 cells that interact with B cells and are associated with antibody production and isotype switching. The immune system 'decides' which T-helper response is the most appropriate for a given pathogen, based on both signals from the innate immune system (costimulatory signals from APCs and cytokines produced by other cell types), the antigen dose and the cytokines produced by the proliferating T cells themselves.

We developed an ordinary differential equation model of T-helper cell differentiation[23] in order to determine the essential factors determining the Th1/2 outcome of an immune response. The model was simplified significantly by making the assumption that the rates of cytokine production and consumption were much faster than the rates of cell proliferation, allowing us to make quasi-steady state assumptions and hence treat the various cytokine concentrations as simple functions of Th1 and Th2 cell numbers. In other words, fast dynamics

drive cytokine concentrations to steady states, which then track slow parameter changes (ie cell numbers). The cytokine concentrations are then eliminated as dynamical variables. This 'slaving' principle reflects, to some extent, the hierarchical structure in the immune system. By separating time-scales we reduce the need to consider the dynamics in full. The interactions we included in the model are illustrated in Figure 4.

Th1/Th2 asymmetries

The model generated a number of interesting results, most of which were rooted in asymmetries in Th1 and Th2 regulation. One feature of particular interest was the induction of switches in the immune response by external means. The model predicted that to switch from a Th2 to a Th1 response required both addition of pro-Th1 cytokines and a reduction in antigen load, which is borne out by data in the literature. Further, it highlighted differences in the regulation of Th1 and Th2 responses: Th1 through apoptosis induced by cell–cell contact, Th2 by negative feedback of the proliferating cells on the APCs. Dynamical switches from Th1 to Th2 responses were also predicted in chronic infections. Interestingly, we also found that under a wide range of parameters, varying the antigen dose induced a switch between two stable states (Th1 and Th2 responses) through an intermediate oscillatory phase, similar to that observed in the TNF model described above. Whether this is a generic feature of cytokine networks is under investigation.

This model did not mimic in full the detailed dynamics of a real T-cell response. Rather, the two-dimensional representation of the system reproduced many of its features and predicted more. These encouraging results reinforce the idea that rather than building detailed dynamical models of systems from scratch, a more coarse-grained approach may be preferable or even necessary in many situations.

Maintaining T-cell memory

In this final example of an ordinary differential equation model, rate equations for the number of resting and cycling memory T-cell pools were derived by balancing proliferation and clearance rates as before. The model was then extended to accommodate different T-cell clones (basically by giving each clone its own set of ordinary differential equations), resulting in a model that could take into account the heterogeneity of the T-cell

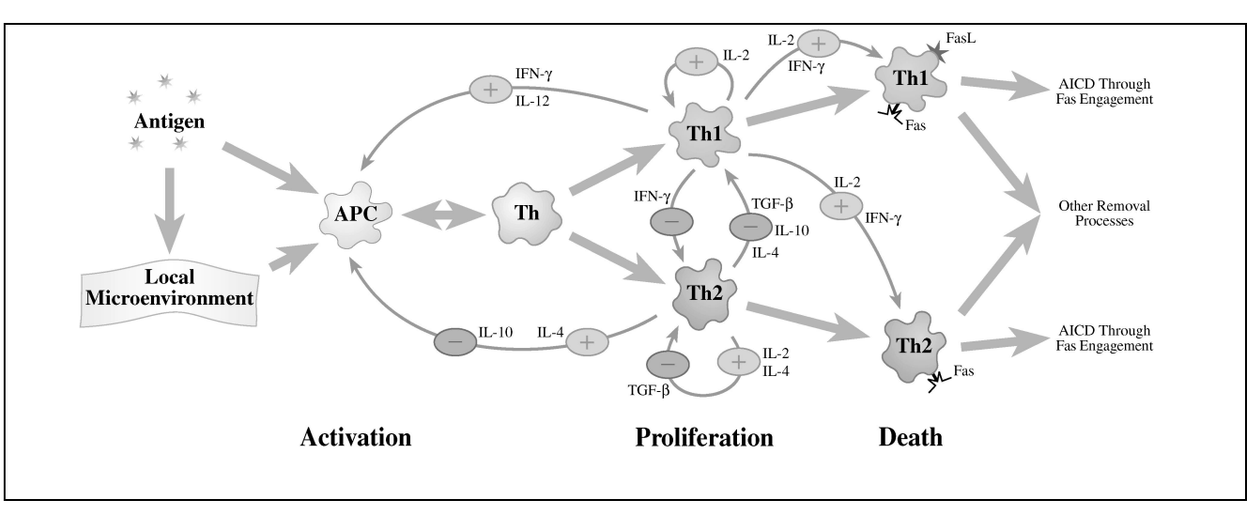

Figure 4: Schematic representation of the interactions governing Th1/2 differentiation, proliferation and death. Arrows labelled with (+) or (−) reflect positive or negative feedback respectively, mediated by cytokines as indicated. AICD is an acronym for activation-induced cell death. Reproduced from Yates *et al.*,[23] with permission from Academic Press

memory population that develops as the organism is successively challenged by different antigens.

Our immune memory is carried in part by a population of T cells that remains approximately constant in number from puberty onwards. This memory T-cell pool is a diverse collection of clones (a clone is a number of cells derived from the same ancestor), each of which is specific for a particular antigen. These cells are ready to respond rapidly upon re-encountering their specific antigen and form the basis of lasting immunity. We maintain memory to many pathogens for years or even our lifetime, and yet the lifetimes of the cells that make up our immune memory may be as short as a few weeks.

Homeostatis and immune memory

The mechanisms that maintain our T-cell pools at a constant size are largely unknown. Experiments suggest that memory can persist in the absence of repeated exposure to antigen or cross-reactive stimulation. Other workers have shown that cytokines produced by other cell types may be sufficient to drive the low levels of proliferation necessary to balance cell loss in the memory compartment. Data from patients with depleted T-cell pools (for example, those who have undergone chemotherapy followed by bone marrow transplantation) show that normal homeostatic numbers of T cells can be reconstituted after a year or two.[24] What is the mechanism at play here? In a recent paper[25] we used the observation that proliferating cells, which make up a small minority of memory cells at any time, are susceptible to programmed death or apoptosis by a mechanism dependent on contact between cells (known as activation-induced cell death, or AICD). Proposing a simple model of the memory pool including resting and cycling cells (Figure 5), we predict that the homeostatic level is insensitive to the lifetime of resting memory cells (which may partly account for the diverse experimental estimates of this quantity) and that the dynamics of the memory pool are determined largely by the properties of the minority of dividing cells. Reconstitution times agree well with the data in the literature. Further, individuals with genetic defects in the AICD pathways have greatly increased numbers of memory T cells, also in agreement with the model.

When we extend this simple model to include the multi-clonal structure of memory it predicts that clonal proportions are preserved under fluctuations in the pool size. This appears to be intuitively the simplest solution to the problem of ensuring the preservation of the full range of clones under various perturbations, including the introduction of new clones (caused by infection by hitherto unencountered pathogens) into a memory pool already full to capacity.

These results highlight a recent shift in our perception of cell dynamics. The rate of turnover of many cell types in mammals is relatively high, particularly in the blood: surely there is an energy cost associated with this apparent continual over-production and removal of cells? The answer may be that it allows us to respond rapidly to changes in our environment or to trauma. Regulation of apoptosis may allow rapid expansion or contraction of cell numbers, while rates of proliferation are constrained by the time it takes cells to divide.

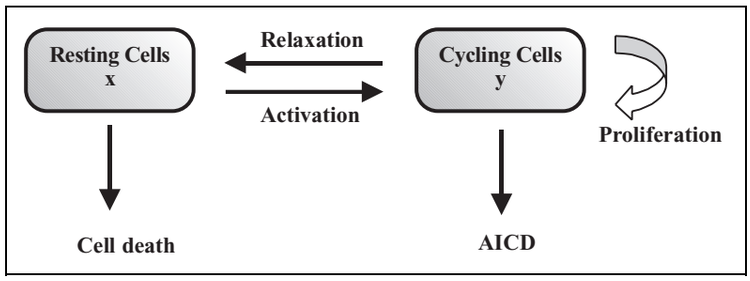

Figure 5: The proposed model for T-cell memory homeostasis, after Yates and Callard.[25] Resting cells become activated by cytokines or other environmental stimuli at rate a, degrade at rate d and are replenished by cells leaving cycle at rate r. Cycling cells divide at rate c and die through contact with other cycling cells at rate f. This leads to the simple rate equations $dx/dt = ry - ax - dx$ and $dy/dt = ax + cy - fy^2 - ra$

Cross-talk between T-cell receptors

Monte Carlo models

Sometimes, the system we are interested in is too complex to easily capture in the form of an ordinary differential equation model. For example, the behaviour of individual agents may be highly complex, or there may be stochastic or heterogeneous spatial elements not easily modelled using differential equations. In such a case, we may still obtain insight into the system using a Monte Carlo simulation. The next model, which has both stochastic and spatial elements, attempts to understand the problem of how T cells discriminate between ligands accurately, and simulates individual T-cell receptors (TCRs) as a Markov chain, with the transition probability between states determined by ligand binding and modifier signals from neighbouring receptors.

Antigen presentation and TCR sensitivity

During an infection, antigen from an infectious organism is captured by an APC, and presented on the cell surface in the peptide groove of major histocompatibility molecules (MHC). The recognition of the peptide–MHC ligand complex by TCR is responsible for maintaining the specificity of the immune response. It is known that 10–200 foreign antigens mixed with perhaps 100,000 self-antigens on the APC are sufficient to trigger T-cell proliferation and effector response.[26] This requires an astonishing degree of accuracy from the TCR which must rapidly discriminate between self and foreign antigens. Even more surprisingly, the TCR discriminates almost exclusively on the basis of the duration of ligand engagement,[27] which is a stochastic event. Previous models of how the T cell achieves its exquisite sensitivity and specificity rely on the concept of kinetic proofreading to explain how TCRs discriminate ligands based on their dissociation time,[28] and the idea of multiple serial encounters by the peptide–MHC complex with different TCR to explain its sensitivity.[29] However, a problem with this scenario is that owing to the stochastic nature of ligand dissociation, the T cell will be very sensitive to both the duration of ligand engagement and the ligand concentration, and it is difficult to see how the T cell can avoid being swamped by false positive signals from the myriad self-antigens presented by the APC.

Recent experiments have documented both positive and negative feedback regulating the response of TCR to ligand.[30] Surprisingly, these feedback effects were not confined to the particular TCR encountering ligand, but appeared to affect neighbouring receptors as well. Encounters of TCR with antagonist ligands (which bind for an intermediate duration) result in recruitment of inhibitor molecules to the receptor's local neighbourhood. Encounters with agonist ligands (which bind for a long duration) result in recruitment of protective molecules to the neighbourhood, which prevent docking of the inhibitor molecules. When we included these neighbour feedback effects in a Monte Carlo simulation of the T cell–APC interface,[31] the model T cell could reliably detect the presence of low densities of foreign peptide with high specificity. Cross-talk between TCR effectively allows the T cell to make more accurate decisions about the nature of the ligands on the APC by pooling information about ligands encountered by different TCR. This observation, coupled with alterations in the degree of receptor cross-talk (especially inhibition) during T-cell maturation, also provides possible solutions to several puzzles in developmental T-cell biology, including how T cells can respond differently to a similar set of antigens presented at different stages, how a single ligand can generate a large T-cell repertoire and why the sensitivity to weak ligands is reduced several hundredfold during T-cell maturation, but the sensitivity to strong ligands remains unchanged. More generally, this study shows how modelling the interactions of the molecules involved in negative and positive feedback with the TCR complex helped reveal its role in

the emergent T-cell properties of sensitivity and specificity.

CONCLUSIONS

Current and future work

There are many avenues still to explore in the projects described above. For example, our models of Th1/2 differentiation are now focusing on the information transferred from infected tissues via APCs to T cells, and how this, along with the dynamics of cytokine and transcriptional factors, influences T helper cell polarisation. In the model of TCR cross-talk, we are exploring the consequences of a more realistic distribution of both self and foreign peptides on the APC; incorporating spatial and mobility constraints governing the interaction of peptide–MHC with TCR; and studying the implications of the model for how altered peptide ligands work. We have found that one has to be prepared to rebuild models from scratch or change focus when new experimental information comes to light, and be rigorous in pursuit of well-defined biological problems.

In summary, our approach is one of close collaboration with experimentalists (ideally with the mathematician actually working in the laboratory) to develop models that can accommodate our uncertainty in our knowledge of the systems we are studying. A good mathematical model will shed light on experimental phenomena and point the way to new experiments. In turn this leads to refinement of the model and the cycle continues.

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References

1. Haurie, C., Dale, D. C. and Mackey, M. C. (1998), 'Cyclical neutropenia and other periodic hematological diseases: A review of mechanisms and mathematical models', *Blood*, Vol. 92, pp. 2629–2640.
2. Shimizu, K. and Gurdon, J. B. (1999), 'A quantitative analysis of signal transduction from activin receptor to nucleus and its relevance to morphogen gradient interpretation', *Proc. Natl Acad. Sci. USA*, Vol. 96, pp. 6791–6796.
3. Callard, R., George, A. J. T. and Stark, J. (1999), 'Cytokines, chaos and complexity', *Immunity*, Vol. 11, pp. 507–513.
4. Segel, L. A. and Lev Bar-Or, R. (1998), 'Immunology viewed as the study of an autonomous decentralised system', in Dasgupta, D., Ed., 'Artificial Immune Systems and their Applications', Springer-Verlag, Berlin, pp. 65–88.
5. Borghans, J. A., Noest, A. J. and de Boer, R. J. (1999), 'How specific should immunological memory be?', *J. Immunol.*, Vol. 163, pp. 569–575.
6. Paul, W. E. (1999), 'Fundamental Immunology', 4th edn, Lippincott-Raven, Philadelphia.
7. Janeway, C. and Travers, P. (2001), 'Immunobiology', 4th edn, Garland, New York.
8. Murray, J. (1993), 'Mathematical Biology', 2nd edn, Springer-Verlag, Berlin.
9. Perelson, A. S. and Weisbuch, G. (1997), 'Immunology for physicists', *Rev. Mod. Phys.*, Vol. 69, pp. 1219–1267.
10. Morel, P. A. (1998), 'Mathematical modelling of immunological reactions', *Frontiers Biosci.*, Vol. 3, pp. 338–347.
11. Barkai, N. and Liebler, S. (1997), 'Robustness in simple biochemical networks', *Nature*, Vol. 387, pp. 913–917.
12. Hartwell, L. H., Hopfield, J. J., Leibler, S. and Murray, A. W. (1999), 'From molecular to modular cell biology', *Nature*, Vol. 402, pp. C47–C50.
13. Oprea, M., van Nimwegen, E. and Perelson, A. S. (2000), 'Dynamics of one-pass germinal center models: Implications for affinity maturation', *Bull. Math. Biol.*, Vol. 62, pp. 121–153.
14. Grakoui, A., Bromley, S. K., Sumen, C. *et al.* (1999), 'The immunological synapse: a molecular machine controlling T-cell activation', *Science*, Vol. 285, pp. 221–227.
15. van den Berg, H. A., Rand, D. A. and Burroughs, N. J. (2001), 'A reliable and safe T cell repertoire based on low-affinity T cell receptors', *J. Theor Biol.*, Vol. 209, pp. 465–486.
16. Rayner, S. A., King, W. J., Comer, R. M. *et al.* (2000), 'Local bioactive tumour necrosis factor (TNF) in corneal allotransplantation', *Clin. Exp. Immunol.*, Vol. 122, pp. 109–116.

17. Chan, C. C. W., Stark, J. and George, A. J. T. (1999), 'Analysis of cytokine network dynamics in corneal allograft rejection', *Proc. R. Soc. B*, Vol. 266, pp. 2217–2223.

18. DsTool (1998), URL: ftp://cam.cornell.edu/pub/dstool/

19. Auto (1997), URL: http://indy.cs.concordia.ca/auto/

20. Content (1997), URL: http://www.can.nl/Systems_and_Packages/Per_Purpose/Special/DiffEqns/Content/GCbody.html

21. Dynamical Systems Software (1997), URL: http://www.maths.ex.ac.uk/~hinke/dss/

22. Feldmann, M. and Maini, R. N. (2001), 'Anti-TNF-α therapy of rheumatoid arthritis: What have we learned?', *Annu. Rev. Immunol.*, Vol. 19, pp. 163–196.

23. Yates, A., Bergmann, C., van Hemmen, J. L. *et al.* (2000), 'Cytokine-modulated regulation of helper T-cell populations', *J. Theor. Biol.*, Vol. 206, pp. 539–560.

24. Godthelp, B. C., van Tol, M. J., Vossen, J. M. and van den Elsen, P. J. (1999), 'T-Cell immune reconstitution in pediatric leukemia patients after allogeneic bone marrow transplantation with T-cell-depleted or unmanipulated grafts: evaluation of overall and antigen-specific T-cell repertoires', *Blood*, Vol. 94, pp. 4358–4369.

25. Yates, A. and Callard, R. (2001), 'Cell death and the maintenance of immunological memory', *Discrete and Continuous Dyn. Sys. B*, Vol. 1, pp. 43–60.

26. Harding, C. V. and Unanue, E. R. (1990), 'Quantitation of antigen-presenting cell MHC class II/peptide complexes necessary for T-cell stimulation', *Nature*, Vol. 346, pp. 574–576.

27. Matsui, K., Boniface, J. J., Steffner, P. *et al.* (1994), 'Kinetics of T-cell receptor binding to peptide/I-Ek complexes: Correlation of the dissociation rate with T-cell responsiveness', *Proc. Natl Acad. Sci. USA*, Vol. 91, pp. 12862–12866.

28. McKeithan, T. W. (1995), 'Kinetic proofreading in T-cell receptor signal transduction', *Proc. Natl Acad. Sci. USA*, Vol. 92, pp. 5042–5046.

29. Valitutti, S., Muller, S., Cella, M. *et al.* (1995), 'Serial triggering of many T-cell receptors by a few peptide–MHC complexes', *Nature*, Vol. 375, pp. 148–151.

30. Germain, R. N. and Stefanova, I. (1999), 'The dynamics of T cell receptor signalling: Complex orchestration and the key roles of tempo and cooperation', *Annu. Rev. Immunol.*, Vol. 17, pp. 467–522.

31. Chan, C., George, A. J. T. and Stark, J. (2001), 'Cooperative enhancement of specificity in a lattice of T-cell receptors', *Proc. Natl Acad. Sci. USA*, Vol. 98, pp. 5758–5763.