Modelling Immunological Systems using PEPA: a preliminary report

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We present preliminary work on modelling aspects of the immune system using process algebra. The problem addressed is how T-helper cell populations respond to co-infections with parasites making conflicting immunological demands. Our goal is to build PEPA models of alternative hypotheses around T-helper cell behaviour and to evaluate those with respect to experimental data.

1 Introduction

Computer Science techniques are increasingly being used to describe biological systems. There are three advantages: the process of formalising the system can lead to deeper understanding through having to make assumptions explicit; formal analysis can be carried out, leading to discovery of previously unknown system properties; and experiments can be carried out *in silico*, usually much more quickly and cheaply than in the laboratory, allowing *in vivo* experiments to be guided towards the most focussed hypothesis tests [7]. Process algebra is one of the modelling methodologies achieving some success in this arena [1, 2, 9, 11] because of its ability to represent systems in an abstract and modular fashion.

Immunological systems are ideally suited to modelling using process algebra. In many ways the immune system is a black box; although many of its inputs and outputs are known, exactly how the system achieves its function is the subject of much investigation. Laboratory experiments have provided large quantities of data, allowing components within the black box to be identified, but there remain many details to be uncovered about the exact details of how components carry out their functions, or on the nature of interaction between components. There are so many potential variables in such systems that exhaustive testing to establish these details is not feasible. Process algebras are ideally suited to describing immunological systems at this level: they may be viewed as networks of (many) interacting components, where the components themselves may have complex, nondeterministic, individual behaviour. In addition, use of process algebra gives access to a range of investigatory techniques, including simulation, verification via logical properties, and transformation from an individual-based discrete state space to a population-based continuous state space via derivation of Ordinary Differential Equations. Through modelling, different hypotheses about the biology of the systems may be investigated, providing focus for future laboratory experiments.

The goal of the current work is the novel application of process algebra to immunological systems. The particular process algebra adopted here is PEPA (Performance Evaluation Process Algebra) [6].

1.1 The Biological Problem

The focus of this work is a particular aspect of how particular components of the immune system, the T-helper cell populations, respond to co-infections with parasites making conflicting immunologi-

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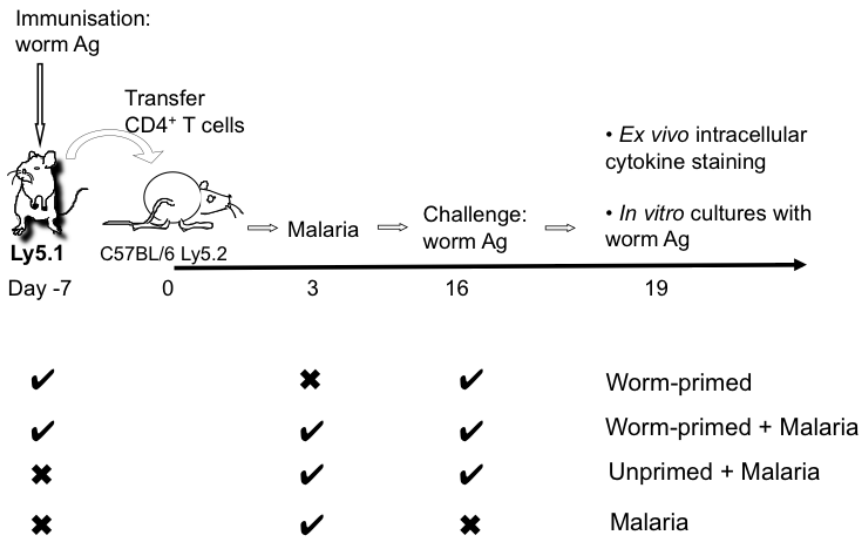

Figure 1: Experimental timeline and groups (from Mahajan et al [8])

cal demands. A full presentation of immunological details is beyond the scope of this paper: see, e.g. Janeway's Immunobiology textbook [10] for a very detailed introduction.

T-helper cells, also known as CD4+ T cells, are central to the modulation of the adaptive immune response. Adaptive immunity is the ability to recognise a pathogen, generate an antigenically tailored response, and remember that pathogen (for a better response next time). There are known to be many different CD4+ cells [13]; here the focus is on T-helper (Th)1 and Th2 cell types. It is now well recognised that the control of helminth infections depends on the presence of CD4+ T cells with a Th2 cytokine profile [3, 4], while most microbial infection as controlled by Th1 CD4+ cells [13]. These contrasting immune responses can interact in helminth-microparasite co-infection and can affect severity and the outcome of disease as Th1 and Th2 responses are mutually inhibitory [5].

Laboratory experiments were carried out [8] to explore the relationship between Th2 responses induced by immunisation with filarial (helminth) antigen and Th1 responses induced by infection with malaria in rodents. One of the questions addressed is the effect of malaria on pre-existing responses to filarial antigens. Essentially, cells were "primed" by exposure to worm antigen and transferred to a congenic mouse, which was exposed to malarial infection, and then given an injection of worm antigen. Figure 1.1 shows this experimental design, with timeline, and with variants to expose the behaviour of the Th1 and Th2 populations.

Experimental results show there is a switch from Th2 to Th1 cytokine profiles. Figure 1.1 gives an indication of Th1 population sizes (defined by production of the cytokine interferon (IFN)-γ) on the left, with Th2 population sizes (defined by production of the cytokine interleukin (IL)-4), on the right. The graph shows multiple results for different filarial antigen. Points indicate particular results, and the lines are the average results. Notice that in mice exposed to malaria infections the level of Th2 falls while the Th1 population increases compared to mice injected with worm antigen only.

It is not known how this change occurs, but there are two alternate hypotheses:

Hypothesis 1: a sub-population of Th1 cells grow out of the Th2 cells.

Hypothesis 2: there is phenotypic switching of individual Th2 cells to Th1 cells

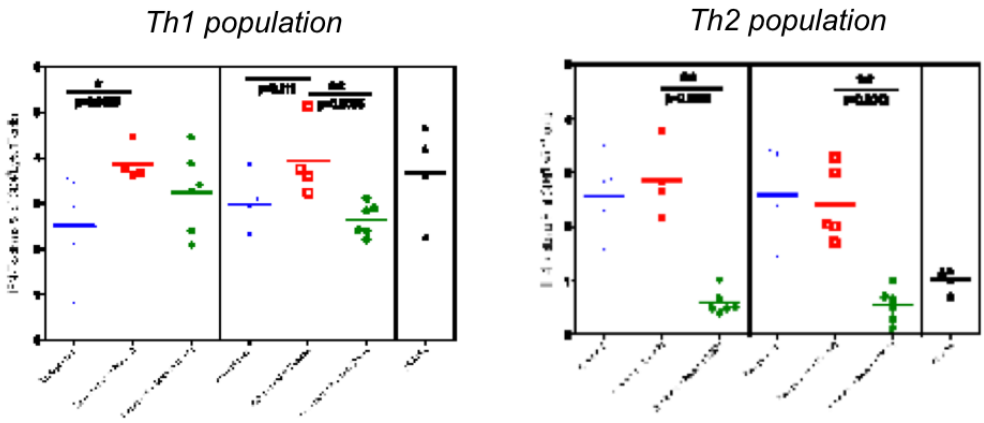

Figure 2: Th1 population (left) and Th2 population (right)

These alternatives can be investigated further using process algebra.

2 PEPA Models

To facilitate description of the event-driven nature of the experiments, models were constructed to represent a single stage of the experiment, e.g. the period between the start of the experiment and exposure to malaria, or the period between malarial infection and the worm boost. The different stages may introduce new behaviour (e.g. switching) or simply change rates to inhibition or promotion of different T-helper cell populations by cytokines such as IL-4 and IFN-γ. Cytokines are not explicitly modelled.

Each experiment is represented by a set of models, run consecutively. In this preliminary work there are four sets of models representing each experimental timeline and group in Figure 1.1. In addition, to investigate the hypothesis above, replicated *in silico* experiments (simulations) were carried out with models incorporating switching behaviour.

The details of the models are not shown here due to space constraints. They may be downloaded from `www.cs.stir.ac.uk/SystemDynamics`.

2.1 Agents

The models are made up of agents which represent cells and the behaviour of the immune system.

There are T-helper cell agents which are either Th1 or Th2. In this model, the functions of Th1 and Th2 are not implemented: the label simply distinguishes the populations. The agents representing Th1 and Th2 have the same range of behaviour. They can recruit naive T cells to become their specific type. This behaviour is controlled by a recruitment rate which will change throughout the timeline depending on the introduction of malarial infection or filarial antigen. T-helper cell agents can divide, producing new T-helper cells with the same type. The division rate is constant and set at one division per week, as suggested by Zhu and Paul [13]. The Th2 cells have a further behaviour of switching which occurs in the Worm-primed + Malaria + Switching experiment only. This is when the Th2 cell switches to become a Th1 cell. This behaviour is controlled by a switching rate (not available from biological data). Zhu and Paul [13] suggest that switching may occur in either direction (Th1 to Th2 and Th2 to Th1), multiple

times. In these preliminary experiments we have restricted our attention to switching from Th2 to Th1 only, with no multiple switching.

Additionally there are naive T cell agents with two recruitment behaviours: they either become Th1 or Th2 cells. This behaviour requires co-operation with the T-helper cell agents of that type. The naive populations throughout the models are set at a level to ensure no resource issues are introduced, i.e. the naive population should never become zero.

2.2 Experiments: initial conditions and model sets

The initial populations of T-helper cells are not available experimentally: instead we are measuring the percentage uplift in the final populations. Two different proportions of initial T-helper cell populations are used (noted in the experimental details below). These distinguish the worm-primed case (20:80 ratio of Th1:Th2) from the unprimed case (50:50 ratio of Th1:Th2).

1. Worm-primed The T-helper cell population is only exposed to the worm antigen.

The initial population of T-helper cells consists of 80% Th2 and 20% Th1. This population takes into account of the first introduction of the worm antigen. The duration of the first stage model is 16 days (384 hours), representing the period in which the populations of Th1 and Th2 grow and recruit at a background level. There is no exposure to Malaria in this model. The resulting population becomes the initial population of the second stage model. The second stage model corresponds to second introduction of worm antigen. The duration of the second stage model is 3 days (72 hours).

The worm-primed experiment population results are used as a base against which we measure the increase/decrease in population of Th cells in the other experiment models.

2. Worm-primed + Malaria This represents when the T-helper cell population is exposed to the worm antigen and malaria infection.

The initial population of T-helper cells consists of 80% Th2 and 20% Th1, as in experiment 1. The duration of the first stage model is 3 days (72 hours), representing background growth and recruitment in Th populations. The second stage model introduces malaria. Practically this is done by increasing the recruit rate for Th1. The duration of the second model is 13 days (312 hours). The third stage model corresponds to the second exposure to worm antigen. The duration of the third model is 3 days (72 hours).

The population results are compared with the worm-primed population results and rates adjusted to be consistent with biological experimental results as seen in Figure 1.1 :-

1. the Th1 population should increase by approximately 55.2% - 57.6%. We are using the two sets of experiments (with different filarial antigen) to provide a range of acceptable results.

2. the Th2 population should either increase by approximately 14.8% or a slightly decrease by 3.2%

3. Worm-primed + Malaria + Switching This repeats experiment (2) and introduces the switching behaviour of the Th2 agent in the second stage model only. The population results are compared with the worm-primed population results as above.

In experiments (2) and (3) the results assist in assessing the two suggested hypotheses (see Section 3).

4. Unprimed + Malaria This represents when the T-helper cell population is only exposed to Malaria and thereafter exposed to the worm antigen.

The initial population of T-helper cells consists of 50% Th2 and 50% Th1. This population takes into account no exposure to the worm antigen or malaria. The duration of the first stage model is 3 days (72 hours), representing background growth and recruitment in Th populations. The second stage model introduces malaria. The duration of the second model is 13 days (312 hours). The third model is introduced due to the first introduction of the worm antigen, therefore different levels of response are encoded in the recruitment rates here (primary exposure rather than secondary exposure). The duration of the third model is 3 days (72 hours). The population results are compared with the worm-primed+malaria population results.

Expected results with comparison to experiment (1) and to mirror biological experimental results :-

1. the Th1 population should increase by approximately 5.6% - 30%
2. the Th2 population should show a significant decrease approximately by 76% to 78%.

3 Analysis and Discussion

The PEPA-Eclipse plugin has been used to simulate the models. The goal of the simulations was to determine the rate of cell growth required under Hypothesis 1, or the rate of Th2-Th1 switching required under Hypothesis 2, that was consistent with empirical data. Note that the sample size of the experimental data is quite small (as seen on Figure 1.1) while we are able to reduce stochastic effects by running repeated simulations and using average population sizes. The output of the PEPA models was hand calibrated initially against the Worm-primed results. The experimental data we have is proportions of Th1 versus Th2 cells in samples taken from mice on day nineteen of a nineteen day experiment (see Figure 1.1).

This produces rates as follows for the worm-primed plus malaria case:

rate description	Hypothesis 1	Hypothesis 2
Th1 recruitment	0.021	0.014
Th2 recruitment	0.021	0.014
malaria contact	0.87	1
worm Ag contact	1	1
switching (Th2 $\rightarrow$ Th1)	0	0.001

It must be noted that many more simulations over a range of possible parameters are still to be explored. Our biological collaborators have indicated that the rates above are not ridiculous; however, there are no confirmatory experimental data available for this congenic system. Further laboratory experiments are required to try to verify the rates suggested above, and relevant laboratory techniques are increasingly available [13].

Our main conclusion at this stage is that much further work is required from this initial starting point. There are many interesting further avenues to explore. The first is to enhance the biological realism of the models. For example, would it be beneficial to include cytokine details explicitly in the model, so that the mutually inhibitory effects of Th1 and Th2 are included directly? Might we want to include spatial aspects in the model, to reflect that recruitment is based on proximity?

We also have experimental data for a slightly different set of experiments, including an additional exposure to worm antigen. Models should be constructed to match this.

There are other investigatory techniques which can be explored. Currently we have only used simulation to produce time series data for the models. For example, it would be instructive to compare the ODEs derived from our models to those proposed by Yates et al [12]. Currently our models are too simplistic to make such a comparison feasible.

Lastly, and most excitingly, the use of process algebra models allows the possibility that we can construct models following completely different experimental design. For example, modelling the situation with an initial malarial infection (or antigen) followed by worm infection. This could then be followed up by wet lab experiments. Our hope would be that these would confirm the *in silico* results, supporting our aim that models are a true representation of the behaviour and function of the immune system.

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