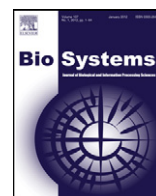




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## *In silico* investigation of novel biological pathways: The role of CD200 in regulation of T cell priming in experimental autoimmune encephalomyelitis

Richard B. Greaves<sup>a,b,\*</sup>, Mark Read<sup>b,c</sup>, Jon Timmis<sup>a,b,c</sup>, Paul S. Andrews<sup>a,b</sup>, James A. Butler<sup>a,b,d</sup>, Bjorn-Ole Gerckens<sup>a,b,d</sup>, Vipin Kumar<sup>e</sup>

<sup>a</sup> Department of Computer Science, Deramore Lane, University of York, UK

<sup>b</sup> York Centre for Complex Systems Analysis (YCCSA), Deramore Lane, University of York, UK

<sup>c</sup> Department of Electronics, University of York, Heslington, York, UK

<sup>d</sup> Centre for Immunology and Infection, Department of Biology, University of York, Heslington, York, UK

<sup>e</sup> Laboratory of Autoimmunity, Torrey Pines Institute for Molecular Studies, La Jolla, USA

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### ABSTRACT

The use of simulation to investigate biological domains will inevitably lead to the need to extend existing simulations as new areas of these domains become more fully understood. Such simulation extensions can entail the incorporation of additional cell types, molecules or molecular pathways, all of which can exert a profound influence on the simulation behaviour. Where the biological domain is not well characterised, a structured development methodology must be employed to ensure that the extended simulation is well aligned with its predecessor. We develop and discuss such a methodology, relying on iterative simulation development and sensitivity analysis. The utility of this methodology is demonstrated using a case study simulation of experimental autoimmune encephalomyelitis (EAE), a murine T cell-mediated autoimmune disease model of multiple sclerosis, where it is used to investigate the activity of an additional regulatory pathway. We discuss how application of this methodology guards against creating inappropriate simulation representations of the biology when investigating poorly characterised biological mechanisms.

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### 1. Introduction

Computational simulation offers a complement to wet-lab techniques by permitting experiments *in silico* that would be difficult or impossible to conduct otherwise (Read et al., 2012). Mechanistic simulations that explicitly represent cells, molecules and spatial compartments permit investigation of cause and effect within biological systems, offering not only a description of key actors in the system, but also of the complex cascade of effects derived from their interactions. In this way, simulation reveals how micro-level events influence macro-level phenomena at the organism level.

In this paper, we develop a methodology that will permit the implementation of extensions within an existing simulation

of the murine immune system in the experimental autoimmune encephalomyelitis (EAE) disease state (Read, 2011). Simulation is increasingly employed in immunology and numerous simulation paradigms have been utilised to address a range of research questions (Forrest and Beauchemin, 2007).

Arguably the most well-established form of biological modelling has been performed using systems of ordinary differential equations (ODEs), in which the population dynamics of cells, molecules and pathogens are represented by equations which describe population expansions, for example by cell proliferation; and contractions such as those due to apoptosis. Prominent studies include the modelling of viral dynamics during HIV-1 infection (Perelson, 2002; Perelson and Nelson, 1999; de Boer et al., 2010) and immune response to influenza (Wu et al., 2011). Cellular automata (CA) have also been employed in modelling the immune system (Kleinstein and Seiden, 2000; Seiden and Celada, 1992). CA describe a system as a lattice of interconnected grid points that each have state, and where the state of a grid point at time  $t + 1$  is dependent on the state of that grid point and of its neighbours at time  $t$ . Cellular Automaton models lack the scale of the real immune system and, along with ODE models, also the true

\* Corresponding author at: Department of Computer Science, Deramore Lane, University of York, UK. Tel.: +44 1904 328912; fax: +44 1904 500159.

E-mail addresses: [richard.greaves@york.ac.uk](mailto:richard.greaves@york.ac.uk) (R.B. Greaves), [mnr101@ohm.york.ac.uk](mailto:mnr101@ohm.york.ac.uk) (M. Read), [jon.timmis@cs.york.ac.uk](mailto:jon.timmis@cs.york.ac.uk) (J. Timmis), [psa@cs.york.ac.uk](mailto:psa@cs.york.ac.uk) (P.S. Andrews), [jab@cs.york.ac.uk](mailto:jab@cs.york.ac.uk) (J.A. Butler), [bjorn@cs.york.ac.uk](mailto:bjorn@cs.york.ac.uk) (B.-O. Gerckens), [vkumar@tpims.org](mailto:vkumar@tpims.org) (V. Kumar).

spatial and temporal representation offered by non-CA agent-based models. Agent-based models (ABM) that permit spatial and temporal description of cell behaviour, and the explicit modelling of the behaviour of individual cells, are finding increased application in a wide variety of immunological contexts. For example, ABM have been used in modelling the epitheliome (Walker et al., 2004), granuloma formation in the lung in response to tuberculosis infection (Segovia-Juarez et al., 2004), thymocyte development in the thymus (Efroni et al., 2007), cytokine roles during TB infection (Marino et al., 2010; Ray et al., 2009), the determinants of effective immune response to *Leishmania major* infection (Dancik et al., 2010) and the formation of Peyer's patches in the intestine (Alden et al., 2012).

However, despite its obvious utility, all simulation is based upon abstraction of the system of interest and encodes the models of biological understanding created via this abstraction. It is this very abstraction that makes the simulation conceptually and computationally tractable. The quality of the resultant simulation model is inherently dependent on the state of knowledge in the biological domain, meaning that it is difficult to create simulations that adequately capture biological processes if these are poorly characterised.

In the post-genomic age, knowledge acquisition in biology has been particularly intense, meaning that ideas of how certain systems work are constantly being refined by the addition of finer detail. This refinement therefore entails a continual characterisation of the domain. This could involve, for the sake of argument, the implication of a new cell type in a regulatory mechanism of interest or even the description of an entirely novel regulatory pathway. There will, therefore, be a need to integrate new knowledge into existing simulations in order to investigate it and to understand its implications for biology. However, this presents potential problems for simulation that has already been rigorously calibrated as the components of the existing simulation represent not only their own behaviour but must also compensate for that of elements of the system not included in the model. If some of these previously omitted elements are now included in the simulation, this will perturb its behaviour. This could potentially entail the complete re-parameterisation (recalibration) of the simulation. However, caution needs to be exercised as the abstraction of a badly characterised pathway may not be correct. It is therefore important that we not only explore the effect of tuning simulation parameters, but also explore multiple models of the added components when developing extensions to existing simulations. Such a rigorous approach to extension development helps ensure that behaviours implemented in the simulation appropriately capture the biology and that baseline simulation behaviours are re-established.

To this end, a methodology for the extension of existing simulations is developed. A newly identified pathway has been implicated in the regulation of autoimmunity in murine experimental autoimmune encephalomyelitis EAE, a murine model of multiple sclerosis (Feuer, 2007; Minas and Liversidge, 2006). However, the mechanisms of action of this pathway are unclear. Therefore, we wish to investigate the different potential mechanisms whereby the pathway may operate. We do this by addition of different representations of the pathway to an existing agent-based simulation of EAE.

The proposed methodology for developing extensions to existing simulations is presented here informally and is discussed in greater detail in Section 4. It consists of three main steps, which are intended to be followed in an iterative manner. The stages in the methodology relate to major concerns in simulation development and are outlined as follows:

- A phase of explicit domain modelling (Andrews et al., 2010) which identifies key actors in the extended system, their relevant behaviours and their relevant interactions with each other

and with components of the existing simulation. The resultant model is implemented in the existing simulation.

- Exploration of the effects of the added mechanism and its parameters.
- Re-assignment of simulation parameter values (recalibration) if needed.

Throughout the paper, we discuss and further develop this methodology in terms of its implications for the existing simulation, a fuller justification of this being presented in Section 4.1. The methodology is developed and demonstrated through a case study focused on the use of simulation to gain deeper understanding of the role of a recently identified regulatory pathway in EAE.

The remainder of the paper is organised as follows. Section 2 provides further background to the work undertaken including a brief overview of the case study simulation and the statistical methods used to explore the simulation parameter space. Section 3 presents the biological domain being explored and how it has been represented in simulation. Section 4 describes the methodology in full, and its application in the present case study. Section 5 evaluates the methodology and concludes the paper.

## 2. Materials and methods

### 2.1. The case study: ARTIMMUS

The biological domain of interest used throughout this paper is experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis, in which mice undergo spontaneous physiological recovery following induction of autoimmunity (Kumar and Sercarz, 2001; Kumar, 2004). The present case study involves ARTIMMUS (Read, 2011), an agent-based simulation of EAE developed and calibrated using a principled methodology that demonstrated it to be a faithful representation of EAE.

ARTIMMUS was developed using explicit domain modelling (Andrews et al., 2010) which provides a principled framework for the development of complex system models and simulation, promoting trust in the simulation and the results emerging from it. In following the process of explicit domain modelling, specific models are created to address specific research questions in a specific domain. The simulation has undergone iterative calibration in which simulation development is guided by continual improvements in the alignment of simulation and *in vivo* behaviours. By following this process, the abstractions and assumptions made during simulation development remain scientifically grounded and are supported by empirical evidence of the appropriateness of their capture of the domain (Read, 2011).

We have identified an additional regulatory pathway believed to be influential in regulating autoimmunity in EAE (Fanchiang et al., 2012). The key cell types involved in this pathway were identified from the literature (Feuer, 2007), but the pathway is otherwise not well characterised. The present case study entails investigating the potential mechanisms through which this pathway regulates autoimmunity in ARTIMMUS.

#### 2.1.1. Assessing simulation behaviour

Extended simulation dynamics are contrasted with the calibrated baseline through various metrics (termed 'responses'), relating to the four T cell populations responsible for autoimmunity and recovery (see Section 3), and the level of autoimmunity experienced (Fig. 1) The sizes of each T cell population expansion (CD4Th1, CD4Th2, CD4Treg and CD8Treg) and the time at which these peaks occur are measured. The number of CD4Th1 cells, which are responsible for autoimmunity, residing in the simulation at 40 days is also measured. These cells are typically no longer present at 40 days in the baseline simulation.

Two further responses are based on the autoimmune severity scores used to grade the extent of disease in laboratory animals. These scores lie on a range of 0 (animal is showing no clinical symptoms of EAE) to 5 (animal is dead) (Kumar et al., 1996), and can vary considerably between individual animals. We measure the maximum scores attained in each simulation, and the score at day 40. Details of how these scores have been fitted in ARTIMMUS can be found in (Read, 2011).

When generating the response landscapes presented in Section 4.2, the median responses across 500 simulation runs were used to assess simulation behaviour.

#### 2.1.2. Statistical methods/analysis

Statistical significance of results is established using the Vargha–Delaney A-test, a non-parametric effect size measure. The A-test scores indicate the probability that a value drawn at random from distribution A will be greater than a value drawn at random from distribution B. The score lies between the values 0.0 and 1.0, with the extreme scores indicating that two distributions are significantly different and 0.5 indicating that the distributions are not statistically different. We assume A-test

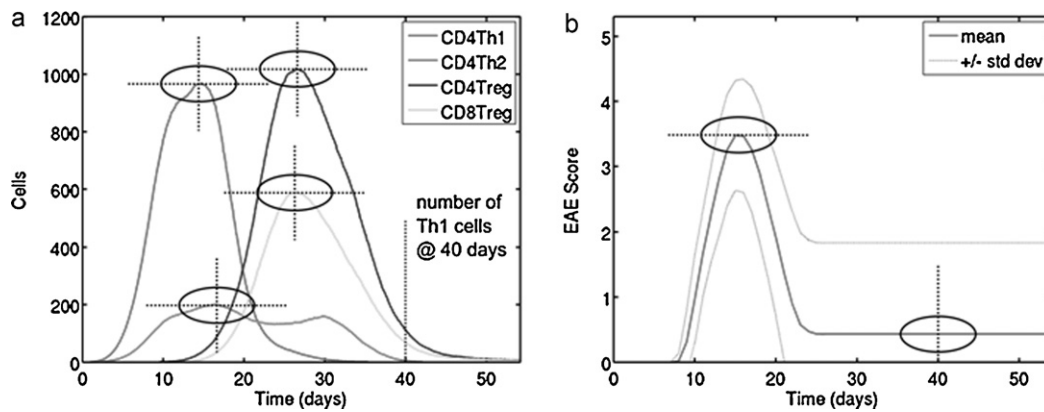


Fig. 1. The responses used to document key aspects of simulation behaviour. (a) T cell effector populations; (b) EAE severity scores.

scores greater than 0.71 or less than 0.29 to indicate scientifically significant effects, in line with the suggested score thresholds (Vargha and Delaney, 2000).

## 2.2. Methods for exploring simulation parameter space

### 2.2.1. Robustness analysis

Robustness analysis is employed to assess the robustness of a simulation to perturbations in the values of individual simulation parameters. During analysis, the parameter of interest is perturbed whilst all other parameters are held at their baseline values. The A-test (discussed in Section 2.1.2) is then used to calculate the effect size of the perturbation in the parameter value on the simulation (full details of the analysis may be found in Read et al., 2012).

### 2.2.2. Global sensitivity analysis

Sensitivity analysis is a form of statistical analysis that attributes variation in system outputs to variation in its inputs. We employ the latin hypercube of McKay et al. (1979), to perform an efficient sampling of parameter space, as a full exploration would be computationally intractable. Simulation parameters are adjusted simultaneously as dictated by the latin hypercube design, thereby revealing compound effects where the influence of one parameter is dependent upon the value of another.

### 2.2.3. Factorial analysis

To fully explore the behaviour of ARTIMMUS over the complete parameter space would be computationally intractable. However, the factorial analysis is a specific instance of a global sensitivity analysis, in which simulation behaviour can be tested against perturbations in a very small set of parameters rather than in all of the simulation parameters.

Simulation responses can be mapped across all combinations of parameter values if there are only very few parameters whose effects we wish to explore. The result could be presented as a three-dimensional response landscape, if for example, one were exploring the effects of just two simulation parameters (see Section 4.2).

## 3. The biological model of the simulation

### 3.1. Experimental autoimmune encephalomyelitis

Experimental autoimmune encephalomyelitis (EAE) is an experimentally induced T cell-mediated autoimmune disease that serves as an animal model of multiple sclerosis (Lublin, 1985). The disease is characterised by damage to the myelin sheath, which coats nerve fibres, resulting in impaired conduction of impulses along the fibres and leading ultimately to paralysis and death. In EAE damage to the myelin sheath is mediated by CD4Th1 cells that are reactive towards various myelin components, for example myelin basic protein (MBP) (van den Bark et al., 1985).

The simulation case study is focussed on the murine model of EAE (Kumar and Sercarz, 2001; Tang et al., 2005). This disease model addresses the mechanisms of spontaneous recovery from EAE which is highlighted as dashed arrows in Fig. 2.

#### 3.1.1. Induction of disease

The disease cycle is informally represented by the circuit of heavy black arrows in Fig. 2. Disease is induced by inoculation of laboratory animals with MBP emulsified in complete Freund's adjuvant (CFA) and followed by injections with pertussis toxin (Pender, 1995). This elicits an aggressive immune response by stimulating dendritic cells (DCs) sufficiently that they can express co-stimulatory molecules. DCs present MBP-derived peptides by phagocytosing (internalising), processing and presenting them as complexes with MHC molecules on their surface. The DCs then migrate to the secondary lymphoid organs (lymph nodes) where they encounter naïve MBP-recognising CD4 T-helper cells, which they bind and activate, allowing the auto-reactive CD4Th1 to proliferate and mature into effectors.

Activated auto-reactive T cells can then migrate through the blood–brain barrier into the CNS. Once inside the CNS, the activated MBP-reactive CD4 Th1-cells set up an inflammatory environment that stimulates microglia and macrophages to secrete tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ). TNF- $\alpha$  causes neuronal death and subsequent release of MBP. Phagocytosis of the released MBP by macrophages leads to the presentation of MBP antigens to further naïve, auto-reactive CD4Th1, which have arisen due to CD4Th proliferation in the cervical lymph nodes. This perpetuates the disease cycle.

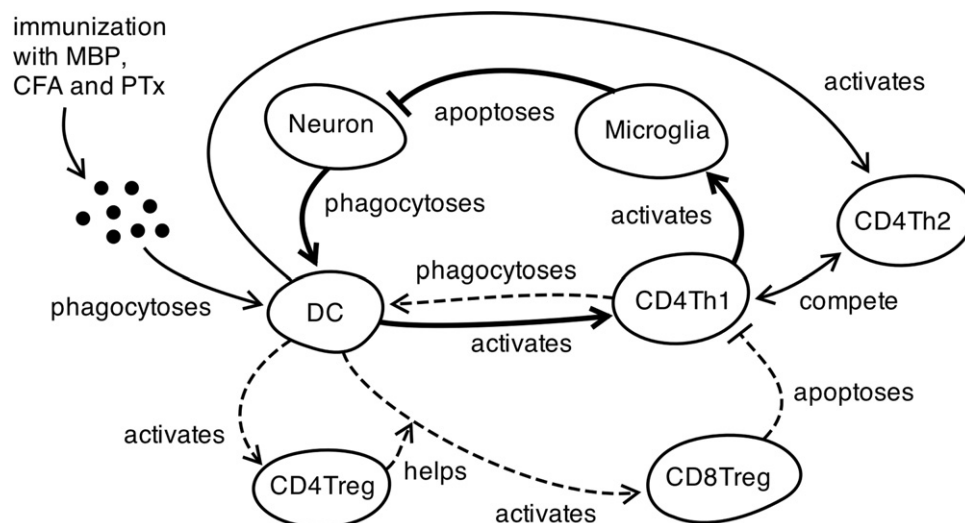
#### 3.1.2. Spontaneous recovery from EAE

Auto-reactive CD4Th1 will ultimately undergo programmed cell death (apoptosis) upon reaching the end of their lifecycle. Apoptotic CD4Th1 can leave the CNS and be phagocytosed by dendritic cells in the lymph nodes (Pender, 1995), which leads to presentation of antigens derived from their T cell receptors (TCRs) by these DCs. TCR fragments when presented on DCs activate the regulatory T cell populations (Kumar and Sercarz, 2001; Tang et al., 2005). Activated CD8Treg are capable of recognising and binding TCR-antigen presented on the surface of auto-reactive CD4Th1 (Kumar and Sercarz, 2001) and kill these by inducing apoptosis (Beeston et al., 2010). This cell-mediated killing serves to regulate the autoimmune response and the population of self-reactive CD4Th1 is thus reduced. Spontaneous recovery from EAE and the return of cellular populations to their resting levels is essentially complete within 50 days (Pender, 1995). This behaviour is reflected in the ARTIMMUS results presented in Fig. 3.

#### 3.1.3. The CD200 pathway

The recently identified regulatory pathway which will be investigated is thought to regulate autoimmune response by reducing the capacity of DCs to stimulate T cell populations. DCs are potent activators of all T cell sub-populations, and so any reduction of their





**Fig. 2.** The cells implicated in the EAE disease and spontaneous recovery cycles and their inter-relationships. The disease cycle is highlighted in thick black lines, the recovery cycle in dashed lines. The thin, solid black lines represent interactions that are part of neither cycle. Figure reproduced from Read (2011).

ability to bring this about could significantly reduce the scale of an immune response.

CD200 and CD200R are cell surface proteins, CD200R being the receptor for the CD200 ligand (Gorczynski et al., 2004). CD200 is expressed on a variety of cells particularly T cells in the immune system and also on neurons in the CNS (Liu et al., 2010). We have shown CD200 to be expressed constitutively on the CD8Treg population responsible for recovery from EAE (Fanchiang et al., 2012). CD200R is similarly widely expressed; microglia and DC being able to express this receptor (Liu et al., 2010). We are interested in DC–T cell priming and so, in line with our iterative development methodology, we begin with a simple model in which only T cell–DC interactions outside the CNS are considered. Upon engagement of CD200R by CD200, a signal is transmitted via the receptor, causing the receiving DC to down-regulate production of certain proteins that are essential to the binding and activation of T cells (Gorczynski

et al., 2004). There now exists considerable experimental evidence for the suppressive effect of CD200 on DC and macrophages (Liu et al., 2010).

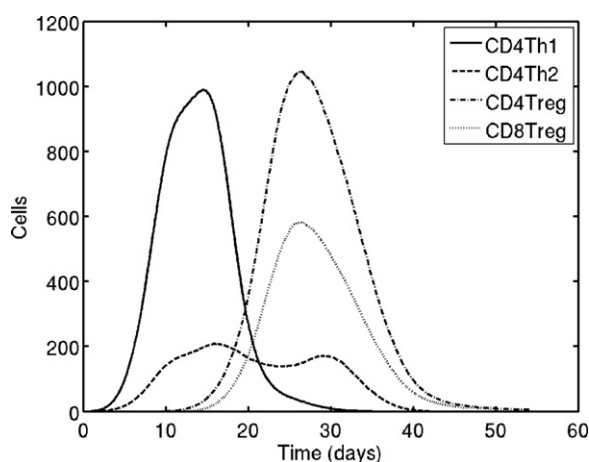
The mechanisms by which Treg-expressed CD200 exerts a regulatory influence on immunogenic DC are not fully characterised. However, the literature does suggest possibilities for exploration beyond MHC and co-stimulus down-regulation. For example, it has been proposed that CD200–CD200R interaction can bring about a change in the cytokine expression profile of the CD200-signalled DC, in effect shifting the cytokine expression from that favouring differentiation of T cells to CD4Th1 cells to one favouring differentiation to CD4Th2 (Jenmalm et al., 2006). This occurs via a down-regulation of the secretion of pro-inflammatory cytokines, IFN- $\gamma$  and IL-17 (Jenmalm et al., 2006) or TNF- $\alpha$  (Minas and Liversidge, 2006), by the negatively signalled DC.

There is also a considerable body of evidence that tryptophan catabolism plays an important immune-modulatory role. Mellor and Munn (1999) observed that cells expressing the enzyme indoleamine 2,3 dioxygenase (IDO), an enzyme involved in tryptophan catabolism, protected the mammalian foetus from maternal T cell attack. Further suggestion of IDO involvement in T cell regulation was provided by the demonstration that IDO plays a role in recovery from EAE (Sakurai et al., 2002). Further studies have shown that IDO expression by macrophages and DCs is stimulated by a variety of cell surface receptor–ligand interactions, including CD200R engagement by CD200, leading to tryptophan catabolism (Fallarino et al., 2004; Minas and Liversidge, 2006; Puccetti and Fallarino, 2008). Tryptophan catabolism serves to inhibit T cell proliferation (Grohmann et al., 2002; Sakurai et al., 2002; Fallarino et al., 2004) and there is some evidence that it also leads to T cell apoptosis (Fallarino et al., 2002; Grohmann et al., 2003).

### 3.2. Modelling the CD200 pathway

In line with the principles of explicit domain modelling (Andrews et al., 2010) the modified behaviours of DC and CD Treg in the simulation are documented in state machine diagrams. These are presented for baseline and extended behaviours of the simulation in Appendix A.

We present several models of the CD200 pathway behaviour, corresponding to two separate iterations of the design methodology. In line with the methodology, the models implemented are



**Fig. 3.** Simulation populations of effector T cells over time. The curves present simulation dynamics averaged over 500 simulation runs and clearly reflect T cell dynamics in the infected mouse: cell populations begin at their resting levels at day 0 when immunisation occurs. The populations of the CD4Th cells then begin to rise, peaking at around 10–15 days, with the CD4Th1 population peaking considerably higher than CD4Th2. By this time, the populations of Tregs will have begun to increase and these will peak around 27–30 days with CD4Treg attaining a higher population than CD8Treg. Recovery from EAE will now be effectively complete and cell populations will fall back to their resting levels (Read, 2011).

kept simple, permitting complexity to be added only when our methodology indicates that this is necessary. We are primarily interested in CD200 effects on T cell priming and so do not represent CD200(R) in the CNS in any of the models implemented. All the models discussed in this section allow for the immediate expression of CD200 by effector CD8Treg with no requirement for local activation. Expression of CD200R by DCs occurs immediately upon their maturation. A down-regulatory CD200–CD200R signal to DCs is assumed to be received whenever DCs come into contact with CD8Treg in simulation space.

The baseline simulation captures recovery and regulation in one pathway, which implicitly accounts for the actions of CD200-mediated regulation which is not explicitly captured. In the extended simulations where behaviour of the CD200 pathway is explicitly captured, the same dynamics of recovery must be provided by two pathways, hence the need for re-balancing the effects of the two regulatory mechanisms.

Since the pathway is poorly characterised it is not possible to obtain values from the literature for the parameters introduced in the new models. As parameters need to be adjusted to compensate for the presence of the new pathway whilst maintaining baseline behaviour, it is necessary to conduct a full analysis of the effects of the parameters introduced by extending the model (Greaves et al., 2012). A factorial analysis of simulation behaviour (see Section 2.2.3) is conducted across the full range of values for both of the added parameters in the first iteration modelling (see Section 3.2.1). The second iteration models (see Section 3.2.2) were explored using a sensitivity analysis across the added parameters relevant to each model.

### 3.2.1. A first iteration model of the CD200 pathway

In this model (see Appendix A for the relevant DC and CD8Treg state machine diagrams), receipt of a down-regulatory (or ‘negative’) signal by an individual DC, triggers that DC to probabilistically down-regulate the expression of Qa-1 and MHC-II molecules (together referred to as MHC) and/or that of co-stimulatory molecules (referred to as CoStim) (Greaves, 2011).

The stipulation that negative signalling probabilistically down-regulates MHC and/or CoStim expression by individually signalled DCs introduces two new parameters into our model: the probability that a negative signal will down-regulate MHC expression and also the probability that negative signalling will down-regulate CoStim expression by DCs; these down-regulatory events occurring either together or separately. We require appropriate values for these new parameters, and these must be defined such that the extended simulation will still produce baseline behaviour.

### 3.2.2. A second iteration model of the CD200 pathway

Further models of immune-regulation are explored in a second iteration of the methodology. These focus on the ideas of tryptophan catabolism and cytokine switching discussed in Section 3.1.3.

Tryptophan catabolism has the effect of reducing the capacity of individual DCs to prime T cells following a negative signal. Initially, mature DCs have the capacity to prime any T cell that binds to them. In the extended model, there is a gradual reduction in the priming capacity of individual DCs by some percentage with each negative signal received.

In ARTIMMUS, cytokines are abstracted as type 1 and type 2 cytokines. Type 1 cytokines are pro-inflammatory cytokines that promote CD4Th differentiation to Th1 cells, with type 2 promoting differentiation to CD4Th2. Cytokine switching is implemented as a complete disabling of type 1 cytokine secretion by negatively signalled DC, thus reducing the concentration of CD4Th1-inducing cytokines surrounding DCs and favouring a type 2 deviation in T cells primed around signalled DCs.

## 4. Results and discussion

### 4.1. A methodology for extending existing simulations

An iterative development procedure based on the principles of explicit domain modelling (Andrews et al., 2010) is employed to ensure that the models developed adequately capture current domain understanding. This section discusses the three principal phases of the methodology and how they integrate to facilitate the development of an extended simulation whose results are consistent with results obtained from the calibrated baseline (unextended) simulation.

#### 4.1.1. Explicit domain modelling and implementation

Simulation implementation is preceded by the construction of models that capture current understanding of the biology (domain) and facilitate the collaborative validation of that understanding. This is important as it provides confidence that simulation based upon these models, is an accurate and appropriate description of the biology (Read et al., 2009a, b). The process of domain modelling can also serve the useful purpose of raising further questions of the domain where there is lack of clarity in biological understanding (Read et al., 2009b).

When characterising descriptions of extensions to simulations there may be little in the literature to guide the development of a more sophisticated model. Therefore, development commences with the design of a very simple extension to the model. This avoids unnecessary complexity and is aligned with the ideas of explicit domain modelling discussed above. The developmental phase is guided by consultation of the literature to discover potential mechanisms through which the biological pathways operate. Domain modelling the possible mechanisms of operation for the simulation extensions ensures that they are understood and that they are reasonable abstractions of the biology. Only once this is achieved should we begin to implement the abstraction into the simulation.

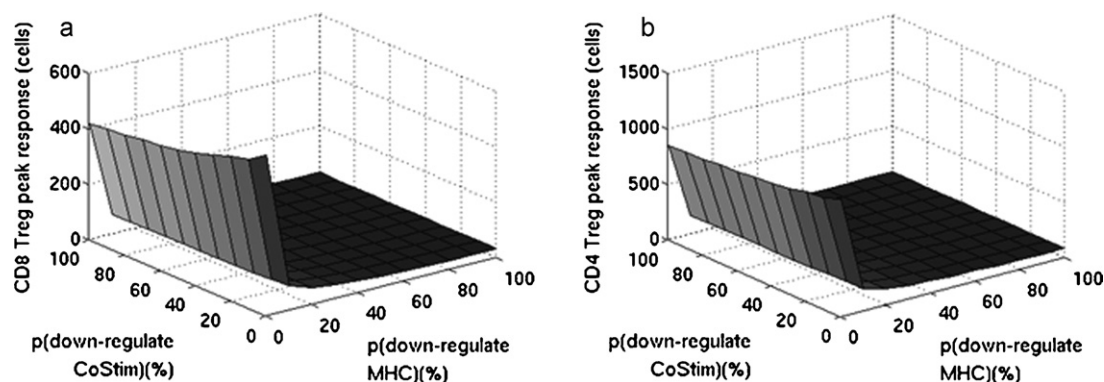
The abstract nature of simulation entails that parameters do not represent exactly the same thing as the corresponding *in vivo* values as they must compensate for other pathways and components that are omitted. This means that when we add details to the simulation that were previously omitted from it, these additions will perturb the behaviour of the original components. A full re-parameterisation (recalibration) of the simulation may be necessary in order to re-establish baseline simulation behaviour. The proposed methodology guards against adopting an inappropriate representation of the domain by evaluating the simple model before deciding whether it is an adequate description of the system. This evaluation is performed through the use of parametric investigation such as sensitivity analyses (described in Section 2.2).

Before a representation of the domain is accepted, it is important to understand as fully as possible the effects that any changes made to the simulation have on its behaviour. Therefore, complexity should be built into the model gradually. In this instance, this is done by implementing the simple representations of the system described in Section 3.2 in the case study simulation, ARTIMMUS. This will facilitate our subsequent exploration of the added CD200 pathway.

#### 4.1.2. Exploration of the effects of the new representation

Addition of novel features to the representation of the domain will inevitably introduce new parameters and these will need to be assigned values. Additionally, existing parameters may need amendment to compensate for the additions to the simulation that were previously abstracted.

Therefore, once the extensions to the simulation are implemented, it is important to explore the effects of any new simulation parameters introduced to the model. One way of doing this is by



**Fig. 4.** Three-dimensional 'response landscape' of (a) peak CD8Treg effector populations and (b) peak CD4Treg effector populations from a factorial analysis of simulation behaviour at values of the two probability parameters between 0% and 100% in steps of 10%.  $p(\text{down-regulate MHC})$  and  $p(\text{down-regulate CoStim})$  represent the probabilities that a single negative signal will down-regulate MHC and CoStim expression respectively by the signalled DC. The baseline simulation is represented as the pairing 0%, 0% in the bottom middle of the plot.

using a global sensitivity analysis employing an efficient sampling technique, for example latin hypercube sampling (McKay et al., 1979). However, if very few new parameters are introduced, it will be possible to explore simulation behaviour over the full range of values of any parameters introduced to the model, referred to here as factorial analysis (Saltelli et al., 2000; see also Section 2.2.3).

The aim of this exploration is to understand how the extended mechanism operates, and based on this, judging whether a full re-parameterisation can re-establish the baseline simulation behaviour. Should this not be possible then it is probable that the representation of the added pathway is inappropriate and should therefore be rejected. However, should re-balancing prove possible, then recalibration (reassignment of all parameter values) of the simulation may still be necessary to exactly restore baseline behaviour. This forms the basis of stage c of the proposed methodology.

#### 4.1.3. Recalibration of simulation

Ultimately the simulation ought to be fully recalibrated to re-establish baseline behaviour. If the extended simulation is judged to be a realistic and representative description of the biology, then one can proceed with recalibration of the simulation. Recalibration prior to this stage risks acceptance of a flawed model which might entail a simulation that is no longer either robust or representative of the domain.

#### 4.2. Results from iteration one of the modelling cycle

In order to explore the effect of the MHC and/or CoStim down-regulation by negatively signalled DC (described in Section 3.2), it is necessary to map the effect of the two probability parameters introduced in this description of the pathway on simulation behaviour. This mapping is performed using a factorial analysis (see Section 2.2.3).

The factorial analysis mapped simulation behaviour for values of the two introduced probability parameters between 0% and 100% in steps of 10%, entailing a mapping of 121 separate simulations with distinct pairings of probability parameter values. Simulation behaviour was summarised in terms of the responses described in Section 2.1.1.

With even a 10% probability that MHC expression could be reduced via negative signalling, there was a considerable effect on T cell effector populations. Both CD8Treg and CD4Treg peak populations were significantly reduced compared to the baseline (data presented in Fig. 4, Supplementary Material). The CD4Th1 peak population was increased by increasing either parameter, though not significantly (data presented in Fig. B1 and

Supplementary Material). No significant changes were found for the maximum EAE severity scores or the EAE Severity at 40 days (data presented in Fig. B2 and Supplementary Material).

Repetition of the factorial analysis with values for the probability parameters between 0% and 1% in steps of 0.1% still showed significant reductions in CD4Treg and CD8Treg populations compared to the baseline even at values of the MHC down-regulation probability as low as 0.3% (data made available as Supplementary Materials).

The model leads to a severe reduction in Treg effector populations, which arises from the impact that the CD200 expressed by CD8Treg has on all T cell populations (illustrated informally in Fig. 5). The results reveal that this model is unlikely to represent the *in vivo* influence of CD200 as the severe reduction in T cell numbers observed is not representative of real-world EAE.

The significant reduction in Treg effector populations is accompanied by a non-significant rise in the population of CD4Th1 cells. This is probably due to the fact that reduction in their priming is off-set by a reduction in the rate of their apoptosis by CD8Treg, of which there are fewer following CD200–CD200R signalling.

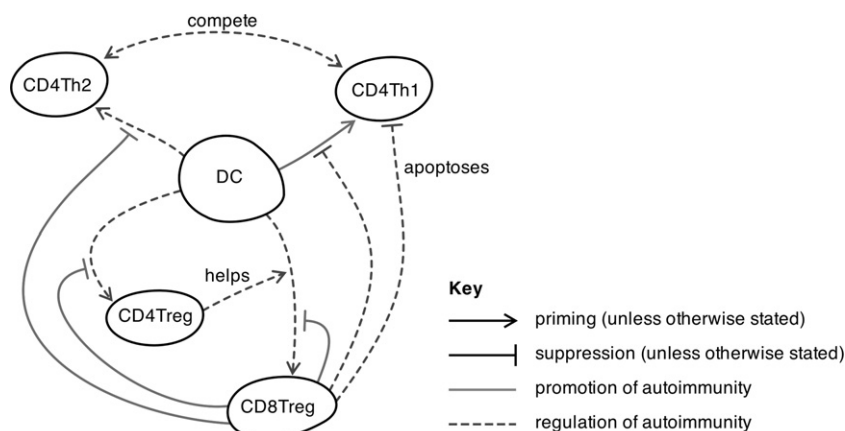
CD8Treg can impact, via the expression of CD200, not only the priming of all other T cell sub-populations, but also their own priming by DCs. This down-regulatory effect tends to be exerted on the DCs priming Treg cells, as few DCs simultaneously prime both CD4Th and CD8Treg cells (Williams et al., 2013).

The factorial analysis reveals that further development of the model is warranted. A number of feasible alternative models, based on this first iteration model, have been proposed (Greaves, 2011). However, one possible further line of investigation concerns the exploration of the potential impacts of cytokine switching and tryptophan catabolism on T cell priming and regulation of autoimmunity as discussed in Section 3.1.3.

#### 4.3. Results from iteration two of the modelling cycle

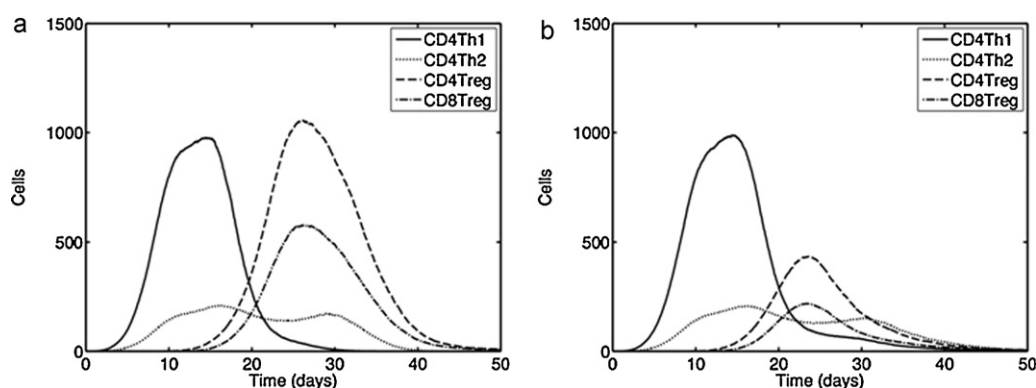
The model employed for the first iteration simulation was an unrealistic representation of the mechanism of action of CD200 outside the CNS. In line with the methodology, we now return to the domain modelling and examine a more sophisticated model of CD200 behaviour, which is presented here. The second iteration model consists of two mechanisms of CD200 action: reduction of negatively signalled DC priming capacity and the switching of cytokine expression by signalled DCs.

Gradual reduction of the capacity for DC to prime T cells had a significant impact on the simulation behaviour (see Fig. 6 and Supplementary Material). The capacity of a DC to prime T cells was reduced by 5% for each negative signal received by that DC. Even



**Fig. 5.** An informal description of the interactions of the different T cell populations of our model with each other and with dendritic cells (DCs). Via CD200 expression, CD8Treg can modulate DC ability to prime all T cell populations, including CD8Treg and can thus significantly impact T cell effector populations. Interactions marked as solid lines tend to promote autoimmunity, whereas those indicated as broken lines serve to reduce it.

Figure reproduced from Read (2011).

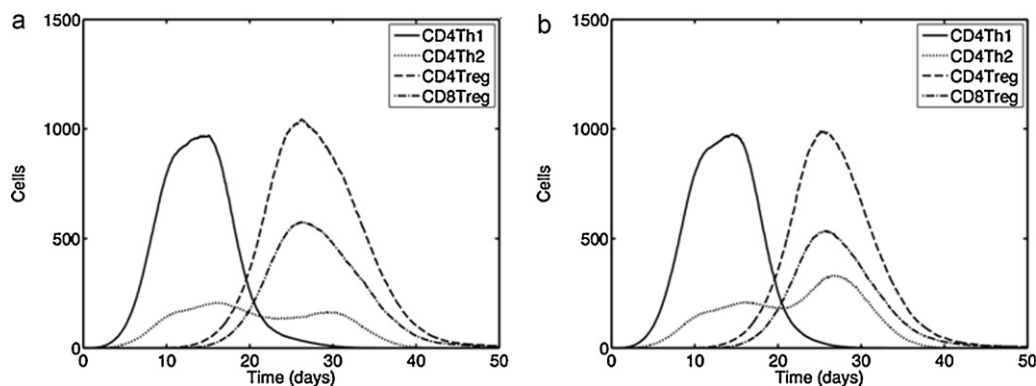


**Fig. 6.** Effector T cell populations (a) in the baseline simulation. The system demonstrates recovery and return to resting cell populations within 50 days after induction of EAE; (b) in the second iteration simulation where the capacity of a dendritic cell to activate T cells is reduced by 5% following each negative signalling event. The populations of Th effectors are largely unaltered by this amendment to the simulation. However, the Treg populations are greatly diminished by the reduction in DC priming capacity.

with a reduction in priming capacity of just 5% per negative signal, Treg populations were substantially reduced (data presented in Fig. 6). No effect was observed on the CD4Th1 and CD4Th2 populations, and similarly loss of DC priming capacity showed no effect on the timing of the attainment of the maximal T cell populations (data presented in Fig. C1 and in the Supplementary Materials).

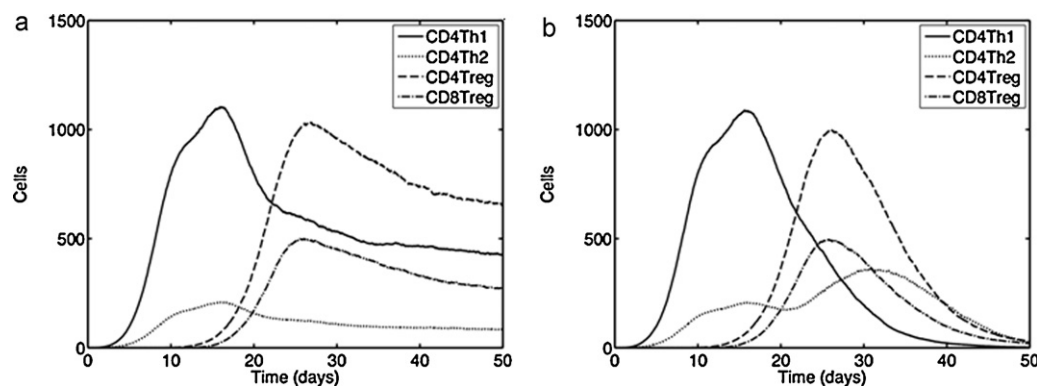
The principle result of CD200-induced switching of DC cytokine secretion profiles was to favour the priming of CD4Th2 cells relative

to the baseline behaviour. This is illustrated in Fig. 7. In the baseline simulation, disabling the ability of CD8Treg to apoptose CD4Th1 leads to elevated populations of all T cell sub-populations beyond day 40 (Fig. 8, panel (a)), a time at which full recovery has usually been effected. The introduction of cytokine switching restores the baseline-like behaviour when CD4Th1 apoptosis by CD8Treg is disabled (Fig. 8, panel (b)). This interesting phenomenon warrants further investigation and so we combine the cytokine switching

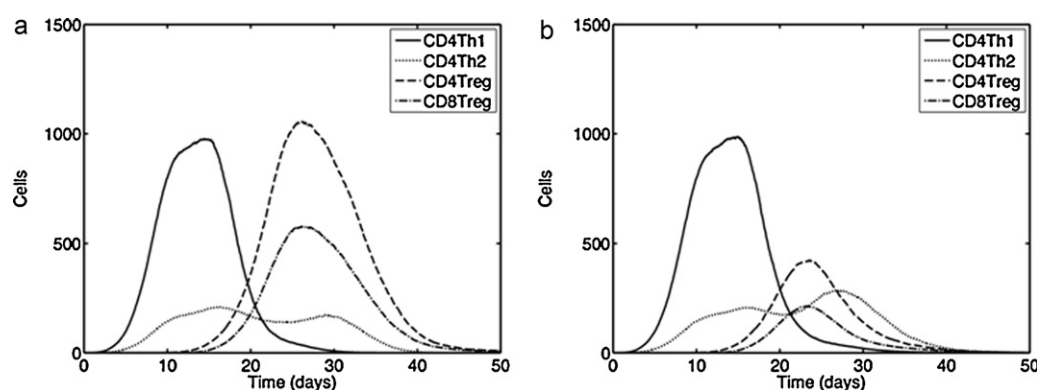


**Fig. 7.** Effector T cell dynamics (a) in the baseline simulation. The system demonstrates recovery and return to resting cell populations within 50 days after inoculation; (b) when CD200-negative signalling of DCs down-regulates their secretion of type 1 cytokines. The population of CD4Th2 effectors is increased throughout the simulation, especially during the recovery phase (days 22–32).





**Fig. 8.** Effector T cell populations (a) in a baseline simulation in which the ability of CD8Treg to suppress CD4Th1 has been suppressed. The populations of all effectors remain elevated at day 40 and do not return to resting levels by day 50 as in the baseline; (b) in a simulation in which DC receiving a CD200-mediated signal suppress expression of type 1 cytokines and abrogation of auto-reactive Th1 cells by CD8Treg has been disabled. Recovery and restoration of effector populations to resting levels by day 50 are restored, but the populations of effector Th2 remain elevated with respect to those in the baseline simulation.



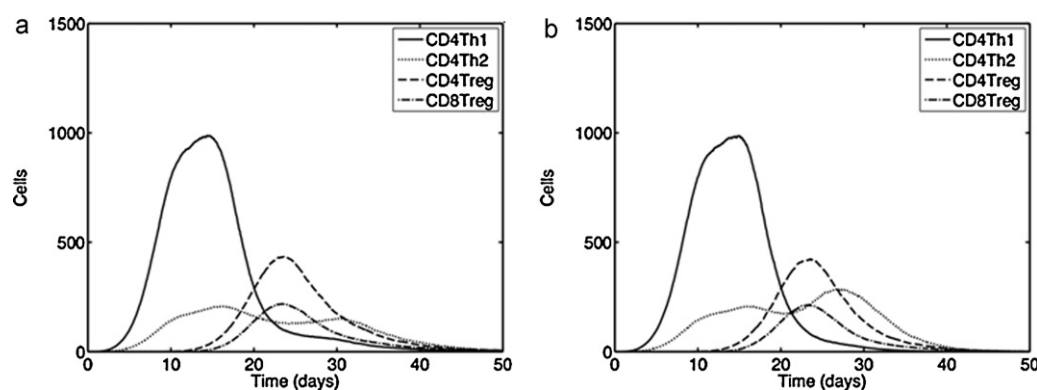
**Fig. 9.** Effector T cell populations (a) in the baseline simulation The system demonstrates recovery and return to resting cell populations within 50 days after inoculation; (b) in a simulation where each CD200 negative signalling event reduces DC priming capacity by 5% and prevents the secretion of type 1 cytokines. The populations of CD4 Th1 effectors are largely unaltered by this amendment to the simulation. However, the Treg populations are greatly diminished by this reduction in DC priming capacity compared to baseline behaviour, and CD4Th2 effector populations are elevated compared to the experiment in which there was reduction in DC priming capacity alone.

mechanism with the reduction of DC priming capacity to investigate the combined effect of these potential mechanisms of CD200 action.

The two new negative signalling mechanisms appear to have opposing effects. Simulation that enables both cytokine switching and the reduction of DC priming capacity simultaneously better reproduces a more qualitatively baseline-like simulation

behaviour, though Treg populations are reduced (data presented in Fig. 9).

Results show that a CD200-mediated reduction in DC priming capacity substantially reduces T cell population expansions. However, CD4Th1 and CD4Th2 populations were much less sensitive to this effect, probably owing to the fact that these populations have already reached their peak population before CD8Treg appear in



**Fig. 10.** Effector T cell populations (a) in the simulation where each negative signalling event reduces DC capacity to prime T cells by 5%. The system demonstrates recovery and return to resting cell populations within 50 days after inoculation. (b) in a simulation where each negative signalling event reduces DC capacity to prime T cells by 5% and prevents secretion of type 1 cytokines. The populations of CD4Th1 effectors are largely unaltered by this amendment to the simulation. However, the Treg populations are greatly diminished by this reduction in DC priming capacity compared to baseline behaviour, and CD4Th2 effector populations are elevated compared to the experiment in which there was reduction in DC priming capacity alone.



large numbers. The outcome of this model of immune regulation is to increase the population of CD4Th1 remaining in the system at day 40 (data presented in Fig. C1). In spite of the potential for negative signalling to reduce the priming of CD4Th by DC, the reduced number of effector CD8Tregs actually results in suppressed rather than enhanced regulation.

However, in contrast, baseline T cell dynamics were less perturbed by the implementation of cytokine switching as illustrated in Fig. 7. Such switching favours type 2 deviation and hence promotes regulatory activity. This behaviour is associated with recovery from EAE (Chen et al., 1998). However, this mechanism on its own may be too severe because the simulation outcome when CD8Treg ability to apoptose CD4Th1 is suppressed is not what is observed in the baseline. It is, however, interesting to note that cytokine switching has the potential to mediate recovery from EAE on its own.

In the combined mechanism simulation, the same reduction in priming capacity now leads to more rapid reduction in populations of CD4Th1 compared to that observed in the experiment in which only DC priming capacity reduction occurs (data presented in Fig. 10). The results suggest that cytokine switching can counter autoimmune behaviour in the system and that Th cell dynamics can be restored to levels more typical of the baseline by combination of the cytokine switching and reduction of DC priming capacity mechanisms.

The results suggest that Treg populations are significantly reduced in the combined mechanism experiment compared to the cytokine switching experiment (illustrated in Fig. D1 and in the Supplementary Materials). However, this occurs alongside an increase in the Th2 population (the type 2 deviation) that is associated with recovery from EAE (Chen et al., 1998). There may, of course, be additional aspects of the biology that would also offset this reduced T cell priming. It is interesting to suggest that recovery from EAE is compatible with reduction in DC priming capacity if the cytokine milieu around the DC is changed to one that is no longer predominantly pro-inflammatory. One could further argue that down-regulation of MHC/CoStim expression on APCs may be a useful mechanism for regulation of autoimmunity in the CNS (which is not currently represented in ARTIMMUS) despite its negative effect with respect to priming of Treg cells. These are, however, outstanding issues that would motivate further development of the simulation as they become more greatly understood. In light of this exploration of the CD200 pathway, investigation of the role of CD200 within the CNS is warranted, but we believe that the combined mechanisms could reproduce the baseline behaviour if all the simulation parameters were recalibrated.

## 5. Conclusions

A methodology for the principled extension of established simulations that will permit the exploration of newly identified aspects of biological systems is presented. In this instance, the case study employed has been EAE, entailing the extension of the ARTIMMUS simulation by addition of three different models of the CD200 regulatory pathway which consider only DC–CD8Treg interactions. The resulting contrasting simulation behaviours lead us to consider the wider implications of extending simulations in computational immunology.

Simulation represents a means of integrating biological data. Once properly calibrated, a parameterised simulation can serve as a tool for formulating and testing hypotheses relating to the domain. It is possible that new pathways or components will be identified as being influential in the system, and simulation provides a means whereby a preliminary exploration of these may be conducted. However, where little is known of the domain, a

structured and principled approach to this exploration is required. An appropriate methodology for such exploration has been presented here.

The proposed methodology utilises both exploration of parameter space (which can be performed systematically) and of abstraction space (which cannot). In contrast, many ODE based approaches attempt to find reaction rates or parameters (de Boer et al., 2010; Perelson and Nelson, 1999 and reviewed in Perelson, 2002). A few also focus on understanding the mechanics of the system or trying to elucidate their mechanisms of action (e.g. Wu et al., 2011). The methodology presented proposes that to understand the potential mechanisms whereby a system operates, it is necessary to explore different models and not just parameters.

The developed methodology has been applied to extending ARTIMMUS, a simulation that has been rigorously developed and calibrated against the real-world system (Read et al., 2012). We are therefore confident that the T cell dynamics of ARTIMMUS constitute a reasonable (in terms of current biological understanding) representation of the domain. The CD200 immuno-regulatory pathway has been identified as having an influential role in the regulation of the ability of dendritic cells to activate T cell populations during autoimmunity (Feuer, 2007; Minas and Liversidge, 2006). In order to conduct a preliminary exploration of the pathway, it was necessary to incorporate an abstraction of that pathway into the current simulation. However, the domain is not well characterised and therefore a rigorous approach was required for implementing models of the pathway. In line with the described methodology, an initial, simple model was developed. Factorial analysis revealed the mechanism to be too simplistic with excessive reduction of the Treg effector populations, irrespective of the values assigned to the probability parameters introduced into the simulation. Therefore two further models are explored. A combination of these produced an overall model of how the CD200–CD200R pathway may regulate autoimmunity that was deemed justifiable on the grounds of the literature, and which was capable of returning close to baseline simulation behaviour.

The strength of the proposed methodology is that exploration of the effects of the chosen representation of the extension is an integral part of the process. By following the iterative development methodology, we have been able to reflect on how the extensions implemented in the simulation are affecting its behaviour. The methodology established that the first iteration model was inappropriate as we could not re-establish baseline simulation behaviour except by disabling the added regulatory pathway in the simulation. We have thus avoided proceeding with further development of an inadequate representation of the CD200–CD200R signalling mechanism.

The severe impact of the first iteration regulatory pathway model on simulation T cell populations prompts us to consider certain important philosophical issues concerning the use of simulation in exploring immunology. Extensions to simulation can rebalance the influence of cells and pathways within that simulation, as stated above, and as such quantitative measures of their influence may change. This can have implications for previous quantitative results and we must therefore be cautious when extracting such results from simulation.

It is clear that strong methodologies are required to guide simulation development to appropriate levels of abstraction. These methodologies must also serve to ensure that simulation adequately captures the domain, that modellers continually evaluate simulation performance against biological observations and that simulation is capable of indicating when something influential, and possibly as yet unidentified *in vivo*, is missing from the simulation. A failure of simulation to fully represent real-world dynamics can further motivate *in vivo* exploration or further development of the simulation. On the other hand, the success of simulation

developed through strong methodologies in replicating biological dynamics affords confidence in their results. This confidence is essential for a true integration of simulation and conventional wet-lab research approaches, which can benefit both biomedical and biological research.

### Acknowledgements

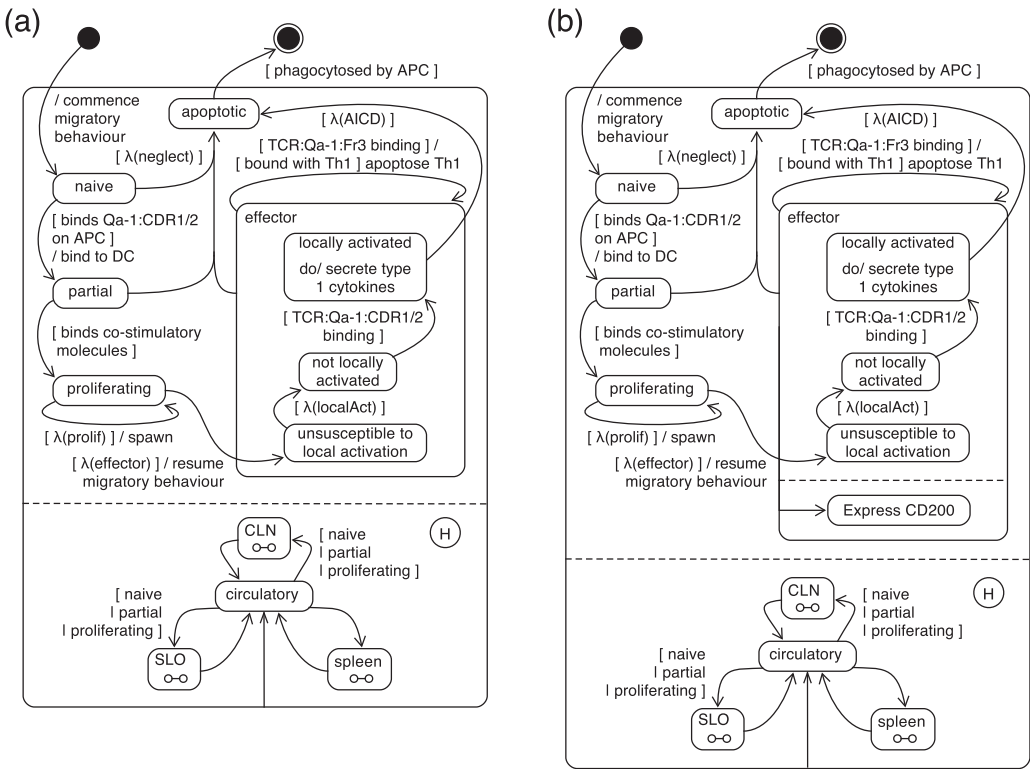
We wish to acknowledge Dr Carl Ritson for allowing us access to the CoSMoS computing cluster at the University of Kent in order to carry out the simulation reported here. Mark Read and Jon Timmis are supported by the EC CoCoRo Project, GA 270382

<http://cocoro.uni-graz.at/drupal/>. Paul Andrews is funded by EPSRC grant “Resilient Futures” grant number EP/I005943/1. Jon Timmis is also financially supported by the Royal Society. Vipin Kumar is supported by grants from the National Institutes of Health, USA and the Multiple Sclerosis National Research Institute, USA.

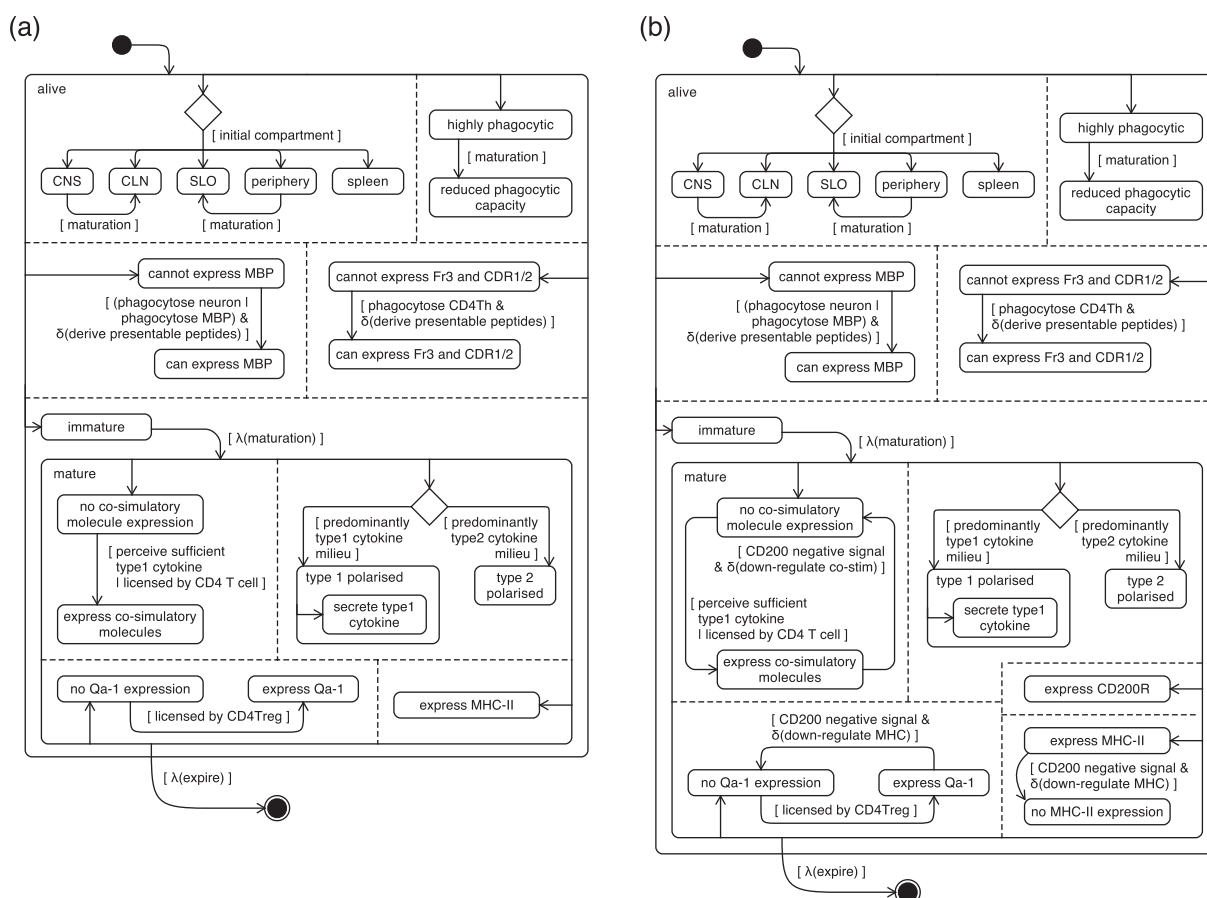
### Appendix A. State machine diagrams

Details of the full domain model and how to interpret the diagrams presented here can be found in [Read \(2011\)](#).

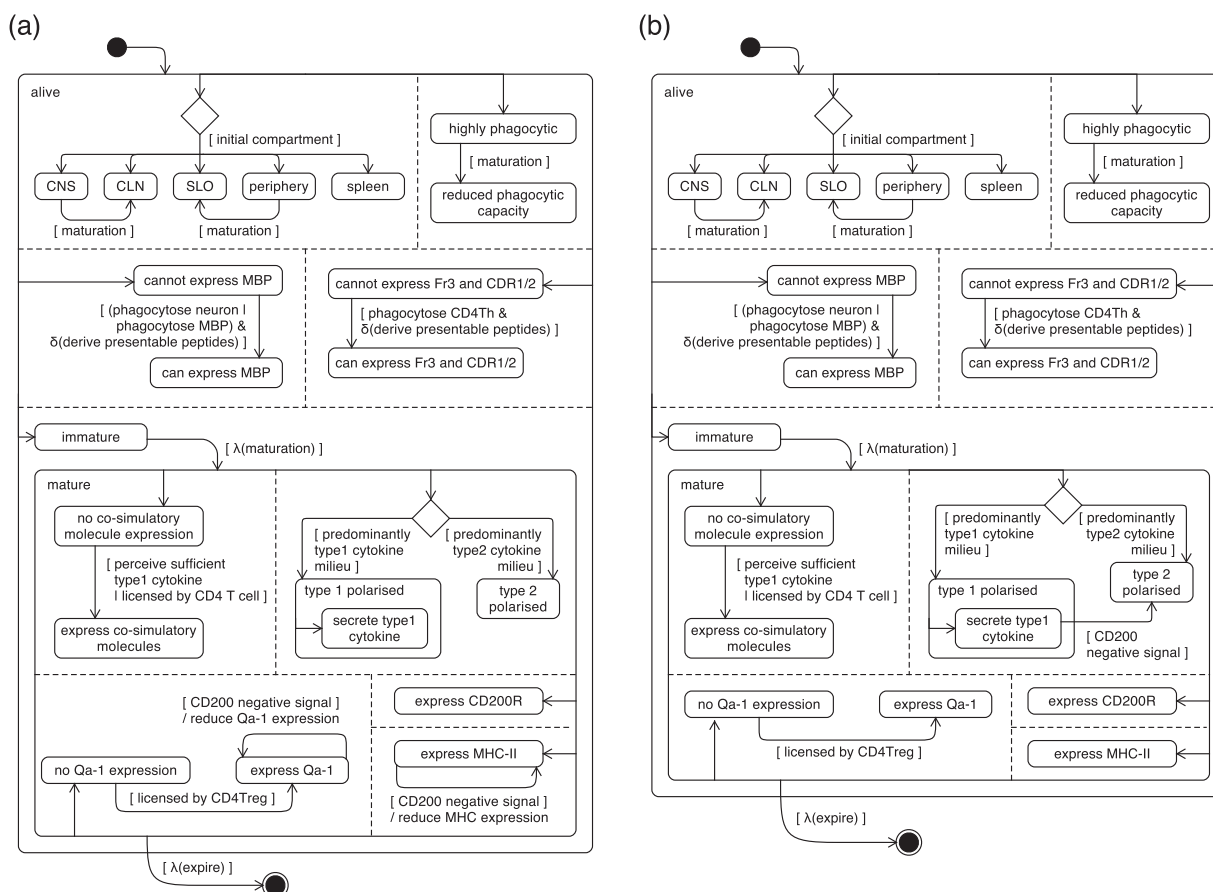
Fig. A1  
Fig. A2  
Fig. A3



**Fig. A1.** State machine diagrams describing the behaviour of (a) CD8Tregs in the baseline simulation; (b) CD8Tregs in the simulations for which CD8Treg are capable of CD200 expression. CD8Treg are capable of expressing CD200 upon them becoming effectors, without any requirement for local activation.



**Fig. A2.** State machine diagrams describing the behaviour of (a) DCs in the baseline simulation (b) DCs in the iteration one model where each negative signalling event probabilistically suppresses expression of MHC and/or CoStim by individual DCs.



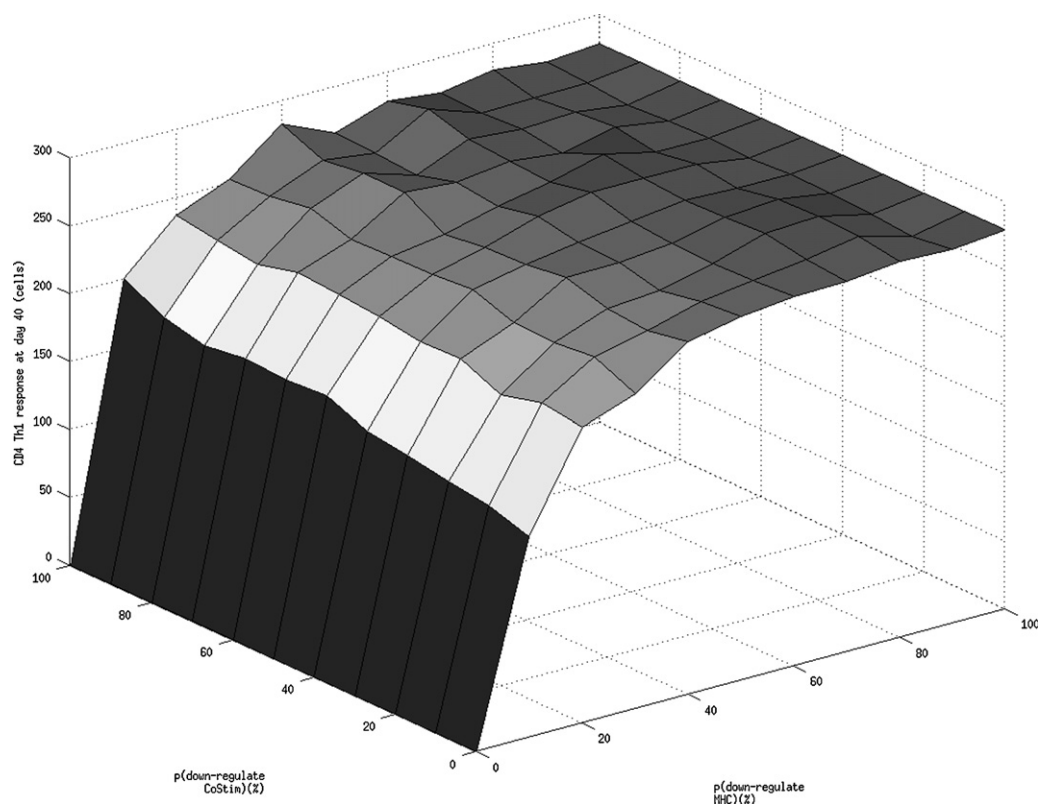
**Fig. A3.** State machine diagrams describing the behaviour of (a) DCs in the simulation where each negative signalling event reduces the priming capacity of individual DCs by 5%; (b) DCs in the simulation where each negative signalling event suppresses secretion of type 1 cytokines by individual DCs.



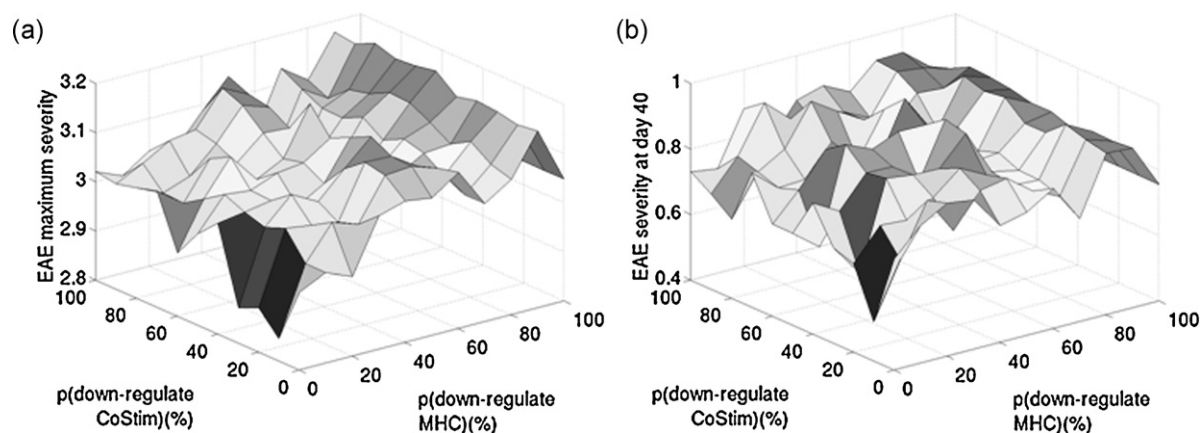
## Appendix B. Factorial analysis results

Fig. B1

Fig. B2



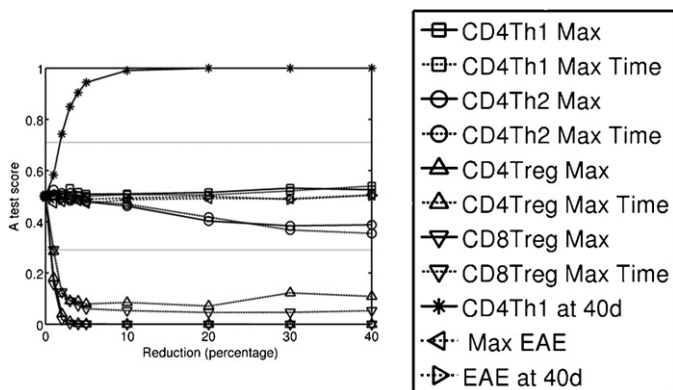
**Fig. B1.** Three-dimensional 'response landscape' of (a) CD4Th1 effector population at day 40 of the simulation from a factorial analysis of simulation behaviour at values of the two probability parameters between 0% and 100% in steps of 10%.  $p(\text{down-regulate MHC})$  and  $p(\text{down-regulate CoStim})$  represent the probabilities that a single negative signal will down-regulate MHC and CoStim expression respectively by the signalled DC. The baseline simulation is represented as the pairing 0%, 0% in the bottom middle of the plot.



**Fig. B2.** Three-dimensional 'response landscape' of (a) maximal EAE severity score and (b) EAE severity at day 40 of the simulation from a factorial analysis of simulation behaviour at values of the two probability parameters between 0% and 100% in steps of 10%.  $p(\text{down-regulate MHC})$  and  $p(\text{down-regulate CoStim})$  represent the probabilities that a single negative signal will down-regulate MHC and CoStim expression respectively by the signalled DC. The baseline simulation is represented as the pairing 0%, 0% in the bottom middle of the plot.

## Appendix C. The effects of DC priming capacity reduction

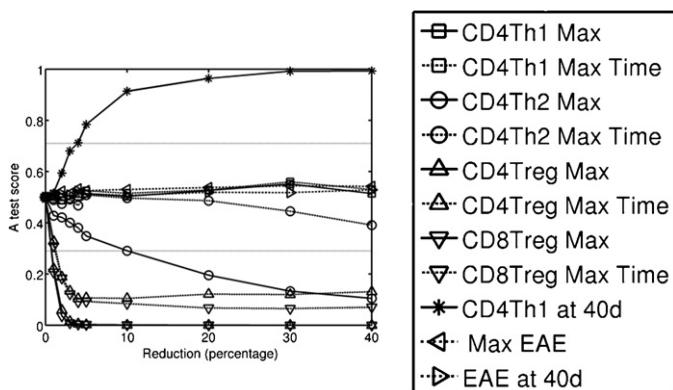
Fig. C1



**Fig. C1.** A sensitivity analysis illustrating the significance of the effect of reducing the DC priming capacity by between 0% and 40%. The Treg effector populations show significant reductions in their numbers with even small reductions in DC priming capacity and the population of CD4Th1 remaining at day 40 is significantly increased.

## Appendix D. The effects of combining DC priming capacity reduction with cytokine switching

Fig. D1



**Fig. D1.** A sensitivity analysis illustrating the significance of the effect of reducing the DC priming capacity by between 0% and 40% whilst cytokine switching is also occurring on signalled dendritic cells. The Treg effector populations show significant reductions in their numbers even for small reductions in DC priming capacity and the population of CD4Th1 remaining at day 40 is significantly increased. The population of Th2 effectors is also reduced by greater reductions in DC priming capacity, but not significantly, compared to the cytokine switching experiment.

## Appendix E. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biosystems.2013.03.007>.

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