

# ARTIMMUS Supporting Materials: Biological Foundations, Domain Model, Platform Model, Parameters and Robustness Analysis

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## **Abstract**

This document provides supporting information for the ARTIMMUS simulation. First, it provides details of the biology represented in the simulation, with references to further information. Second, the ARTIMMUS domain model is presented. This is a comprehensive graphical model depicting the biology that ARTIMMUS is intended to simulate. Third, is the platform model. This expands on the domain model by providing implementation-specific details, and abstractions. Fourth, a full listing of ARTIMMUS's parameters is provided. Lastly, the numerical results of a complete robustness analysis applied to ARTIMMUS parameters is detailed.

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# 1 The biology of EAE underpinning ARTIMMUS

Experimental autoimmune encephalomyelitis (EAE) is an animal autoimmune disease that arises through the direction of immunity towards the central nervous system (CNS). It is one of the earliest developed animal models of disease, and has been widely used as a model for studying multiple sclerosis [Baker & Jackson 2007, Baxter 2007, Goverman 2009, Zamvil & Steinman 1990]. EAE is studied in a variety of animals including, but not exclusively, mice, rats, guinea pigs and monkeys. The clinical course of EAE varies with animal and with the immunization protocol used to induce it, and across the different models a wide range of disease phenotypes can be induced, from acute to relapsing to chronic paralysis, of varying degrees of severity [Baker & Jackson 2007, Baxter 2007]. Depending on the protocol used to induce it, EAE can be fatal for a portion of experimental animals. ARTIMMUS simulates the model of EAE used in the Kumar laboratory [Kumar & Sercarz 2001, Kumar 2004], which is studied in mice.

EAE involves direction of immunity towards the myelin sheath, an insulatory material that coats the neurons of the CNS, and which is necessary for their proper function. The disease is typically induced through two methods: the administration of myelin derivatives, such as myelin basic protein (MBP), in conjunction with adjuvants that strongly stimulate the immune system into action; or through the adoptive transfer of myelin specific effector T cells from one animal already induced into disease into another [Zamvil & Steinman 1990].

The susceptibility of experimental animals to clinical disease following induction of EAE varies considerably. Some do not present clinical symptoms at all, whilst others perish. The Kumar lab grades the severity of EAE experienced by an experimental mouse on a scale of 0 to 5: 0, no symptoms; 1, flaccid tail; 2, hind limb weakness; 3, hind limb paralysis; 4, whole body paralysis; 5, death [Kumar *et al.* 1996]. Figure 1 demonstrates the variation in autoimmune severities experienced by mice undergoing various interventions. In the EAE model employed in the Kumar lab animals frequently experience spontaneous recovery from autoimmune symptoms, as may be seen on the figure.

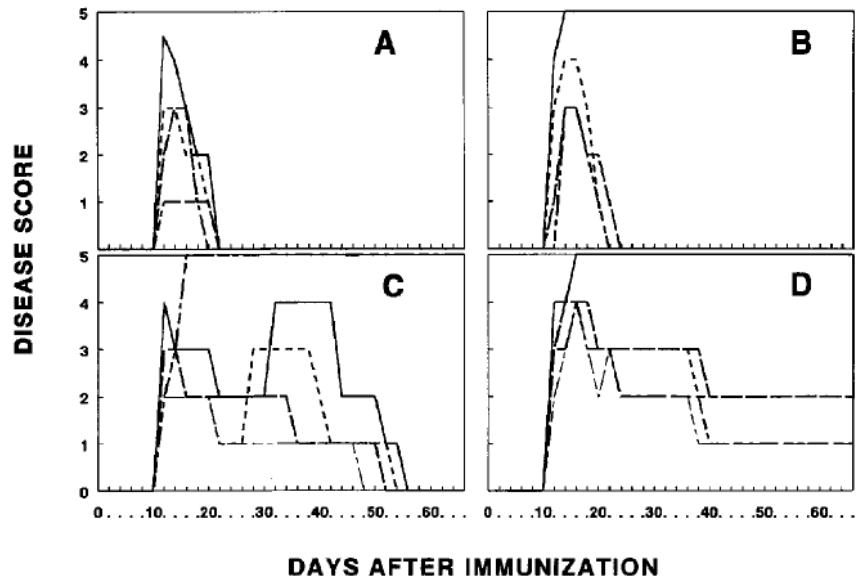


Figure 1: The progression of EAE in individual mice amongst four experimental groups, with five mice in each group. Group A experiences onset and then physiological recovery from disease. Groups B, C and D have received various interventions that interfere with the animal's ability to recover from clinical symptoms. Note the considerable variation in disease progression experienced by mice of the same experimental group, having undergone the exact same immunization procedure. Figure reproduced from [Kumar *et al.* 1996].

Figure 2 depicts the major cell types that are involved in the Kumar laboratory's EAE model and its associated recovery. Their roles in mediating autoimmunity and subsequent recovery are discussed in the following sections.

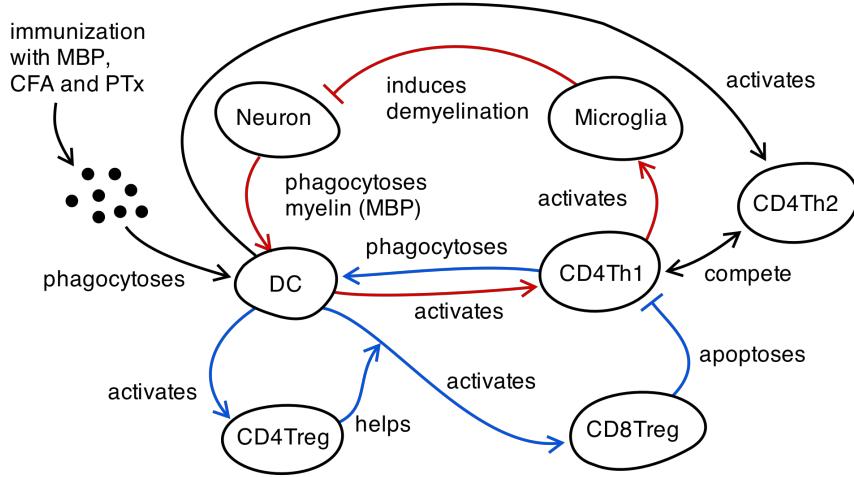


Figure 2: Abstract depiction of the major cell types involved in EAE autoimmunity and its associated recovery. Red arrows indicate interactions leading to autoimmunity, blue arrows indicate regulatory activity that counters the autoimmune response.

## 1.1 EAE autoimmunity

EAE is induced through sub-cutaneous injection of MBP, leading dendritic cell (DC) phagocytosis of MBP and subsequent display of MHC-II:MBP on DC cell surfaces. The adjuvants accompanying immunization for EAE, CFA and PTx<sup>1</sup>, induce DCs to adopt a highly immunogenic phenotype, making them strong primers of CD4Th1 cell immune responses [Menezes *et al.* 2007].

DCs residing in the periphery, having phagocytosed MBP and been induced into maturity by adjuvant, migrate to the local lymph nodes, and there prime populations of naive MBP-specific CD4Th cells [Goverman 2009]. These cell populations enter their proliferative cycles, and adopt either CD4Th1 or CD4Th2 polarizations. Having been exposed to adjuvant, the DCs generally promote the adoption of CD4Th1 polarizations. However polarization adoption is probabilistic, and some CD4Th cells will still adopt a CD4Th2 phenotype [Ando *et al.* 1989, Menezes *et al.* 2007]. Upon completion of their proliferative cycles, these T cells differentiate into effector T cells, migrate out of the lymph nodes, and rejoin the circulatory system, eventually reaching the CNS compartment.

CD4Th1 and CD4Th2 effector cells are able to cross the blood-brain barrier and gain entry to the CNS. There they are locally activated by APCs presenting MHC-II:MBP. Hereafter CD4Th1 cells commence the secretion of pro-inflammatory cytokines [Ando *et al.* 1989, Menezes *et al.* 2007]. The proinflammatory cytokines simulate local macrophages and microglia into the secretion of TNF- $\alpha$ , reactive oxygen species (ROS), and nitric oxide (NO), which promote demyelination [Hendriks *et al.* 2005, Raivich & Banati 2004, Tambuyzer *et al.* 2009].

Demyelination leads to myelin phagocytosis by macrophages, microglia and DCs. Macrophages and microglia present MHC-II:MBP following this phagocytosis, and thereby provide further stimulus for local activation of encephalitogenic<sup>2</sup> CD4Th1 and CD4Th2 infiltrates. The proinflammatory cytokine milieu induces these macrophages, microglia and DCs to up-regulate co-stimulatory molecule expression, hence adopting immunogenic phenotypes. The DCs migrate to the cervical lymph nodes (CLN), and there present MHC-II:MBP [Goverman 2009].

This presentation of MBP in the CLN by DCs provides further stimulus for the generation of MBP-specific CD4Th cell populations. Once a CD4Th1 presence is established in the CNS compartment, MBP-presenting DCs will prime further populations of these same cells in the CLN, and hence autoimmunity becomes self-perpetuating, potentially persisting for long after the T cell proliferation in the peripheral lymph nodes resulting from immunization have ceased.

## 1.2 EAE regulation

Many mice induced into EAE spontaneously recover from autoimmune symptoms, even when the mechanisms mediating regulation are interfered with, see figure 1. The Kumar lab has identified a network of cells that has

<sup>1</sup>CFA, complete Freund's adjuvant, consists of the inactivated bacterium *mycobacterium tuberculosis*. PTx, pertussis toxin, is a protein produced by the bacterium *mordetella pertussis*. Both are powerful immunopotentiators.

<sup>2</sup>*Encephalitis* relates to inflammation of the tissues of the brain.

a regulatory effect on autoimmunity, and mediates recovery from disease. This regulatory network is indicated on figure 2 by blue arrows. The Kumar lab has characterised two forms of regulatory T cell (Treg) that play a significant role in recovery from EAE, CD4Tregs and CD8Tregs [Kumar 2004]. Experiments to deplete or incapacitate these cells results in labored recovery from autoimmune symptoms [Kumar *et al.* 1996, Beeston *et al.* 2010], whereas their artificial premature activation following induction for EAE protects recipients from autoimmunity [Tang *et al.* 2007]. Further, the adoptive transfer of activated CD4Tregs or CD8Tregs into mice prior to immunization protects them from subsequent attempts to induce EAE [Tang *et al.* 2006, Kumar 1998, Kumar *et al.* 2001]. This section details how these two Treg cell populations operate within the mouse immune system to mediate recovery from autoimmunity.

Through the natural course of their lifecycles, CD4Th1 cells die of AICD, entering apoptosis and being subsequently phagocytosed by APCs, such as DCs [Kabelitz *et al.* 1993]. The majority of encephalitogenic CD4Th1 cells use the V $\beta$ 8.2 TCR gene segment to encode their TCRs [Menezes *et al.* 2007]. DCs derive and present two peptides from two regions of these TCRs: framework 3 (Fr3), and complementarity determining region 1/2. MHC-II:Fr3 complexes are presented by DCs, which prime populations of CD4Tregs [Kumar 1998, Smith *et al.* 2010].

CD4Treg cells secrete IL-2 and INF- $\gamma$  cytokines necessary for the induction of Qa-1:CDR1/2 expression on DCs [Tang *et al.* 2006], a process known as *licensing*. Qa-1 is a form of non-classical MHC-I molecule that presents a substantially smaller repertoire of peptides than classical MHC-I can. Qa-1:CDR1/2 presentation and CD4Treg cytokine secretion leads to the priming of CD8Treg populations<sup>3</sup> [Smith *et al.* 2009, Kumar 2004].

For around 8 hours following differentiation into effector cells, V $\beta$ 8.2 TCR CD4Th1 cells express Qa-1:CDR1/2 complexes, for which CD8Treg cells are specific. Binding between these two cells leads to apoptosis induction in CD4Th1 cells by CD8Tregs [Beeston *et al.* 2010, Tang *et al.* 2006]. At the population level, this rise in CD8Treg population number leads to a reduction in the CD4Th1 cells that mediate self-perpetuating autoimmunity: recently primed CD4Th1 cells originating from the CLNs are induced into apoptosis, and hence the loop of self-perpetuating autoimmunity is broken.

The MBP-specific CD4Th2 cells escape regulation by CD8Treg cells, and as a result of their unhindered expansion the immune response deviates in a type 2 direction [Kumar 2004, Kumar & Sercarz 2001, Madakamutil *et al.* 2003]. In the context of EAE, CD4Th2 cells, and the cytokines they secrete, do not promote demyelination and instead serve to counter the pro-inflammatory encephalitogenic context in the CNS [Kumar 1998].

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<sup>3</sup>CD8Treg cells that are specific for Qa-1 have been implicated in maintaining self-tolerance and countering autoimmune behaviour in a variety of diseases by regulating self-reactive T cells [Lu *et al.* 2006, Kim *et al.* 2010].

## 2 Domain Model

This section presents a domain model of EAE, based on the biology of the previous section, largely expressed using the unified modelling language (UML). The model is presented in a top-down manner, comprising three layers that depict the system at different levels of abstraction. They are:

1. A system-level overview of the domain model. This highly abstract layer details how the cells of the domain model interact in order to produce system-level behaviours, and how these behaviours are believed to correspond to phenomena observed in the real-world domain. This modelling stage does not make use of any UML notation.
2. Modelling of system *perspectives*: decompositions of EAE's complex onset and recovery. This modelling layer describes in greater detail the cell-level events and interactions that together constitute system-wide behaviours marking each stage of disease and recovery. These models are expressed using UML activity and class diagrams.
3. Cell-level dynamics. This layer represents the lowest-level modelling in the domain model, detailing the dynamics of individual entities in the system. UML state machine diagrams have been used in expressing these models.

Note that the following abstractions of the biology outlined above have been made in the present domain model:

- 'CNS Macrophage' refers to macrophage and microglia in the CNS.
- Demyelination of neurons is abstracted as neuronal apoptosis. Phagocytosis of 'apoptotic' neurons leads to MHC:MBP presentation on APCs.

### 2.1 Relationship between real domain and domain model

Figure 3 delineates the system of interest, and is termed an *expected behaviours* diagram. It does not conform to any UML notation. The diagram abstractly indicates those entities of the real domain that are represented in the domain model, how the interactions between them result in system-wide behaviours, and how these system-wide behaviours are believed to constitute the phenomena observed in the real domain. Boxes annotated with '<<expected>>' tags represent emergent phenomena that are expected to manifest at the large-scale from low-level interactions of entities within the system. The entities themselves, and an abstract indication of their interactions with one another, are represented at the bottom of the diagram.

Experimentation carried out within Kumar's lab has lead to observations that categorize into three distinct phenomena. EAE as resulting from sub-cutaneous immunisation with myelin basic protein (MBP), complete Freund's adjuvant (CFA), and pertussis toxin (PTx), leads to damage of the central nervous system (CNS). This leads to paralysis in the subject. Following the induction of EAE, the majority of experimental animals experience physiological recovery from paralysis; no experimental intervention is administered in facilitating recovery. Lastly, mice having undergone recovery from autoimmunity are resistant to subsequent attempts to induce paralysis with similar immunisation.

It is hypothesised that paralysis following immunisation with MBP, CFA and PTx is the result of T cell autoimmunity targeting myelin as expressed by cells in the CNS. Myelin is expressed by a variety of cells, and the domain model abstracts these into a single cell titled the *neuron*. It is believed that MBP is phagocytosed by dendritic cells (DCs) which prompts them to prime MBP-specific T cells. Multiple T cell sub-species may comprise these populations, including CD4Th1, CD4Th2 and CD4Th17<sup>4</sup> cells. The domain model abstracts the actions of CD4Th1 and CD4Th17 cells into a single entity, termed the *CD4Th1* cell. CD4Th1 and CD4Th2 cells suppress the priming of one another's populations, largely through the secretion of type 1 and type 2 cytokines. There are numerous types of cytokine secreted by each cell type, and their effects are complex. They have been collectively abstracted into two domain model entities, *type 1 cytokine*, and *type 2 cytokine*.

Whilst both CD4Th1 and CD4Th2 cells gain access to the CNS, CD4Th2 cells do not contribute to demyelination. Upon infiltrating the CNS, CD4Th1 cells induce the activation of macrophages and microglia that reside there. Microglia ordinarily reside in the CNS, and the inflammatory context that follows the infiltration of CD4Th1 cells to the CNS results in the recruitment of macrophages to the site. These various APCs have been abstracted in the domain model, and are represented as a single cell type termed the *CNS macrophage*. The activation of CNS

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<sup>4</sup>The role of CD4Th17 cells in EAE has not been explicitly investigated by the Kumar lab, but it is acknowledged that they likely do mediate autoimmune behaviour in their model. For reference on CD4Th17 in EAE, see [Zepp *et al.* 2011].

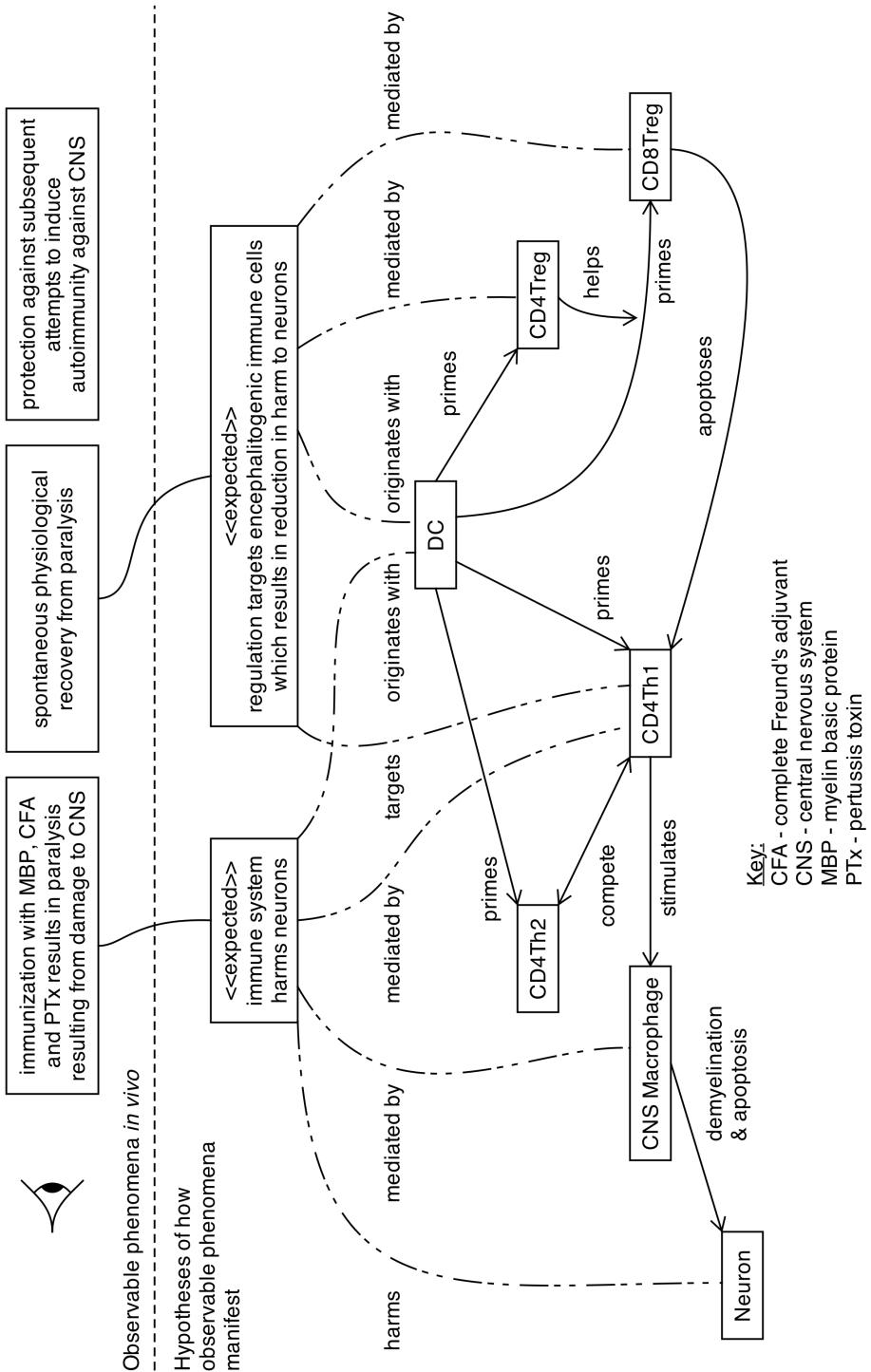


Figure 3: The *expected behaviours* diagram depicting the phenomena observed in the real domain, and the behaviours manifesting from cellular interactions believed to be responsible for them.

macrophages leads to the secretion of pro-inflammatory molecules such as TNF- $\alpha$ , nitric oxide, and reactive oxygen species. The domain model abstracts these molecules into a single entity titled *soluble demyelinating agent* (SDA). SDA is harmful to neurons, leading to demyelination: the stripping of myelin from neurons. Neuronal myelin is not explicitly represented in the domain model, which instead represents damage to neurons as their entering apoptosis.

The spontaneous recovery from paralysis observed *in vivo* is believed to be the result of CD8Treg cells inducing apoptosis in MBP-specific CD4Th1 cells. The physiological turnover of CD4Th1 cells leads to their phagocytosis by DCs, and the subsequent priming of CD4Treg populations by those DCs. CD4Treg cells license DCs to prime CD8Treg populations; it has been established that the absence of CD4Treg cells in mice induced into EAE results in increased paralysis and delayed recovery from autoimmunity [Kumar *et al.* 1996]. It is believed that the role of these CD4Treg cells is to induce the expression of Qa-1 in DCs, required for the priming of CD8Treg populations. These CD8Tregs have been categorised as CD8 $\alpha\alpha$  TCR $\alpha\beta$  regulatory T cells by the Kumar laboratory, and are simply termed *CD8Treg* cells in this domain model.

The cell-level events that lead to protection against subsequent attempts to induce EAE are currently unknown. This domain model makes no claim concerning the manifestation of this phenomenon, and it is not explicitly represented in ARTIMMUS.

The inter-cellular interactions outlined above, and indicated on figure 3, are done so at a very abstract level of detail. The following sections examine how these cellular behaviours manifest in system-wide emergent behaviours, termed *perspectives*, in greater detail. EAE paralysis and its associated recovery are characterised by considerable complexity, and to facilitate exploration of how the cellular interactions contribute to the expected behaviours outlined in figure 3 each behaviour is divided into two *perspectives*. The resultant four *perspectives* describe the collective consequences of cellular interactions, and may be considered as four stages of EAE and its recovery. They are:

1. The *initial establishment of autoimmunity* in the CNS following immunisation.
2. The *self-perpetuation of autoimmunity*.
3. The *establishment of regulation* that results in the apoptosis of CD4Th1 cells.
4. The *type 2 deviation of autoimmune response* that results from regulatory activity and ultimately leads to the termination of both autoimmune and regulatory immune responses.

These are described below. The following section describes the spatial abstraction of an experimental animal.

### 2.1.1 Spatial representation in domain model

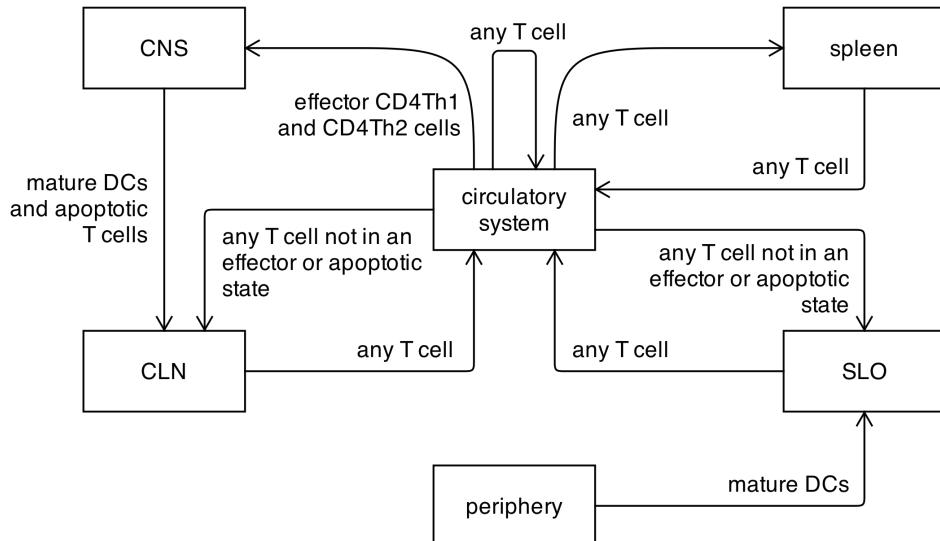


Figure 4: The spatial components of the domain model, and the manner in which the cells of the domain model may migrate between them.

The present domain model abstracts the physical space of a real-world experimental animal into six compartments, depicted in figure 4. The migratory patterns of cells between spatial compartments is indicated.

The *periphery* represents the sub-cutaneous region of the experimental animal where immunisation with myelin basic protein (MBP), complete Freund's adjuvant (CFA) and pertussis toxin (PTx) are administered for the induction of EAE. Immunogenic type 1 polarised DCs expressing MHC-II:MBP peptides migrate from the periphery to the draining lymph nodes upon maturation. No other cells are considered to enter the periphery for the purposes of this domain model.

The *SLO* (secondary lymphoid organ) compartment is an abstraction of the lymph nodes that drain the region of the mouse where immunisation for EAE is administered. In addition to the DCs that migrate to the SLO following immunisation, this compartment contains a small number of ordinary DCs. Any T cell that is not in either an effector or apoptotic state can migrate into this compartment from the circulatory system, and any T cell in any state may migrate from it back into the circulatory system.

The *CNS* compartment represents the central nervous system of an experimental animal. It contains neurons, CNS macrophages, and DCs. Effector CD4Th1 and CD4Th2 cells are able to migrate into this compartment from the circulatory system, however CD4Treg and CD8Treg cells are not. Neurons and CNS macrophages permanently reside in this compartment, and DCs migrate from the CNS to the CLN upon maturation.

The cervical lymph nodes (CLNs) of an experimental animal are lymph nodes in the neck that drain the CNS. These are represented in the domain model by the *CLN* compartment. It contains a small number of permanently residential DCs, and the mature DCs that migrate from the CNS compartment. Apoptotic CD4Th1 and CD4Th2 cells are able to migrate from the CNS compartment into the CLN. Any T cell that is not in either an effector or apoptotic state is able to migrate into the CLN from the circulatory system. Any T cell, in any state, is able to migrate from the CLN into the circulatory system.

The *spleen* compartment represents the spleen of an experimental animal. It contains a large number of permanently residential DCs. Any T cell in any state may enter or leave this compartment via the circulatory system.

The *circulatory system* compartment is the domain model's representation of the circulatory system of an experimental animal. Only T cells may reside in this compartment. It is through this compartment that T cells are able to migrate to the other compartments represented in the domain model, with the exception of a direct connection from the CNS to the CLN and from the periphery to the SLO.

## 2.2 Modelling perspectives

The following sections depict the manifestation of EAE *perspectives*, decompositions of the disease into four stages covering its onset and recovery, from cellular-level events and interactions. This is accomplished through the use of UML activity and class diagrams. Only standard UML notation is employed in class diagrams, however several additions have been made to the standard UML activity diagram notation, as described here.

Figure 5b denotes a *propagation* relationship, conceived to demonstrate that an entity may perform an action that has some consequence elsewhere, but without wishing to imply that this activity stops. This relationship is indicated by an arrow originating from a line that is perpendicular to the border of the activity, but does not lie in contact with it. In the example, activity A leads to activity B. The occurrence of activity B has consequences represented in activities C and D, which may be considered as new paths of execution. However, the activity B does not terminate. Consequences C and D may each occur any number of times whilst activity B is undertaken. New occurrences of C and D cease once the transit from B to E is made. For example, activity B might represent a cell in a proliferating state, in which case action C could represent the creation of a daughter cell. That daughter cell would go on to interact with other entities in the system, independently of the proliferating cell. Several daughter cells may be created as a result of proliferation, before the proliferative activity ceases.

Figure 5c denotes a relationship that can be *interrupted* or down-regulated. Activity A leads to activity B, however activity C has the consequence of either fully or partially preventing that transit. For example, activity A could represent an activity undertaken by a population of cells, and that activity could lead to some consequence in another population of cells. Activity C may represent the secretion of some molecule that interferes with the ability of the population represented by A having the stated consequence on the population represented by B. The interference is not necessarily absolute, it may be partial. Continuing the example, it may be that as a result of C, A has a diminished ability to affect B, rather than that the relationship is completely prevented.

Figure 5d depicts a *contributory* relationship. Activity A leads to a decision, B, after which either activity D or E will commence. The nature of that decision is influenced by activity C. C does instigate the decision (A leads to the decision), it only influences it when it occurs. In the example, the contributory influence of C on B is shown as being propagative, meaning that C may continue to influence many occurrences B, until a sequential transition away from C occurs.

The activities depicted on the activity diagrams that follow are largely cellular state changes and interactions that take place in particular compartments of the domain model. Dotted lines, representing activity diagram 'swim

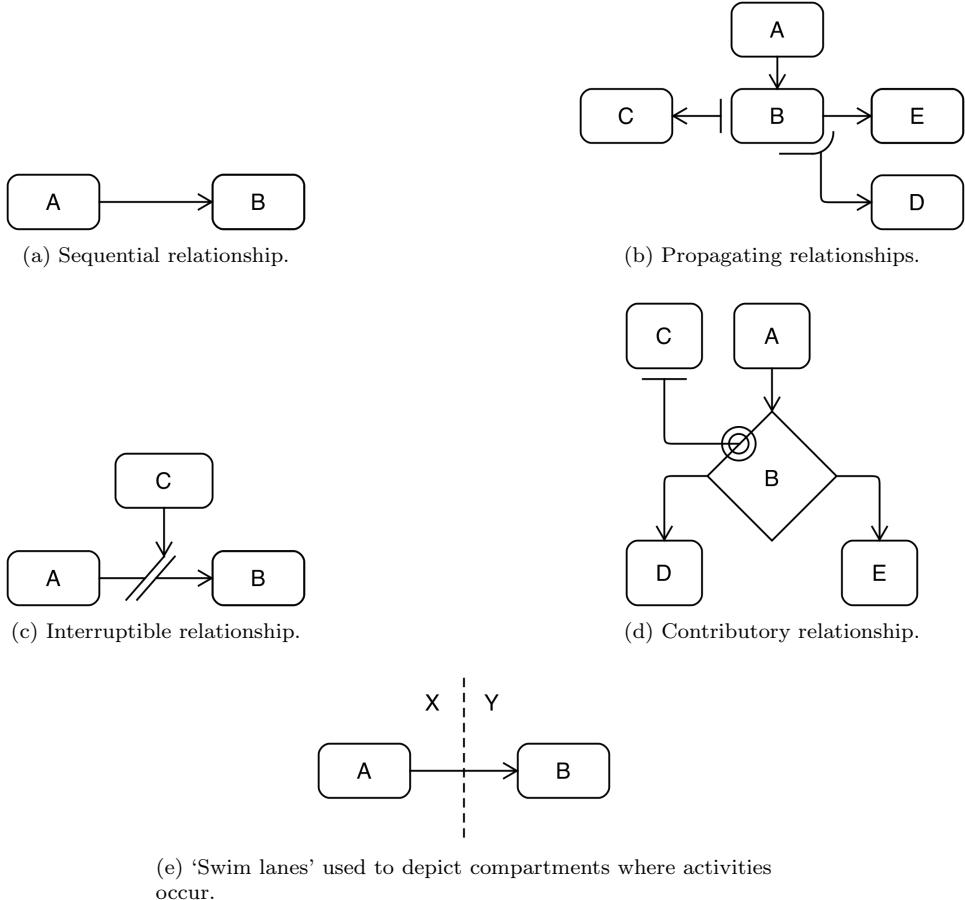


Figure 5: The types of relationship used in activity diagrams.

lanes', have been used to segregate groups of activities in accordance to the compartments in which they take place. This is demonstrated in figure 5e, in which activity A takes place in compartment X, and gives rise to activity B taking place in compartment Y.

Activities on the activity diagrams that follow are typically expressed at the level of a single cell. However, there are in all cases many such cells undergoing similar processes, and this is indicated in the text that accompanies the diagrams.

Note that some of the *perspectives* depicted are cyclic in nature, and hence do not contain end states. Start states have been given for cyclic diagrams in order to facilitate exploration of the diagram in the text.

The following sections model each of the four perspectives in turn. Section 2.2.1 depicts the *initial establishment of autoimmunity* in the CNS following immunisation. This leads to the *self-perpetuation of autoimmunity*, modelled in section 2.2.2. The manner in which the autoimmune response incites the regulatory response is described in section 2.2.3. Lastly, recovery is marked by a *type 2 deviation of the autoimmune response*, and this is depicted in section 2.2.4.

### 2.2.1 Initial establishment of autoimmunity

This section describes the onset of EAE autoimmunity, detailing the events that lead from immunisation to the apoptosis of neurons in the CNS, as depicted in figure 6. Note that whilst the figure depicts activities at the single-cell level, there are in all cases many such cells undergoing similar processes.

EAE is induced in experimental animals through the sub-cutaneous administration of myelin basic protein (MBP), complete Freund's adjuvant (CFA), and pertussis toxin (PTx). MBP is phagocytosed by DCs resident in the periphery where immunisation occurs, and this leads to their expression of MHC-II:MBP complexes. CFA and PTx are powerful immunopotentiators, and induce a type 1 polarisation in DCs, prompting them to upregulate co-stimulatory molecules. Following maturation, each DC will migrate to the draining lymph nodes, represented in

the domain model as the *secondary lymphoid organ* (SLO) compartment.

Once in the SLO compartment, type 1 polarised DCs secrete type 1 cytokine. They come into contact with MBP-specific CD4Th cells, which bind to the MHC-II:MBP complexes they express. This binding delivers signal 1 to the T cells. The type 1 cytokines secreted by the DC prompts the CD4Th cells to primarily adopt type 1 polarisations, leading to their differentiation into CD4Th1 cells. Note that the adoption of either type 1 or type 2 polarisations by CD4Th cells is probabilistic, and not absolute. Some proportion of the CD4Th population will differentiate into CD4Th2 cells, despite the secretion of type 1 cytokine by the DCs on which they prime. CD4Th2 cells are not believed to be of any significant consequence at this early stage of disease onset, and as such their actions are not explicitly detailed on the diagram. However, their behaviours are largely identical to that of CD4Th1 cells, as discussed in more detail in sections 2.2.4 and 2.3.1 below.

The CD4Th1 cells bind with the co-stimulatory molecules expressed by the DCs on which they prime, and derive signal 2. Receipt of both signals 1 and 2 leads a T cell to enter its proliferative cycle. During this cycle a CD4Th1 cell will divide and produce a naive daughter cell, a process termed *spawning* in this domain model. Whilst in its proliferative cycle, a single CD4Th1 cell may produce multiple daughter cells. The majority of these naive daughter cells will immediately bind the MHC-II:MBP complexes expressed by the priming DC, and follow a similar sequence of events as the parent cell. Those that do not begin priming on the same DC as their parents assume migratory behaviour.

Once the proliferative cycle is complete, a CD4Th1 cell will differentiate into an effector T cell and will resume migratory behaviour, leaving the SLO compartment. These cells will eventually migrate into the CNS compartment. The CNS compartment is populated with CNS macrophages, some proportion of which express MHC-II:MBP complexes. This expression is the result of physiological turnover of neurons in the CNS, which the CNS macrophages residing there will phagocytose. The infiltrating CD4Th1 cells bind with MHC-II:MBP as expressed on these CNS macrophages, and become locally activated. Local activation prompts CD4Th1 cells to secrete type 1 cytokines, which stimulate CNS macrophages to secrete soluble demyelinating agents (SDA). In sufficient concentration, SDA is harmful to neurons, and leads to their apoptosis.

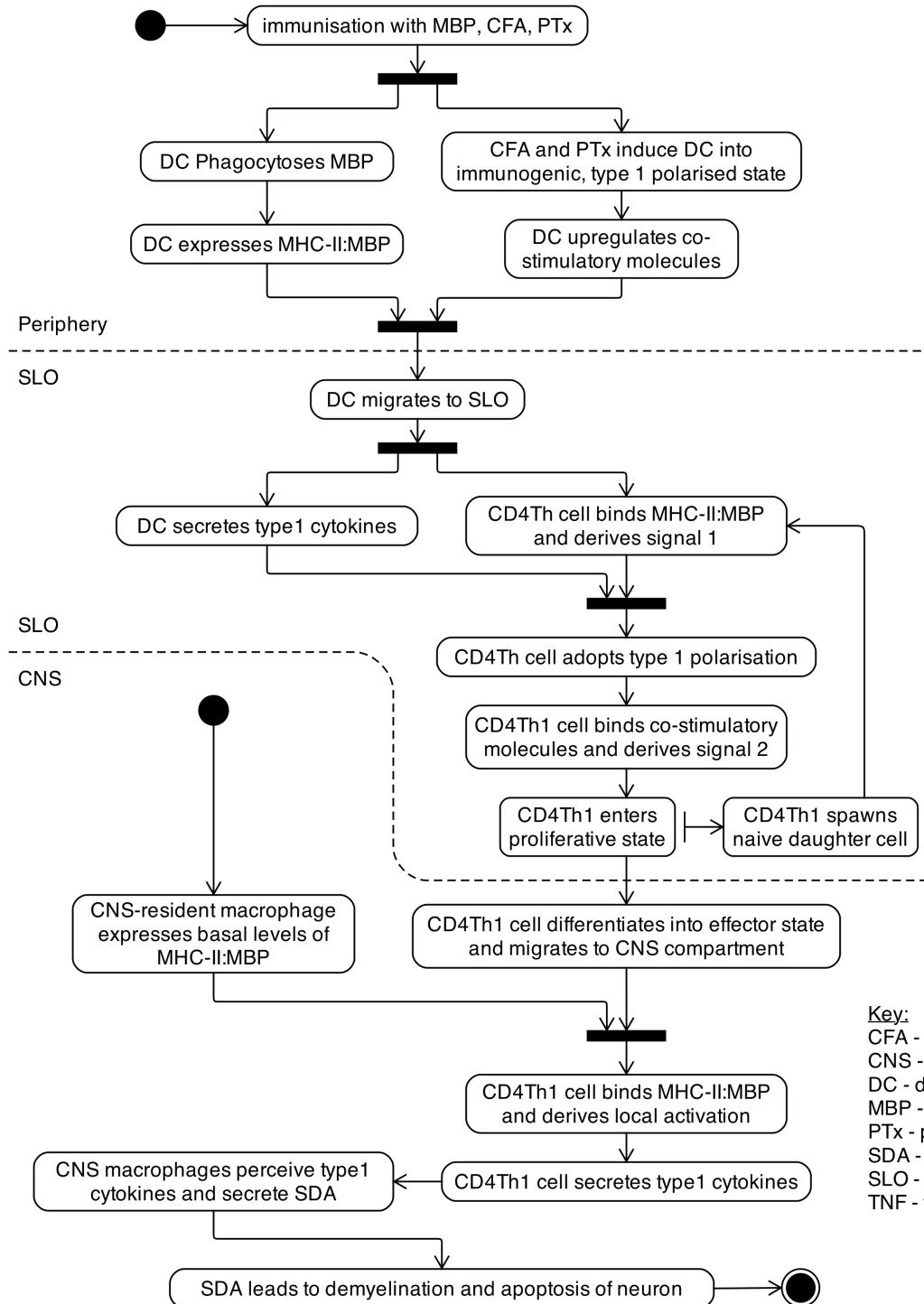


Figure 6: UML activity diagram depicting the cellular interactions and events that lead to neuronal apoptosis in the CNS following immunisation for EAE.

**Key:**

- CFA - complete Freund's adjuvant
- CNS - central nervous system
- DC - dendritic cell
- MBP - myelin basic protein
- PTx - pertussis toxin
- SDA - soluble demyelinating agents
- SLO - secondary lymphoid organ
- TNF - tumor necrosis factor

## 2.2.2 Self-perpetuation of autoimmunity

Following the initial establishment of autoimmunity, a series of events lead to its self-perpetuation. The key events and interactions between cells that constitute this behaviour are presented as an activity diagram in figure 7. Due to the self-perpetuating nature of this autoimmunity the diagram has no end state.

Apoptotic neurons in the CNS compartment are phagocytosed by resident DCs, and by CNS macrophages. Note that any single apoptotic neuron can only be phagocytosed by either a DC or a CNS macrophage; the use of the fork relationship here represents the requirement for both DCs and CNS macrophages to phagocytose neurons in order for autoimmunity to self-perpetuate. Both DCs and CNS macrophages present MHC-II:MBP complexes as a result of phagocytosis. The type 1 cytokines secreted by CD4Th1 infiltrates in the CNS induce type 1 polarisation in DCs, prompting their expression of co-stimulatory molecules.

Upon maturation, CNS-resident DCs migrate to the CLN, where they present MHC-II:MBP to naive and proliferating CD4Th populations, delivering signal 1 to them. Their type 1 polarisation leads DCs to secrete type 1 cytokine. As a result of this, proliferating CD4Th cells preferentially adopt type 1 polarisations and differentiate into CD4Th1 cells. Again, this preferential adoption of type 1 polarisations is not absolute, even in the presence of DCs secreting type 1 cytokine some CD4Th cells will adopt CD4Th2 polarisations. CD4Th2 cells play no role in the self-perpetuation of the autoimmune response, and as such are not indicated on the diagram. The co-stimulatory molecules expressed by the DCs on which CD4Th cells prime deliver signal 2 to them, inducing these T cells to enter their proliferation cycles. Proliferating CD4Th cells spawn naive daughter cells, many of which are immediately primed on the same DC as their parent cells. Upon completion of their proliferative cycles, CD4Th1 cells differentiate into effector cells and resume migratory behaviour. This leads them to infiltrate the CNS compartment.

Effector CD4Th1 cells entering the CNS compartment are locally activated by CNS macrophages expressing MHC-II:MBP. Following local activation, a CD4Th1 cell secretes type 1 cytokine, which in turn stimulates CNS macrophages to secrete soluble demyelinating agents (SDA). In sufficient concentration, SDA is harmful to neurons, and results in their apoptotic death. These apoptotic neurons are phagocytosed by CNS-resident DCs and CNS macrophages. Hence, autoimmunity self-perpetuates.

Figure 8 depicts a UML class diagram of the cells and molecules involved in both the instigation and self-perpetuation of autoimmunity. Immunisation with MBP, CFA, and PTx results in MBP being phagocytosed by DCs that are induced into immunogenic<sup>5</sup> phenotypes. This interaction is modelled such that a single instance of MBP, CFA and PTx can only have these effects on a single DC, but that any single DC may engage in this relationship with zero or more instances of MBP, CFA and PTx. DCs also derive MBP from the phagocytosis of apoptotic neurons. A single DC or CNS macrophage may phagocytose zero or more neurons, and a neuron can be phagocytosed by at most one DC, or one CNS macrophage, but not both. DCs secrete type 1 cytokines if they are type 1 polarised, however they do not if they are type 2 polarised. As such, any single DC may secrete zero to many type 1 cytokine molecules. A single instance of a type 1 cytokine molecule is secreted by either a DC or a CD4Th1 cell, but not both. In addition to CFA and PTx, type 1 cytokine can induce a type 1 polarisation in a DC. Many type 1 cytokine molecules are required for this induction, and since this interaction does not destroy the molecule, a single type 1 molecule may engage in this relationship with several distinct DCs before it decays, however it may only do so with a single DC at a time.

DCs that have phagocytosed MBP may present MHC-II:MBP complexes upon maturation. Such DCs are able to prime many CD4Th cells, and as such are capable of presenting many MHC-II:MBP complexes. Any particular DC may present between zero and many co-stimulatory molecules, depending on whether expression has been induced. Perception of either CFA and PTx, or sufficient type 1 cytokine prompts this expression of co-stimulatory molecules. A co-stimulatory molecule is expressed by exactly one DC. Co-stimulatory molecules deliver signal 2 to CD4Th cells, and prime them to proliferate. However, a CD4Th cell is not necessarily a recipient of signal 2 in its lifespan. Co-stimulatory molecules have been modelled such that a single instance is sufficient to deliver signal 2 to a T cell. A CD4Th cell is either a recipient of signal 2, or it is not, and it cannot receive the signal more than once. Likewise, MHC-II:MBP is modelled in such a manner that a single instance is sufficient to deliver signal 1 to a CD4Th cell. A single instance of MHC-II:MBP is also sufficient to locally activate a CD4Th cell. Signal 1 can be delivered to a CD4Th1 cell only once, whereas local activation may occur multiple times. An MHC-II:MBP molecule is able to engage in these relationships with only one CD4Th cell at a time. However, it may participate in many such events with many different CD4Th cells over the course of its existence, since CD4Th cells that differentiate into effector cells migrate away from the DC and allow other cells to bind with it.

Upon receipt of signal 2, a CD4Th cell enters its proliferative cycle, during which time it may spawn between zero and many daughter cells. A daughter cell has only a single parent cell which spawned it. If a CD4Th cell

<sup>5</sup>Expressing MHC:peptide complexes in conjunction with co-stimulatory molecules.

perceives sufficient type 1 cytokine upon receipt of signal 2, then it adopts a CD4Th1 polarisation. Many type 1 cytokine molecules are required for this induction, and since the perception of cytokine molecules does not result in their destruction, any single molecule may engage in multiple such relationships. CD4Th1 cells secrete many type 1 cytokine molecules upon being locally activated. Simultaneous perception of sufficient numbers of type 1 cytokine molecules can stimulate a CNS macrophage into secreting SDA. Only CNS macrophages secrete this cytokine in this domain model, and as such a SDA molecule must have been secreted by exactly one CNS macrophage. Simultaneous perception of sufficient SDA induces apoptosis in a neuron.

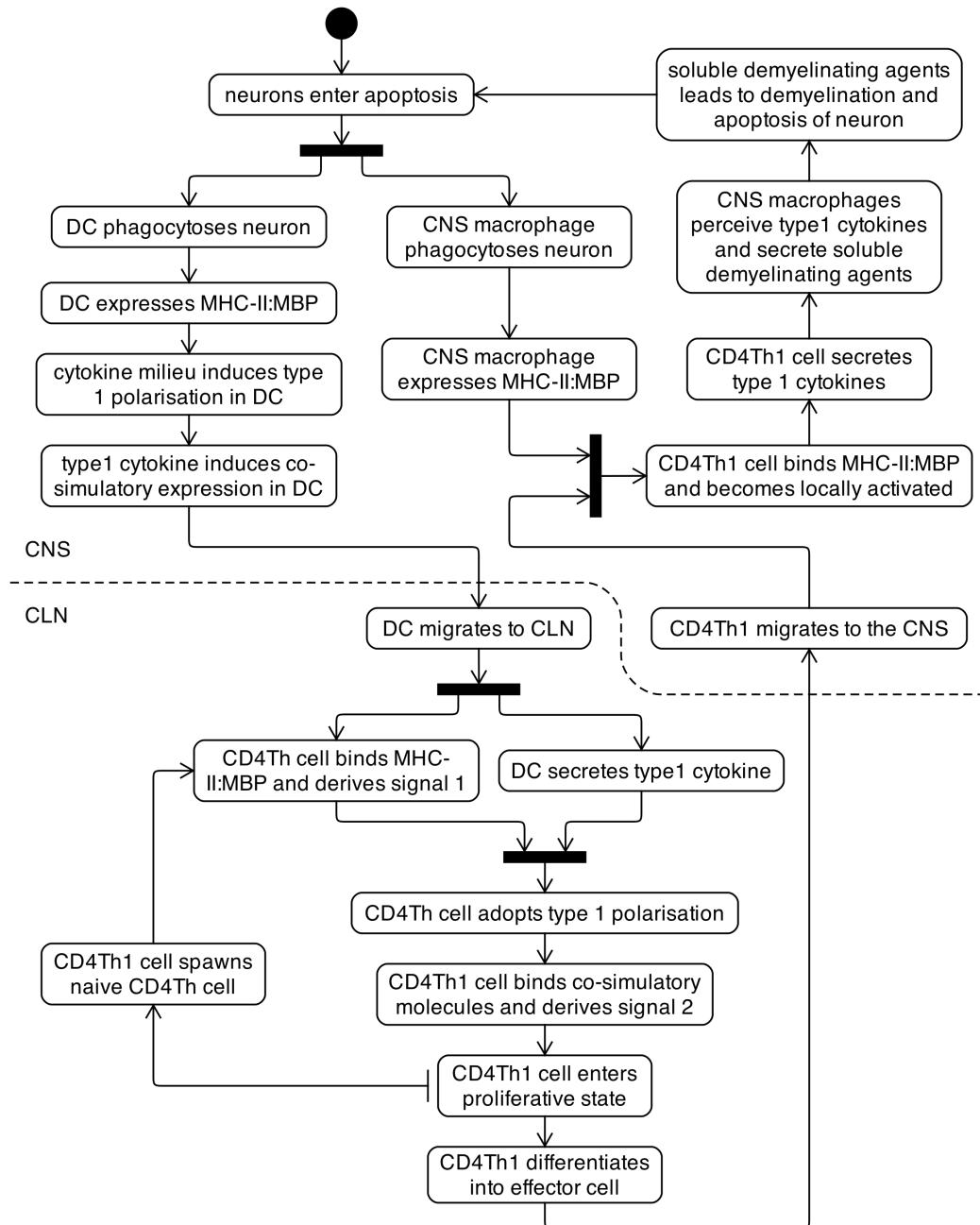


Figure 7: UML activity diagram depicting the cellular interactions and events that lead to the self-perpetuation of autoimmunity following neuronal apoptosis resulting from immunisation for EAE.

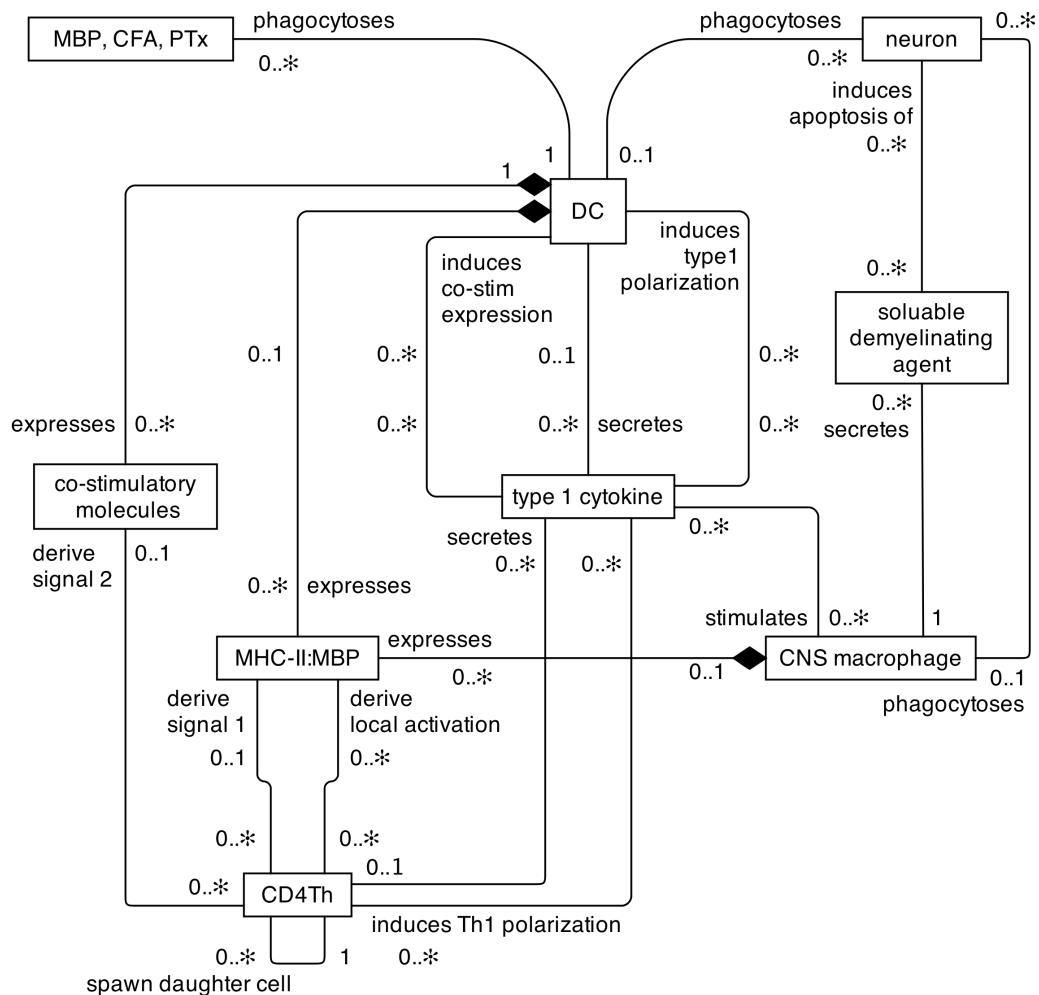


Figure 8: UML class diagram depicting the relationships between entities of the domain model involved in the establishment and perpetuation of the autoimmune response.

### 2.2.3 Establishment of regulation

The physiological lifecycle of T cells eventually leads all T cells to enter apoptosis, including MBP-specific CD4Th cells. It is the apoptosis of CD4Th cells that leads to the instigation of the regulatory T cell response that ameliorates autoimmune activity, as depicted on figure 9. Following their apoptosis, these CD4Th cells are phagocytosed by DCs. There is no constraint over which compartment a CD4Th cell resides in upon entering apoptosis, and they may be phagocytosed by any DC. CD4Th cells contain Fr3 and CDR1/2 peptides, which are components of the cell's T cell receptor (TCR). Upon phagocytosis of a CD4Th cell, a DC processes these peptides and is able to present them on MHC complexes. A pro-inflammatory cytokine milieu induces co-stimulatory molecule expression on a DC.

If the DC resided in the spleen during its immature state, then it remains there following maturation. Likewise CLN and SLO resident DCs do not migrate elsewhere upon maturation. If the DC resided in the CNS, then it migrates to the CLN. These migratory details are not indicated on the figure in the interest of reducing its complexity. Once mature, a DC will express MHC-II:Fr3, which leads to the delivery of signal 1 to naive CD4Treg cells that encounter it. The co-stimulatory molecules expressed by the DC deliver signal 2 to CD4Treg cells, causing them to enter their proliferative cycles. During this cycle any single CD4Treg may produce a number of naive CD4Treg daughter cells, which will either begin priming on the same DC as the parent cell, or resume migratory activity. Upon completion of its proliferative cycle, a CD4Treg will differentiate into an effector cell. As an effector cell, binding with MHC-II:Fr3 on a DC allows the CD4Treg to license the DC for Qa-1 molecule expression. Hence, the DC on which the CD4Treg was priming becomes licensed, however an effector CD4Treg may license any MHC-II:Fr3 expressing DC.

Once licensed for Qa-1 expression, DCs that have phagocytosed CD4Th cells are able to express Qa-1:CDR1/2 complexes. Naive CD8Treg cells that encounter the DC may derive signal 1 through binding with these complexes. Signal 2 is derived through binding with co-stimulatory molecules expressed on the DC. As with CD4Tregs, CD8Tregs enter their proliferative cycles upon receipt of both signals 1 and 2, and produce naive daughter CD8Treg cells during this process. Upon completion of the proliferative cycle, a CD8Treg will differentiate into an effector cell and resume migratory activity.

CD4Th1 cells express Qa-1:CDR1/2 for around 8 hours following their differentiation into effector cells. During this period, an effector CD8Treg may bind with the CD4Th1 cell, and induce it into apoptosis. Apoptotic CD4Th1 cells, like any apoptotic CD4Th cell, may be phagocytosed by a DC which derives Fr3 and CDR1/2 peptides from it. Both CD4Treg and CD8Treg cells secrete type 1 cytokine following local activation, and this contributes to the pro-inflammatory cytokine milieu that induces DCs into expressing co-stimulatory molecules necessary for the priming of further Treg populations. Hence, the regulatory immune response perpetuates, as is reflected in the lack of a terminating state on figure 9.

Figure 10 depicts a class diagram describing the static relationships between entities involved in the instigation and perpetuation of the regulatory immune response. The response is instigated through the phagocytosis of CD4Th (including both CD4Th1 and CD4Th2) cells by DCs. Any particular DC may phagocytose between zero and many CD4Th cells over the course of its lifespan. A CD4Th cell is phagocytosed by exactly one DC. This prompts the DC to express MHC-II:Fr3. Qa-1:CDR1/2 complexes are expressed only after a DC has been licensed by a CD4Treg, as discussed below. Expression is only possible on DCs that have phagocytosed at least one CD4Th1 cell, in which case many such complexes are expressed. Otherwise none are. A MHC-II:Fr3 complex is expressed by exactly one DC, and the same holds for Qa-1:CDR1/2 cells.

An MHC-II:Fr3 complex can bind with at most one CD4Treg cell at a time, but over the course of its existence may bind with many different CD4Treg cells. Such binding events deliver signal 1 to CD4Treg cells. A CD4Treg can receive signal 1 at most once. The same relationships hold for Qa-1:CDR1/2 complexes and CD8Treg cells.

Having received signal 1, both CD4Treg and CD8Treg cells can receive signal 2 if the DC to which they are bound expresses co-stimulatory molecules. A DC must be induced into expressing co-stimulatory molecules, and this only occurs upon the perception of a sufficient concentration of type 1 cytokines. A DC is only induced into co-stimulatory molecule expression at most once, after which these molecules are expressed for the remainder of its mature lifespan. Co-stimulatory molecule expression is induced by the simultaneous perception of sufficient concentration of type 1 cytokine, once a DC is mature. Perception by cells does not destroy cytokine molecules, and a particular type 1 cytokine may be perceived by any number of DCs during its existence. When and if expression is induced, a DC will express many co-stimulatory molecules. Co-stimulatory molecules are modelled in such a manner that a single instance is sufficient to deliver signal 2 to a T cell upon binding. As with signal 1, T cells can only receive signal 2 at most once, and they may not receive it at all during their lifespans.

Upon receipt of both signals 1 and 2, both CD4Tregs and CD8Tregs enter their proliferative cycles. During this time they may spawn any number of naive daughter T cells, though only one cell may be spawned at a time. A T

cell has only one parent T cell.

Proliferating T cells differentiate into effector cells once their proliferative cycle completes. An effector CD4Treg cell licenses a DC for Qa-1 expression upon binding with MHC-II:Fr3 complexes as expressed by that DC. This binding also locally activates the CD4Treg. A DC can be licensed for Qa-1 expression at most once, after which time it may express Qa-1 molecules for the remainder of its lifespan as a mature cell. This does not however prevent any number of effector CD4Tregs binding with a particular DC, and hence deriving local activation. A CD4Treg may license any number of DCs.

Both CD8Treg and CD4Treg cells secrete type 1 cytokines upon being locally activated. They may be locally activated any number of times after their differentiation into effector cells. If locally activated, these cells secrete many type 1 cytokines. A particular type 1 cytokine molecule can only be secreted from one source, in this case either a CD4Treg or a CD8Treg (other sources not indicated on the diagram).

Effector CD8Treg cells may bind with Qa-1:CDR1/2 complexes as expressed by CD4Th1 cells, a binding from which they derive local activation. Following this binding, a CD8Treg cell will induce apoptosis in the CD4Th1 cell. A CD4Th1 cell can be induced into apoptosis by a CD8Treg at most once, and a CD8Treg cell can induce apoptosis in any number of CD4Th1 cells.

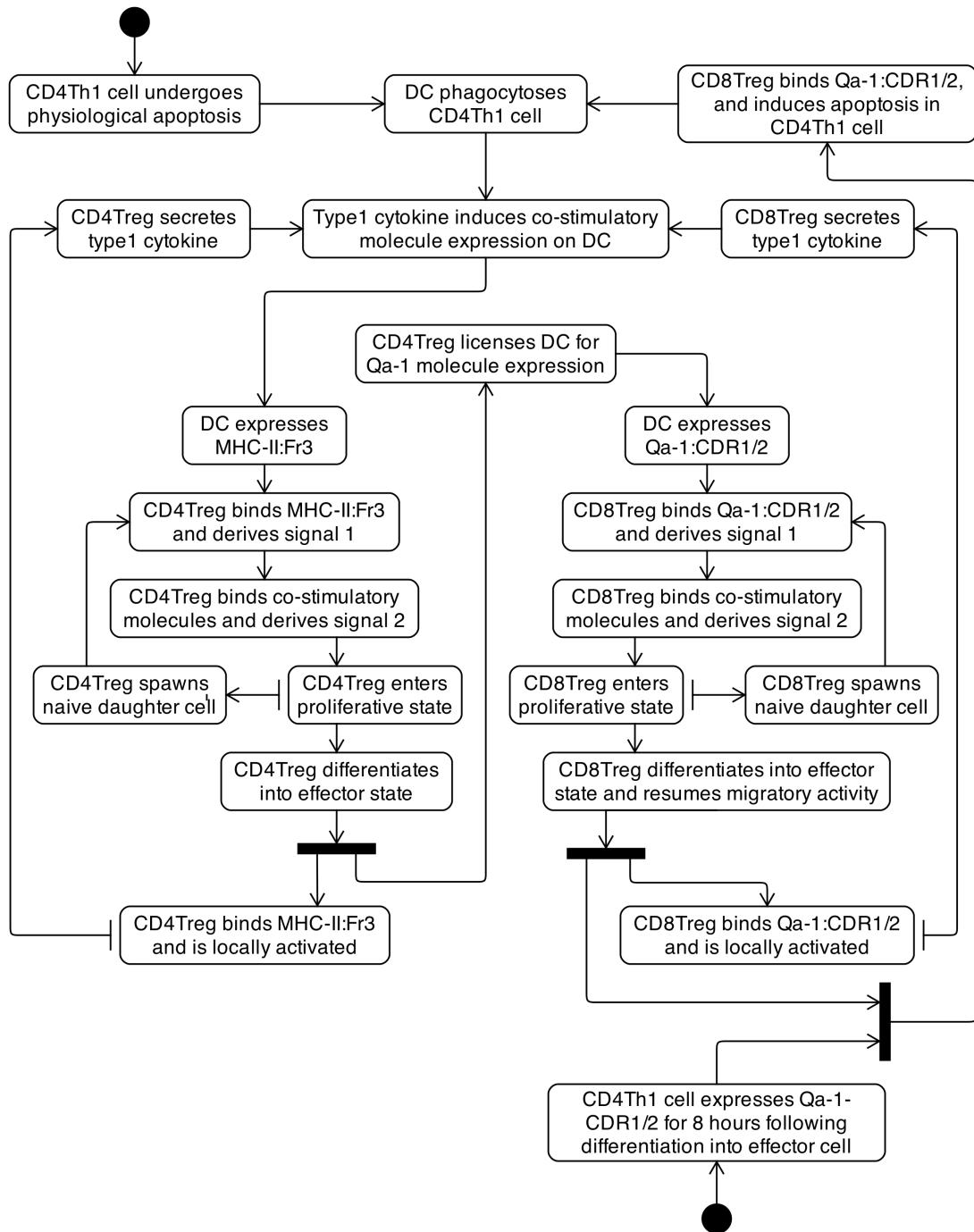


Figure 9: UML activity diagram depicting the cellular interactions and events that lead to the instigation and perpetuation of the regulatory immune response.

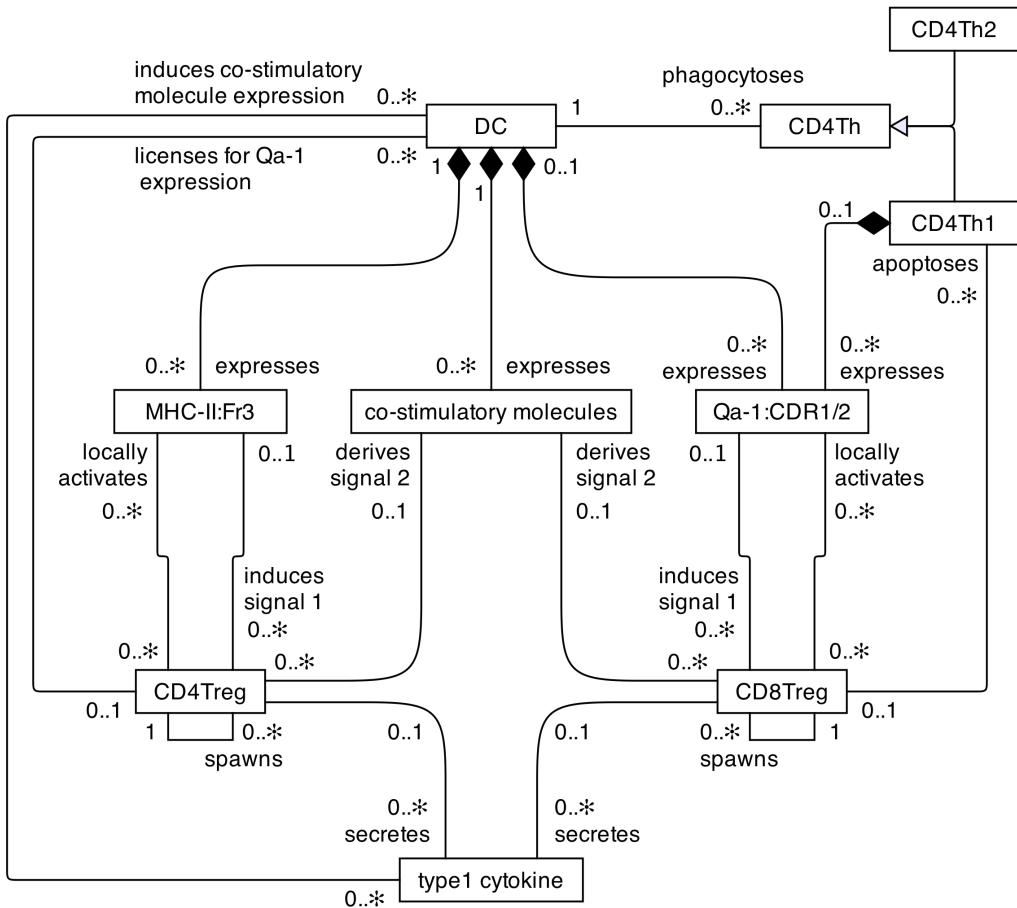


Figure 10: UML class diagram depicting the relationships between entities of the domain model involved in the instigation and perpetuation of the regulatory immune response.

#### 2.2.4 Type 2 deviation of the autoimmune response

This section describes the last of the four stages of EAE and its associated recovery: the deviation of the autoimmune response in a type 2 direction. Figure 11 depicts an activity diagram showing the cellular interactions and events that lead to the type 2 deviation. Once more the actions depicted here are cyclic, type 2 deviation does not occur as a single atomic action within the system, but emerges as a gradual shift in behaviours spanning multiple populations of cells. However, the autoimmune response does eventually terminate, and hence an end state is indicated.

Immunisation for EAE induces a heavily type 1 response, with CFA and PTx prompting DCs to adopt a type 1 polarisation which causes priming CD4Th cells to preferentially adopt a CD4Th1 polarisation. As noted above, a DC's influence on the polarisation adopted by priming CD4Th cells is not absolute; although the majority of CD4Th cells primed on type 1 DCs adopt CD4Th1 polarisations, some still differentiate into CD4Th2 cells. Both CD4Th1 and CD4Th2 cells migrate to the CNS compartment, where they secrete type 1 and type 2 cytokines respectively. In the early stages of EAE, CD4Th1 cells outnumber their CD4Th2 counterparts, and as such the CNS cytokine milieu is composed primarily of type 1 cytokine. In sufficient concentration, type 1 cytokine leads to neuronal apoptosis.

DCs in the CNS phagocytose apoptotic neurons, and upon maturation adopt either a type 1 or type 2 polarisation, depending on the balance of type 1 and type 2 cytokines in their local vicinity. Likewise, by default a DC will not express co-stimulatory molecules, these are induced in the presence of sufficient type 1 cytokine. A DC that expresses both MHC:peptide complexes and co-stimulatory molecules is immunogenic, and is able to prime T cell populations. Absence of co-stimulatory molecules renders a DC tolerogenic, in which case it induces anergy in T cells, preventing their proliferation and differentiation. Immunogenic DCs are either type 1 or type 2 polarising, which influences the polarisation adopted by the CD4Th cells that they prime.

The physiological turnover of CD4Th cells leads to their phagocytosis by DCs, and the subsequent priming of CD4Treg and CD8Treg populations. During the ~8 hours immediately following their differentiation into effector cells, CD4Th1 cells are susceptible to regulation, wherein CD8Tregs induce apoptosis in CD4Th1 cells. At a population level this regulatory action serves to reduce the number of CD4Th1 cells that infiltrate the CNS compartment. CD8Treg cells do not regulate CD4Th2 cells, which continue to enter the CNS and secrete type 2 cytokine. The reduction in CD4Th1 cells results in the cytokine milieu in the CNS shifting towards a type 2 dominance. DCs able to present MBP will adopt type 2 polarisations, and prime predominantly CD4Th2 cells. Once more, this adoption of type 2 polarisations by the CD4Th population is not absolute, and some CD4Th1 cells will continue to be primed, however many of these will be subject to regulation. As the quantity of CD4Th1 cells infiltrating the CNS reduces, so too does the quantity of type 1 cytokine being secreted there. This results in a reduction in neuronal apoptosis, and hence a reduction in MBP-presenting DCs migrating to the CLN. DCs migrating from the CNS to the CLN will fail to express co-stimulatory molecules, hence becoming tolerogenic and unable to prime T cell populations.

As the number of CD4Th cells primed in the system reduces, so too does the number of DCs presenting MHC-II:Fr3, and consequently Qa-1:CDR1/2. The regulatory immune response terminates alongside the autoimmune response, and experimental animals recovery from paralysis.

Figure 12 denotes a class diagram of the cells and molecules involved in type 2 deviation, and indicates relationships between these entities, and the numbers of entities that engage in such relationships over time. The central component of type 2 deviation of the autoimmune response is the DC, which primes T cells populations. Any particular T cell primes only on a single DC at a time. In the majority of cases a T cell entering proliferation will complete this cycle whilst bound to the same DC, however should the DC expire, a proliferating T cell will continue migratory behaviour until either differentiating into an effector cell, or binding to another DC expressing MHC:peptide complexes for which it is specific. If a DC is not presenting MHC:peptide complexes then it cannot prime T cells, otherwise it may prime many.

The expression of MHC:peptide complexes requires that a DC have phagocytosed either a neuron for expression of MBP peptides, or a CD4Th cell for the expression of Fr3 and CDR1/2 peptides. Any particular apoptotic cell must be phagocytosed by exactly one APC (CNS macrophages are not indicated on figure 12, since they are not directly related to type 2 deviation of the immune response). The phagocytosis of Treg cells is not indicated on the diagram since they contain no peptides pertaining to EAE.

A pre-requisite for priming of T cells is that the DC express co-stimulatory molecules, a single instance of which is modelled here as being sufficient to induce signal 2 in a single T cell at a time. Co-stimulatory molecule expression is induced in DCs through the perception of type 1 cytokine. Many such cytokine molecules are required for this induction. A type 1 cytokine molecule is secreted by only one cell, which is either a DC, a locally activated effector CD4Th1, CD4Treg (not shown on figure 12) or CD8Treg cell.

An immunogenic DC is either type 1 or type 2 polarized, and this influences the polarization adopted by CD4Th

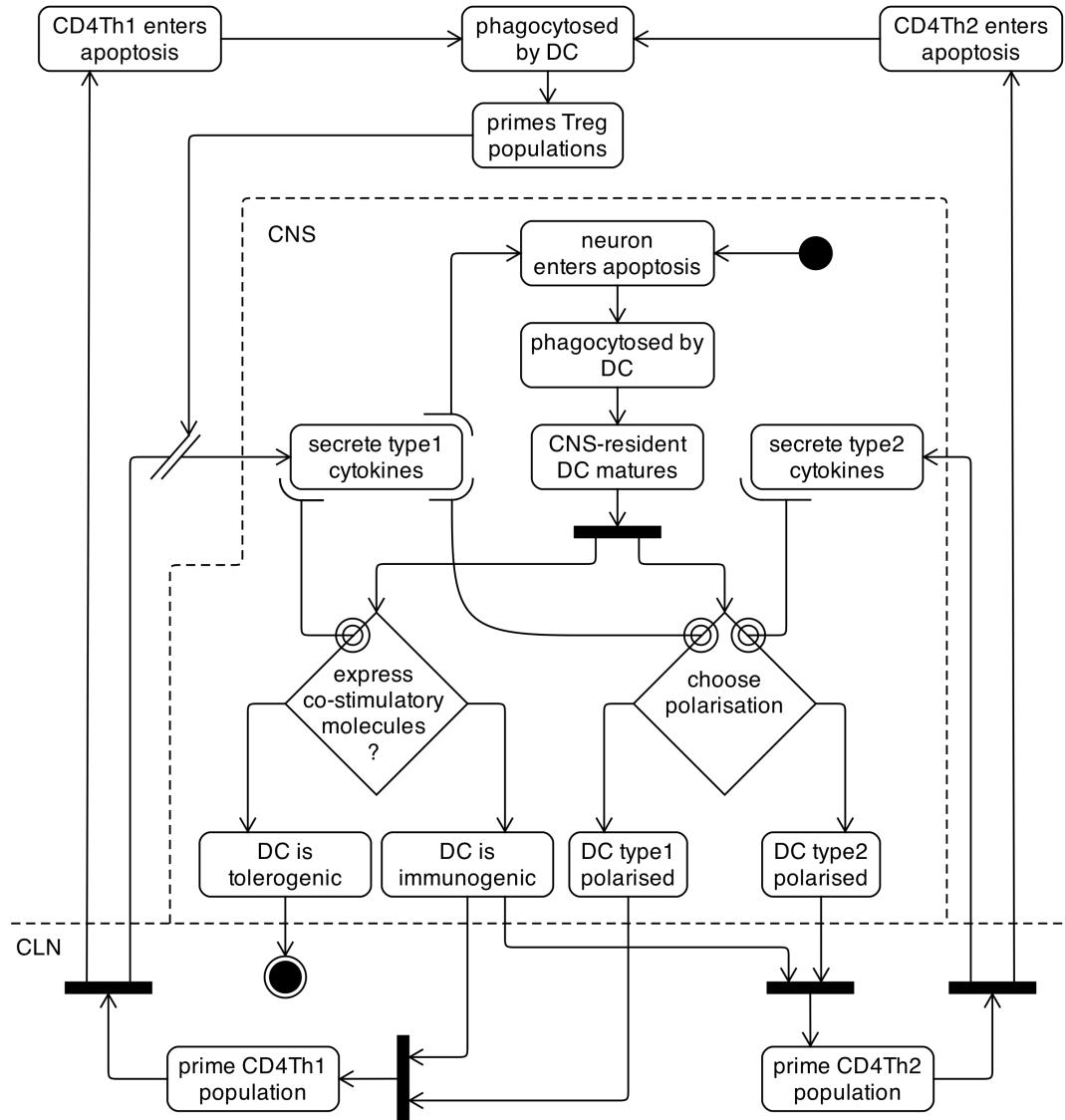


Figure 11: UML activity diagram depicting the cellular interactions and events that lead to a type 2 deviation of the immune response.

cells that it primes. Type 1 polarized DCs secrete type 1 cytokine, whereas type 2 polarized DCs do not secrete any cytokines. The polarization that a DC adopts is based on the balance of type 1 versus type 2 cytokine in its immediate vicinity upon maturation. Cytokines molecules are not destroyed following their perception by cells, and as such they may influence anywhere between zero and many cells.

Neurons are indirectly induced into apoptosis by CD4Th1 cells, through the secretion of type 1 cytokine and stimulation of CNS macrophages. Owing to this indirect relationship, a single neuron may be induced into apoptosis through the actions of at least one CD4Th1 cell, and a single CD4Th1 cell is responsible for inducing apoptosis in any number of neurons.

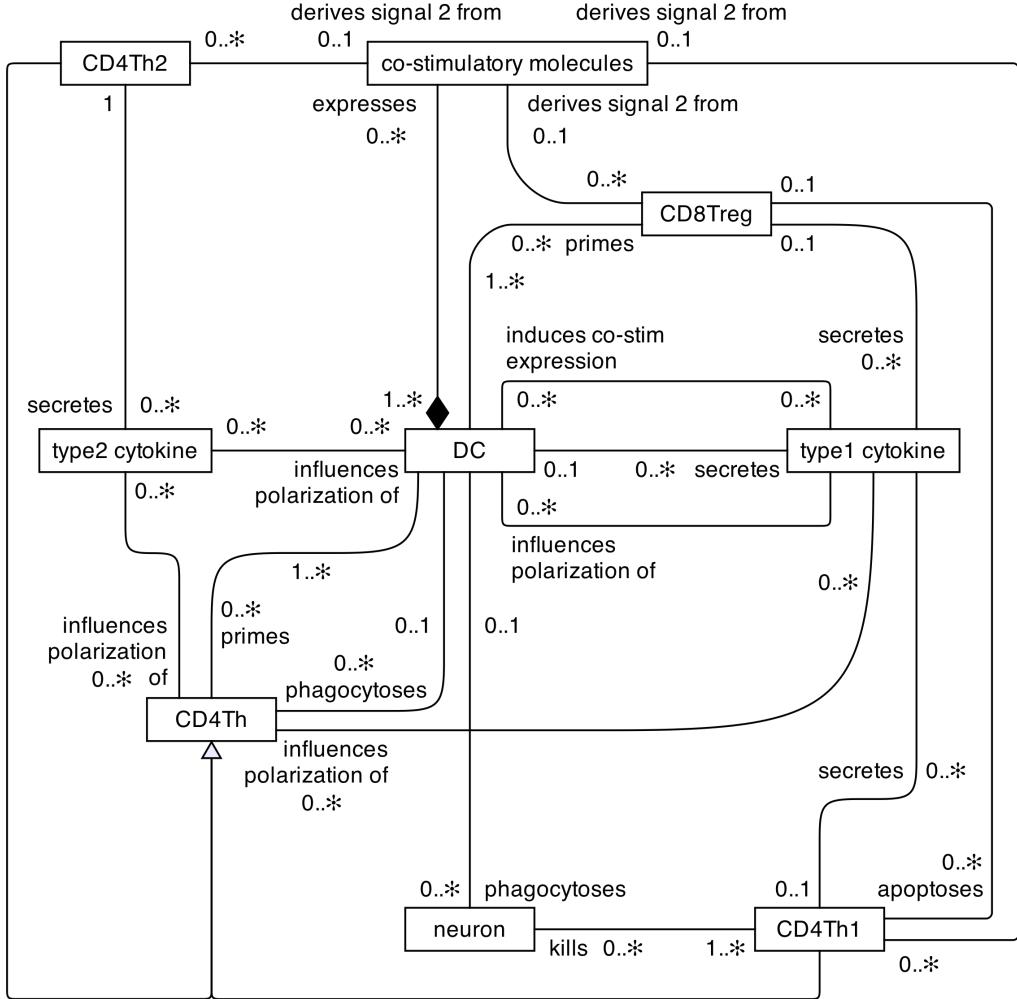


Figure 12: UML class diagram depicting the relationships between entities of the domain model that lead to the type 2 deviation of the autoimmune response.

## 2.3 Depicting single-entity dynamics

The following sections detail the complete dynamics for the various physical entities included in this domain model, at the single-entity level. Dynamics are expressed using standard UML state machine diagram notation. In facilitating the expression of transition guards, the following symbols have been used:

‘&’: logical conjunction. ( $A \& B$ ) evaluates to true when both  $A$  and  $B$  are true, and false otherwise.

‘|’: logical disjunction. ( $A | B$ ) evaluates to true when either  $A$  or  $B$  are true, and false otherwise.

‘ $\delta(\text{condition})$ ’: used to indicate probabilistic events. Will evaluate to true with some probability.

‘ $\lambda(\text{condition})$ ’: used to indicate temporal events. Will evaluate to true when some period of time has elapsed.

Section 2.3.1 examines the single-cell dynamics of the various T cells represented in the domain model. Section 2.3.2 explores the dynamics of dendritic cells and CNS macrophages, and neurons are the subject of section 2.3.3. Lastly, section 2.3.4 focusses on the dynamics of MBP and the various cytokines represented in the domain model.

### 2.3.1 T cell dynamics

Figures 13, 14 and 15 depict state machine diagrams of CD4Th, CD4Treg and CD8Treg cells respectively. The three T cell types represented in the domain model share many similarities. They commence their life cycles in

a naive state, in the circulatory system, and assume migratory behaviour. All naive T cells can migrate into the SLO, the CLN, and the spleen from the circulatory system, and vice versa.

Migratory behaviour continues until a T cell encounters a DC presenting MHC:peptide complexes for which it is specific. Thereupon the T cell will cease migratory behaviour and bind to the APC. It derives signal 1, leading it to enter a partially activated state. In the case of CD4Th cells, a polarisation is adopted at this point. These cells become either CD4Th1 or CD4Th2 cells, depending on the local cytokine milieu, largely influenced by whether or not the DC secretes type 1 cytokine. A predominantly type 1 cytokine milieu increases the probability that a CD4Th1 polarisation be adopted, whereas lack of type 1 cytokine promotes CD4Th2 polarisation.

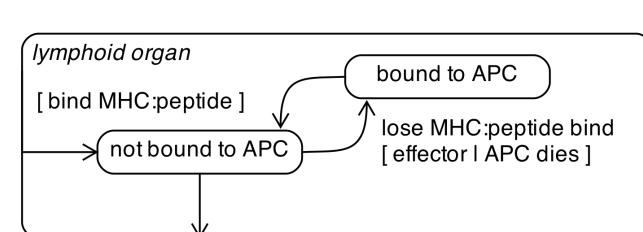
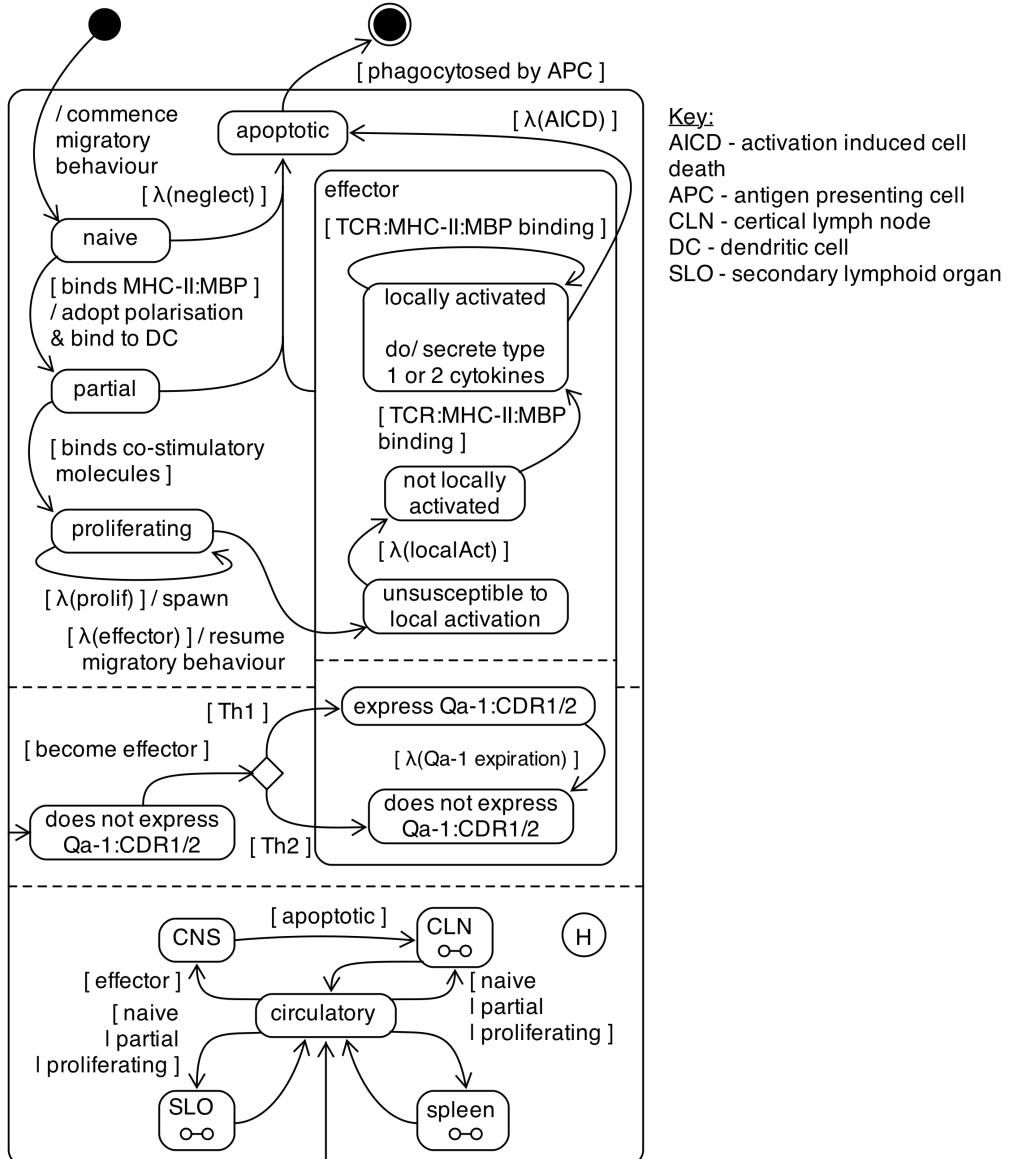
If the DC to which the T cell binds expresses co-stimulatory molecules, then the T cell derives signal 2, and enters a proliferative state. Whilst in either a naive or partially activated state, a T cell may survive some period of time without receiving signal 1 and then signal 2 before dying of neglect. This is represented on the state machine diagrams as  $\lambda(\text{neglect})$ .

T cells remain in a proliferative state for some period of time, represented as  $\lambda(\text{effector})$  before they differentiate into effector cells. Whilst proliferating, a T cell divides and creates naive daughter cells of the same type, a process termed *spawning* in this domain model. The time required for this to occur is represented as  $\lambda(\text{prolif})$ . Daughter T cells are created in the same locale as their parents, indicated by the *H* state in the state machine diagrams.

Effector T cells detach from the DCs on which they prime, and resume migratory behaviour. If primed in either the CLN, SLO or spleen compartments, these T cells re-enter the circulatory system. Effector T cells lack the adhesion molecules required for them to migrate back into the CLN or SLO compartments, however they may re-enter the spleen. Effector CD4Th1 and CD4Th2 cells are able to migrate into the CNS compartment, where they remain until they enter apoptosis, upon which they may migrate into the CLN.

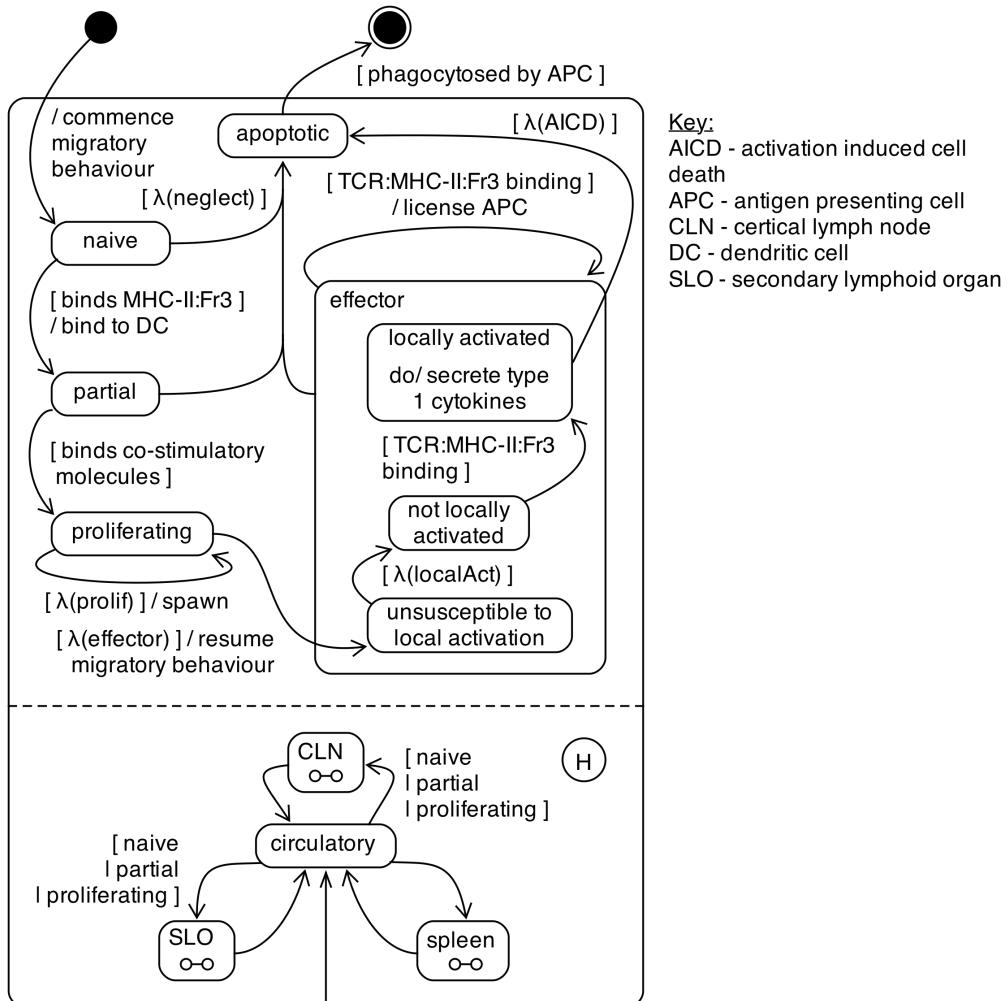
Effector T cells require local activation before they may secrete cytokines. It requires some time following differentiation into an effector cell before a T cell may be locally activated through TCR:MHC:peptide interaction. TCR:MHC:peptide interaction prior to the elapse of this time, represented by  $\lambda(\text{localAct})$ , may allow a T cell to perform some effector function, but not cytokine secretion. In the case of CD4Treg cells, TCR:MHC-II:Fr3 binding at any point during their effector cycles results in the CD4Treg cell attempting to license the DC with which it is bound. For CD8Treg cells, TCR:Qa-1:CDR1/2 binding with a CD4Th1 cell results in the induction of apoptosis in the CD4Th1 cell. After  $\lambda(\text{localAct})$  has elapsed, TCR:MHC:peptide binding induces cytokine secretion in effector T cells. CD4Th1, CD4Treg and CD8Treg cells secrete type 1 cytokine, whereas CD4Th2 cells secrete type 2 cytokine.

As with naive and partially activated T cells, effector T cells must receive stimulation from TCR:MHC:peptide binding regularly to avoid entering apoptosis through neglect. For those cells that reach locally activated states, effector lifespan is still limited, and persistent stimulation of this form leads to activation induced cell death (*AICD*). Apoptotic T cells are eventually phagocytosed by APCs.

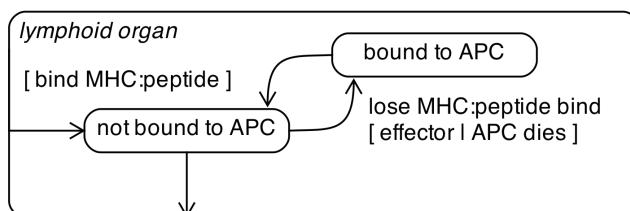


(b) Decomposition of the lymphoid organ states: the SLO, CLN and spleen.

Figure 13: State machine diagram depicting the dynamics of CD4Th cells.

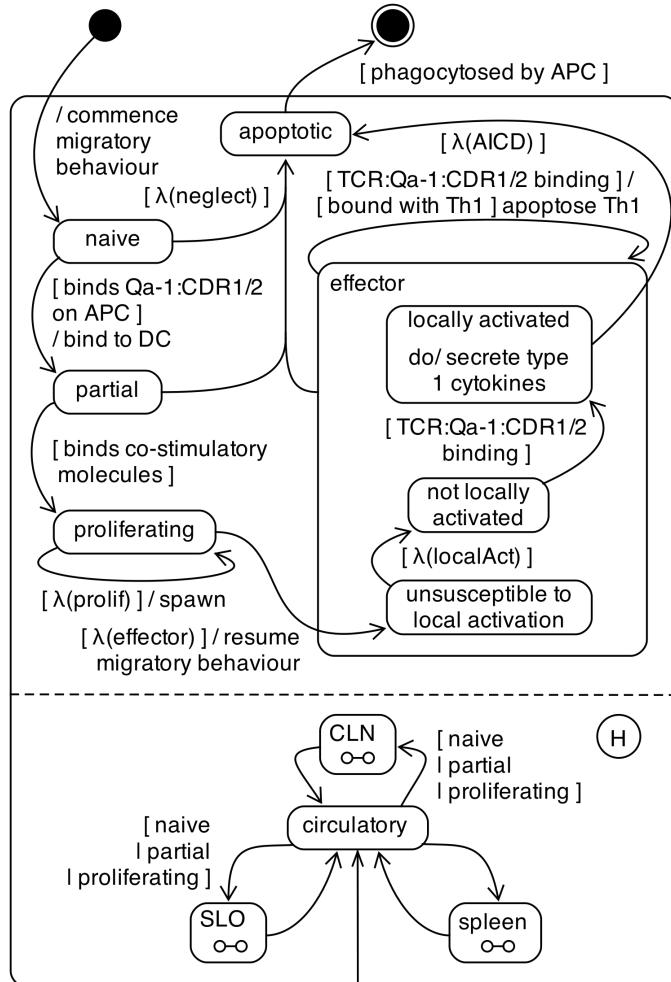


(a) Dynamics of CD4Treg cells.

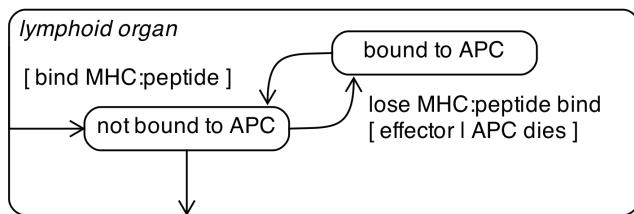


(b) Decomposition of the lymphoid organ states: the SLO, CLN and spleen.

Figure 14: State machine diagram depicting the dynamics of CD4Treg cells.



(a) Dynamics of CD8Treg cells.



(b) Decomposition of the lymphoid organ states: the SLO, CLN and spleen.

Figure 15: State machine diagram depicting the dynamics of CD8Treg cells.

### 2.3.2 DC and CNS macrophage dynamics

Dendritic cells are responsible for priming T cell populations. Their dynamics are depicted on figure 16. Dendritic cells begin their existence in an immature state, and mature some time later, represented by  $\lambda(\text{maturation})$ .

In an immature state a DC is highly phagocytic, and the capacity to perform phagocytosis is reduced following the maturation of the cell. DCs may reside in five compartments in the domain model: the CNS, CLN, SLO, periphery, and the spleen. If the DC is in any of the CLN, SLO or spleen during their immature lifespan, then they do not migrate to away from these compartments upon maturation. However, upon maturation DCs residing in the CNS or the periphery migrate to the CLN and the SLO respectively.

For a DC to present MHC:peptide complexes, it must be capable of presenting both the MHC and peptide components. The peptide components considered in this domain model are MBP, Fr3 and CDR1/2. MBP is derived from phagocytosis of a neuron or from direct phagocytosis of MBP following immunisation. Fr3 and CDR1/2 are derived from the TCRs of CD4Th cells. In addition to phagocytosis of such cells, capacity to present peptides is probabilistic: only a very small proportion of phagocytosis events lead to the derivation of presentable peptides. This is represented as  $\delta(\text{derive presentable peptides})$  on the diagram.

A DC is able to express MHC-II molecules upon maturation. Qa-1 molecules can only be expressed once the DC has been licensed to do so by a CD4Treg cell. By default, a mature DC does not express co-stimulatory molecules upon maturation. Expression is induced by the perception of a sufficient concentration of type 1 cytokine, and may be induced through licensing by either a CD4Th cell or a CD4Treg cell. Co-stimulatory molecule expression may be induced at any point following maturation.

Upon maturation a DC adopts either a type 1 or type 2 polarisation. Type 1 polarised DCs secrete type 1 cytokine. The decision of which polarisation to adopt is entirely dependent on the balance of type 1 versus type 2 cytokine in the location of the DC upon maturation.

DCs expire once their lifespan is exceeded, represented as  $\lambda(\text{expire})$ .

CNS macrophages exist only in the central nervous system (CNS). The only MHC:peptide complex that they present is MHC-II:MBP, and in a similar manner to DCs, the presentation requires the phagocytosis of a neuron, and is probabilistic. Some small proportion of CNS macrophages express MBP immediately, represented by  $\delta(\text{basal expression})$ . This is to reflect the fact that the physiological turnover of neurons, which is not in itself represented in the domain model, will result in their phagocytosis by CNS macrophages, and the presentation of MHC-II:MBP complexes.

CNS macrophages exist in immature and mature states. Whilst immature they are more phagocytic than when mature. Maturation occurs some time into their lifespan, represented by  $\lambda(\text{maturation})$ , but may also be induced through perception of a sufficient concentration of type 1 cytokine. Perception of sufficient concentration of type 1 cytokine induces SDA secretion in CNS macrophages. Both immature and mature CNS macrophages are able to express MHC-II molecules.

CNS macrophages do not exist indefinitely, and expire after some period of time, represented by  $\lambda(\text{expire})$ .

### 2.3.3 Neuron dynamics

Neurons reside exclusively in the CNS compartment. Their dynamics are depicted on figure 18. All neurons express MBP. They are alive until perception of sufficient concentration of SDA induces their apoptotic death. Apoptotic neurons are eventually phagocytosed by an APC.

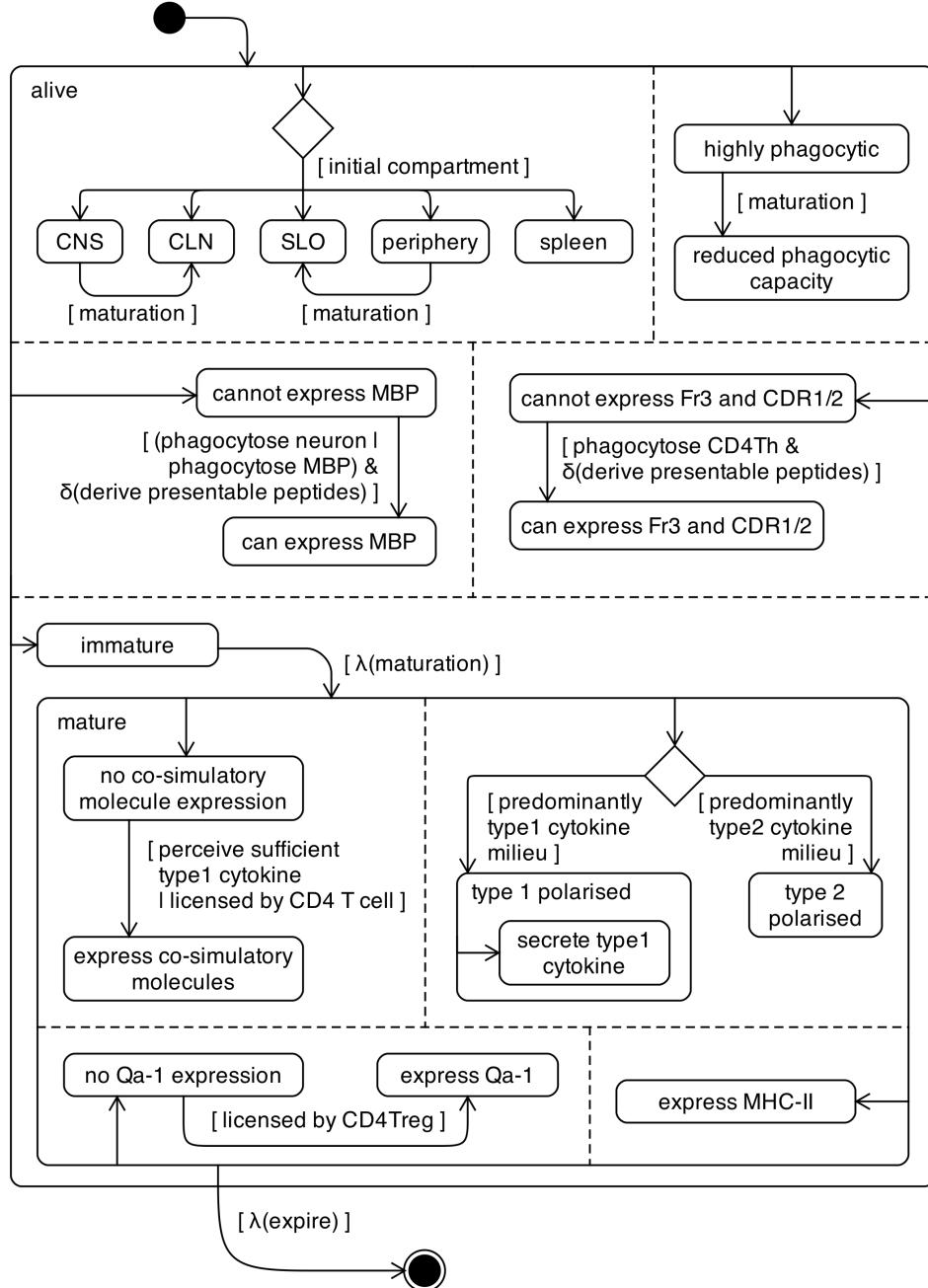


Figure 16: State machine diagram depicting the dynamics of dendritic cells.

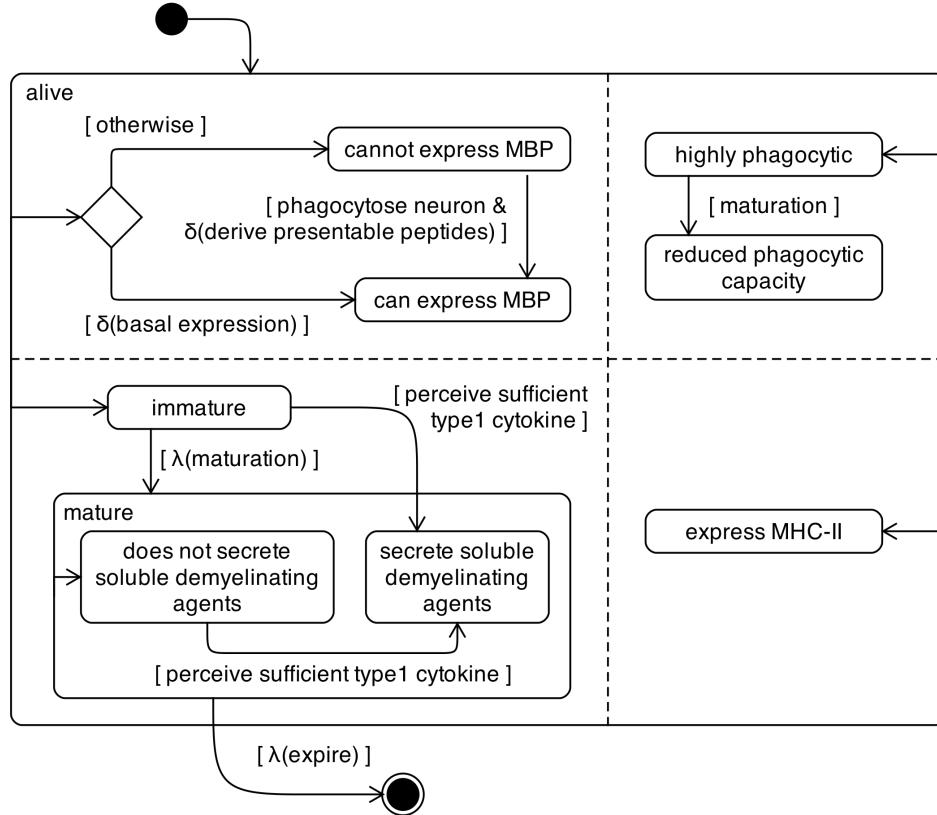


Figure 17: State machine diagram depicting the dynamics of CNS macrophages.

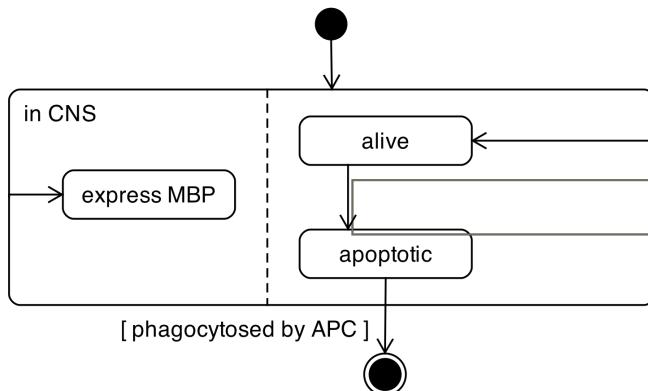


Figure 18: State machine diagram depicting the dynamics of neurons.

### 2.3.4 Cytokine and MBP dynamics

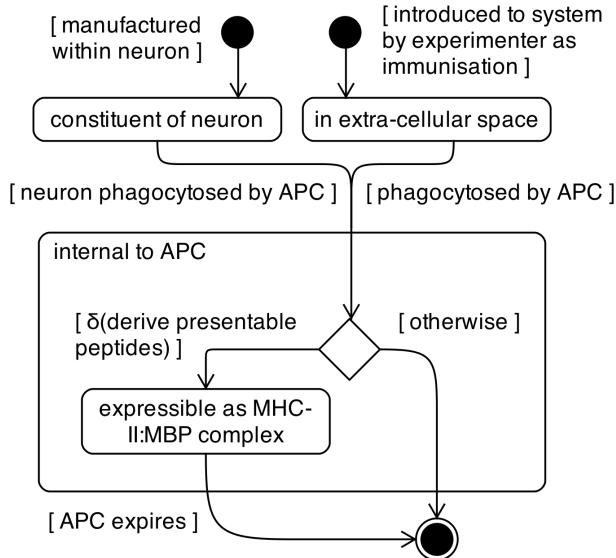


Figure 19: State machine diagram depicting the dynamics of myelin basic protein (MBP).

Myelin basic protein (MBP) is the substance for which encephalitogenic T cells are specific; it is integral to autoimmune activity. Type 1 and type 2 cytokines influence a variety of cellular behaviours that dictate the progression of autoimmunity and recovery. Although these entities of the domain model do not strictly carry state, state machine diagrams have been used to depict the system from their perspectives.

MBP dynamics are depicted on figure 19. It is introduced to the system through two means: it is manufactured and expressed by neurons, or it is administered into the system by an experimenter. Neurons that enter apoptosis are eventually phagocytosed by an APC. Likewise, MBP residing in extra-cellular space as a result of immunisation is eventually phagocytosed by an APC.

Once internal to the APC, there is a probability that MBP will be successfully processed for presentation as MHC-II:MBP complexes. This is represented as  $\delta(\text{derive presentable peptides})$ . If MBP is successfully presented on MHC, then the MBP molecule is destroyed when the APC expires, if it is not, then it is considered to be destroyed immediately; it is of no further consequence to the system.

Type 1 cytokine, depicted in figure 20, is secreted by a variety of cells: CD4Th1 cells, CD4Treg cells, CD8Treg cells, and dendritic cells (DCs). Once secreted it exists in extra-cellular space for some period of time before it decays. Whilst in extra-cellular space, a type 1 cytokine molecule may be perceived by a variety of cells, and may influence their subsequent behaviours. Note that the induction of behavioural changes in cells requires the simultaneous perception of multiple cytokine molecules. Perception of a cytokine molecule by a cell does not destroy the molecule, and it continues to exist in extra-cellular space after dis-engaging from the cell.

Perception of sufficient type 1 cytokines by a CD4Th cell can result in it adopting a CD4Th1 polarisation, however this adoption is preferential, not absolute. The probability of a CD4Th1 polarisation being adopted is represented as  $\delta(\text{type 1})$ . If type 1 cytokine is perceived as a sufficiently dominant constituent of the local cytokine milieu, then a maturing DC will adopt a type 1 polarisation. Type 1 cytokine perception also induces co-stimulatory molecule expression in mature DCs. Lastly, sufficient concentrations of type 1 cytokine induce SDA secretion in CNS macrophages.

The dynamics of type 2 cytokine, and its influence on other cells of the domain model, are depicted on figure 21. The dynamics of type 2 cytokine are identical to those of type 1 cytokine, with the exception that it is secreted by CD4Th2 cells only. Its influences, however, differ. It has two influences over cells of the domain model. When perceived in sufficient concentration, it induces a preference for CD4Th cells to adopt type 2 polarisations, again this preference is not absolute. It may also induce type 2 polarisation in DCs if perceived as a sufficiently dominant constituent of the local cytokine milieu.

Soluble demyelinating agent (SDA), figure 22, is secreted only by CNS macrophages and exists in the CNS compartment. When perceived in sufficient concentration it induces apoptosis in neurons.

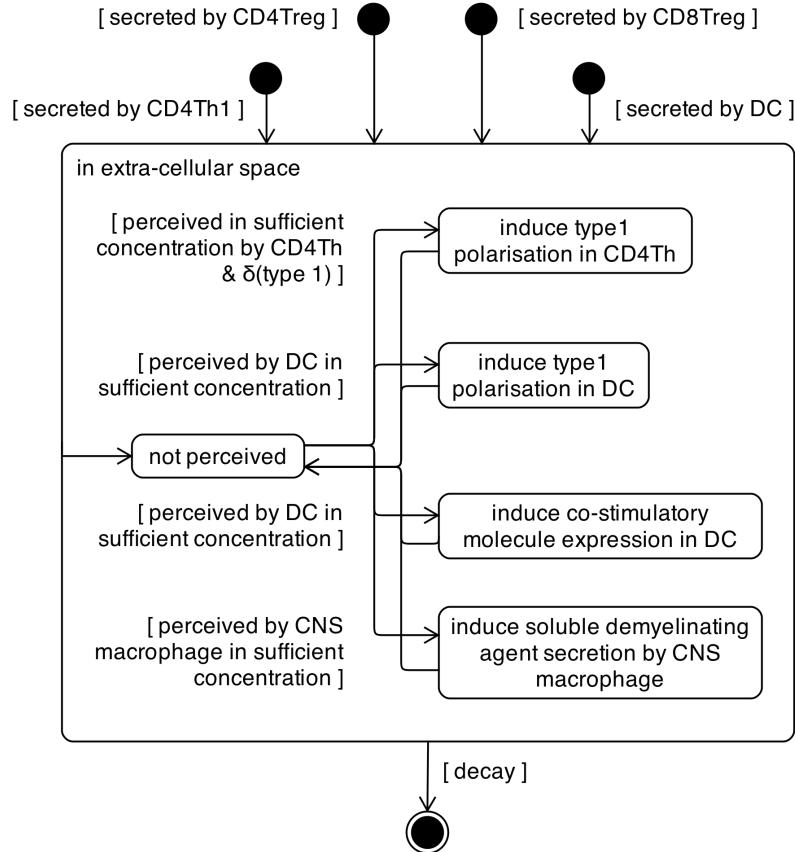


Figure 20: State machine diagram depicting the dynamics of type 1 cytokine, and its influence on other cells of the domain model.

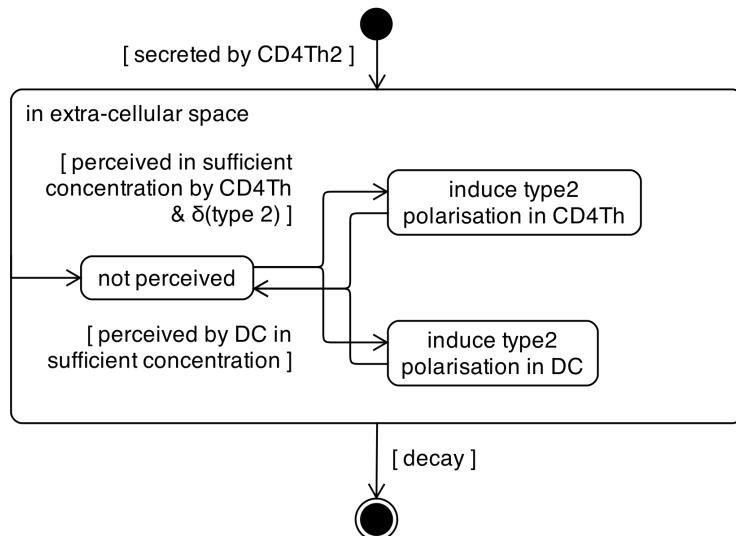


Figure 21: State machine diagram depicting the dynamics of type 2 cytokine, and its influence on other cells of the domain model.

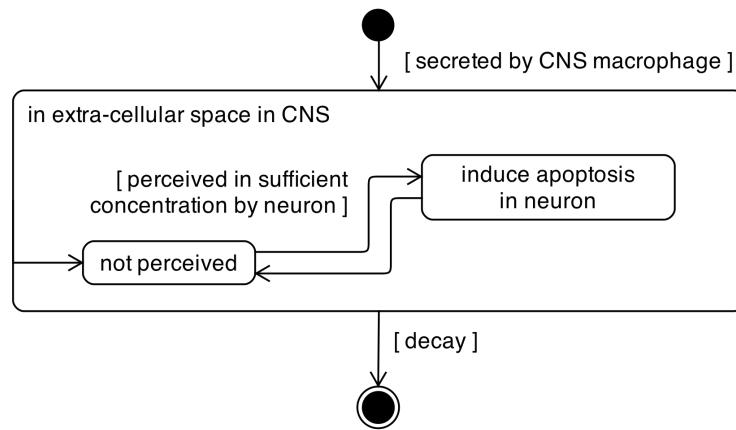


Figure 22: State machine diagram depicting the dynamics of SDA, and its influence on other cells of the domain model.

## 2.4 Temporal and numerical aspects of EAE

The three layers of modelling outlined above describe the cellular interactions and events that together constitute EAE onset and recovery, but they do not parameterise these dynamics. Table 1 outlines various temporal aspects to EAE. It depicts the major population-level events that characterise EAE, and the times at which these are observed to occur *in vivo*. Also indicated are the times associated with certain cell-level events. The following items describe probabilistic and numerical details concerning the cells involved in EAE.

- There exists around  $\frac{1}{10}$  the density of DCs in the CNS as exist in the SLO and CLN compartments.
- At the peak of the regulatory immune response, there exist around  $\frac{2}{3}$  the number of CD8Tregs as CD4Tregs.
- There exist around  $\frac{1}{7}$  the number of CNS macrophages in the CNS as neurons.
- A DC is around 7 times the size of a T cell.
- CD4Th1 population expansion following immunisation is around 10 to 50 fold.
- Increase of DCs in the SLO compartment following immunisation is around 2 to 4 fold.
- The probability that the phagocytosis of a cell leads to the successful derivation and presentation of peptides is only a few percent.

There are a great many other aspects to the present domain model that require parameterisation, however these details are not known in the domain and can hence not be specified at this time. They are the subject of calibration activities later in the document.

Time	Event
0 days	immunisation with MBP, CFA and PTx in the periphery
3-5 days	Detectable proliferation of CD4Th1 and CD4Th2 cells in the SLO
5-7 days	Detectable infiltration of CD4Th1 and CD4Th2 cells in the CNS
10-15 days	Visible paralysis of mouse
10 days	Detectable proliferation of CD4Treg and CD8Treg cells in CLN
30 days	CD4Th cells no longer found in CNS
30-40 days	Recovery from EAE

(a) Times at which population-level events occur in EAE. The times given are estimates, not exact figures.

Event	Time
Duration of Qa-1:CDR1/2 expression on CD4Th1 cells following differentiation into effector cells	8 hours
Time required for spawning of daughter CD4Th1, CD4Treg and CD8Treg cells	1 day
Time required for spawning of daughter CD4Th2 cell	1.5 day
Time T cell spent in proliferative state	5 days
AICD death occurs in CD4Th1, CD4Treg and CD8Treg effector cells	5 days
AICD death occurs in CD4Th2 effector cells	8 days
Time a naive T cell spends in lymph node	12 hours
Time a naive T cell spends in the spleen	5 hours
Time a naive T cell spends in the circulatory system	30 min

(b) The times associated with cell-level events in EAE. The times given are estimates, not exact figures.

Table 1: Temporal aspects to EAE.

### 3 Platform Model

This section describes the ARTIMMUS platform model, which is derived from the above domain model, and makes and adds implementation-specific assumptions and details necessary for software implementation.

The individual cell-level behaviours of the domain model are expressed as state machine diagrams, and it is these that form the specifications for the simulation platform: cellular behaviours are coded on the basis of state machine diagrams. The other diagrams, which cover system-level behaviours, describe the expected result of the large scale simulation of individual cells. These diagrams are in no way used as specification for code in the simulation platform; these behaviours should emerge as a result of cellular interactions represented in the state machine diagrams.

#### 3.1 Simulation architecture

The ARTIMMUS simulation platform is developed in Java, and within the MASON<sup>6</sup> simulation framework [Balan *et al.* 2003, Luke *et al.* 2004]. Java belongs to the object oriented programming paradigm, wherein logical entities in the program are explicitly represented and are responsible for maintaining their own state. MASON provides facilities for spatial representation, visualisation, and inspection of state within the simulation. It provides a simulation engine that manages the execution of simulation agents. Time is discretized into *time steps*; every time step the state of every cell and compartment in the simulation is updated.

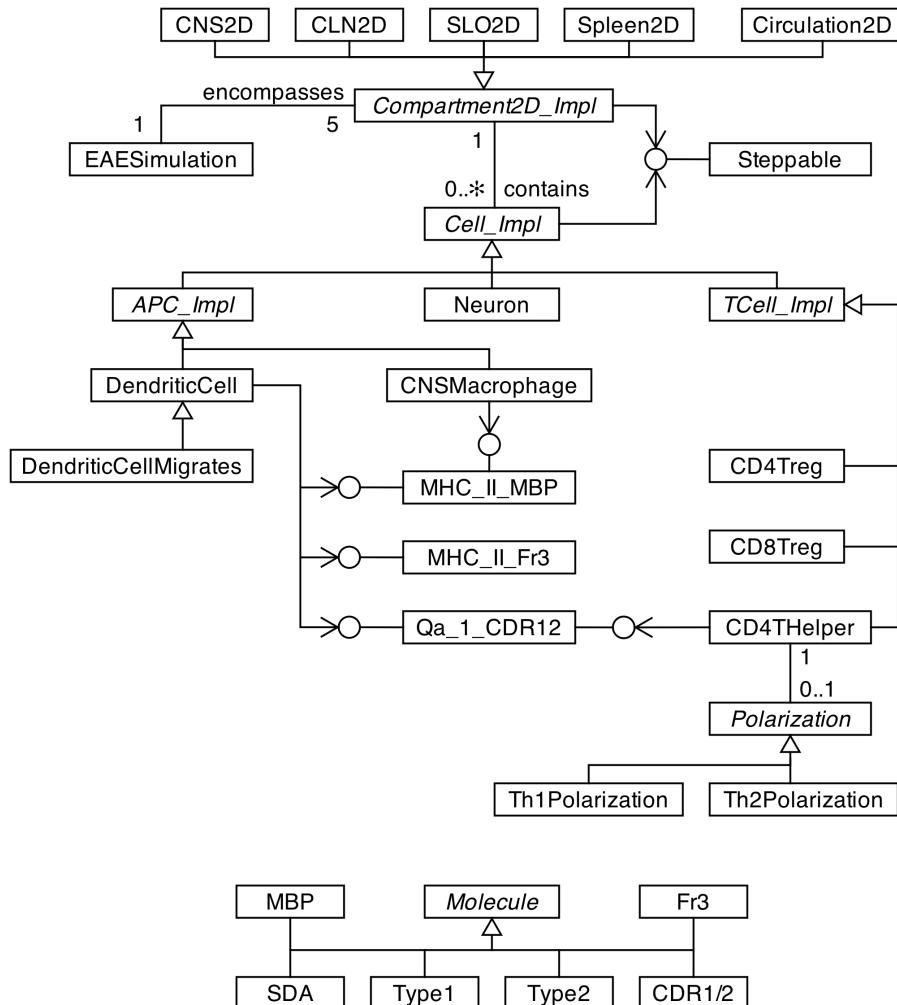


Figure 23: Class diagram depicting organisation of classes in the simulation platform in terms of inheritance hierarchies and interface implementations. The majority of associations are not shown, in aid of readability.

<sup>6</sup>Multi-Agent Simulator of Neighborhoods (MASON),  
<http://www.cs.gmu.edu/~eclab/projects/mason/>.

Figure 23 depicts the organisation of classes in the simulation platform, focussing on inheritance and implementation of interfaces; for clarity, the majority of associations are not shown, as these may be derived from the domain model presented above. The simulation platform makes extensive use of abstract classes, inheritance, and interface implementation. The simulation ultimately consists of cells all of which inherit from `Cell_Impl`, and spatial compartments in which they exist, which inherit from `Compartment2D_Impl`. Both these abstract classes implement `Steppable`, the MASON interface required for execution within the simulation engine. The class `EAEimulation` is the driver of the simulation, and is responsible for its initialisation.

There are many behavioural aspects common to multiple cell types. Such behaviours, such as maintaining the state of a T cell, are coded as far up the hierarchy tree as possible. This coding practice reduces redundant code, and leaves a single point of code maintenance should it ever need amendment. Where cells are concerned, all classes encoding cellular behaviour are abstract, with the exception of those that may actually be instantiated to directly represent a cell of the domain model. A similar programming pattern is used for coding the simulation's spatial compartments. These share many commonalities, which are implemented in `Compartment2D_Impl` and inherited in the concrete subclasses. In either case, abstract classes are often used to provide default behaviours which are overridden by specific exceptions where necessary.

The simulation platform makes use of interfaces with respect to the MHC:peptide complexes that cells may express. This serves to reduce the complexity of code. For example, a CD8Treg is specific for Qa-1:CDR1/2, which may be expressed by either a DC or a CD4Th cell. From the perspective of the `CD8Treg` class, it need only establish whether an instance of a cell implements the `Qa_1_CDR12` interface to know whether they may interact through this complex, rather than querying whether the cell is an instance of the `CD4THelper` or `Dendritic Cell` types.

The `Molecule` abstract class is the superclass for all the cytokines (Type1, Type2 and SDA) and peptides (Fr3, CDR1/2 and MBP) of the system. Cytokines are not represented as individual agents in the simulation. Rather, their concentrations in space are maintained by `Compartment2D_Impl` and represented as real numbers; this is discussed in further detail in section 3.2. The concrete cytokine classes are programmed using the singleton pattern, and as such references to these singletons are used when cells either secrete cytokines into the compartment or request their concentration. These singleton instances are also referred to by cells when being phagocytosed by APCs; the APCs need not know what form of cell is being phagocytosed to establish which peptides they contain, these references to these singleton peptides are passed directly to the APC by the cell being phagocytosed at the time.

## 3.2 Spatial representation

Physical space is explicitly represented within ARTIMMUS through use of a two dimensional lattice grid, graphically depicted in figure 24. The lattice grid consists of four layers, one for each type of entity explicitly represented in the simulation. There is one layer to maintain the location of cells, and one for each of the cytokine types: type 1 cytokine, type 2 cytokine, and SDA.

Cells occupy grid spaces in the lattice, and may move between them. There are two sizes of cell considered in the simulation: T cells, and all other cells. T cells are assumed to be  $\frac{1}{7}$  the size of all other cells. A single grid space is equivalent to the area of a single larger cell. As such, a soft upper limit of 7 T cells may occupy a single grid space, however for all other cells only a single cell may occupy a given grid space.

Cytokines (including SDA) diffuse around the grid, in accordance to algorithm 1, which is adapted from [Andrews & Timmis 2006]. Diffusion is performed at each time step in the simulation, and is implemented as an atomic operation. At each time step the majority of cytokine contents of a particular grid space are randomly diffused to one of its eight neighbours. Cytokine concentrations are represented as real numbers, however diffusion operates only on whole numbers, moving quantities of multiples of 1.0 to neighbours at a time. Concentrations less than 1.0 remain in the same grid space.

With respect to cells, lattice grids are toroidal only in the horizontal dimension. Cells migrating into a compartment are placed at the top of the lattice grid, those moving off the bottom of the grid are considered to have migrated elsewhere. With respect to cytokines, grid spaces are both vertically and horizontally toroidal.

## 3.3 Immunisation mechanism

Immunisation for EAE is accomplished *in vivo* through the administration of MBP, PTx and CFA. These immunisation substances do not find explicit representation within the simulation, which instead represents immunisation through the appearance of MBP-presenting immunogenic type 1 polarising DCs in the SLO compartment. Hence, the periphery compartment of the domain model is not represented in the platform model, and is not implemented in ARTIMMUS.

---

**Algorithm 1:** Cytokine diffusion algorithm. Designed as an atomic operation, all of the cytokine molecules present in a grid space at the start of the operation are distributed amongst its neighbours. Algorithm has been adapted from [Andrews & Timmis 2006].

---

```

input: grid, lattice grid containing cytokine concentrations
output: grid, lattice grid containing cytokine concentrations following diffusion

newgrid  $\leftarrow$  grid.clone
for x  $\leftarrow$  1 to grid.width do
    for y  $\leftarrow$  1 to grid.height do
        quantity  $\leftarrow$  newgrid[x][y]
        if quantity  $\neq$  0 then
            share  $\leftarrow$  [quantity  $\div$  8]                                //  $\div$  represents modulus operation
            grid[x][y]  $\leftarrow$  quantity                                         // reduce by ‘quantity’
            grid[x - 1][y - 1]  $\leftarrow$ + share                               // increase by ‘share’
            grid[x][y - 1]  $\leftarrow$ + share
            grid[x + 1][y - 1]  $\leftarrow$ + share
            grid[x - 1][y]  $\leftarrow$ + share
            grid[x + 1][y]  $\leftarrow$ + share
            grid[x - 1][y + 1]  $\leftarrow$ + share
            grid[x][y + 1]  $\leftarrow$ + share
            grid[x + 1][y + 1]  $\leftarrow$ + share
            remainder  $\leftarrow$  quantity - (8  $\times$  share)
            repeat remainder times                                // select random gridspace from the neighbourhood
                xi  $\leftarrow$  (random(3) - 1) + x
                yi  $\leftarrow$  (random(3) - 1) + y
                grid[xi][yi]  $\leftarrow$ + 1

```

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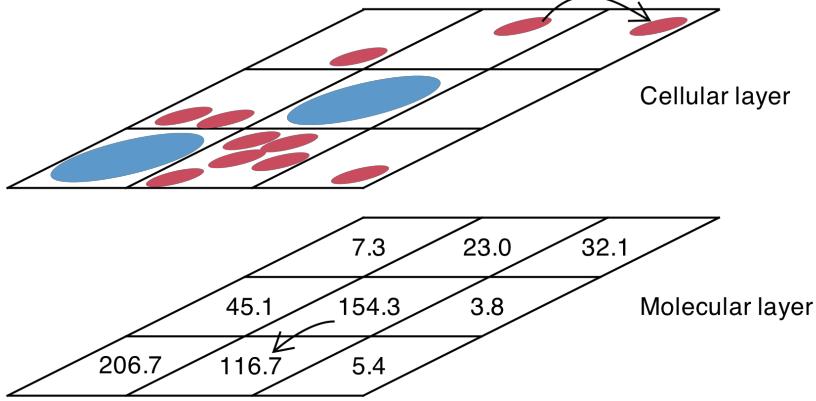


Figure 24: The lattice grid based spatial representation of ARTIMMUS. Each grid space in the lattice may contain cells and molecules, such as cytokines, with bespoke layers representing the spatial occupancy of each within the lattice. The ability of cells to move and for molecules to diffuse between grid spaces is indicated.

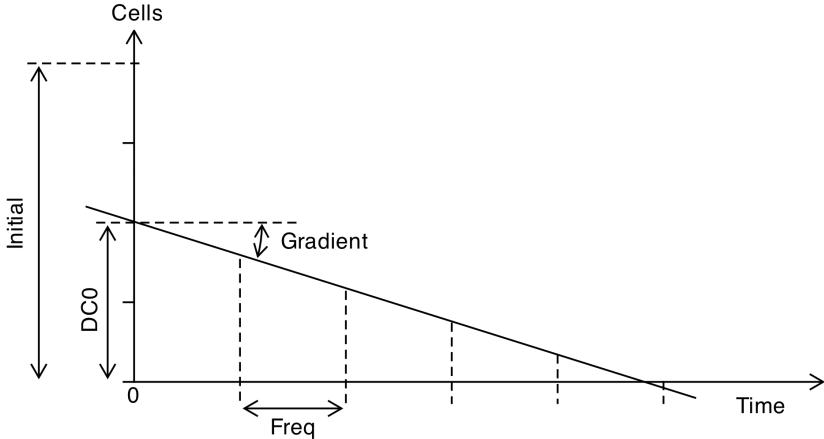


Figure 25: ARTIMMUS’s immunization mechanism, and how it is parameterised. The label “*Simulation\_immunizationLinear*” has been omitted from parameter names.

The immunisation mechanism, depicted in figure 25, is parameterised through 4 parameters: *Simulation\_immunizationDC0*, *Simulation\_immunizationLinearFreq*, *Simulation\_immunizationLinearGradient*, and *Simulation\_immunizationLinearInitial*. The last specifies the number of immunisation DCs placed into the SLO compartment at time zero, as a one-off event. The remainder parameterise a linearly reducing number of DCs that are added to the SLO periodically. The period is defined by *Simulation\_immunizationLinearFreq*. *Simulation\_immunizationDC0* and *Simulation\_immunizationLinearGradient* describe the level of DCs inserted at time zero, and the rate of linear decay. Every *Simulation\_immunizationLinearFreq* hours, the value described by these two parameters, given the current simulation time, is rounded to the nearest whole number of DCs which are then placed in the SLO.

### 3.4 Cellular turnover

Neurons enter apoptosis upon exposure to a sufficient concentration of SDA in a single time step. Upon phagocytosis by an APC, a new neuron is placed at the exact same location as that which preceded it.

The basal size of T cell populations are homeostatically maintained, through a mechanism that acts independently of any proliferative activities, which reflects the activities of the thymus in creating new T cells. The probability that a naive T cell is inserted into the simulation at each time step is calculated as the initial population size of the particular T cell type, divided by the mean lifespan of a naive T cell, multiplied by the proportion of an hour that a single time step represents. T cells created in this manner are inserted into a random location in the circulatory system.

Like neurons, DCs and CNS macrophages are replaced by new cells upon entering apoptosis. The immature cells that replace such apoptotic cells are placed at random locations within the compartments where they originated from.

### 3.5 Periodic migration of DCs

There exist two forms of DC in the simulation: those capable of migratory behaviour, and those that are not. Non-migratory DCs are those placed in the spleen, SLO and CLN compartments upon simulation initialisation. Migratory DCs exist in the CNS as immature cells, and move into the CLN compartment upon maturation.

This migratory behaviour is periodic, DCs remain in the CNS compartment as immotile immature cells for some predetermined period of time, randomly determined for each individual DC, before commencing migration to the CLN compartment. Once in the CLN migratory DCs continue to follow the blood flow for some randomly determined period of time before coming to a rest somewhere in the compartment.

### 3.6 Migratory behaviour

Compartments in ARTIMMUS are represented as lattice grids. In each time step a motile<sup>7</sup> cell may move to any of the eight grid spaces surrounding the one in which it currently resides, or it may remain stationary. Cell movements are calculated by considering horizontal and vertical movements independently. In the horizontal plane, a cell has equal probabilities of moving left, right, or remaining stationary.

Cells migrating into a compartment are placed at the top of the lattice grid. Conversely, traversing beyond the bottom of the lattice grid is interpreted as the cell leaving the compartment. The probabilities of vertical movement, being a move down, up, or remaining vertically stationary, are dictated by the time required for a T cell to migrate through the compartment. The times required for migration through a compartment are compartment-specific, and are represented by parameters of the form *CLN\_timeToCrossOrgan*, many of which hold literature defined values. Figure 26 indicates how the probabilities of a cell performing a particular vertical movement are derived, based on a compartment's height, and how many time steps a cell will generally remain in a compartment for.

### 3.7 Probabilistic timing of events

Many of the cellular state transitions described in the domain model are implemented to occur after some probabilistically determined period of time. For example, on average DCs remain immature for a particular period of time, however individual DCs differ in the exact durations they spend in this state. Such state transitions are implemented through use of normal distributions, as depicted in figure 27. When a cell enters a particular state, event A, a probability distribution is used to select an absolute time in the future for the transition to the following state, event B. Hence, such distributions describe the duration of time cells spend in particular states. They are parameterised by a mean and standard deviation<sup>8</sup>. Note that, since a normal distribution is used to describe the durations of time spent in particular states, it is possible that event B will be selected to have occurred before event A. This is guarded against by converting all events occurring in the past into events occurring 1 hour after event A.

### 3.8 Local activation of T cells

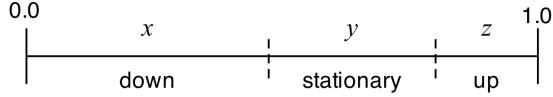
The domain model stipulates that effector T cells cannot secrete cytokines until they have been locally activated. The intended meaning of local activation is that the T cell has left the location at which it was primed and is re-stimulated at the site of the immune response. In order to prevent T cells in ARTIMMUS from being locally activated by the APCs on which they prime, a time limitation has been placed on their ability to be locally activated. T cells can only derive local activation some time after differentiation into effector cells, parameterised by *TCell\_timeLocalActivationDelay*.

### 3.9 Polarisation selection by DCs and CD4Th cells

Upon maturation DCs become either type 1 or type 2 polarising. Type 1 polarising DCs secrete type 1 cytokine that induces preferential selection of CD4Th1 polarisations by CD4Th cells. The polarisation selected by a DC is

<sup>7</sup>Not all cells are motile. T cells are motile only when not bound to an APC, neurons are never motile, and APCs are motile only in certain states.

<sup>8</sup>The parameters of ARTIMMUS that describe standard deviations actually describe twice the standard deviation. Hence, their values indicate that ~95% of transitions must occur within a particular period of time surrounding the mean.



height of compartment in grid spaces =  $\phi$

time to cross compartment =  $\omega$

time slice, as a proportion of an hour =  $\tau$

time steps in which cell must cross compartment =  $\delta = \frac{\omega}{\tau}$

proportion of total time steps that result in a net downwards movement =  $\alpha = \frac{\phi}{\delta}$

There are three constraints on the probabilities of moving down ( $x$ ), up ( $z$ ), or remaining stationary ( $y$ ): all possible movements must sum to 1.0; any upwards movement must be reflected in the probability of downwards movement, in order to traverse the compartment in  $\delta$  time steps; and the probability of remaining stationary is the mean of probabilities of moving up and down. This last constraint is an assumption made to make the equations solvable. The constraints are mathematically expressed as follows:

$$x + y + z = 1.0 \quad (1)$$

$$x = z + \alpha \quad (2)$$

$$y = \left( \frac{x+z}{2} \right) \quad (3)$$

Derivation of how to calculate  $z$ ,  $y$ , and  $x$ :

$$z = x - \alpha \quad (4)$$

$$y = \left( \frac{x+z}{2} \right) = \left( \frac{x+x-\alpha}{2} \right) = \left( \frac{2x-\alpha}{2} \right) \quad (5)$$

$$\begin{aligned} x + y + z &= 1.0 \\ x + \left( \frac{2x-\alpha}{2} \right) + (x-\alpha) &= 1 \\ 2x + (2x-\alpha) + 2x - 2\alpha &= 2 \\ 6x - 3\alpha &= 2 \\ x &= \left( \frac{2-3\alpha}{6} \right) \\ x &= \left( \frac{1-1.5\alpha}{3} \right) \end{aligned} \quad (6)$$

Figure 26: The calculation of probabilities that a migrating cell, such as a T cell, will move either downwards, upwards, or remain at its current vertical level in a particular time step. Probabilities are represented within the range 0.0 to 1.0. The probabilities of moving down, up, or remaining vertically stationary are represented by  $x$ ,  $z$  and  $y$  respectively. The calculations are derived through consideration of a compartment's height, and how long a cell has to traverse this distance.

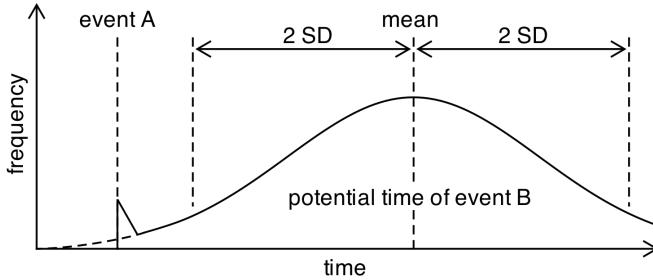


Figure 27: Depiction of how the absolute time of events is determined through a normal distribution of possibilities. When event A occurs, the absolute time at which event B is to take place is derived from a normal distribution of relative times in the future. Simulation parameters specifying the mean and  $2 \times$  the standard deviation describe the distribution. Event B cannot take place in the past, hence absolute time for B that are less than the time of event A are converted into future times.

dependent on the local cytokine milieu at the time of maturation. If the total proportion of type 2 cytokine in the cytokine milieu is less than the figure represented by the parameter *DendriticCell\_cytokineType2PolarizationRatio* then a type 1 polarisation is adopted. Otherwise a type 2 polarisation is adopted, including the case that there is a complete absence of any type 1 or type 2 cytokine in the milieu.

CD4Th cells adopt either a CD4Th1 or CD4Th2 polarisation upon entering their proliferative cycles. One more, the decision of which polarisation to adopt is based on the local cytokine milieu in the grid space where the cell resides. If the proportion of type 1 cytokine in the milieu is greater than or equal to 80%<sup>9</sup>, then a type 1 polarisation is preferentially adopted. Otherwise, including the case that there exist no cytokines in the milieu, a type 2 polarisation is preferred. The adoption of a polarisation is probabilistic, with the probability in each case parameterised through *CD4THelper\_diff08* and *CD4THelper\_diff00* respectively.

### 3.10 Simulation initialisation

Simulation initialisation, at time zero, is done in a manner that minimises simulation artifacts arising from cells starting in identical states and locations. DCs and CNS macrophages are created to be part way through their lifespans as immature cells; this prevents large quantities of these cells entering maturity at unrealistically similar times.

The initial populations of naive T cells are placed in randomly determined compartments, excluding the CNS. Some proportion of migratory DCs are placed in the CNS as immature cells, whereas the remainder are placed in the CLN compartment as mature cells. The exact proportion is dictated by the proportion of a DCs entire lifespan that is spent in an immature state.

### 3.11 Cytokine decay

In addition to diffusing around compartmental space, cytokines also decay over time. This has been implemented in discrete time as follows:

$$C_{t+\tau} = C_t \left( \frac{1}{2} \right)^{\frac{\tau}{\lambda}} \quad (7)$$

Where  $\tau$  is the duration of time represented by a time step, in hours (e.g., 0.125),  $\lambda$  is the decay rate, also in hours, and  $C_t$  is the concentration of cytokine in a particular grid space at time  $t$ . Cytokine concentrations, represented as real numbers, subject to this implementation of decay will never reach a concentration of exactly 0.0. As such, at each time step, the concentration of cytokine in a particular grid space is set to 0.0 if it is less than the value indicated by the parameter *Molecule\_decayThreshold*. This thresholding adds realism to the simulation; cytokines secreted by cells *in vivo* do not have infinitely far reaching influence.

<sup>9</sup>Represented at 0.8, percentages are scaled onto the range between 0.0 and 1.0.

### 3.12 T cell specificity

Upon contact of a T cell with a cell expressing MHC:peptide for which it is specific, a binding between the two cells is probabilistically determined. This reflects the range of specificities that *in vivo* T cells have for particular MHC:peptide complexes. A successful binding will lead to further interactions between the cells. In the case of T cells induced into their proliferative cycles, the T cells become immotile, bound to the APC for the duration of their proliferative cycle. In contrast, an unsuccessful binding does not lead to cellular interaction.

The determination of whether a binding is successful is conducted when the two cells first contact one another, as indicated by their occupancy of either neighbouring or the same grid spaces, and is probabilistic in nature. All T cells that are not created through proliferation are assigned specificities upon creation, represented as a real number randomly selected to lie between the values indicated by the simulation parameters *TCell\_specificityLowerLimit* and *TCell\_specificityUpperLimit*. These two parameters obey the constraint:

$$0.0 \leq \text{TCell\_specificityLowerLimit} \leq \text{TCell\_specificityUpperLimit} \leq 1.0$$

Daughter cells arising from proliferation inherit the specificities of their parent cells. This specificity represents the probability that contact with a neighbouring cell for which a T cell is specific will result in a successful binding.

## 4 Parameters

The following table, table 2, is a comprehensive list of all the parameters to the ARTIMMUS simulation platform. It lists their name, type, default value following calibration, and a note describing its function. Parameters are grouped according to their function; for example, the names of all parameters relating to T cells begin with the text “*TCell\_*.” Some parameters are overridden by others, for example, *TCell\_ProliferationMean* applies to all T cells except for CD4Th2 cells, for which the parameter value is provided by *Th2Polarization\_proliferationMean*. The prefixes that parameter names are assigned are related to the abstract and concrete classes in the simulation platform’s design, indeed it is in these classes that the parameters are held in the simulation platform. As such, parameters relating to abstract classes may have influence over several concrete cell classes, as indicated by the class diagram of figure 23. Hence, parameters starting with the text “*APC\_*” apply to both CNS macrophages and DCs.

Details of how these parameters were calibrated can be found in [Read 2011, Read *et al.* 2011] and the ‘Determining Disease Intervention Strategies using Spatially Resolved Simulations’ manuscript currently under consideration for publication.

Table 2: The standard parameters of the ARTIMMUS simulation platform. The table details their types, default values (following calibration), and what they parameterise. Note that to aid transparency parameter names relate to their function, and are presented in a hierarchical manner. For example, the first two parameters in the table are *APC\_immatureDurationMean* and *APC\_immatureDurationStdDev*.  $\mathbb{R}$  refers to the real numbers,  $\mathbb{N}$  refers to the natural numbers. Percentages are indicated in the range 0.0 to 1.0, indicated by the notation (% / 100).  $2 \times \mathbb{N}$  indicates even numbers.

Parameter details	Description
<i>APC</i>	
<i>immatureDurationMean</i> type: $0.0 \leq \mathbb{R}$ default: 48.0 hours	The mean of the normal distribution of times from which the duration that an APC spends in an immature state before maturing is selected from.
<i>immatureDurationStdDev</i> type: $0.0 \leq \mathbb{R}$ default: 24.0 hours	$2 \times$ the standard deviation of the normal distribution of times from which the duration that an APC spends in an immature state before maturing is selected from.
<i>timeOfDeathMean</i> type: $0.0 \leq \mathbb{R}$ default: 110.0 hours	The mean of the normal distribution of times from which an APCs mature lifespan is selected from.
<i>timeOfDeathStdDev</i> type: $0.0 \leq \mathbb{R}$ default: 48.0 hours	$2 \times$ the standard deviation of the normal distribution of times from which an APCs mature lifespan is selected from.
<i>probabilityPhagocytosisToPeptide</i> type: $0.0 \leq \mathbb{R} \leq 1.0$ default: 0.02 (% / 100)	The probability (scaled between 0.0 and 1.0) that the phagocytosis of a cell by an APC will lead to the derivation of presentable peptides.
<i>CD4THelper</i>	
<i>diff00</i> type: $0.0 \leq \mathbb{R} \leq 1.0$ default: 0.05 %/100	The probability (scaled between 0.0 and 1.0) that a CD4Th cell will adopt a type 1 polarization if type 1 cytokine comprises less than 80% of the cytokine milieu in the grid space where the cell resides.
<i>diff08</i> type: $0.0 \leq \mathbb{R} \leq 1.0$ default: 0.85 (%/100)	The probability (scaled between 0.0 and 1.0) that a proliferating CD4Th cell will adopt a type 1 polarization if type 1 cytokine comprises 80% or more of the cytokine milieu in the grid space where the cell resides.
<i>CD4Treg</i>	
<i>type1SecretedPerHourWhenActivated</i> type: $0.0 \leq \mathbb{R}$ default: 10.0 molecules	The quantity of type 1 cytokine secreted per hour by locally activated effector CD4Treg cells.
<i>CD8Treg</i>	
<i>type1SecretedPerHourWhenActivated</i> type: $0.0 \leq \mathbb{R}$ default: 10.0 molecules	The quantity of type 1 cytokine secreted per hour by locally activated effector CD8Treg cells.
<i>cd8TregToCD4ThelperSpecificityDropOff</i> type: $0.0 \leq \mathbb{R} \leq 1.0$ default: 1.0 (%/100)	The probability (scaled between 0.0 and 1.0) that a successful binding between an effector CD8Treg and a Qa-1 expressing CD4Th cell will lead to the former inducing the apoptosis of the latter.
<i>CNSCell</i>	
<i>apoptosisSDAThreshold</i> type: $0.0 \leq \mathbb{R}$ default: 5.0 molecules	The threshold quantity of SDA above which a neuron occupying the same grid space is induced into apoptosis.
<i>Circulation</i>	
<i>width</i> type: $2 \times \mathbb{N}$ default: 62 grid spaces	The width of the circulation compartment in terms of grid spaces.
<i>height</i> Continued on Next Page...	The height of the circulation compartment in terms of grid spaces.

Table 2 – Continued

Parameter details	Description
<i>type</i> : $2 \times \mathbb{N}$ <i>default</i> : 40 grid spaces	
<i>timeToCrossOrgan</i> <i>type</i> : $0.0 \leq R$ <i>default</i> : 5.0 hours	The average time required for a T cell to cross the circulation compartment.
<i>CLN</i>	
<i>width</i> <i>type</i> : $2 \times \mathbb{N}$ <i>default</i> : 50 grid spaces	The width of the CLN compartment in terms of grid spaces.
<i>height</i> <i>type</i> : $2 \times \mathbb{N}$ <i>default</i> : 50 grid spaces	The height of the CLN compartment in terms of grid spaces.
<i>timeToCrossOrgan</i> <i>type</i> : $0.0 \leq R$ <i>default</i> : 12.0 hours	The average time required for a naive T cell to migrate through the CNS compartment.
<i>CNS</i>	
<i>width</i> <i>type</i> : $2 \times \mathbb{N}$ <i>default</i> : 50 grid spaces	The width of the CNS compartment in terms of grid spaces.
<i>height</i> <i>type</i> : $2 \times \mathbb{N}$ <i>default</i> : 50 grid spaces	The height of the CNS compartment in terms of grid spaces.
<i>timeToCrossOrgan</i> <i>type</i> : $0.0 \leq R$ <i>default</i> : 20.0 hours	The average time for an apoptotic T cell to migrate through the CNS compartment.
<i>CNSMacrophage</i>	
<i>basalMBPExpressionProbability</i> <i>type</i> : $0.0 \leq R \leq 1.0$ <i>default</i> : 0.2 (%) / 100)	The probability that a newly created CNS macrophage will express MHC-II:MBP complexes.
<i>type1RequiredForActivation</i> <i>type</i> : $0.0 \leq R$ <i>default</i> : 2.5 molecules	The threshold quantity of type 1 cytokine above which a CNS macrophage occupying the same grid space is induced into SDA secretion.
<i>sdaSecretedPerHourWhenStimulated</i> <i>type</i> : $0.0 \leq R$ <i>default</i> : 100.0 molecules	The quantity of SDA secreted per hour by an activated CNS macrophage.
<i>phagocytosisProbabilityImmature</i> <i>type</i> : $0.0 \leq R \leq 1.0$ <i>default</i> : 0.7 (%) / 100)	The probability that contact between an immature CNS macrophage and an apoptotic cell will result in the former phagocytosing the latter.
<i>phagocytosisProbabilityMature</i> <i>type</i> : $0.0 \leq R \leq 1.0$ <i>default</i> : 0.3 (%) / 100)	The probability that contact between a mature CNS macrophage and an apoptotic cell will result in the former phagocytosis the latter.
<i>DendriticCell</i>	
<i>type1RequiredForActivation</i> <i>type</i> : $0.0 \leq R$ <i>default</i> : 2.0 molecules	The minimum quantity of type 1 cytokine required for the induction of co-stimulatory molecule expression in a mature DC occupying the same grid space.
<i>phagocytosisProbabilityImmature</i> <i>type</i> : $0.0 \leq R \leq 1.0$ <i>default</i> : 1.0 (%) / 100)	The probability that contact between an immature DC and an apoptotic cell will result in the former phagocytosing the latter.
<i>phagocytosisProbabilityMature</i> <i>type</i> : $0.0 \leq R \leq 1.0$ <i>default</i> : 0.3 (%) / 100)	The probability that contact between a mature DC and an apoptotic cell will result in the former phagocytosing the latter.
<i>type1SecretedPerHourImmunized</i> Continued on Next Page...	The quantity of type 1 cytokine secreted per hour of an immunogenic DC.

Table 2 – Continued

Parameter details	Description
type: $0.0 \leq R$ default: 10.0 molecules	
<i>cytokineType2PolarizationRatio</i> type: $0.0 \leq R \leq 1.0$ default: 0.17 (% / 100)	The minimum proportion of type 2 cytokine comprising the cytokine milieu required for the adoption of a type 2 polarization in a DC occupying the same grid space.
<i>DendriticCellMigrates</i>	
<i>lengthOfTimeMovingFollowingMigration</i> type: $0.0 \leq R$ default: 3.5 hours	The length of time that a migratory DC is motile for after migrating into a lymphoid compartment from its original compartment.
<i>Molecule</i>	
<i>molecularHalfLife</i> type: $0.0 \leq R$ default: 30 min	The half life of secreted cytokines in hours.
<i>decayThreshold</i> type: $0.0 \leq R$ default: 0.01	The threshold concentration of cytokine in a grid space below which the concentration is set to 0.0.
<i>Simulation</i>	
<i>immunizationLinearFreq</i> type: $0.0 \leq R$ default: 6.0 hours	The periodicity with which immunization DCs are inserted into the SLO compartment, whilst this process persists.
<i>immunizationLinearInitial</i> type: IN default: 14 cells	The number of immunization DCs placed in the SLO compartment at time zero (time at which immunization for EAE is administered).
<i>immunizationLinearDC0</i> type: $0.0 \leq R$ default: 2.0 cells	The number of immunization DCs placed in the SLO compartment as a result of immunization for EAE decreases linearly over time. This parameter dictates the number at time zero.
<i>immunizationLinearGradient</i> type: $R \leq 0.0$ default: -0.005 cells/hour	The number of immunization DCs placed in the SLO compartment as a result for immunization for EAE decreases linearly over time. This parameter dictates the gradient of linear decrease.
<i>numCD4Th</i> type: IN default: 40 cells	The basal number of naive CD4Th cells in the simulation.
<i>numCD4Treg</i> type: IN default: 30 cells	The basal number of naive CD4Treg cells in the simulation.
<i>numCD8Treg</i> type: IN default: 30 cells	The basal number of naive CD8Treg cells in the simulation.
<i>numCNS</i> type: IN default: 500 cells	The number of neurons in the simulation.
<i>numCNSMacrophage</i> type: IN default: 75 cells	The number of CNS macrophages in the simulation.
<i>numDC</i> type: IN default: 10 cells	The number of DCs permanently residing in the CLN and SLO compartments in the simulation.
<i>numDCCNS</i> type: IN default: 40 cells	The number of migratory DCs in the CNS compartment at the start of the simulation.

Continued on Next Page...

Table 2 – Continued

Parameter details	Description
<i>numDCSpleen</i> type: $\mathbb{N}$ default: 100 cells	The number of DCs permanently residing in the spleen compartment.
<i>SLO</i>	
<i>width</i> type: $2 \times \mathbb{N}$ default: 50 grid spaces	The width of the SLO compartment in terms of grid spaces.
<i>height</i> type: default: 50 grid spaces	The height of the SLO compartment in terms of grid spaces.
<i>timeToCrossOrgan</i> type: $0.0 \leq \mathbb{R}$ default: 12.0 hours	The average time required for a naive T cell to migrate through the SLO compartment.
<i>Spleen</i>	
<i>width</i> type: $2 \times \mathbb{N}$ default: 62 grid spaces	The width of the SLO compartment in terms of grid spaces.
<i>height</i> type: $2 \times \mathbb{N}$ default: 40 grid spaces	The height of the SLO compartment in terms of grid spaces.
<i>timeToCrossOrgan</i> type: $0.0 \leq \mathbb{R}$ default: 5.0 hours	The average time required for a naive T cell to migrate through the Spleen compartment.
<i>TCell</i>	
<i>apoptosisNaiveMean</i> type: $0.0 \leq \mathbb{R}$ default: 30.0 hours	The mean of the normal distribution of times from which the durations that naive T cells can survive without receiving MHC:peptide stimulation are selected from.
<i>apoptosisNaiveStdDev</i> type: $0.0 \leq \mathbb{R}$ default: 17.0 hours	$2 \times$ the standard deviation of the normal distribution of times from which the durations that naive T cells can survive without receiving MHC:peptide stimulation are selected from.
<i>apoptosisPartialMaturityMean</i> type: $0.0 \leq \mathbb{R}$ default: 12.0 hours	The mean of the normal distribution of times from which the durations that partially activated T cells can Survive without receiving MHC:peptide stimulation are selected from.
<i>apoptosisPartialMaturityStdDev</i> type: $0.0 \leq \mathbb{R}$ default: 6.0 hours	$2 \times$ the standard deviation of the normal distribution of times from which the durations that partially activated T cells can survive without receiving MHC:peptide stimulation are selected from.
<i>AICDMean</i> type: $0.0 \leq \mathbb{R}$ default: 60.0 hours	The mean of the normal distribution of times from which the durations that effector T cells can survive for, given frequent local activation, are selected from.
<i>AICDStdDev</i> type: $0.0 \leq \mathbb{R}$ default: 56.0 hours	$2 \times$ the standard deviation of the normal distribution of times from which the durations that effector T cells can survive for, given frequent local activation, are selected from.
<i>becomeEffectorMean</i> type: $0.0 \leq \mathbb{R}$ default: 60.0 hours	The mean of the normal distribution of times from which the durations that T cells spend in proliferating states are selected from.
<i>becomeEffectorStdDev</i> type: $0.0 \leq \mathbb{R}$ default: 56.0 hours	$2 \times$ the standard deviation of the normal distribution of times from which the durations that T cells spend in proliferating states are selected from.
<i>proliferationMean</i> Continued on Next Page...	The mean of the normal distribution of times from which the durations required for proliferating T cells to produce naive daughter cells are selected from. This parameter does not apply to CD4Th2 cells.

Table 2 – Continued

Parameter details	Description
<i>type: <math>0.0 \leq R</math></i> default: 19.2 hours	
<i>proliferationStdDev</i> type: $0.0 \leq R$ default: 9.6 hours	$2 \times$ the standard deviation of the normal distribution of times from which the durations required for proliferating T cells to produce naive daughter cells are selected from. This parameter does not apply to CD4Th2 cells.
<i>cellsPerGridspace</i> type: $1 \leq N$ default: 7 cells	The maximum number of T cells that fit into a single simulation grid space.
<i>specificityUpperLimit</i> type: $0.0 \leq R \leq 1.0$ default: 0.9 %/100	The upper limit of specificities that T cells may have for their cognate MHC:peptide complexes.
<i>specificityLowerLimit</i> type: $0.0 \leq R \leq 1.0$ default: 0.5 %/100	The lower limit of specificities that T cells may have for their cognate MHC:peptide complexes.
<i>timeLocalActivationInducedEffect... ...FunctionFor</i> type: $0.0 \leq R$ default: 48.0 hours	The length of time that effector T cells survive for following each local activation event, before entering apoptosis through neglect.
<i>timeLocalActivationDelay</i> type: $0.0 \leq R$ default: 10.0 hours	The length of time that must elapse following differentiation into effector cells before a T cell is susceptible to local activation.
<i>Th1Polarization</i>	
<i>mhcUnExpressionDelayMean</i> type: $0.0 \leq R$ default: 8.0 hours	The mean of the normal distribution of times from which the durations of Qa-1 expression by effector CD4Th1 cells are selected from.
<i>mhcUnexpressionDelayStdDev</i> type: $0 \leq R$ default: 2.0 hours	$2 \times$ the standard deviation of the normal distribution of times from which the durations of Qa-1 expression by effector CD4Th1 cells are selected from.
<i>type1SecretedPerHourWhenActivated</i> type: $0.0 \leq R$ default: 100.0 molecules	The quantity of type 1 cytokine secreted per hour by locally activated effector CD4Th1 cells.
<i>Th2Polarization</i>	
<i>proliferationMean</i> type: $0.0 \leq R$ default: 28.8 hours	The mean of the normal distribution of times from which the durations required for proliferating CD4Th2 cells to produce naive daughter cells are selected from.
<i>proliferationStdDev</i> type: $0.0 \leq R$ default: 19.2 hours	$2 \times$ the standard deviation of the normal distribution of times from which the durations required for proliferating CD4Th2 cells to produce naive daughter cells are selected from.
<i>type2SecretedPerHourWhenActivated</i> type: $0.0 \leq R$ default: 100.0 molecules	The quantity of type 2 cytokine secreted per hour by locally activated effector CD4Th2 cells.

## 5 Robustness Analysis

This section describes the results of the Robustness analysis as applied to ARTIMMUS. The experimental procedure can be found in [Read *et al.* 2011], and the ‘Determining Disease Intervention Strategies using Spatially Resolved Simulations’ manuscript currently under consideration for publication. Details are also given in [Read 2011]

The analysis is applied to ‘responses’ that measure simulation behaviour. Full details may be found in [Read 2011]. In summary, 13 responses are used in analysing ARTIMMUS. These are:

- The peak number of each T cell population found at any point during simulation, for each of CD4Th1, CD4Th2, CD4Treg and CD8Treg populations.
- The times at which these peaks occur.
- The maximum EAE disease severity score obtained at any point in time.
- The EAE disease severity score at 40 days post-induction of EAE.

The robustness analysis identifies the boundaries each side of calibrated default values at which parameter perturbations result in significant deviations in these responses. For the T cell responses the Vargha-Delaney A test is used (see [Read *et al.* 2011, Read 2011]). For the EAE disease score related responses the A test measure is used, and in addition  $\pm 1.0$  in average disease score is considered significant.

At the time of writing the author knows of no reason to question the values that the simulation’s parameters have been assigned. However, were reasons to question simulation parameter values to arise in the future, this analysis can indicate where implications on simulation results exist. Although the focus of this section is on arbitrarily determined parameter values, a summary of robustness indices for all simulation parameters with respect to all simulation responses is presented in table 3. and appendix section 5.1 provides a full robustness analysis, including robustness boundaries and indices, with respect to each response.

It may be seen from table 3 that some simulation responses are highly fragile with respect to parametric perturbation, with several examples of perturbations of less than 2% being sufficient to result in significant deviation in simulation behaviours. There are many parameters for which perturbations cause significant deviation in behaviours for all simulation responses.

The findings of this section reveal that, in comparison, the simulation is relatively robust with respect to perturbation of its arbitrarily assigned parameters. Most cause significant deviations in simulation behaviour for at most half the responses, but require perturbations of around 40% or more to do so. Noteworthy exceptions are *CNS\_height*, *CNS\_width*, and *CNSMacrophage\_sdaSecretedPerHourWhenStimulated*. Small perturbations of less than 10% result in significant behavioural deviations in nearly all responses. These results point to the criticality of the rate of neuronal apoptosis in the system, to which all three parameters directly contribute.

Table 3: Summary of parameter robustness indexes, ordered by total rank. Responses are indicated as follows: 1M, *CD4Th1 Max*; 1MT, *CD4Th1 Max Time*; 2M, *CD4Th2 Max*; 2MT, *CD4Th2 Max Time*; 4M, *CD4Treg Max*; 4MT, *CD4Treg Max Time*; 8M, *CD8Treg Max*; 8MT, *CD8Treg Max Time*; Th40, *CD4Th1 at 40 Days*; MEA, *Max EAE*; E40A, *EAE at 40 Days*. Significant deviation is indicated through the *A* test. ME and E40 represent *Max EAE* and *EAE at 40 Days*, with significant deviations in response behaviour defined as a change of at least 1.0 in the mean EAE score. Not-a-number values, representing no significant deviation in behaviour, are marked with a period for clarity. Response columns show the smallest of the two robustness indexes for each parameter-response combination. The ‘total’ is the sum of ranks for each parameter across all responses, with small response indexes being ranked highest.

Rank	Parameter Name	1M	1MT	2M	2MT	4M	4MT	8M	8MT	Th40	MEA	E40A	ME	E40	Total
1	CNSMacrophage_sdaSecretedPerHourWhenStimulated	5.64	11.97	2.99	5.74	4.84	9.01	6.67	9.22	16.26	1.55	1.86	1.41	0.89	28
2	CNSCellApoptosisSDAThreshold	5.26	13.78	3.27	5.20	5.27	11.88	6.63	13.03	14.70	1.45	2.04	1.36	0.97	30
3	CNS.height	6.53	14.79	4.02	6.72	5.82	10.99	7.40	12.67	17.45	3.66	3.83	3.31	1.82	56
4	Molecule.molecularHalfLife	7.44	16.73	4.17	7.76	6.79	12.80	8.73	13.93	25.29	2.22	2.58	2.02	1.23	64
5	Simulation_numCNSMacrophage	12.12	23.71	6.79	13.13	13.18	18.65	13.97	19.00	42.02	3.41	3.60	3.08	1.71	106
6	APC.timeOfDeathMean	7.30	26.84	7.62	35.80	4.27	42.36	5.79	47.02	26.88	11.79	24.68	11.87	11.76	115
7	Simulation_ImmunizationLinearDC0	6.67	16.71	9.02	43.58	8.72	22.22	12.88	21.38	43.09	13.94	24.95	15.90	14.13	124
8	CNS.width	9.27	23.01	4.66	7.02	7.20	16.40	8.12	17.10	.	3.90	3.93	3.25	1.87	150
9	Simulation_ImmunizationLinearGradient	16.92	22.76	15.16	44.10	12.95	36.71	16.24	39.82	54.15	33.83	50.24	34.83	37.75	184
10	Simulation_numCNS	43.41	56.83	27.59	38.11	34.59	45.09	36.73	46.67	.	6.92	11.51	7.55	5.41	253
11	Simulation_numDCCNS	39.70	51.70	23.84	39.09	16.67	63.58	22.29	57.64	112.10	65.01	138.00	71.18	55.11	262
12	Th1Polarization.type1SecretedPerHourWhenActivated	38.04	62.49	38.01	44.88	35.32	53.98	39.64	56.07	477.10	26.07	141.30	27.92	48.21	263
13	TCell.proliferationMean	9.28	9.18	9.81	.	3.54	5.58	5.88	5.91	15.59	18.24	.	19.17	.	270
14	TCellCellsPerGridspace	8.98	47.96	10.41	33.17	8.56	39.20	9.61	42.67	37.56	22.03	.	24.78	.	272
15	TCell.becomeEffectorMean	18.22	24.94	20.64	47.50	9.53	14.98	17.27	14.94	29.03	28.88	.	29.29	.	286
16	Simulation_ImmunizationLinearFreq	10.31	.	16.13	36.11	26.81	33.46	35.57	37.20	.	23.16	33.20	20.72	16.67	290
17	TCell.AICDMean	8.81	.	8.54	24.62	12.10	.	13.92	.	99.74	9.80	17.71	10.61	10.26	322
18	Simulation_numCD4Th	48.10	41.43	54.33	74.73	45.91	42.25	57.20	41.97	79.81	62.24	.	64.00	243.80	330
19	TCell.timeLocalActivationInducedEffectorFunctionFor	14.59	65.05	19.02	49.56	17.56	68.72	21.95	53.73	.	22.52	.	23.26	31.35	336
20	CNSMacrophage.type1RequiredForActivation	58.00	127.50	50.32	75.85	71.60	108.30	80.11	106.30	96.71	34.70	58.82	36.37	35.39	341
21	Circulation.height	61.06	46.95	36.99	182.60	32.55	43.99	39.21	45.58	29.27	46.44	.	51.30	.	361
22	CD4THelper.diff08	5.66	47.23	2.69	92.33	8.84	90.82	10.18	92.70	.	16.58	.	16.93	.	365
23	APC.probabilityPhagocytosisToPeptide	32.53	65.77	16.92	25.85	9.78	60.98	12.01	74.93	49.54	.	.	.	.	441
24	SLO.width	67.65	44.32	56.01	162.80	48.82	44.10	137.80	44.75	.	119.50	.	130.90	.	485
25	DendriticCell.type1SecretedPerHourImmunized	88.89	.	60.43	1834.00	84.73	91.87	86.42	91.50	.	93.41	889.50	93.30	604.80	487
26	Simulation_numCD8Treg	94.96	76.89	.	.	37.63	73.86	24.09	73.60	53.27	.	99.01	.	97.46	498
27	DendriticCell.phagocytosisProbabilityImmature	76.65	91.47	53.31	70.32	54.63	92.77	61.30	92.95	.	92.48	.	93.67	.	517
28	TCell.timeLocalActivationDelay	64.22	75.41	58.56	325.50	88.58	57.11	81.86	59.87	.	93.46	.	96.19	.	518
29	Simulation_numCD4Treg	.	83.07	.	.	30.89	47.23	55.04	48.05	91.56	.	98.98	.	97.94	530
30	TCellApoptosisNaiveMean	38.20	53.26	49.23	.	50.61	54.93	.	63.73	.	65.05	.	68.47	.	555
31	CNSMacrophage.basalMBPExpressionProbability	76.59	95.18	78.32	96.19	82.58	.	85.40	.	.	83.73	.	83.49	285.70	565
32	CNSMacrophage.phagocytosisProbabilityMature	.	.	78.38	90.65	83.66	.	.	.	24.24	56.55	28.15	25.25	588	
33	Circulation.timeToCrossOrgan	82.13	.	59.66	.	44.98	78.82	57.61	76.69	.	69.59	.	70.98	.	591
34	DendriticCell.cytokineType2PolarizationRatio	81.25	83.80	49.16	.	77.75	82.52	79.53	82.23	484.50	.	.	.	.	606
35	Molecule.decayThreshold	752.70	.	235.20	.	664.70	3561.00	760.10	3721.00	87.05	1317.00	.	1283.00	.	628
36	CLN.width	198.40	57.31	51.53	.	48.45	39.93	59.73	.	.	.	.	.	.	665
37	Th2Polarization.proliferationMean	41.66	42.96	45.21	.	34.15	36.36	.	.	.	.	.	.	.	674
38	Th2Polarization.type2SecretedPerHourWhenActivated	.	.	87.03	94.89	369.10	495.30	409.80	545.80	97.59	.	.	.	.	684
39	APC.immatureDurationMean	54.25	.	53.55	.	32.42	50.52	.	57.96	.	.	.	.	.	690
40	Spleen.height	.	.	160.00	.	157.90	131.60	163.10	57.75	140.70	.	.	.	.	719
41	TCell_specificityLowerLimit	.	86.34	.	.	88.54	60.03	73.45	61.09	.	.	.	.	.	731
42	Th1Polarization.mhcUnExpressionDelayMean	93.54	69.20	.	.	47.22	32.11	81.03	33.11	17.57	.	.	.	97.70	732
43	Spleen.width	82.59	.	.	.	.	131.40	.	160.60	140.00	.	.	.	.	733
44	SLO.height	.	82.59	.	.	.	.	.	.	141.20	.	.	.	.	745
45	CD8Treg.cd8TregToCD4ThelperSpecificityDropOff	99.80	92.76	.	.	.	.	.	72.78	.	.	.	99.82	.	756
46	DendriticCell.type1RequiredForActivation	99.93	99.77	.	.	98.85	97.67	.	97.33	.	.	.	.	.	764
47	Simulation_numDCSpleen	.	.	.	.	50.17	.	50.76	.	82.67	.	.	.	.	799
48	CLN.height	.	.	.	.	45.65	.	114.80	.	45.61	.	.	.	.	801
49	CNSMacrophage.phagocytosisProbabilityImmature	.	.	.	.	87.80	.	.	46.66	.	52.72	.	.	.	809
50	TCell.proliferationStdDev	.	.	.	.	75.77	86.54	.	90.68	.	.	.	.	.	820
51	TCell_AICDStdDev	.	.	.	.	40.80	.	42.35	.	31.36	.	37.63	.	.	832
52	TCell_specificityUpperLimit	.	.	.	.	.	.	.	.	.	.	.	.	.	842
53	CD4THelper.diff00	.	.	1345.00	.	.	.	.	1060.00	.	.	.	.	.	863
54	Simulation_numDC	.	.	.	.	342.10	.	467.00	.	.	.	.	.	.	885
55	Circulation.width	.	.	.	.	.	.	.	.	126.50	.	.	.	.	891
56	TCell_becomeEffectorStdDev	.	.	.	.	52.67	.	.	.	.	.	.	.	.	899
57	Simulation_ImmunizationLinearInitial	.	937.50	.	.	.	.	.	.	.	.	.	.	.	900
58	DendriticCell.phagocytosisProbabilityMature	.	.	.	.	.	89.19	.	.	.	.	.	.	.	903
59	APC.immatureDurationStdDev	.	.	.	.	.	.	.	.	.	.	.	.	.	936
60	APC.timeOfDeathStdDev	.	.	.	.	.	.	.	.	.	.	.	.	.	936
72	CD4Treg.type1SecretedPerHourWhenActivated	.	.	.	.	.	.	.	.	.	.	.	.	.	936
72	CD8Treg.type1SecretedPerHourWhenActivated	.	.	.	.	.	.	.	.	.	.	.	.	.	936
72	CLN.timeToCrossOrgan	.	.	.	.	.	.	.	.	.	.	.	.	.	936
72	CNS.timeToCrossOrgan	.	.	.	.	.	.	.	.	.	.	.	.	.	936

Continued on Next Page...

Table 3 – Continued

Rank	Parameter Name	1M	1MT	2M	2MT	4M	4MT	8M	8MT	Th40	MEA	E40A	ME	E40	Total
72	DendriticCellMigrates.lengthOfTimeMovingFollowing...	.	.	.	.	.	.	.	.	.	.	.	.	.	936
72	SLO.timeToCrossOrgan	.	.	.	.	.	.	.	.	.	.	.	.	.	936
72	Spleen.timeToCrossOrgan	.	.	.	.	.	.	.	.	.	.	.	.	.	936
72	TCellApoptosisNaiveStdDev	.	.	.	.	.	.	.	.	.	.	.	.	.	936
72	TCellApoptosisPartialMaturityMean	.	.	.	.	.	.	.	.	.	.	.	.	.	936
72	TCellApoptosisPartialMaturityStdDev	.	.	.	.	.	.	.	.	.	.	.	.	.	936
72	Th1Polarization.mhcUnExpressionDelayStdDev	.	.	.	.	.	.	.	.	.	.	.	.	.	936
72	Th2Polarization.proliferationStdDev	.	.	.	.	.	.	.	.	.	.	.	.	.	936

Table 4: Robustness indexes for parameters pertaining to compartmental dimensions. Responses are indicated as follows: 1M, *CD4Th1 Max*; 1MT, *CD4Th1 Max Time*; 2M, *CD4Th2 Max*; 2MT, *CD4Th2 Max Time*; 4M, *CD4Treg Max*; 4MT, *CD4Treg Max Time*; 8M, *CD8Treg Max*; 8MT, *CD8Treg Max Time*; Th40, *CD4Th1 at 40 Days*; MEA, *Max EAE*; E40A, *EAE at 40 Days*. Significant deviation is indicated through the *A* test. ME and E40 represent *Max EAE* and *EAE at 40 Days*, with significant deviations in response behaviour defined as a change of at least 1.0 in the mean EAE score.

Parameter Name	1M	1MT	2M	2MT	4M	4MT	8M	8MT	Th40	MEA	E40A	ME	E40
CLN.height	.	.	.	.	44.60	59.28	122.30	59.79	45.61	.	.	.	.
CLN.width	198.40	57.31	51.53	.	44.12	.	53.26	48.73	.	.	.	.	.
CNS.height	6.53	14.79	4.02	6.72	5.81	12.57	7.44	11.92	17.45	3.66	3.83	3.31	1.82
CNS.width	9.27	23.01	4.66	7.02	7.25	17.88	8.35	20.97	.	3.90	3.93	3.25	1.87
Circulation.height	61.06	46.95	36.99	182.60	34.30	43.86	39.66	45.19	29.27	46.44	.	51.30	.
Circulation.width	.	.	.	.	.	.	126.50	.	.	.	.	.	.
SLO.height	82.59	.	.	.	.	.	131.00	.	160.60	140.00	.	141.20	.
SLO.width	67.65	44.32	56.01	162.80	47.34	45.93	141.30	45.71	.	119.50	.	130.90	.
Spleen.height	.	.	160.00	.	160.50	57.50	165.10	58.37	140.70	.	.	.	.
Spleen.width	.	.	.	.	50.39	35.94	73.76	34.69	.	.	.	.	.

Table 5: Robustness indexes for parameters specifying initial cell numbers. Responses indicated as in table 4.

Parameter Name	1M	1MT	2M	2MT	4M	4MT	8M	8MT	Th40	MEA	E40A	ME	E40
Simulation.numCD4Th	48.10	41.43	54.33	74.73	47.15	43.88	58.67	43.05	79.81	62.24	.	64.00	243.80
Simulation.numCD8Treg	94.96	76.89	.	.	39.77	75.10	23.54	78.07	53.27	.	99.01	.	97.46
Simulation.numCD4Treg	.	83.07	.	.	30.90	48.09	56.81	47.05	91.56	.	98.98	.	97.94
Simulation.numDCSpleen	.	.	.	.	51.47	.	51.16	.	82.67	.	.	.	.
Simulation.numDC	.	.	.	.	335.60	.	456.60	.	.	.	.	.	.

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Table 6: Robustness indexes for parameters pertaining to T cell-APC interactions. Responses indicated as in table 4

Parameter Name	1M	1MT	2M	2MT	4M	4MT	8M	8MT	Th40	MEA	E40A	ME	E40
CNSMacrophage.phagocytosisProbabilityImmature	.	.	.	.	87.70	.	.	.	.	46.66	.	52.72	.
CNSMacrophage.phagocytosisProbabilityMature	.	.	78.38	90.65	83.48	.	.	.	.	24.24	56.55	28.15	25.25
DendriticCell.phagocytosisProbabilityImmature	76.65	91.47	53.31	70.32	53.52	93.54	60.78	93.36	.	92.48	.	93.67	.
DendriticCell.phagocytosisProbabilityMature	.	.	.	.	.	86.09	.	.	.	.	.	.	.
TCell.specificityLowerLimit	.	86.34	.	.	93.33	60.70	74.95	62.75	.	.	.	.	.
TCell.specificityUpperLimit	.	.	.	.	42.14	.	43.10	.	.	.	.	.	.

Table 7: Robustness indexes for cytokine secretion and decay. Responses indicated as in table 4.

Parameter Name	1M	1MT	2M	2MT	4M	4MT	8M	8MT	Th40	MEA	E40A	ME	E40
CNSMacrophage.sdsSecretedPerHourWhenStimulated	5.64	11.97	2.99	5.74	4.79	9.29	6.71	9.68	16.26	1.55	1.86	1.41	0.89
DendriticCell.type1SecretedPerHourImmunized	88.89	.	60.43	1834.00	85.23	.	87.52	.	.	93.41	889.50	93.30	604.80
CD4Treg.type1SecretedPerHourWhenActivated	.	.	.	.	.	.	.	.	.	.	.	.	.
CD8Treg.type1SecretedPerHourWhenActivated	.	.	.	.	.	.	.	.	.	.	.	.	.
Th1Polarization.type1SecretedPerHourWhenActivated	38.04	62.49	38.01	44.88	35.75	57.56	40.50	55.19	477.10	26.07	141.30	27.92	48.21
Th2Polarization.type2SecretedPerHourWhenActivated	.	.	87.03	94.89	384.30	587.30	455.00	540.30	97.59	.	.	.	.
Molecule.decayThreshold	752.70	.	235.20	.	673.80	.	808.60	.	87.05	1317.00	.	1283.00	.

Table 8: Robustness indexes for parameters specifying standard deviations of timing distributions. Responses indicated as in table 4.

Parameter Name	1M	1MT	2M	2MT	4M	4MT	8M	8MT	Th40	MEA	E40A	ME	E40
APC.immatureDurationStdDev	.	.	.	.	.	.	.	.	.	.	.	.	.
APC.timeOfDeathStdDev	.	.	.	.	.	.	.	.	31.36	.	37.63	.	.
TCelL.AICDStdDev	.	.	.	.	.	.	.	.	.	.	.	.	.
TCelL.apoptosisNaiveStdDev	.	.	.	.	.	.	.	.	.	.	.	.	.
TCelL.apoptosisPartialMaturityStdDev	.	.	.	.	67.85	.	.	.	.	.	.	.	.
TCelL.becomeEffecterStdDev	.	.	.	.	62.05	88.80	.	94.55	.	.	.	.	.
TCelL.proliferationStdDev	.	.	.	.	.	.	.	.	.	.	.	.	.
Th2Polarization_proliferationStdDev	.	.	.	.	.	.	.	.	.	.	.	.	.

### 5.0.1 Compartmental dimensions

Table 4 shows the robustness indices for parameters specifying compartmental dimensions. Altering the heights and widths of compartments, independently of any other parameter, adjusts the density of cells in the simulation.

In the case of the CNS compartment this has a marked effect on many of the simulation's responses; apoptotic neurons incite self-perpetuating autoimmunity which in turn stimulates the expansion of regulatory T cells. Altering *CNS\_height* and *CNS\_width* adjusts the density of cells in the CNS compartment. When these parameters are decreased, the same quantity of SDA, secreted by the same number of CNS macrophages, reaches more neurons. There exists less physical space over which to dissipate, increasing its concentration. As such the rate at which neurons enter apoptosis increases. The simulation is highly sensitive to perturbation of these two parameters, with perturbations of less than 5% resulting in significant behavioural changes in numerous responses.

The other compartmental dimension parameters generally require perturbations of at least a third of the default value in order to attain significant behavioural changes, and in many cases no significant change is affected.

A common trend in compartments where T cell priming takes place, the CLN, SLO and spleen, is for perturbation of a compartment's width to have a greater effect on a simulation response than the same perturbation of its height. This is believed to be a simulation artifact, resulting from the manner in which cells move within the compartment. Cells migrate through a compartment from top to bottom. Hence, a migratory T cell will always explore the full height of a compartment. The same is not true for a compartment's width, where the probability of a T cell moving left, right or remaining stationary are the same. On average, a T cell will traverse down through a compartment in a straight line, exploring relatively little of the width. Reducing a compartment's width means that DCs are packed horizontally closer together, and this increases the probability that T cells will encounter DCs expressing MHC:peptide complexes for which they are specific.

The same phenomenon can explain why the simulation is significantly less robust towards perturbation of *Circulation\_height* than *Circulation\_width*. Decreasing the width of the circulatory compartment, where significant CD4Th1 apoptosis by CD8Treg cells occurs, increases the probability that cells will interact with one another. This results in increased CD4Th1 regulation of CD8Treg cells, which in turn increases the degree of stimulation for Treg priming at that point in time. Hence, the peaks of Treg population sizes are increased.

In summary, these results indicate that altering the density of cells in the CNS is critical to the behaviour of the simulation, but less so for other compartments.

### 5.0.2 Seeding of simulation with cells

Table 5 summarises simulation robustness with respect to those parameters that dictate the initial number of cells in the simulation, and that were arbitrarily assigned baseline values. The initial number of T cells in the simulation, dictated by *Simulation\_numCD4Th*, *Simulation\_numCD4Treg* and *Simulation\_numCD8Treg*, was arbitrarily chosen, though the ratio between Treg cells and CD4Th1 cells was informed by the domain expert. Robustness analysis finds that perturbations of around 40% or more are typically required to affect significant changes in behaviour.

*Simulation\_numDC* and *Simulation\_numDCSpleen* were also assigned arbitrary values, though the ratio between them was informed by the domain expert. The former is unable to affect significant changes in simulation behaviour unless perturbed by several times the default value. These results do not cause concern. The latter requires perturbation of 50%, and this affects only three responses, pertaining to the maximum number of effector CD4Treg and CD8Tregs attained, and the number of CD4Th1 cells remaining at 40 days.

These parameters influence the initial density of cells in the simulation. In the case of T cells, reducing the density prolongs the typical length of time required for immune responses to instigate, since the probabilities of T cells encountering immunogenic APCs are reduced. It is noted that the simulation does not encompass any notion of chemokine secretion by immunogenic APCs, which has the effect of attracting T cells to immunogenic APCs. At present immune response instigation relies on random movement of T cells through secondary lymphoid compartments where APCs reside. Were the density of MBP-specific CD4Th cells, CD4Treg or CD8Treg cells in the simulation to be found inconsistent with *in vivo* findings and cause concern, implementation of this chemokine attractant mechanism may compensate for high numbers of T cells. The same applies were the number of DCs in the spleen found to be artificially high.

In summary, at present, the robustness of the simulation with respect to these parameters does not cause concern.

### 5.0.3 T cell-APC interaction

The parameters *TCell\_specificityUpperLimit*, *TCell\_specificityLowerLimit*, *DendriticCell\_phagocytosisProbabilityImmature*, *DendriticCell\_phagocytosisProbabilityMature*, *CNSMacrophage\_phagocytosisProbabilityImmature* and

*CNSMacrophage\_phagocytosisProbabilityMature* have somewhat arbitrarily defined parameter values. Table 6 summarises the robustness indices of these parameters.

The simulation is relatively robust with respect perturbation of the APC phagocytosis parameters, in most cases no significant deviation in behaviour occurs, and where it does, perturbations of over 50% are typically required. These robustness indices are not considered problematic. It is noteworthy that *CNSMacrophage\_phagocytosisProbabilityMature* is able to increase the mean level of EAE experienced at 40 days by 1.0 when increased by 25% of its default value. The lack of any such deviation in behaviour for *CNSMacrophage\_phagocytosisProbabilityImmature* indicates that phagocytosis, and hence peptide derivation, by mature CNS macrophages is important for maintaining neuronal apoptosis. Figure 28 shows the effector T cell dynamics and the mean progression of EAE over time for *CNSMacrophage\_phagocytosisProbabilityMature* values of 20% and 40%. It may be observed that whilst the difference in T cell population dynamics appears minor, the EAE severities differ substantially. Mature APCs are highly motile, and are more likely to contact apoptotic neurons, which are stationary. Whilst increasing this parameter by 20% may reduce the phagocytosis of apoptotic CD4Th1 cells and neurons by dendritic cells, the lack of substantial difference in T cell priming patterns suggests that this effect is minor. Rather than explain the increased EAE severity through the increased priming of CD4Th1 cells, it is likely that increased phagocytic activity in mature CNS macrophages is resulting in increased MHC-II:MBP expression by these cells, and hence recent CD4Th1 infiltrates in the CNS are able to derive local activation more quickly. Earlier local activation of CD4Th1 cells will result in, at a population level, increased type 1 cytokine secretion, which prompts more widespread SDA secretion by CNS macrophages, and hence increased neuronal apoptosis.

Phagocytic activity of mature CNS macrophages is hence shown to impact neuronal apoptosis in the CNS. However, should future experimentation indicate that this parameter is incorrectly set, the implications for ARTIMMUS are not overly severe. This parameter's range of effects does not extend to T cell population dynamics, rather, it is limited to the rate of neuronal apoptosis. As such, the *in silico* EAE severity scoring mechanism would likely require re-calibration.

The simulation also displays robustness with respect to perturbation of T cell specificity related parameters. *TCell\_specificityUpperLimit* perturbation is found to be significant with respect to only two responses, when perturbed by over 40%. *TCell\_specificityLowerLimit* affects significant changes only when perturbed by over 60% of its default value.

#### 5.0.4 Cytokine secretion and decay

Table 7 summarises simulation robustness with respect to perturbation of parameters specifying cellular cytokine secretion and cytokine decay. Perturbation of *CD4Treg\_type1SecretedPerHourWhenActivated* and *CD8Treg\_type1SecretedPerHourWhenActivated*, which were assigned arbitrary values significantly less than *Th1Polarization\_type1SecretedPerHourWhenActivated*, is shown to be inconsequential to simulation behaviour. *Th2Polarization\_type2SecretedPerHourWhenActivated* is shown to significantly alter behaviour when perturbed by at least 85%.

Perturbation of both *Th1Polarization\_type1SecretedPerHourWhenActivated* and *CNSMacrophage\_sdaSecretedPerHourWhenStimulated* incites significant deviation in simulation behaviour for all responses. In the former case a perturbation of at least 25% is required, in the latter 1% is sufficient to increase the level of EAE severity experienced at 40 days by 1.0. *CNSMacrophage\_sdaSecretedPerHourWhenStimulated* is directly related to the rate of neuronal apoptosis, which in turn drives both autoimmune and hence regulatory responses. *Th1Polarization\_type1SecretedPerHourWhenActivated* may be considered a 'second order' parameter in influencing the rate of neuronal apoptosis, type 1 cytokine being required for the activation of CNS macrophages. These results are significant, and point to the importance of balancing parameters that lead to neuronal apoptosis such that the behaviours observed *in vivo* are accurately replicated in the simulation. Together, parameters describing the secretion rates of cytokines, cell sensitivities to cytokines, and *Molecule\_molecularHalflife* dictate the physical reach of cytokine effects. Should it be found that the two arbitrarily determined cytokine secretion parameters discussed here contribute to an unrealistic diffusion of cytokine effect in the simulation, then many other parameters will need recalibration to ensure that this discrepancy is corrected. These include the other parameters directly involved in neuronal apoptosis: *CNSCell\_apoptosisSDAThreshold*, *APC\_probabilityPhagocytosisToPeptide*, *Simulation\_numCNS*, and *Simulation\_numCNSMacrophage*.

*Molecule\_decayThreshold* represents the minimum concentration of cytokine that may exist in a grid space before it is considered to be zero; without this feature the simulation's decay mechanism would allow cytokine concentrations to approach but never reach zero. This parameter was assigned an arbitrary value intended to be many orders of magnitude smaller than the smallest quantity of cytokine secreted by any cell. Parameter perturbation is found to significantly alter simulation behaviour for several responses, however a perturbation of at least 85% is required, and in most cases the required perturbation is several hundred percent. It may be concluded

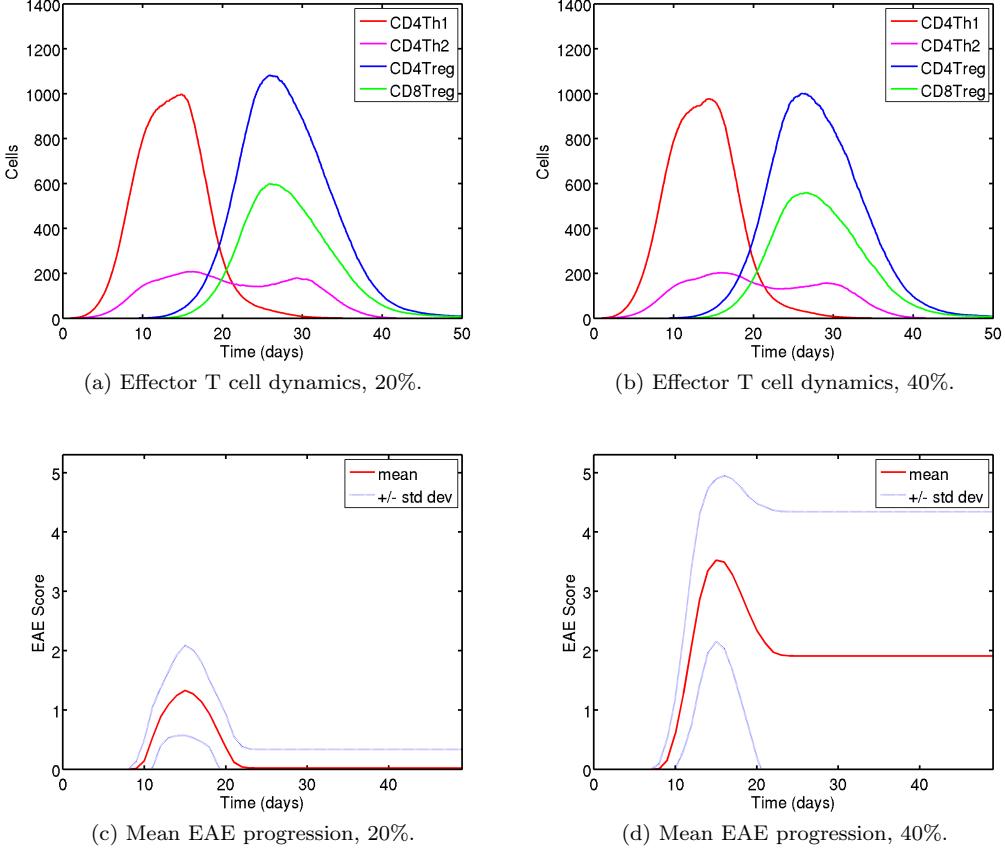


Figure 28: Changes in the phagocytic potential of mature CNS macrophages has a marked effect on the mean progression of EAE, although relatively little effect is seen in actual T cell dynamics. This is believed to be due to the time taken for effector CD4Th1 cells to derive local activation in the CNS: increased CNS macrophage phagocytic activity leads to increased expression of MHC-II:MBP.

that simulation behaviour is not critically defined by the exact value held by this parameter.

### 5.0.5 Standard deviation related parameters.

The duration of time that cells spend in particular states are typically drawn from a normal distribution, specified by a mean and standard deviation. The mean values are based on domain-specific knowledge, or are tuned in order to align simulation behaviour with that observed *in vivo*. Parameters specifying standard deviations, listed in table 8 have been arbitrarily assigned values that are far from zero but less than those held by the corresponding mean parameter.

Table 8 summarises robustness indices for arbitrarily assigned standard deviation parameters. This analysis indicates that perturbation of these parameters is largely inconsequential to simulation behaviour. *TCell\_AICDStdDev* is found to exert influence on the *max EAE* response when perturbed by around 30% or more. Figure 29 depicts the mean severity of EAE experienced under *TCell\_AICDStdDev* parameter values of 0 and 60 hours, and confirms this parameter's marked effect on the maximum severity of EAE reached. The result may be explained through the requirement for a CD4Th1 cell to reach the CNS and become locally activated before contributing to autoimmune activity. To illustrate with a hypothetical scenario, were *TCell\_AICDStdDev* to be set to zero, and the average time required for a CD4Th1 to migrate into the CNS compartment and become activated be 40 hours, then the average time that each CD4Th1 cell would perform effector function for would be 20 hours. Increasing *TCell\_AICDStdDev* to 60 hours, many cells would remain in effector state for significantly longer than 60 hours, and many would enter apoptosis prior to reaching the CNS.

For any one CD4Th1 cell, increasing values for *TCell\_AICDStdDev* that further reduce its effector life-span below the 40 hours required to reach the CNS does not further decrease its contribution to autoimmunity; it makes

no contribution. However, the other extreme is that a cell experiencing a similarly extended lifespan *would* provide additional contribution to autoimmune activity. Since some proportion of a CD4Th1 cell's effector lifespan is spent migrating to the CNS and awaiting local activation, increases in autoimmune contribution resulting from increases in  $TCell\_AICDStdDev$  outweigh the reduced contribution of cells that apoptose before being locally activated in the CNS.

Should the default value of 56 hours for  $TCell\_AICDStdDev$  be deemed inappropriate, the *in silico* EAE severity scoring mechanism would require re-calibration to reflect the fact that the rate of neuronal apoptosis is reasonably dependent on this parameter.

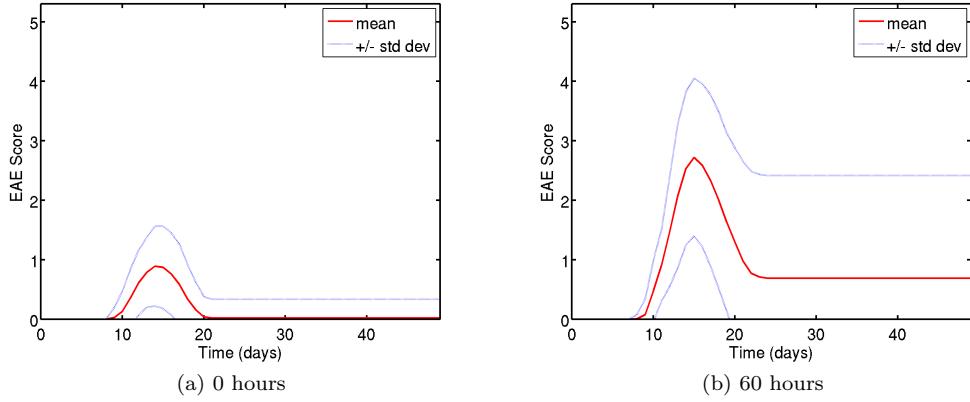


Figure 29: The mean severity of EAE experienced under parameter values of 0 and 60 hours for  $TCell\_AICDStdDev$ .

### 5.1 Full robustness analysis data

This section presents the full results of applying the robustness analysis to the ARTIMMUS simulation. Data is presented for each response in turn, in the following order:  $CD4Th1\ Max$ ,  $CD4Th1\ Max\ Time$ ,  $CD4Th2\ Max$ ,  $CD4Th2\ Max\ Time$ ,  $CD4Treg\ Max$ ,  $CD4Treg\ Max\ Time$ ,  $CD8Treg\ Max$ ,  $CD8Treg\ Max\ Time$ ,  $CD4Th1\ at\ 40\ days$ ,  $Maximum\ EAE$ , and  $EAE\ at\ 40\ days$ . Two further responses are presented hereafter,  $Maximum\ EAE\ A\ test$  and  $EAE\ at\ 40\ days\ A\ test$ , which employ the A test on  $Maximum\ EAE$  and  $EAE\ at\ 40\ days$  responses to determine when a scientifically significant change in simulation behaviour has taken place. Data is presented in tables, with each table presenting the simulation's parameters, the robustness index, lower and upper indexes, the lower and upper boundaries at which significant deviation in simulation behaviours take place, the default value for each parameter. Parameters are ranked according to their robustness indexes.

Table 9: Robustness indexes for parameters with respect to the *CD4Th1 Max* response. RI, robustness index; LI, lower index; UI, upper index; LB, lower boundary; DV, default value; UB, upper boundary. Results indicating no significant deviation in behaviour are marked with a period.

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
CNSCell_apoptosisSDAThreshold	5.263	5.263	7.752	4.737(+)	5	5.388(−)	1
CNSMacrophage_sdaSecretedPerHourWhenStimulated	5.638	7.201	5.638	92.8(−)	100	105.6(+)	2
CD4THelper_diff08	5.66	6.334	5.66	0.7962(−)	0.85	0.8981(+)	3
CNS_height	6.53	6.53	7.432	46.73(+)	50	53.72(−)	4
Simulation_immunizationLinearDC0	6.672	7.838	6.672	1.843(−)	2	2.133(+)	5
APC_timeOfDeathMean	7.3	7.3	9.124	102(−)	110	120(+)	6
Molecule_molecularHalflife	7.439	9.284	7.439	0.4536(−)	0.5	0.5372(+)	7
TCell_AICDMean	8.806	8.806	11.16	54.72(−)	60	66.69(+)	8
TCell_cellsPerGridspace	8.98	8.98	.	6.371(−)	7	.	9
CNS_width	9.273	9.273	12.56	45.36(+)	50	56.28(−)	10
TCell_proliferationMean	9.282	11.97	9.282	16.9(+)	19.2	20.98(−)	11
Simulation_immunizationLinearFreq	10.31	10.31	11.67	5.382(+)	6	6.7(−)	12
Simulation_numCNSMacrophage	12.12	14.29	12.12	64.28(−)	75	84.09(+)	13
TCell_timeLocalActivationInducedEffectorFunctionFor	14.59	14.59	23.67	40.99(−)	48	59.36(+)	14
Simulation_immunizationLinearGradient	16.92	16.92	21.62	-0.005846(−)	-0.005	-0.003919(+)	15
TCell_becomeEffectorMean	18.22	26.21	18.22	44.27(−)	60	70.93(−)	16
APC_probabilityPhagocytosisToPeptide	32.53	32.53	64.08	0.01349(−)	0.02	0.03282(+)	17
Th1Polarization_type1SecretedPerHourWhenActivated	38.04	38.04	509.8	61.96(−)	100	609.8(+)	18
TCell_apoptosisNaiveMean	38.2	38.2	104.4	18.54(−)	30	61.32(+)	19
Simulation_numDCCNS	39.7	40.88	39.7	23.65(−)	40	55.88(+)	20
Th2Polarization_proliferationMean	41.66	41.66	.	16.8(+)	28.8	.	21
Simulation_numCNS	43.41	43.41	.	283(−)	500	.	22
Simulation_numCD4Th	48.1	48.1	83.79	20.76(−)	40	73.51(+)	23
APC_immatureDurationMean	54.25	54.25	98.42	21.96(+)	48	95.24(−)	24
CNSMacrophage_type1RequiredForActivation	58	76.5	58	0.5875(+)	2.5	3.95(−)	25
Circulation_height	61.06	.	61.06	.	50	80.53(−)	26
TCell_timeLocalActivationDelay	64.22	64.22	258.4	3.578(−)	10	35.84(−)	27
SLO_width	67.65	.	67.65	.	50	83.82(−)	28
CNSMacrophage_basalMBPExpressionProbability	76.59	76.59	175.6	0.04683(−)	0.2	0.5511(+)	29
DendriticCell_phagocytosisProbabilityImmature	76.65	76.65	.	0.2335(−)	1	.	30
DendriticCell_cytokineType2PolarizationRatio	81.25	81.25	.	0.03188(−)	0.17	.	31
Circulation_timeToCrossOrgan	82.13	.	82.13	.	5	9.107(−)	32
SLO_height	82.59	.	82.59	.	50	91.29(−)	33
DendriticCell_type1SecretedPerHourImmunized	88.89	88.89	860.5	1.111(−)	10	96.05(+)	34
Th1Polarization_mhcUnExpressionDelayMean	93.54	93.54	.	0.5165(+)	8	.	35
Simulation_numCD8Treg	94.96	94.96	.	1.513(+)	30	.	36
CD8Treg_cd8TregToCD4ThelperSpecificityDropOff	99.8	99.8	.	0.001967(+)	1	.	37
DendriticCell_type1RequiredForActivation	99.93	99.93	.	0.001395(−)	2	.	38
CLN_width	198.4	.	198.4	.	50	149.2(−)	39

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Table 9 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
Molecule_decayThreshold	752.7	.	752.7	.	0.01	0.08527(–)	40
APC_immatureDurationStdDev	.	.	.	.	24	.	72
APC_timeOfDeathStdDev	.	.	.	.	48	.	72
CD4THelper_diff00	.	.	.	.	0.05	.	72
CD4Treg_type1SecretedPerHourWhenActivated	.	.	.	.	10	.	72
CD8Treg_type1SecretedPerHourWhenActivated	.	.	.	.	10	.	72
CLN_height	.	.	.	.	50	.	72
CLN_timeToCrossOrgan	.	.	.	.	12	.	72
CNS_timeToCrossOrgan	.	.	.	.	20	.	72
CNSMacrophage_phagocytosisProbabilityImmature	.	.	.	.	0.7	.	72
CNSMacrophage_phagocytosisProbabilityMature	.	.	.	.	0.3	.	72
Circulation_width	.	.	.	.	50	.	72
DendriticCellMigrates_lengthOfTimeMovingFollowingMigration	.	.	.	.	3.5	.	72
DendriticCell_phagocytosisProbabilityMature	.	.	.	.	0.3	.	72
SLO_timeToCrossOrgan	.	.	.	.	12	.	72
Simulation_immunizationLinearInitial	.	.	.	.	14	.	72
Simulation_numCD4Treg	.	.	.	.	30	.	72
Simulation_numDC	.	.	.	.	10	.	72
Simulation_numDCSpleen	.	.	.	.	100	.	72
Spleen_height	.	.	.	.	50	.	72
Spleen_timeToCrossOrgan	.	.	.	.	5	.	72
Spleen_width	.	.	.	.	50	.	72
TCell_AICDStdDev	.	.	.	.	56	.	72
TCell_apoptosisNaiveStdDev	.	.	.	.	17	.	72
TCell_apoptosisPartialMaturityMean	.	.	.	.	12	.	72
TCell_apoptosisPartialMaturityStdDev	.	.	.	.	6	.	72
TCell_becomeEffectorStdDev	.	.	.	.	56	.	72
TCell_proliferationStdDev	.	.	.	.	9.6	.	72
TCell_specificityLowerLimit	.	.	.	.	0.5	.	72
TCell_specificityUpperLimit	.	.	.	.	0.9	.	72
Th1Polarization_mhcUnExpressionDelayStdDev	.	.	.	.	2	.	72
Th2Polarization_proliferationStdDev	.	.	.	.	19.2	.	72
Th2Polarization_type2SecretedPerHourWhenActivated	.	.	.	.	100	.	72

Table 10: Robustness indexes for parameters with respect to the *CD4Th1 Max Time* response. RI, robustness index; LI, lower index; UI, upper index; LB, lower boundary; DV, default value; UB, upper boundary. Results indicating no significant deviation in behaviour are marked with a period.

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
TCell_proliferationMean Continued on Next Page...	9.177	9.177	11.47	17.44(-)	19.2	21.4(+)	1

Table 10 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
CNSMacrophage_sdaSecretedPerHourWhenStimulated	11.97	11.97	19.41	88.03(–)	100	119.4(+)	2
CNSCell_apoptosisSDAThreshold	13.78	14.78	13.78	4.261(+)	5	5.689(–)	3
CNS_height	14.79	16.28	14.79	41.86(+)	50	57.4(–)	4
Simulation_immunizationLinearDC0	16.71	17.84	16.71	1.643(–)	2	2.334(–)	5
Molecule_molecularHalflife	16.73	16.73	31.33	0.4164(–)	0.5	0.6566(+)	6
Simulation_immunizationLinearGradient	22.76	22.76	38.57	-0.006138(–)	-0.005	-0.003071(–)	7
CNS_width	23.01	.	23.01	.	50	61.51(–)	8
Simulation_numCNSMacrophage	23.71	23.71	29.6	57.22(–)	75	97.2(+)	9
TCell_becomeEffectorMean	24.94	24.94	45.57	45.04(+)	60	87.34(–)	10
APC_timeOfDeathMean	26.84	.	26.84	.	110	139.5(–)	11
Simulation_numCD4Th	41.43	41.43	55.51	23.43(+)	40	62.2(–)	12
Th2Polarization_proliferationMean	42.96	42.96	.	16.43(–)	28.8	.	13
SLO_width	44.32	44.32	51.27	27.84(–)	50	75.64(+)	14
Circulation_height	46.95	46.95	.	26.53(–)	50	.	15
CD4THelper_diff08	47.23	47.23	.	0.4485(+)	0.85	.	16
TCell_cellsPerGridspace	47.96	47.96	.	3.642(–)	7	.	17
Simulation_numDCCNS	51.7	51.7	.	19.32(–)	40	.	18
TCell_apoptosisNaiveMean	53.26	53.26	213.3	14.02(–)	30	93.98(+)	19
Simulation_numCNS	56.83	56.83	.	215.9(–)	500	.	20
CLN_width	57.31	57.31	.	21.34(–)	50	.	21
Th1Polarization_type1SecretedPerHourWhenActivated	62.49	62.49	.	37.51(–)	100	.	22
TCell_timeLocalActivationInducedEffectorFunctionFor	65.05	65.05	.	16.78(–)	48	.	23
APC_probabilityPhagocytosisToPeptide	65.77	65.77	.	0.006846(–)	0.02	.	24
Th1Polarization_mhcUnExpressionDelayMean	69.2	69.2	.	2.464(+)	8	.	25
TCell_timeLocalActivationDelay	75.41	75.41	364	2.459(–)	10	46.4(–)	26
Simulation_numCD8Treg	76.89	76.89	249.6	6.933(+)	30	104.9(–)	27
Simulation_numCD4Treg	83.07	83.07	496.2	5.079(+)	30	178.9(–)	28
DendriticCell_cytokineType2PolarizationRatio	83.8	83.8	.	0.02755(–)	0.17	.	29
TCell_specificityLowerLimit	86.34	86.34	.	0.06832(+)	0.5	.	30
DendriticCell_phagocytosisProbabilityImmature	91.47	91.47	.	0.08532(–)	1	.	31
CD8Treg_cd8TregToCD4ThelperSpecificityDropOff	92.76	92.76	.	0.07239(+)	1	.	32
CNSMacrophage_basalMBPExpressionProbability	95.18	95.18	.	0.009648(–)	0.2	.	33
DendriticCell_type1RequiredForActivation	99.77	99.77	.	0.00461(–)	2	.	34
CNSMacrophage_type1RequiredForActivation	127.5	.	127.5	.	2.5	5.687(–)	35
Simulation_immunizationLinearInitial	937.5	.	937.5	.	14	145.3(–)	36
TCell_AICDMean	.	.	.	.	60	.	72
Simulation_immunizationLinearFreq	.	.	.	.	6	.	72
APC_immatureDurationMean	.	.	.	.	48	.	72
Circulation_timeToCrossOrgan	.	.	.	.	5	.	72
SLO_height	.	.	.	.	50	.	72
DendriticCell_type1SecretedPerHourImmunized	.	.	.	.	10	.	72
Molecule_decayThreshold	.	.	.	.	0.01	.	72

Continued on Next Page...

Table 10 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
APC. <i>immatureDurationStdDev</i>	.	.	.	.	24	.	72
APC. <i>timeOfDeathStdDev</i>	.	.	.	.	48	.	72
CD4THelper. <i>diff00</i>	.	.	.	.	0.05	.	72
CD4Treg. <i>type1SecretedPerHourWhenActivated</i>	.	.	.	.	10	.	72
CD8Treg. <i>type1SecretedPerHourWhenActivated</i>	.	.	.	.	10	.	72
CLN. <i>height</i>	.	.	.	.	50	.	72
CLN. <i>timeToCrossOrgan</i>	.	.	.	.	12	.	72
CNS. <i>timeToCrossOrgan</i>	.	.	.	.	20	.	72
CNSMacrophage. <i>phagocytosisProbabilityImmature</i>	.	.	.	.	0.7	.	72
CNSMacrophage. <i>phagocytosisProbabilityMature</i>	.	.	.	.	0.3	.	72
Circulation. <i>width</i>	.	.	.	.	50	.	72
DendriticCellMigrates. <i>lengthOfTimeMovingFollowingMigration</i>	.	.	.	.	3.5	.	72
DendriticCell. <i>phagocytosisProbabilityMature</i>	.	.	.	.	0.3	.	72
SLO. <i>timeToCrossOrgan</i>	.	.	.	.	12	.	72
Simulation. <i>numDC</i>	.	.	.	.	10	.	72
Simulation. <i>numDCSpleen</i>	.	.	.	.	100	.	72
Spleen. <i>height</i>	.	.	.	.	50	.	72
Spleen. <i>timeToCrossOrgan</i>	.	.	.	.	5	.	72
Spleen. <i>width</i>	.	.	.	.	50	.	72
TCell. <i>AICDStdDev</i>	.	.	.	.	56	.	72
TCell. <i>apoptosisNaiveStdDev</i>	.	.	.	.	17	.	72
TCell. <i>apoptosisPartialMaturityMean</i>	.	.	.	.	12	.	72
TCell. <i>apoptosisPartialMaturityStdDev</i>	.	.	.	.	6	.	72
TCell. <i>becomeEffectorStdDev</i>	.	.	.	.	56	.	72
TCell. <i>proliferationStdDev</i>	.	.	.	.	9.6	.	72
TCell. <i>specificityUpperLimit</i>	.	.	.	.	0.9	.	72
Th1Polarization. <i>mhcUnExpressionDelayStdDev</i>	.	.	.	.	2	.	72
Th2Polarization. <i>proliferationStdDev</i>	.	.	.	.	19.2	.	72
Th2Polarization. <i>type2SecretedPerHourWhenActivated</i>	.	.	.	.	100	.	72

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Table 11: Robustness indexes for parameters with respect to the *CD4Th2 Max* response. RI, robustness index; LI, lower index; UI, upper index; LB, lower boundary; DV, default value; UB, upper boundary. Results indicating no significant deviation in behaviour are marked with a period.

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
CD4THelper. <i>diff00</i>	2.688	3.205	2.688	0.8228(+)	0.85	0.8729(-)	1
CNSMacrophage. <i>sdaSecretedPerHourWhenStimulated</i>	2.992	2.992	3.343	97.01(-)	100	103.3(+)	2
CNSCell. <i>apoptosisSDAThreshold</i>	3.267	3.267	4.1	4.837(+)	5	5.205(-)	3
CNS. <i>height</i>	4.018	4.018	4.854	47.99(+)	50	52.43(-)	4

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Table 11 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
Molecule_molecularHalflife	4.171	4.171	5.513	0.4791(–)	0.5	0.5276(+)	5
CNS_width	4.657	4.657	6.711	47.67(+)	50	53.36(–)	6
Simulation_numCNSMacrophage	6.788	7.189	6.788	69.61(–)	75	80.09(+)	7
APC_timeOfDeathMean	7.617	7.617	9.935	101.6(–)	110	120.9(+)	8
TCell_AICDMean	8.544	8.544	10.33	54.87(–)	60	66.2(+)	9
Simulation_immuneActivationLinearDC0	9.022	9.022	10.91	1.82(–)	2	2.218(+)	10
TCell_proliferationMean	9.809	11.93	9.809	16.91(+)	19.2	21.08(–)	11
TCell_cellsPerGridspace	10.41	10.41	.	6.271(–)	7	.	12
Simulation_immuneActivationLinearGradient	15.16	17.62	15.16	-0.005881(–)	-0.005	-0.004242(+)	13
Simulation_immuneActivationLinearFreq	16.13	16.13	37.34	5.032(+)	6	8.24(–)	14
APC_probabilityPhagocytosisToPeptide	16.92	16.92	19.48	0.01662(–)	0.02	0.0239(+)	15
TCell_timeLocalActivationInducedEffectorFunctionFor	19.02	19.02	65.13	38.87(–)	48	79.26(+)	16
TCell_becomeEffectorMean	20.64	29.22	20.64	42.47(–)	60	72.38(–)	17
Simulation_numDCCNS	23.84	26.32	23.84	29.47(–)	40	49.53(+)	18
Simulation_numCNS	27.59	27.59	93.45	362(–)	500	967.3(–)	19
Circulation_height	36.99	.	36.99	.	50	68.49(–)	20
Th1Polarization_type1SecretedPerHourWhenActivated	38.01	38.01	.	61.99(–)	100	.	21
Th2Polarization_proliferationMean	45.21	45.21	.	15.78(+)	28.8	.	22
DendriticCell_cytokineType2PolarizationRatio	49.16	49.16	.	0.08643(+)	0.17	.	23
TCell_apoptosisNaiveMean	49.23	49.23	156.7	15.23(–)	30	77.02(+)	24
CNSMacrophage_type1RequiredForActivation	50.32	54.99	50.32	1.125(+)	2.5	3.758(–)	25
CLN_width	51.53	51.53	.	24.23(+)	50	.	26
DendriticCell_phagocytosisProbabilityImmature	53.31	53.31	.	0.4669(–)	1	.	27
APC_immatureDurationMean	53.55	53.55	.	22.29(+)	48	.	28
Simulation_numCD4Th	54.33	54.33	103.4	18.27(–)	40	81.36(+)	29
SLO_width	56.01	56.01	97.02	22(+)	50	98.51(–)	30
TCell_timeLocalActivationDelay	58.56	58.56	212	4.144(+)	10	31.2(–)	31
Circulation_timeToCrossOrgan	59.66	.	59.66	.	5	7.983(–)	32
DendriticCell_type1SecretedPerHourImmunized	60.43	60.43	.	3.957(+)	10	.	33
CNSMacrophage_basalMBPExpressionProbability	78.32	78.32	132.1	0.04335(–)	0.2	0.4643(+)	34
CNSMacrophage_phagocytosisProbabilityMature	78.38	78.38	.	0.06485(+)	0.3	.	35
Th2Polarization_type2SecretedPerHourWhenActivated	87.03	92.12	87.03	7.879(–)	100	187(+)	36
Spleen_height	160	.	160	.	50	130(–)	37
Molecule_decayThreshold	235.2	.	235.2	.	0.01	0.03352(+)	38
CD4THelper_diff00	1345	.	1345	.	0.05	0.7226(–)	39
Th1Polarization_mhcUnExpressionDelayMean	.	.	.	.	8	.	72
Simulation_numCD8Treg	.	.	.	.	30	.	72
Simulation_numCD4Treg	.	.	.	.	30	.	72
TCell_specificityLowerLimit	.	.	.	.	0.5	.	72
CD8Treg_cd8TregToCD4ThelperSpecificityDropOff	.	.	.	.	1	.	72
DendriticCell_type1RequiredForActivation	.	.	.	.	2	.	72
Simulation_immuneActivationLinearInitial	.	.	.	.	14	.	72

Continued on Next Page...

Table 11 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
SLO_height	.	.	.	.	50	.	72
APC_immatureDurationStdDev	.	.	.	.	24	.	72
APC_timeOfDeathStdDev	.	.	.	.	48	.	72
CD4Treg_type1SecretedPerHourWhenActivated	.	.	.	.	10	.	72
CD8Treg_type1SecretedPerHourWhenActivated	.	.	.	.	10	.	72
CLN_height	.	.	.	.	50	.	72
CLN_timeToCrossOrgan	.	.	.	.	12	.	72
CNS_timeToCrossOrgan	.	.	.	.	20	.	72
CNSMacrophage_phagocytosisProbabilityImmature	.	.	.	.	0.7	.	72
Circulation_width	.	.	.	.	50	.	72
DendriticCellMigrates_lengthOfTimeMovingFollowingMigration	.	.	.	.	3.5	.	72
DendriticCell_phagocytosisProbabilityMature	.	.	.	.	0.3	.	72
SLO_timeToCrossOrgan	.	.	.	.	12	.	72
Simulation_numDC	.	.	.	.	10	.	72
Simulation_numDCSpleen	.	.	.	.	100	.	72
Spleen_timeToCrossOrgan	.	.	.	.	5	.	72
Spleen_width	.	.	.	.	50	.	72
TCell_AICDStdDev	.	.	.	.	56	.	72
TCell_apoptosisNaiveStdDev	.	.	.	.	17	.	72
TCell_apoptosisPartialMaturityMean	.	.	.	.	12	.	72
TCell_apoptosisPartialMaturityStdDev	.	.	.	.	6	.	72
TCell_becomeEffectorStdDev	.	.	.	.	56	.	72
TCell_proliferationStdDev	.	.	.	.	9.6	.	72
TCell_specificityUpperLimit	.	.	.	.	0.9	.	72
Th1Polarization_mhcUnExpressionDelayStdDev	.	.	.	.	2	.	72
Th2Polarization_proliferationStdDev	.	.	.	.	19.2	.	72

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Table 12: Robustness indexes for parameters with respect to the *CD4 Th2 Max Time* response. RI, robustness index; LI, lower index; UI, upper index; LB, lower boundary; DV, default value; UB, upper boundary. Results indicating no significant deviation in behaviour are marked with a period.

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
CNSCell_apoptosisSDAThreshold	5.2	5.2	6.986	4.74(+)	5	5.349(–)	1
CNSMacrophage_sdaSecretedPerHourWhenStimulated	5.742	5.742	6.124	94.26(–)	100	106.1(+)	2
CNS_height	6.717	7.156	6.717	46.42(+)	50	53.36(–)	3
CNS_width	7.021	7.021	11.46	46.49(+)	50	55.73(–)	4
Molecule_molecularHalflife	7.759	7.759	8.434	0.4612(–)	0.5	0.5422(+)	5
Simulation_numCNSMacrophage	13.13	13.13	13.49	65.15(–)	75	85.12(+)	6
TCell_AICDMean	24.62	24.62	32.68	45.23(–)	60	79.61(+)	7
APC_probabilityPhagocytosisToPeptide	25.85	25.85	58.4	0.01483(–)	0.02	0.03168(+)	8

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Table 12 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
TCell_cellsPerGridspace	33.17	33.17	.	4.678(–)	7	.	9
APC_timeOfDeathMean	35.8	35.8	.	70.62(–)	110	.	10
Simulation_immuneActivationLinearFreq	36.11	36.11	.	3.834(–)	6	.	11
Simulation_numCNS	38.11	38.11	96.73	309.4(–)	500	983.6(–)	12
Simulation_numDCCNS	39.09	39.09	57.43	24.37(–)	40	62.97(+)	13
Simulation_immuneActivationLinearDC0	43.58	43.58	.	1.128(+)	2	.	14
Simulation_immuneActivationLinearGradient	44.1	.	44.1	.	-0.005	-0.002795(+)	15
Th1Polarization_type1SecretedPerHourWhenActivated	44.88	44.88	.	55.12(–)	100	.	16
TCell_becomeEffectorMean	47.5	47.5	.	31.5(–)	60	.	17
TCell_timeLocalActivationInducedEffectorFunctionFor	49.56	49.56	.	24.21(–)	48	.	18
DendriticCell_phagocytosisProbabilityImmature	70.32	70.32	.	0.2968(–)	1	.	19
Simulation_numCD4Th	74.73	74.73	.	10.11(+)	40	.	20
CNSMacrophage_type1RequiredForActivation	75.85	86.19	75.85	0.3452(+)	2.5	4.396(–)	21
CNSMacrophage_phagocytosisProbabilityMature	90.65	90.65	.	0.02806(+)	0.3	.	22
CD4THelper_diff08	92.33	92.33	.	0.06518(–)	0.85	.	23
Th2Polarization_type2SecretedPerHourWhenActivated	94.89	94.89	.	5.113(–)	100	.	24
CNSMacrophage_basalMBPExpressionProbability	96.19	96.19	387.3	0.007622(–)	0.2	0.9745(+)	25
SLO_width	162.8	.	162.8	.	50	131.4(+)	26
Circulation_height	182.6	.	182.6	.	50	141.3(–)	27
TCell_timeLocalActivationDelay	325.5	.	325.5	.	10	42.55(–)	28
DendriticCell_type1SecretedPerHourImmunized	1834	.	1834	.	10	193.4(–)	29
TCell_proliferationMean	.	.	.	.	19.2	.	72
Th2Polarization_proliferationMean	.	.	.	.	28.8	.	72
DendriticCell_cytokineType2PolarizationRatio	.	.	.	.	0.17	.	72
TCell_apoptosisNaiveMean	.	.	.	.	30	.	72
CLN_width	.	.	.	.	50	.	72
APC_immatureDurationMean	.	.	.	.	48	.	72
Circulation_timeToCrossOrgan	.	.	.	.	5	.	72
Spleen_height	.	.	.	.	50	.	72
Molecule_decayThreshold	.	.	.	.	0.01	.	72
CD4THelper_diff00	.	.	.	.	0.05	.	72
Th1Polarization_mhcUnExpressionDelayMean	.	.	.	.	8	.	72
Simulation_numCD8Treg	.	.	.	.	30	.	72
Simulation_numCD4Treg	.	.	.	.	30	.	72
TCell_specificityLowerLimit	.	.	.	.	0.5	.	72
CD8Treg_cd8TregToCD4ThelperSpecificityDropOff	.	.	.	.	1	.	72
DendriticCell_type1RequiredForActivation	.	.	.	.	2	.	72
Simulation_immuneActivationLinearInitial	.	.	.	.	14	.	72
SLO_height	.	.	.	.	50	.	72
APC_immatureDurationStdDev	.	.	.	.	24	.	72
APC_timeOfDeathStdDev	.	.	.	.	48	.	72
CD4Treg_type1SecretedPerHourWhenActivated	.	.	.	.	10	.	72

Continued on Next Page...

Table 12 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
CD8Treg_type1SecretedPerHourWhenActivated	.	.	.	.	10	.	72
CLN_height	.	.	.	.	50	.	72
CLN_timeToCrossOrgan	.	.	.	.	12	.	72
CNS_timeToCrossOrgan	.	.	.	.	20	.	72
CNSMacrophage_phagocytosisProbabilityImmature	.	.	.	.	0.7	.	72
Circulation_width	.	.	.	.	50	.	72
DendriticCellMigrates_lengthOfTimeMovingFollowingMigration	.	.	.	.	3.5	.	72
DendriticCell_phagocytosisProbabilityMature	.	.	.	.	0.3	.	72
SLO_timeToCrossOrgan	.	.	.	.	12	.	72
Simulation_numDC	.	.	.	.	10	.	72
Simulation_numDCSpleen	.	.	.	.	100	.	72
Spleen_timeToCrossOrgan	.	.	.	.	5	.	72
Spleen_width	.	.	.	.	50	.	72
TCell_AICDStdDev	.	.	.	.	56	.	72
TCell_apoptosisNaiveStdDev	.	.	.	.	17	.	72
TCell_apoptosisPartialMaturityMean	.	.	.	.	12	.	72
TCell_apoptosisPartialMaturityStdDev	.	.	.	.	6	.	72
TCell_becomeEffectorStdDev	.	.	.	.	56	.	72
TCell_proliferationStdDev	.	.	.	.	9.6	.	72
TCell_specificityUpperLimit	.	.	.	.	0.9	.	72
Th1Polarization_mhcUnExpressionDelayStdDev	.	.	.	.	2	.	72
Th2Polarization_proliferationStdDev	.	.	.	.	19.2	.	72

Table 13: Robustness indexes for parameters with respect to the *CD4 Treg Max* response. RI, robustness index; LI, lower index; UI, upper index; LB, lower boundary; DV, default value; UB, upper boundary. Results indicating no significant deviation in behaviour are marked with a period.

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
TCell_proliferationMean	3.539	3.539	3.799	18.52(+)	19.2	19.93(−)	1
APC.timeOfDeathMean	4.273	4.273	4.392	105.3(−)	110	114.8(+)	2
CNSMacrophage_sdaSecretedPerHourWhenStimulated	4.842	6.025	4.842	93.97(−)	100	104.8(+)	3
CNSCell_apoptosisSDAThreshold	5.274	5.274	6.125	4.736(+)	5	5.306(−)	4
CNS_height	5.822	5.822	6.523	47.09(+)	50	53.26(−)	5
Molecule_molecularHalflife	6.794	6.933	6.794	0.4653(−)	0.5	0.534(+)	6
CNS_width	7.202	7.202	9.32	46.4(+)	50	54.66(−)	7
TCell_cellsPerGridspace	8.562	8.562	.	6.401(−)	7	.	8
Simulation_immunizationLinearDC0	8.725	8.725	9.881	1.825(−)	2	2.198(+)	9
CD4THelper_diff08	8.844	8.844	9.845	0.7748(−)	0.85	0.9337(+)	10
TCell_becomeEffectorMean	9.525	9.525	21.13	54.28(−)	60	72.68(+)	11
APC_probabilityPhagocytosisToPeptide	9.778	9.778	10.68	0.01804(−)	0.02	0.02214(+)	12

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Table 13 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
TCell_AICDMean	12.1	12.1	13.02	52.74(–)	60	67.81(+)	13
Simulation_immuneActivationLinearGradient	12.95	18.51	12.95	-0.005926(–)	-0.005	-0.004353(+)	14
Simulation_numCNSMacrophage	13.18	13.25	13.18	65.06(–)	75	84.89(+)	15
Simulation_numDCCNS	16.67	16.67	20.75	33.33(–)	40	48.3(+)	16
TCell_timeLocalActivationInducedEffectorFunctionFor	17.56	17.56	48.79	39.57(–)	48	71.42(+)	17
Simulation_immuneActivationLinearFreq	26.81	26.81	30.71	4.392(+)	6	7.843(–)	18
Simulation_numCD4Treg	30.89	30.89	32.79	20.73(–)	30	39.84(+)	19
APC.immatureDurationMean	32.42	32.42	37.5	32.44(+)	48	66(–)	20
Circulation_height	32.55	.	32.55	.	50	66.28(–)	21
Th2Polarization_proliferationMean	34.15	34.15	.	18.96(+)	28.8	.	22
Simulation_numCNS	34.59	34.59	72.22	327.1(–)	500	861.1(–)	23
Th1Polarization_type1SecretedPerHourWhenActivated	35.32	35.32	693.8	64.68(–)	100	793.8(+)	24
Simulation_numCD8Treg	37.63	37.63	44.89	18.71(+)	30	43.47(–)	25
TCell_specificityUpperLimit	40.8	40.8	.	0.5328(–)	0.9	.	26
Circulation_timeToCrossOrgan	44.98	.	44.98	.	5	7.249(–)	27
CLN_height	45.65	45.65	74.06	27.18(+)	50	87.03(–)	28
Simulation_numCD4Th	45.91	45.91	84.8	21.64(–)	40	73.92(+)	29
Spleen_width	47.22	47.22	141.1	26.39(–)	50	120.6(–)	30
CLN_width	48.45	48.45	49.1	25.77(+)	50	74.55(–)	31
SLO_width	48.82	48.82	99.03	25.59(+)	50	99.51(–)	32
Simulation_numDCSpleen	50.17	50.17	.	49.83(–)	100	.	33
TCell_apoptosisNaiveMean	50.61	50.61	.	14.82(–)	30	.	34
TCell_becomeEffectorStdDev	52.67	52.67	.	26.51(+)	56	.	35
DendriticCell_phagocytosisProbabilityImmature	54.63	54.63	.	0.4537(–)	1	.	36
CNSMacrophage_type1RequiredForActivation	71.6	92.85	71.6	0.1787(+)	2.5	4.29(–)	37
TCell_proliferationStdDev	75.77	.	75.77	.	9.6	16.87(+)	38
DendriticCell_cytokineType2PolarizationRatio	77.75	77.75	.	0.03782(–)	0.17	.	39
CNSMacrophage_basalMBPExpressionProbability	82.58	82.58	355.4	0.03485(–)	0.2	0.9108(+)	40
CNSMacrophage_phagocytosisProbabilityMature	83.66	83.66	.	0.04901(+)	0.3	.	41
DendriticCell_type1SecretedPerHourImmunized	84.73	84.73	627	1.527(–)	10	72.7(+)	42
CNSMacrophage_phagocytosisProbabilityImmature	87.8	87.8	.	0.08541(+)	0.7	.	43
TCell_specificityLowerLimit	88.54	88.54	.	0.05732(–)	0.5	.	44
TCell_timeLocalActivationDelay	88.58	88.58	219.6	1.142(–)	10	31.96(–)	45
DendriticCell_type1RequiredForActivation	98.85	98.85	643.9	0.023(+)	2	14.88(–)	46
Spleen_height	157.9	.	157.9	.	50	129(–)	47
Simulation_numDC	342.1	.	342.1	.	10	44.21(+)	48
Th2Polarization_type2SecretedPerHourWhenActivated	369.1	.	369.1	.	100	469.1(–)	49
Molecule_decayThreshold	664.7	.	664.7	.	0.01	0.07647(–)	50
CD4THelper_diff00	.	.	.	.	0.05	.	72
Th1Polarization_mhcUnExpressionDelayMean	.	.	.	.	8	.	72
CD8Treg_cd8TregToCD4ThelperSpecificityDropOff	.	.	.	.	1	.	72
Simulation_immuneActivationLinearInitial	.	.	.	.	14	.	72

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Table 13 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
SLO_height	.	.	.	.	50	.	72
APC_immatureDurationStdDev	.	.	.	.	24	.	72
APC_timeOfDeathStdDev	.	.	.	.	48	.	72
CD4Treg_type1SecretedPerHourWhenActivated	.	.	.	.	10	.	72
CD8Treg_type1SecretedPerHourWhenActivated	.	.	.	.	10	.	72
CLN_timeToCrossOrgan	.	.	.	.	12	.	72
CNS_timeToCrossOrgan	.	.	.	.	20	.	72
Circulation_width	.	.	.	.	50	.	72
DendriticCellMigrates_lengthOfTimeMovingFollowingMigration	.	.	.	.	3.5	.	72
DendriticCell_phagocytosisProbabilityMature	.	.	.	.	0.3	.	72
SLO_timeToCrossOrgan	.	.	.	.	12	.	72
Spleen_timeToCrossOrgan	.	.	.	.	5	.	72
TCell_AICDStdDev	.	.	.	.	56	.	72
TCell_apoptosisNaiveStdDev	.	.	.	.	17	.	72
TCell_apoptosisPartialMaturityMean	.	.	.	.	12	.	72
TCell_apoptosisPartialMaturityStdDev	.	.	.	.	6	.	72
Th1Polarization_mhcUnExpressionDelayStdDev	.	.	.	.	2	.	72
Th2Polarization_proliferationStdDev	.	.	.	.	19.2	.	72

Table 14: Robustness indexes for parameters with respect to the *CD4Treg Max Time* response. RI, robustness index; LI, lower index; UI, upper index; LB, lower boundary; DV, default value; UB, upper boundary. Results indicating no significant deviation in behaviour are marked with a period.

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
TCell_proliferationMean	5.584	5.584	5.836	18.13(–)	19.2	20.32(+)	1
CNSMacrophage_sdaSecretedPerHourWhenStimulated	9.006	9.006	14.99	90.99(–)	100	115(+)	2
CNS_height	10.99	17.94	10.99	41.03(+)	50	55.49(–)	3
CNSCell_apoptosisSDAThreshold	11.88	13.28	11.88	4.336(+)	5	5.594(–)	4
Molecule_molecularHalflife	12.8	12.8	19.96	0.436(–)	0.5	0.5998(+)	5
TCell_becomeEffectorMean	14.98	14.98	.	51.01(+)	60	.	6
CNS_width	16.4	16.4	16.84	41.8(+)	50	58.42(–)	7
Simulation_numCNSMacrophage	18.65	18.65	22.78	61.01(–)	75	92.09(+)	8
Simulation_immunizationLinearDC0	22.22	22.22	.	1.556(+)	2	.	9
Spleen_width	32.11	32.11	63.56	33.94(–)	50	81.78(+)	10
Simulation_immunizationLinearFreq	33.46	33.46	.	3.992(–)	6	.	11
Simulation_immunizationLinearGradient	36.71	60.12	36.71	-0.008006(+)	-0.005	-0.003164(+)	12
TCell_cellsPerGridspace	39.2	39.2	.	4.256(–)	7	.	13
CLN_width	39.93	39.93	.	30.03(–)	50	.	14
Simulation_numCD4Th	42.25	42.25	77.48	23.1(+)	40	70.99(–)	15
APC_timeOfDeathMean	42.36	42.36	.	63.4(–)	110	.	16

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Table 14 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
Circulation_height	43.99	49.09	43.99	25.45(–)	50	72(+)	17
SLO_width	44.1	44.1	62.38	27.95(–)	50	81.19(+)	18
Simulation_numCNS	45.09	45.09	.	274.6(–)	500	.	19
Simulation_numCD4Treg	47.23	47.23	69.81	15.83(+)	30	50.94(–)	20
Th1Polarization_type1SecretedPerHourWhenActivated	53.98	53.98	.	46.02(–)	100	.	21
TCell_apoptosisNaiveMean	54.93	54.93	71.98	13.52(–)	30	51.59(+)	22
TCell_timeLocalActivationDelay	57.11	57.11	.	4.289(–)	10	.	23
TCell_specificityLowerLimit	60.03	60.03	.	0.1999(+)	0.5	.	24
APC_probabilityPhagocytosisToPeptide	60.98	60.98	.	0.007804(–)	0.02	.	25
Simulation_numDCCNS	63.58	63.58	89.9	14.57(–)	40	75.96(+)	26
TCell_timeLocalActivationInducedEffectorFunctionFor	68.72	68.72	.	15.02(–)	48	.	27
Simulation_numCD8Treg	73.86	73.86	114.8	7.841(+)	30	64.44(–)	28
Circulation_timeToCrossOrgan	78.82	.	78.82	.	5	8.941(+)	29
DendriticCell_cytokineType2PolarizationRatio	82.52	82.52	.	0.02972(–)	0.17	.	30
TCell_proliferationStdDev	86.54	.	86.54	.	9.6	17.91(–)	31
CD4THelper_diff08	90.82	90.82	.	0.078(–)	0.85	.	32
DendriticCell_type1SecretedPerHourImmunized	91.87	91.87	.	0.8128(–)	10	.	33
DendriticCell_phagocytosisProbabilityImmature	92.77	92.77	.	0.0723(–)	1	.	34
DendriticCell_type1RequiredForActivation	97.67	97.67	578.8	0.04653(–)	2	13.58(+)	35
CNSMacrophage_type1RequiredForActivation	108.3	.	108.3	.	2.5	5.207(–)	36
Spleen_height	131.6	.	131.6	.	50	115.8(+)	37
Th2Polarization_type2SecretedPerHourWhenActivated	495.3	.	495.3	.	100	595.3(–)	38
Molecule_decayThreshold	3561	.	3561	.	0.01	0.3661(–)	39
TCell_AICDMean	.	.	.	.	60	.	72
APC_immatureDurationMean	.	.	.	.	48	.	72
Th2Polarization_proliferationMean	.	.	.	.	28.8	.	72
TCell_specificityUpperLimit	.	.	.	.	0.9	.	72
CLN_height	.	.	.	.	50	.	72
Simulation_numDCSpleen	.	.	.	.	100	.	72
TCell_becomeEffectorStdDev	.	.	.	.	56	.	72
CNSMacrophage_basalMBPExpressionProbability	.	.	.	.	0.2	.	72
CNSMacrophage_phagocytosisProbabilityMature	.	.	.	.	0.3	.	72
CNSMacrophage_phagocytosisProbabilityImmature	.	.	.	.	0.7	.	72
Simulation_numDC	.	.	.	.	10	.	72
CD4THelper_diff00	.	.	.	.	0.05	.	72
Th1Polarization_mhcUnExpressionDelayMean	.	.	.	.	8	.	72
CD8Treg_cd8TregToCD4ThelperSpecificityDropOff	.	.	.	.	1	.	72
Simulation_immuneActivationLinearInitial	.	.	.	.	14	.	72
SLO_height	.	.	.	.	50	.	72
APC_immatureDurationStdDev	.	.	.	.	24	.	72
APC_timeOfDeathStdDev	.	.	.	.	48	.	72
CD4Treg_type1SecretedPerHourWhenActivated	.	.	.	.	10	.	72

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Table 14 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
CD8Treg_type1SecretedPerHourWhenActivated	.	.	.	.	10	.	72
CLN_timeToCrossOrgan	.	.	.	.	12	.	72
CNS_timeToCrossOrgan	.	.	.	.	20	.	72
Circulation_width	.	.	.	.	50	.	72
DendriticCellMigrates_lengthOfTimeMovingFollowingMigration	.	.	.	.	3.5	.	72
DendriticCell_phagocytosisProbabilityMature	.	.	.	.	0.3	.	72
SLO_timeToCrossOrgan	.	.	.	.	12	.	72
Spleen_timeToCrossOrgan	.	.	.	.	5	.	72
TCell_AICDStdDev	.	.	.	.	56	.	72
TCell_apoptosisNaiveStdDev	.	.	.	.	17	.	72
TCell_apoptosisPartialMaturityMean	.	.	.	.	12	.	72
TCell_apoptosisPartialMaturityStdDev	.	.	.	.	6	.	72
Th1Polarization_mhcUnExpressionDelayStdDev	.	.	.	.	2	.	72
Th2Polarization_proliferationStdDev	.	.	.	.	19.2	.	72

Table 15: Robustness indexes for parameters with respect to the *CD8Treg Max* response. RI, robustness index; LI, lower index; UI, upper index; LB, lower boundary; DV, default value; UB, upper boundary. Results indicating no significant deviation in behaviour are marked with a period.

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
APC.timeOfDeathMean	5.794	5.794	6.062	103.6(–)	110	116.7(+)	1
TCell_proliferationMean	5.881	8.698	5.881	17.53(+)	19.2	20.33(–)	2
CNSCell_apoptosisSDAThreshold	6.628	6.628	7.897	4.669(+)	5	5.395(–)	3
CNSMacrophage_sdaSecretedPerHourWhenStimulated	6.674	6.752	6.674	93.25(–)	100	106.7(+)	4
CNS_height	7.4	7.4	7.626	46.3(+)	50	53.81(–)	5
CNS_width	8.118	8.118	10.5	45.94(+)	50	55.25(–)	6
Molecule_molecularHalflife	8.731	8.731	9.419	0.4563(–)	0.5	0.5471(+)	7
TCell_cellsPerGridspace	9.613	9.613	.	6.327(–)	7	.	8
CD4THelper_diff08	10.18	10.18	11.72	0.7635(–)	0.85	0.9497(+)	9
APC_probabilityPhagocytosisToPeptide	12.01	12.01	14.29	0.0176(–)	0.02	0.02286(+)	10
Simulation_immuneActivationLinearDC0	12.88	12.99	12.88	1.74(–)	2	2.258(+)	11
TCell_AICDMean	13.92	13.92	14.77	51.65(–)	60	68.86(+)	12
Simulation_numCNSMacrophage	13.97	13.97	15.22	64.52(–)	75	86.42(+)	13
Simulation_immuneActivationLinearGradient	16.24	22.65	16.24	-0.006133(–)	-0.005	-0.004188(+)	14
TCell_becomeEffectorMean	17.27	17.27	23.7	49.64(–)	60	74.22(–)	15
TCell_timeLocalActivationInducedEffectorFunctionFor	21.95	21.95	.	37.46(–)	48	.	16
Simulation_numDCCNS	22.29	22.29	36.09	31.08(–)	40	54.43(+)	17
Simulation_numCD8Treg	24.09	24.09	27.19	22.77(–)	30	38.16(+)	18
Simulation_immuneActivationLinearFreq	35.57	35.57	60.17	3.866(+)	6	9.61(–)	19
Th2Polarization_proliferationMean	36.36	36.36	.	18.33(+)	28.8	.	20

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Table 15 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
Simulation_numCNS	36.73	36.73	84.14	316.3(–)	500	920.7(–)	21
Circulation_height	39.21	.	39.21	.	50	69.6(–)	22
Th1Polarization_type1SecretedPerHourWhenActivated	39.64	39.64	.	60.36(–)	100	.	23
TCell_specificityUpperLimit	42.35	42.35	.	0.5189(–)	0.9	.	24
APC_immatureDurationMean	50.52	50.52	55.64	23.75(+)	48	74.71(–)	25
Simulation_numDCSpleen	50.76	50.76	.	49.24(–)	100	.	26
Simulation_numCD4Treg	55.04	59.46	55.04	12.16(+)	30	46.51(–)	27
Simulation_numCD4Th	57.2	57.2	121.2	17.12(–)	40	88.46(+)	28
Circulation_timeToCrossOrgan	57.61	.	57.61	.	5	7.881(–)	29
CLN_width	59.73	59.73	63.65	20.14(+)	50	81.83(–)	30
DendriticCell_phagocytosisProbabilityImmature	61.3	61.3	.	0.387(–)	1	.	31
TCell_specificityLowerLimit	73.45	73.45	.	0.1327(–)	0.5	.	32
DendriticCell_cytokineType2PolarizationRatio	79.53	79.53	.	0.03481(–)	0.17	.	33
CNSMacrophage_type1RequiredForActivation	80.11	.	80.11	.	2.5	4.503(–)	34
Spleen_width	81.03	.	81.03	.	50	90.52(–)	35
TCell_timeLocalActivationDelay	81.86	81.86	275.6	1.814(–)	10	37.56(–)	36
CNSMacrophage_basalMBPExpressionProbability	85.4	85.4	.	0.02919(–)	0.2	.	37
DendriticCell_type1SecretedPerHourImmunized	86.42	86.42	.	1.358(–)	10	.	38
DendriticCell_phagocytosisProbabilityMature	89.19	89.19	.	0.03243(+)	0.3	.	39
CLN_height	114.8	.	114.8	.	50	107.4(–)	40
SLO_height	131.4	.	131.4	.	50	115.7(–)	41
SLO_width	137.8	.	137.8	.	50	118.9(–)	42
Spleen_height	163.1	.	163.1	.	50	131.5(–)	43
Th2Polarization_type2SecretedPerHourWhenActivated	409.8	.	409.8	.	100	509.8(–)	44
Simulation_numDC	467	.	467	.	10	56.7(+)	45
Molecule_decayThreshold	760.1	.	760.1	.	0.01	0.08601(–)	46
TCell_apoptosisNaiveMean	.	.	.	.	30	.	72
TCell_proliferationStdDev	.	.	.	.	9.6	.	72
DendriticCell_type1RequiredForActivation	.	.	.	.	2	.	72
TCell_becomeEffectorStdDev	.	.	.	.	56	.	72
CNSMacrophage_phagocytosisProbabilityMature	.	.	.	.	0.3	.	72
CNSMacrophage_phagocytosisProbabilityImmature	.	.	.	.	0.7	.	72
CD4THelper_diff00	.	.	.	.	0.05	.	72
Th1Polarization_mhcUnExpressionDelayMean	.	.	.	.	8	.	72
CD8Treg_cd8TregToCD4ThelperSpecificityDropOff	.	.	.	.	1	.	72
Simulation_immuneActivationLinearInitial	.	.	.	.	14	.	72
APC_immatureDurationStdDev	.	.	.	.	24	.	72
APC_timeOfDeathStdDev	.	.	.	.	48	.	72
CD4Treg_type1SecretedPerHourWhenActivated	.	.	.	.	10	.	72
CD8Treg_type1SecretedPerHourWhenActivated	.	.	.	.	10	.	72
CLN_timeToCrossOrgan	.	.	.	.	12	.	72
CNS_timeToCrossOrgan	.	.	.	.	20	.	72

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Table 15 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
Circulation_width	.	.	.	.	50	.	72
DendriticCellMigrates_lengthOfTimeMovingFollowingMigration	.	.	.	.	3.5	.	72
SLO_timeToCrossOrgan	.	.	.	.	12	.	72
Spleen_timeToCrossOrgan	.	.	.	.	5	.	72
TCell_AICDStdDev	.	.	.	.	56	.	72
TCell_apoptosisNaiveStdDev	.	.	.	.	17	.	72
TCell_apoptosisPartialMaturityMean	.	.	.	.	12	.	72
TCell_apoptosisPartialMaturityStdDev	.	.	.	.	6	.	72
Th1Polarization_mhcUnExpressionDelayStdDev	.	.	.	.	2	.	72
Th2Polarization_proliferationStdDev	.	.	.	.	19.2	.	72

Table 16: Robustness indexes for parameters with respect to the *CD8Treg Max Time* response. RI, robustness index; LI, lower index; UI, upper index; LB, lower boundary; DV, default value; UB, upper boundary. Results indicating no significant deviation in behaviour are marked with a period.

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
TCell_proliferationMean	5.911	5.911	6.219	18.07(–)	19.2	20.39(+)	1
CNSMacrophage_sdaSecretedPerHourWhenStimulated	9.218	9.218	20.23	90.78(–)	100	120.2(+)	2
CNS_height	12.67	.	12.67	.	50	56.34(–)	3
CNSCell_apoptosisSDAThreshold	13.03	13.03	13.8	4.348(+)	5	5.69(–)	4
Molecule_molecularHalflife	13.93	13.93	28.95	0.4304(–)	0.5	0.6448(+)	5
TCell_becomeEffectorMean	14.94	14.94	.	51.04(+)	60	.	6
CNS_width	17.1	17.1	20.9	41.45(+)	50	60.45(–)	7
Simulation_numCNSMacrophage	19	19	27.91	60.75(–)	75	95.93(+)	8
Simulation_immunizationLinearDC0	21.38	21.38	.	1.572(+)	2	.	9
Spleen_width	33.11	33.11	70.29	33.45(–)	50	85.14(+)	10
Simulation_immunizationLinearFreq	37.2	37.2	.	3.768(–)	6	.	11
Simulation_immunizationLinearGradient	39.82	57.97	39.82	-0.007899(+)	-0.005	-0.003009(+)	12
Simulation_numCD4Th	41.97	41.97	73.05	23.21(+)	40	69.22(–)	13
TCell_cellsPerGridspace	42.67	42.67	.	4.013(–)	7	.	14
SLO_width	44.75	44.75	68.55	27.62(–)	50	84.28(+)	15
Circulation_height	45.58	50.81	45.58	24.59(–)	50	72.79(+)	16
Simulation_numCNS	46.67	46.67	.	266.7(–)	500	.	17
APC_timeOfDeathMean	47.02	47.02	.	58.28(–)	110	.	18
Simulation_numCD4Treg	48.05	48.05	69.65	15.59(+)	30	50.9(–)	19
TCell_timeLocalActivationInducedEffectorFunctionFor	53.73	53.73	.	22.21(–)	48	.	20
Th1Polarization_typeISecretedPerHourWhenActivated	56.07	56.07	.	43.93(–)	100	.	21
Simulation_numDCCNS	57.64	57.64	99.66	16.94(–)	40	79.87(+)	22
Spleen_height	57.75	57.75	131.7	21.13(–)	50	115.9(+)	23
TCell_timeLocalActivationDelay	59.87	59.87	398.1	4.013(–)	10	49.81(–)	24

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Table 16 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
TCell_specificityLowerLimit	61.09	61.09	.	0.1946(+)	0.5	.	25
TCell_apoptosisNaiveMean	63.73	63.73	77.7	10.88(–)	30	53.31(+)	26
Simulation_numCD8Treg	73.6	73.6	118.9	7.921(+)	30	65.67(–)	27
APC_probabilityPhagocytosisToPeptide	74.93	74.93	.	0.005014(–)	0.02	.	28
Circulation_timeToCrossOrgan	76.69	.	76.69	.	5	8.834(+)	29
DendriticCell_cytokineType2PolarizationRatio	82.23	82.23	.	0.03021(–)	0.17	.	30
TCell_proliferationStdDev	90.68	.	90.68	.	9.6	18.31(–)	31
DendriticCell_type1SecretedPerHourImmunized	91.5	91.5	.	0.8497(–)	10	.	32
CD4THelper_diff08	92.7	92.7	.	0.06202(–)	0.85	.	33
DendriticCell_phagocytosisProbabilityImmature	92.95	92.95	.	0.07054(–)	1	.	34
DendriticCell_type1RequiredForActivation	97.33	97.33	579.6	0.05345(–)	2	13.59(+)	35
CNSMacrophage_type1RequiredForActivation	106.3	.	106.3	.	2.5	5.158(–)	36
Th2Polarization_type2SecretedPerHourWhenActivated	545.8	.	545.8	.	100	645.8(–)	37
Molecule_decayThreshold	3721	.	3721	.	0.01	0.3821(–)	38
TCell_AICDMean	.	.	.	.	60	.	72
Th2Polarization_proliferationMean	.	.	.	.	28.8	.	72
TCell_specificityUpperLimit	.	.	.	.	0.9	.	72
APC_immatureDurationMean	.	.	.	.	48	.	72
Simulation_numDCSpleen	.	.	.	.	100	.	72
CLN_width	.	.	.	.	50	.	72
CNSMacrophage_basalMBPExpressionProbability	.	.	.	.	0.2	.	72
DendriticCell_phagocytosisProbabilityMature	.	.	.	.	0.3	.	72
CLN_height	.	.	.	.	50	.	72
SLO_height	.	.	.	.	50	.	72
Simulation_numDC	.	.	.	.	10	.	72
TCell_becomeEffectorStdDev	.	.	.	.	56	.	72
CNSMacrophage_phagocytosisProbabilityMature	.	.	.	.	0.3	.	72
CNSMacrophage_phagocytosisProbabilityImmature	.	.	.	.	0.7	.	72
CD4THelper_diff00	.	.	.	.	0.05	.	72
Th1Polarization_mhcUnExpressionDelayMean	.	.	.	.	8	.	72
CD8Treg_cd8TregToCD4THelperSpecificityDropOff	.	.	.	.	1	.	72
Simulation_immuneActivationInitial	.	.	.	.	14	.	72
APC_immatureDurationStdDev	.	.	.	.	24	.	72
APC_timeOfDeathStdDev	.	.	.	.	48	.	72
CD4Treg_type1SecretedPerHourWhenActivated	.	.	.	.	10	.	72
CD8Treg_type1SecretedPerHourWhenActivated	.	.	.	.	10	.	72
CLN_timeToCrossOrgan	.	.	.	.	12	.	72
CNS_timeToCrossOrgan	.	.	.	.	20	.	72
Circulation_width	.	.	.	.	50	.	72
DendriticCellMigrates_lengthOfTimeMovingFollowingMigration	.	.	.	.	3.5	.	72
SLO_timeToCrossOrgan	.	.	.	.	12	.	72
Spleen_timeToCrossOrgan	.	.	.	.	5	.	72

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Table 16 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
TCell_AICDStdDev	.	.	.	.	56	.	72
TCell_apoptosisNaiveStdDev	.	.	.	.	17	.	72
TCell_apoptosisPartialMaturityMean	.	.	.	.	12	.	72
TCell_apoptosisPartialMaturityStdDev	.	.	.	.	6	.	72
Th1Polarization_mhcUnExpressionDelayStdDev	.	.	.	.	2	.	72
Th2Polarization_proliferationStdDev	.	.	.	.	19.2	.	72

Table 17: Robustness indexes for parameters with respect to the *CD4Th1at40d* response. RI, robustness index; LI, lower index; UI, upper index; LB, lower boundary; DV, default value; UB, upper boundary. Results indicating no significant deviation in behaviour are marked with a period.

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
CNSCell_apoptosisSDAThreshold	14.7	14.7	.	4.265(+)	5	.	1
TCell_proliferationMean	15.59	.	15.59	.	19.2	22.19(+)	2
CNSMacrophage_sdaSecretedPerHourWhenStimulated	16.26	.	16.26	.	100	116.3(+)	3
CNS_height	17.45	17.45	.	41.27(+)	50	.	4
Th1Polarization_mhcUnExpressionDelayMean	17.57	17.57	.	6.595(+)	8	.	5
Molecule_molecularHalflife	25.29	.	25.29	.	0.5	0.6265(+)	6
APC_timeOfDeathMean	26.88	26.88	.	80.43(+)	110	.	7
TCell_becomeEffectorMean	29.03	29.03	48.65	42.58(+)	60	89.19(+)	8
Circulation_height	29.27	.	29.27	.	50	64.63(+)	9
TCell_cellsPerGridspace	37.56	37.56	.	4.371(+)	7	.	10
Simulation_numCNSMacrophage	42.02	.	42.02	.	75	106.5(+)	11
Simulation_immuneLinearDC0	43.09	43.09	.	1.138(+)	2	.	12
CLN_height	45.61	.	45.61	.	50	72.81(+)	13
APC_probabilityPhagocytosisToPeptide	49.54	49.54	.	0.01009(+)	0.02	.	14
Simulation_numCD8Treg	53.27	53.27	.	14.02(+)	30	.	15
Simulation_immuneLinearGradient	54.15	.	54.15	.	-0.005	-0.002292(+)	16
APC_immatureDurationMean	57.96	57.96	.	20.18(+)	48	.	17
CD8Treg_cd8TregToCD4ThelperSpecificityDropOff	72.78	72.78	.	0.2722(+)	1	.	18
Simulation_numCD4Th	79.81	79.81	.	8.075(+)	40	.	19
Simulation_numDCSpleen	82.67	82.67	.	17.33(+)	100	.	20
Molecule_decayThreshold	87.05	87.05	.	0.001295(+)	0.01	.	21
Simulation_numCD4Treg	91.56	91.56	375.3	2.532(+)	30	142.6(+)	22
CNSMacrophage_type1RequiredForActivation	96.71	96.71	.	0.08234(+)	2.5	.	23
Th2Polarization_type2SecretedPerHourWhenActivated	97.59	97.59	.	2.41(+)	100	.	24
TCell_AICDMean	99.74	.	99.74	.	60	119.8(+)	25
Simulation_numDCCNS	112.1	.	112.1	.	40	84.84(+)	26
Circulation_width	126.5	.	126.5	.	50	113.3(+)	27
Spleen_height	140.7	.	140.7	.	50	120.3(+)	28

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Table 17 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
<i>SLO_height</i>	160.6	.	160.6	.	50	130.3(+)	29
<i>Th1Polarization_type1SecretedPerHourWhenActivated</i>	477.1	.	477.1	.	100	577.1(+)	30
<i>DendriticCell_cytokineType2PolarizationRatio</i>	484.5	.	484.5	.	0.17	0.9936(+)	31
<i>CD4THelper_diff00</i>	1060	.	1060	.	0.05	0.5798(+)	32
<i>CNS_width</i>	.	.	.	.	50	.	72
<i>Spleen_width</i>	.	.	.	.	50	.	72
<i>Simulation_immuneActivationLinearFreq</i>	.	.	.	.	6	.	72
<i>SLO_width</i>	.	.	.	.	50	.	72
<i>Simulation_numCNS</i>	.	.	.	.	500	.	72
<i>TCell_timeLocalActivationInducedEffectorFunctionFor</i>	.	.	.	.	48	.	72
<i>TCell_timeLocalActivationDelay</i>	.	.	.	.	10	.	72
<i>TCell_specificityLowerLimit</i>	.	.	.	.	0.5	.	72
<i>TCell_apoptosisNaiveMean</i>	.	.	.	.	30	.	72
<i>Circulation_timeToCrossOrgan</i>	.	.	.	.	5	.	72
<i>TCell_proliferationStdDev</i>	.	.	.	.	9.6	.	72
<i>DendriticCell_type1SecretedPerHourImmunized</i>	.	.	.	.	10	.	72
<i>CD4THelper_diff08</i>	.	.	.	.	0.85	.	72
<i>DendriticCell_phagocytosisProbabilityImmature</i>	.	.	.	.	1	.	72
<i>DendriticCell_type1RequiredForActivation</i>	.	.	.	.	2	.	72
<i>Th2Polarization_proliferationMean</i>	.	.	.	.	28.8	.	72
<i>TCell_specificityUpperLimit</i>	.	.	.	.	0.9	.	72
<i>CLN_width</i>	.	.	.	.	50	.	72
<i>CNSMacrophage_basalMBPExpressionProbability</i>	.	.	.	.	0.2	.	72
<i>DendriticCell_phagocytosisProbabilityMature</i>	.	.	.	.	0.3	.	72
<i>Simulation_numDC</i>	.	.	.	.	10	.	72
<i>TCell_becomeEffectorStdDev</i>	.	.	.	.	56	.	72
<i>CNSMacrophage_phagocytosisProbabilityMature</i>	.	.	.	.	0.3	.	72
<i>CNSMacrophage_phagocytosisProbabilityImmature</i>	.	.	.	.	0.7	.	72
<i>Simulation_immuneActivationLinearInitial</i>	.	.	.	.	14	.	72
<i>APC_immatureDurationStdDev</i>	.	.	.	.	24	.	72
<i>APC_timeOfDeathStdDev</i>	.	.	.	.	48	.	72
<i>CD4Treg_type1SecretedPerHourWhenActivated</i>	.	.	.	.	10	.	72
<i>CD8Treg_type1SecretedPerHourWhenActivated</i>	.	.	.	.	10	.	72
<i>CLN_timeToCrossOrgan</i>	.	.	.	.	12	.	72
<i>CNS_timeToCrossOrgan</i>	.	.	.	.	20	.	72
<i>DendriticCellMigrates_lengthOfTimeMovingFollowingMigration</i>	.	.	.	.	3.5	.	72
<i>SLO_timeToCrossOrgan</i>	.	.	.	.	12	.	72
<i>Spleen_timeToCrossOrgan</i>	.	.	.	.	5	.	72
<i>TCell_AICDStdDev</i>	.	.	.	.	56	.	72
<i>TCell_apoptosisNaiveStdDev</i>	.	.	.	.	17	.	72
<i>TCell_apoptosisPartialMaturityMean</i>	.	.	.	.	12	.	72
<i>TCell_apoptosisPartialMaturityStdDev</i>	.	.	.	.	6	.	72

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Table 17 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
Th1Polarization_mhcUnExpressionDelayStdDev	.	.	.	.	2	.	72
Th2Polarization_proliferationStdDev	.	.	.	.	19.2	.	72

Table 18: Robustness indexes for parameters with respect to the *Max EAE* response. RI, robustness index; LI, lower index; UI, upper index; LB, lower boundary; DV, default value; UB, upper boundary. Results indicating no significant deviation in behaviour are marked with a period.

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
CNSCell_apoptosisSDAThreshold	1.361	1.361	1.597	4.932(+)	5	5.08(–)	1
CNSMacrophage_sdaSecretedPerHourWhenStimulated	1.412	1.846	1.412	98.15(–)	100	101.4(+)	2
Molecule_molecularHalflife	2.022	2.736	2.022	0.4863(–)	0.5	0.5101(+)	3
Simulation_numCNSMacrophage	3.081	3.741	3.081	72.19(–)	75	77.31(+)	4
CNS_width	3.247	3.247	4.32	48.38(+)	50	52.16(–)	5
CNS_height	3.306	3.306	3.478	48.35(+)	50	51.74(–)	6
Simulation_numCNS	7.553	7.553	8.636	462.2(–)	500	543.2(+)	7
TCell_AICDMean	10.61	10.61	12.25	53.63(–)	60	67.35(+)	8
APC.timeOfDeathMean	11.87	11.87	14.68	96.95(–)	110	126.1(+)	9
Simulation_immuneActivationLinearDC0	15.9	15.99	15.9	1.68(–)	2	2.318(+)	10
CD4THelper_diff08	16.93	16.93	.	0.7061(–)	0.85	.	11
TCell_proliferationMean	19.17	.	19.17	.	19.2	22.88(–)	12
Simulation_immuneActivationLinearFreq	20.72	20.72	36.19	4.757(+)	6	8.171(–)	13
TCell_timeLocalActivationInducedEffectorFunctionFor	23.26	23.26	107.8	36.84(–)	48	99.73(+)	14
TCell_cellsPerGridspace	24.78	24.78	.	5.265(–)	7	.	15
Th1Polarization_type1SecretedPerHourWhenActivated	27.92	27.92	58.19	72.08(–)	100	158.2(+)	16
CNSMacrophage_phagocytosisProbabilityMature	28.15	28.15	32.94	0.2155(–)	0.3	0.3988(+)	17
TCell_becomeEffectorMean	29.29	29.29	59.28	42.43(–)	60	95.57(–)	18
Simulation_immuneActivationLinearGradient	34.83	34.83	41.15	-0.006741(–)	-0.005	-0.002943(+)	19
CNSMacrophage_type1RequiredForActivation	36.37	42.72	36.37	1.432(+)	2.5	3.409(–)	20
TCell_AICDStdDev	37.63	37.63	.	34.93(–)	56	.	21
Circulation_height	51.3	.	51.3	.	50	75.65(–)	22
CNSMacrophage_phagocytosisProbabilityImmature	52.72	52.72	.	0.331(–)	0.7	.	23
Simulation_numCD4Th	64	64	252	14.4(–)	40	140.8(+)	24
TCell_apoptosisNaiveMean	68.47	68.47	.	9.458(–)	30	.	25
Circulation_timeToCrossOrgan	70.98	.	70.98	.	5	8.549(–)	26
Simulation_numDCCNS	71.18	71.18	75.32	11.53(–)	40	70.13(+)	27
CNSMacrophage_basalMBPExpressionProbability	83.49	83.49	244.6	0.03301(–)	0.2	0.6891(+)	28
DendriticCell_type1SecretedPerHourImmunized	93.3	93.3	594.6	0.6696(–)	10	69.46(+)	29
DendriticCell_phagocytosisProbabilityImmature	93.67	93.67	.	0.06329(–)	1	.	30
TCell_timeLocalActivationDelay	96.19	96.19	273.9	0.381(–)	10	37.39(–)	31
SLO_width	130.9	.	130.9	.	50	115.4(–)	32

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Table 18 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
SLO_height	141.2	.	141.2	.	50	120.6(–)	33
Molecule_decayThreshold	1283	.	1283	.	0.01	0.1383(–)	34
Simulation_numCD4Treg	.	.	.	.	30	.	72
Simulation_numCD8Treg	.	.	.	.	30	.	72
Th1Polarization_mhcUnExpressionDelayMean	.	.	.	.	8	.	72
CLN_height	.	.	.	.	50	.	72
APC_probabilityPhagocytosisToPeptide	.	.	.	.	0.02	.	72
APC_immatureDurationMean	.	.	.	.	48	.	72
CD8Treg_cd8TregToCD4THelperSpecificityDropOff	.	.	.	.	1	.	72
Simulation_numDCSpleen	.	.	.	.	100	.	72
Th2Polarization_type2SecretedPerHourWhenActivated	.	.	.	.	100	.	72
Circulation_width	.	.	.	.	50	.	72
Spleen_height	.	.	.	.	50	.	72
DendriticCell_cytokineType2PolarizationRatio	.	.	.	.	0.17	.	72
CD4THelper_diff00	.	.	.	.	0.05	.	72
Spleen_width	.	.	.	.	50	.	72
TCell_specificityLowerLimit	.	.	.	.	0.5	.	72
TCell_proliferationStdDev	.	.	.	.	9.6	.	72
DendriticCell_type1RequiredForActivation	.	.	.	.	2	.	72
Th2Polarization_proliferationMean	.	.	.	.	28.8	.	72
TCell_specificityUpperLimit	.	.	.	.	0.9	.	72
CLN_width	.	.	.	.	50	.	72
DendriticCell_phagocytosisProbabilityMature	.	.	.	.	0.3	.	72
Simulation_numDC	.	.	.	.	10	.	72
TCell_becomeEffectorStdDev	.	.	.	.	56	.	72
Simulation_immuneActivationLinearInitial	.	.	.	.	14	.	72
APC_immatureDurationStdDev	.	.	.	.	24	.	72
APC_timeOfDeathStdDev	.	.	.	.	48	.	72
CD4Treg_type1SecretedPerHourWhenActivated	.	.	.	.	10	.	72
CD8Treg_type1SecretedPerHourWhenActivated	.	.	.	.	10	.	72
CLN_timeToCrossOrgan	.	.	.	.	12	.	72
CNS_timeToCrossOrgan	.	.	.	.	20	.	72
DendriticCellMigrates_lengthOfTimeMovingFollowingMigration	.	.	.	.	3.5	.	72
SLO_timeToCrossOrgan	.	.	.	.	12	.	72
Spleen_timeToCrossOrgan	.	.	.	.	5	.	72
TCell_apoptosisNaiveStdDev	.	.	.	.	17	.	72
TCell_apoptosisPartialMaturityMean	.	.	.	.	12	.	72
TCell_apoptosisPartialMaturityStdDev	.	.	.	.	6	.	72
Th1Polarization_mhcUnExpressionDelayStdDev	.	.	.	.	2	.	72
Th2Polarization_proliferationStdDev	.	.	.	.	19.2	.	72

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Table 19: Robustness indexes for parameters with respect to the *EAE at 40d* response. RI, robustness index; LI, lower index; UI, upper index; LB, lower boundary; DV, default value; UB, upper boundary. Results indicating no significant deviation in behaviour are marked with a period.

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
CNSMacrophage_sdaSecretedPerHourWhenStimulated	0.8865	.	0.8865	.	100	100.9(+)	1
CNSCell_apoptosisSDAThreshold	0.9662	0.9662	.	4.952(+)	5	.	2
Molecule_molecularHalflife	1.227	.	1.227	.	0.5	0.5061(+)	3
Simulation_numCNSMacrophage	1.714	.	1.714	.	75	76.29(+)	4
CNS_height	1.822	1.822	.	49.09(+)	50	.	5
CNS_width	1.874	1.874	.	49.06(+)	50	.	6
Simulation_numCNS	5.405	.	5.405	.	500	527(+)	7
TCell_AICDMean	10.26	.	10.26	.	60	66.15(+)	8
APC_timeOfDeathMean	11.76	.	11.76	.	110	122.9(+)	9
Simulation_immuneActivationLinearDC0	14.13	.	14.13	.	2	2.283(+)	10
Simulation_immuneActivationLinearFreq	16.67	16.67	.	5(+)	6	.	11
CNSMacrophage_phagocytosisProbabilityMature	25.25	.	25.25	.	0.3	0.3758(+)	12
TCell_timeLocalActivationInducedEffectorFunctionFor	31.35	.	31.35	.	48	63.05(+)	13
CNSMacrophage_type1RequiredForActivation	35.39	35.39	.	1.615(+)	2.5	.	14
Simulation_immuneActivationLinearGradient	37.75	.	37.75	.	-0.005	-0.003113(+)	15
Th1Polarization_type1SecretedPerHourWhenActivated	48.21	.	48.21	.	100	148.2(+)	16
Simulation_numDCCNS	55.11	.	55.11	.	40	62.05(+)	17
Simulation_numCD8Treg	97.46	97.46	.	0.7634(+)	30	.	18
Th1Polarization_mhcUnExpressionDelayMean	97.7	97.7	.	0.1842(+)	8	.	19
Simulation_numCD4Treg	97.94	97.94	.	0.6187(+)	30	.	20
CD8Treg_cd8TregToCD4ThelperSpecificityDropOff	99.82	99.82	.	0.001759(+)	1	.	21
Simulation_numCD4Th	243.8	.	243.8	.	40	137.5(+)	22
CNSMacrophage_basalMBPExpressionProbability	285.7	.	285.7	.	0.2	0.7714(+)	23
DendriticCell_type1SecretedPerHourImmunized	604.8	.	604.8	.	10	70.48(+)	24
CD4THelper_diff08	.	.	.	.	0.85	.	72
TCell_proliferationMean	.	.	.	.	19.2	.	72
TCell_cellsPerGridspace	.	.	.	.	7	.	72
TCell_becomeEffectorMean	.	.	.	.	60	.	72
TCell_AICDStdDev	.	.	.	.	56	.	72
Circulation_height	.	.	.	.	50	.	72
CNSMacrophage_phagocytosisProbabilityImmature	.	.	.	.	0.7	.	72
TCell_apoptosisNaiveMean	.	.	.	.	30	.	72
Circulation_timeToCrossOrgan	.	.	.	.	5	.	72
DendriticCell_phagocytosisProbabilityImmature	.	.	.	.	1	.	72
TCell_timeLocalActivationDelay	.	.	.	.	10	.	72
SLO_width	.	.	.	.	50	.	72
SLO_height	.	.	.	.	50	.	72
Molecule_decayThreshold	.	.	.	.	0.01	.	72
CLN_height	.	.	.	.	50	.	72

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Table 19 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
APC_probabilityPhagocytosisToPeptide	.	.	.	.	0.02	.	72
APC_immatureDurationMean	.	.	.	.	48	.	72
Simulation_numDCSpleen	.	.	.	.	100	.	72
Th2Polarization_type2SecretedPerHourWhenActivated	.	.	.	.	100	.	72
Circulation_width	.	.	.	.	50	.	72
Spleen_height	.	.	.	.	50	.	72
DendriticCell_cytokineType2PolarizationRatio	.	.	.	.	0.17	.	72
CD4THelper_diff00	.	.	.	.	0.05	.	72
Spleen_width	.	.	.	.	50	.	72
TCell_specificityLowerLimit	.	.	.	.	0.5	.	72
TCell_proliferationStdDev	.	.	.	.	9.6	.	72
DendriticCell_type1RequiredForActivation	.	.	.	.	2	.	72
Th2Polarization_proliferationMean	.	.	.	.	28.8	.	72
TCell_specificityUpperLimit	.	.	.	.	0.9	.	72
CLN_width	.	.	.	.	50	.	72
DendriticCell_phagocytosisProbabilityMature	.	.	.	.	0.3	.	72
Simulation_numDC	.	.	.	.	10	.	72
TCell_becomeEffectorStdDev	.	.	.	.	56	.	72
Simulation_immuneActivationLinearInitial	.	.	.	.	14	.	72
APC_immatureDurationStdDev	.	.	.	.	24	.	72
APC_timeOfDeathStdDev	.	.	.	.	48	.	72
CD4Treg_type1SecretedPerHourWhenActivated	.	.	.	.	10	.	72
CD8Treg_type1SecretedPerHourWhenActivated	.	.	.	.	10	.	72
CLN_timeToCrossOrgan	.	.	.	.	12	.	72
CNS_timeToCrossOrgan	.	.	.	.	20	.	72
DendriticCellMigrates_lengthOfTimeMovingFollowingMigration	.	.	.	.	3.5	.	72
SLO_timeToCrossOrgan	.	.	.	.	12	.	72
Spleen_timeToCrossOrgan	.	.	.	.	5	.	72
TCell_apoptosisNaiveStdDev	.	.	.	.	17	.	72
TCell_apoptosisPartialMaturityMean	.	.	.	.	12	.	72
TCell_apoptosisPartialMaturityStdDev	.	.	.	.	6	.	72
Th1Polarization_mhcUnExpressionDelayStdDev	.	.	.	.	2	.	72
Th2Polarization_proliferationStdDev	.	.	.	.	19.2	.	72

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Table 20: Robustness indexes for parameters with respect to the *Max EAE A Test* response. RI, robustness index; LI, lower index; UI, upper index; LB, lower boundary; DV, default value; UB, upper boundary. Results indicating no significant deviation in behaviour are marked with a period.

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
CNSCell_apoptosisSDAThreshold	1.452	1.452	1.472	4.927(+)	5	5.074(-)	1

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Table 20 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
CNSMacrophage_sdaSecretedPerHourWhenStimulated	1.549	1.604	1.549	98.4(–)	100	101.5(+)	2
Molecule_molecularHalflife	2.22	2.352	2.22	0.4882(–)	0.5	0.5111(+)	3
Simulation_numCNSMacrophage	3.411	3.456	3.411	72.41(–)	75	77.56(+)	4
CNS_height	3.664	3.827	3.664	48.09(+)	50	51.83(–)	5
CNS_width	3.896	3.896	4.093	48.05(+)	50	52.05(–)	6
Simulation_numCNS	6.915	6.915	8.834	465.4(–)	500	544.2(+)	7
TCell_AICDMean	9.797	9.797	13.17	54.12(–)	60	67.9(+)	8
APC.timeOfDeathMean	11.79	11.79	16.35	97.03(–)	110	128(+)	9
Simulation_immuneActivationLinearDC0	13.94	13.94	17.24	1.721(–)	2	2.345(+)	10
CD4THelper_diff08	16.58	16.58	.	0.7091(–)	0.85	.	11
TCell_proliferationMean	18.24	.	18.24	.	19.2	22.7(–)	12
TCell_cellsPerGridspace	22.03	22.03	.	5.458(–)	7	.	13
TCell_timeLocalActivationInducedEffectorFunctionFor	22.52	22.52	98.96	37.19(–)	48	95.5(+)	14
Simulation_immuneActivationLinearFreq	23.16	23.16	35.75	4.611(+)	6	8.145(–)	15
CNSMacrophage_phagocytosisProbabilityMature	24.24	24.24	34.52	0.2273(–)	0.3	0.4036(+)	16
Th1Polarization_type1SecretedPerHourWhenActivated	26.07	26.07	61.19	73.93(–)	100	161.2(+)	17
TCell_becomeEffectorMean	28.88	28.88	57.06	42.67(–)	60	94.24(–)	18
TCell_AICDStdDev	31.36	31.36	.	38.44(–)	56	.	19
Simulation_immuneActivationLinearGradient	33.83	33.83	42.12	-0.006691(–)	-0.005	-0.002894(+)	20
CNSMacrophage_type1RequiredForActivation	34.7	42.87	34.7	1.428(+)	2.5	3.367(–)	21
Circulation_height	46.44	.	46.44	.	50	73.22(–)	22
CNSMacrophage_phagocytosisProbabilityImmature	46.66	46.66	.	0.3734(–)	0.7	.	23
Simulation_numCD4Th	62.24	62.24	267.2	15.1(–)	40	146.9(+)	24
Simulation_numDCCNS	65.01	65.01	80.51	14(–)	40	72.21(+)	25
TCell_apoptosisNaiveMean	65.05	65.05	.	10.48(–)	30	.	26
Circulation_timeToCrossOrgan	69.59	.	69.59	.	5	8.48(–)	27
CNSMacrophage_basalMBPExpressionProbability	83.73	83.73	264.4	0.03254(–)	0.2	0.7289(+)	28
DendriticCell_phagocytosisProbabilityImmature	92.48	92.48	.	0.07523(–)	1	.	29
DendriticCell_type1SecretedPerHourImmunized	93.41	93.41	620.2	0.6591(–)	10	72.02(+)	30
TCell_timeLocalActivationDelay	93.46	93.46	265.5	0.6538(–)	10	36.55(–)	31
SLO_width	119.5	.	119.5	.	50	109.7(–)	32
SLO_height	140	.	140	.	50	120(–)	33
Molecule_decayThreshold	1317	.	1317	.	0.01	0.1417(–)	34
Th1Polarization_mhcUnExpressionDelayMean	.	.	.	.	8	.	72
CLN_height	.	.	.	.	50	.	72
APC_probabilityPhagocytosisToPeptide	.	.	.	.	0.02	.	72
Simulation_numCD8Treg	.	.	.	.	30	.	72
APC_immatureDurationMean	.	.	.	.	48	.	72
CD8Treg_cd8TregToCD4ThelperSpecificityDropOff	.	.	.	.	1	.	72
Simulation_numDCSpleen	.	.	.	.	100	.	72
Simulation_numCD4Treg	.	.	.	.	30	.	72
Th2Polarization_type2SecretedPerHourWhenActivated	.	.	.	.	100	.	72

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Table 20 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
Circulation_width	.	.	.	.	50	.	72
Spleen_height	.	.	.	.	50	.	72
DendriticCell_cytokineType2PolarizationRatio	.	.	.	.	0.17	.	72
CD4THelper_diff00	.	.	.	.	0.05	.	72
Spleen_width	.	.	.	.	50	.	72
TCell_specificityLowerLimit	.	.	.	.	0.5	.	72
TCell_proliferationStdDev	.	.	.	.	9.6	.	72
DendriticCell_type1RequiredForActivation	.	.	.	.	2	.	72
Th2Polarization_proliferationMean	.	.	.	.	28.8	.	72
TCell_specificityUpperLimit	.	.	.	.	0.9	.	72
CLN_width	.	.	.	.	50	.	72
DendriticCell_phagocytosisProbabilityMature	.	.	.	.	0.3	.	72
Simulation_numDC	.	.	.	.	10	.	72
TCell_becomeEffectorStdDev	.	.	.	.	56	.	72
Simulation_immuneActivationLinearInitial	.	.	.	.	14	.	72
APC_immatureDurationStdDev	.	.	.	.	24	.	72
APC_timeOfDeathStdDev	.	.	.	.	48	.	72
CD4Treg_type1SecretedPerHourWhenActivated	.	.	.	.	10	.	72
CD8Treg_type1SecretedPerHourWhenActivated	.	.	.	.	10	.	72
CLN_timeToCrossOrgan	.	.	.	.	12	.	72
CNS_timeToCrossOrgan	.	.	.	.	20	.	72
DendriticCellMigrates_lengthOfTimeMovingFollowingMigration	.	.	.	.	3.5	.	72
SLO_timeToCrossOrgan	.	.	.	.	12	.	72
Spleen_timeToCrossOrgan	.	.	.	.	5	.	72
TCell_apoptosisNaiveStdDev	.	.	.	.	17	.	72
TCell_apoptosisPartialMaturityMean	.	.	.	.	12	.	72
TCell_apoptosisPartialMaturityStdDev	.	.	.	.	6	.	72
Th1Polarization_mhcUnExpressionDelayStdDev	.	.	.	.	2	.	72
Th2Polarization_proliferationStdDev	.	.	.	.	19.2	.	72

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Table 21: Robustness indexes for parameters with respect to the *EAE at 40d A Test* response. RI, robustness index; LI, lower index; UI, upper index; LB, lower boundary; DV, default value; UB, upper boundary. Results indicating no significant deviation in behaviour are marked with a period.

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
CNSMacrophage_sdaSecretedPerHourWhenStimulated	1.862	.	1.862	.	100	101.9(+)	1
CNSCell_apoptosisSDAThreshold	2.041	2.041	.	4.898(+)	5	.	2
Molecule_molecularHalflife	2.577	.	2.577	.	0.5	0.5129(+)	3
Simulation_numCNSMacrophage	3.599	.	3.599	.	75	77.7(+)	4

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Table 21 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
CNS.height	3.827	3.827	.	48.09(+)	50	.	5
CNS.width	3.934	3.934	.	48.03(+)	50	.	6
Simulation_numCNS	11.51	.	11.51	.	500	557.5(+)	7
TCell_AICDMean	17.71	.	17.71	.	60	70.62(+)	8
APC.timeOfDeathMean	24.68	.	24.68	.	110	137.1(+)	9
Simulation_immunizationLinearDC0	24.95	.	24.95	.	2	2.499(+)	10
Simulation_immunizationLinearFreq	33.2	33.2	.	4.008(+)	6	.	11
Simulation_immunizationLinearGradient	50.24	.	50.24	.	-0.005	0.002488(+)	12
CNSMacrophage_phagocytosisProbabilityMature	56.55	.	56.55	.	0.3	0.4696(+)	13
CNSMacrophage_type1RequiredForActivation	58.82	58.82	.	1.029(+)	2.5	.	14
Simulation_numCD4Treg	98.98	98.98	.	0.3068(+)	30	.	15
Simulation_numCD8Treg	99.01	99.01	.	0.2977(+)	30	.	16
Simulation_numDCCNS	138	.	138	.	40	95.22(+)	17
Th1Polarization_type1SecretedPerHourWhenActivated	141.3	.	141.3	.	100	241.3(+)	18
DendriticCell_type1SecretedPerHourImmunized	889.5	.	889.5	.	10	98.95(+)	19
CD4THelper_diff08	.	.	.	.	0.85	.	72
TCell_proliferationMean	.	.	.	.	19.2	.	72
TCell_cellsPerGridspace	.	.	.	.	7	.	72
TCell_timeLocalActivationInducedEffectorFunctionFor	.	.	.	.	48	.	72
TCell_becomeEffectorMean	.	.	.	.	60	.	72
TCell_AICDStdDev	.	.	.	.	56	.	72
Circulation_height	.	.	.	.	50	.	72
CNSMacrophage_phagocytosisProbabilityImmature	.	.	.	.	0.7	.	72
Simulation_numCD4Th	.	.	.	.	40	.	72
TCell_apoptosisNaiveMean	.	.	.	.	30	.	72
Circulation_timeToCrossOrgan	.	.	.	.	5	.	72
CNSMacrophage_basalMBPExpressionProbability	.	.	.	.	0.2	.	72
DendriticCell_phagocytosisProbabilityImmature	.	.	.	.	1	.	72
TCell_timeLocalActivationDelay	.	.	.	.	10	.	72
SLO_width	.	.	.	.	50	.	72
SLO_height	.	.	.	.	50	.	72
Molecule_decayThreshold	.	.	.	.	0.01	.	72
Th1Polarization_mhcUnExpressionDelayMean	.	.	.	.	8	.	72
CLN_height	.	.	.	.	50	.	72
APC_probabilityPhagocytosisToPeptide	.	.	.	.	0.02	.	72
APC_immatureDurationMean	.	.	.	.	48	.	72
CD8Treg_cd8TregToCD4ThelperSpecificityDropOff	.	.	.	.	1	.	72
Simulation_numDCSpleen	.	.	.	.	100	.	72
Th2Polarization_type2SecretedPerHourWhenActivated	.	.	.	.	100	.	72
Circulation_width	.	.	.	.	50	.	72
Spleen_height	.	.	.	.	50	.	72
DendriticCell_cytokineType2PolarizationRatio	.	.	.	.	0.17	.	72

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Table 21 – Continued

Parameter Name	RI (%)	LI (%)	UI (%)	LB	DV	UB	Rank
<i>CD4THelper_diff00</i>	.	.	.	.	0.05	.	72
<i>Spleen_width</i>	.	.	.	.	50	.	72
<i>TCell_specificityLowerLimit</i>	.	.	.	.	0.5	.	72
<i>TCell_proliferationStdDev</i>	.	.	.	.	9.6	.	72
<i>DendriticCell_type1RequiredForActivation</i>	.	.	.	.	2	.	72
<i>Th2Polarization_proliferationMean</i>	.	.	.	.	28.8	.	72
<i>TCell_specificityUpperLimit</i>	.	.	.	.	0.9	.	72
<i>CLN_width</i>	.	.	.	.	50	.	72
<i>DendriticCell_phagocytosisProbabilityMature</i>	.	.	.	.	0.3	.	72
<i>Simulation_numDC</i>	.	.	.	.	10	.	72
<i>TCell_becomeEffectorStdDev</i>	.	.	.	.	56	.	72
<i>Simulation_immuneActivationLinearInitial</i>	.	.	.	.	14	.	72
<i>APC_immatureDurationStdDev</i>	.	.	.	.	24	.	72
<i>APC_timeOfDeathStdDev</i>	.	.	.	.	48	.	72
<i>CD4Treg_type1SecretedPerHourWhenActivated</i>	.	.	.	.	10	.	72
<i>CD8Treg_type1SecretedPerHourWhenActivated</i>	.	.	.	.	10	.	72
<i>CLN_timeToCrossOrgan</i>	.	.	.	.	12	.	72
<i>CNS_timeToCrossOrgan</i>	.	.	.	.	20	.	72
<i>DendriticCellMigrates_lengthOfTimeMovingFollowingMigration</i>	.	.	.	.	3.5	.	72
<i>SLO_timeToCrossOrgan</i>	.	.	.	.	12	.	72
<i>Spleen_timeToCrossOrgan</i>	.	.	.	.	5	.	72
<i>TCell_apoptosisNaiveStdDev</i>	.	.	.	.	17	.	72
<i>TCell_apoptosisPartialMaturityMean</i>	.	.	.	.	12	.	72
<i>TCell_apoptosisPartialMaturityStdDev</i>	.	.	.	.	6	.	72
<i>Th1Polarization_mhcUnExpressionDelayStdDev</i>	.	.	.	.	2	.	72
<i>Th2Polarization_proliferationStdDev</i>	.	.	.	.	19.2	.	72

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