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EXPLORING IMMUNE MEMORY 'IN-MACHINA'

THESIS PRESENTED FOR THE DEGREE OF
MASTER OF SCIENCE (M.SC.)

SUBMITTED TO: SCHOOL OF COMPUTER SCIENCE

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January, 2001

Content

1. Abstract

The immune system protects the living body from infectious pathogens. To do that, it employs highly complex molecular mechanisms for recognition of the pathogen, initiation and regulation of an adequate response and memorization of the pathogen for possible future cases of re-infection. The ability of the immune system to produce faster and more efficient immune responses against re-infecting pathogens is called immune memory. The phenomenon and the underlying molecular principles of immune memory are constantly investigated by immunologists using both in-vivo and in-vitro experimental models. Nevertheless, many questions regarding the memory feature of the immune system still remain unsolved.

Some of these questions are addressed in this work by conducting experiments 'in-machina', i.e. within a computational model of the immune system. The model developed in this work is a cellular automaton model. It consists of the main cells and molecules of the immune system and simulates the spatial organization and local interactions that forge the immune response. The model is based on current perceptions of the basic principles of the immune system. The model was implemented in an Immune System Simulation (ISS) software, which is a general tool for conducting a broad range of immune system experiments in a highly controlled, reproducible and low-cost environment.

The simulation restores the basic features of the immune system, including development of primary and secondary immune responses against a virulent pathogen and production of immune memory. It is calibrated to reproduce experimental data regarding the real kinetics of the immune response.

The analysis of the characteristics of immune memory within the simulation system yielded some new insights on the biological immune system. It is shown that the memory phenomenon is more likely to be manifested in an 'affinity-based' immune system, i.e., a system which requires a strict recognition criteria for triggering a response, and that the development of immune memory is beneficial for regulating autoimmunity. In addition, it is shown that an efficient immune memory will develop only if the production of memory cells during the primary response exceeds a given threshold. This 'vaccination threshold' ensures a successful secondary response in every case of subsequent challenge. This threshold may have a biological equivalent that can be used to design and evaluate vaccine paradigms.

Memory cells are shown to be of significance not only for protection against re-infections. Memory cells are shown to be crucial for the success of the primary immune response against virulent pathogens, and the kinetics of the primary response is significantly effected by the amount of memory cells produced. Moreover, the simulation predicts that survival of up to 5% of activated T cells is sufficient for efficient primary and secondary responses, an observation that supports experimental findings from theoretical considerations.

Finally, the effect of the immune system recognition repertoire on the outcome of the immune response is analyzed. It is shown that repertoire changes occurring during the immune response, when the system is adapting to protect the body from the infecting pathogens, are the dominant factor in determining the outcome of the immune response, surpassing the importance of the initial lymphocyte receptor repertoire. Thus, the system demonstrates that an initial immune repertoire that is generated in a random fashion does

not affect the ability of any individual immune system to produce efficient and robust immune responses.

The results produced by the simulation system presented in this work improve our understanding of some known characteristics of the immune system. Furthermore, they predict certain behaviors that can be specifically verified using experimental paradigms. These predictions can be used to point out possible directions for future experimental research. The developed simulation system can be further enhanced to include additional components of the immune system and expand the range of questions that can be addressed with it.

More generally, this work emphasizes the contribution of mathematical and computational modeling to the study of complex biological systems such as the immune system. Interdisciplinary research, combining knowledge from both experimental and theoretical methodologies, allows a better understanding of the underlying principles of these systems.

2. Introduction

2.1 Immune System Overview

The vertebrate immune system is designed to protect the body and maintain its integrity by identifying and eliminating foreign or harmful pathogens. Among these pathogens are bacteria, viruses, fungi, different kinds of parasites, toxins, proteins and other molecules or cells from external or internal origin. Elements that can trigger an immune response are generally referred to as *antigens*. The living body has several physical and physiological defense mechanisms against foreign invaders[1-3]. The first barrier is the skin. Other barriers are epithelial cells, which separate internal systems from the outer environment, fluid and mocus flows, which eliminate foreign materials, and hydrolytic enzymes (e.g. lysozime in the tears), which neutralize bacteria and viruses. Another immunological line of defense is the *innate immune system*, which is the non-specific arm of the immune system. It consists mainly of a class of leukocytes (white blood cells) called 'phagocytes'. Phagocytes are cells that engulf and destroy extra-cellular cells and materials, clearing the system of both debris and pathogens. Examples of phagocytes are neutrophils and macrophages. Another important role of the innate immune system is to present peptide fragments of the engulfed pathogen on their cell surfaces, serving as an antigen presenting cells (APC) for the specific arm of the immune system, the *acquired immune response*. The acquired immune response is the most sophisticated and complex part of the immune system. It consists of a network of leukocytes and a variety of chemicals and molecules with complex signaling and regulation mechanisms aimed to control and optimize the immune response. The key cells of the acquired immune response are the *lymphocytes*. Lymphocytes are cells that are able to specifically recognize and respond to antigens. Antigen recognition is achieved by using a diverse set of molecules, called *antigen receptors*, which are located on the lymphocyte's surface, and are able to bind to antigenic protein fragments (*epitopes)*. Each lymphocyte carries a receptor of a single specificity and therefore binds a specific antigen. The binding between the receptor and the antigen is specific. The strength of the binding is termed *affinity*. The affinity is dependent on the chemical and spatial characteristics of the receptor and the antigenic epitope, and of various adhesion molecules located on the surfaces of lymphocytes and APCs. Antigen recognition by lymphocytes can initiate a cascade of events and interactions involving secretion of chemical molecules, cellular proliferation and differentiation that will result in elimination of the antigen and immunization of the body against future encounters with the same antigen or similar antigens. The affinity-based recognition causes the acquired immune response to be slow relatively to non-specific response mechanisms of the innate immune system, but the specificity allows the system to efficiently eliminate the foreign antigen, without causing autoimmune damage to the body's self antigens. Lymphocytes are created in the bone marrow. They are separated into two compartments, named according to their development sites: T cells, which develop within the *thymus*, and B cells, which mature in the *bursa* in birds and in *Peyer's patches* or the bone marrow in mammals. T cells are further separated into *T-Helper* cells and *T-Killer cells*, the latter also called Cytotoxic T Lymphocytes (*CTL*) cells. After their maturation, lymphocytes enter the circulation of the lymph and blood systems. Most of the lymphocytes circulate through lymphoid organs like the spleen or the lymph nodes. Lymphocytes can be also found in the blood and in peripheral tissues.

The different types of lymphocytes have different functions. B lymphocytes are responsible for the *humoral* immune response, namely antibody production against extra-

cellular antigens. CTLs are responsible for the *cellular* immune response, namely a response involving killing body cells which were infected by intra-cellular antigens. T-Helper cells, as implied by their name, help other types of lymphocytes to produce an effective response. The 'Help' signals are given and regulated through chemical binding of different membrane molecules, and through secretion of various signaling molecules named *cytokines*.

In order to handle the huge variety of possible antigens, and to avoid self-attacks, the immune system must be able to maintain and regulate a diverse repertoire of antigen receptors, adjusting it to the environmental changing conditions. Doing that, the immune system employs many processes that are interesting from a computational point of view. Some of these processes involve combinatorial genetics, optimization of effectiveness, learning , environmental adaptation and memory.
The diversity of lymphocyte receptors is generated by somatic gene rearrangements. Different parts of the receptor are encoded by sets of gene segments. During the lymphocyte's development, one member of each set of gene segments is joined randomly to the others by DNA recombination. This results in an exponential number of possible combinations and a huge diversity of the receptor structures. The initial repertoire of antigen receptors is constantly modified by two processes: the first is the constant cellular death of short-lived lymphocytes and generation of new ones. The second is the proliferation of antigen-specific lymphocyte clones occurring after the initiation of the immune response. This c*lonal expansion* narrows the total repertoire diversity until the foreign antigen is cleared. Most of the lymphocytes in these clones die, but some survive as long-lived memory cells, representing the immunological history of the body in the lymphocytes repertoire.

2.2 Immune Memory Overview

The immune system can 'remember' previously encountered antigens for a long time. This memory feature is demonstrated by the ability to produce a faster and more efficient secondary immune response against these antigens, and is the underlying principle of vaccination against diseases commonly used by modern medicine. Vaccination has successfully eradicated several human infectious diseases in the last century, while for many other important diseases there is still no effective vaccine. Therefore, new vaccination types and various routes of administration are subject to continuous research. The specific requirements for successful vaccination vary according to the nature of the infecting organism [1]. For extra-cellular organisms, antibody provide the most important defense mechanism, while for control of intra-cellular pathogens an effective CTL response is also essential. More generally, an effective vaccine must be safe (i.e. should not itself cause illness) and give a sustained protection (of few years) against the live pathogen with high success rate at the population level.

The molecular mechanisms of immunological memory are not yet fully understood [4, 5]. It is commonly believed that memory is maintained by long-lived memory cells that are generated during the primary immune response. Memory lymphocytes improve the recall response by both increasing the frequency of antigen specific cells and by employing a faster and more precise response mechanism against the antigen.

The developmental process and characteristics of B and T memory cells are subjects of constant biological research. The response dynamics of T lymphocytes includes three phases. In the initial activation and proliferation phase antigen specific cells become activated, differentiate into effector cells and expand rapidly. In the second phase, the death phase, most of the activated cells die and disappear. In the last phase, the memory

phase, a stable pool of memory cells is generated, and this pool survives for a long time [5]. It is difficult to distinguish memory T cells from activated T cells on the basis of cell surface markers. The distinct characteristics of memory T cells , their signaling pathways and activation mechanisms are not fully understood. It was experimentally shown that memory T cells that developed in one animal can be transferred to another animal and protect it against a specific antigen for a long time [6]. It was also shown that that memory T cells that were produced in the primary response exhibit cytotoxic activity in-vitro [7].

Another aspect of understanding memory T lymphocytes is elucidating the precise differentiation pathway of these cells. One view is that there is a linear differentiation process of effector T cells into memory T cells [8]. According to this model, part of the activated T cells die and others become memory cells. The factors determining whether the fate of the cell will be death or survival as a memory cell are also investigated . Antigen affinity of the T cell receptor may play a role in this decision, where cells with higher affinity survive as memory cells [9]. It is also possible that the selection is stochastic [10]. A second model for memory T cell differentiation claims that a naive (non-activated) cell 'decides' whether to become an activated/effector cell or a memory cell according to environmental factors such as the level of infection or the combination of several stimuli [11, 12]. An expansion of that view is that the differentiation decision is made a few times during the cell's life cycle : with each consequent stage of differentiation towards an effector cell the cell's potential to become a memory cell decreases [13].

B lymphocyte development during the immune response occurs in two discrete pathways [14]. The first pathway involves a rapid expansion of short-lived *plasma cells*, which secrete antibodies against the antigen. The second pathway involves expansion and maturation of long-lived memory B cells in the B-cell-rich microenvironments of lymph nodes named *germinal centers [15]*. It is not clear whether plasma and memory B cells develop from the same naive B cell ancestors, or from distinct precursors [16].

A long debate exists among immunologists regarding the ability of memory cells to survive without persisting antigen stimulation. Although there are experimental evidence for the ability of memory cells to survive in an antigen-free environment [6, 17], there are alternative views that envision long-term memory as the result of continuous stimulation by persisting antigen [18, 19].

The immune system has to retain its diversity in order to cope with a variety of different emerging antigens, while adjusting the immune repertoire to previously encountered antigens in order to allow a better recall response. Balancing coverage of antigen space with response efficiency improvement, is a non-trivial computational task for this biological system.

2.3 Immune System Models

The biological immune system has some unique features that make it appealing for mathematical modeling: it is a highly distributed system, it carries out a complex recognition and classification task, it evolves and matures using combinatorial, evolutionary and adaptation mechanisms and it is able to 'remember'.
The benefit of modeling is bi-directional: immunologists can gain insights into the principles underlying the immune system by examining them within the scope of a theoretical model, while mathematicians and computer scientists may utilize some of these principles in order to improve computational algorithms.
Immune system models can be generally separated into three groups:

1. Applied models, using principles and metaphors of the immune system for solving computational or engineering problems.
2. Continuous models, describing the dynamics of the immune system by sets of differential equations.
3. Discrete models, describing immune process as a series of interactions in discrete time steps, or utilizing combinatorial methods to predict properties of the immune system .

The following section gives a brief overview of few representative models of each group.

2.3.1 Applied Models

The underlying principles of the immune system have inspired researchers from fields other than immunology [20]. Works employing these principles can be found in computer science, engineering and even political science.

Forrest et al. have employed immune-based mechanisms for improving computer security and change-detection algorithms [21-24]. These works were inspired by the *negative selection* process occurring in the thymus during T lymphocytes development in order to eliminate self-reacting lymphocytes. For computer security purposes, a set of 'change-detectors' is constructed and tested against the protected data (bit-strings are used as detectors for static data, while series of system calls are used as detectors for Unix processes). Only detectors that cannot recognize the 'self' data survive and these are used to monitor against non-authorized changes, intrusions or computer viruses.

The idea of using the immune system metaphor to build an anti-virus protection for computer systems was commercially adopted by IBM in their anti-virus software [25, 26]. Immune network hypothesis was used by Ishida [27] and by Ishiguru [28] for fault diagnosis applications. Another use of the immune networks was made by Hunt et al. [29] for machine learning and pattern recognition applications.

2.3.2 Continuous Models

A large portion of the theoretical works in immunology utilizes systems of differential equations in order to describe the dynamics of lymphocytes and the interactions between them. The system's description may include equations and parameters for generation and death rates of lymphocytes, lymphocytes proliferation rates, transitions between resting/activated states or between naive/memory phenotypes, transitions of the response between humoral and cellular activity, antigen increase and elimination rates, etc.

Among the issues that were addressed using this approach are the maturation of the humoral immune response exhibited by B cell proliferation and differentiation using clonal selection and somatic hypermutations [30, 31], the effect of feedback in monitoring, balancing and improving the immune response [32], the role of cross-reactive stimulation in maintaining immune memory [33], the threshold ratio between T-h memory cells and antigen dose needed to establish T cell memory [34], and a thorough description of antiviral immune response during hepatitis B and influenza infections [35, 36].

A sub-class of models are based on the *idiotypic network* hypothesis, suggested by Jerne [37]. This hypothesis describes the immune system as a regulated network of molecules and cells that are able to recognize antigens as well as one another. During an immune response, a set of antibodies (Ab1) is created against the foreign antigen. Since an antibody carries protein molecules that are recognizable by the immune system, there would be production of *anti-idiotypic* antibodies (Ab2) reacting 'against' Ab1. Similarly, Ab3 antibodies would be produced against Ab2, and so forth. This network of idiotypic interactions is claimed to have a role in regulating the immune response. An example for idiotypic-network based model is the 'B model' for B-cell clonal dynamics, proposed in [38].

2.3.3 Discrete Models

One sub-class of discrete models uses probability and optimization techniques to estimate characteristics of the immune system and to predict its behavior.

Agur et al. proposed to analyze the strategy of the immune system as an optimization problem [39, 40]. The problem addressed in this work was what should be the optimal mutation rate of the B cell receptors in order to maximize the probability that the required antibody will be generated before the pathogen kills the host. Using dynamic programming methods, a globally optimal strategy of a step-function mutation rate was analytically demonstrated.

A different attitude was proposed by Pereleson et al. in a model called 'Shape Space' [41]. In this model, the immunological receptors are geometrically described as points in a multi-dimensional space. Each dimension of this space is a binding parameter like length, width, charge etc. Each receptor is able to bind to epitopes within a small 'recognition ball' surrounding its complement in the shape space. The model deals with several aspects of the immune repertoire such as how large should this repertoire be in order to be complete, and what is the probability of recognition of foreign vs. self antigens.

A second sub-class of discrete models are cellular-automata models. These models are discrete in both space and time, and the dynamics of the immune system is described by deterministic rules of the cells, molecules and their local interactions. Agur introduced the concept of conducting biological experiments 'in-machina' (i.e., within computer simulation) [42]. These type of experiments can be used to qualitatively examine immunological questions by fast, reproducible and cheap means, before planning the real *in-vitro* or *in-vivo* experiments.

The advantage of a cellular automaton model is in the direct correlation between the biological terms and the components and processes of the model. The approximations made in order to make the model simple enough to be implemented can be biologically reasoned and their influence on the reliability of the model can be estimated. When such mathematical approximations are made in order to solve differential equations models, they can obscure the relation of the results to the biological system.

Celada and Seiden described a cellular automaton model of the immune response [43-45] that includes antigen presenting cells, B and T lymphocytes, antigens and antibodies. This model was used to study affinity maturation and hypermutation of B cell, as well as virus-host competition in disease and immune states. However, immune memory related questions were not handled in these earlier works.

2.4 Research Objectives

The primary objective of the research is to explore the nature of immune memory, utilizing a large-scale cellular automaton model of the acquired immune response. The aim is to define the requirements for development of efficient immune memory and elucidate the main factors influencing it. Specifically, the research questions are defined as follows:

- What is the relation between the affinity of the lymphocyte-antigen interaction and the efficiency of the immune response.
- What is the relation between memory T cell production in the primary response and the efficiency of the secondary response.
- What is the role of memory T lymphocytes in the primary immune response.
- How does the initial immunolgical repertoire effect the efficiency of the immune response, and what is the additional contribution of the changes in the repertoire during the response.
- What model of memory T cells differentiation is better for an efficient immune response.

The efficiency of the immune response is assessed in this work mostly by measuring the speed of the response, i.e. the time from the antigen injection until its complete elimination by the immune system, and the magnitude of the antigen expansion. Additional factors like the delay from the antigen injection to the triggering of the response, to the peak of the response and to the final relaxation of the response are also measured and utilized.

All of these questions are subject to continuos immunological research, and do not have decisive biological answers. However, these questions were not handled so far in a mathematical modeling work. To explore this extensive set of questions within a computational model, the model has to be large-scaled, to include various immune system components in a modular structure, and to allow conducting controlled 'in-machina' experiments. The discrete cellular automaton model developed for this research is therefore suitable:

- It can produce a realistic quantitative description of immunological processes.
- It enables to trace the dynamics and organization of the system during experiments.
- It is highly modular, robust and easily expandable.
- The conducted experiments have a straightforward biological equivalence, and therefore their results can be used to point out possible directions for further, more complicated in-vivo or in-vitro experiments.

3. Methods

3.1 Immune System Simulation Model

3.1.1 Overview

Immune system simulations were conducted using a software package developed for this purpose. The Immune System Simulation (ISS) software is a general tool for investigating various qualitative and quantitative aspects of the immune system, its organization and dynamics. The software implements a cellular automaton model of the acquired immune response. The details of the implemented model are described below. Many of the biological assumptions in the model are based on a review by P. Matzinger [46]. Immune memory modeling is based on a review by R. Ahmed and D. Gray [5].

3.1.2 The Simulated world

The 'world' in which simulations are executed is constructed of two main parts (*Figure 1*):

- The *cell nodes grid* , which is rectangular lattice of nodes. Each *cell node* contains one tissue cell, and may contain immune entities (antigens, antibodies, dendritic cells, B lymphocytes, or T lymphocytes). A cell node has four neighboring nodes. The lattice has periodic boundaries to form a surface of a torus.
- The lymph nodes array, which is a vector of nodes, representing the lymphatic system. Each lymph node may contain immune entities. Each lymph node is connected to the next one to form a cyclic circulation.

The cell nodes grid and the lymph nodes array are connected: the cell nodes grid is divided into equal-size areas. Each lymph node is mapped and linked to such a distinct area. Immune entities can move from a cell node to the lymph node mapped to that area, and backwards. This structure enables the lymph node to be a local center for immune interactions [47].

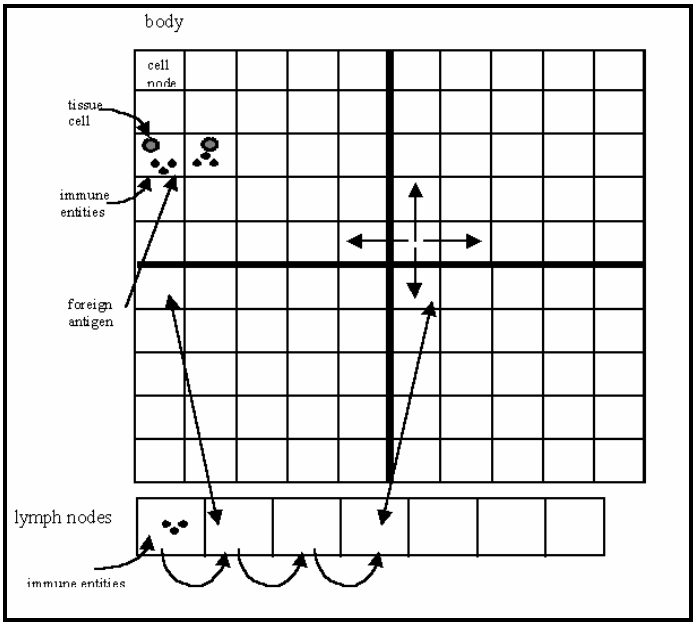

Figure 1: Simulated World

3.1.3 Simulated immune entities

The simulation is constructed of immune 'entities'. Each entity has several attributes that define its current state, and a transition function, or state machine, that determines its dynamic behavior.

The model includes implementation of the following entities:

- B lymphocytes
- T-Helper lymphocytes
- T-Killer lymphocytes (CTL)
- Dendritic cells (APC)
- Antibodies
- Antigens
- Body tissue cells

3.1.3.1 Lymphocytes

Each simulated lymphocyte (B or T) has a specific *receptor*, able to bind to antigens or other immune entities.

Lymphocytes have a *state* indicator, that may have one of four values:

- *RESTING* – the basal state in which lymphocytes are created and in which they stay in the absence of antigen stimulus.
- *BOUND* – the state in which the lymphocyte receptor is bound to an antigen with sufficient affinity.
- *ACTIVATED* – the state in which the lymphocyte performs its effector function.
- *TOLERATED* – the state in which the lymphocyte, instead of being activated, becomes tolerated by an antigen. This state is sometimes referred to as *anergy* [48].

Lymphocytes have also an indication for their *experience*: a lymphocyte can either be *VIRGIN* (naive or not yet activated), or *EXPERIENCED* (memory). Naive and memory lymphocytes in the biological immune system are distinguishable by cell surface markers and by the cytokines which they secrete[49].

3.1.3.2 Antigen Presenting Cells

Antigen Presenting Cells (APC) perform antigen capturing and antigen presentation to T lymphocytes. There are two simulated types of APCs:

- Dendritic cells, which perform non-specific capturing of both extra-cellular and intra-cellular antigens. These cells are considered to be 'professional' APC, able to present antigens to both types of T cells.
- B lymphocytes, which perform specific capturing of extra-cellular antigens only. These cells serve as APC to T-helper cells.

During antigen capturing, APCs are able to record the current level of antigen *inflammation* in their local environment.

3.1.3.3 Antibodies

Antibodies are secreted by B lymphocytes, and carry the immunological receptor of their secreting cell. Antibodies are able to eliminate encountered extra-cellular antigens. This is a simplification of the biological function of the antibody, that by binding to the antigen, enables its elimination by phagocytes.

3.1.3.4 Antigens

Antigens are the entities which may trigger an immune response. An antigen has an indication for its *origin*, which may be *SELF* (body's own antigen) or *FOREIGN* (external invader).

An antigen also carries a specific 'signature', named *peptide*, which can be identified by the immune system.

An antigen may be *intra-cellular*, i.e., penetrate tissue cells in the body like a virus, or *extra-cellular*, i.e., reside in the fluid circulation outside tissue cells, like bacteria.

3.1.3.5 Tissue cells

Tissue cells construct the simulated 'body'. A tissue cell has a *life* indicator, indicating its vitality. The life indicator may be affected by a harmful antigen. A tissue cell *state* may be *ALIVE* for a regular living cell, *NECROTIC_DEATH* for a cell which has died as a result of antigenic damage, or *APOPTOTIC_DEATH* for an infected cell which was killed by the immune system.

A tissue cell also has an *inflammation* indicator, indicating the level of antigen inflammation in its local environment. The inflammation is calculated according to the state of the cell and the states of its adjacent tissue cells. This feature of the model stands for the activity of stress-related molecules (like Heat-Shock Proteins) or intra-cellular organelles and components (like mitochondria, mannose or RNA) [50].

3.1.4 Principles of simulation

The simulation model is based on the principles of cellular automata [44, 51]: it is discrete in both space and time, and the dynamics of the system is described by deterministic rules of the immune entities and their local interactions.

In the initialization phase, immune entities (dendritic cells, T-Helper, T-Killer and B lymphocytes, self and foreign antigens, tissue cells) are created and spread over the cell and lymph nodes:

- One tissue cell is located in each cell node.
- Lymphocytes' receptors are generated by a random process.
- Peptides of antigens are generated by a random process.
- Initial locations of dendritic cells, lymphocytes and self antigens are selected randomly.

- Foreign antigens are periodically injected into specific location in the cell nodes grid.

Simulation proceeds in discrete time units. In each time unit, each immune entity performs one simulation step, according to its current state, its local environment and its logic of deterministic and stochastic transition rules. Steps of an immune entity are, for example, transition from one node to another, interaction with other entities, cloning, death, etc.

3.1.5 Simulated immune processes

3.1.5.1 Matching process

The basic process underlying every specific interaction between simulated immune entities is the *matching process*, i.e., the binding of two immunological receptors or peptides. Peptides and receptors are represented in the model by fixed-length strings over a finite alphabet. In order to decide whether binding was successful or not, a *matching rule* is applied on the two strings to calculate the matching probability. In the model, the following matching rule is used:

Let A,B be strings over finite alphabet Σ : $A=a_1a_2..a_n$, $B=b_1b_2..b_n$, $a_i,b_i \in \Sigma$

and let $s_i = \begin{cases} 1 & if\,(a_i = b_i) \\ 0 & if\,(a_i \neq b_i) \end{cases}$, $score = \sum_{i=1}^{n} s_i$

score denotes the number of matching letters of A and B.

$$\text{match\_probability(score)} = \begin{cases} 0 & score < r * M \\ \dfrac{score - r * M}{M * (1 - r)} & r * M <= score <= M \\ 1 & score > M \end{cases}$$

where integer M>0 is the maximal matching threshold, and 0<r<=1 is a constant defining the minimal matching threshold, r*M (see *Figure 2*).

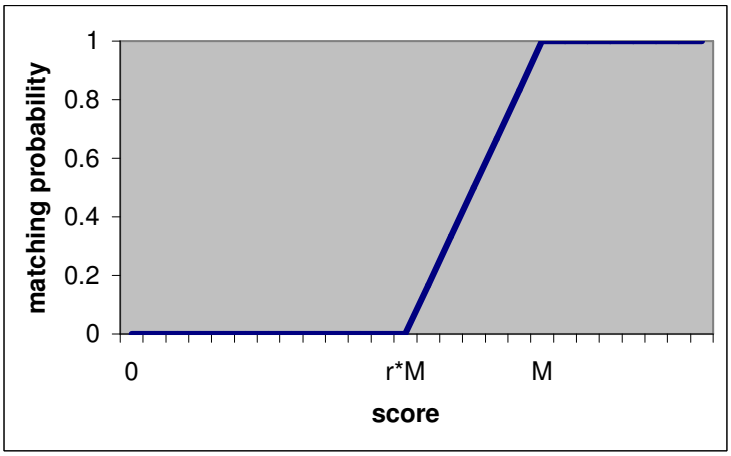

Figure 2: Matching Function

3.1.5.2 Interaction signals

Immune entities in the model communicate through direct interaction signals. There are four types of interaction signals:

- BIND signal – given when a successful binding of lymphocyte's receptor to an antigen has been accomplished. Non-activated T lymphocytes receive this signal from an APC (dendritic cell or B cell). B lymphocytes and antibodies receive this signal directly from the antigen. Activated T-Killers receive this signal from the infected tissue cell.
- CO-STIMULATION signal – given by the APC after the BIND signal, in order to activate T-Helper lymphocytes. This signal is given only if antigen inflammation level is high enough, in order to distinguish between harmful and harmless antigens [52].
- HELP signal – given by T-Helper lymphocyte in order to activate B or T-Killer lymphocytes.
- KILL signal – given in order to force an immune entity to die. T-Killer lymphocytes give this signal to infected tissue cells. Antibodies give this signal to extra-cellular antigens. All immune entity may induce their own death by this signal.

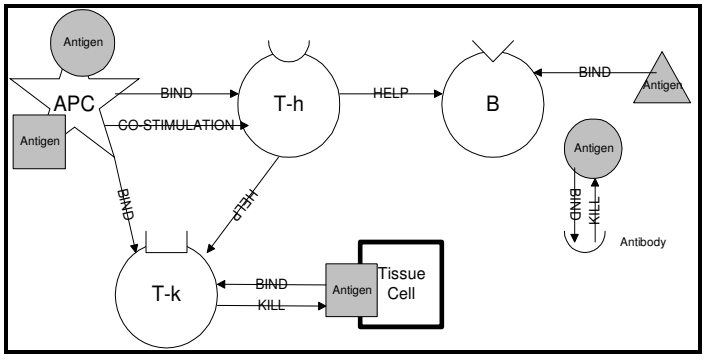

Figure 3: Interaction Signals. BIND, CO-STIMULATIN, HELP and KILL signals between APC, lymphocytes, antigens and tissue cells.

3.1.5.3 Immune response process

The immune processes simulated by the model can be separated into several distinct phases (in each phase, the biological rationale and reference is given in parenthesis):

1. Lymphocytes generation and negative selection.

   Lymphocytes are generated with random receptors. In order to simulate the maturation process of T lymphocytes T cells may go through a 'negative selection' process. The aim of negative selection is to eliminate self-reactive T lymphocytes. Negative selection is simulated by testing each newly generated T lymphocyte against the pool of self antigens. Successful binding commits the tested lymphocyte to die. The selection is not absolute: there is a 'miss' probability enabling self reactive lymphocytes to escape negative selection.
   (*The biological negative selection process occurs in the thymus. In this process self-reactive T lymphocytes that are able to bind to self antigens expressed in the thymus, are eliminated during their development phase in order to prevent possible auto-immunity. Since not all self antigens*

are expressed in the thymus, mature self reactive T cells can avoid the selection and enter the immune system circulation [53]*).*

2. Infection.

   Infection is simulated by injection of foreign antigens to a specific location in the cell nodes grid. The injected antigens act according to their activity rules – they may move around the cell nodes grid, penetrate tissue cells, clone and mutate, affect the tissue's cell vitality, and finally kill the tissue cell. The foreign antigen used in this work for most of the experiments has four phases of operation: in the first phase, it randomly moves across the cell nodes grid. Than, it penetrates a tissue cell and waits. In the next phase it starts replicating, and finally it starts decreasing the *life* indicator of the tissue cell, eventually causing the necrotic death of the cell, after which it leaves the tissue cell and starts again with the initial phase. Meanwhile, tissue cells sense the level of inflammation in their local environment – each tissue cell has an inflammation indicator. The value of this indicator is periodically updated according to the cell's state and the states of its adjacent cells. When a tissue cell undergoes an 'abnormal' death, i.e. death caused by harmful antigen, the value of the inflammation indicator is maximized. Otherwise, while the cell is alive, the inflammation indicator's value is calculated by averaging the inflammation levels of neighbor cells, divided by some diffusion factor.
   (*Natural antigens may have various 'strategies' in order to survive and breed in the body* [54]. *A tissue cell that was infected by a harmful antigen may activate chemical alarm signals that inform the innate immune system about the local inflammation* [50]).

3. Antigen capturing and presentation.

   Antigens are captured by antigen presenting cells (dendritic and B cells). A search for antigens can use a heuristic for targeted movement towards higher antigenic load. Dendritic cells are able to capture both intra-cellular and extra-cellular antigens, and capturing is non-specific, while B cells are able to capture only extra-cellular antigens, which specifically match their receptor. When an antigen is encountered, its peptide is recorded by the APC. In addition, the level of inflammation in the location of the capture is recorded. After a successful capture, the APC moves to the draining lymph node, where it may present the antigen to T lymphocytes: extra-cellular antigens are presented to T-Helper cells, and intra-cellular antigen are presented to T-Killer cells. (*Figure 4*, [46]).

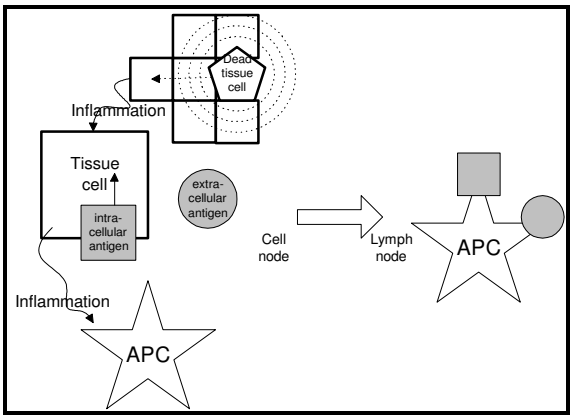

Figure 4: Antigen Capturing. Intra-cellular and extra-cellular antigens are captured by an APC, that records the inflammation level in the cell node, and moves to the draining lymph node.

4. T-Helper cells activation.

   In the lymph node, APCs activate T-Helper cells by two signals: BIND and CO-STIMULATION. When an APC encounters a T-Helper cell with a receptor matching the presented extra-cellular antigen, it gives the lymphocyte a BIND signal. When the T-Helper is bound to an antigen, the APC may give it also a CO-STIMULATION signal, causing its activation. The co-stimulation is given only if the APC's recorded level of inflammation is above a given threshold. These two signals can be given by two different APCs. Following activation, the T-Helper becomes an effector cell, able to give HELP signals to other lymphocytes. If the CO-STIMULATION signal is not given to a naive T-helper cell in conjunction with a BIND signal, it becomes tolerant (*Figure 5*, [46]).

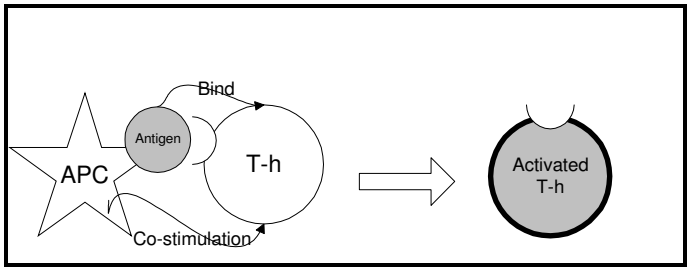

Figure 5: T-Helper Activation. T-h is activated by BIND and CO-STIMULATION signals given by an APC.

5. T-Killer cells activation.

   T-Killer cells are activated in the lymph node. Activation requires two signals: BIND and HELP. A BIND signal is supplied by an APC presenting an intra-cellular antigen to which the T-Killer's receptor matches. After the T-Killer cell is bound to the antigen, a HELP signal may be given by an activated T-Helper cell with specificity to the same antigen. After activation, the T-Killer cell performs its effector function of killing infected tissue cells. (*Figure 6, [55]).*

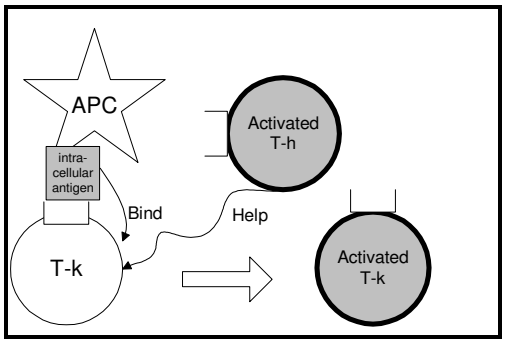

Figure 6: T-Killer Activation. T-k cell is activated by BIND signal given by an APC and HELP signal given by an activated T-h cell

6. T-Killer cells effector function.

   An effector T-Killer cell kills encoutered tissue cells that are infected by an antigen matching its receptor. Tissue cell's death induced by T-Killer lymphocyte is a programmed death (*apoptosis*), which does not cause inflammation signals. (*Figure 7*, [46])

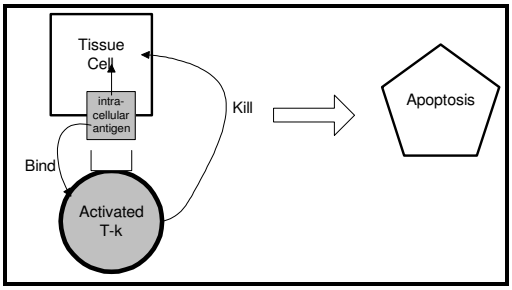

Figure 7: T-Killer Effector Function. An effector T-k cell gives a KILL signal to an infected tissue cell, causing its qpoptotics death.

7. B cells activation.

   B cell activation requires two signals: BIND and HELP. The BIND signal is given directly by the antigen, which is specifically captured by the B cell in the cell nodes grid. Following antigen capturing, the B cell moves to the draining lymph node, where it can get the HELP signal from an activated T-Helper cell with specificity to the same antigen. After activation, the B cell performs its effector function by secreting antibodies. (*Figure 8*, [46]).

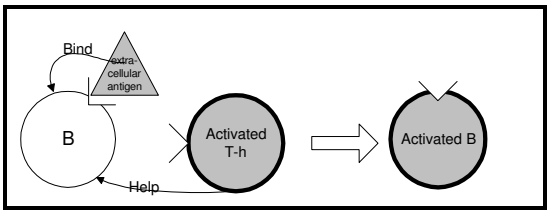

Figure 8: B Cell Activation. A B cell is activated by BIND signal given by an antigen and HELP signal given by an activated T-h cell.

8. B cells effector function.

   An effector B cell resides in the lymph node and secretes specific antibodies. The secreted antibodies move to the cell nodes grid, killing matching extra-cellular antigens. (*Figure 9)*

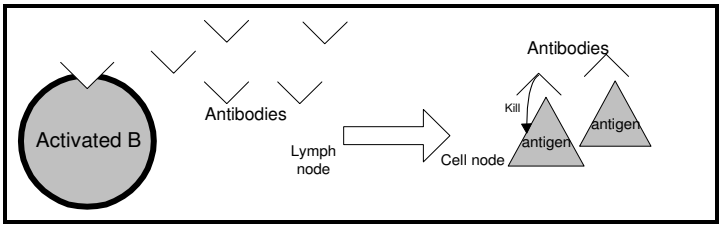

Figure 9: B Cell Effector Function. A activated B cell secretes antibodies, which kills extra-cellular antigens.

9. Cloning and life cycle.

   An activated lymphocyte is able to periodically clone copies of itself by cell division. The cloned lymphocytes are already in activated state. Cloning is augmented after successful effector interaction (e.g., T-Helper that gives a HELP signal or T-Killer that kills a tissue cell), with direct correlation to the affinity of the interaction.
   Antigens may also clone according to their activity rules.

   Every lymphocyte has a limited life span, defined by its *half-life* parameter. Each simulation time unit, the viability of each lymphocyte is determined by calculating its *life_probability* function, defined by:

   life_probabilily(t) = $e^{\frac{-\ln 2}{T_{1/2}} * t}$, where $T_{1/2}$ is the half-life parameter, and t is the age of the lymphocyte.

   A global 'homeostasis' mechanism monitors the total number of living lymphocytes, and creates new lymphocytes when natural death caused quantities to drop below the basal level.

10. Memory differentiation.

    During the immune response, a lymphocyte may differentiate into a memory cell. A memory cell has a much longer life span, and it may also have a faster activation mechanism: a resting memory T-Helper lymphocyte may be activated only by the first activation signal of antigen binding (without co-stimulation), and as a result, T-Killer and B cells activation is also faster.

    Memory differentiation decision is made according to the employed *memory model*, and to the memory *survival function*. Two major memory models are implemented: in the linear differentiation model (*Figure 10*, [8]), activated lymphocytes differentiate into memory cells or die, either by a random selection or according to some criteria, such as antigen affinity: a lymphocyte with higher average antigen affinity has higher probability to become a memory cell.

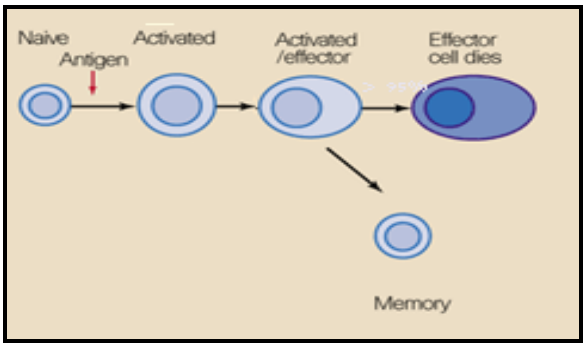

Figure 10: Linear Memory Differentiation (Taken from [5])

In the second 'decreasing potential' model (*Figure 11*, [13]), naive cells can become activated/effector cells, or memory cells, either by random selection or according to environmental factors such as level of antigen inflammation. Differentiation can occur several times during the cell's life. Increasing differentiation into an effector cell results in decreasing potential to become a memory cell and survive.

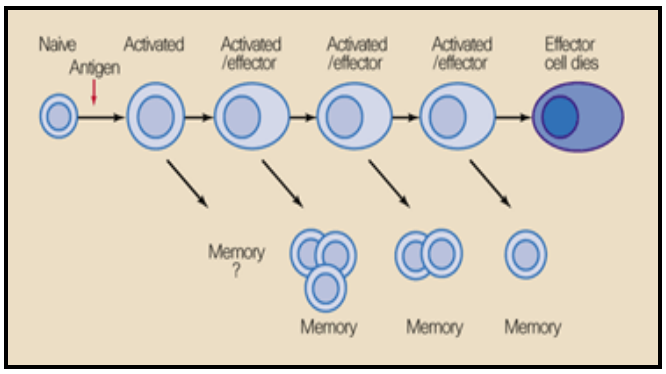

Figure 11: Decreasing Potential Differentiation (Taken from [5])

The memory survival function is used to calculate the probability of a lymphocyte to become a memory cell. When memory differentiation decision is made randomly (in either one of the memory models), the survival function is merely a uniform distribution probability function with a given expectation. When decision is made according to some heuristic criteria, the following exponential survival function is used:

$$\text{survive\_probability}(val)=\begin{cases} 0 & val < MIN \\ C^{val-MAX} & MIN \le val \le MAX \\ 1 & val > MAX \end{cases}$$

where MIN is minimal threshold constant, MAX is maximal threshold constant and C is an exponent base constant. The function's input value, val, is determined by the differentiation criteria: it may be average affinity if criteria is best affinity, antigen load if criteria is inflammation level, etc.

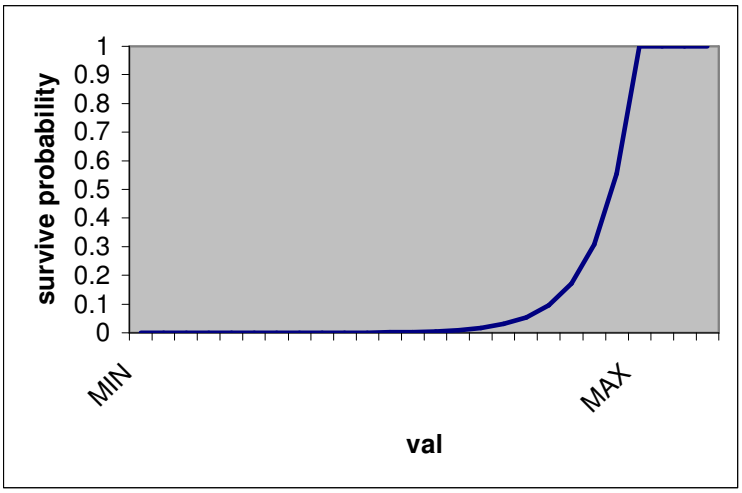

Figure 12: Memory Survival Function

3.1.5.4 Activity rules

The process of the immune response, described in the previous section was specifically implemented by sets of activity rules for each immune entity. These activity rules define the state transition of the entities . The activity rules for the different immune entities are described in detail in Appendix A.

3.1.6 Simulation parameters

Many of the model's parameters can be set per simulation. A detailed list of the parameters is presented in Appendix A.

3.1.7 Simplifying assumptions

The main simplifying assumptions underlying the model are:

- The innate immune system is simplified to perform only the antigen presentation function.
- Binding process between lymphocytes' antigen receptors and antigenic epitopes is simplified to be a string matching process.
- Complex generation and maturation processes of lymphocytes are not implemented. Thymus function is simplified to be a probabilistic elimination of self reactive T cells.
- Signaling mechanism through cytokines is not implemented.
- Functionality of antibodies is implemented only as direct antigen elimination.
- Functionality of tissue cells is implemented only as intra-cellular antigen presentation and cell damage signaling.

3.2 Experiments Execution

A *simulation* is a single execution of the simulation program with a unique set of parameters. An *experiment* is multiple executions of the same simulation, carried out with different randomly generated initial conditions. The experiment's results are obtained by averaging and analyzing the results of the repeated simulations.
In a typical experiment simulations are repeated 50 times.
Details of the output files generated by the simulation system, and the graphical tools for simulation monitoring are given in Appendix A.

Averaging the experiments results can be done in two ways:

- Calculating the average and standard deviation of the results files of all simulations. This calculations yields an average result file, containing an average of the simulations' snapshots for each time unit.
- Analyzing each result file separately, drawing the relevant data out, and averaging the analyzed data.

For most experiments, the second analysis was used. The parameters drawn out of the results file for the experiments were:

- The latency from antigen injection to the beginning of the response ($T^{Th}_{start}, T^{Tk}_{start}, T^{B}_{start}, T^{Ab}_{start}$ - time to the beginning of Th, Tk, B cells and antibodies responses).
- The latency from antigen injection to the peak of the response ($T^{Th}_{peak}, T^{Tk}_{peak}, T^{B}_{peak}, T^{Ab}_{peak}$ - time to the peak of Th, Tk, B cells and antibodies responses).
- The duration of the response from antigen injection until antigen elimination (T^{Ag}_{end}).
- The duration of the response from antigen injection until response relaxation ($T^{Th}_{end}, T^{Tk}_{end}, T^{B}_{end}, T^{Ab}_{end}$ - time to the end of Th, Tk, B cells and antibodies responses).
- The maximal amplitudes of the lymphocyte/antibodies response ($A^{Th}, A^{Tk}, A^{B}, A^{Ab}$ – amplitudes of Th, Tk, B cells and antibodies responses).
- The maximal amplitude of the antigen (A^{Ag}).

4. Results

4.1 Simulation System Verification and Calibration

4.1.1 Development of an immune response

After antigen injection, the simulated immune system develops a humoral and cellular immune response against the antigen. The response is indicated by the activation and proliferation of antigen-specific T-h lymphocytes in the local lymph nodes of the infected area. The activated T-h cells supply activation signals for T-k and B lymphocytes, which proliferate as well. T-k cells kill infected tissue cells, while B cells secrete antibodies which eliminate extra-cellular antigens. This immune response eliminates the foreign antigen and generates antigen-specific memory T cells. After antigen elimination, the immune response is gradually terminated. Subsequent injections of the same antigen result in a secondary and a tertiary immune responses which are triggered faster and eliminate the antigen earlier. A typical immune response dynamics is illustrated in *Figure 13*.

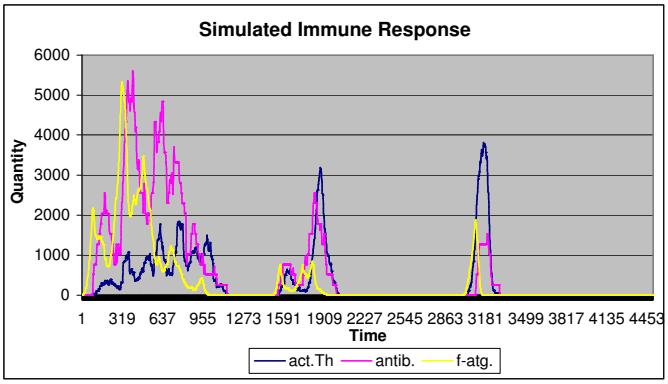

Figure 13: A typical simulated immune response. Dynamics of antigen, activated T-h lymphocytes and antibodies in a primary, secondary and tertiary immune response. A dose of 10 antigens was injected in time units 0, 1500 and 3000.

4.1.2 Development of efficient immune memory

In order to use the simulated system for studying immune memory, it is imperative to show that development of immune memory does in fact lead to an improved immune response in a consequent antigen challenge. To verify the contribution of immune memory to the improved efficiency of the secondary immune responses, antigen elimination time with and without immune memory was compared. Two experiments of 50 simulations each were conducted. In the first experiment the simulation system did not produce any memory cells. In the second experiment 2-3% of the activated T cells survived as memory cells. All other parameters were the same for both experiments. The results are presented in *Figure 14*. Without employing any immune memory mechanism there is no significant difference between the primary and the secondary responses. When memory T cells are produced during the primary response, the secondary response eliminates the antigen 20% faster. The results indicate that the simulation system reproduces the memory feature of the immune system.

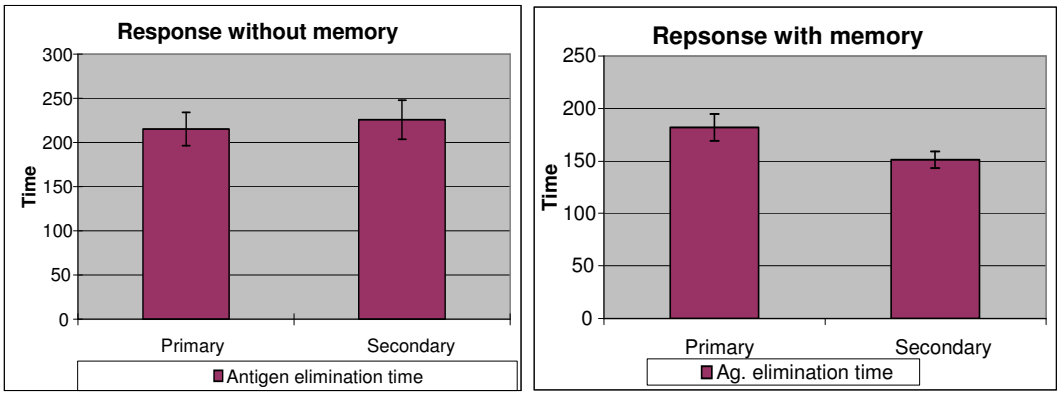

Figure 14: Primary and secondary immune responses with and without memory.
Antigen elimination time in the primary and in the secondary immune responses when memory lymphocytes are not produced (Left) and when memory T lymphocytes are produced (Right). The simulation parameters are detailed in appendix B (Table 5). The antigen was less virulent that in consequent experiments. Each bar represent an average of 50 simulations. $p < 0.05$ (right graph).

4.1.3 Time scale calibration

The primary immune response, and specifically T cell response in in-vivo systems, can be divided into three distinct phases: lymphocytes activation and expansion, which typically lasts about 7 days, lymphocytes death, which occurs between days 7 and 30, and finally generation of stable memory pool, that can persist for many years [5]. Quantitative experimental data about the time kinetics of the immune response were taken from [56], where activated CD8 T cells ('T-killer' cells) against Lymphocytic Choriomeningitis Virus (LCMV) infection were monitored *in-vivo* in mice. In these essays, viral clearance was accomplished in 8 to 10 days, and T cells dynamics in the primary response was rapid expansion during the first 8 days, followed by gradual decline in the number of antigen-specific T cells.

Similar data are described in [57] for the dynamics of T-helper cells in vivo against Pigeon Cytochrome C (PCC) antigen. This essay also compares the dynamics of primary and secondary responses, showing a faster activation phase of 3-4 days during the secondary response (*Figure 15*).

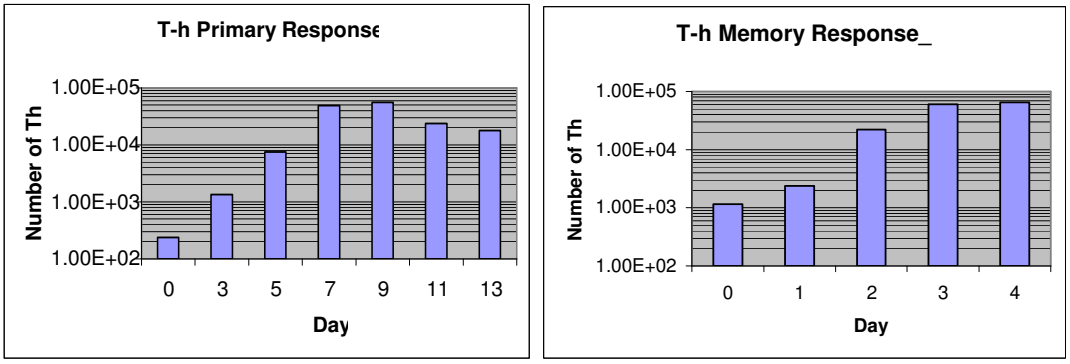

Figure 15: In-vivo data. dynamics of antigen specific Th and Tk cells in primary and secondary response (data from [57])

The above data were used as a reference for assessing the compatibility of the simulation time dynamics to the real biological system. Since simulation time is measured by discrete time units, the equivalent of a simulation time unit was calculated to be approximately 0.3 hour. Using this time scale, antigen clearance in simulated primary response was achieved in 8-9 days. The activation and proliferation phase of T cells lasted for 6-8 days, while the death phase lasted for additional 2-3 days. In the simulated secondary response, antigen

clearance was achieved in 3-4 days and the activation phase lasted for about 2-3 days. Results of the simulated dynamics of T cells response are shown in *Figure 16*.

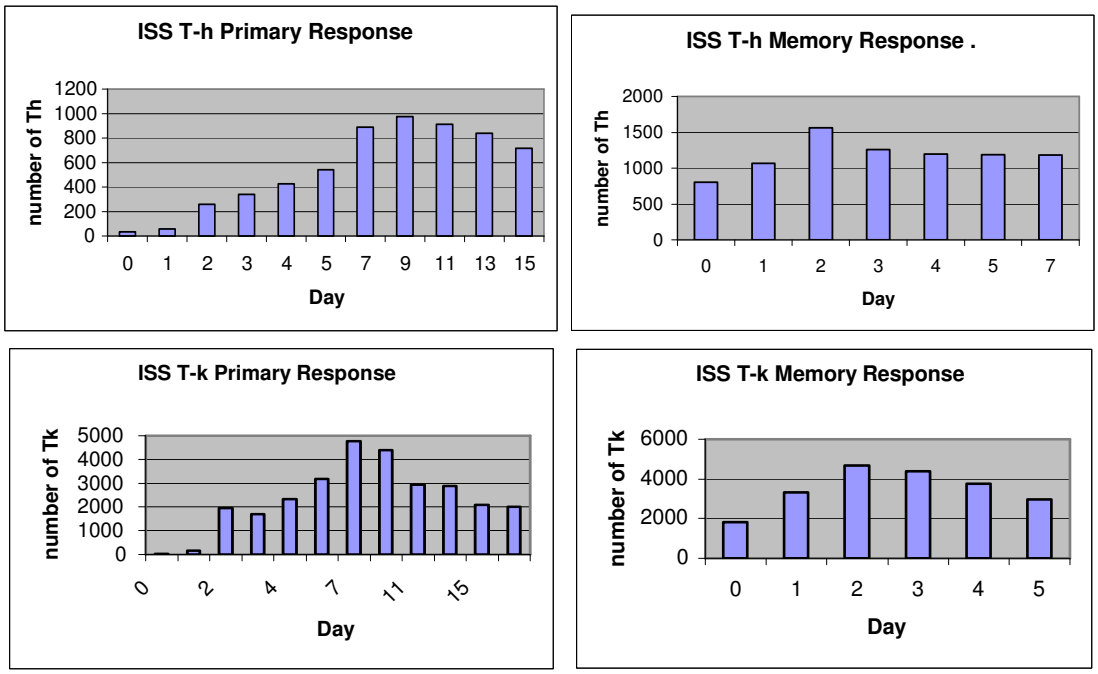

Figure 16: In-machina data. Dynamics of the simulated antigen specific Th and Tk cells in the primary and secondary responses. The time scale is 0.3 hours per simulation time unit. For antigen specific T-h cells (top figures), in the primary response, the activation and proliferation phase is 6-8 days long with expansion rate of ~50-fold. The death phase is 2-3 days long. In the secondary response, the proliferation phase last for an additional 2-3 days and the expansion rate is 4-6-fold. For antigen specific T-k cells (bottom figures) the time dynamics are similar, while expansion rates are ~420-fold during the primary response, and rise an additional 9-12-fold during the secondary response. Antigen clearance in the primary response was achieved after 8-9 days, and in the secondary response – after 3-4 days.

4.1.4 Calibration of immune entities quantities

In order to determine reasonable initial quantities of immune entities, a calibration experiment was conducted: initial quantities of T, B and dendritic cells were varied from 100 to 2000, and the latency from antigen injection to the beginning of antibody response and to antigen elimination was measured. Antigen quantity in these experiment was set to 10 antigens per injection. The results are presented in *Table 1:* Small initial quantities result in a very long response. The response is naturally shorter as the initial quantities are enlarged. The immune response produced by the initial quantity of 1000 cells is 670 time unit long, which is equal to 8.4 days. The initial quantity of T-helper cells, T-killer cells, B cells and dendritic cells was therefore set to 1000 to correspond the calibrated time scale.. This value is small compared to the real magnitude of the biological immune system – the amount of T cells in the human body is estimated to be 10^{12} cells, with estimated $25x10^6$ different receptors [58]. In mice the size of the T cell pool is about $2x10^8$ cells, and the size of the B cell pool is about $1.5x10^6$ cells [59]. The simulation system therefore simulates a small fragment of tissue.

Initial quantity	T^{Ab}_{start}	T^{Ag}_{end}
100	316.4 (48.74)	>4500
400	130.2 (21.92)	>4500
700	85.33 (14.57)	1873.33 (220.06)
1000	62.04 (3.51)	670.35 (53.81)
1500	44.7 (6.07)	282.8 (54.94)
2000	44 (5.75)	119.8 (15.42)

Table 1: Calibration of initial quantities of immune entities.
Time latencies from antigen injection to the beginning of antibody production and to the antigen elimination. Initial quantities values refer to quantities of T,B and dendritic cells. Results are the average of 10 simulation runs for each quantity, with SEM values in parenthesis. Time is given in simulation units.

4.1.5 Proliferation rates of immune entities

During the activation and proliferation phase of the immune response, there is a significant expansion of the lymphocyte population, mainly due to expansion of antigen specific lymphocytes. The extent of this proliferation is firmly dependent on the characteristics of the experimental system (type and dose of antigen, labeling and measuring methods). For the total population of T-helper cells, the reported expansion factor is 4-5-fold in the primary response and in the secondary response [9]. The reported expansion of antigen specific T-helper cells is 250-1200-fold in the primary response, and an additional 70-fold expansion occurs in the secondary response [5, 9, 57, 60]. For T-killer cells, the total expansion is reported to be 10-fold, while antigen specific cells expand 100-5000-fold in the primary response, and 5-100-fold in the secondary response [5, 56]. The expansion rates in the simulated immune system are limited by the computational capacity and are therefore smaller. However, the dynamics of clonal expansion with time, as well as ratios between the primary and secondary responses and between the total expansion and the antigen-specific expansion are kept in the simulation system. The expansion of T-helper cells in the simulation was typically 2-3-fold. Antigen specific T-helper cells expanded ~50-fold during the primary response, and 4-6-fold

during the secondary response. T-killer cells expanded faster, the total expansion being 14-15-fold, and the specific expansion being ~420-fold during the primary response, with an additional 9-12 fold expansion during the secondary response. Results of total Th and Tk expansion are graphically shown in *Figure 16*.

4.1.6 Narrowing of the immune repertoire

During the development of an immune response and the expansion of antigen-specific lymphocytes the repertoire of immunological receptors is narrowed and antigen-specific clones dominate the immune repertoire, making it less diverse and more focused on the antigenic challenge. Experimental data about clonal dominance suggest that at the peak of the primary response 50-70% of the activated T-killer cells are antigen-specific [56]. Antigen-specific T-helper cells become even more dominant, being 80% of the activated Th population during the peak of primary response, and up to 95% during the peak of the memory response [57]. The simulation system obtains similar results, with 60% specific Tk cells during primary response, growing to 80% during secondary response, and 40-65% specific Th cells during the primary and the memory response, respectively. A typical repertoire narrowing during a simulated immune response is illustrated in *Figure 17*.

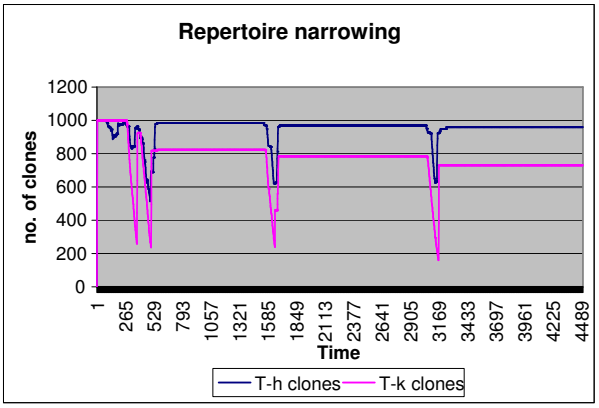

Figure 17: Repertoire narrowing of T cell clones during an immune response.
A typical dynamics of T-h and T-k clones during primary, secondary and tertiary immune response. Antigen-specific clones of T cells proliferate and dominates 40-80% of the total T cell population.

4.2 Insights on immune memory

4.2.1 Interaction affinity and response efficiency

The objective of this experiment was to understand the relation between the affinity of the lymphocyte-antigen interaction and the efficiency of the secondary response. This was achieved by varying the maximal matching threshold used for the matching function. This threshold defines how many matching bits are required for a definite match. Different matching thresholds produce matching functions with different matching probabilities.

For each matching threshold, the actual matching probability for the given peptide length and the matching rule was evaluated by simulating 10^6 matching interactions between random peptides. The resulting probabilities are listed in *Table 2*.

Max match threshold	Actual matching probability
18	0.267666
20	0.150377
22	0.075206
23	0.033477

Table 2: Relation between matching threshold and actual matching probability

The simulation parameters are given in Appendix B (*Table 5)* and the results are summarized in *Figure 18*. The matching threshold was modified from 18 to 23. With a low matching threshold of 18-20, lymphocytes are activated quickly and antigen elimination is achieved after a short time (87-97 time units). However, the secondary and tertiary responses are similar to the primary response and immune memory is not developed. With a high matching threshold of 22-23, the primary response is slower, but a significant memory effect is demonstrated: the secondary response eliminates the antigen 2-folds faster and reduces the maximal antigen amplitude by half. The tertiary responses is even more efficient with an additional 1.3-fold speedup. The interpretation of this result is that a strict matching criteria favors positive selection of high affinity lymphocytes which survive as memory cells, and ensure an improved recall responses. In addition, a strict matching rule extends the duration of the primary response to allow development of enough memory cells.

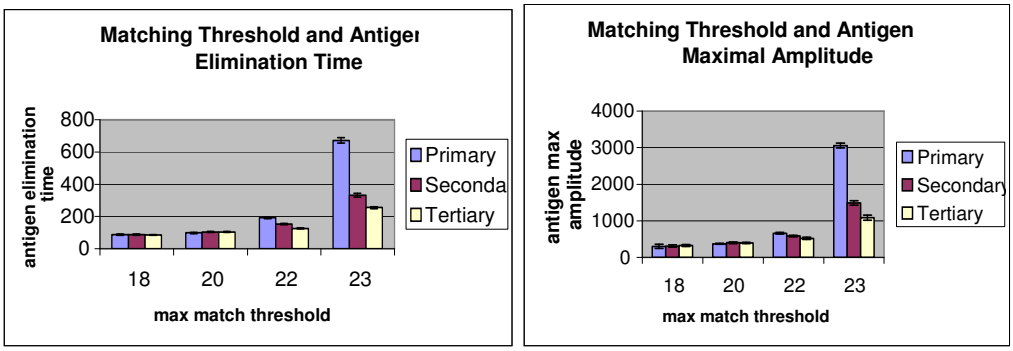

Figure 18: Matching threshold and efficiency of the immune response..
Antigen elimination time (Left) and antigen maximal amplitudes (Right) in primary, secondary and tertiary responses, using different maximal matching threshold.

Although a permissive matching rule does not allow immune memory to develop, *Figure 18* shows that it enables efficient primary, secondary and tertiary responses. In absolute

values of antigen elimination times and amplitudes the secondary and tertiary responses with a permissive matching rule are better than the corresponding responses with a strict matching rule. This apparent paradox is resolved when considering the tradeoff between fast antigen elimination and autoimmunity. During the immune response effector lymphocytes and antibodies may be targeted against the body's self antigens. This self-attack, named autoimmunity, may result from self antigens that are cross-reactive with the foreign antigen, or self antigens that are found in the inflammation area and trigger an immune response due to the inflammatory context. A permissive matching rule, in addition to fast elimination of foreign antigens, may also lead to elimination of many of the self antigens. A strict matching rule requires longer time periods to eliminate the foreign antigen, but the response is more precise, and self-damage is better regulated. This tradeoff is demonstrated in *Figure 19* - the elimination time of the foreign antigen and the overall response time are longer as the affinity requirement is more strict (left figure). On the other hand, the kinetics of autoimmunity is also slower (right figure). In *Figure* 20, the gradual degeneration of self antigens during the primary, secondary and tertiary responses is plotted against the simulation time, using different matching thresholds. It appears that immune memory is beneficial for the regulation of autoimmunity. When the matching threshold is low and no significant memory is generated, the number of self antigens declines steeply during the short time of the primary response. Both the secondary and tertiary responses cause the elimination of an additional major fraction of the remaining self antigens. With a higher matching threshold the primary response is much longer, causing about the same damage to self antigens more gradually, and producing antigen specific memory cells. These memory cells are most likely to be re-activated in the secondary and tertiary responses. The memory responses are shorter and much more targeted on the foreign antigen, causing significantly less damage to self. The matching threshold therefore has an important impact on the generation of immune memory and on regulation of autoimmunity.

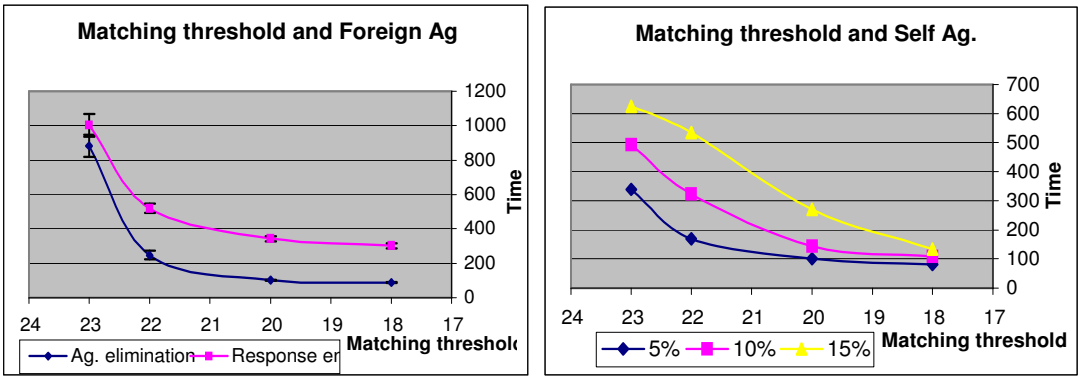

Figure 19: Matching threshold and antigen elimination.
(Left) The relation between the maximal matching threshold, the foreign antigen elimination and the total duration of the primary immune response. (Right) The relation between the maximal matching threshold and the self antigen degeneration time in the primary immune response. Each line represent the duration from the beginning of the response until a fixed percentage of self antigen (5%, 10%, 15%) is eliminated by autoimmunity. Each point is an average of 50 simulation runs with the same parameters.

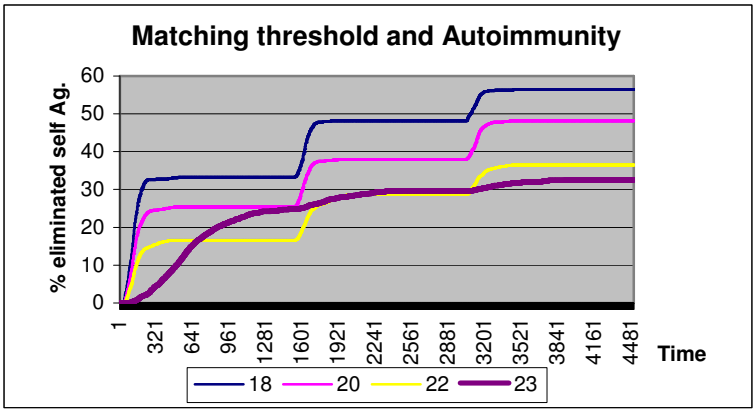

Figure 20: Matching threshold and autoimmunity.
Number of self antigens during primary, secondary and tertiary immune responses with different maximal matching thresholds. 100 harmless extra-cellular self antigens were randomly generated during initialization. Foreign antigen was injected in time units 0, 1500 and 3000. Each line represent and average of 50 simulation runs with the same parameters. Simulation parameters are detailed in Appendix B (Table 5).

4.2.2 The memory threshold for efficient secondary response

The result of the previous experiment implies that when the matching rule is strict enough the primary response will be slower, more memory cells will develop and subsequent responses will be more efficient. The objective of this experiment was to define the relation between the quantity of memory T-h cells generated in the primary response and the efficiency of the secondary response. The efficiency of the secondary response was taken in this experiment as the difference between the antigen elimination time in the primary and secondary responses.

The efficiency of the response was plotted against the quantity of memory T-h cells generated in the primary response for 50 simulation runs with the same parameters (Fig. 21). There is an evident correlation between the quantity of memory cells and the efficiency of the secondary response. The results show that when the quantity of memory cells generated in the primary response is very small, the secondary response is not necessarily more efficient and the difference in response duration is either positive or negative. As the number of memory cells increases the secondary response eliminates the antigen faster. Interestingly, there is a threshold value of memory T-h cells that ensures that the secondary response is more efficient. This number signifies a phase transition - if the number of memory cells generated in the primary response is equal to or greater than this threshold, the difference in the responses duration is always a positive number, indicating that the secondary response is efficient. This phenomenon occurs for the two matching thresholds (22 and 23), indicating that this behavior of the system is general. Thus, if enough memory is produced during the primary response the ability of the system to produce an efficient secondary response is robust.

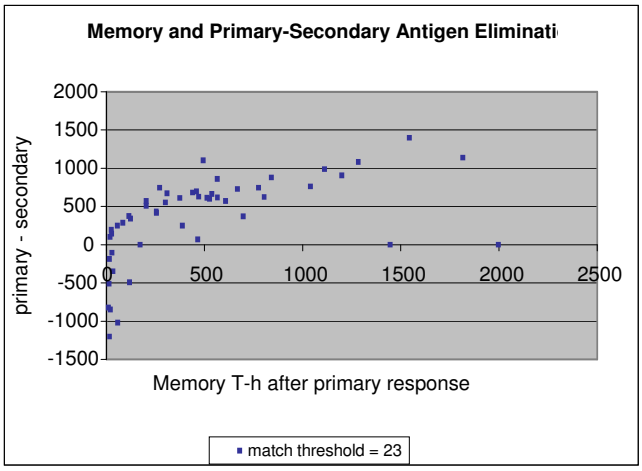

Figure 21: Memory survival threshold for efficient secondary response.
Results of 50 simulation executions with a strict matching rule (matching threshold=23). Y axis indicates the efficiency of the secondary response, denoted by the difference between antigen elimination time in primary and secondary responses. X axis indicates the quantity of T-h cells generated during primary response.

There is a positive linear correlation between the duration of the primary response and the quantity of memory cells produced during this response (data not shown).The data presented in *Figure 21* were used to estimate the threshold quantity of memory T-h cells required for an efficient vaccination in the simulation system. This threshold was used to calculate the minimal required duration of the primary response. The results are listed in *Table 3*.

Max matching threshold	Memory Th threshold (with p-values)	Primary response duration threshold (with p-values)
23	244.44 (7.65E-09)	229.91 (1.39E-14) time units 69 hours
22	36.77 (2.5E-10)	18.74 (7.43E-17) time units 5.6 hours

Table 3: Th Memory and response duration thresholds for efficient vaccination.
Results are based on the experiments described in Figure 21. Threshold values were calculated by linear regression, and the p-values are given in parenthesis. translation of simulation time units to hours is based on the scaling of 0.3 hour per time unit.

4.2.2.1 *The role of memory cells in the primary response*

Memory lymphocytes are usually associated with the recall responses against an antigen that the immune system has already encountered before. However, since a memory cell is a lymphocyte with a longer life expectancy and possibly a faster activation mechanism, it is reasonable to assume that once a lymphocyte has differentiated into a memory cell it can always be re-activated. If the antigen infection is cleared relatively slowly, the re-activation of the memory cell may be part of the primary immune response. In order to assess the effect of memory lymphocytes on the primary immune response, the dynamics of the immune response against a virulent antigen was monitored without any employment of memory mechanism (*Figure 22*). Surprisingly, without immune memory the system cannot eliminate the infection, and the immune entities fluctuate with strong correspondence to the antigen's fluctuations. This result indicates that memory lymphocytes are crucial for the success of the primary response against a virulent antigen.

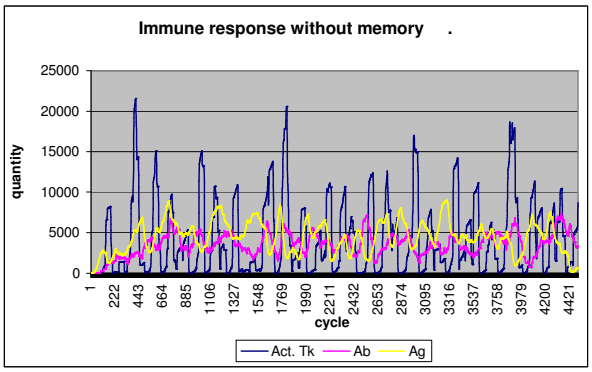

Figure 22: Immune response without a memory mechanism.
A typical dynamics of the antigen, antibodies and activated T-k lymphocytes. parameters of the simulations are as listed in Table 5(Appendix B), except for memory T cells survival rate, which was set to 0. A dose of 10 antigens was injected in simulation time units 0, 1500 and 3000.

The next goal was to define the relation between the production of memory lymphocytes and the efficiency of the primary response. To do that, the duration of antigen elimination was measured with different probabilities of memory T-h and T-k survival. The results are presented in *Figure 23*. Seven experiments (50 simulation runs in each experiment) were conducted. In all of the experiments differentiation of T lymphocytes into memory cells was linear with random selection. The survival probability for T-h and T-k lymphocytes varied from 0.01 to 0.2. As the survival probability is higher, more memory cells are produced and the antigen elimination time in the secondary response is shorter. However, the same relation appears in the primary response as well, meaning that the newly produced memory lymphocytes are likely to be re-activated during the primary response, contributing to the elimination of the antigen in this response. When a threshold level of T cell survival probability of approximately 0.05 was reached there was no further improvement in the primary response. This result indicates that in addition to the contribution of memory cells there are also other factors that determine the efficiency of the response, and uncontrolled production of memory cells is not necessarily beneficial for the improvement of the response.

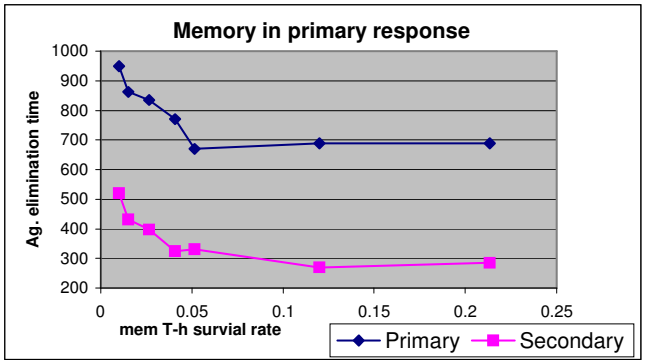

Figure 23: memory survival probabilities and responses duration.
Antigen elimination time in the primary and secondary responses with seven different survival rates for memory T cells. Each point is an average of 50 simulation runs.

4.2.3 The significance of the initial immune repertoire

Following its initial generation, the immune repertoire is going through changes. New lymphocytes are randomly generated and put in the system's circulation concomitant with death of lymphocytes, either by 'naturally' terminating their life cycle or by ending their activation phase during an immune response. To check the effect of the initial immune repertoire on changes that occur later, the following experiment was conducted: two sets of simulations (50 runs in each set) with the same input parameters were executed. In the first set, the initial lymphocyte population was randomly generated in each simulation run, the same way it was done for all the previously described experiments. In the second set, the exact same initial lymphocyte population was used for all simulation runs. The equivalent in-vivo experiment would be using 50 genetically different (allogeneic) animals for the first experiment, and 50 genetically identical (syngeneic) animals for the second one.

The results of the two sets were very similar to each other (*Figure 24*) in all of the parameters measured. The similarity is not only in the general dynamics of antigen elimination but also in parameters related to the immune repertoire like the average lymphocyte-antigen affinity and the number of lymphocyte clones. The implication is that the repertoire changes occurring during the immune response are far more dominant in determining the outcome of the immune response and the generation of immune memory than the initial repertoire of these receptors. Thus, differences in the initial immune repertoire are not imperative to the outcome of the immune response in the simulation system.

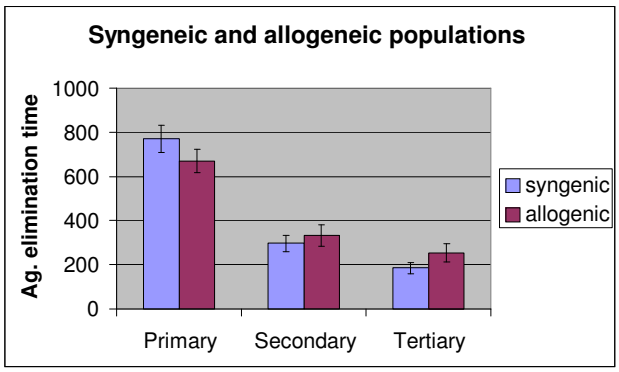

Figure 24: A comparison between syngeneic and allogeneic populations. Antigen elimination time in the primary, secondary and tertiary responses in two experiments: in the 'syngeneic' experiment the same initial lymphocytes population was used for all 50 runs. In the 'allogeneic' experiment different initial populations were generated for every simulation run.

4.2.4 Comparison of different memory differentiation models

Three different models for T lymphocyte memory differentiation were used in the following experiments: linear differentiation of memory cells by highest affinity selection, linear differentiation of memory cells by random selection and decreasing potential differentiation by random selection. For each model several different survival rates for memory T cells were used. The simulation parameters are given in Appendix B.

In the linear differentiation models, an activated lymphocyte is chosen at the end of its activation phase either to die or to differentiate into a long-lived memory cell. The selection criteria may be the affinity between the lymphocyte and the antigen or simply a random selection. In all experiments, the antigen was successfully eliminated in the primary response, and the secondary and tertiary responses started, reached their peak, eliminated the antigen and ended faster than the primary response. Results are illustrated and compared in *Figure 25*. These results were obtained for two different maximal thresholds of the memory survival function.

In the decreasing potential model a lymphocyte is chosen to differentiate into an effector cell or a memory cell several times during its life cycle. Each consequent differentiation step into an effector cell decreases the cell's potential to become a memory cell and survive. The selection is random and several different survival probabilities were used. The results show that when the survival probability was low (0.05), the results were similar to the results obtained with the linear differentiation models, as illustrated in *Figure 25*.

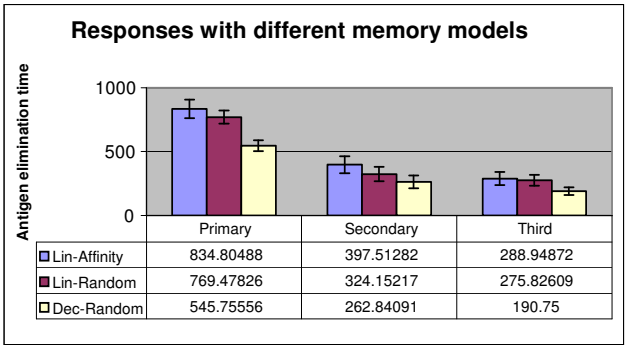

Figure 25: Comparison of immune responses with different memory models.
Results are the average of 50 simulation runs, with parameters described in Appendix B. Antigen elimination times in the linear memory differentiation by affinity and by random selection, and in the decreasing potential differentiation by random selection.

5. Discussion

5.1 Research Main Results

The results of the experiments conducted in this research using the Immune System Simulation (ISS) tool can be categorized into two sub-sets:

1. Results that restore biological phenomena or data reported in the literature. This type of results was used for the calibration and verification processes of the model, aimed to show its biological relevance.
2. Results that gives new insights on the immune system, suggesting a novel point of view on currently debatable biological phenomena.

The following results restore existing biological data:

- Primary and recall infections of virulent antigen cause the activation, development and regulation of humoral and cellular immune responses against the antigen.
- During the primary immune response memory T cells develop. These cells ensure an efficient secondary response. The dynamics of the primary and secondary T cell response over time is similar to in-vivo experiments described in the literature.
- During the primary and the secondary immune responses antigen-specific T cell clones proliferate and expand. Proliferation rates and diversity changes of T cells are equivalent to literature in-vivo experiments.

The following results are new insights on the immune system:

- An immune system with affinity-based interactions is more likely to express the immune memory phenomenon: the secondary immune response is more efficient as the lymphocyte-antigen matching required for activation is more strict. A strict matching criteria favors positive selection of high affinity lymphocytes, and extends the duration of the primary response to allow development of memory cells, while keeping autoimmunity relatively low in subsequent responses.
- Immunization by primary exposure to the antigen is successful only if the primary immune response is significant enough to produce a threshold amount of memory T cells. If the minimal required memory is not produced, the secondary response will not be improved. But if enough memory cells are produced, the system expresses a very robust immunization against subsequent antigen stimulation. The simulation system is used to estimate this 'vaccination threshold'. The biological equivalent of this threshold can be used to design and evaluate vaccine paradigms.
- Memory lymphocytes may have an important contribution to the success of the immune system to eliminate the antigen in the primary response, as well as in the subsequent responses. Without any memory cells, the primary response against a virulent antigen may fail to eliminate the antigen. As more activated T cell survive as memory cells the primary response significantly improves. The simulation predicts that survival of up to 5% of activated T cells is sufficient for efficient

primary and secondary responses, an observation that supports experimental findings from theoretical considerations.

- Repertoire changes occurring during the immune response are more dominant in determining the outcome of the immune response than the initial random generation of these receptors. This may explain how any individual immune system adapts itself to respond efficiently against a broad range of antigens, although its initial repertoire of receptors was created in a random fashion.

5.2 Biological Interpretation Of The Results

A computational model of a biological system can contribute to a better understanding of the modeled biological phenomena. To achieve this goal, the biological relevance of the model must be proven, by showing that quantitative data, as well as qualitative principles, are restored within the model. Furthermore, the model should be capable of giving new insights and predictions about the biological system, which correspond to debatable issues in the literature. This section associates the main results of this work with current biological research, showing that insights gained from this work may contribute to a better understanding of the immune system, and suggesting possible experiments to test the theoretical results.

5.2.1 Requirements for an efficient secondary immune response

In the experiments described in the previous chapter, the efficiency of the secondary immune response was evaluated by assessing the speed and the magnitude of the response. The efficiency of the secondary response is a measure for the success of the immunization, and it is influenced by many different factors. Some of these factors were explored using the simulation system, and the obtained results may be used to define some of the requirements for development of an efficient immune memory and thus for the development of efficient immunization paradigms.

5.2.1.1 Interaction affinity

The matching rule used for the simulated interactions between immune entities has a major effect on the overall outcome of the immune response. The matching rule directly determines the matching probability, as shown in *Table 2*, and the matching probability is the equivalent to the bonding strength or interaction strength, determining whether activation signals will be triggered. One can hypothesize that a less strict matching rule with a lower threshold for a definite match should yield an improved response. With such a matching rule, more lymphocytes will be activated, and the antigen will be eliminated faster. The results of the matching threshold experiment, shown in *Figure 18*, prove this hypothesis to be generally correct: the antigen elimination time decreases with the matching threshold. For example, in the primary response, the most strict matching rule (threshold=23) yields a long response of 670 time units (~8.3 days) from infection to antigen elimination, while the less strict matching rule (threshold=18) yields a short response of only 87 time units (~1.1 days).

But, taking into account that activated lymphocytes may also bind to self antigens, a permissive matching rule may result in elimination of a large portion of the body's self antigens. Indeed, it was shown in *Figure 20* that a permissive matching rule causes major degeneration of self antigens in a very short time in both primary and memory responses. When a strict matching rule is used, the damage to self antigens in the primary response is more gradual, and more important – the damage to self antigens in the memory responses is highly controlled. Therefore, the matching rule balances the speed of the response, namely how fast it clears the foreign antigen and the preciseness of the response, namely how much damage is caused to self antigens by autoimmunity.

Figure 18 demonstrates that improved secondary and tertiary responses are produced only when a strict matching rule is used, while no memory effect is demonstrated with a permissive matching rule. This result implies that the phenomenon of immune memory is more likely to be expressed in an immune system with affinity-based recognition. A strict matching criteria favors positive selection of high affinity lymphocytes which

survive as memory cells, and ensures an improved recall responses. In addition, a strict matching rule extends the duration of the primary response to allow development of enough memory cells, while keeping the damage to self antigens relatively low. A permissive matching rule, on the other hand, allows a fast activation of lymphocytes against both foreign and self antigens. The primary response is short, and it involves activation of a small number of lymphocytes and generation of small quantities of memory cells. Antigen is indeed eliminated very quickly, but the response does not produce efficient vaccination. The following memory responses are still efficient against the foreign antigen, but at the same time the body's self antigens are significantly damaged.

In the natural immune system the primary immune response is a long-lasting process on a biochemical scale. Elimination of an antigen usually takes days, and the immune response proceeds even after the clinical disease has terminated. The simulation showed that this slow time course is of crucial importance to the development of immunization against subsequent challenges. The requirement for a very strict affinity recognition between the lymphocyte and the antigen serves this purpose. It was shown here that the stringent matching criteria are rate-limiting in the primary response but at the same time crucial for the efficient secondary response. The simulation shows that an immune system not based on stringent recognition would eliminate antigen but would not exhibit the hallmark characteristics of immune memory. In addition, such an immune system would severely damage the body's self antigen by autoimmune attacks. In other words, the simulation suggests that the slow kinetics of the primary response are in the basis of the development of immune memory and that this memory has an important role in regulating autoimmunity in subsequent responses.

5.2.1.2 memory threshold

Within a single experiment, the quantities of memory cells vary between different simulation executions. This allows to estimate the correlation between the amount of memory cells produced during the primary response, and the efficiency of the secondary response. Such correlation is illustrated in *Figure 21,* where a larger quantity of memory T-h cells causes the secondary response to be faster relative to the primary response. The simulation shows that there is a threshold amount of memory cells that guarantees a better secondary response. If this amount of memory cells is generated than the organism is necessarily vaccinated against the antigen, and therefore this threshold was termed 'vaccination threshold'. This 'vaccination threshold' for memory T-h cells was estimated in the simulation system (*Table 3*). Since there is a linear relation between the response duration and the quantity of produced memory cells, there is an implied threshold for the duration of the primary response.

The emerging picture is therefore as follows: in order for the immune system to develop a successful immunity, the primary antigen challenge must be able to trigger a significant immune response, which will generate enough memory cells for efficient vaccination. In order to do that, the primary response must last a minimal amount of time. Indeed, it is known that the primary immune response against most of the virulent antigens lasts between several days to more than as week, with antigen elimination occurring well after the clinical symptoms of the disease have disappeared [1]. In addition, getting clinical symptoms of a disease undeniably ensures production of vaccination against the same pathogen in people with intact immune systems. In the living body, when symptoms of

the disease induced by the antigen are expressed, the 'vaccination threshold' is most likely achieved, and the body is immunized against future infections of the same antigen.

In the simulation system, the 'vaccination threshold' is strongly dependent on antigen type and on parameters of T-h proliferation and differentiation, and putting all parameters into the system, it can be used to evaluate vaccine doses and protocols.

5.2.1.3 memory differentiation model

Several different immune memory models were used in the experiments: linear differentiation by highest antigen affinity, linear differentiation by random selection and decreasing potential differentiation by random selection. In addition, a control experiment without any memory mechanism was conducted.

Three experiments using different immune memory models were compared, as illustrated in *Figure 25*. The main differences between these three experiments are in the absolute duration of primary, secondary and tertiary responses. When the ratios between primary, secondary and tertiary responses are examined, all three experiments yield similar results – antigen elimination time in the secondary response is 2.1-2.4 times shorter than in the primary response, and antigen elimination time in the tertiary response is 1.2-1.4 times shorter than in the secondary response. Other parameters, like maximal amplitudes of the antigen, show similar behavior. This similarity between the differentiation models can be understood when examining the number of different T-h or T-k clones actively participating in the immune response. During the activation and proliferation phase, the immune repertoire is narrowed and becomes dominated by a few clones with high antigen affinity. The variation of the antigen affinities among these clones is low, and lymphocytes of these clones are selected under any of the tested selection rules. The only important factor is the memory survival probability, and since probabilities were chosen in all experiments to be similar, the outcome is also similar.

The control experiment demonstrated that without any immune memory mechanism, there is no improvement is recall responses. When the infectious antigen is virulent, i.e. spreads fast and causes severe damage to tissue cells, the simulated immune response cannot eliminate the antigen, as illustrated in *Figure 22*. This result is explained by the role of memory cells in the primary response, as will be discussed later. When the antigen is less virulent the immune system is able to cope with it successfully, but there is no significant improvement in the efficiency of secondary or tertiary responses, as illustrated in (*Figure 14).*

5.2.2 The role of memory cells in the primary response

In the model, memory cells were shown to have a major role in keeping the immune system alert against attacks of previously encountered antigens and allowing the system to respond faster and more efficiently against subsequent challenges by these antigens.

Since a memory cell is a lymphocyte with a longer life expectancy and possibly a faster activation mechanism, it is reasonable to assume that once a lymphocyte has differentiated into a memory cell it can always be re-activated. If the antigen infection is cleared relatively slowly, the re-activation of the memory cell may be part of the primary immune response. The results from the simulation experiments show that not only do memory cells participate in the primary immune response, but they have a crucial role in determining the efficiency of this response as well. This observation that memory cells participate and effect the outcome of the primary immune response is new, yet it can be easily understood when examining the nature of immune memory development. As *Figure 22* demonstrates, without any memory cells the simulated immune system fails to eliminate a virulent infectious antigen. This is because a lymphocyte becomes activated only for a limited period, during which the invading antigen may not be completely cleared. When the lymphocyte reaches its 'decision point', it either dies or becomes a memory cell. In the former case, an antigen specific lymphocyte dies and goes out of the immune circulation. In the latter case, the antigen specific lymphocyte remains in the local environment of the infection for as long as it takes, waiting to be re-activated and to re-attack the antigen. Therefore, the overall ability of the simulated immune system to overcome the antigen in the primary response depends on the amount of memory T cells generated during this response.

This dependency is demonstrated in *Figure 23* - the time from infection to antigen elimination in the primary response is 30% shorter when the survival probability of memory T cells is raised from 0.01 to 0.05. When the survival probability reaches a threshold of approximately 0.05 there is no further improvement in the primary response. This result indicates that the contribution of memory cells is not the sole factor determining the efficiency of the response, and unregulated production of memory cells is not necessarily beneficial for the response. Indeed, it was experimentally found that the survival ratio of antigen-specific T cells during the final stages of the immune response is about 0.01-0.05 [5, 56]. It therefore appears that the simulation system captures a genuine behavior of the immune system and provides a theoretical support for it .

Recently, Jacob and Baltimore described a novel transgenic mouse system which enables discrimination between naive, activated and memory T cells in-vivo using a recombinant expression system that irreversibly marks antigen-stimulated T cells [7]. This system was used to monitor the distinct populations of T cells at different phases of the immune response to Lymphocytic Choriomeningitis Virus (LCMV). They found that 8 days after infection, during the acute phase of the response (corresponding to the primary immune response in the simulation), a subset of the antigen-specific T-k cells displayed characteristics of LCMV-specific memory cells, both in terms of their ability for long-term growth and their ability to adaptively transfer immunity against LCMV infection. These memory cells were only a small fraction of the total LCMV-specific T-k cells during the acute response, whereas during the memory phase, more than 30 days after infection, these were the only LCMV-specific cells found. Interestingly, during the acute phase of the response these cells exhibited cytotoxic activity in-vitro that was identical to activated T cells. It therefore seems likely that these memory cells possessed the ability to actively participate in the ongoing immune response, but this was not shown in the experimental work. The work of Jacob and Baltimore thus provides experimental support to the predictions of the simulation. The theoretical results, on their part,

provide a possible explanation to the experimental observation and suggest that the memory cells actively participate in the acute phase of the response, contributing to its efficiency. On the basis of the theoretical prediction it is now possible to design an experiment confirming the actual participation of memory cells in the acute phase using the experimental system described, by specifically eliminating the memory population during the acute phase and monitoring the kinetics of the response.

5.2.3 The significance of the initial immune repertoire

The influence of the initial immune repertoire on the generation of immune memory was assessed. Specifically, the question of the relative contribution of the initial receptor repertoire vs. the contribution of repertoire alterations during the response was addressed by comparing the differences in the outcome of an antigen challenge in simulated syngeneic and allogeneic populations.

In the simulation system, lymphocyte receptors are generated by a random process, in which each of the possible receptors has an equal probability to be generated. The random generation process guarantees a non-biased diversity of the immune repertoire. Since the possible antigens are not known when the generation process occurs, the system must be capable of developing a response against any harmful antigen. Using a receptor of 23 binary bits, with a strict matching rule demanding two strings to be completely equal for a definite match, the estimated probability for a successful match is 0.03 (*Table 2).* An initial population of 1000 lymphocytes, each with the above antigen matching probability, guarantees that potentially there is always a matching lymphocyte in the repertoire. But, considering also the spatial conditions needed for a successful match and for lymphocyte activation–(that requires antigen capturing and presentation to the matching T-h cell, the presence and activation of matching T-k or B cell in the same lymph node, and the interaction between the effector T-k cells or antibodies with the antigen), the actual probability for triggering an immune response is much lower. Still, this probability was sufficient to enable the system to respond efficiently against an antigen with a random specificity.

Following its initial generation, the immune repertoire is going through changes. during an immune response antigen-specific clones proliferate and dominate the repertoire. After the response terminates most of these lymphocytes die and some survive as memory cells. In addition, each non-memory lymphocyte has a finite life expectancy. Therefore lymphocytes are constantly dying and new lymphocytes are constantly generated by homeostasis mechanisms. All of these changes shape the immune repertoire and determine its diversity. As demonstrated in *Figure 24,* the measurements of the immune response in a syngeneic population with 50 identical initial repertoires is very similar to that of an allogeneic population with 50 unique initial repertoires.

The implication of this result is that the changes in the structure of the repertoire occurring during the simulation are more dominant in determining the outcome of the immune response than the initial generation of the immune receptors. The initial specific immune repertoire has a minor impact on the efficiency of the response providing that the repertoire is sufficiently diverse. The dynamics of clonal expansion and the narrowing of the repertoire are imperative to the generation of the immune response. Thus, the well-accepted conception that the initial immune repertoire is generated in a random fashion was shown not to affect the ability of any individual immune system to generate efficient and robust immune responses. In other words, the simulation system shows that any intact immune system will eliminate an antigen and develop immune memory regardless of its specific initial lymphocyte repertoire. Indeed, every healthy individual that overcomes a pathogen infection becomes vaccinated against it without

any dependence on his initial lymphocyte receptor pool. Therefore, vaccinations against common diseases achieve high success rates in healthy populations [2].

This 'in-machina' experiment can be directly verified in-vivo by accurately measuring parameters of the primary and secondary immune responses and comparing their distribution and variance in syngeneic and allogeneic populations.

5.3 Pros And Cons Of The Computational Approach

5.3.1 Pros of the simulation system

The implementation of the immune system simulation model is a general tool for investigating various aspects of the immune system within a computer simulation. The simulation system benefits from the general advantages of models since it is a low-cost tool that can be used to construct highly controlled and repeatable 'in-machina' experiments. Thus, the system can be used for exploring both qualitative principles and quantitative phenomena of mechanisms in the immune system. The unique characteristics of the simulation system give it some additional benefits:

1. Generality and Flexibility.
   The system supplies a high degree of generality and flexibility by allowing the user to control over 70 input parameters. These parameters are used to determine the 'configuration' of the simulated model, to precisely tune the quantitative behavior of the system and to determine the dynamics of the injected foreign antigen.
2. Realism.
   Incorporation of many different immunological mechanisms makes the simulation system realistic and robust. The experiments and their results have a straightforward biological equivalence.
3. Modularity and Expandability.
   The system was designed in a modular object-oriented way, which allows its expansion and modification. Expansion of the simulation scale is simply achieved by using a more powerful computing platform.
4. Ease of use.
   The simulation system is very easy to use. It is suitable for being used by researchers from other disciplines that do not possess any programming skills.

5.3.2 Cons of the simulation system

The immune system simulation model was initially designed to capture only a narrow portion of the broad and complex nature of the biological immune system. It is possible to use the simulation system to qualitatively assess biological assumptions before constructing a more complex and expensive laboratory experiment. But in order to produce meaningful quantitative results, the simulation system must be tuned to accurately imitate a specific experimental model and predict its results. This might be a non-trivial challenge: the simulation system is flexible enough to allow its user to vary a large number of input parameters. Trying to comprehensively estimate all of the parameters from literature data is hard to do since experimental models usually investigate only a few parameters while others are not monitored. In addition there are large variations in reported data between different experimental models.

Beside parameter tuning, the simulation model is coded with a set of deterministic and stochastic rules. These rules define, within the simplifying assumptions of the model, how the immune system works. Formulating these rules from the current biological knowledge is difficult to do – many aspects of the biological immune system are not thoroughly understood. Some questions in immunology are only answered theoretically, and others are subjects for continuous scientific debates, where contradicting

assumptions are presented and often supported by contradicting experimental data. In order to allow the simulation to work, these controversial questions can be addressed by two possible ways: the first is to support simulation of different views through additional input parameters, which might make the system harder to tune. The second is to choose the more common hypothesis and implement it, hoping that the simulation results will not be wrongly biased. In this work, most 'hard-coded' assumptions are based on common theories of immunology, simplified to make implementation and investigation possible. More debatable issues, like the nature of immune memory, were left for the users of the simulation system to determine using the input parameters.

The relatively small capacity of the simulation system might be a drawback. In the highly-complex immune system, some characteristics may emerge only when the number of immune entities and size of the body are few orders of magnitude larger. The simulation system handles this issue not by intending to mimic the complete system on a smaller scale, but instead by simulating small subsystems – few tissue cell, several lymph nodes and immune system cells and molecules.

5.3.3 Future enhancements and directions

The simulation system may be further developed to incorporate additional immune mechanisms. Enhancements will make the implemented model more realistic, and will enable exploring other aspects of the immune system. It should be mentioned, however, that as the model becomes more realistic, it also becomes more complicated, as there are more parameters and more possible behavior patterns. Therefore, enhancements should be modular, by allowing to use each of the system's component independently, and carefully constructing a more complex simulation system by putting few components together.

A first possible enhancement has to do with signaling between immune entities. The current model includes a very simplified signaling method, in which direct 'binary' signals are transferred between different cells (APCs, lymphocyes, tissue cells). In the natural immune system, a highly complex network of chemical signals, mediated by proteins called *cytokines*, is used to regulate and control the activity of the immune cells. Cytokines are secreted and recognized by immune system cells. There are more than 20 type of cytokines, and at least 90 different cytokine-mediated activities have been recognized [2]. Some of these activities encourage activation and proliferation and others suppress the immune response. Cytokine-mediated signals are determined by the protein concentrations, and therefore are not binary signals. Incorporation of cytokine signaling in the simulation system would make the process of decision making by cells more complex and more realistic.

Another gross simplification made by the model is the nature of antibodies. Simulated antibodies are plain specific antigen-eliminating molecules with a given existence time. In fact, different types of antibodies can be found in the living body (the major human types are named IgG, IgM, IgA, IgE and IgD), and the mixture of these types, produced during an immune response has a crucial effect on the overall outcome. Expanding the variety of antibody response in the simulation may help exploring the humoral arm of the acquired immune response.

Another possible enhancement involves elaborating the binding process between receptors and antigenic epitopes, which is currently implemented as binary string matching. The actual strength of the binding depends on the spatial and chemical structures of the receptors, and in order to simulate it, complex representation and generation processes of the receptors should be employed.

The representation of the tissue as three-dimensional space, instead of the implemented two-dimensional lattice may also expose new aspects of the spatial organization and dynamics of the system.

And finally, an artificial distinction was made in the model between tissue cells, which can be infected by antigens, and immune system cells which react against antigens. One of the main challenges of immunology in recent years is understanding a pathogen that attacks the lymphocytes themselves, the HIV. Simulating virus infection of the immune system cells may be used to explore aspects of AIDS.

The simulation system may be used in the future to investigate different immunological questions, like preferable infection strategies for foreign antigens, the nature of self tolerance mechanisms, the course of auto-immune diseases, and even aspects of immune system evolutionary development.

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7. Appendix A: The Simulation System Details

7.1 Activity rules

Each immune entity has a set of activity rules, defining its state transition. The following section describes in detail the activity rules for the different immune entities:

Tissue cell

- If *state* is *ALIVE*: update *inflammation* indicator by averaging the inflammation levels of the neighbors, and divide it by the propagation factor.
- If *state* is *NECROTIC_DEATH*: update *inflammation* indicator to the maximal level.
- If state is APOPTOTIC_DEATH: die without leaving any trace and (specifically, without changing the *inflammation* indicator).
- If *state* is *NECROTIC_DEATH* or *APOPTOTIC_DEATH*, and sufficient time (*RECOVERY_TIME*) has passed since death – recover and set *state* to *ALIVE*.

Dendritic cell

- If located in a *CELL_NODE*:
  - Move across the cell nodes grid for *DENDRITIC_MOVE_TIME*, than try to capture an antigen. Movement time is used to guarantee equitable distribution of dendritic cells across the cells nodes grid. Movement may be random or heuristic towards higher antigen load.
  - If there are extra-cellular antigens in the cell node, capture one of them randomly.
  - If there are intra-cellular antigens attached to the tissue cell, capture one of them randomly.
  - If an antigen was captured - record the local inflammation level and move to the draining lymph node, otherwise – move to adjacent node, and try to capture an antigen there.
- If located in a *LYMPH_NODE*:
  - Stay in the lymph node for DENDRITIC_LYMPH_WAIT_TIME.
  - If an extra-cellular antigen was captured, and if a matching T-Helper cell is present: present the antigen to the T-Helper cell, giving it a BIND signal. If activation conditions are fulfilled, activate the T-Helper cell by giving it a CO-STIMULATION signal.
  - If an intra-cellular antigen was captured, and if a matching T-Killer cell is found in the lymph node: present the antigen to the T-Killer cell, giving it BIND signal. If there is also a matching activated T-Helper cell in the lymph, transfer the HELP signal from the T-Helper cell to activate the T-Killer cell.

T-Helper cell

- Make life/death decision according to the cell's *HALF_LIFE* parameter.
- If *state* is *RESTING* (initial state):
  - Traverse the cyclic lymph nodes array, waiting T_HELPER_REST_WAIT_TIME in each lymph node for antigen presentation by APC.
  - If a matching antigen was presented, and BIND signal was given – divert to BOUND state.
- If *state* is *BOUND* (bound to an antigen presented by an APC):
  - Wait T_HELPER_BOUND_TIME for activation signal from APC.
  - If activation signal was given: divert to ACTIVATED state.
  - If activation signal was not given: divert to TOLERATED state.
- If *state* is *ACTIVATED* (effector cell):
  - If there is a matching B cell in the lymph node, give it an activation HELP signal.
  - If there is a matching T-killer cell in the lymph node, give it an activation HELP signal (through the APC).
  - Clone daughter cells after successful effector interaction and reiterate every T_HELPER_CLONE_PERIOD.
  - Make memory differentiation decision according to the employed memory model.
- If *state* is *TOLERATED* (got *BIND* signal without activation signal):
  - Wait T_HELPER_TOLERANCE_TIME, and revert to *RESTING* state.

T-Killer cell

- Make life/death decision according to the cell's *HALF_LIFE* parameter.
- If located in a *LYMPH_NODE*:
  - Is state is RESTING (initial state):
    - Traverse the cyclic lymph nodes array, waiting T_KILLER_REST_WAIT_TIME in each lymph node for antigen presentation by an APC.
    - If a matching antigen was presented, and BIND signal was given – divert to BOUND state.
  - If state is BOUND (bound to an antigen presented by an APC):
    - Wait T_KILLER_BOUND_TIME for activation signal from T-Helper cell.
    - if activation signal was given: divert to ACTIVATED state.
    - if activation signal was not given: divert to TOLERATED state.
  - If state is ACTIVATED (effector cell):
    - Move to a random cell node in the local environment of the lymph node, and perform effector function.

- If state is TOLERATED (got BIND signal without activation signal):
  - Wait T_KILLER_TOLERANCE_TIME, and revert to RESTING state.
- If located in a CELL_NODE (effector cell):
- Move across the cell nodes grid for T_KILLER_ACTIVATED_TIME, encountering infected tissue cells. Movement may be random or heuristic, towards higher antigen load.
- if an infected cell with a matching antigen was encountered, give the tissue cell a KILL signal, causing it an apoptotic death.
- Clone daughter cells after successful effector interaction and reiterate every T_KILLER_CLONE_PERIOD.
- Make memory differentiation decision according to the employed memory model.

B cell

- Make life/death decision according to the cell's *HALF_LIFE* parameter.
- If located in a *CELL_NODE*:
  - Move across the cell nodes grid for B_CELL_MOVE_TIME, then try to capture an antigen. Movement time is used to guarantee equitable distribution of B cells across the cells nodes grid. Movement may be random or heuristic, towards higher antigen load.
  - If there are extra-cellular antigens in the cell node, capture one of them randomly.
  - If an antigen was captured - record the local inflammation level and move to the draining lymph node, otherwise – move to adjacent node, and try to capture an antigen there.
- If located in a *LYMPH_NODE*:
  - If state is RESTING (initial state):
    - Move to a random cell node in the local environment of the lymph node.
  - If state is BOUND (antigen was captured):
    - Stay in the lymph node for B_CELL_LYMPH_WAIT_TIME.
    - If a matching T-Helper cell is found in the lymph node: present the antigen to the T-Helper cell, giving it BIND signal. If activation conditions are fulfilled, activate the T-Helper cell.
    - If an activating HELP signal was given by a matching effector T-Helper cell, divert to ACTIVATED state.
  - If state is ACTIVATED (effector cell):
    - Secrete antibodies
    - Clone daughter cells with possible mutations every B_CELL_CLONE_PERIOD.
    - Make memory differentiation decision according to the employed memory model.

Antibody

- If located in a *LYMPH_NODE* (initial state):
  - Move to a random cell node in the local environment of the lymph node.
- If located in a *CELL_NODE*:
  - Move across the cell nodes grid, looking for matching extra-cellular antigens. Movement may be random of heuristic, towards higher antigen load.
  - If a matching antigen was found – eliminate it.
- Die after *ANTIBODY_LIFE_TIME*.

Antigen (harmful)

- If *state* is *Phase 0* (move phase):
  - Move across cell nodes grid for ANTIGEN_1_MOVE_TIME.
  - Try to infect a tissue cell. If successful: divert to Phase 1, otherwise – keep moving.
- If *state* is *Phase 1* (infect phase):
  - Wait in infected cell node for ANTIGEN_1_CELL_WAIT_TIME. Than, Divert to Phase 2.
- If *state* is *Phase 2* (clone phase):
  - Clone daughter antigens with possible mutations, for ANTIGEN_1_CLONE_TIME.
    Than, divert to Phase 3.
- If *state* is *Phase 3* (kill phase):
  - Decrease the tissue cell's life indicator, until the cell is dead.
  - Leave the tissue cell and revert to Phase 0.

7.2 Simulation parameters

Many of the model's parameters can be set per simulation. The following section specifies these parameters, their meaning and their default values in the simulation system.

No	Parameter name	Parameter meaning	default value
1.	*LATTICE_SIZE_X*	Horizontal size of cell nodes grid	128
2.	*LATTICE_SIZE_Y*	Vertical size of cell nodes grid	128
3.	*LYMPH_NODE_RATIO*	Ratio between number of cell nodes and number of lymph nodes	64
4.	*THYMUS_MISS_PROBABILITY*	Probability of auto-reactive T cell to escape negative selection in the thymus	0.2
5.	*THYMUS_ENABLED*	Enable/Disable flag of thymus negative selection mechanism	Dis
6.	*SIGNAL_2_ENABLED*	Enable/Disable flag of second signal for lymphocyte activation	En
7.	*HUMORAL_ENABLED*	Enable/Disable flag of humoral arm of immune response	En
8.	*CELLULAR_ENABLED*	Enable/Disable flag of cellular arm of immune response	En
9.	*TH_MEM_ENABLED*	Enable/Disable flag of improved response mechanism of T-h memory cells	En
10.	*HERISTIC_MOVE_ENABLED*	Enable/Disable flag of heuristic movement of immune entities across cell nodes grid according to antigen load	En
11.	*NUMBER_OF_T_HELPERS*	Initial quantity of T-helper cells	1000
12.	*NUMBER_OF_T_KILLERS*	Initial quantity of T-killer cells	1000
13.	*NUMBER_OF_B_CELLS*	Initial quantity of B cells	1000
14.	*NUMBER_OF_DENDRITICS*	Initial quantity of Dendritic cells	1000
15.	*NUMBER_OF_SELF_ANTIGENS*	Initial quantity of self antigens	0
16.	*PEPTIDE_ALPHABET_SIZE*	Number of different possible symbols in a peptide string	2
17.	*PEPTIDE_LENGTH*	Length of a peptide string	23
18.	*PEPTIDE_MATCH_THRESHOLD* *MATCH_MIN_CONST*	Parameters (M, r) for the peptides matching function	23 2/3
19.	*MAX_LIFE_VALUE*	Initial (maximal) *life* value of tissue cell	10
20.	*DEATH_VALUE*	Value of *life* indicator which means tissue cell death	0
21.	*RECOVERY_TIME*	Time from tissue cell death to recovery	300
22.	*MAX_INFLAM_VALUE*	Maximal *inflammation* value, set by a tissue cell after necrotic death	100
23.	*INFLAM_PROPAGATION_FACTOR*	The factor by which the *inflammation* value is divided when propagated to a neighbor tissue cell	2
24.	*DENDRITIC_MOVE_TIME*	Number of time units in which a dendritic cell moves across the cell nodes grid, looking for antigen	5
25.	*DENDRITIC_LYMPH_WAIT_TIME*	Number of time units in which a dendritic cell waits in the lymph node after capturing an antigen	5
26.	*INFLAM_THRESHOLD*	The inflammation threshold of the APC, determining if a second signal will be given by the APC to the T-helper	1
27.	*T_KILLER_BOUND_TIME*	Number of time units in which a T-killer cell stays bound to a presented antigen, waiting for a second signal	10
28.	*T_KILLER_ACTIVATED_TIME*	Number of time units in which a T-killer cell stays activated	100

No	Parameter name	Parameter meaning	default value
29.	*T_KILLER_REST_WAIT_TIME*	Number of time units in which a T-killer cell in resting state waits in each lymph node for an APC presenting a peptide	2
30.	*T_KILLER_TOLERANCE_TIME*	Number of time units in which a T-killer cell stays in tolerated state (inactive) after receiving signal one without signal two	1
31.	*T_KILLER_CLONE_SIZE*	Number of T-killer cells cloned each time unit by an activated T-killer cell	5
32.	*T_KILLER_CLONE_PERIOD*	Number of time units between consequent T-killer cloning	50
33.	*T_KILLER_MUTATION_RATE*	Mutation rate of cloned T-killer cells	0
34.	*T_KILLER_MEMORY_MODEL*	Memory model chosen for T-killer cells	Linear-random
35.	*T_KILLER_MEMORY_SURVIVAL_RATE*	Survival rate of memory T-killers for random memory differentiation	0.05
36.	*T_KILLER_MEMORY_MIN_THRESHOLD* *T_KILLER_MEMORY_MAX_THRESHOLD* *T_KILLER_MEMORY_POWER_BASE*	Parameters (MAX,MIN,C) for survival function of memory T-killers (heuristic memory differentiation)	0 24 1.8
37.	*T_KILLER_MEMORY_HALF_LIFE*	Half life time of a memory T-killer cell	Infinite
38.	*T_KILLER_CLONED_HALF_LIFE*	Half life time of a regular T-killer cell	4000
39.	*T_HELPER_BOUND_TIME*	Number of time units in which a T-helper cell stays bound to a presented antigen, waiting for a second signal	10
40.	*T_HELPER_ACTIVATED_TIME*	Number of time units in which a T-helper cell stays activated	100
41.	*T_HELPER_REST_WAIT_TIME*	Number of time units in which a T-helper cell in resting state waits in each lymph node for an APC presenting a peptide	2
42.	*T_HELPER_ACT_WAIT_TIME*	Number of time units in which an activated T-helper cell waits in each lymph node	10
43.	*T_HELPER_TOLERANCE_TIME*	Number of time units in which a T-helper cell stays in tolerated state (inactive) after receiving signal one without signal two	1
44.	*T_HELPER_CLONE_SIZE*	Number of T-helper cells cloned each time unit by an activated T-helper cell	5
45.	*T_HELPLER_CLONE_PERIOD*	Number of time units between consequent T-helper cloning	50
46.	*T_HELPER_MUTATION_RATE*	Mutation rate of cloned T-helper cells	0
47.	*T_HELPER_MEMORY_MODEL*	Memory model chosen for T-helper cells	Linear-random
48.	*T_HELPER_MEMORY_SURVIVAL_RATE*	Survival rate of memory T-helpers for random memory differentiation	0.05
49.	*T_ HELPER _MEMORY_MIN_THRESHOLD* *T_ HELPER _ MEMORY _MAX_THRESHOLD* *T_ HELPER _MEMORY_POWER_BASE*	Parameters (MAX,MIN,C) for survival function of memory T-helpers (heuristic memory differentiation)	0 24 1.8
50.	*T_HELPER_MEMORY_HALF_LIFE*	Half life time of a memory T-helper cell	Infinite
51.	*T_HELPER_CLONED_HALF_LIFE*	Half life time of a regular T-helper cell	4000
52.	*B_CELL_ACTIVATED_TIME*	Number of time units in which a B cell stays activated	5
53.	*B_CELL_MOVE_TIME*	Number of time units in which a B cell moves across the cell nodes grid, looking for antigen	10
54.	*B_CELL_LYMPH_WAIT_TIME*	Number of time units in which a B cell waits in the lymph node after capturing an antigen	10
55.	*B_CELL_CLONE_SIZE*	Number of B cells cloned each time unit by an activated B cell	2
56.	*B_CELL _CLONE_PERIOD*	Number of time units between consequent	1

No	Parameter name	Parameter meaning	default value
		B cell cloning	
57.	*B_CELL_NUMBER_OF_ANTIBODIES*	Number of antibodies produced each time unit by an activated B cell	3
58.	*B_CELL_MUTATION_RATE*	Mutation rate of cloned B cells	0
59.	*B_CELL_MEMORY_MODEL*	Memory model chosen for B cells	Linear-random
60.	*B_CELL_MEMORY_SURVIVAL_RATE*	Survival rate of memory B cells for random memory differentiation	0
61.	*B_CELL _MEMORY_MIN_THRESHOLD* *B_CELL _ MEMORY _MAX_THRESHOLD* *B_CELL _MEMORY_POWER_BASE*	Parameters (MAX,MIN,C) for survival function of memory B cells (heuristic memory differentiation)	0 0 1.8
62.	*B_CELL_MEMORY_HALF_LIFE*	Half life time of a memory B cell	Infinite
63.	*B_CELL_CLONED_HALF_LIFE*	Half life time of a regular B cell	2000
64.	*ANTIBODY_LIFE_TIME*	Life time of an antibody	100
65.	*ANTIGEN_0_MOVE_TIME*	Number of time units in which anitgen_0 (self antigen) moves across cell nodes grid before attaching to a tissue cell	3
66.	*ANTIGEN_0_CELL_WAIT_TIME*	Number of time units in which anitgen_0 (self antigen) waits in the tissue cell	3
67.	*ANTIGEN_1_MOVE_TIME*	Number of time units in which anitgen_1 (foreign antigen) moves across cell nodes grid before attaching to a tissue cell	5
68.	*ANTIGEN_1_CELL_WAIT_TIME*	Number of time units in which anitgen_1 (foreign antigen) waits in the tissue cell before starting cloning itself	5
69.	*ANTIGEN_1_CLONE_TIME*	Number of time units in which anitgen_1 (foreign antigen) clones itself in the tissue cell, before starting killing the cell	5
70.	*ANTIGEN_1_KILL_TIME*	Number of time units in which anitgen_1 (foreign antigen) effect the *life* variable of the tissue cell in order to kill it	3
71.	*ANTIGEN_1_CLONE_SIZE*	Number of antigen_1 copies cloned each time unit by antigen_1 in cloning phase	1
72.	*ANTIGEN_1_MUTATION_RATE*	Mutation rate of cloned antigen_1	0
73.	*ANTIGEN_INJECTION_X*	Antigen_1 horizontal injection position	64
74.	*ANTIGEN_INJECTION_Y*	Antigen_1 vertical injection position	64
75.	*ANTIGEN_INJECTION_QUANTITY*	Injected quantity of antigen_1	10
76.	*ANTIGEN_INJECTION_INTERVAL*	Time interval for repeated injections of antigen_1	1500

Table 4 : Simulation parameters and their default values

7.3 Simulation output

Several output files are created during simulation execution:

- The *record* file (a file with .rec extension) contains the simulation parameters and additional data needed for restoring the exact same simulation in 'restore' mode.
- The *result* file (a file with .rsl extension) contains snapshots of simulation state in each time unit. The data in this file include the number of dead tissue cells and of T-h, T-k and B cells in various states, the number of antibodies and of antigens, and different statistics about lymphocytes clones, affinities and memory survival.
- The *trace* file (a file with .trc extension) contains a more detailed description of the simulation dynamics, for debugging purposes.

During execution of a simulation, the states and dynamics of the immune entities can be visualized using different *graphic views*. A graphic view displays a specific profile of both the cell nodes grid and the lymph nodes array. In addition, it enables to examine various data of particular node, and of the simulation in general.

The available profiles for cell nodes grid:

- Tissue cells vitality: displays *life* indicator for each tissue cell.
- Inflammation level: displays *inflammation* indicator for each tissue cell.
- Immune entities (T-killers, B cells, Antibodies, Antigens): displays locations and concentrations.

On each cell nodes profile, the following *events* can be displayed:

- Necrotic death of a tissue cell by an antigen.
- Apoptotic death of a tissue cell by a T-Killer cell.
- Elimination of an antigen by an antibody.

The available profiles for lymph nodes array:

- Inflammation level: displays inflammation levels recorded by APCs.
- T-Helper and T-Killer cells: displays locations and concentrations of *RESTING*, *BOUND*, *ACTIVATED* and *TOLERATED* cells.
- B cells: displays locations and concentrations of *RESTING*, *BOUND* and *ACTIVATED* B cells.

On each lymph nodes profile, the following *events* can be displayed:

- T-Helper, T-Killer and B cell activation.
- T-Helper and T-Killer cell toleration.

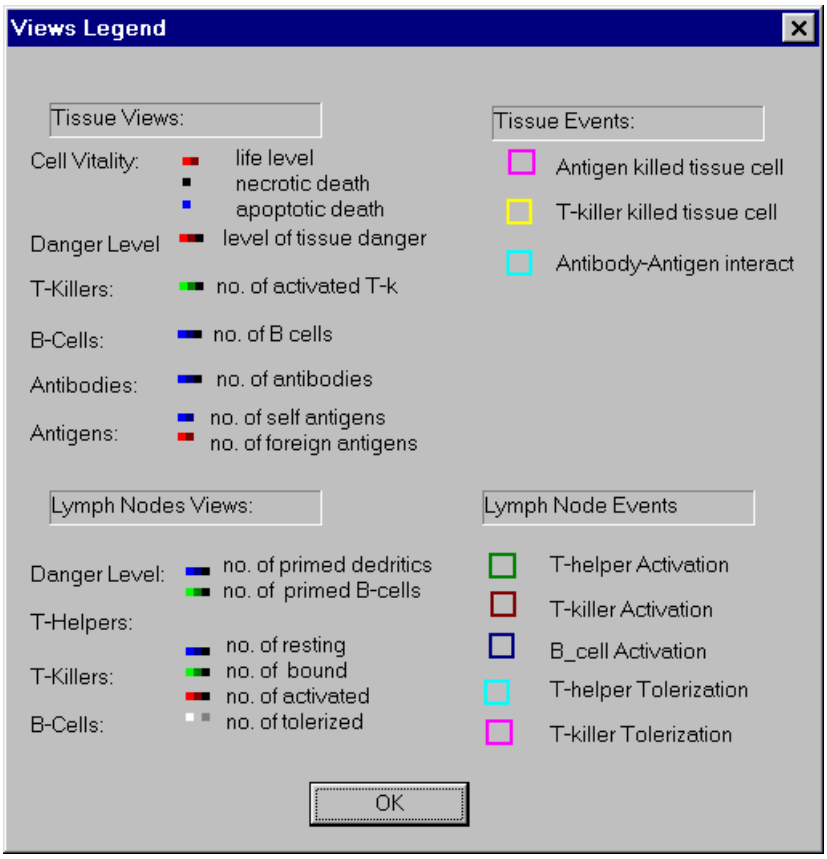

Figure 26: Graphic Views Legend

The following data regarding the simulation are displayed in the graphic view:

- Number of dead tissue cells (necrotic and apoptotic death).
- Number of lymphocytes (T-Helper, T-Killer or B cells): *RESTING*, *ACTIVATED* and *MEMORY*.
- Number of antibodies.
- Number of self and foreign antigens.
- Statistics about actual matching probability.
- Statistics about actual memory survival of T-h, T-k and B cells.
- Statistics about clones and affinities of T-h, T-k and B cells.

The following data regarding a selected cell node are displayed in the graphic view:

- *Life* and *inflammation* indicators of the tissue cell.
- Existence of attached antigen.
- Number of dendritic cells.
- Number of B cells.
- Number of activated T-killer cells.
- Number of antibodies.
- Number of extra-cellular antigens.

The following data regarding a selected lymph node are displayed in the graphic view:

- Number of T-Helper, T-Killer and B cells: *RESTING, BOUND* and *ACTIVATED.*
- Number of dendritic cells.

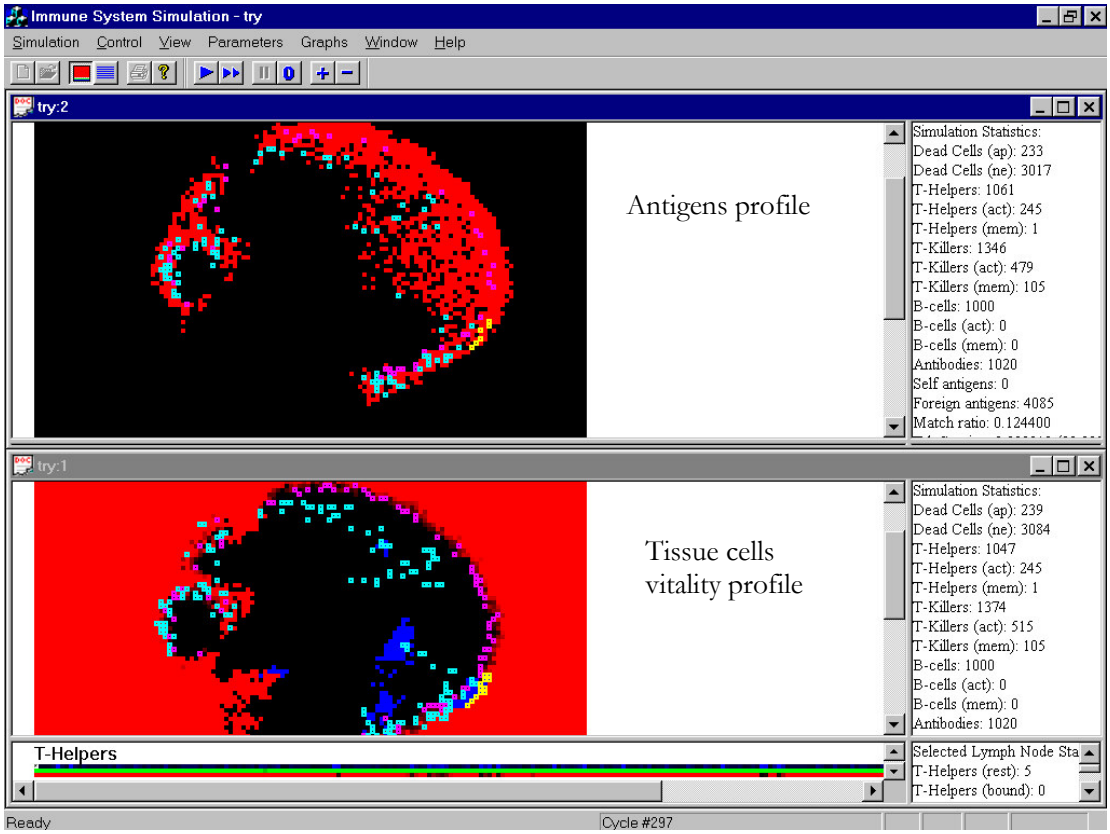

Figure 27: Graphic View Example

7.4 Technical Details

The ISS code was written in C++, compiled with Microsoft Visual C++ 6.0 compiler for Windows95 platform, and run on a Pentium II workstation. The random numbers generator used for random operations was R250, A fast "shift-register" generator with a long (2^250) period. The algorithm is described in [61]. The code for the random numbers generator was obtained from [62]. Results were analyzed and visualized using MS-Excel 97 and Matlab 5.2.

8. Appendix B: Detailed Parameters

No	Parameter name	A	B	C	D	E
1.	LATTICE_SIZE_X	128				
2.	LATTICE_SIZE_Y	128				
3.	NUMBER_OF_T_HELPERS	1000				
4.	NUMBER_OF_T_KILLERS	1000				
5.	NUMBER_OF_B_CELLS	1000				
6.	NUMBER_OF_DENDRITICS	1000				
7.	NUMBER_OF_SELF_ANTIGENS	0				
8.	ANTIGEN_INJECTION_X	64				
9.	ANTIGEN_INJECTION_Y	64				
10.	ANTIGEN_INJECTION_QUANTITY	10				
11.	ANTIGEN_INJECTION_INTERVAL	1500				
12.	PEPTIDE_LENGTH	23				
13.	PEPTIDE_MATCH_THRESHOLD	18 ; 20 ; 22 ; 23	23	23	23	23
14.	T_KILLER_ACTIVATED_TIME	100				
15.	T_KILLER_CLONE_SIZE	5				
16.	T_KILLER_MEMORY_MODEL	Linear-rand.	Linear-rand.	Linear-avg. affinity.	Linear-rand.	Parallel-rand.
17.	T_KILLER_MEMORY_SURVIVAL_RATE	0.02	0.02; 0.025; 0.03; 0.1; 0.17	-	0.02 ; 0.18	0.05 ; 1
18.	T_KILLER_MEMORY_MIN_THRESHOLD	-	-	0		
19.	T_KILLER_MEMORY_MAX_THRESHOLD	-	-	20 ; 24	-	-
20.	T_KILLER_MEMORY_POWER_BASE	1.8				
21.	T_HELPER_ACTIVATED_TIME	100				
22.	T_HELPER_CLONE_SIZE	5				
23.	T_ HELPER _MEMORY_MODEL	Linear-rand.	Linear-rand.	Linear-avg. affinity.	Linear-rand.	Parallel-rand.
24.	T_ HELPER _MEMORY_SURVIVAL_RATE	0.045	0.026; 0.04; 0.05; 0.12; 0.21	-	0.03 ; 0.23	0.05 ; 1
25.	T_HELPER_MEMORY_MIN_THRESHOLD	-	-	0	-	-
26.	T_ HELPER_MEMORY_MAX_THRESHOLD	-	-	20 ; 24	-	-
27.	T_ HELPER _MEMORY_POWER_BASE	1.8				
28.	B_CELL_ACTIVATED_TIME	5				
29.	B_CELL_CLONE_SIZE	2				
30.	B_CELL_NUMBER_OF_ANTIBODIES	3				
31.	B_CELL_MEMORY_SURVIVAL_RATE	0				

Table 5 : Simulation parameters used for experiments. For all other parameters default values, listed in Table 4, were used.
A: parameters of interaction affinity, memory threshold and initial repertoire experiments
B: parameters of memory cells in the primary response experiments
C: parameters of linear differentiation by highest affinity selection experiments
D: parameters of linear differentiation by random selection experiments
E: parameters of decreasing potential differentiation by random selection experiments