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SIMMUNE, a tool for simulating and analyzing Immune System behavior *

M. Meier-Schellersheim, G. Mack
II.Institut für Theoretische Physik
Universität Hamburg†

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Abstract

We present a new approach to the simulation and analysis of immune system (IS) behavior. The simulations that can be performed with our software package called SIMMUNE are based on immunological data that describe the behavior of IS agents (cells, molecules) and the IS's challengers (bacteria, viruses) on a microscopical (i.e. agent-agent interaction) scale by defining cellular stimulus response mechanisms. All processes within the simulated IS are based on these mechanisms. Since the behavior of the agents in SIMMUNE can be very flexibly configured, its application is not limited to IS simulations.
We outline the principles of SIMMUNE's multiscale analysis of emergent structure within the simulated IS that allow the identification of immunological contexts using minimal *a priori* assumptions about the higher-level organization of the IS.
Keywords: immune system simulation, locally interacting agents, multiscale analysis.

1 Introduction

For quite a long time immunological research limited itself mainly to the investigation of molecular details of cellular mechanisms within the immune system (IS). The structure of immune responses was believed to be rather simple: Upon infection of the organism IS agents had to 'recognize' the foreign material that caused the infection, the *pathogen*, with highly specific receptors and then remove everything within the organism that would bind to these receptors. To

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†email martin.meier-schellersheim@desy.de, mack@lienhard.desy.de

make this principle work, the IS only had to ensure that it did not produce receptors complementary to components of host organism.
From this point of view, developing a vaccine against some kind of disease thus simply meant searching for the right (harmless) fragment of the pathogen that could be presented to the IS as an *antigen* (a substance that provokes an immune response). Having recognized this fragment once, the IS would memorize this knowledge through maintaining a large enough number of the right receptors and then upon contact with the real pathogen roll out its arsenal of defense quickly enough to avoid a spreading of the pathogen within the organism.
In many cases vaccine development is more complicated. The IS normally looks for more than just one signal (the antigen) before it produces a full response. Given that the IS can hardly rule out every receptor that fits to material of the own organism without crippling its own functionality because of the degeneracy of receptors [1] it is understandable that it should ask for more information.
(For a mathematical discussion of the problem of immune receptor specificity see for example [1].)
Providing the right *adjuvans*, the biochemical context within which the antigen is seen by the IS, is important. Adjuvants often contain fragments of classical pathogens, i.e. pathogens the organism and its ancestors have been accustomed to for a long time. Being confronted to these pathogen fragments the IS more readily switches into 'defense mode'.
Unfortunately, within certain contexts the IS readily accepts the organism's own material as antigenic. Autoimmune diseases like diabetes are examples where the IS, once it finds itself in a certain context, attacks its own host.

Cohen [2] formulated the *cognitive paradigm* postulating that cognitive abilities - enabling the IS to select its response according to the context of the presentation of antigen - were an indispensable ingredient of IS behavior.
Grossman [4] explained why we should investigate the IS's context recognition and proposed some models for appropriate cell behavior.
Segel and Bar-Or [5] investigated the question how the IS's cells might be able to optimize their contribution to immune responses with the help of feedback mechanisms and compared the IS to other systems of decentralized organizing agents. Segel [6] also presented the idea of a *diffuse informational network* of cytokines encoding the molecular context the IS finds itself in. Atlan and Cohen [7] pointed out that the IS must achieve the ability to extract – in a cognitive process – *meaning* from the wealth of *information* its cells gather via their receptors.
Often, the term 'recognition' in an immunological sense refers to the ability of the IS to provide receptors that can bind to the surface of foreign material with high enough affinity to direct an effective immune response against this material. Recognition of this kind comes down to the question whether molecular

[1] IS receptors usually fit to more than just one single molecular structure. The neccessity to be able to provide receptors to virtually any (foreign) molecular structure the IS might get confronted to, prevents the IS from destroying every receptor that fits to the material of its host organism.

shapes are complementary while the ability to act context dependent requires the IS's agents to mutually coordinate the processing of the molecular signals which they receive from their milieu.
How could the IS's information processing work to achieve context recognition? What is the nature of the context, that make the IS respond in a protective (or harmful) manner? How is the IS provided with the specific context that would activate an immune response to some threatening disease?
To be able to start answering these questions, we need to investigate how the agents of the IS exchange information, how they influence each other's states, how their reactions to combined signals differ from their reactions to these signals when occurring at different times. Further, what is the spatial scope of specific signals, *i.e.*, do they influence only their direct neighborhood or do they spread over larger areas of the organism?

The software package SIMMUNE which we want to introduce here was designed to facilitate simulations dealing with these questions.
SIMMUNE simulates the IS on the agent level, *i.e.* on the level of interactions between cells and molecules. The analysis that is performed on the simulations, however, operates on multiple scales; this is described in section 3.4. Application of the approach of identifying immunological contexts by multiscale analysis to more elaborate simulations will be described in a forthcoming publication [15].

In section 2 we will briefly describe some of the classical methods of IS modelling to be able to point out the differences and similarities between them and the approach presented here. In section 3 our software package SIMMUNE will be introduced. We will give an overview over its structure and present some simple examples of SIMMUNE applications, as well as some of the methods to analyze the simulations. Section 3 concludes with some remarks on the limitations of the current version of SIMMUNE.
In section 3.3.2 we will describe some immunological mechanisms as far as they are implemented in the example simulations. Descriptions of immunological mechanisms refer to the way they are implemented in the simulations which we present here. In those cases where they differ fundamentally from their real biological counterparts we will mention this.

2 Modelling IS Behavior

The possibilities of tracing directly the complex sequences of interactions in real ISs of living organisms are very limited. However, Jenkins [9] has presented a method of monitoring the activities of selected cell clones *in situ*, i.e. in the living organism. (A definition of 'cell clones' as they are implemented in our simulations will be given in chapter 3.3 .)
Various techniques of IS modelling have been developed that allow to investigate theoretically different aspects of immunology. Perelson and Weisbuch [1] have provided a comprehensive survey of this area.

2.1 Reaction-Kinetics Models

The great complexity of IS behavior is due to the large number of different types of IS agents (cells, molecules) that can interact with each other in various ways. Deriving the system's behavior from the interactions between its many constituents is one of the goals of theoretical immunology.
Abstracting from the specific details of interaction between the IS's agents one can formulate systems of coupled differential equations describing how the time development of an agent's concentration in the modelled organism depends on the concentrations of other types of agents. We call these models *reaction-kinetics* models as they bear resemblence to models of reaction kinetics in chemistry. A very simple example for such a system is the following.

$$\begin{aligned} \frac{dI}{dt} &= p_{infect} IC - p_{kill} IK - d_I I \\ \frac{dK}{dt} &= p_{resp} IK - d_K K \\ \frac{dC}{dt} &= s - p_{infect} IC - d_C C \end{aligned}$$

Using as a shortcut notation I, K and C for agent names as well as for their concentrations, the equations above describe a situation that may be interpreted as follows: Infectious agents of type I transform agents of type C into new agents of type I upon contact with a rate p_{infect}. Agents of type I get removed (killed) upon contact with agents of type K with a rate p_{kill}. Agents K proliferate upon contact with I with a rate p_{resp}. Agents of all three types die naturally at their specific rates d_I, d_K, d_C. C type agents are produced at a constant rate s. K could be considered to be an immune system cell type

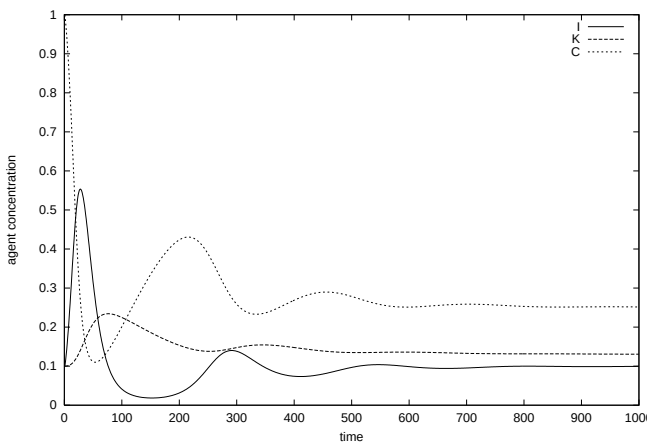

Figure 1: time development of agent concentration in the simple IS model

being produced as a response to the appearance of the infectious I. C may be

presenting any possible type of target cell for the pathogen I.
Integrating this system of equations yields different kinds of time development for I, K and C depending on the parameters p_{infect}, p_{kill}, p_{resp}, s, the death rates d_x and of course the initial values of I, K and C.
Fig. 1 shows the time development of the agents for one set of parameters (see appendix). We see how at first the number of infected agents I grows while the number of 'healthy' agents C decreases. Then, as the response from the IS agents K grows, the number of infected agents declines while C recovers. Finally the system ends up in a steady state that may be interpreted as a chronic infection: Infectious agents persist even though the IS constantly prepares agents of type K to suppress the infection.
Other sets of parameters may lead to stronger oszillations of the agents concentrations. Fig. 2 shows in a half-logarithmic plot a system that settles down into a steady 'chronic infection' state after having gone through states where the concentration of I is very low.

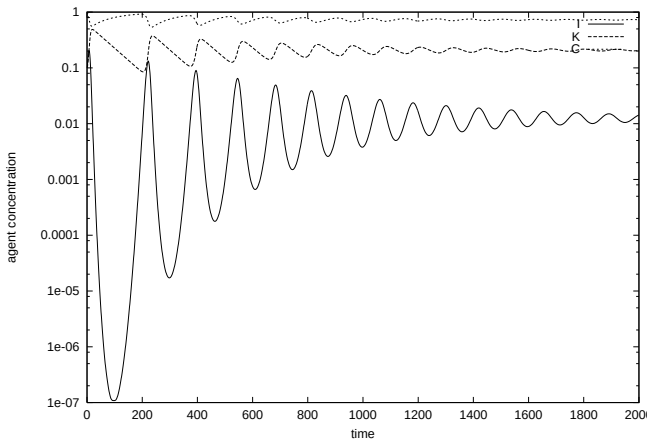

Figure 2: The model IS settles down into 'cronic infection' after oscillations with very low concentrations of I.

Fig. 2 illustrates one of the limits of applicability of simulation results of the *reaction-kinetics* approach: In a real system of interacting entities at a given time all agents of a certain type may have dissappeared due to destructive interaction with other agents (in our example, such an interaction is for example the suppression of I by K). In the *reaction-kinetics* approach there are no individual agents. Even agent types with unrealistically low concentrations may experience a 'comeback'. Of course, this problem can be avoided by appropriate setup of the model, but it points out one of the weaknesses of the approach: *Information* is *global* in *reaction-kinetics* models. In contrast, information in nature is something *local*.
In physics, this principle of *locality* is one of the major foundations of modern

theories. It lies at the heart of field theory and can be used as a starting point for entering Einstein's General Theory of Relativity.
In immunology, the locality of information processing by the IS's agents may prove to play an important role too.

2.2 Automata

Automata models of the IS neglect the microscopic details of IS behavior. They identify a set of characteristic states of the IS (like 'in rest' or 'with infection') together with transition rules that define how the automaton may switch from one state to another.
At discrete timesteps the state of the automaton is evaluated and the rules are applied to define the automaton's state for the next timestep.
Atlan and Cohen [10] investigated the effects of suppressor T cells using a neural network automaton.

2.3 Cellular Automata

Cellular automata (CA) were invented to investigate how simple building blocks could locally cooperate to produce aggregates with interesting behavior. The building blocks are automata living on a grid. Their rules define how the change in state of a single automaton at the next time step depends on his own current state and the states of his direct neighbor automata.
Clearly, the most fascinating aspect of CA modelling is the fact that even rather simple transition rules together with strictly *local* interactions can lead to very complex behavior of automata aggregates. An overview and classification of different types of CAs can be found in [11].
In immunology we encounter a similar situation – all IS activities are based on the actions of cells reacting to their direct neighbor cells and molecules. There is no central supervision of immune responses. Nevertheless the IS manages to coordinate the actions of its constituents over larger spans of space and time.

Celada and Seiden [12] developed a CA model of the IS where the cells are simulated by automata that may (as a modification of the usual CA concept of static correspondence between information and position) carry their state information with them while they are moving on a 2-dimensional grid. Depending on the agents (cells, molecules) they encounter they may change their state *e.g.* from *naive* to *activated*.
With their simulation program called IMMSIM Celada and Seiden were able to investigate a number of IS phenomena, for example *Affinity maturation and hypermutation [...] of the humoral immune response* [13].

3 SIMMUNE

SIMMUNE is an attempt to derive IS behavior from immunological data that describe the behavior of the cells of the IS on a microscopical level by defining cellular stimulus response mechanisms.
A cellular stimulus response mechanism (*cellular mechanism* for short) consists of a description of a set of stimuli a cell needs to experience before it performs certain actions, and a description of those actions.
Metaphorically, a mechanism thus may be considered to be a set of conditional actions: The cell checks whether certain conditions are fulfilled and if they are, it performs certain actions.
In SIMMUNE, the condition part of a mechanism can consist of an arbitrary number of conditions that can be combined through logical *AND* or *AND NOT*. The action part may consist of one or more actions. Fig. 3 illustrates the concept of a cellular mechanism.

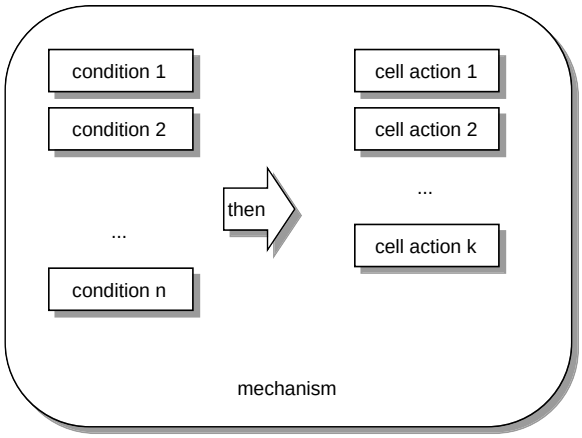

Figure 3: cellular mechanism

Fig. 4 presents an example of a cellular mechanism. There, the stimuli consist of the $B{:}C$ complex and the D molecule on the cell's surface.

3.1 Components of SIMMUNE

3.1.1 Celltypes

Like in cellular automata models, in SIMMUNE too cells are individual entities that interact with each other only locally. The cells live on a 3-dimensional grid. Each cell in a SIMMUNE model belongs to a certain cell type that is defined by the set of mechanisms according to which cells of a this type act. SIMMUNE cell types need not be equivalent to cell types as they are identified

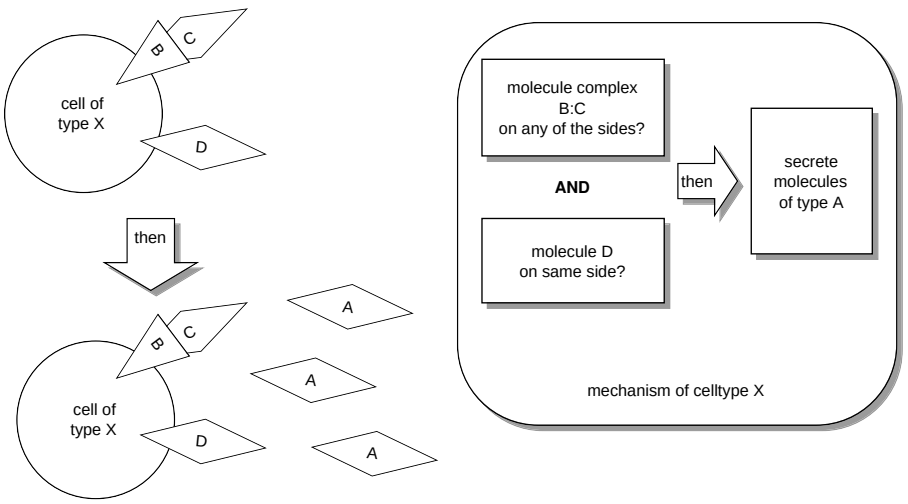

Figure 4: an example of a cellular mechanism

in experimental immunology.[2]
It is important to notice that mechanisms in SIMMUNE do not define cell *states* (that would be described by a fixed set of attributes, like certain types of molecules on the cell's surface) but cell *behavior*, while the usual approach in CA immune system models is to define a set of cell states and the rules how cells may switch from one state to another (cellular actions simply being the process of changing the cell's state).
Depending on the stimuli they receive from their environment, cells in SIMMUNE may change their attributes in various ways according to the mechanisms of their type. They may express receptor molecules on their surface, incorporate material from the extracellular milieu, secrete certain messenger substances, kill neighbor cells or move into a certain direction. They may also have part of their mechanisms modified (for example as a consequence of viral attack) without completely changing their type.[3]

In SIMMUNE several conditions, each checking a simple attribute of the cell's state may be combined in the condition part of a mechanism. A condition for a given cell action may be fulfilled in many different ways – all of which may bear specific information for other mechanisms.
Fig. 5 illustrates how the mechanism based approach of SIMMUNE is able to provide more flexibility in describing cell behavior than IS models that com-

[2]Interestingly enough the categorization of IS cells into rigidly distinct cell types has often been a controversial subject in immunology. Currently this is the case for T-helper1 vs. T-helper2 cells.

[3] Here, the analogy between sets of cellular mechanisms in SIMMUNE and the genetic encoding of cellular behavior in real biological systems is obvious.

pletely define cell states and transitions between them.

Besides its mechanisms, cell type properties in SIMMUNE include typical (mean) lifetime and the size of the cells.

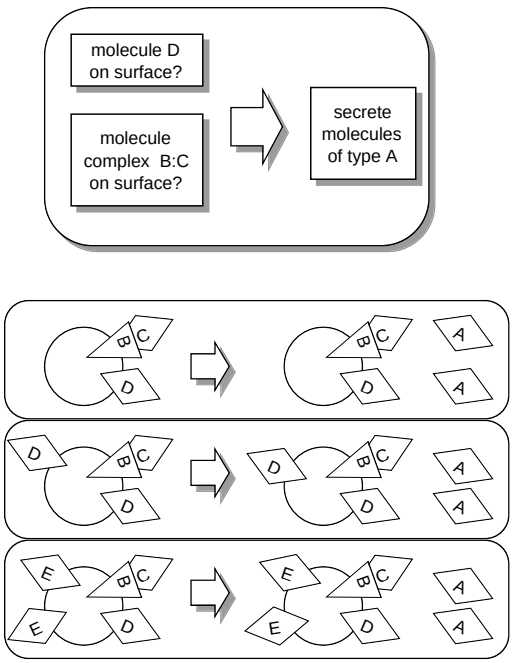

Figure 5: different ways of satisfying common conditions

3.1.2 Molecules

In SIMMUNE cells are the basic units of signal- and information processing. The signals they receive consist of molecules from the extracellular milieu that bind to the receptor molecules which the cells display on their surfaces. (Molecules also perform signal- and information processing simply by forming aggregates according to their mutual binding possibilities. In real ISs certain kinds of molecular aggregates are responsible of attacking cellular pathogens as a first line of defense before the IS has build up its complete arsenal of effector cells. The death of a cellular pathogen as a reaction to contact with the above mentioned molecular aggregates would, however, be encoded as a *cellular* action in SIMMUNE.) The significant properties of a molecule hence are described by the set of its possibilities to bind to other molecules.
A part of a molecule that is visible to the milieu and hence can be used to bind

the molecule to receptors is called an *epitope*. Even though one usually only refers to the binding sites of 'normal' molecules as epitopes, we treat receptor binding sites just as 'normal' molecule binding sites and call them epitopes too. The binding possibilities of any molecule are thus defined by the binding possibilities of its epitopes.
Other molecule properties in SIMMUNE include the typical lifetime of the molecule before it desintegrates into its fragment molecules and the types of fragments that are produced upon desintegration of the molecule.

3.1.3 Compartments

The effects of many interactions in the IS are local by their nature. An example is the cell-cell communication via contact receptors. But moving molecules or cells may influence all those parts of the organism which they can access.
To provide the right specific milieus for the different tasks it has to fulfill, the IS uses different compartments. For example, as mentioned above, the IS needs to avoid producing too many cells with receptors with high affinity for material of the own organism. It achieves this by establishing a central 'school' compartment, the *thymus*, where cells of a certain very important IS celltype, the *T-cells*, are tested to be useful and to be not too self-reactive before they are allowed to start their immune activities.
Another wellknown type of IS compartment are the *lymph nodes*. They are used to bring together the different cell types of the IS for information exchange.
Every type of IS cell may be considered to see a different aspect of an antigen. Hence, in order to exploit all the information available about the antigen, the IS needs to provide the lymph nodes as meeting points for the different cells carrying different pieces of information. From a simplified and abstract point of view, compartments simply gather certain kinds of agents while excluding others. A simulation with SIMMUNE may comprise different compartments.

3.2 Operation of SIMMUNE

Besides the properties of cell and molecule types, SIMMUNE lets its user define the properties of the compartments within the simulated IS. Dimensions of the compartments, diffusion rates of molecules and cells within the compartments as well as initial concentrations of the different types of agents can be given. Furthermore, the exchange of agents between the different compartments can be regulated: which kinds of agents are allowed to pass from one compartment to another and at which rate.
SIMMUNE offers a graphical user interface that can be used to watch agents' concentrations and the spatial distribution of cells or manipulate the running simulation by injecting new cells or molecules.

3.3 Example Applications

3.3.1 Local vs. Global Interaction between Agents

This example is meant to demonstrate with a very simple model differences between a *reaction-kinetics* simulation and a simulation with locally interacting agents. Five types of cells interact as illustrated in Fig. 6.
ID0 cells proliferate upon contact with cells of type *OC*. Upon contact with certain *cytokines* (signal molecules) they transform into cells of type *ID1* or *ID2*, depending on the kind of cytokine they register. *ID1* and *ID2* themselves produce the cytokines C_1 and C_2 that make their precursor $ID0$ switch into their own state respectively. *AID* cells kill all *ID* type cells upon contact.

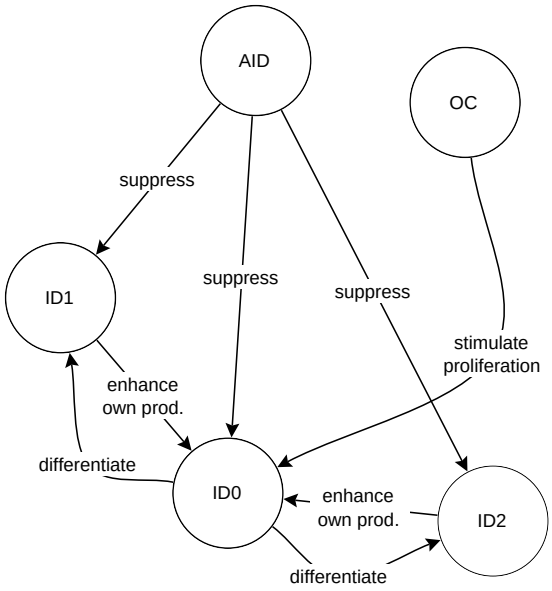

Figure 6: Network of cell interactions in the 'Local vs. Global Interaction' simulation

Translation of this interaction network into a system of coupled differential equations leads to a situation with an unstable equilibrium between the two cell types *ID1* and *ID2*. Because of the positive feedback between *ID1/ID2* and *ID0* small deviations from equal concentrations of both types may push the system into nearly exclusive production of one type.

Simulation of this system with locally interacting agents yields a different behavior. The cytokines that are secreted by *ID1* and *ID2* do not instantly spread all over the compartment. If the cytokines' lifetimes are short and diffusion is not too strong, *ID1* and *ID2* act with their cytokines only on *ID0* cells that are located within a small neighborhood of themselves.
The parameters of the simulation are given in the appendix.

Fig. 10 in the appendix shows a cut through the compartment after the simulation has reached a state of dynamic equilibrium. Neither *ID1* nor *ID2* have achieved global dominance. Instead, the two competitors cluster in areas with a diameter of typically 5-10 cells. Another interesting effect is that the *AID* cells do not appear inside of these clusters. Although they perform free random moves (controlled only by the availability of free space) they remain on the clusters' surfaces. One is reminded of the situation of a water drop on a hot stove: while the cells on the surface of the cluster get killed, those cells inside the cluster manage to survive.

3.3.2 A simple Immune System

Here we demonstrate a simple IS model simulation. The simulation includes five types of cells, three of which may go through differentiations during their lifetimes. Furthermore one type of virus is part of the model.
Most of the cellular mechanisms in this model are strong simplifications of the processes in real ISs. The reason why we present this simulation is that all of its behavior results from direct cell-cell respectively cell-molecule interactions and that it suffices as an example simulation that allows to describe how we analyze the behavior of the simulations and to explain the notion of an IS *context*. We will return to this in section 3.4 .

The first cell type *OC* is an organism cell not belonging to the IS. Cells of this type divide at a certain (low) rate. This reproduction is controlled by *contact inhibition*: *OC* cells have receptors on their membranes that are able to bind to receptors of the same type on the membranes of neighbor cells. If such a cell finds too many of these receptor complexes on its surface, it refrains from dividing.
OC cells are the target of the virus *V*. Upon contact with *V* the *OC* type cells get infected and start producing new viruses that are kept inside the cells. After some time however the cells burst and release their virus content to the extracellular milieu. Even though infected cells do not constitute a completely new cell type in immunological terminology they will be called *IC* type cells here to facilitate the discussion of the model and to identify them in the diagrams.
IC cells besides producing new viruses present virus epitopes on *MHC1* receptor molecules on their surface. They do not proliferate and have a shorter lifespan than *OC* cells.

T-Cells appear in three states of activity. As naive T-cells *NT* and activated T-killer (*TK*) or T-helper (*TH*) cells. *NT* cells possess two mechanisms. The first makes them express receptor molecules *TCR* on their surfaces, the second mechanism induces their transformation into *TK* or *TH* cells and proliferation (reproduction). Using a rather simplified T-cell activation scheme, we define the stimulus for this transformation to be the presence of a *TCR* being bound to a receptor molecule of type *MHC1* that besides its own epitopes presents an additional antigen epitope.

A receptor molecule *TCR* possesses a binding site that uses two epitopes. Both of these are selected at random. This means their binding possibilities (i.e. which epitopes they can bind to) are arbitrarily chosen. Each *NT* cell once in its lifetime selects its own (random-) epitopes. All *TCR* molecules produced by this cell will possess these two epitopes. When the cell transforms into a *TK* or *TH* cell it keeps this epitope choice; if such a cell divides, both daughtercells also will use these two epitopes for their receptors. Thus all *TK* cells stemming from a common *NT* cell will bind with their receptors to the same *MHC1*/antigen complexes. These cells are said to constitute a *clone*.
A *TCR* to be able to bind to a *MHC* must be able to bind to the *MHC*'s own epitopes as well as to the antigen epitope which the *MHC* presents.
Naive T-cells (*NT*) are stimulated to change their type and become (activated) *TK* or *TH* cells upon registering a *TCR:MHC* complex. *TK* cells react to such complexes by killing the cell that presents the *MHC* receptor. This is the way the IS tries to remove infected cells (*IC*) before they are able to release their virus load to the milieu.
The *TCR*s of *TH* cells bind not to *MHC1* but to similar membrane molecules called *MHC2* that are used by B-cells (see below). Similar to the contact inhibition of *OC* cells, *TK* and *TH* cells have a contact mediated mechanism that controlls their proliferation. [4] They have receptors (called *FAS*) and their counterparts (*FAS-ligand*) on their surface. An activated T-cell that finds a *FAS:FAS-ligand* complex on its surface commits suicide.

Besides the T-cell response that needs direct cell-cell contact between IS effector cells and infected cells, the IS uses cells that – after having been activated – secrete molecules which may bind to the virus particles to mark them for later destruction. These cells are called B-cells and the marker molecules they produce are called *antibodies* (*AB*). Similar to the T-cells, the B-cells exist in our simulation as a pre-activation type *NB* (naive B-cell) and as activated B-cells of type *B*.
The *NB* cells express on their surface B-cell receptors called *BCR*. The epitopes of these receptors are randomly determined – analogously to the random epitope selection of *NT* cells. Some of the *NB* cells may possess *BCR* molecules on their surface that are able to bind to the virus *V* while others may possess receptors that bind to molecules that are used by the cells of the IS. The *NB* cells possess mechanisms that make them present the epitopes of anything which got bound by their *BCR* to the extracelular milieu with the help of membrane molecules called *MHC2*. If a naive B-cell encounters a T-helper cell *TH* that has *TCR* which bind to the *MHC2* of the B-cell (including the additional epitopes the *MHC2* presents) the B-cell gets activated. Activated B-cells start secreting antibodies AB that have the same random epitopes that were used by the *BCR*s of the naive cell. These antibodies are hence capable of binding to the virus that activated the T-helpers. The viruses that are marked for destruction by *AB*s

[4] Knowledge about the mechanisms that are used by *real* ISs to controll T-cell proliferation seems to be still rather limited.

are removed by cells of type *macrophage*. These cells have receptors that fit to a non-random binding site of antibodies. In this way they are able to destroy the viruses that are bound to antibodies.

Fig. 7 shows the behavior of this simplified IS. The system starts with a certain concentration of OC that quickly enters a plateau concentration. Then the virus V is injected at a high dose and infection spreads: the concentration of infected cells IC grows. After a while, infected cells meet NT cells that possess the appropriate receptors to react to the MHC presenting the virus-epitope. T-helpers and -killers appear. T-cell proliferation stops at a certain concentration of T-cells as the encounters between T-cells and thus the *FAS:FAS-ligand* induced T-cell death get frequent.

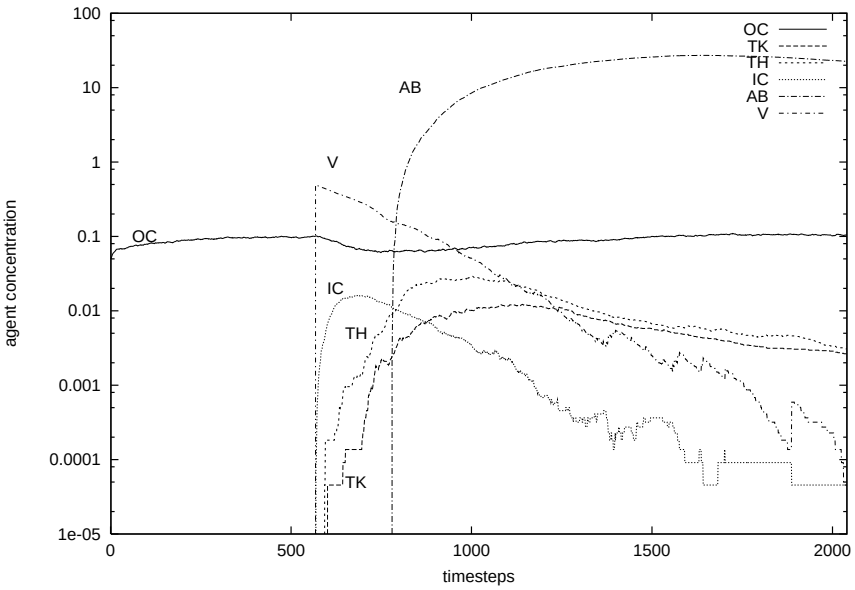

Figure 7: time dependency of the concentration of organism cells (OC), T-killer cells (TK), T-helper cells (TH), infected cells (IC), antibodies (AB) and virus (V) in the simple IS model.

B-cells, once being activated by T-helpers, start producing AB. As the T-cell concentration grows, infected cells are effectively removed from the system. High antibody concentration allows the removal of so many viruses that the rate of infection of OC finally gets low enough to let the organism experience reconvalescence - the OC concentration returns to its (contact-inhibition controlled) plateau. As in reality, the antibodies in our simulation not only mark viruses for destruction, they also may block the binding sites of the viruses and prevent them from attaching themselves to their target cells IC.

3.3.3 B-Cell Activation

B-cells in real ISs may be activated in two different ways. One of them depends on the assistance of T-helper cells, but B-cells are also capable of responding to certain kinds of antigens directly, i.e. without the assistance of T-cells. These antigens need to possess several indentical epitopes at the right distance from each other as to allow a simultaneous binding of more than one B-cell antigen receptor (BCR). Polysaccharides as they appear on the membranes of bacteria have this property. The aggregation of several antigen receptors on the membrane of a B-cell triggers a cascade of intracellular mechanisms of the B-cell that leads to its activation. The cell will differentiate into an antibody secreting cell and will proliferate.
This T-cell independent B-cell activation features an interesting dose-response curve. Instead of inducing a stronger response, very large concentrations of an antigen lead to an attenuated B-cell activation. The reason for this is that if the antigen is present in abundance, the probability is rather high that each of the receptors gets bound by an antigen molecule of its own. The probability of B-cell receptor aggregation decreases. (For a mathematical discussion of this effect as well as for a list of references to work on real ISs see [1].)
In our computer-experiment we inject a large dose of antigen (AG) concentrated at one of the walls of the compartment. While the antigen diffuses we investigate the B-cell activation by looking at the concentration of the molecules A that are produced by the activated B-cells in slices of the compartment parallel to the gradient of the antigen concentration.

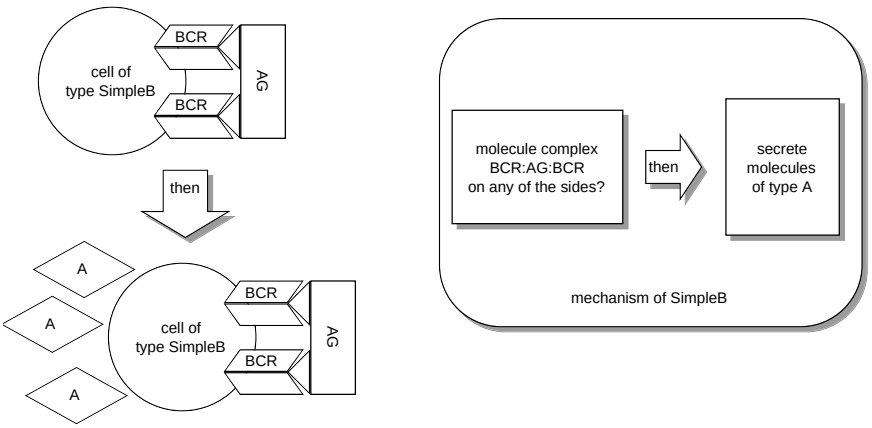

Figure 8: mechanism of simple B-cell: cross linking of two B-cell receptors makes the B-cell secrete A.

In the simulation simple B-cells are created with the ability to distinguish signals from six different sides. They possess one important mechanism which

is illustrated in Fig. 8.

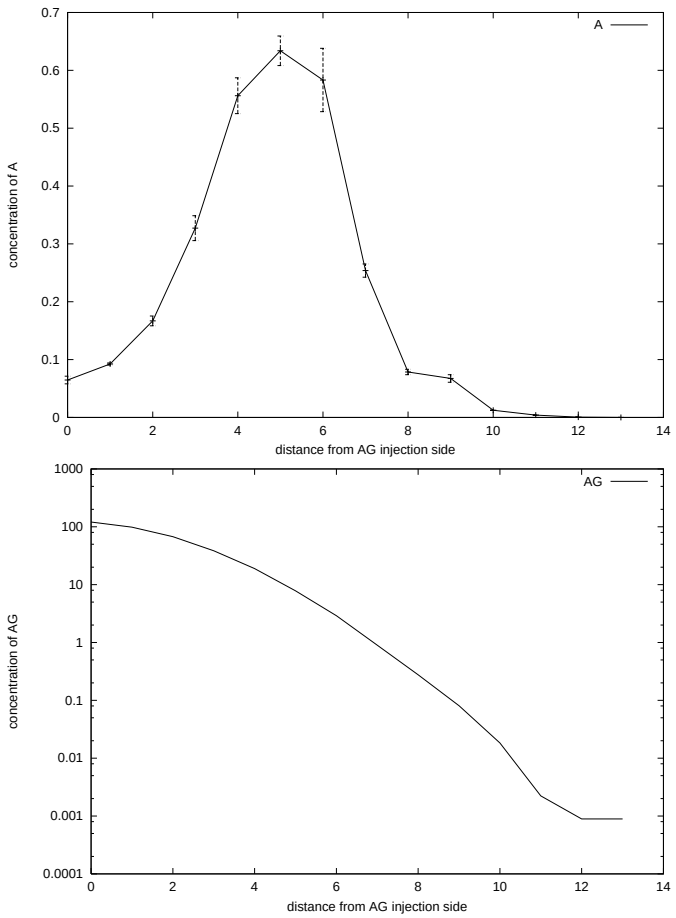

Figure 9: Spatial variation of the concentration of molecules A produced by activated B-cells and of the concentration of activating antigen AG.

The binding of the AG-epitopes by the receptors is reversible (with equal reaction constants for binding and release of AG) and the A molecules desintegrate after a certain mean lifetime. The AG molecules are stable. The resulting concentration of A thus indicates how successfully receptor-antigen-receptor aggregates are produced. Fig. 9 shows the number of AG and A molecules per cell as a function of the distance from the wall of the compartment where the high antigen concentration was initially injected. The maximum of the concentration of A is located around the area where 10 AG molecules per cell can be found. This corresponds – as expected – to just below 2 AG molecules per side of the cell.

3.4 Simulation Analysis

The main purpose of SIMMUNE is to provide a tool to investigate how context adaptive behavior of the IS might emerge from local cell-cell and cell-molecule interactions.
The most obvious kind of context dependent behavior of the IS can be found in the ability of single cells to react differently to a stimulus when it comes in combination with other stimuli as opposed to an isolated event. Thus, mechanisms with several conditons already encode context dependent behavior.
More difficult is the analysis of context recognition that emerges from cooperations between several cells. Cooperations between cells manifest themselves as correlations between the actions of these cells. A very primitive example from the simulation of section **3.3.2** would be the correlation between the *'kill'*-action of T-killer cells and the *'die'*-action of infected cells.
Whether a cell-cell interaction is of just local importance or part of a network of interdependent cell actions cannot be decided on the microscopical level of single cells. It requires to analyze on multiple scales the correlations between cell actions.
Analyzing the behavior of a system on multiple scales means identifying on each scale collections of objects that may be regarded as *single* composite objects on a coarser scale. This identification of composite objects is based on the recognition of common attributes or coherent behavior of the component objects. In order to allow an analysis that involves a series of scales that emerge from each other, the rules for the composition of objects must be applicable not only to the elementary objects of the original, finest scale but also to the composite objects of coarser scales.
In our case, the elementary objects are cell actions. Collections of them are identified to constitute composite objects if they are spatially and temporally correlated. Iterating the process by applying the correlation analysis to the objects of the first coarse scale may yield a further scale that is coarser than the first in two aspects: The components of its objects are themselves composite objects and the correlations between the constituents of its objects involve larger spatial and temporal distances.
Starting from single-cell behavior we enter the scale of cell-cell cooperations by looking for correlations between cell actions. Looking for correlations between cell cooperations takes us to the next scale. Repeated application of this process might lead to a scale that directly describes the macroscopical behavior of the IS.
The network of interactions of our example simulation from section **3.3.2** leads to two (yet simple) coarse scales: On the first we register the correlations between the actions of IS effector cells like T-killer cells and non-IS cells like infected cells. The second coarse scale contains for example the correlation between T-cell activation and the appearance of activated B-cells.
Having explained how we proceed in order to look for context dependent behavior of the IS we are now able to give a definition of what we call a 'recognized immunological context': An immunological context that is recognized by the

IS[5] leads to a response of the IS that can be traced on multiple scales as described above. It can be distinguished from other contexts that are descibed by different patterns of correlation.

Recently, the principle of identifying biological contexts on different scales by iterated clustering of data has successfully been applied to the infrared analysis of human blood serum by Werner et al. [16].

Usually, models about higher level cooperative effects within the IS (like automata models that describe the behavior of the IS in terms of predefined states) must be based on assumptions that try to bridge the scale gap between the scale of direct cellular interactions and overall behavior of the IS.
We do not need to make such assumptions. Cooperative phenomena are detected using methods of statistical analysis.
Of course we pay a price for this: Since SIMMUNE is entirely based on direct cell-cell or cell-molecule interactions we need to make assumptions about cellular mechanisms whenever we make use of aspects of single-cell behavior that are not yet understood in detail.

3.5 Limitations of SIMMUNE

Due to the faithful modelling of cell behavior, computer power requirements of SIMMUNE are quite high. With currently available single processor computer power and memory the maximum number of cells (per computer processor) that can participate in a simulation is practically not larger than *500.000*.
Recently Smith et al. [14] presented a technique called *lazy evaluation* to simulate quite realistic clone numbers and sizes. *Lazy evaluation* makes use of the fact that out of the large number of clones only a small fraction is actually able to interact with a given antigen and the presenting cells of the IS. Only this fraction will be activated to proliferate and play an important role in the immune response. By simulating only this fraction while limiting its overall concentration to the value it would have with the full cell repertoire being existent, major memory and cpu-time savings can be achieved. To accelerate the simulation of those models that do not need to consider idiotypic networks (see [17] or *e.g.* [1]) future versions of SIMMUNE may adopt this technique.
Another limitation of SIMMUNE will not be circumvented by increasing computer power or techniques as lazy evaluation: As mentioned in the foregoing section, simulations with SIMMUNE sometimes have to involve mechanisms and parameters that have not yet been established by experiment. This often makes quantitative predictions difficult. On the other hand, the need for detailed descriptions of cellular mechanisms may point out gaps in immunological knowledge that have not yet been sufficiently investigated.

[5]Context dependent behavior of single cells as we mentioned above would be encoded in cellular mechanisms. We want to distinguish between such context recognition and the context recognition that emerges from multi-cellular cooperation.

3.6 Beyond Immunology

Since all celltypes are user-defined and the basic cellular actions of SIMMUNE are typically as general as 'expression of (user-defined) membrane receptors' or 'movement along gradients of concentrations of molecules', the software may be used to simulate populations of cells of any kind. It has already been used to simulate systems of neurons with some of the molecular structure of synaptic gaps between them.

4 Conclusion

Besides providing its user with the possibility to define in detail the behavior and properties of cells and molecules in simulations of the IS, the mechanism based approach of SIMMUNE yields the fundament for a new kind of simulation analysis in immunology. Multiscale correlation analysis of cellular actions may allow the automated classification of immunological contexts that have not yet been considered until now.
Institutes interested in working with SIMMUNE may contact us to receive a copy of the program together with a manual.

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A Simulation Parameters and Diagrams

Parameters for *reaction-kinetics* simulations from **2.1**:

figure	p_{infect}	p_{kill}	p_{resp}	s	d_I	d_K	d_C	I_0	K_0	C_0
Fig. 1	0.3	0.5	0.1	0.01	0.01	0.01	0.01	0.1	0.1	1.0
Fig. 2	0.3	1.0	0.8	0.01	0.01	0.01	0.01	0.1	0.1	1.0

Parameters for the simulation from **3.3.1**:

	OC	$ID0$	$ID1$	$ID2$	AID	C_1	C_2
initial concentration	0.05	0.01	0.005	0.005	0.01	0	0
av. lifespan (timesteps)	∞	∞	∞	∞	∞	100	100

molecular diffusion rate: 0.0001
cellular diffusion rate: 0.01
(The diffusion rate defines the per timestep probability for agents to get transported to a neighbor grid point. In this simulation cellular diffusion replaces active cell movement. The product of molecular diffusion rate and molecular lifespan defines the *mean* range of a molecular signal emitted by a cell.)

compartment dimensions: 80 x 80 x 10

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Figure 10: clusters in feedback simulation from **3.3.1**; symbols: $ID0$ o, $ID1$ $*$, $ID2$ $+$,AID $\#$