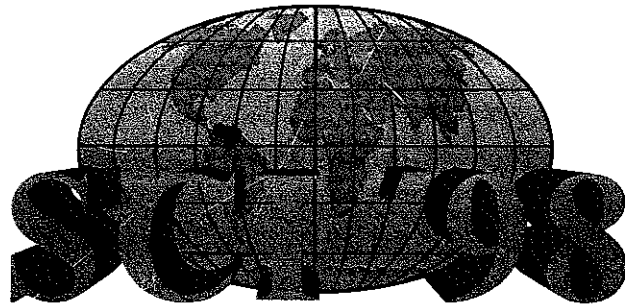**World Multiconference on SYSTEMICS, CYBERNETICS AND INFORMATICS**

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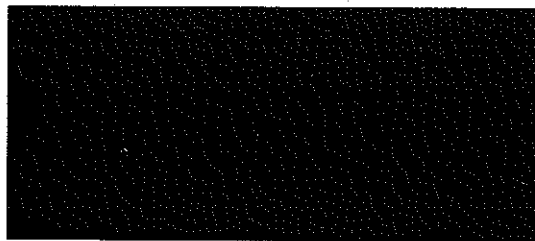Systems, Analysis and Synthesis)

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A Multiagent System to Model and Simulate In-Vitro Experimentations

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ABSTRACT

The models of immune mechanisms which can be simulated on computers are numerous. They can be based on a mathematical approach and mainly on differential equations. For this global approach, the problem is to determine the influence of a cell population on an other cell population This approach is particularly well adapted to the in-vivo phenomena simulation. In this case, the number of cells taken into account is very important ($n > 10^{12}$). Another model consists in the local description of a cell's behavior, and in the description of its receptors. The simulation manages to determine interactions between the cells. Therefore, global phenomena are seen as the emergence of all the individual interactions.

This last approach started in the early nineties with the work of Forrest on the receptor description and Seiden & Celada on the humoral response and thymus activity. The main advantages of such an approach are the modularity and its incremental aspect. The modularity allows a quite simple addition or removal of agents. The incremental aspect is the ability to easily improve the cell-agent model. This is the reason why the studies have been quickly extended by Smith on vaccine efficacy [SMI97a], by Seiden's team on rheumatoid factors [STE97] and Ballet on humoral response against HIV virus [BAL97] [BAL98b]. By now, these models have no geometrical constraints. Therefore we have decided to develop a multiagent system doted with geometrical constraints. Thus, we are able to simulate in-vitro experimentations into which the geometrical aspect is important. Thanks to the simple geometrical aspect, this study demonstrates that it is geometrical restraints, this study demonstrates that it is possible to simulate several in-vitro experimentations. We present in this paper our simulator and three in-machina experimentations. Each of them are compared with the real in-vitro tests.

Keywords: Multiagent System, Immunology, Simulation, Immunodosage, Immun Complex, B-CD5 Cell.

1. INTRODUCTION

Here is the list of the different sections we develop in this paper Firstly, the multiagent system is described in details. We continue with the presentation of our cell-agent. Their receptors, the signal interpretation and their behavior. After that, we deal with the interaction and receptor model. Thanks to this model, we are able to define complex cell-agents with various receptors on their surface. It is possible for each cell-agent to have multiple and temporary links. We also simulate the molecule internalization into a cell-agent. The cell internal description can be complicated and can express numerous behaviors.

Then, we expose the results of simulations such as an immunodosage test, the immun complex formation (with rabbit's antibodies) and B-CD5 cell apoptosis. All of these results are compared to real in-vitro experimentations and show their good qualitative validity. Moreover, we observe in simulation some identical geometrical configurations as in real experiments.

The third part of this paper deals with the limitations of our model.

As a conclusion, we describe the prospects of the model and its potential applications in immune research and education We think that this multiagent system could be used for the preparation of in-vitro experimentations and to show the students various immune phenomena.

2. MULTIAGENT MODEL

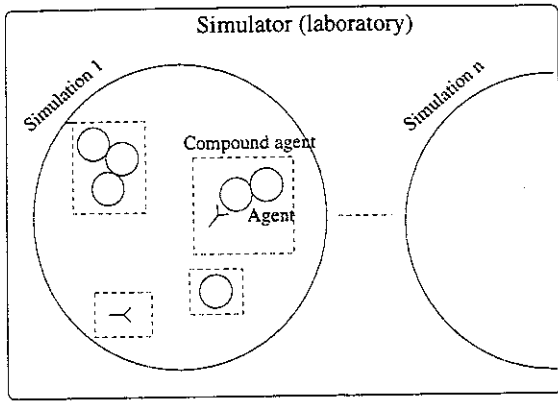In this section, we describe the basis mechanisms of the simulator.

Figure 1 The simulator and its components.

The simulator is made up of elementary agents grouped in compound agents. These agents evolve into a bi-dimensional environment. A compound agent is made up of a set of elementary agents. The elementary agents have their own behaviors and receptors. A behavior is an algorithm which describe the way an agent reacts according to the environment stimuli and its internal state. The stimuli come from receptors and the internal state depends on the past of each agent. In practice, each compound agent lives one after another and make the elementary agents live (Figure 1). For an elementary agent, to live means executing its behavior algorithm. Every agent which belong to one compound agent are physically bound by their receptors. To sum up, we can say that a compound agent is considered as a single entity concerning its moves but is a mosaic of agents when talking about individual behaviors. The moves are calculated according to the influences between the receptors. During simulations, the compound agents merge at the time of creation of one or several binds or, on the contrary, divide when bind breaks occur (Figure 2).
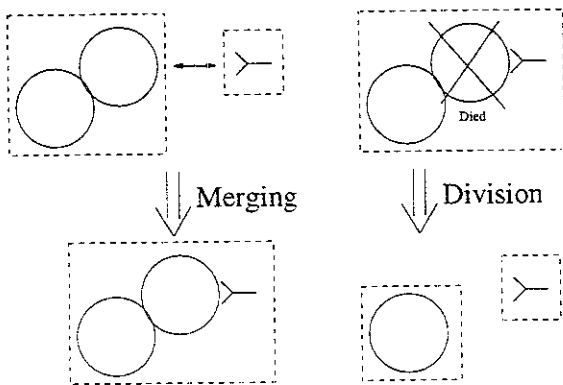

Figure 2 Merging and division of compound agents

Now, we are going to describe the agents, what they represent and how they are modeled

3. AGENT MODEL

An agent represents a cell or a molecule from the immune system. With regard to a cell, the agent is doted of a group of receptors on its surface as well as an often complex internal behavior (Figure 3). In the case of the agent representing a molecule, it is doted with a set of epitopes. Every agent is subject to the environmental rules. These rules only consist in subjecting the agents through their receptors to the influence of receptors of all every other agent. This influence involve a moving of agents (relocation and rotation). The environment physical rules into which the agents evolve are explained in the following section. Moreover, according to the stimuli they received thanks to their receptors, the agents modify their behavior and internal state. For instance, the interleukine B cell receptors involve the division of this cell when bound to the interleukine agent's receptor.

Thus, the agents only communicate through their receptors. As previously seen, even if the agent can bound together, they keep their own behavioral independance. A complex made up of several agents is also an agent (compound agent) doted with a center of gravity and a mass. This mass is the sum of masses of all its components. A compound agent is a single entity when talking about environment rules.
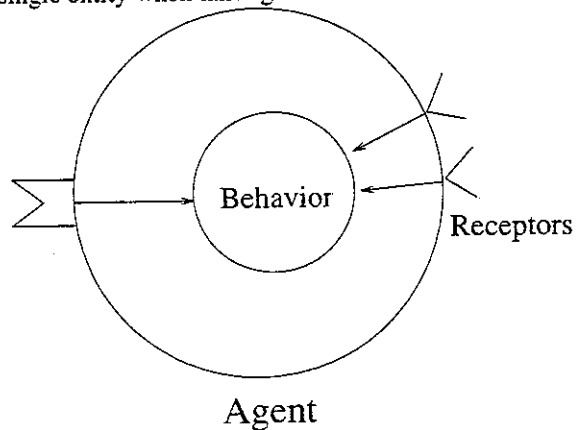

Figure 3. Agent description.

The binds are temporary. In fact, a break of bind may occur in three cases. Firstly, when one of the cells dies, the different bound receptors are free out and are able to bind again. The second case happens when the duration of the bind reaches a determined value. Lastly, in a case of molecule bound to a receptor, the agent internalize the receptor and its molecule. The agent takes care of the internalization. Then, the

internalized receptor and molecule are both deleted. A new creation of one or several receptors can occur.
The interactions between agents are based on the receptor influences. These interactions are detailed in the following section.

4. INTERACTIONS

Interactions between agents are made by their receptors. The influence of a receptor on another one is determined by their affinity and distance into the environment. An Euclidian distance or a Hamming distance value the affinity between two receptors [STE94] [DER97b]. In these cases, the receptor is represented by a p dimensional vector. For example, the antigen presentation can use the Seiden and Celada mechanism [SEI92a] [SEI92b]. Each receptor on the agent's surface acts on the compound agent moving. Therefore, a resultant is calculated from all the influences of the agent's receptors belonging to the compound agent. The compound agent moves according to the resultant (Figure 4).
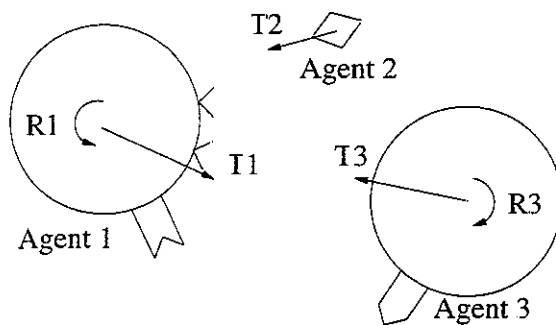
T = Translatory   R = Rotation

Figure 4. Interactions between agents.

As soon as the agents are described, it only remains to put them into their environment and to observe the result of both their behavior and interaction. The next section shows several examples of simulations representing real in-vitro experimentations.

5. SIMULATIONS AND RESULTS

Firstly, the aim is to verify that our simulator gives good results on a simulation of a simple in-vitro experimentation. Then, we demonstrate that with our simulator, biologists can create, try and check tests. Thanks to the simulator, they can design different tests and discuss about them before they do the real in-vitro experimentations.
We also think that the simulation is an important educational tool offering the students the ability to visualize the immune mechanisms. Besides, they can observe the impact of one or several parameters on the results and this quickly and cheaply.
Beyond the mistakes of measurement which appear in every in-vitro test, the simulation can give the criteria of accuracy for various kinds of experimentations.
We present hereafter three simulations of in-vitro experimentations. The first one is an immunodosage test commonly used for detecting diseases. The second simulation reproduces a classical immune test which is the complex forming of antigens – antibodies. The third simulation deals with the impact of the B cell agglutination on their behaviors.

5.1 IMMUNODOSAGE

The immunodosage using competition is used to detect the presence of antibodies directed against a given antigen. Effectively, it is possible to verify the contamination of an entity with, for example, a virus by testing the presence of antibodies directed against this virus. This can even be done without the appearance of the symptoms of the disease. It is the case for the Elisa Test which belongs to the variety of tests used for the detection of a person infected with the HIV virus.
We begin this section with the description of agents which are used for the simulation of immunodosage using competition, for instance RIA or Elisa. We continue with the drawing of a calibration curve.
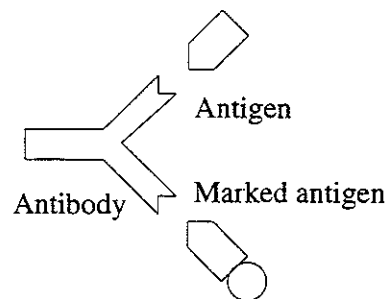

Figure 5. Agents of immunodosage.

For this simulation, we are using three different kinds of agents. The first one corresponds to antibodies fixed on the back side of the test tube. The second one represents the antigen to be detected (unmarked antigen) and the third type is the marked antigen (Figure 5). The antigens have a strong affinity with the variable parts of the antibodies.

Here is the description of the simulation. Firstly, we put the antibody agents in circle into the virtual test tube. These latest are fixed and they cannot move. Then, we put the marked and unmarked antigens and we observe the evolution of the number of marked

3

antigens bound to antibodies. The simulation stops when bind is no longer possible.

The curve thus obtained is a calibration curve (Figure 6).

This curve presents some strong similarities with the curve obtained in-vitro for this kind of dosage.

Therefore, our simulator allows to get a curve found during in-vitro experimentation, at least concerning the qualitative study. This is the reason why we found it interesting to continue in this way and to simulate other experimentations. The test describe in the following section consist in the simulation of immun complex formation. This simulation goes further than the immunodosage since in addition to the qualitative curve, we observe geometrical formations linked to the antibody and antigen morphologies
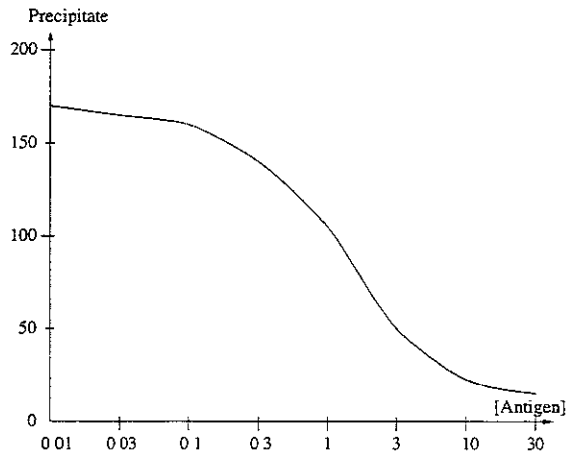

Figure 6 Curve of calibration.

5.2 IMMUNE COMPLEX FORMATION

The antibody – antigen bind leads to the formation of complexes which produce a precipitate. Thus, it is possible to determine the equivalent area, that is to say the area where the epitope concentration (antigens' receptors) is the same as the paratope concentration (antibodies' receptors). In this area, the quantity of precipitate reaches its maximum.

In pratice, a group of experimentations are made, each of them having the same antibody concentration, but different antigen concentrations. Usually, the goal of such test is to find out the area of equivalence. Since the number of antibodies is known, it is possible to determine the number of antigens
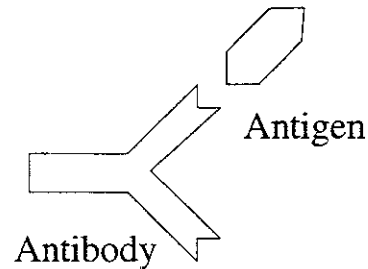

Figure 7 Agents of immune complex

For example, the quantity of precipitate is measured by immunonephelometry. This method uses the property of the immune complex to diverge the light of a laser beam [REV95].
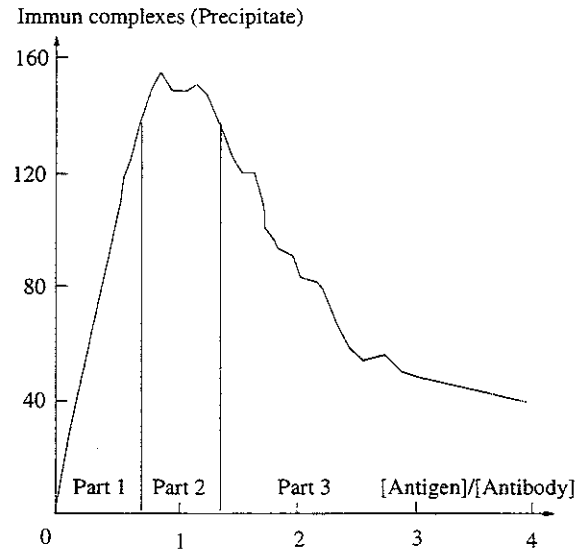

Figure 8 Curve of precipitate.

This experimentation is a classic immunological test and thanks to our simulator we are able to find again the main in-vitro results. We begin with the description of agents and we end with the simulation result study. This study is twofold. The first part focus on the quantity of precipitate observed in-machina and the second part aims at the geometrical structures created in-machina

We just need two kinds of agents for this experimentation. Antibody agents and bivalent antigen agents are required (Figure 7). In the case of monovalent antigens there is no precipitate. The receptors of antigen and antibody agent are complementary, that is they have a strong affinity.

During the simulations, we put a constant quantity of antibodies and increasing number of antigens. The simulation stops when there is no more receptor to bind. As soon as the simulation is over, we evaluate

the quantity of precipitate created. For this matter, every antigen bound with two antibodies are added.

For each in-machina experimentation, the quantity of immune complex obtained is memorized. This quantity is put on the graphic which give the number of immune complex according to the ratio antigens / antibodies (Figure 8). Again, we find the three area observed in-vitro, that is the linear area of excess of antibody (Figure 8-Part 1), the top of the curve in the equivalence area (Figure 8-Part 2) and the decreasing area of excess of antigens (Figure 8-Part 3).

We also find a group of geometrical structures made by the immun complex according to the ration antigens / antibodies (Figure 9). Then, the simulator can also be used to find various structural configurations.
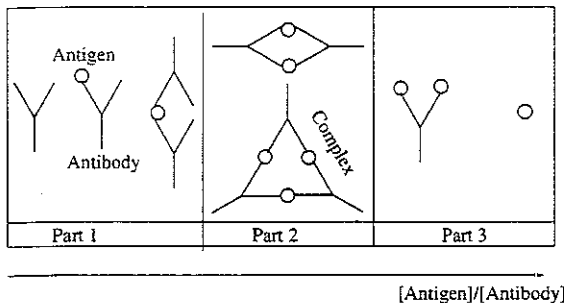

Figure 9. Immun complex formations

The results we have presented here are well known by biologists and we just have reproduced them thanks to our simulator. In the next in-machina experimentation, we use the simulation to test an assumption which has to be verified in-vitro. We see that the geometrical structures influence the results of the simulation. Effectively, interesting results of the impact of geometrical constraints are given in the following section for a set of experimentations made in the laboratory of immunology of Brest. We have simulated these in-vitro tests using our simulator.

5 3 B-CD5 APOPTOSIS

This experimentations which are made in-vitro by the laboratory of immunology of Brest aim at the determination of the CD5 and CD72 receptors utility on B cells [JAM96]. A detailed description of the in-machina experimentation made by [BAL98a] is available. In this paper, we keep to the study of the geometrical constraint impact on the results.

The experimentation we present here consists in measuring the impact of the injection of an antibody directed against the B cell CD5 receptors in two different moments. Thus, it is necessary to do two in-machina tests. In the first test, there is no anti-CD5

antibodies put into the virtual test tube. In the second test, they are placed when the number of B cell is at its maximum.
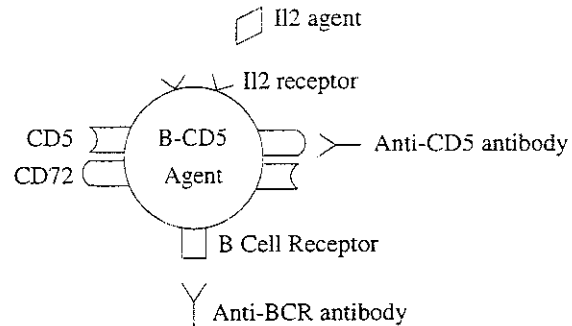

Figure 10. Agents implied in B-CD5 in-machina apoptosis experimentation.

In addition to the anti-CD5 antibody agents and the B cells, the simulation uses anti-BCR (B Cell Receptor) antibody agents which activate the B cells and interleukine 2 agents which are the growth factors of the B cells (Figure 10).
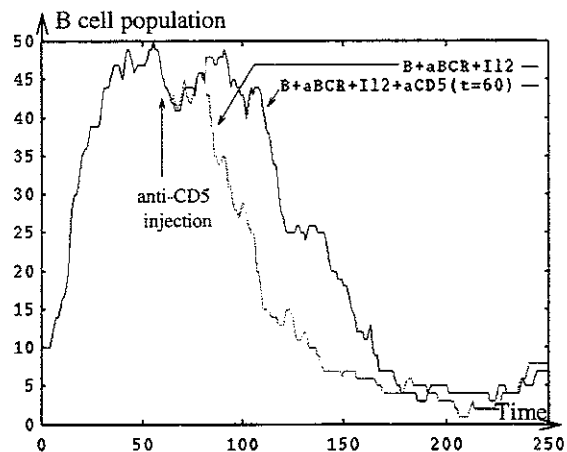

Figure 11 Evolution of the B-CD5 population

As the time goes by, the curve of B cell population is different if the anti-CD5 antibodies are put at the maximum of the proliferation (t=60) (Figure 11).

The assumption is that the anti-CD5 antibodies keep the B cells alive allowing them a latter apoptosis.

However, the simple action of the anti-CD5 antibody on a B cell does not explain the importance of the upholding of the population of B cells after the injection of anti-CD5 antibodies. Thanks to the simulation, we can say that at the maximum of the B cell population curve, the cells are agglutinated in complexes. Then, they strongly stimulate each other. The cells on the border of the complexes are the only one to be less simulated. This means that they rapidly

undergo the apoptosis. The dead cells do not stimulate the other cells any longer. Thus a chain reaction follows which leads to a dramatic decrease of the number of living B cells. On the contrary, if some anti-CD5 antibodies are injected just before the drop of the curve, they stimulate *a priority* the cells on the border of the complexes. Therefore, this delays the chain reaction (Figure 12).

These in-machina results have to be verified with a real experimentation. In fact, the simulation brings elements of reflection but today, it does not provide a ready-made solution. Moreover, the simulator has its own limits that we develop in the following section.
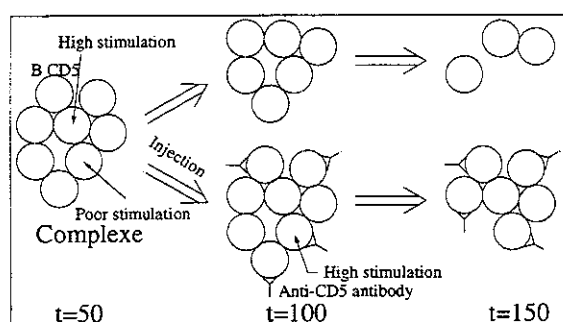

Figure 12. Anti-CD5 antibodies injection impact on B-CD5 cells.

6. LIMITS OF OUR SIMULATOR

A living cell is very complex. Today, it is not possible to entirely model it. Numerous biochemical mechanisms occur inside a cell. These one are not directly taken into account by our simulator. The modeling of its interior has to be done by a virtual cell or agent programmer in order to build the internal mechanisms. This is also the accuracy limit of our simulator. As for the simulation the apoptosis of B-CD5 cells, the internal biochemical evolution is globally modeled. The purpose of the next step of the simulator development is to increase the accuracy of the simulator by providing a group of agents composing a cell. The cell thus becomes a multiagent system.

Furthermore, during a simulation, all the parameters are known. On the contrary, during an in-vitro and *a fortiori* in-vivo experimentation, hazardous phenomena can happen. This is the reason why simulations cannot replace in-vitro and in-vivo experimentations. However the knowledge of all the parameters of the simulation allows to verify whether the known mechanisms can explain the studied phenomena or not. It is the case in the simulation of immun complex formation as well as in the

simulation of B-CD5 cell apoptosis. Finally, in-machina experimentations give some indications and main directions without pretending to exactly reproduce the reality. This is one the reasons why, our results are qualitative and not quantitative and they have to be taken cautiously. We must not forget that two different behaviors, two different mechanisms can have the same global result.

7. CONCLUSION

We have seen that with our immunological simulator, it is possible to reproduce various in-vitro experimentations. We find qualitative and structural similarities between the in-machina and in-vitro experimentation. We think that our simulator can work for numerous other tests to reproduce them, to create new ones, to test assumptions or to prepare in-vitro experimentions. The firsts to be interested in our simulator are the medical and biology students since it brings them flexibility and speed while allowing the visualization of the inter-cellular mechanisms. Besides, this simulator can be interesting for researchers in immunology or in other biological fields to test their assumptions and to prepare their experimentations. It can also help them to present their works in a visual and dynamic way. Finally the simulator should be interesting for dosages or tests of combination of molecules in pharmacy.

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