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Large Scale Agent-Based Modeling of the Humoral and Cellular Immune Response

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Abstract. The Immune System is, together with Central Nervous System, one of the most important and complex unit of our organism. Despite great advances in recent years that shed light on its understanding and in the unraveling of key mechanisms behind its functions, there are still many areas of the Immune System that remain object of active research. The development of in-silico models, bridged with proper biological considerations, have recently improved the understanding of important complex systems [1,2]. In this paper, after introducing major role players and principal functions of the mammalian Immune System, we present two computational approaches to its modeling; i.e., two in-silico Immune Systems. (i) A large-scale model, with a complexity of representation of $10^6 - 10^8$ cells (e.g., APC, T, B and Plasma cells) and molecules (e.g., immunocomplexes), is here presented, and its evolution in time is shown to be mimicking an important region of a real immune response. (ii) Additionally, a viral infection model, stochastic and light-weight, is here presented as well: its seamless design from biological considerations, its modularity and its fast simulation times are strength points when compared to (i). Finally we report, with the intent of moving towards the virtual lymph node, a cost-benefits comparison among Immune System models presented in this paper.

1 Introduction

The theory of clonal selection, formalized by Nobel Laureate F. M. Burnet (1959, whose foundation are in common with D. Talmage’s idea (1957) of a cellular selection as the basis of the immune response), suggests that among all possible cells, B and T lymphocytes, with different receptors circulating in the host organism, only those who are actually able to recognize the antigen will start to proliferate by duplication (cloning). Hence, when a B cell is activated by binding an antigen, it produces many clones, in a process called clonal expansion. The resulting cells can undergo somatic hypermutation, and then they can give rise to offspring B cells with mutated receptors. During the immune activity, antigens compete with these new B cells, with their parents and with other clones. The higher the affinity of a B cell to bind to available antigens, the more likely it will clone. This results in a Darwinian process of variation and selection, called affinity maturation. By increasing the size of those cell populations through clonal expansion, and through the production of cells with longer lifetime expectation, and then establishing a defense over time (immune memory), the immune system (IS) assures the organism a higher specific responsiveness to recognized antigenic attacks. In particular, on recognition, memory lymphocytes are produced. Plasma B cells, deriving from stimulated B lymphocytes, are in charge of the production of antibodies targeting the antigen. This mechanism is usually observed in population of lymphocytes in two subsequent antigenic infections. More in detail, the first exposition to the antigen triggers the primary response; in this phase the antigen is recognized and the memory is developed. During the second response, that occurs when the same antigen is found again, as a result of the stimulation of the cells already specialized and present as memory cells, a rapid and more abundant production of antibodies is observed. The secondary response can be elicited from any antigen, which is similar, although not necessarily identical, to the original one, which established the memory. This is known as cross-reactivity.

In this article we present two new computational models capable of capturing fundamental aspects of the IS, at two different complexity levels. A high-complex model, large scale agent-based, that embeds all of the entities and all of the interaction detailed above; thanks to this computational model we have successfully reproduced many IS processes and behaviors. In a second, low-complexity model, we show how a stochastic model based on Gillespie algorithm, captures major behaviors of the IS during a viral infection, even if we are borderline with the definition of “well-mixed” solution. The paper is structured as follows: next Section (S2) details the high-complexity agent-based model; it spans from the introduction of role-playing entities in the model, to real simulations and discussion. Section 3 details the low-complexity IS model; there, after the introduction of process algebra concepts adopted, it is presented a viral infection model based on π -calculus. Conclusions (S4) end the paper and give a cost-benefit comparison of the two models in order to pave the way for the *whole lymph node simulation*.

2 Agent-Based Modeling

Research on the IS dynamics, in the last two decades, has produced several mathematical and computational models. Different approaches include differential equation based models [3,4,5], cellular automata models [6,7], classifier systems [8], genetic algorithms [9], network/computational models [10,11,12] and agent-based models [13,14,15,16], which seem to be the best suited abstraction to handle the great complexity of IS reality.

The first model here presented is based on the deterministic agent-based paradigm; the high-complexity of this model comes from the fact that it can be considered a *large scale* model and it is extremely realistic; indeed, there are totally $10^6 - 10^8$ cells (e.g., APC, T, B and Plasma cells) and molecules (e.g., immunocomplexes) involved in this model: all of the major role-players of the IS are embedded and represented in our model. It is worth remarking the centrality of the scale problem, as choosing a proper model scale have recently allowed for important improvements in the IS simulation [17,18,19,20]. Much like the nervous system, the IS performs pattern recognition tasks and, additionally, it retains memory of the antigens to which it has been exposed. To detect an antigen, the IS activates a particular recognition process that involves many role-players. An overall view of IS role-players includes: antigens (Ag), B lymphocytes (B), plasma B cells (PLB), antigen presenting cells (APC), T helper lymphocytes (Th), immunocomplexes (IC) and antibodies (Ab). The Ag is the target of the immune response. Th and B lymphocytes are responsible for the discrimination of the self-nonsel, while PLBs produce antibodies able to label the Ags to be taken by the APCs, which represent the wide class of macrophages. Their function is to present the phagocytised antigens to T helper cells for activation. The ICs are Ab–Ag ties ready to be phagocytised by the macrophages. All of the role-playing entities here described are encoded in our model: each agent has a type (i.e.: Ag, B, PLB, etc.) and those typical features that characterize the type (e.g., the Ag has a unique code, or bit string, that will determine whether there will be a bind with a complementary entity); each agent belongs to a population (e.g., the Ags, Bs, etc.) whose size is plotted in order to quantify group presence, affinity driven interactions, mutations, clonal selection and all of the processes detailed above.

2.1 Immuno Responses

The IS mounts two different responses against pathogenic entities: the humoral response, mediated by antibodies, and the cellular one, mediated by cells. Along with the aforementioned entities, the IS includes the T killer cells, the Epithelial cells or generic virus-target cells, and various lymphokines. These components are necessary to activate the cellular response. One can, from an abstract point of view, envision two types of precise interaction rules: specific interactions, that occur when an entity binds to another by means of receptors; and, non-specific interactions, that occur when two entities interact without any specific recognition process. Only a specific subset of mature lymphocytes will respond to a

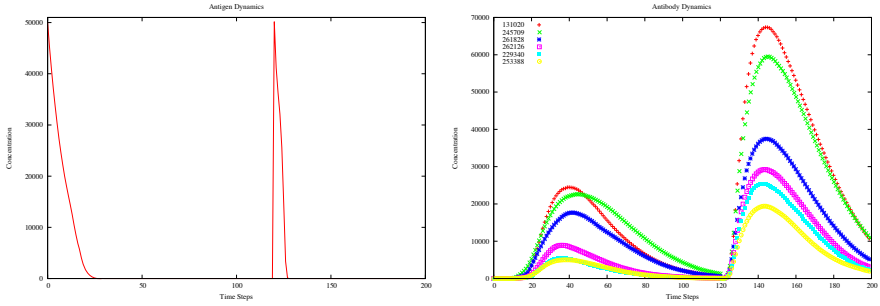


Fig. 1. Immunization - Antigen and Antibody dynamics. Injections of antigens at time steps 0 and 120.

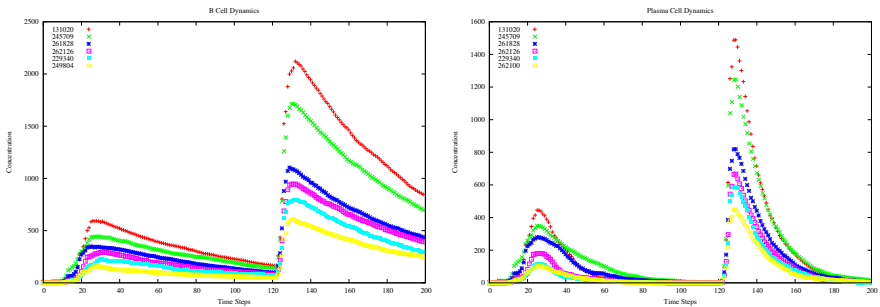


Fig. 2. Immunization - B Cell and Plasma cell dynamics

given Ag, specifically those bearing the receptors that will bind the Ag. Binding usually occurs on a small patch on the Ag (receptor or antigenic determinant or epitope), and the antigen-binding sites on T cell receptors and antibodies (paratopes or idiotypes). Thus, immune recognition of Ags comes from the specific binding of antigen-to-antigen receptors on B and T cells. Hence, the immune response derives its specificity from the fact that Ags select the clones. A non-active B or T cell that has never responded to an Ag before is called virgin or naive. When a naive, mature lymphocyte bearing receptors of the appropriate affinity, binds an Ag (in combination with other signals, usually cytokines), it responds by: proliferating, i.e., cloning itself and, in turn, expanding the population of cells bearing those receptors; the produced clones will differentiate into effector cells that will produce an appropriate response (antibody production for a B cell; cytotoxic responses or help responses for a T cell), and memory cells that will be ready (somewhat like the naive cell) to encounter the Ag in the future and respond in the same way (but this time with many more cells).

Switching from theory right to simulations, we present the results of our agent-based encoding with 18 bits; its representation capability is of the order of 10^6 cells/molecules. Fig. 1 shows time track of antigen, and antibody population

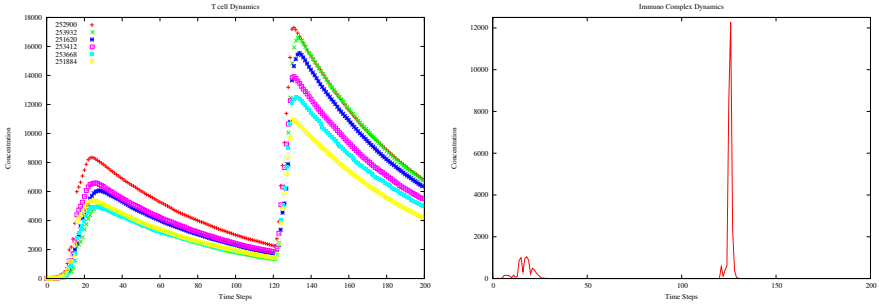


Fig. 3. Immunization - T cell and Immune complexes dynamics

of a system injected with the antigen at time steps 0 and 120. Figure 2 shows primary and secondary immune responses of B lymphocyte and Plasma cell population. Figure 3 reports T cell population and Immunocomplexes. We have three main immune response types to a given antigen: immune response by T killer (cytotoxic) lymphocyte; by T helper lymphocyte; and, by B lymphocyte. In Figure 2, we can see the immune response performed by lymphocytes of class B: the free Ag selects a B lymphocyte, whose receptors match its own. These two entities bind together. A B lymphocyte, which has -internalized- and transformed the Ag, shows on its surface fragments of Ags bound to a protein coded by MHC molecule of class II. The mature T helper lymphocyte can, now, bind to the complex antigen-protein, visible on the B lymphocyte. Such a binding frees the interleukin IL2, which in turn allows the B lymphocyte to clone and differentiate. The cellular cloning goes on as long as the B lymphocytes are stimulated by T helper lymphocytes. Mature PLBs free their receptors, Abs, which bind free Ags, creating ICs. In turn, they will be phagocytised by APCs (the “garbage collectors” of the IS). Other mature B lymphocytes stay in the system as B memory cells. Lastly, the IS comprises the hypermutation phenomena observed during the immune responses: the DNA portion coding for Abs is subjected to mutation during the proliferation of the B lymphocytes. This gives the IS the ability to generate diversity. We should underline that, even if the knowledge on the various mechanisms of the immune system is quite advanced, the relative importance of its components with respect to each specific task is not deeply understood.

2.2 Cross-Reactivity and Epithelial Cell Signaling

Now we show the molecular and cellular population dynamics correlated with two further events involved in the clonal selection principle: cross-reactivity and epithelial cell signaling. Cell concentrations give us a clear picture of the learning and immunization processes that occurred during immune responses. To do this, we need some recollection about a model extended by our agent-based model: the Celada-Seiden model [7]; the manner is a robust computational model based

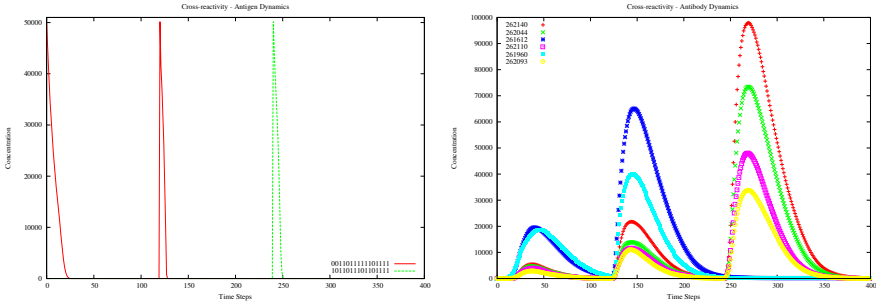


Fig. 4. Cross-Reactivity, Antigen and Antibody dynamics

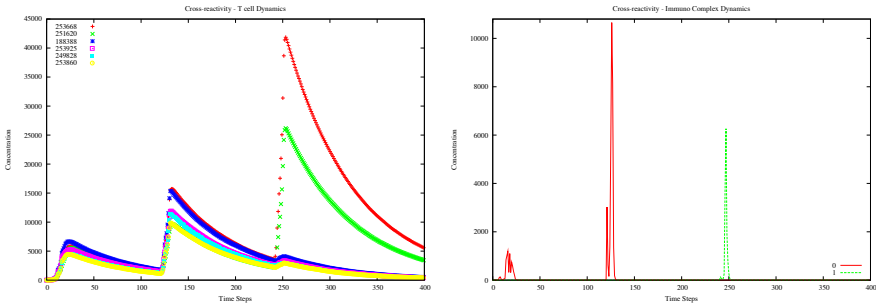


Fig. 5. Cross-Reactivity, T cell and Immunocomplexes dynamics

on the cellular automata paradigm that has been validated with in vitro and in vivo experiments. In particular, it has been shown that Celada-Seiden model can reproduce real phenomena of the immune response. The model includes the following seven entities: Ags, B cells, PLB, APC, T-helper cells, IC, and Ab. The chemical interactions between receptors (the bindings) are mimicked as stochastic events. The probability that two receptors interact is determined by a bit-to-bit matching over the bit strings representing them. Every cell can be in one of the allowed states (e.g., a B cell can be Active, Internalized, Exposing, Stimulated or Memory according to whether it has bound an antigen or not, if it expose the MHC/peptide complex, if it duplicates or if it is considered a memory B cell) and successful interactions between two entities produce a cell-state-change. The cellular automata of the Celada-Seiden’s model has an underlying regular, hexagonal, two-dimensional lattice. Each site incorporates many entities, which interact in loci and diffuse to adjacent sites and then move randomly. Every site of the automaton includes a large number of bit strings accounting for the definition of the various entities and for their states (i.e., both receptors and cell-states). In our agent-based simulation, initially there are neither PLBs nor Abs, nor ICs in the Host, given a specific class of antigens. The plot of Fig. 4 (left plot) shows the three injections of antigens. In the first two injections,

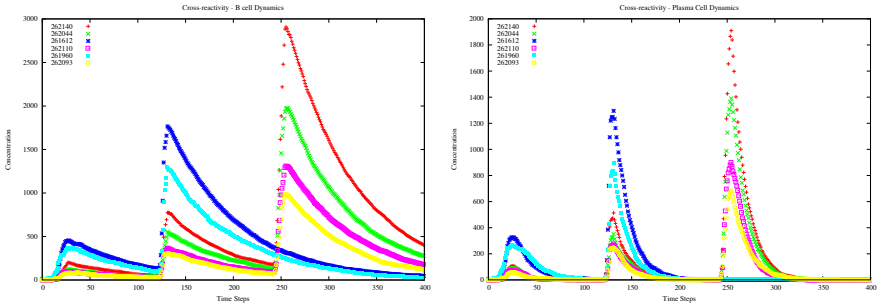


Fig. 6. Cross-Reactivity, B Cell and Plasma cell dynamics

we insert the same antigen, namely the binary string (0011011111101111). The third time we inject a - mutated - antigen (two bits underwent a simulated flip mutation), namely the binary string (1011011101101111). It is of note how the cross-reactivity, observed in nature is here reproduced: Fig. 4 (right plot), Fig. 5 and Fig. 6 present the dynamics of cell populations under control, presenting important similarities with real immunological responses.

Moving towards larger and then more complex (but closer to the reality) systems, we have enriched our IS simulation scenario: we indeed simulated a 20 bit encoded IS. With such an encoding, we have been able to simulate more than 10^8 cells and molecules. We have simulated such an environment for 400 time steps. Fig. 7(a) presents those Ags introduced in the system, while Fig. 7(b) shows the Interferon response released by lymphocytes, a countermeasure that the IS has dynamically adopted. It is of note how the Interferon response is triggered rightly after the introduction of Ags, with a diversified intensity. Such intensity variation is motivated by the primary and secondary responses and cross-reactivity reaction.

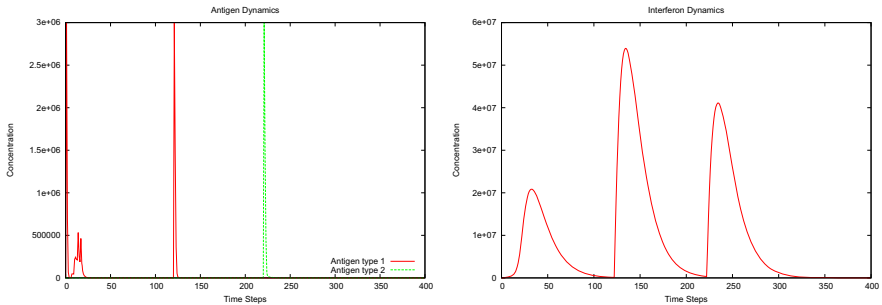


Fig. 7. (a) Antigen Dynamics, as observed after its introduction in the 20 bits simulation environment. (b) Interferon response, dynamically triggered after the Ag detection.

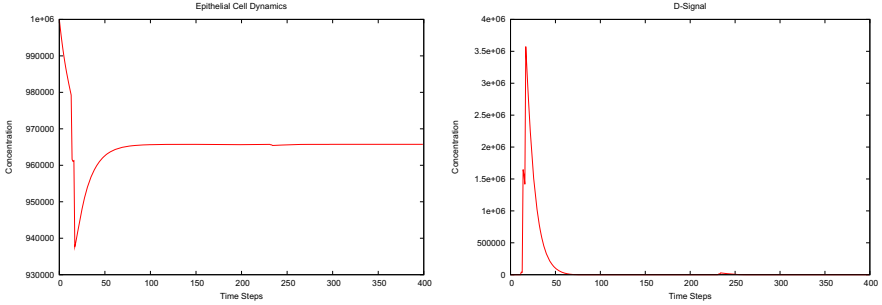


Fig. 8. (a) Epithelial cells in the IS; the change in time of this cell population is a key component in the natural IS. (b) D-signal propagated by epithelial cells to warn other IS components through a signaling mechanism.

In Fig. 8(a) it is presented how the number of epithelial cells changes in time: these cells are part of our natural IS. It has been validated that these cells represent not only a mechanical barrier in our IS, but they are also enabled to communicate the infection through an ad-hoc signal as described in [21,22]; in fact a “warning signal”, namely the *D-signal* is used to propagate the information that something uncommon is taking place. Fig. 8(b) presents the D-signal in object, as observed within this 20 bit simulation.

3 Modeling and Simulation by Stochastic π -Calculus

Biological entities, such as proteins or cells, are social entities (i.e., they act as an organized group), and life depends on their interactions. We can then reduce a biological system to a network of entities interacting among each others in a particular way, i.e., a way in which each elementary process is coded and controlled. The stochastic π -calculus has been recently used to model and simulate a range of biological systems; in particular, stochastic-based models [23] and more in general statistics-based models [24], have recently improved our understanding of key components of the immune system. In this section we want to show the new features of this approach, the pros and cons, and finally the results obtained in simulating HBV infections.

In recent years, there has been considerable research on designing programming languages for complex parallel computational tasks. Interestingly, some of this research is also applicable to biological systems, which are typically highly complex and massively parallel systems. In particular, a mathematical programming language known as the stochastic π -calculus [25] has been recently used to model and simulate many biological systems. One of the main benefits of this calculus is its ability to model large systems incrementally, by composing simpler models of subsystems in an intuitive way (i.e., modularity). Such *in silico* experiments can be used to formulate testable hypotheses on the behavior of biological systems, as a guide to future experiments *in vivo*. Currently available simulators for the stochastic π -calculus are implemented based on standard

theory of chemical kinetics, using an adaptation of the Gillespie algorithm [26]. There has already been substantial research on efficient implementation techniques for variants of the π -calculus, in the context of programming languages for parallel computer systems. However, this research does not take into account some specific properties of biological systems, which differ from most computer systems in fundamental ways. A key difference is that biological systems are often composed of large numbers of processes with identical (or equivalent) behavior, such as thousands of proteins of the same type. Another difference is that the scope (the environment where the interaction can actually take place) of private interaction channels is often limited to a relatively small number of processes, usually to represent the formation of complexes. In general *two fundamental intuitions* are shared by all of these proposals: 1. *molecules* (individual biological agents, in general) can be abstracted as *processes*; 2. molecular *interactions* can be modeled as *communications*. Here we introduce SPiM [27], the calculus simulator used in our experiments, and our simulation of infection, i.e., the HBV virus, based on the Perelson’s model [28].

3.1 SPiM

Proceeding from the concept that molecules are represented as processes and interactions are communications, we can see that the syntax of processes and environments in SPiM is a subset of the syntax of the stochastic π -calculus (SPi) with the additional constraint that each choice of action is defined separately in the environment. Stochastic behavior is incorporated into the system by associating to each channel x its corresponding interaction rate given by $\rho(x)$, and by associating each delay τ_r with a corresponding rate r . Each rate characterizes an exponential distribution, such that the probability of a reaction with rate r occurring within the time t is given by $F(t) = 1 - e^{-rt}$. The average duration of the reaction is given by the mean $1/r$ of this distribution. A machine term V consists of a set of private channels Z , a store S and a heap H . The heap keeps track of the number of copies of identical species, while the store records the activity of all the reactions in the heap. The system is executed according to the reduction rules of the stochastic π -machine [25]. The rules rely on a construction operator $V \oplus P$, which adds a machine process P to a machine term V (cf. Table 1 for detail).

For the calculus dialect implemented by SPiM, Phillips et al. proved a formal equivalence between an ad-hoc graphical notation and a SPiM program [29]. This is very interesting because people without programming background can formalize a complete and correct model in π -calculus without knowing SPiM: for instance, biologists can detail a biological system using this graphical notation and, SPiM is particularly suited to simulate a complete biological system without building a full mathematical model of the interactions. Together with good features there are also some drawbacks; in particular, SPiM is suitable when all rates are known, and they do not change at running time: this is a very strict hypothesis, because many biological systems have entities that change their rate of interaction at running time or in some particular situations. E.g., in the

Table 1. Syntax of processes and environments in SPiM. The syntax is a normal form for the stochastic π -calculus, in which each choice of actions can only occur at the top level of a definition. For convenience, C is used to denote a restricted choice νnM and D is used to denote the body of a definition. For each definition of the form $X(m) = D$ it is assumed that $fn(D) \subset m$.

P,Q ::=	0	Null	E ::=	Empty
	X(n)	Instance	E,X(m)=P	Process
	P — Q	Parallel	E,X(m)= νnM	Choice
	νxP	Restriction	$\pi ::= ?x(m)$	Input
M ::=	0	Null	!x(n)	Output
	$\pi.P + M$	Action	τ_r	Delay

immune system, the rate of interaction, the affinity, between B-cell and Antigen can change over the time because a B-cell can undergo hypermutations and then altering its receptor and so its affinity with the Antigen [30].

3.2 Modeling HBV Infection with SPiM

Here we present a stochastic pi-calculus model of infection caused by hepatitis B virus (HBV). The simulation is based on the basic model of virus infection proposed in [28]. The choice of the Perelson model is justified by the fact that this model was tested in vivo and found a broad consensus where HBV is concerned [31]. The model considers a set of cells susceptible to infection (i.e., target cells), T which, through interactions with a virus V , become infected. Infected cells I are assumed to produce new virus particles at a constant average rate p and to die at rate δ , per cell. The average lifespan of a productively infected cell is $1/\delta$, and so if an infected cell produces a total of N virions during its lifetime, the average rate of virus production per cell, $p = N\delta$. Newly produced virus particles, V , can either infect new cells or be cleared from the Host at rate c per virion. HBV infection was modeled with these Ordinary Differential Equations (ODE):

$$dT/dt = s - dT - \beta VT \quad (1)$$

$$dI/dt = \beta VT - \delta I \quad (2)$$

$$dV/dt = pI - cV \quad (3)$$

Target cells become infected at a rate taken proportional to both the virions concentration and the uninfected cell concentration (βVT); while infected cells (I) are produced at the same rate (βVT) and it is assumed that they are depleted at rate δ per cell. Finally, it is assumed that free virions are produced at a constant rate, p , per cell and cleared at constant rate, c . We have simulated an HBV infection with a high number of virions in blood; we have set virions at 20% of target cells as we used a population of 5×10^3 target cells and 10^3 virions. This ratio corresponds to the scenario in which the infection is growing,

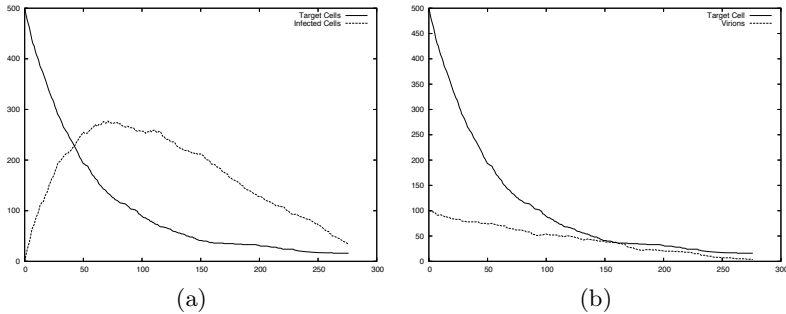


Fig. 9. Simulation of HBV infection using a stochastic π -calculus model. In (a), we report the variation over time (x-axis) of the number of target and infected cells (y-axis); in (b), we present the variation over time (x-axis) of the number of target cells and virions (y-axis).

rapidly, possibly degenerating in a chronic status. We have set the infection rate at a relatively low rate, but from the analysis of the plot in Fig. 9, we can see that the number of virions is big enough to kill target cells. Moreover, infected cells rapidly grow with respect to healthy ones; it is clear that after a first phase where initial virions infect target cells, thanks to the growth of infected cells, the growth in terms of number of virions comes along. This observation is confirmed by Perelson studies, where, after an initial steady state, virions grow proportionally to the number of infected cells. As in Perelson model, the immune system response is implicitly considered by setting the rate of death for virions and infected cells; although there is an *in-vivo* assessment of this model, in general, fine-grained simulation should take into account several other boundary conditions, like cell type and pharmacological treatment.

4 Conclusions

In this paper we have given a broad understanding of the Immune System and we have presented two models for its simulation (i.e., a large-scale agent-based model, and a simpler stochastic one), each model has been introduced together with its strength key points, and with its pertinence context. It is worth mentioning in our conclusions, which are the *modeling factors* that have to be taken into account in the choice of a modeling approach for the IS. If the choice is between a more complex agent-based model versus a stochastic simpler model, there are three factors that play a major role in the modeling outcome: (i) simulation time, (ii) model precision and accuracy, and (iii) model applicability. Where simulation time is concerned, stochastic models have surely to be preferred; in fact, models here outlined base their evolution process on the evaluation of a stochastic function; e.g., the Gillespie algorithm used in SPiM, guarantees light binaries and

execution times in the order of *seconds* on a desktop computer for a small model, such as the one we presented on HBV. As far as agent-based models are concerned, execution times are generally larger of at least one order of magnitude, moving towards *minutes* and, according to the number of molecules involved in the simulations, maybe towards *hours*. It is also interesting how, for agent-based and cellular automata models, there is a direct mapping (through an opportune scaling factor) between simulated time steps and real-world timing; the latter, can provide interesting insights about the reliability of an IS simulation and its biological plausibility (e.g., a model where humoral immune response is seen within the same day of the infection has to be preferred when compared to another model where the same response can be observed only after a simulation time that corresponds to one year in real-world timing). The second factor that has to be considered in the modeling is the precision and the accuracy: if stochastic models provide faster answers, with agent-based models we can track the behavior of the single cell/molecule involved in the system and then we have a significantly higher precision in the model controlling. This means, for instance, that we can operate single element alterations at run-time (e.g., an unexpected mutation), without the need of hard-coding this event in our model specification (as one has to do in π -calculus and in process algebra in general). Moreover, in agent-based models, we can tune affinity and the equivalent of reaction rate constants at run-time. Finally, we can track the behavior of a family of agents involved in the system and then study how different cell populations interact one versus the other. These interesting features of agent-based models, come with a price, that is the longer execution time discussed above. The last factor here discussed, is the model applicability and its pertinence: with this argument we want to highlight the fact that not all the modeling approaches can be really extended towards the perfect virtual simulation that has a 1:1 mapping with reality. With respect to this, it is worth noting that when the spatial characteristic of the IS has to be simulated, stochastic models based on the Gillespie algorithm loose one of their theoretical axes, that is the fact that all of the molecules in the simulation are in a “well-mixed” context. Recent extensions of the Gillespie algorithm have been proposed to account for the spatial information [32]; in an example in which there are areas where Antigens are the majority and the immune response begins, then there are regions where almost nothing happens, and finally there are other regions where naive lymphocytes are the majority, it seems clear that the spatial information has an important role.

In conclusion, where SPiM based modeling of the IS is concerned, its features are definitely preferable when either *light computation* or system *modularity* are more important than *model precision and accuracy* and when the spatial aspect of the system does not play an important role. Finally, moving towards *the virtual lymph node*, agent-based models, and in particular the 20 bit model here presented, seems a valuable approach for the simulation of this (very)large scale system, made of a number of cells/molecules in the order of 10^8 , that can interact among eachothers in a spatially aware context where different regions are devoted to different functions. In the following we give some insights about

possible applications of such a system. *Biological transferability and applicability* of a very-large scale system are important and wide; where *prevention therapies* are concerned, a verified computational model could be employed in the development of new *vaccines*. The idea would be to perform a simulation on a number of new molecules (e.g., molecule $[A]$ and molecule $[B]$) and study how they interact with the Host, i.e.: $f(\text{new_molecule})$ and among eachothers, as the effect could be additive ($f([A] + [B]) = f([A]) + f([B])$), neutral, i.e.: molecules designed for a competing aim ignore eachothers resulting in $f([A] + [B]) = f([A]) = f([B])$, or even disruptive. With a verified IS computational model, we could even study time-series of the simulated response – a practical application would be (i) to modulate vaccine injection schedule in order to have and enhanced immunization; (ii) drug resistance could be studied in terms of time-series as well. To move towards such beautiful scenarios, we are currently considering the conversion of the Binary epitope into a realistic one, built on top of the amino acid alphabet; with respect to the latter point we are investigating an alternative epitope library employing an accepted framework [33] for the (i) *Single-* and (ii) *Multi-Objective* modeling approach aimed at a design of the antibody complementary determining regions that is (iii) extended with the notion of functional *Robustness* [34] at the epitope binding task.

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