SIMULATION OF IMMUNE SYSTEM RESPONSE TO BACTERIAL CHALLENGE

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ABSTRACT

Immune system (IS) simulations have several applications, such as biological theory testing or as a complement in the development of improved drugs. This paper presents an agent based approach to simulate the IS response to bacterial infection challenge. The agent simulator is implemented in a discrete time and two-dimensional space, and composed by two layers: a) a specialized cellular automata responsible for substance diffusion and reactions; and b) the layer where agents move, act and interact. The IS model focuses upon low level cellular receptor interactions, receptor diversity and genetic-ruled agents, aiming to observe and study the resultant emergent behavior. The model reproduces the following IS behavioral characteristics: specificity and specialization, immune memory and vaccine immunization.

INTRODUCTION

Experimental immunology research is a difficult and expensive research field, where the systematic clinical trials of new drugs follow a complex and strict protocol (Emerson and Rossi 2007). As such, computational models of the immune system (IS) can be a valuable tool to understand the effects of a new drug, as well as to test or validate immunologic theories.

In agent-based modeling (ABM), a system is modeled as a group of independent decision-making agents that evaluate its current situation and make decisions on the basis of a rule set (Chen et al. 2004). Cellular automata (CA) (Wolfram 2002) are the simplest form of ABM, and are based on an environment with non-moving agents, discrete in space and time. Agents can range from simple propositional logic based agents (Remondino 2003) to learning agents (Bonabeau 2002) (e.g., using neural networks or evolutionary algorithms).

Several ABM models to perform IS simulation already exist, each presenting a different focus, approach and features. *ImmSim*, one of the most referenced and peer reviewed IS simulators, is based on a CA with probabilistic rules (Kohler et al. 2000), presenting concepts later used in other models, such as entities moving from site to site. The fundamental concepts of this model were explored in 1992 (Celada and Seiden 1992). The *AbAIS (Agent-based Artificial Immune System)* introduced a hybrid approach supporting the evolution of an heterogeneous population of genetic-ruled agents over a CA environment (Grilo et al. 2001). *Simmune* brought the modeling focus on low level molecular interactions (Meier-Schellersheim and Mack 1999), although with limited results regarding IS simulation. Event driven IS modeling was introduced in *CAFISS*, a platform which used multithreaded asynchronous updating of the simulation (Tay and Jhavar 2005), where each IS cell instance runs in its own thread; although realistic, this is a computationally expensive approach. The concern on specialized engines to manage physical and chemical interactions was underlined by the *Sentinel* platform, used in the evaluation of several immunological memory theories (Robbins and Garrett 2005).

In this paper we discuss an agent-based model of the IS and bacteria, and present several simulations of the IS response to bacterial attack under different circumstances. The model is developed using LAIS, a framework for simulation of biological systems in general and the IS in particular (Fachada 2008), which gathers and improves on important features of previous models, offering a versatile and accessible modeling approach. Simulations concern immune memory, specificity and specialization; a vaccine simulation is also presented.

THE IMMUNE SYSTEM

The exact function of the IS is still a source of active debate, but it can be stated that its physiologic function is to protect individuals against infections (Abbas and Lichtman 2006) caused by pathogenic agents. At the same time the IS must distinguish self from non-self, in order to avoid self inflicted damage. Auto-immune diseases are the consequence of failure to perform such distinction. The IS is gifted with learning and memory features: it remembers previous challenges with specific pathogens, and deals with them more effectively in subsequent encounters. The defense mechanism of an indi-

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vidual consists of innate and adaptive immunity, which work together to provide protection against infections.
Innate immunity is the first line of defense against infections; its performance does not depend of prior contact with potential threats. Innate immunity cells, such as macrophages, recognize generic pathogen-associated molecular patterns in the surface of microbes, destroying them via phagocytosis (i.e., by engulfing them) (Abbas and Lichtman 2006).
Microbial adversaries can rapidly evolve strategies to evade innate immunity mechanisms. Adaptive immunity is the evolutionary answer of vertebrate animals, allowing the body to adapt to first time invasions, remembering and handling them more effectively in the future. Lymphocytes are adaptive immunity agents which can challenge particular invaders through the recognition of the unique receptors they express, known as antigens. There are two main types of lymphocytes, which differ in function and type of antigen receptor: B cells and T cells.
The B cell produces antibody molecules complementary to a given antigen in its native form; it plays a central role in humoral immunity, the protection against extracellular microbes. When a macrophage detects a microorganism covered (opsonized) with antibody, the probability of successful phagocytosis increases substantially. The B cell is activated when its receptor (BCR), a superficial antibody, binds specific antigen.
The T cell is the main actor in cell-mediated immunity (CMI), which provides protection against intracellular microbes. T cells are subdivided in Th (helper) cells, which assist macrophages and B cells, and Tc (cytotoxic) cells, which kill infected cells. Th cells may also help the activation of Tc lymphocytes. The T cell receptor (TCR) is more complex, and will not bind to native antigen; instead, it binds a complex formed by an MHC molecule and an antigen derived peptide. MHC (Major Histocompatibility Complex) is a genetic receptor of body cells, involved in antigen presentation to T cells; MHC class I is present in all nucleated cells and is recognized by Tc cells; MHC class II exists on antigen-presenting cells (mainly B cells, macrophages, dendritic cells), and is recognized by Th cells (Roitt and Delves 2001). Cells that present antigenic peptides to Th cells via MHC class II, such as macrophages and B cells, are known as Antigen Presenting Cells (APC). Macrophages process antigen for presentation after microbe phagocytosis, while B cells do the same after engulfing BCRs binding antigen.
When a B cell presents antigen to a Th cell, the latter is stimulated to secrete cytokines (mediators of immune and inflammatory reactions (Abbas and Lichtman 2006)), which in turn increase B cell proliferation and differentiation. B cells either become plasma cells, which secrete antibody, or long-lived memory B cells, which allow a more effective response in future challenges by the same microorganism. After a few days, some of the antigen activated B cells undergo a process called somatic hypermutation, which consists of high-frequency mutations in antibody specificity; B cells producing higher affinity antibodies after mutation have an increased chance of survival, leading to affinity maturation of the humoral immune response. When a Th cell recognizes the antigenic peptide + MHC class II complex on the surface of a macrophage, it releases IFN-γ, a cytokine which helps the macrophage destroy phagocyted, but still living microbes. Several bacteria, such as *Listeria*, *Mycobacterium tuberculosis* or *M. leprae*, survive inside macrophages, requiring external macrophage activation by Th cells in order to be properly eliminated.
These are important aspects of IS dynamics and adversarial strategies, and illustrate the variety of ways in which different components interact in order achieve their goal. However, this introduction doesn't even begin to reflect the true complexity of what is at stake; it serves only to contextualize the reader and to establish an underlying natural agent-based structure, further justifying the use of agent-based approaches for modeling the IS.

THE LAIS SIMULATION FRAMEWORK

The LAIS framework is a multi-threaded agent-based simulation platform, offering a set of tools for the simulation of biological systems. The platform is implemented in Java and makes use of following open source libraries: a) the Repast Agent Simulation Toolkit (North et al. 2006) classes that provide or simplify spatial organization and visualization, event scheduling and simulation output (e.g., charts, CSV files, movies); and b) the Simple XML serialization library[1] that provides simple class development and instantiation using XML. The platform will be available on Sourceforge early 2009.
The two main actors in the LAIS framework are the substances and the agents. The simulator is organized in two layers: a) a specialized cellular automaton (CA) responsible for substance diffusion, reaction and degradation; and, b) the agent layer where the agents move and act. The communication between these layers occurs when agents produce or consume substances, or when an agent action depends on the underlying substances. Current implementation restricts the simulation to discrete time and two-dimensional space.

Substances

Substances are uniquely identified by a 64 bit string, allowing a repertoire of $2^{64} \approx 10^{19}$ different substances. In the model specification it is possible to attribute specific biological functions to different bit substrings. The biological affinity between substances primarily depends on the existence of complementary zones, i.e., regions where

[1] `http://simple.sourceforge.net/`

the biological substances can "fit" with each other. To mimic the IS, the bit string of substances that model IS antibodies are composed by: a) a constant region responsible for secondary functions such as macrophage binding or complement fixation, and b) a variable region which is used to determine the binding affinity with the antigen. The biological affinity is implemented by the Hamming distance between two substance bitstrings (Celada and Seiden 1992).
LAIS represents the substances as real valued concentrations, allowing to: a) model diffusion and reaction phenomena in the CA layer; and b) simulate the substances present on the agent surface, in the agent layer. Antigens are modeled as substances and thus differentiated from pathogenic agents themselves.
New substances can be dynamically created during simulation as the result of: a) different substances produced by mutation of cloning agents; and, b) substance merging. Merging can be either affinity dependent, such as in the antigen-antibody complex formation or independent, such as in the case of the complex formed by MHC and the antigen peptide.
The simulator offers the possibility to group the substances into families in order to: a) simplify the process of tracking substances with similar functions, e.g. in B cell response, where a multitude of different antibodies are temporarily produced; and, b) allow the definition of substance merging rules affecting specific families. In the latter case, model specification is considerably facilitated and the substance merging simulation becomes computationally feasible.

Agents

Agents have a set of conditional rules which evaluate state, superficial substance concentration and the local CA cell, analyzing local substance concentration, as well as substances displayed by other agents. These rules are grouped in lists of rules; each list of rules is associated to a list of actions. A "rule list - action list" mapping is called a "gene". In order to perform the actions in a list, all the rules in the associated gene rule list must yield true. Fig. 1 shows the schematics of a LAIS agent. Rules and actions are hard-coded Java classes, but accept instantiation parameters, making them flexible. The grouping of rules and actions with different instantiation parameters permits a vast range of behaviors. If a particular behavior cannot be achieved using available rules and actions, it is relatively simple to code additional ones, following specific interfaces. The agent set of genes (each one being a "rule list - action list" mapping) can be referred as the agent's genotype. Evolution takes place when an agent creates another agent, either by a cloning process (e.g., cellular division) or by producing a different type of agent (e.g., an infected immune cell producing viruses). In such cases, rules and actions are also cloned. These have a mutation parameter which can modify referenced substances. Agent movement is controlled by these rules; movement can be random, inertial (higher probability of moving forward) or substance dependent (simulating chemotaxis, cell movement directed by a chemical concentration gradient (Abbas and Lichtman 2006)). LAIS supports the exchange of genetic code (at gene level), a feature introduced in AbAIS, allowing models to represent realistic evolving systems.

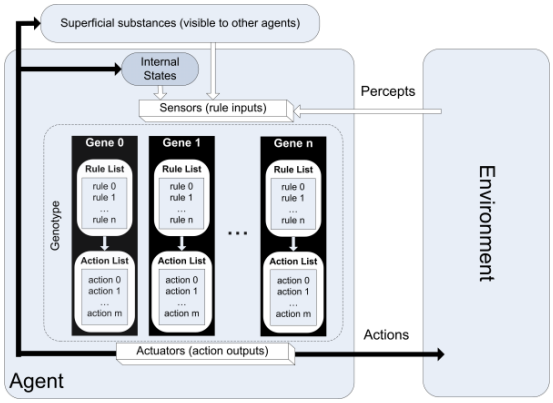


Figure 1: The LAIS agent model.

MODELING CONSIDERATIONS

One of the most complex challenges when developing a IS model is to find a balance between scale, granularity and computational feasibility. Features that are included in the model should not only be theoretically and experimentally sound, but also relevant for in the context of the simulations to perform. Knowledge gaps, incomplete data and excluded system features imply that models are incomplete, always abstract to some point. However, an incomplete model can still do a good job of simulating reality. Biological systems can also work without various parts; they are robust, having redundant features and components with overlapping functions. As such, if a model captures the principal components of a biological system, there is no reason why it cannot yield realistic simulations (Cohen 2007).

Having the previous paragraph into consideration, four types of entities were modeled in order to perform a simulation of immune response to bacterial challenge: APCs, B cells, Th cells and a phagocytosis resistant bacteria agent. The most relevant soluble substances for this experiment consisted in three cytokines, a single bacterial antigen, and a variety of antibodies and antigen + antibody complex, not known at the beginning of the simulation, as its production is a consequence of the immune response. Other substances, such as MHC Class II, are only present in the agent surface, but are of critical importance in the overall simulation. The behavior of IS agent models follows the description discussed in

the IMMUNE SYSTEM section, while the model bacteria consists of an agent who's replication rate is higher than the death rate, so if left alone in the simulation environment would grow indefinitely; naturally, limited resources would stop this from occurring in reality. Detailed model implementation can be found in (Fachada 2008).

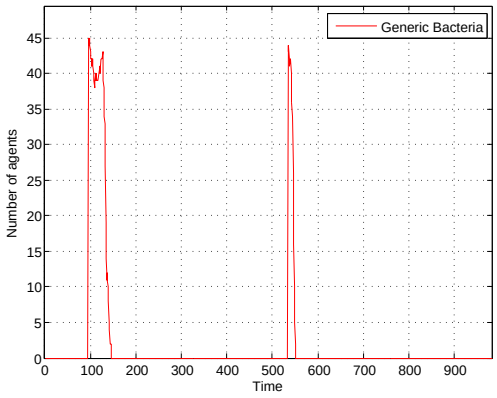
EXPERIMENTS AND RESULTS

Immune memory

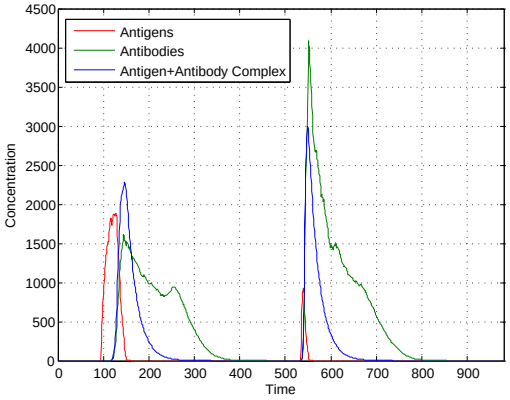
The establishment of memory against previous infections is one of the most important characteristics of the adaptive immune system. This property is responsible for enhanced immune responses to recurrent infections (Abbas and Lichtman 2006).

Bacteria are inserted for the first time in the simulation environment at tick 95 (fig. 2a). The immune response ensues, with APCs performing phagocytosis. APCs are unable to kill the ingested bacteria, but the release of IL-12 cytokine and presentation of MHC Class II + antigen complex activates specific Th cells. These release IL-2, which induces self-proliferation and proliferation of B cells, a process called clonal expansion (i.e., the multiplication of cells specific for the invader). Th cells continue to proliferate, and become effector cells, i.e., cells which produce IFN-γ. This cytokine will in turn help the APCs "kill" the phagocyted bacteria. Antigen specific B cells also take part in the initial activation of Th cells, as they also express MHC Class II + antigen complex, after engulfing soluble antigen. However, they only become activated after IL-2 signaling. Some of the activated B cells become antibody producing plasma cells, while others will go into somatic hypermutation state and others become memory. The antibodies produced by plasma cells act in two fronts by *a*) opsonizing the bacteria, helping APCs perform phagocytosis, and *b*) directly neutralizing bacteria when concentration is high enough. B cells in somatic hypermutation undergo affinity maturation, in which only high affinity clones are able to survive; the surviving clones then become antigen specific activated, plasma and memory cells. When the concerted response is in place, bacteria starts to be removed from the environment, lasting 51 ticks from initial insertion to full neutralization.

When all bacteria are eliminated, the immune response is hampered by the negative feedback rules in the agents. All traces of the first bacterial challenge, except for the long-lived memory cells, are cleared by tick 500 (fig. 2). At tick 533, a new dose of bacteria is introduced in the simulation environment (fig. 2a); it's possible to observe that they resist less when compared to the primary attack, surviving 18 ticks. The secondary response had a shorter delay between antigen deployment and antibody production, and the production of antibody was higher, as can be observed in fig. 2b. These results compare favorably with literature descriptions (Abbas and Lichtman 2006, Roitt and Delves 2001).



(a) Number of bacteria.



(b) Substance concentration by family.

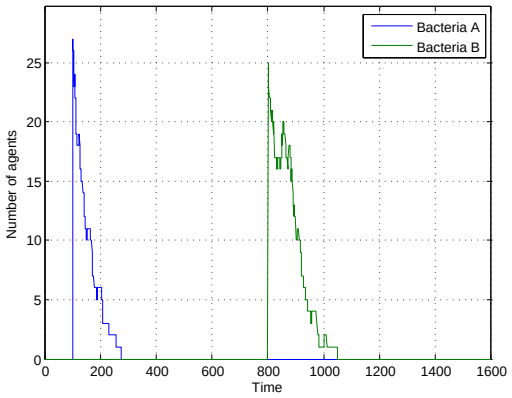
Figure 2: Dynamics during the establishment of immune memory.

Specificity and specialization

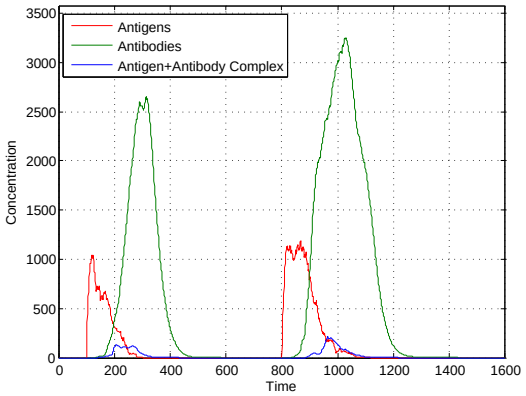
Like memory, specificity and specialization are two important properties of the adaptive immune system. Specificity is the ability to recognize and respond to a variety of microorganisms, while specialization refers to the fact that responses for distinct microbes are optimized for defense against these microbes (Abbas and Lichtman 2006). This experiment aims to demonstrate these two characteristics.

In order to verify specificity and specialization in the model, two types of bacteria, A and B, are introduced at simulation ticks 50 and 800, respectively (fig. 3a). The bacteria are distinguished only by their superficial antigen. The response to the first challenge leads to the creation of specific memory against bacteria A. When the secondary challenge occurs, there is no evident improvement in the quality of the secondary response (fig. 3b), with bacteria B surviving slightly longer (fig. 3a). This occurs because memory cells created during the

first challenge are specific for bacteria A, not recognizing bacteria B during the secondary response.
Comparing the antibody response (fig. 3b) with results from literature (Abbas and Lichtman 2006, Roitt and Delves 2001), it is possible to conclude that the model yields the expected results.



(a) Number of bacteria.

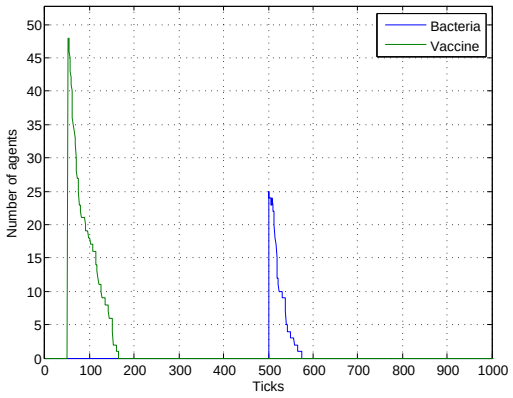


(b) Substance concentration by family.

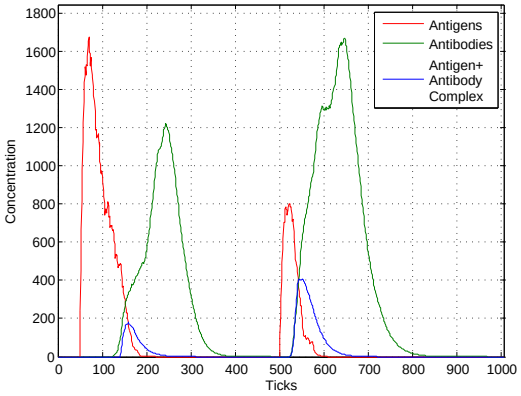
Figure 3: Specific memory created for bacteria A does not recognize bacteria B.

Vaccine simulation

Vaccination uses the memory property of the immune system to achieve a state of immunization against a given pathogen. The first stages of vaccine testing constitute a perfect opportunity for the use of simulators, which can provide preliminary indications on the effectiveness and safety of new drugs. The use of killed organisms as a vaccine is one of the most common, although with limited effectiveness (Roitt and Delves 2001). In this experiment, a virtual vaccine based on dead (non-replicating) bacteria is used to immunize the host, and its effectiveness measured (fig. 4).
The vaccine, compose of 50 non-replicating bacteria, is introduced at tick 50 (fig. 4a (in the figure the number does not reach 50 due to the simulation mechanism:



(a) Number of bacteria.



(b) Substance concentration by family.

Figure 4: Vaccine composed of attenuated bacteria causes a state of immunization.

first the vaccine is deployed, then cells perform their steps - some vaccine disappears - and only then are the charts updated). The immune response is eventually mounted, removing the vaccine from the system and creating memory cells in the process. At tick 500, 25 units of "live" replicating bacteria are deployed in the simulation environment (fig. 4a). The IS recognizes the invader and the response is almost immediate when compared with the initial injection of vaccine (fig. 4b). The immune response removes the bacteria without difficulty, asserting the effect of vaccination using "dead" microorganisms in the presented model.

CONCLUSIONS

This paper presents an agent based approach to simulate the immune system response to bacterial challenge. It aims to demonstrate LAIS framework capabilities for this type of simulations. Three simulation scenarios were designed to test the implemented model for immune memory, vaccine immunization and the specificity and specialization behavioral characteristics present in the IS. Results show that: a) LAIS framework archi-

tecture provides all the necessary flexibility to specify the IS model in a biological meaningful way; and b) the implemented IS model can reflect all these three characteristics and, thus, be further calibrated and validated against experimental data.

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REFERENCES

Abbas A. and Lichtman A., 2006. *Basic immunology: functions and disorders of the immune system.* Saunders Elsevier, 2nd edition, updated edition 2006-2007 ed.

Bonabeau E., 2002. *Agent-based modeling: Methods and techniques for simulating human systems. Proceedings of the National Academy of Sciences*, 99, no. 3, 7280–7287.

Celada F. and Seiden P., 1992. *A Computer Model of Cellular Interactions in the Immune System. Immunology Today*, 13, no. 2, 56–62.

Chen K.; Calzone L.; Csikasz-Nagy A.; Cross F.; Novak B.; and Tyson J., 2004. *Integrative Analysis of Cell Cycle Control in Budding Yeast. Molecular Biology of the Cell*, 15, no. 8, 3841–3862.

Cohen I., 2007. *Modeling Immune Behavior for Experimentalists. Immunological Reviews*, 216, no. 1, 232–236.

Emerson A. and Rossi E., 2007. *ImmunoGrid - The Virtual Human Immune System Project. Stud Health Technol Inform*, 126, 87–92.

Fachada N., 2008. *Agent-based Simulation of the Immune System.* Master's thesis, Instituto Superior Técnico, Lisboa.

Grilo A.; Caetano A.; and Rosa A., 2001. *Agent based Artificial Immune System.* In *Proc. GECCO-01, Vol. LBP.* 145–151.

Kohler B.; Puzone R.; Seiden P.; and Celada F., 2000. *A systematic approach to vaccine complexity using an automaton model of the cellular and humoral immune system. I. Viral characteristics and polarized responses. Vaccine*, 19, no. 7-8, 862–76.

Meier-Schellersheim M. and Mack G., 1999. *SIMMUNE, a tool for simulating and analyzing immune system behavior.* Tech. rep., Institut für Theoretische Physik, Universität Hamburg.

North M.; Collier N.; and Vos J., 2006. *Experiences Creating Three Implementations of the Repast Agent Modeling Toolkit. ACM Transactions on Modeling and Computer Simulation*, 16, no. 1, 1–25.

Remondino M., 2003. *Emergence of Self Organization and Search for Optimal Enterprise Structure: AI Evolutionary Mehtods Applied to Agent Based Process Simulation.* In *Proceedings 15th European Simulation Symposium.* 229–236.

Robbins M. and Garrett S., 2005. *Evaluating Theories of Immunological Memory Using Large-Scale Simulations.* In C. Jacob; M.L. Pilat; P.J. Bentley; and J. Timmis (Eds.), *Artificial Immune Systems*, Springer Berlin / Heidelberg, *Lecture Notes in Computer Science*, vol. 3627, chap. 16. 193–206. URL `http://dx.doi.org/10.1007/11536444_15`.

Roitt I.M. and Delves P.J., 2001. *Essential Immunology.* Blackwell Publishing, 10th ed. URL `www.roitt.com`.

Tay J. and Jhavar A., 2005. *CAFISS: a Complex Adaptive Framework for Immune System Simulation.* In *Proceedings of the 2005 ACM symposium on Applied Computing.* ACM Press New York, NY, USA, 158–164.

Wolfram S., 2002. *A New Kind of Science.* Wolfram Media Inc.