Review

Characterizing emergent properties of immunological systems with multi-cellular rule-based computational modeling

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The immune system is comprised of numerous components that interact with one another to give rise to phenotypic behaviors that are sometimes unexpected. Agent-based modeling (ABM) and cellular automata (CA) belong to a class of discrete mathematical approaches in which autonomous entities detect local information and act over time according to logical rules. The power of this approach lies in the emergence of behavior that arises from interactions between agents, which would otherwise be impossible to know *a priori*. Recent work exploring the immune system with ABM and CA has revealed novel insights into immunological processes. Here, we summarize these applications to immunology and, particularly, how ABM can help formulate hypotheses that might drive further experimental investigations of disease mechanisms.

Introduction

In recent years, computational analysis, coupled with high-throughput experimental data, has facilitated the study of complex biological phenomena [1]. In particular, systems biology-based approaches are enabling the synthesis of vast amounts of literature through the construction of computational models (see Glossary) for probing biological function [2]. Examples of these approaches include genome-scale reconstructions of metabolic networks [3], differential equation-based kinetic models of signaling networks [4] and statistical inference models of cellular network organization [5]. Once constructed and validated, models can be perturbed in different ways (e.g. inputs can be altered to mimic different environments) to facilitate exploration of network features [6]. These *in silico* (or dry-laboratory) experiments are complementary to traditional wet-laboratory experimental approaches [7]. Moreover, computational modeling, which often requires less time and cost, aids in facilitating experiments and/or measurements that can be infeasible in a laboratory setting, all the while generating novel insights and hypotheses for further research and development.

Glossary

Adaptive (adapting): a property of an agent or system that exhibits behavioral changes (e.g. in response to perturbations to its surrounding environment); to display features of memory.
Agent: a discrete unit with defined rules governing its behavior or response; agents can interact with other agents and their surrounding environment.
Agent-based modeling (ABM): a modeling framework simulating systems in discrete time and space; agent-based models include agents, rules, time steps and environments; agents can be mobile and are characterized by asynchronous behaviors, that is, they update their states independently of one another.
Autonomous: acting independently of others.
Cellular automata (CA): a related approach to ABM; also simulates systems in discrete time and space; cells or entities are fixed in position (immobile) and change state over a time period; synchronous updating of agent states is applied in classical CA. It is important to note that the word 'cellular' in CA does not imply a biological cell, but, rather, signifies a discrete entity or element.
Complex system: a system comprised of numerous interconnected parts without a central organizing structure; associations or interactions between parts lead to global behaviors; dynamic and non-linear.
Computational model: general term used for describing a computer program that simulates a natural phenomenon such as a biological process.
Continuous: describes transitions between an infinite number of states; non-discrete.
Deterministic: lacking randomness; an agent or system whose future state is fully determined by the current state in which it lies.
Discrete (discretization): describes transitions between a countable number of states; a set of isolated points in time and/or space; non-continuous.
Dry-lab vs. Wet-lab: terms used for differentiating between *in silico* computational work and classical experimental work with, and handling of, biological samples.
Emergence: the global behaviors or patterns that arise through 'self-organization' and that could not have otherwise been characterized *a priori*.
Environment: the topology of the simulation or 'world' space; can be 1D, 2D or 3D.
Identifiable: unique and having own history of interactions, activities or states.
IF-THEN-ELSE: conditional programming statement.
Multi-cellular: related to a property of a biological system consisting of two or more cells.
Non-linear: as in a non-linear system, in which the output does not scale according to the behaviors of components that comprise the system.
Rules (rule-set): a set of logical decision heuristics which govern agents' activities; literature-derived or based on qualitative observations of the system.
Self-organizing (self-organization): the ability to exhibit organization that drives phenotype without a global organizing structure; based on component interactions of a system.
Sensitivity analysis: related to quantifying the relationship between variations in input parameters and resulting effects on model outputs.
Stochastic (stochasticity): random; a process that is driven by probabilistic outcomes.
von Neumann or Moore neighborhoods: environment in classical 2D square-lattice CA models; von Neumann scheme refers to grid elements orthogonal (North, South, East and West) from the element of interest; Moore scheme refers to all eight grid elements (North, South, East, West, Northwest, Northeast, Southwest and Southeast) surrounding the element of interest.

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Examples of complex biological phenomena include tissue morphogenesis [8], immune mechanisms against disease [9] and neurological function [10]. Usually, these processes are comprised of numerous interconnected components, and the set of associations or interactions between these components ultimately gives rise to the global behavior that is observed (i.e. the 'end-stage' phenotype) [10]. For example, the connections between billions of neurons in the human brain together lead to consciousness, sensory perception and thought. No one neuron is individually responsible for these human qualities; rather, the network of interacting neurons yields complex phenotypes [10].

Similarly, the immune response to disease invokes a multi-cellular cascade comprised of heterogeneous cell-types interacting with each other for clearance of foreign particles [11]. For instance, the process of $CD4^+$ T-cell activation in the lymph node (LN) is attributed to the 'interaction' between $CD4^+$ T cells and dendritic cells (DCs). More specifically, binding kinetics between T-cell receptors (TCRs) and peptide-major histocompatibility complexes (pMHCs), concentration of antigen on the surface of DCs and effects of co-stimulatory molecules all have a role in driving T-cell activation, thereby constituting the 'interaction' that occurs between T cells and DCs [11,12]. T-cell activation does not occur when T cells and DCs are in isolation and do not experience this 'interaction'.

For the study of these types of complex systems, there exist numerous mathematical approaches, including nonlinear dynamics and differential equations (see Ref. [10] for an overview on complex systems analysis in the biological and chemical sciences). However, here, we present a specific class of computational techniques consisting of agent-based modeling (ABM) and cellular automata (CA) for the study of complex multi-cellular immunological systems. After the introduction of these methods, we specifically discuss the impact of ABM on the field of immunology.

Box 1. Differentiating between ABM and CA

ABM and CA are rule-based computational approaches that can be used to simulate multi-cellular immunological systems in discrete space and time. Here, we highlight a few specific differences between these techniques.

An important distinguishing characteristic between ABM and 'classical' CA is the presence of mobile agents in the former approach [63]. Although elements in a classical CA model are allowed to change state during a simulation, they are fixed in position. In ABM, agents can move and interact with other agents and/or the environment [63]. Another difference between ABM and CA lies in the process of state transitions of entities. Agent-based models are characterized by asynchronous behaviors, as individual agents can update their states independently of one another [64]. However, classical CA models are synchronous in their updating of states of grid elements. In other words, states of all elements in the lattice are updated at the end of a time step in parallel [65].

Furthermore, in 2D agent-based models, the environment is discretized into micro-compartments or a lattice of sites on a Cartesian coordinate space [9,16,66]. Typically, the composition of the environment by which a particular mobile agent is influenced is dependent on the micro-compartment in which the agent resides at any given time-point. Micro-compartments can hold a variety of information, including concentration values (of cytokines), presence or absence of stationary cell-types (such as DCs) and spatial coordinates [9,66]. In a classical 2D square-lattice CA, the environment surrounding a particular cell is described in terms of either the von Neumann or Moore neighborhoods (see Glossary) [67].

Finally, in ABM, rule-sets can include both stochastic and deterministic elements [17]. As an example of stochasticity in ABM, it has been postulated that T cells exhibit random motility in the lymph node as they scan the environment in search of cognate antigen [51]. Random variables can thus be used to dictate the 'heading' of agents in a simulation to mimic this behavior. Stochastic rule-sets also help account for the fact that tissue-scale simulations do not necessarily consider all of the internal processes of an individual cell. In contrast, deterministic rule-sets imply that a similar configuration of the agent and/or its neighbors at a particular time step always yields the same new state at the next time step [21]. See supplementary material I for an immunological example of a deterministic rule. Classical CA models are defined by the incorporation of strictly deterministic rule-sets [20]. Notably, other 'modified' CA approaches have included probabilistic rule-sets [20].

A class of computational approaches to study immunological systems

ABM (otherwise known as individual-based modeling [13]) is a computational platform in which discrete autonomous units or agents detect local surrounding information and take action at each of several discrete time steps, according to a set of logical rules [8,9,14–16]. Explicitly, the defining characteristics of agent-based models are agents, rules, discrete time steps and the spatial environment [9,14]. Rules are either obtained from experimental literature or are formulated as a set of hypothesized interactions based on a qualitative understanding of systems-level behavior [14,17,18]. For example, two-photon microscopy can shed light on temporal interactions of $CD4^+$ T cells with DCs [19], and these data can in turn be used to formulate rules governing the time a T-cell agent interacts with a DC within an agent-based model. Given the importance of multi-cellular interactions in the immune response to disease, we generally present examples in which immune cells are represented as agents (i.e. computational modeling at the tissue-scale). However, agent-based models can be readily extended to other biological scales of interest (e.g. cellular-scale and organism-scale behaviors can be equivalently simulated). Therefore, depending on the biological application and scale of interest, agents and the corresponding environment can be modeled as animals in a forest, cells in a LN or cytokines near a site of infection.

CA is a related rule-based modeling approach for simulating systems in discrete time and space [20]. CA models are also comprised of a grid of elements (or lattice of cells), but these elements are fixed in space and capable of changing state over the period of a simulation. The state of an element (a selection of one in a finite number of total states) at a subsequent time step is dependent on the element's own state and/or the states of its neighbors at the current time step [20,21]. See Box 1 and supplementary material I for additional details on ABM and CA approaches.

Ultimately, ABM and CA are specific computational frameworks that facilitate the inference of systemic properties. We present a comprehensive catalog of agent-based and CA immunological models in Table 1 and in supplementary material II. Although we label these models distinctly as 'agent-based' or 'CA', we wish to emphasize that these modeling approaches are similar as they are both rule-based computational techniques that involve a dis-

Table 1. A catalog of immunological models related to cancer, pathogenic infection and general immune processes

Model No.	Scale(s) modeled	Agents (e.g. cell-types)	World (i.e. environment)	Model description	Refs
Examples of cancer models					
Cancer #1	Tissue	• B cells • T-helper (T_H) cells • T-killer cells • T-suppressor (T_S) cells • Tumor cells (with and without antigen)	Peripheral tissue	• Cellular automata (CA) model for characterizing interactions between tumor and immune cells • Concentrations of cell-types discretized as low or high • Results presented as a list of steady-state fixed points consisting of high or low concentrations of cell-types and corresponding to cancerous or non-cancerous states	[60]
Cancer #2	Tissue	• Natural killer cells • Cytotoxic T lymphocytes (CTLs) • Tumor cells	Nutrient-accessible tissue	• Hybrid CA-partial differential equation (PDE) model • Described interactions between a growing tumor and the immune (innate and adaptive) system • PDEs were solved for concentrations of nutrient species and the solutions were fed into CA to evaluate cell activities, such as motility and division	[61]
Cancer #3	Tissue	• B cells • T cells • Plasma B cells • Antigen-presenting cells (APCs) • Normal cells • Tumor cells	Peripheral tissue	• Tumor-immunity system CA model for simulating tumor-immune system growth • Generated visual images of the tumor growth process, matching well with Gompertz growth dynamics of tumor-immune system • Eventual goal is to introduce drug therapies into model	[62]
Examples of pathogen models					
Pathogen #1	Cellular and tissue	• B cells (naïve, stimulated and memory cells) • Antibody-secreting B cells • $CD4^+$ T_H cells (naïve, effector and memory cells) • $CD8^+$ CTLs (naïve, effector and memory cells) • APCs • Epithelial cells ('target' and 'virus-infected' cells)	Germinal center, lymph node or peripheral tissue	• CA model for exploring features of cell-mediated and humoral immunity during primary and secondary immune responses to antigenic stimulation • Model characterized dynamics of B cells (including affinity maturation), T_H cells, CTLs, APCs, target epithelial cells and antibody-secreting B cells • Based on a parallel computing version of Ref. [20]	[42]
Pathogen #2	Molecular and tissue	• B cells (naïve, stimulated and memory cells) • Antibody-secreting B cells • $CD4^+$ T_H cells (effector [T_H1 and T_H2] and memory cells) • $CD8^+$ CTLs • APCs • Epithelial cells ('target' and 'virus-infected' cells)	Lymph node	• CA model characterizing cell-mediated and humoral immunity • Set of viral antigens varied in their parameter values of infectivity, growth rate and lethal threshold levels (within epithelial cells) • Response with and without vaccination evaluated under conditions of cell-mediated, humoral or combined cell-mediated and humoral immunity	[31]
Pathogen #3	Tissue	• T cells (combined $CD4^+$ and $CD8^+$) • Macrophages (resting, activated, infected, chronically infected and dead cells)	Alveolar lung tissue	• Agent-based model of granuloma formation after *Mycobacterium tuberculosis* infection in the lung • Parameters defined for containment and dissemination scenarios, and granuloma dynamics observed for each condition at days 12, 25, 50, 100 and 200 • Results included a switch in correlation between parameters associated with intracellular bacterial growth rate and granuloma size during initial and later infection periods • Strong correlation between T-cell movement and granuloma size (or extracellular bacteria) also observed	[9]

Table 1 (*Continued*)

Model No.	Scale(s) modeled	Agents (e.g. cell-types)	World (i.e. environment)	Model description	Refs
Examples of general immunological models					
General immunology #1	Tissue	• T_H cells (naïve and effector [T_H1 and T_H2] cells)	Lymph node	• CA model of T_H-cell polarization into T_H1 and T_H2 subsets • Varying antigen densities, presence or absence of a cell death term and differing probabilities of T_H1 versus T_H2 activation evaluated	[37]
General immunology #2	Cellular	• T cells (T-cell receptors) • APCs (pMHC) • Adhesion and 'dummy' molecules	Immunological synapse	• Agent-based model of TCR microclustering in establishing T-cell activation • Effects of null, antagonist, weak agonist and strong agonist peptides promoting differing levels of TCR activation considered • Suggested T-cell activation is context-dependent wherein TCRs interact with a heterogeneous mix of peptides rather than just agonist peptides	[30]
General immunology #3	Tissue	• $CD4^+$ T cells (naïve, activated and effector cells) • $CD8^+$ T cells (naïve, activated, effector and memory cells) • APCs (DCs) • Licensed DCs	Lymph node	• Agent-based model analyzing the effects of chemotactic strength in T-cell and DC interactions in the lymph node • Total distance, search time (to find a DC), and transit time (to scan the entire node) for T cells after entering the lymph node tracked • Sensitivity to anatomic arrangement of afferent lymphatics and high-endothelial venules investigated • 'Short-term persistence' of T cells, that is, T cells have a higher probability of maintaining their direction of travel over small time-scales, incorporated into the rule-set	[14]
General immunology #4	Cellular and tissue	• B cells (naïve, memory and plasma cells) • T_H cells • APCs • Antibodies	Thymus and peripheral tissue	• CA model of the humoral immune response • Role of model parameters in generating experimentally observed outcomes explored • Positive and negative selection of thymus, B-cell, T-cell and antibody dynamics to primary and secondary antigenic injection and antibody response to varying antigenic dose explored • Autoimmune reactions with and without B-cell anergy and an idiotypic network by varying B-cell and antibody interaction strength and introducing B-cell anergy also simulated	[20]

cretization in time and space. In the following sections, we focus this review to describe the extension of ABM to immunology and demonstrate the potential of ABM to provide novel insights into otherwise complex immunological questions.

Need for systems analysis of immunology

The immune response to disease is a multi-cellular phenomenon that is formed through interactions between diverse cell-types in addition to a wide array of other factors (e.g. inflammatory cytokines). The scope and complexity of the immune system necessitates the use and development of computational methods for its analysis. For instance, as part of the mature TCR repertoire, there are $\sim 10^7$ different TCRs present in humans [22]. Similarly, more than 15% of the genes in the human genome can be linked to immune function [23]. In addition, the immune system is capable of self-organizing and adapting to various environmental perturbations or exogenous stimuli, including a wide range of foreign antigens to which it has not been previously exposed. The immune system can also discriminate between self and foreign antigens, and can develop memory to more effectively counteract recurring antigenic stimulation [11].

The behavior that arises from this self-organization and adaptation is termed emergence (i.e. a phenomenon that is a result of the interactions among components of a system and that is otherwise indiscernible when individual components are observed in action independently of one another) [6,24]. Consider, for example, the flocking behavior of birds, a classic example of an emergent system simulated in the 'Boids' model [25]. Each individual bird

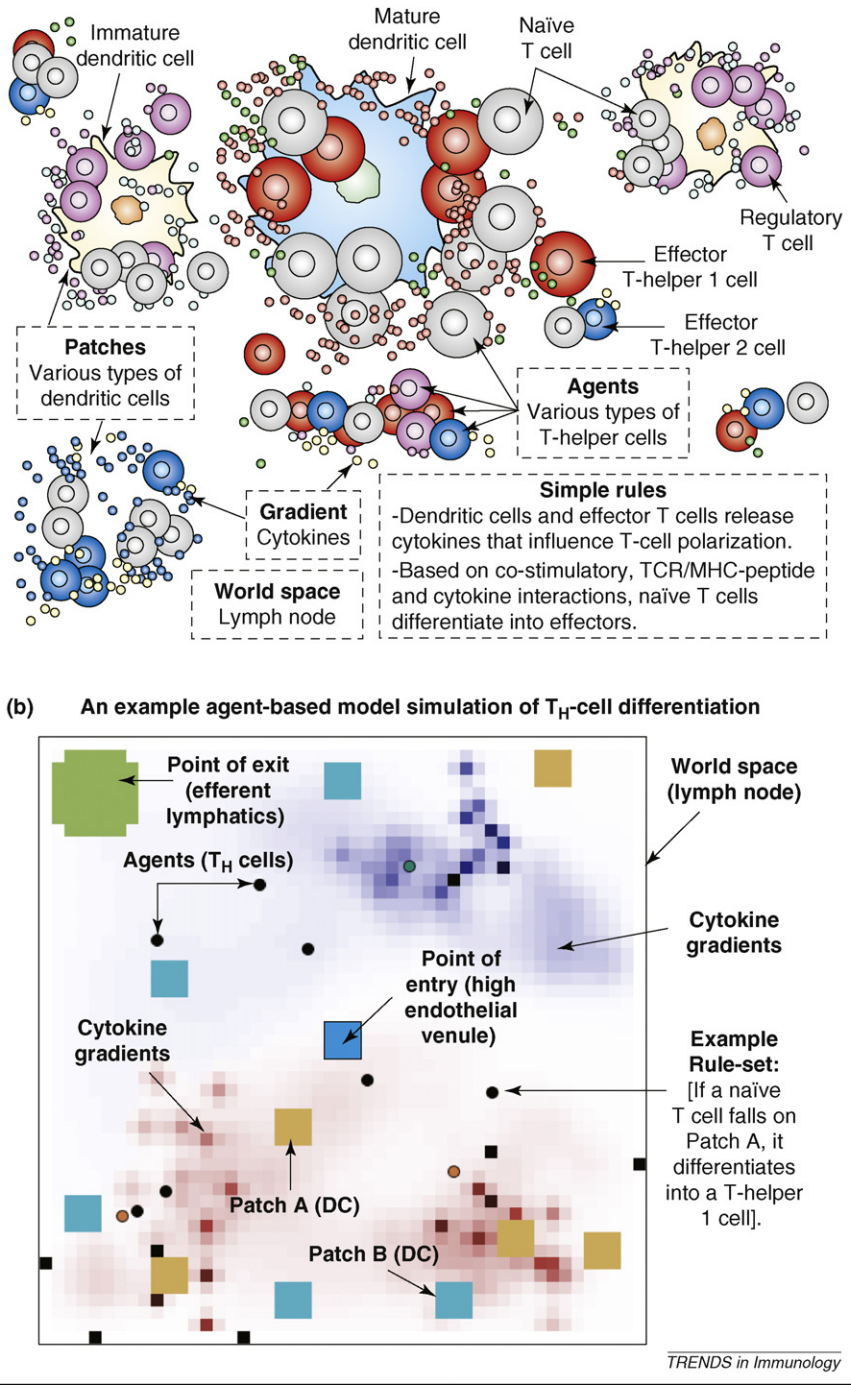

Figure 1. Application of ABM to an immunological system. **(a)** An immunological process is depicted in ABM terminology. The process of T-helper (T_H)-cell differentiation via the interaction between naïve T cells and DCs within a lymph node is highlighted as the immunological process of interest. Effector subtypes include T-helper 1 (T_H1) cells, T-helper 2 (T_H2) cells and regulatory T cells (T_{REG}). Individual T cells are represented as agents, DCs are stationary with fixed spatial area and are a part of the lymph node environment in which the T cells reside, the cytokine processes are included through a diffusion gradient and the set of interactions between the T cells and other T cells, DCs and cytokine processes is captured by the rule-set. **(b)** An agent-based model simulation of T_H-cell differentiation (programmed in NetLogo) is shown. Each of the components of the immunological process of interest is simulated using an agent-based model, including individual autonomous 'agents,' the environment (i.e. the world space, which can be described as discrete spatial units or 'patches'), diffusive components (e.g. a concentration gradient) and rule-sets.

takes action according to three simple rules that enable it to maintain separation from, match the heading and speed of and keep to the center of the other birds in its local environment [25]. Each bird completes these tasks with knowledge of the behavior of other birds in its local environment, and ultimately, the complex behavior of flocking 'self-organizes' without a global organizing structure or 'leader' bird.

A systems biology-based approach towards immunological processes is thus appropriate. A multi-cellular computational modeling framework with the ability to simulate non-linear and dynamic behavior, track individual cells that are heterogeneous in nature, and synthesize cell–cell and cell–environment interactions is needed. The modeling framework should also allow for cells to develop memory of various prior interactions and adapt as needed to the

external environment. Furthermore, the framework should permit visualization of emergent phenomena that result from the combined interactions between cells of the immune system. As ABM is useful in characterizing properties of systems that exhibit such complex behavior, it is well-suited for addressing key challenges in immunology.

Application of ABM to immunology

ABM has been used extensively to further our understanding of systems in several different disciplines, including biology, ecology, economics and sociology [8,16,26–28]. In immunology, the ABM approach has facilitated the study of the immune response to a variety of disease conditions, including bacterial and viral infections, autoimmune disorders and cancer (see Table 1 and supplementary material II). Here, we briefly describe the approach of modeling an immunological system using ABM by illustrating the process of T-helper (T_H)-cell differentiation into T-helper 1 (T_H1) and T-helper 2 (T_H2) effector subtypes via the interaction between naïve T cells and DCs within a LN (Figure 1). In Figure 1a, the immunological process of T_H-cell differentiation is depicted in ABM terminology. We show naïve T cells, effector T_H1 and T_H2 cells, regulatory T cells (T_{REG}) and immature and mature DCs in addition to cytokines released by T cells and DCs. Each of these component types is represented as a feature associated with an agent-based model. For example, all T cells are represented as discrete autonomous entities or agents, whereas DCs are indicated as patches (discrete stationary spatial units that comprise the LN environment). Cytokine diffusion can be modeled as a gradient, with highest concentrations of cytokine present around secreting cells. Note that cytokine concentration can be treated as a continuous variable as was done in [9]. Discretization of a partial differential equation (PDE) capturing diffusion in time and space allows tracking of the concentration of cytokine factors within each patch of the LN [9].

In Figure 1b, an example agent-based model simulation of T_H-cell differentiation in the LN (programmed in NetLogo [29]) is shown for a particular time step. The immunological system is modeled using an ABM framework that includes agents, an environment, diffusive components and rules. The final output of this system (represented in the agent-based model) would be the global macroscopic behavior that is a result of all the underlying interactions (e.g. the total number of T_H1, T_H2 and T_{REG} cells present in the LN after a defined period of time). See Box 2 for details on agent-based model development and iterative refinement.

Expanding upon features of agent-based models

With discrete units interacting spatially and temporally, agent-based models can readily simulate complex, unexpected, non-linear and dynamical behaviors typically observed in the immune response [9,14,30]. A unique aspect of ABM compared with other computational strategies is the inclusion of agents whose behaviors are dictated by logical rules. Here, we highlight features of ABM that make this approach particularly useful for simulating immunological systems. In the process, we also characterize differences between ABM and other modeling approaches (including differential equations and traditional object-oriented methods). See Box 3 and supplementary material I for additional advantages and disadvantages of the ABM approach.

Tracking and adaptive behavior of agents

The discrete unit, entity or agent in an agent-based model can be classified as being identifiable [21]. In other words, although all agents of the same sub-type can be equipped with identical rule-sets, the history of interactions and activities are unique to each agent [31]. This allows for the tracking of individual agents as they progress through their life-cycles. For example, after activation within the LN, a naïve T cell agent develops into a blast cell with a characteristic increase in cell diameter and undergoes several divisions before evolving into an effector sub-type [19]. This progression through the life-cycle can be tracked for every cell in the simulation using ABM, thereby allowing the user to ascertain exactly which cells are in their initial blast

Box 2. An iterative cycle of agent-based model development and refinement

The agent-based model-building process can be divided into the following steps: (i) mining of published literature; (ii) determining biological scale of interest; (iii) problem definition, including delineation of initial and boundary conditions; (iv) explicit listing of assumptions; (v) formulation of a comprehensive rule-set (see supplementary material I on defining global and local variables); (vi) parameter estimation; (vii) programming and construction of the agent-based model; (viii) observation of global phenotypic behavior; (ix) qualitative or quantitative experimental validation and (x) elucidation of novel insights and possible future extensions [66]. Development of an agent-based model is closely linked to experimental observations, and published literature is typically mined after every step. See Refs [17,21,66,68] for more detailed reviews on ABM methodology.

Defining the problem, stating assumptions and describing the boundary conditions are important first steps in model development. Depending on the biological scale of interest, various types of experiments can provide the information needed to devise a rule-set and parameterize the model. Figure I shows examples of experimental observations and/or analyses that can be used to construct the model. The model needs to be validated with a different set of experimental literature from the one used in formulating the rule-set and parameters [17]. Also, any new hypotheses or observations of emergent phenomena using the ABM framework can aid in driving new experimental programs, therefore constituting a strategy of 'model-driven biological discovery'. Computational models typically require less time and cost than the execution of laboratory experiments, and they can enable measurements of aspects of a system that are not capable of being tracked *in vitro* or *in vivo*. Thus, an investigative, iterative and hypothesis-driven approach towards characterizing emergent properties of complex biological processes can be implemented.

In ABM, a particular rule-set effectively constitutes the collection of hypotheses about the corresponding system at the time of model construction. As more experimental data become available, rule-sets can be modified and improved. Rules that dictate interactions (between components of a system) that have not been experimentally determined, and yet simulate previously characterized emergent phenomena, can serve as hypotheses for future wet-laboratory investigations. Finally, as with any computational model, the outputs to the agent-based model should be analyzed in tandem with rules, assumptions and boundary conditions that dictate the model-building process.

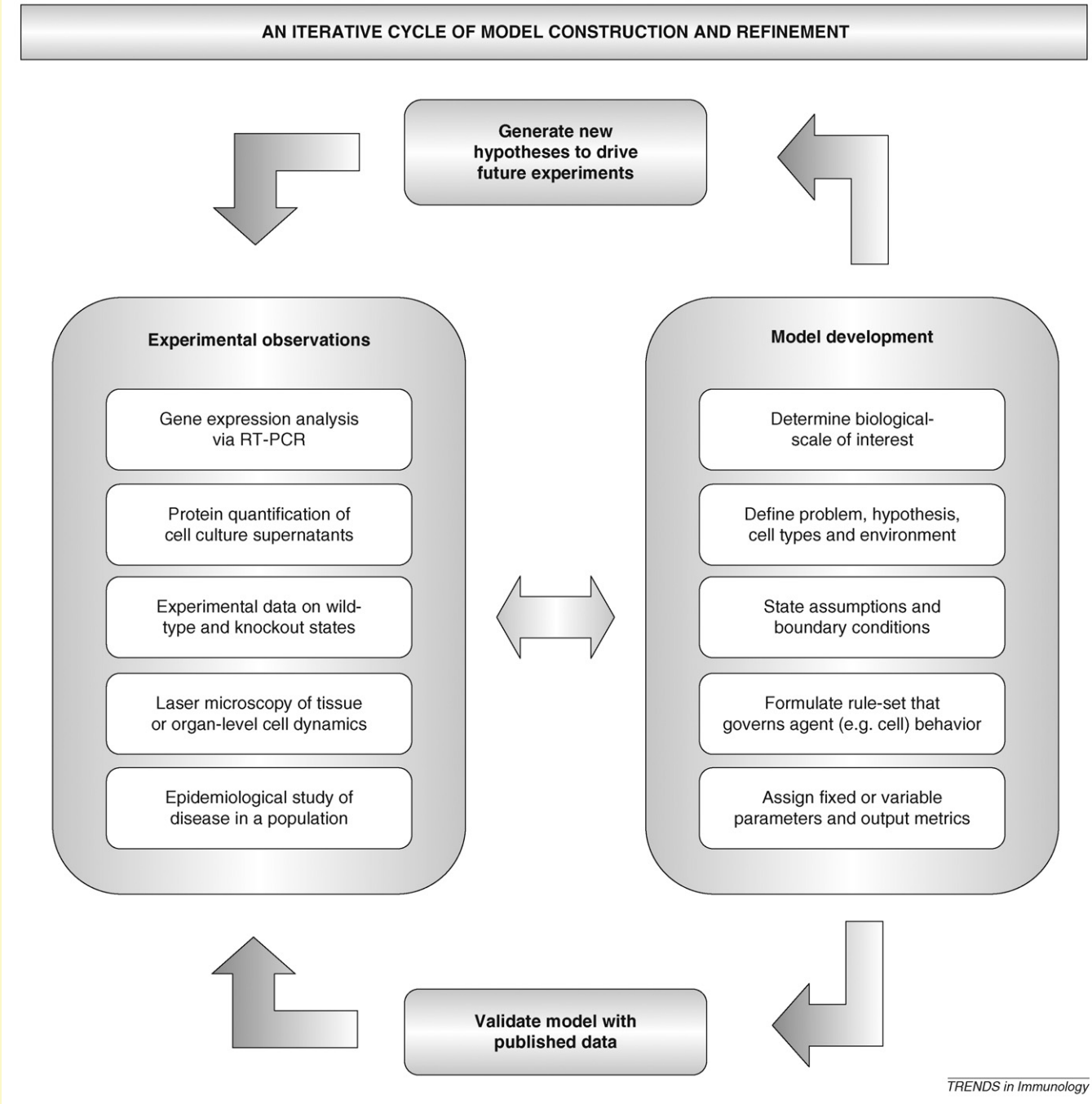

Figure I. Model building and iterative refinement in concurrence with experimentation. The key steps involved in model construction are highlighted. Experimental observations are needed to implement a rule-set, iteratively refine the model and also validate model observations and hypotheses.

stages as opposed to those cells that are in their final round of division. The ability to track individual cells also distinguishes the ABM approach from traditional differential equation-based modeling. Often, differential equation-based models assume well-mixed, homogeneous populations of cells, thus implying that all cells within a population are alike. In contrast, the ABM approach treats cells of the same and of different types as discrete entities [9,31,32].

In addition, each agent can be allowed to develop memory of various prior interactions based on its individual history of activities [21]. For example, a 'counter' can be initialized as a local variable for a particular set of agents belonging to the same cell-type (e.g. type 'A'). Every instance in which an agent of type 'A' detects an interaction with another agent of a different cell-type 'B', the 'counter' is incremented by '+1'. The specifics of this interaction are dependent on the underlying rule-set (e.g. whether the interaction occurs when the two cell-types share the same micro-compartment or are present in adjacent micro-compartments). In this way, the number of prior interactions between each agent of type 'A' and agents of type 'B' can be tracked. Supposing the 'counter' crosses a particular threshold, an agent of type 'A' can undergo a state change, with the new state governed by an altered rule-set. This

Box 3. Advantages and limitations of the ABM approach

Here, we briefly summarize advantages and limitations associated with the ABM approach for simulating immunological systems. See 'Expanding upon features of agent-based models' in main text and supplementary material I for more details.

Advantages

- *Comprehensibility*: ABM is more appealing to non-mathematicians because of the use of logical rules as opposed to a rigorous mathematical formalism [34]. Software packages such as NetLogo [29], StarLogo [69] and Repast [70] have graphical outputs mimicking the actual structure of a model system and these are more visually accessible to biologists and clinicians than, for example, differential equation-based concentration profiles.
- *Identifiability*: an individual agent is 'identifiable' [21], with a unique history of interactions and activities [31]. This feature enables distinguishing between agents of the same cell-type and tracking as they progress through their respective life-cycles.
- *Adaptability*: when allowed to develop memory of prior interactions based on its individual history of activities, an agent can 'adapt' by undergoing a state transition and being governed by an altered rule-set [21].
- *Local interactions*: at a given time step, the decision-making ability of agents is dependent on the presence of other agents in the vicinity and the composition of the environment. Thus, simulating local cell–cell and cell–environment interactions is possible with ABM.
- *Spatial compartmentalization*: because the environment is modeled on a Cartesian coordinate space [16], compartments can be created as necessary. Movement of agents can be governed by rule-sets that incorporate information on spatial restrictions.
- *Clarification of discrepancies*: ABM constitutes a systems biology-based approach that synthesizes data and literature for formulation of a rule-set and generation of testable hypotheses. This process can aid in clarifying conflicting sources of information.

Limitations

- *Parameter estimation*: it is often difficult to locate key model parameters, including rate constants, in published literature [66]. In these cases, parameters need to be estimated, either via additional laboratory experiments or theoretical approaches, including sensitivity analysis and optimization procedures [66,71].
- *Computationally intensive*: because of the numbers of agents and interactions, ABM can be computationally intensive in terms of run-time and memory [32,42]. For example, simulations of immune processes can comprise thousands of agents, and as a result, require several hours of run-time and gigabytes of memory on standard desktop computers. Furthermore, rule-sets that include stochastic elements (e.g. randomized scanning of agents) requiring multiple simulations to determine whether global behavior observed on any one run is reproducible can increase computational time considerably. Importantly, there are methods for overcoming these limitations (see supplementary material I).

strategy allows for adaptive behavior of individual agents within the system.

The ability to account for adaptive behavior of agents makes ABM attractive for simulating immunological processes because the immune system itself is described as being adaptive to foreign antigens that it encounters over time [11]. This adaptive power of agents (i.e. their ability to be reactive to external perturbations and proactively change their state or behavior) within the ABM framework differentiates them from strictly reactive 'objects' associated with traditional object-oriented programming (OOP) [33]. In other words, whereas objects are static in the sense that they retain their identities (i.e. class-types), agents are dynamic and can alter their identities over the course of a simulation. For example, in OOP, a naïve T-cell 'object' remains a naïve T-cell 'object' throughout a simulation, and the methods associated with it do not change. In contrast, in ABM, a naïve T-cell 'agent' might evolve into an effector T-cell 'agent' that is governed by an altered rule-set.

Formulation of logical rules

Rules in agent-based models are a collection of clear, concise statements, often written as 'IF-THEN-ELSE' relationships [15,34]. A hypothetical rule might take on the following form: 'IF a naïve T cell (agent 1) detects a foreign antigen on the surface of a DC (stationary and part of the environment) by residing in the same micro-compartment at a given time step, THEN agent 1 is allowed to change state to become an activated T cell, ELSE agent 1 remains a naïve T cell'. Other examples of rules used in an agent-based model include: 'activated T cells proliferate at a doubling period of 8 h for 4 divisions' and 'if a naïve, cognate $CD4^+$ T cell comes in contact with a mature or licensed DC, or a naïve $CD8^+$ T cell comes in contact with a licensed DC, the T cell binds with probability equal to 75%' [14].

Rules can also involve the presence or absence of cytokines within the environment, the concentrations of intracellular species (within each cell or agent) or the flux of ligands into and out of a system [35]. In addition, they can describe known evolutionary characteristics of the immune response, such as the ability of the immune response to adapt as part of host-pathogen interactions. For instance, development of memory T-cell subsets with lower activation thresholds compared to their naïve counterparts enables the immune system to mount a robust secondary response to recurring antigenic stimulation [11,36]. In this way, rules can dictate the life-cycle progression for agents in a simulation.

Insights into immunological processes

In recent years, agent-based and CA models have explored key immunological processes, including T-cell and DC interactions in the LN [14], T_H-cell polarization [37], germinal center reactions and high-affinity B-cell selection [38,39], dynamics of HIV infection [40], positive and negative selection of T cells in the thymus [41] and primary or secondary immune responses to recurring antigenic stimulation [20,39,42] (see Table 1 and supplementary material II). Here, we highlight a few of these models, focusing specifically on the topic of T_H-cell priming and differentiation. We categorize our discussion into three different scenarios: (i) instances in which model observations have been validated or refined by experiments, (ii) instances in which models have generated novel hypotheses that still need to be verified by future experimental efforts and (iii) instances in which the development of new models in the future would improve our understanding of biological processes.

Model results validated and refined: switching T_H-cell polarization

One situation in which model observations have been validated and refined subsequently by experimental data pertains to T_H-cell polarization. Various microbial infec-

tions cause a divergence in effector T_H response as a means towards protective immunity [37,43,44]. For example, experimental studies in mice show that C57 Black/6 (C57BL/6) strains primarily invoke a T_H1 response to *Leishmania major* infection to confer resistance, whereas susceptible BALB/c (Bagg albino) strains elicit a T_H2 outcome [45,46].

A simple CA model explored populations of effector T_H cells under varying antigen densities, the presence or absence of a T-cell death term and differing probabilities of T_H1 versus T_H2 activation from an antigen source [37] (Table 1, 'General immunology #1'). A key observation of the model was that once polarization was achieved in the LN (i.e. the dominance of a single effector population emerged), it was not possible to switch the response towards the suppressed outcome by altering the probability of activation such that the opposite result was favored [37].

This observation can be validated qualitatively with independent experiments assessing *L. major* burden in BALB/c mice at the end of five weeks [47]. Interleukin-12 (IL-12) administered to BALB/c mice during the first week of infection had 15-times-reduced parasite burden (compared to saline-treated mice). By contrast, when IL-12 treatment was delayed by one week, polarization towards a T_H2 response was achieved by the end of the first week and, consequently, only a two-times reduction in parasite burden was observed [47]. However, other experiments have indicated that a combination therapy comprising the anti-leishmanial drug 'Pentostam' (which reduces antigen load) in addition to IL-12 causes a shift from the dominant T_H2 outcome to a T_H1 response in BALB/c mice [48]. Therefore, this result has refined the CA model observation. Although the experimental findings were generated independently of the CA model, this particular situation speaks to how immunological models can yield hypotheses, inform decisions and drive future experimental work on a particular system of interest.

In this example, model observations were validated with experimental data at the tissue-scale (T_H1 versus T_H2 polarization), whereas the input parameters were defined at the cellular-scale (antigen density). It is important to differentiate between experimental datasets that are used for constructing the rule-set and those employed for purposes of validation [17].

Model results to be verified experimentally: T-cell receptors and peptides

A recent agent-based model exploring the interactions between TCRs and peptides presented on major histocompatibility complexes (MHCs) is an example of a model that needs to be further explored and validated experimentally. Specifically, the priming of naïve T_H cells by DCs to differentiate into effector T_H1 and T_H2 subtypes depends on several signaling mechanisms and the formation of the immunological synapse [11,43]. One of the key interactions is between TCRs and the peptides on the MHCs [11].

A cellular-scale agent-based model explored T-cell priming in the context of heterogeneous peptide populations via the concept of TCR clustering in the immunological synapse [30] (Table 1, 'General immunology #2'). The model simulated a peptide repertoire consisting of nulls, antagonists, weak agonists and strong agonists that promoted differing levels of TCR activation. A few observations that were reported from the agent-based model included: (i) small numbers of strong agonist among a larger population of nulls, antagonists and weak agonists resulted in T-cell priming; (ii) peptide signatures that were slightly below an activating threshold could become activating if strong agonists were added; and (iii) non-activating peptide signatures were not necessarily composed of all null peptides, but, rather, of varying degrees of all peptide types [30].

A key hypothesis that emerged from the model is that T-cell priming is context dependent, that is, the entire signature of peptides on a DC that interacts with TCRs confers activation, not just the strong agonist peptide and TCR associations [30]. As with many agent-based models, future wet-laboratory work, perhaps requiring new experimental protocols or technologies, is required to address and validate these findings. However, experiments have shown that 200–300 foreign pMHCs in a large population of 100 000 self pMHCs are adequate to prime naïve T cells [49,50].

This model is an example of the ABM approach being applied to cellular-scale phenomena, that is, interactions occurring between molecules present on the surface of whole cells. This example demonstrates that ABM is readily extendible to different biological scales. In Table 1 and supplementary material II, we provide information on the biological scale(s) specific to every agent-based and CA immunological model that we catalog.

Future models: examples of other factors and processes involved in T_H-cell polarization

Agent-based models have characterized emergent properties of the immunological response and yielded new hypotheses requiring further validation. In some cases, these *in silico* observations have been confirmed experimentally. Nevertheless, there are numerous aspects of the immunological response that have yet to be examined and that are well suited for computational analysis. For example, by simultaneously perturbing multiple complex interactions that facilitate polarization of the T_H-cell response, agent-based models can indicate combination therapies that would effectively bias immune responses to clear microbial pathogens.

Beyond the interactions described in the preceding sections, there are several other factors that influence activation and differentiation of T_H-cell responses. For example, the generation of effector subtypes is dependent on affinity, avidity and time of interaction between TCRs and peptides on MHCs, all the while given a frequency of interaction between specific T cells and DCs of 1 in 10^5 [19,51–53]. Agent-based models can be used to effectively determine sensitivity of these parameters towards formulating a dominant T_H-cell response. Furthermore, given the probability of T-cell and DC interactions, in addition to the ability of DCs to sample ~5000 T cells per hour [51], an agent-based model can begin to probe crowding and competition around DCs and the resultant effects on T-cell activation. The spatial effects around DCs can be visualized using the ABM approach.

Molecules, such as pathogen-associated molecular patterns (PAMPs), binding to pattern recognition receptors (PRRs) on the surface of DCs or other inflammation-associated tissue factors can also influence DC maturation and the ultimate differentiation of T_H cells towards a particular subtype [43]. In addition, T_{REG} cells can act to suppress an effector T_H response [43,44]. Agent-based models can thus be used to assess the dynamics of generating a protective immune response when including the selective bias of PAMP molecules and the inhibitory effects of T_{REG} cells as part of the underlying rule-set. The heterogeneity of the immune response can be captured using ABM as many different cell-types can be accounted in the model.

Concluding remarks

As immunological research has expanded in recent years, the need for a multi-scale, systems biology-based approach to quantitatively interrogate immunological systems has arisen [54]. The immune response is achieved through a series of interactions from the genetic level up to the whole body, and it can span multiple time scales ranging from a millisecond to a lifetime [54]. Integrating behaviors across multiple biological scales of organization remains a central challenge in systems biology [55] and is important to understanding the development of the immune response against disease. ABM is an approach that enables integration across scales by incorporating rule-sets at one level to characterize emergence of behavior at a higher level of organization [56]. For example, cellular-scale interactions between cell-surface molecules can lead to activation and proliferation of a particular cell-type, which has implications at the tissue level.

Moreover, understanding immune mechanisms in healthy individuals and how the immune system adapts under different disease challenges is crucial for targeted disease therapy. Several disease-causing organisms have evolved effective mechanisms to evade host immune responses. For example, the opportunistic pathogen *Pseudomonas aeruginosa* secretes proteases, such as alkaline protease and elastase, to hinder the normal activity of T cells, natural killer cells, phagocytes and inflammatory cytokines [57]. Similarly, *Leishmania* parasites can persist lifelong in patients by employing several evasion strategies [58]. Therefore, it is vital to comprehend the complicated interaction networks at the multi-cell level to efficiently engineer novel therapeutics against organisms that continually evade and resist normal immune function.

In this review, we have presented ABM and CA as one class of computational tools to pursue a systems biology-based approach to studying immunology. These approaches attempt to simulate multi-cellular (or tissue-scale) immunological behaviors by representing individual cells as discrete autonomous agents, each with its own set of logical rules. The modeling techniques, however, are also extendible to other biological scales of interest [30,59]. Ultimately, ABM is proving to be a powerful approach to describe the inherent complexities of the immune response, to study the attributes of a complex system (i.e. adaptability, self-organization and emergence) and, coupled with experimental observations, to generate novel hypotheses and drive discovery.

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Supplementary data

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References

1 Kitano, H. (2002) Computational systems biology. *Nature* 420, 206–210
2 Reed, J.L. *et al.* (2006) Towards multidimensional genome annotation. *Nat. Rev. Genet.* 7, 130–141
3 Chavali, A.K. *et al.* (2008) Systems analysis of metabolism in the pathogenic trypanosomatid Leishmania major. *Mol. Syst. Biol.* 4, 177
4 Hoffmann, A. *et al.* (2002) The IkappaB-NF-kappaB signaling module: temporal control and selective gene activation. *Science* 298, 1241–1245
5 Woolf, P.J. *et al.* (2005) Bayesian analysis of signaling networks governing embryonic stem cell fate decisions. *Bioinformatics* 21, 741–753
6 Aderem, A. (2005) Systems biology: its practice and challenges. *Cell* 121, 511–513
7 Kitano, H. (2002) Systems biology: a brief overview. *Science* 295, 1662–1664
8 Robertson, S.H. *et al.* (2007) Multiscale computational analysis of *Xenopus* laevis morphogenesis reveals key insights of systems-level behavior. *BMC Syst. Biol.*,1, 46 DOI: 10.1186/1752-0509-1-46
9 Segovia-Juarez, J.L. *et al.* (2004) Identifying control mechanisms of granuloma formation during M. tuberculosis infection using an agent-based model. *J. Theor. Biol.* 231, 357–376
10 Ottino, J.M. (2003) Complex systems. *AIChE J.* 49, 292–299
11 Abbas, A.K. and Lichtman, A.H. (2003) *Cellular and Molecular Immunology*. Saunders
12 Lanzavecchia, A. and Sallusto, F. (2002) Progressive differentiation and selection of the fittest in the immune response. *Nat. Rev. Immunol.* 2, 982–987
13 Grimm, V. (1999) Ten years of individual-based modelling in ecology: what have we learned and what could we learn in the future? *Ecol. Modell.* 115, 129–148
14 Riggs, T. *et al.* (2008) A comparison of random vs. chemotaxis-driven contacts of T cells with dendritic cells during repertoire scanning. *J. Theor. Biol.* 250, 732–751
15 An, G. (2004) In silico experiments of existing and hypothetical cytokine-directed clinical trials using agent-based modeling. *Crit. Care Med.* 32, 2050–2060
16 Bailey, A.M. *et al.* (2007) Multi-cell agent-based simulation of the microvasculature to study the dynamics of circulating inflammatory cell trafficking. *Ann. Biomed. Eng.* 35, 916–936
17 Thorne, B.C. *et al.* (2007) Agent-based modeling of multicell morphogenic processes during development. *Birth Defects Res. C Embryo Today* 81, 344–353
18 Grant, M.R. *et al.* (2006) Simulating properties of *in vitro* epithelial cell morphogenesis. *PLOS Comput. Biol.* 2, e129
19 Miller, M.J. *et al.* (2004) Imaging the single cell dynamics of CD4+ T cell activation by dendritic cells in lymph nodes. *J. Exp. Med.* 200, 847–856
20 Seiden, P.E. and Celada, F. (1992) A model for simulating cognate recognition and response in the immune system. *J. Theor. Biol.* 158, 329–357
21 Macal, C.M. and North, M.J. (2005) Tutorial on agent-based modeling and simulation. In *Winter Simulation Conference* (Kuhl, M.E. *et al.*, eds), DOI: 10.1109/WSC.2005.1574234 (http://ieeexplore.ieee.org/xpls/abs_all.jsp?arnumber=1574234)
22 Arstila, T.P. *et al.* (1999) A direct estimate of the human alphabeta T cell receptor diversity. *Science* 286, 958–961
23 Hackett, C.J. *et al.* (2007) Immunology research: challenges and opportunities in a time of budgetary constraint. *Nat. Immunol.* 8, 114–117
24 Ottino, J.M. (2004) Engineering complex systems. *Nature* 427, 399
25 Reynolds, C.W. (1987) Flocks, herds, and schools: a distributed behavioral model. *SIGGRAPH* 21, 25–34
26 Topping, C.J. and Odderskaer, P. (2004) Modeling the influence of temporal and spatial factors on the assessment of impacts of pesticides on skylarks. *Environ. Toxicol. Chem.* 23, 509–520

27 Macy, M.W. and Sato, Y. (2002) Trust, cooperation, and market formation in the U. S. and Japan. *Proc. Natl. Acad. Sci. U. S. A.* 99 (Suppl 3), 7214–7220
28 LeBaron, B. (2002) Short-memory traders and their impact on group learning in financial markets. *Proc. Natl. Acad. Sci. U. S. A.* 99 (Suppl 3), 7201–7206
29 Sklar, E. (2007) Software review: NetLogo, a multi-agent simulation environment. *Artif. Life* 13, 303–311
30 Casal, A. *et al.* (2005) Agent-based modeling of the context dependency in T cell recognition. *J. Theor. Biol.* 236, 376–391
31 Kohler, B. *et al.* (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, 862–876
32 Chao, D.L. *et al.* (2004) A stochastic model of cytotoxic T cell responses. *J. Theor. Biol.* 228, 227–240
33 Wooldridge, M. and Ciancarini, P. (2001) Agent-oriented software engineering: the state of the art. In *Agent-Oriented Software Engineering* (Ciancarini, P. and Wooldridge, M.J., eds), pp. 55–82, Springer
34 Vodovotz, Y. *et al.* (2004) Mathematical models of the acute inflammatory response. *Curr. Opin. Crit. Care* 10, 383–390
35 Athale, C. *et al.* (2005) Simulating the impact of a molecular 'decision-process' on cellular phenotype and multicellular patterns in brain tumors. *J. Theor. Biol.* 233, 469–481
36 Williams, M.A. *et al.* (2008) Rapid culling of the CD4+ T cell repertoire in the transition from effector to memory. *Immunity* 28, 533–545
37 Brass, A. *et al.* (1994) A cellular automata model for helper T cell subset polarization in chronic and acute infection. *J. Theor. Biol.* 166, 189–200
38 Meyer-Hermann, M.E. *et al.* (2006) An analysis of B cell selection mechanisms in germinal centers. *Math. Med. Biol.* 23, 255–277
39 Celada, F. and Seiden, P.E. (1996) Affinity maturation and hypermutation in a simulation of the humoral immune response. *Eur. J. Immunol.* 26, 1350–1358
40 Zorzenon dos Santos, R.M. and Coutinho, S. (2001) Dynamics of HIV infection: a cellular automata approach. *Phys. Rev. Lett.* 87, 168102
41 Morpurgo, D. *et al.* (1995) Modelling thymic functions in a cellular automaton. *Int. Immunol.* 7, 505–516
42 Bernaschi, M. and Castiglione, F. (2001) Design and implementation of an immune system simulator. *Comput. Biol. Med.* 31, 303–331
43 Kapsenberg, M.L. (2003) Dendritic-cell control of pathogen-driven T-cell polarization. *Nat. Rev. Immunol.* 3, 984–993
44 Kaiko, G.E. *et al.* (2008) Immunological decision-making: how does the immune system decide to mount a helper T-cell response? *Immunology* 123, 326–338
45 von Stebut, E. and Udey, M.C. (2004) Requirements for Th1-dependent immunity against infection with Leishmania major. *Microbes Infect.* 6, 1102–1109
46 von Stebut, E. (2007) Cutaneous Leishmania infection: progress in pathogenesis research and experimental therapy. *Exp. Dermatol.* 16, 340–346
47 Sypek, J.P. *et al.* (1993) Resolution of cutaneous leishmaniasis: interleukin 12 initiates a protective T helper type 1 immune response. *J. Exp. Med.* 177, 1797–1802
48 Nabors, G.S. *et al.* (1995) Switch from a type 2 to a type 1 T helper cell response and cure of established Leishmania major infection in mice is induced by combined therapy with interleukin 12 and Pentostam. *Proc. Natl. Acad. Sci. U. S. A.* 92, 3142–3146
49 Yates, A. *et al.* (2001) An approach to modelling in immunology. *Brief. Bioinform.* 2, 245–257
50 Harding, C.V. and Unanue, E.R. (1990) Quantitation of antigen-presenting cell MHC class II/peptide complexes necessary for T-cell stimulation. *Nature* 346, 574–576
51 Miller, M.J. *et al.* (2004) T cell repertoire scanning is promoted by dynamic dendritic cell behavior and random T cell motility in the lymph node. *Proc. Natl. Acad. Sci. U. S. A.* 101, 998–1003
52 Rogers, P.R. and Croft, M. (1999) Peptide dose, affinity, and time of differentiation can contribute to the Th1/Th2 cytokine balance. *J. Immunol.* 163, 1205–1213
53 Lanzavecchia, A. and Sallusto, F. (2000) Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells. *Science* 290, 92–97
54 Kirschner, D.E. *et al.* (2007) Toward a multiscale model of antigen presentation in immunity. *Immunol. Rev.* 216, 93–118
55 Ridgway, D. *et al.* (2006) Accommodating space, time and randomness in network simulation. *Curr. Opin. Biotechnol.* 17, 493–498
56 Young, D. *et al.* (2008) Systems biology of persistent infection: tuberculosis as a case study. *Nat. Rev. Microbiol.* 6, 520–528
57 Kharazmi, A. (1991) Mechanisms involved in the evasion of the host defence by Pseudomonas aeruginosa. *Immunol. Lett.* 30, 201–205
58 Bogdan, C. and Rollinghoff, M. (1998) The immune response to Leishmania: mechanisms of parasite control and evasion. *Int. J. Parasitol.* 28, 121–134
59 Woolf, P.J. and Linderman, J.J. (2003) Untangling ligand induced activation and desensitization of G-protein-coupled receptors. *Biophys. J.* 84, 3–13
60 Chowdhury, D. *et al.* (1991) A discrete model for immune surveillance, tumor immunity and cancer. *J. Theor. Biol.* 152, 263–270
61 Mallet, D.G. and De Pillis, L.G. (2006) A cellular automata model of tumor-immune system interactions. *J. Theor. Biol.* 239, 334–350
62 Hu, R. and Ruan, X. (2003) A simple cellular automaton model for tumor-immunity system. In *International Conference on Robotics, Intelligent Systems and Signal Processing (Proceedings of the 2003 IEEE)*, pp. 1031–1035, DOI:10.1109/RISSP.2003.1285731 (http://ieeexplore.ieee.org/xpls/abs_all.jsp?arnumber=1285731)
63 Torrens, P.M. and Benenson, I. (2005) Geographic automata systems. *Int. J. Geogr. Inf. Sci.* 19, 385–412
64 Bonabeau, E. (1997) From classical models of morphogenesis to agent-based models of pattern formation. *Artif. Life* 3, 191–211
65 Schonfisch, B. and de Roos, A. (1999) Synchronous and asynchronous updating in cellular automata. *Biosystems* 51, 123–143
66 Thorne, B.C. *et al.* (2007) Combining experiments with multi-cell agent-based modeling to study biological tissue patterning. *Brief. Bioinform.* 8, 245–257
67 Kari, J. (2005) Theory of cellular automata: a survey. *Theor. Comput. Sci.* 334, 3–33
68 Grimm, V. and Railsback, S.F. (2005) *Individual-Based Modeling and Ecology*. Princeton University Press
69 Resnick, M. (1996) StarLogo: an environment for decentralized modeling and decentralized thinking. In *Conference on Human Factors in Computing Systems*, pp. 11–12, ACM
70 North, M.J. *et al.* (2006) Experiences creating three implementations of the repast agent modeling toolkit. *ACM Trans. Model. Comput. Simul.* 16, 1–25
71 Calvez, B. and Hutzler, G. (2005) Parameter space exploration of agent-based models. In *Knowledge-Based Intelligent Information and Engineering Systems, Pt 4. Proceedings* 3684, 633–639