Multiscale modelling in immunology: a review

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Abstract

One of the greatest challenges in biomedicine is to get a unified view of observations made from the molecular up to the organism scale. Towards this goal, multiscale models have been highly instrumental in contexts such as the cardiovascular field, angiogenesis, neurosciences and tumour biology. More recently, such models are becoming an increasingly important resource to address immunological questions as well. Systematic mining of the literature in multiscale modelling led us to identify three main fields of immunological applications: host–virus interactions, inflammatory diseases and their treatment and development of multiscale simulation platforms for immunological research and for educational purposes. Here, we review the current developments in these directions, which illustrate that multiscale models can consistently integrate immunological data generated at several scales, and can be used to describe and optimize therapeutic treatments of complex immune diseases.

Key words: multiscale modelling; immunology; mathematical modelling; inflammation; host–virus interaction; computational methods

Introduction

Multiscale modelling (MSM) aims at integrating the mathematical and computational description of processes operating at variable spatial, temporal and organizational levels. In the past 20 years, it has become an established resource to investigate diverse physiopathological processes. Examples can be found in the cardiovascular field [1], neural systems [2, 3], tumour growth [4], vascular formation [5], epidermal wound healing [6–9] and tissue engineering [10]. Previous reviews devoted to MSM research provided an insightful historical reconstruction [11] and a general survey of MSM resources and communities [12], and recapitulated applications to angiogenesis [13] and tumour biology [14].

Systematic literature mining reveals that one of the most recent and rapidly developing trends in MSM research is given by immunological applications (Figure 1). In this context, attempts are being made to deepen our understanding of infection and inflammatory processes, and to suggest improved treatments of widespread immunological disorders. Here, we aim to review the state-of-the-art of this growing body of knowledge, and to discuss current challenges and future perspectives.

This work is organized as follows. In the first section, we provide a minimal background on immunological processes and modelling techniques commonly considered in single-scale models, which form the building blocks of multiscale models. In the second section, we organize the published works in three
Levels of immunological organization

Complex biological systems such as the human body are composed by basic building blocks organized into hierarchical structures. Molecules and macromolecules—such as lipids, proteins and DNA—are organized into cells, which are arranged in tissues, organs, organ systems and finally in the whole organism [15, 16]. These hierarchical layers are regarded as the different ‘levels of biological organization’ [17]. Such organizational levels are intertwined with a flow of information feedback, both bottom-up and top-down, making impossible the identification of a privileged level of causation [17–19].

In this section, we exemplify key immunological processes occurring at different levels of biological organization (Figure 2), along with the corresponding modelling techniques (Table 1). The examples below provide a minimal background on the elements most frequently considered in immunological modelling. For simplicity, the spatial scales are broadly classified in three main groups: host–virus interactions; inflammatory diseases/treatment; development of software.

Microscopic level

At the molecular level, fundamental immunological events involve the recognition of ‘danger’ signals by innate immune cells, which can sense molecular fragments derived both from pathogens [24] and from host cells, possibly damaged as a result of infection [25]. A key molecular interaction is the binding of antigens to major histocompatibility complex (MHC) class molecules in antigen-presenting cells such as dendritic cells (DC). The binding affinity between a given antigen and MHC molecules was found to correlate with several immune diseases [26], suggesting that this parameter critically characterizes the immune response at the systems level.

At the microscopic, intracellular level, we point to the importance of immune-specific signalling pathways activated in innate and adaptive immune cells during immune reactions. Among many, we mention the Toll-like receptor signalling, responsible for innate immune responses; the Nuclear Factor *κ* B (NF-κB) signalling pathway, a master regulator of inflammation; the transduction of pro- and anti-inflammatory cytokines such as Tumour Necrosis Factor alpha (TNF-*) and interleukin (IL)-10, respectively; the intracellular network driving the differentiation of naïve T helper cells. The topology of many immunological pathways are available in online repositories [27–29], and some key immune-related pathways have already been the target of mathematical modelling, particularly NF-κB [30–32] and T helper differentiation [33, 34]. Such models, generally based on ordinary and partial differential equations (ODEs, PDEs), Boolean networks and stochastic approaches, can reproduce qualitative and quantitative features of the transduction process, and can be integrated in multiscale descriptions of the immune response. For critical reviews on advantages and disadvantages of the above modelling formalisms, the reader can refer to the work by Germain et al. [35] and Narang et al. [36].

In the context of host–virus interactions, it is worth mentioning the molecular machineries regulating the different stages of the intracellular life cycle of viruses, such as the replication of viral genomes and the assembly of new virions. These mechanisms are currently major targets in the design of antiviral therapies and have been mathematically modelled in several works [37, 38].

Mesoscopic level

The mesoscopic scale sets the stage for local interactions within immune cells and between immune and non-immune cells. These interactions globally coordinate recruitment of immune cells to the site of infection and antimicrobial activity. Cell–cell interactions are mediated by a variety of contact molecules and soluble mediators.
such as interleukins and chemokines, whose deregulation is a feature of most immune diseases. A prototypical cell–cell interaction is the immunological synapse between DC and T cells that mediates antigen presentation [39, 40].

From the modelling point of view, processes at the mesoscopic scale are commonly described within the framework of population dynamics with its deterministic, stochastic and age-structured variants [41]. In addition to continuous...
approaches, discrete approaches that allow achieving a single-cell resolution, such as cellular automata (CA) and agent-based models (ABM), have become increasingly popular [42]. Furthermore, several works have been devoted to describe mathematically the process of chemotaxis, the movement of cells in response to biochemical signals. In these models, reaction-diffusion equations are used to couple the space-time distribution of a motile cell population with the space-time distribution of a chemoattractant, as in the well-known Keller–Segel model [43, 44].

In a pathological context, important interactions occur between effector immune cells with tumours and with viruses, which motivated intensive modelling work reviewed, for example, by Efthimiou et al. [45] and Louz et al. [46].

Macroscopic level
At the macroscopic scale, it is important to underline the fact that immune system processes typically involve multiple organs, such as bone marrow, spleen, thymus and lymph nodes. Lymph nodes play a particularly important role in shaping the nature of an immune response. A lymph node is roughly from a few millimeters to 1–2 centimeters long [47] and involves a complex anatomy where antigen-presenting cells interact with T cells and B cells in specific compartments. In the human body, there are ~600 lymph nodes, and those closest to a given site of infection are first engaged in the infection dynamics [26]. However, in systemic infections, many distant lymph nodes are potentially affected.

The different organs involved in an immune response are connected by a network of lymphatic and blood vessels, given that a fundamental macroscopic feature of immune system processes is the global migration and circulation of immune cells throughout the body. On this topic, it is worth noting that a remarkable imbalance still exists in terms of effort spent for the modelling of lymphatic system in contrast to the cardiovascular system [48].

At the macroscopic scale, one of the most evident immunological manifestations is tissue inflammation, with its characteristic features of swelling, increased blood flow, tissue damage and ensuing involvement of multiple organs. Although attempts have been made [49–51], comprehensive models of inflammatory processes remain a far goal.

MSM in immunology

Literature analytics
We started our review of multiscale models in immunology by analysing publication trends in PubMed (http://www.ncbi.nlm.nih.gov/pubmed). We first generated a reference trend provided by the number of publications containing the term ‘multiscale’ in the title and/or abstract, observing a seemingly exponential growth over the past 20 years (Figure 1A).

We then included in our search criteria key terms related to the immunological field, such as ‘immunology’, ‘inflammation’, ‘infection’ and similar, obtaining 103 papers. Further manual curation led us to rule out 57 papers that, while still complying with our search criteria, were not sufficiently relevant to our scope. Thus, we selected a total of 46 papers devoted to multiscale approaches in immunology. Although this represents a small fraction in the field of MSM, a closer look reveals a marked increase in the publication rate over past years (Figure 1A).

In an effort to organize this body of knowledge, we looked for commonalities in the immunological questions addressed, and identified three main research directions: modelling of host–virus interactions, modelling of inflammation/immunotherapy and development of immune system simulation platforms (Figure 1B).

In the following sections, we review a selection of works representative of each direction. The selection was guided mainly by the following criteria: (i) diversity of immunological application domains and subdomains; (ii) diversity of modelling techniques; (iii) relatively recent publication, with particular emphasis on the past 5 years.

Host–virus interactions

Initial models of host–virus interactions were developed within the framework of population dynamics, considering the interactions between the virus, its target cells and infected cells. An important application of such models is the quantitative reproduction of the viral decline induced by antiviral drugs, particularly in the context of HIV, influenza virus and hepatitis C virus (HCV) infection. However, population dynamics models have limited explanatory power because they neglect the intracellular mechanism of action of antiviral agents.

To overcome this limitation, several multiscale approaches have been proposed. For instance, Heldt et al. [58, 52] developed a multiscale model of influenza virus infection including processes at both the extra- and the intracellular level. At the extracellular level, the model describes the infection dynamics in a cell population, considering the abundance of infected and uninfected cells, their apoptotic counterparts and the infectious viral particles. At the intracellular level, the virus synthesizes its proteins, replicates its genome and assembles new virions. The two levels are coupled via the age-dependent virus production rate, which depends on the internal state of the infected cell and determines the number of virions released into the extracellular space. In turn, the extracellular level controls the number of infected cells and their lifespan. The whole model, which amounts to a mixed system of ordinary and linear PDEs, was able to fit experimental data across the two scales. It was then used to study how interfering with specific steps of the viral life cycle affects virus production, which led to several intriguing predictions. For example, it is predicted that inhibitors of viral transcription, replication, protein synthesis, nuclear export and assembly/release are most effective in decreasing virus titers, whereas targeting virus entry primarily delays infection. Another prediction is that the success of antivirals may strongly depend on the dynamics of virus-induced apoptosis and on the host’s immune response, reported as a constant parameter in the model.

Similarly to the model described above, Rong et al. [53] developed a multiscale model of HCV infection considering both the extra- and the intracellular scale. At the extracellular scale, a standard ODE model of the viral infection is used [54]. This comprises three differential equations representing the dynamics of target cells, infected cells and the virus. The model was extended to include intracellular processes of viral replication: intracellular viral production, degradation and secretion. Unlike the standard population dynamics model, the multiscale model predicted three different phases of viral decline on antiviral therapy. The slopes of these phases reflect, respectively, the rate of serum viral clearance, the rate of loss of intracellular viral RNA (vRNA) and the rate of loss of intracellular replication. These kinetic parameters, difficult to estimate directly, can instead be estimated by matching the experimental response to
antiviral treatment with an analytical function derived from the multiscale model.

Still within a population dynamics modelling framework, Guedj et al. [55] analysed the in vivo viral decline following administration of daclatasvir, an anti-hepatitis C drug currently in development. Beyond the standard models that only consider the level of cell infection and virus in the serum, the multiscale model used here takes into account critical features of intracellular HCV vRNA replication phenomena such as production, degradation and assembly/secretion, each with its given rate. The model could mimic the experimental viral decline only when assuming that daclatasvir can block at the same time vRNA synthesis and virion assembly/secretion, a finding also supported by further in vitro experiments that allow measurements at both the intra- and extracellular scale.

Overall, the models of host–virus interactions described above show that the integration of processes at the inter- and intracellular scale allow investigating the precise mechanism of action of antiviral agents, thereby enhancing the explanatory and predictive power of models based only on the population level. In point of fact, a common limitation of such models is that, by omitting the mechanistic details of the immune response, one is precluded the possibility to clarify the crucial contribution of different immune weapons to the antiviral activity.

Tuberculosis infection is yet another domain that motivated modelling work at both single and multiple scales (reviewed by Marino et al. [56], Chang et al. [57]). Here, we report about a case study provided by Fallahi-Sichani et al. [58], who investigated the evolution of granulomas, aggregates of immune cells and bacteria in the lungs whose composition reflects success or failure of the host to contain tuberculosis infection. More specifically, the authors explored the role of TNF-α and of its receptor TNFR in shaping the dynamics of granulomas. The model integrates processes at the molecular, cellular and tissue scale. At the molecular scale, the kinetics of TNF-α-TNFR binding and trafficking is modelled with non-linear ODEs for each individual cell. The cellular and tissue-scale dynamics is given by a two-dimensional agent-based model, whose dynamical rules accounted for chemotactic recruitment of immune cells to the site of infection, intracellular and extracellular growth of bacteria, phagocytosis, cell death and apoptosis, T cell cytotoxic and regulatory functions, activation of macrophages by T cells and caseation. The linkage between different scales is achieved via TNF-α-induced cell responses (i.e. apoptosis and NF-κB activation) those are modelled as Poisson processes with rate parameters computed as functions of the molecular concentrations obtained from the intracellular ODE model. After pattern-based validation of the model using available literature, sensitivity analysis led to establish ranges of the model parameters driving different outcomes of granuloma evolution, such as uncontrolled bacterial growth, containment or clearance. These results may give new insight on which biological parameters are most important in driving therapeutic success. The model by Fallahi-Sichani et al. is a remarkable attempt to include multi-organ processes, as well as an explicit description of the immune response. On the other hand, the description of the infection microenvironment is restricted to a biased selection of a few known signalling molecules such as TNF-α, which at this stage neglects the contribution of a range of other pro- and anti-inflammatory mediators.

**Inflammation and immunotherapy**

Multiscale approaches have been applied to describe several steps leading to the generation of an immune response, such as antigen presentation and T helper cell polarization, and to simulate immunotherapy of various inflammatory diseases.

Klinke [59] developed a multiscale model based on differential equations to describe the information transfer from several DC subsets to T helper cells under the effect of a critically important cytokine, IL-12, composed by different subunits. The model considers three anatomical sites: blood, lung epithelium and the lymph nodes. These sites set the stage for three main processes. The first process is the trafficking of several DC subsets in the lung epithelium. The second process is the DC exposure to the signalling molecules in lung microenvironment, comprising IL-4, interferon gamma (IFN-γ) and prostaglandin E2. The effect of these signals is considered to be dependent on the DC maturation stage via age-structured differential equations. Finally, in the third step, DCs migrate to the lymph nodes, where they promote the differentiation of T helper cells through the secretion of the different IL-12 subunits. The model allowed simulating how the specific instructional signals received by DCs in the lung epithelium modulate the DC production of IL-12 in the lymph nodes, and therefore the downstream effects in terms of T helper cell polarization. An important feature of this model is the attempt to account for several DC subsets, as well as for multiple signals present in the lung microenvironment. However, IL-12 is considered the only driver of T helper cell polarization, which is an over-simplification of the complex DC-T helper cells crosstalk in the lymph nodes.

Multiscale models have been proposed to better characterize the events driving T helper cell polarization [60], by coupling cell intrinsic and extrinsic factors at the single cell level [61]. At the intracellular level, the model comprises a Boolean circuit describing the activation dynamics of a minimalistic yet non-trivial gene regulatory network driving Th1 and Th2 polarization. The intracellular processes are ‘individually embedded’ in each CD4 T cell so to translate local cytokine signals with the explicit differentiation phenotype in an agent-based model through the activation of the represented genes. The resulting automaton was able to reproduce dynamic observations at the cell population level of type I hypersensitivity, still remaining compatible with a realistic gene expression profile at the microscopic level [62]. The study identifies the time frequency of 2 weeks as the one to avoid when administering chemotherapy in cancer patient, otherwise risking an anaphylactic shock. This case study further demonstrates that intracellular and extracellular scale can consistently be integrated provided that (i) the intracellular molecular networks are biologically sound and allow for relevant asymptotic regimens and (ii) the stable dynamics at the lower level can be rationally translated into an action (the rule) at the upper level. It must be pointed out, however, that this latter requirement is a strong one and could prevent the use of such seemingly powerful approach in practice.

Recently, an enhancement of such model has been proposed to overcome the limitations deriving from the lack of differentiation processes into key T helper cell subtypes such as Th17 and T regulatory cells [63]. The updated model now describes T helper cell differentiation in four main subclasses (Th1, Th2, Th17 and Treg) in which the boolean gene regulation network consists of 40 nodes, and it is able to describe a coherent behaviour in terms of cell population and cytokine dynamics when challenged with different immunological stimuli.

Somewhat at the border between physiology and therapy, Chen et al. [64, 65] published a differential equation-based model recapitulating key aspects of a humoral immune response happening at multiple scales. The intracellular scale describes detailed molecular mechanisms controlling internalization and
processing of antigens in DCs for subsequent presentation to T cells. The cellular scale accounts for the kinetics and reciprocal interactions of DC, T cells and B cells. Finally, a whole-body compartment accounts for the pharmacokinetics of a putative therapeutic protein. The model, when used to simulate the immune response against a hypothetical antigen, was able to reproduce established phenomena such as immune memory to secondary antigen challenges. From a therapeutic perspective, an interesting application of the model stems from the possibility to predict the induction of antidrug antibodies by the immune system, a side effect that often undermines therapeutic efficacy.

In a disease context, we consider three case studies devoted respectively to Crohn’s disease, liver inflammation and tumour immunotherapy.

Dwivedi et al. [66] presented a multiscale model of Crohn’s disease, an inflammatory bowel disease that results in a pro-inflammatory environment in the mucosal layer of the gastrointestinal (GI) tract. In particular, the authors focused on the pathological role of IL-6 over-expression in this disease. The model is structured in modules. An intracellular module describing the IL-6 transduction pathway, an organ-level module that includes the GI tract and the liver. These two organs are viewed as homogenous cell populations connected through the blood, included as an additional compartment. As a third module, the authors consider a pharmacodynamic model of monoclonal antibodies targeting the cytokine IL-6 or its cognate receptor in either the membrane-bound (IL-6Rα) or soluble form (sIL-6Rα). The parameters of each module were first estimated independently using available data at each scale. The resulting estimations were then used as initial values for a global, multiscale calibration. The multiscale model was validated independently using available clinical data, and then used for simulating alternative treatment options involving blockade of IL-6, IL-6Rα and/or the IL-6/sIL-6Rα complex. Interestingly, simulations predicted the dual inhibition of IL-6/sIL-6Rα complex in addition to IL-6 or IL-6Rα as an improved therapeutic strategy. Thus, this example illustrates the potential of multiscale approaches for exploring how pharmacological perturbations at the molecular level can propagate to higher-level physiopathological manifestations. A limiting factor in this model might be the selection of IL-6 as the dominant player characterizing the inflammatory microenvironment, thus neglecting the role of other potentially important mediators of the immune regulation (e.g. TNF-α, IL-12).

Dutta-Moscato et al. [67] published an agent-based model for the formation of liver fibrosis as a result of chronic liver inflammation. The cell-agents, consisting of parenchymal and inflammatory cells, interact through the exchange of pro- and anti-inflammatory agents (TNF-α, Transforming Growth Factor beta 1, High Mobility Group Box 1), and the rules of these interactions are extracted from the literature. In addition to biochemical processes, the model also describes the mechanical forces exerted by the cell agents on each other, which allows to assess the effect of inflammation on tissue elasticity, a parameter that is decreased in the generation of fibrosis. A patch of healthy liver was modelled as a 2D layer of hepatocytes arranged in lobules, structures that shape the liver microarchitectures. The model parameters were calibrated to maintain a baseline equilibrium of this regular structure in the absence of injury. Then, tissue injury was simulated as the appearance of zones containing dead hepatocytes, which in turn activates an inflammatory cascade leading to recruitment of immune cells (monocytes and neutrophils) and to proliferation of myofibroblasts depositing collagen, a mechanism contributing to the formation of fibrosis. Simulations could mimic histopathological features of fibrosis development, as obtained from animal models. Finally, the model was used to compare potential anti-fibrotic therapies. Important features of this model are the detailed description of the tissue microarchitecture, along with the inclusion of the tissue biomechanics. The model is limited to the local inflammatory microenvironment, and may neglect the involvement of other organs or contributions participating in the inflammatory processes.

While multiscale approaches have been applied to address several questions in tumour biology, few of these works have been devoted to model tumour-immune interactions. On this topic, Klinke [68] illustrated how diverse spatial and temporal scales contribute to shape tumour-immune interactions, taking the anti-tumour effects mediated by IL-12 as a guiding example. In the author’s opinion, our inability to relate information from different scales may greatly limit the development of successful antitumour treatments.

Alemani et al. [69] propose a hybrid method to simulate the tumour growth and its relationship with the immune system. Interestingly, the model describes the molecular details of the tumour microenvironment using a lattice Boltzmann (LB) method. LB is a simulation technique developed in computational fluid dynamics that models a fluid of particles that propagate and collide over a discrete lattice mesh [70]. The immune system is described at the cellular level with a lattice-gas cellular automata (LGCA) method, already used for cancer immunoprevention vaccine simulations in mice [71]. The authors claim that combining LB and LGCA overides the limitations of a classical CA-PDE approach and can reproduce early stage avascular tumours, finger-like tumours patterns and tumour shapes with border irregularity. A limitation of such model may be represented by the lack of details in describing the immune compartment.

Altogether, the case studies presented in this section show that multiscale models provide a valuable methodology as well as a set of tools for studying the progression of complex inflammatory diseases and the effect of therapeutic manipulations.

Software and resources

An additional research line, emerging from our literature review, aims to develop open-source and freely available software and resources for multiscale simulations of immunological processes, as well as for educational purposes, that we will briefly report here. Remarkable efforts have been spent to provide biologists with tools and comprehensive approaches that can help in defining multiscale scenarios and simulating effectively related problems [72]. In this direction, MSM methodologies, including distributed modelling, have been proposed [73, 74], and their performances recently investigated from an architectural and computational point of view to offer informed decision support [75]. Such efforts have also led to the definition of dedicated multiscale environments and infrastructures [76], as well as for high-level programming languages such as the MSM Language [77].

Among the stand-alone immunology-related simulating platforms, we could identify seven available tools: IMMIM/MC-ImmSim [78-81]; the Basic Immune Simulator (BIS) [82, 83]; the Multiscale Systems Immunology (MSI) [84]; the fully-integrated immune response model (FIRM) [85]; the LINDSAY 3D modelling and visualization environment [86]; the Enteric Immunity Simulator - MultiScale Modeling platform (ENISI MSM) [87]; Simmune [88]. The main characteristics of these tools are summarized in Table 2.

Generally, these platforms use cellular agents at the cellular scale and describe their interactions through the exchange of signalling molecules. Most tools allow simulating processes
Table 2. Examples of simulation and visualization platforms for multiscale modelling in immunology

<table>
<thead>
<tr>
<th>Tool</th>
<th>Modelling approach</th>
<th>Programming language</th>
<th>Scope</th>
<th>Scales involved</th>
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</table>
| BIS [75, 76] | Agent-based | Java | Study of the interactions between the cells of the innate and adaptive immune system | • Parenchymal tissue  
• Secondary lymphoid tissue  
• Lymphatic/humoral circulation  
• Intracellular (signalling pathways)  
• Cellular (Cell movement and subtypes)  
• Intercellular (Cytokine diffusion)  
• Tissue (Inflammation and lesions) |
| ENISI MSM [80] | ODE Agent-based | Java (object-oriented) | Immunologist-friendly platform to simulate and visualize signalling pathways, metabolic networks, gene-regulatory networks, cytokine and chemokine diffusions, cell movement, tissue compartments, including lesion formation and discriminates among five anatomical sites: the site of immune recognition, the T and B cell compartments within the lymphoid tissue, the lung and the blood. | • Multi-organ structure  
• Circulating cells dynamics  
• Soluble factors (generic inflammatory signals)  
• Molecular/intracellular (gene regulatory networks)  
• Molecular/intercellular (soluble mediator signalling)  
• Cellular (population dynamics)  
• Organ (lymph nodes)  
• Anatomical context/generic tissue  
• Lymph node  
• Immunological agents (cells/antibodies/viruses) |
| FIRM [78] | Integration of published models | Mathematica 7.0; Matlab | Expandable framework for integration of heterogeneous subset models to simulate immune responses | • Multi-organ structure  
• Circulating cells dynamics  
• Soluble factors (generic inflammatory signals) |
| ImmSim/C-ImmSim [71, 72, 74] | Agent-based | C | Simulation of the humoral and cellular response of a mammalian immune system | • Molecular/intracellular (gene regulatory networks)  
• Molecular/intercellular (soluble mediator signalling)  
• Cellular (population dynamics)  
• Organ (lymph nodes)  
• Anatomical context/generic tissue  
• Lymph node  
• Immunological agents (cells/antibodies/viruses) |
| LINDSAY Composer [79] | Agent-based | Various object-oriented graphical programming interface for the end user | Agent-based three-dimensional modelling and advanced visualization environment for virtual physiology simulation | • Environment (physical spatial volume)  
• Vasculature  
• Cell  
• Soluble factors |
| MSI [77] | Agent-based | Python/C++ | Modelling of the early immune response to vaccination by an agent based immune response simulation that incorporates realistic biophysics and intracellular dynamics | • Intra/intercellular signalling  
• Cells/cell dynamics  
• Medium/environment/tissue |
| Simmune [81] | Agent-based | C | Modelling and simulation of multi-cellular biological systems at the molecular and cellular scale. | • Parenchymal tissue  
• Secondary lymphoid tissue  
• Lymphatic/humoral circulation  
• Intracellular (signalling pathways)  
• Cellular (Cell movement and subtypes)  
• Intercellular (Cytokine diffusion)  
• Tissue (Inflammation and lesions) |

happening in several anatomical sites connected through a compartment describing the blood and/or the lymphatic system.

IMMSIM/C-ImmSim is a model representing both innate and adaptive (humoral and cytotoxic) immune response to a variety of pathogens ranging from virus, bacteria and malignant cells. It integrates intracellular events in the form of Boolean gene regulation networks with mesoscopic cellular dynamics through signals.

BIS is able to simulate basic cell types, mediators and antibodies in three zones of activity representing parenchymal tissue/infection site, secondary lymphoid tissue and the lymphatic/humoral circulation.

The MSI project is an object-oriented modular simulation framework written in C++ and Python designed to incorporate realistic biophysics and intracellular dynamics. The software implements a modular design that allows for flexible configuration of components and initialization of parameters, thus allowing for simulations of processes occurring over different temporal and spatial scales.

Differently from other strategies, FIRM is the only attempt to integrate previously published models within a single simulator, and discriminates among five anatomical sites: the site of immune recognition, the T and B cell compartments within the lymphoid tissue, the lung and the blood.

Interestingly, among the most recent tools, the LINDSAY Composer, makes it possible to integrate immune system processes with other physiological systems, such as the circulatory, endocrine, or nervous system, thereby providing the first step towards whole-body simulations.

The ENISI MSM platform integrates the existing Complex Pathway Simulator COPASI [89] and ENISI [90], an agent-based simulator for modelling gut immunity, to study cases that are not accessible with single-scale models, specifically facilitating the connection of intracellular molecular events with major changes in tissue architecture and pathology occurring at the cellular and tissue levels.

Simmune provides an interface for model building and parameter entry, for automated construction of complex molecular interaction networks from the input of binary molecular interactions, as well as visual outputs. It also permits creating models across varying scales of biological resolution, from intracellular molecular networks to individual cell behaviour to the activity of groups of simulated cells. It is worth mentioning that, although originally developed for simulating immune responses, Simmune has no attributes that are unique to immunology and, according to its authors, it is applicable to other biological contexts.
Conclusions and perspectives

As compared with other fields such as heart physiology, angiogenesis and tumour biology, MSM is a relatively new approach in the context of immunology [91]. The recent developments reviewed in this work show that MSM allows addressing complex immunological questions involving host-virus interactions, inflammatory processes and immunotherapy. However, many challenges lie ahead before fully exploiting the potential of this approach. Important lessons learned from our review concern with common limitations of existing models at the different immunological scales. At the microscopic, intracellular scale, most existing works consider immune transduction pathways promoting or dampening inflammation as isolated cascades. Yet, the behaviour of immune cells in a complex inflammatory microenvironment is ultimately determined by the simultaneous engagement of multiple, interlinked pathways [92]. Future multiscale models should aim to describe how different immune transduction pathways may produce synergistic and antagonistic interactions, and how these interactions propagate to higher scales.

At the mesoscopic scale, notwithstanding some efforts [93–95], a persistent issue is the lack of systematic and quantitative reconstructions of the intercellular communication networks between immune cells, often limited to a few established signalling molecules. As compared with signal transduction networks, intercellular communication networks would have relatively few nodes (cell types) but many possible edges (signals) linking any two nodes, mathematically resulting in multi-graphs. We envision that a systematic analysis of the topology of such multi-graphs would allow for an unbiased selection of critical signals, which could be then included in multiscale models.

At the macroscopic scale, little modelling work has been done to account for the precise geometry and compartmentalization of the lymph node [96–98]. Similarly, the biomechanics of lymphoid tissue and of lymphatic ducts system [99, 100] has received comparatively less attention than in other fields such as bone regeneration and epidermal wound healing, where the mechano-biological framework has found interesting applications [101, 102]. The mechano-biological framework allows coupling the tissue mechanics with cellular processes such as proliferation and differentiation. In immunology, this may be particularly suitable to describe the macroscopic deformation undergone by lymph nodes during an immune response, providing a feedback to the intracellular scale through mechano-transduction processes.

Despite the role of lymph nodes as key anatomical sites, it has been remarked that, out of over $10^{11}$ immune system cells in constant circulation between the blood and lymphatics, only a 10% of activated cells travel to the lymph nodes on a regular basis, whereas the remaining fraction circulates to other organs such as spleen, lung, liver and bone marrow [103, 104]. To capture the full spectrum of immune system dynamics in a model, it will be necessary to include these compartments in addition to the lymph nodes [26].

Another lesson learned from immunological applications of multiscale models is the general, implicit assumption that immune system processes are primarily driven by immune cells while it is well known that, for example, epithelial cells can impose key decisions on innate and adaptive immune cells [105]. More generally, one of the most difficult challenges we face is to take into account established interactions between the immune and other systems, which would reflect the biological notions of ‘epimmunome’ and ‘neuroimmunology’.

In conjunction with problems pertaining to immunological applications, it is important to mention the well-known technical difficulties arising from bridging different modelling paradigms at different scales (e.g. discrete and continuous, deterministic and stochastic models, fast and slow characteristic times, etc.). Further issues involve the management of numerical instabilities deriving from models formulated at different scales; problems concerning parameter estimation; sensitivity of simulation results to large numbers of parameters; control and optimization problems; large computational requirements; standardization and re-usability of the models developed. For a discussion of several of these issues, the reader may refer to the work by Qu et al. [106].

One more consideration that comes out from latest research is that MSM of the immune system is likely unachievable at this time in anything but discrete (i.e. low granularity) models at least for what concerns the mesoscopic scale. This has pros and cons. The well-known benefits of working with ABM have to be weighted with the disadvantage coming from the fact that the granularity of discrete models encapsulates general concepts more than quantitative terms. Therefore, though good for assessing how well separate concepts fit together, such MSM is less likely to achieve high accuracy and may lead investigators down a wrong path.

With the explosion of the -omics data, a fundamental open problem in MSM is to link data from genomics, transcriptomics and metabolomics studies to higher-level phenotypic characteristics. Our review attests a huge gap separating the modelling and the immunoinformatics communities. Recently, two extensive reviews have been published specifically devoted to immunoinformatics resources [107, 108], the latter particularly concerned with methods across multiple scales. We hope that the increasing availability of databases and user-friendly resources will promote the convergence of MSM and omics data.

Multiscale immunological models directly target a wide range of pathologies with the hope to help assessing the efficacy of treatments, and to optimize therapeutic regimens. In this respect, such models clearly fall within the scope of computational systems medicine in particular when considering the possibility to tailor the models on patient-specific immunological signatures. Notably, these words fully resonate with the mission of the Virtual Physiological Human initiative, which, among other goals, aims at integrating whole organ models into a unified computer model of the human organism able to predict the response to therapeutic perturbations of different physiological and pathological conditions [109].

In conclusion, despite the many technical challenges and open problems, we expect that multiscale approaches will make important contributions to immunology in the coming years [110].

Key Points

- Immune system processes embrace multiple levels of biological organization, from the molecular to the organism scale.
- Multiscale modelling (MSM) is increasingly used to address complex questions in immunology, where it must still unlock its potential.
- We identify three emerging fields of MSM applications in immunology: host-virus interactions, inflammatory diseases and their treatment and development of simulation platforms.
- Recent multiscale models can consistently explain immunological data from several scales, and can be used to simulate immunotherapies.
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