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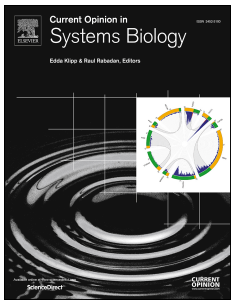

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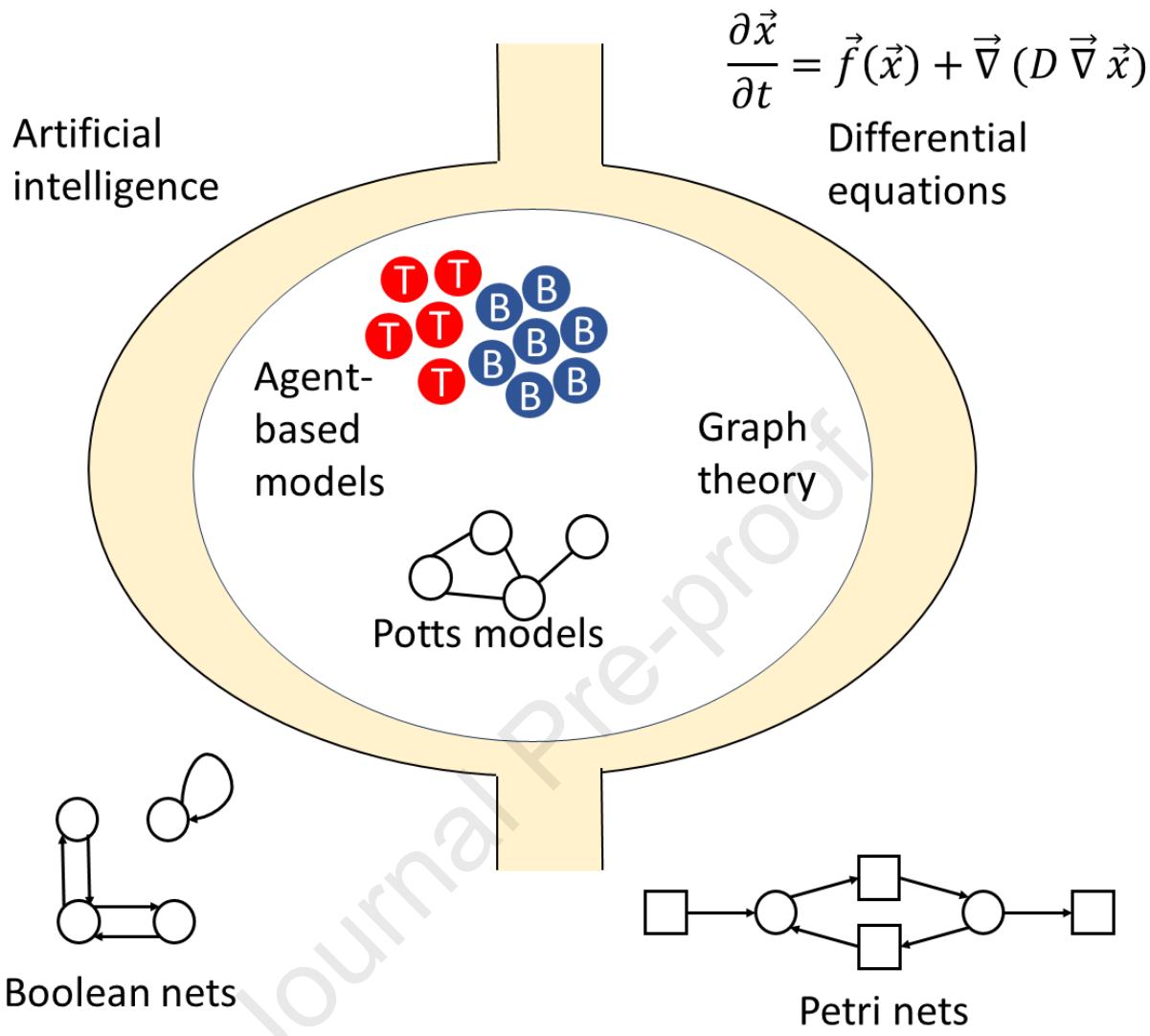

Graphical Abstract

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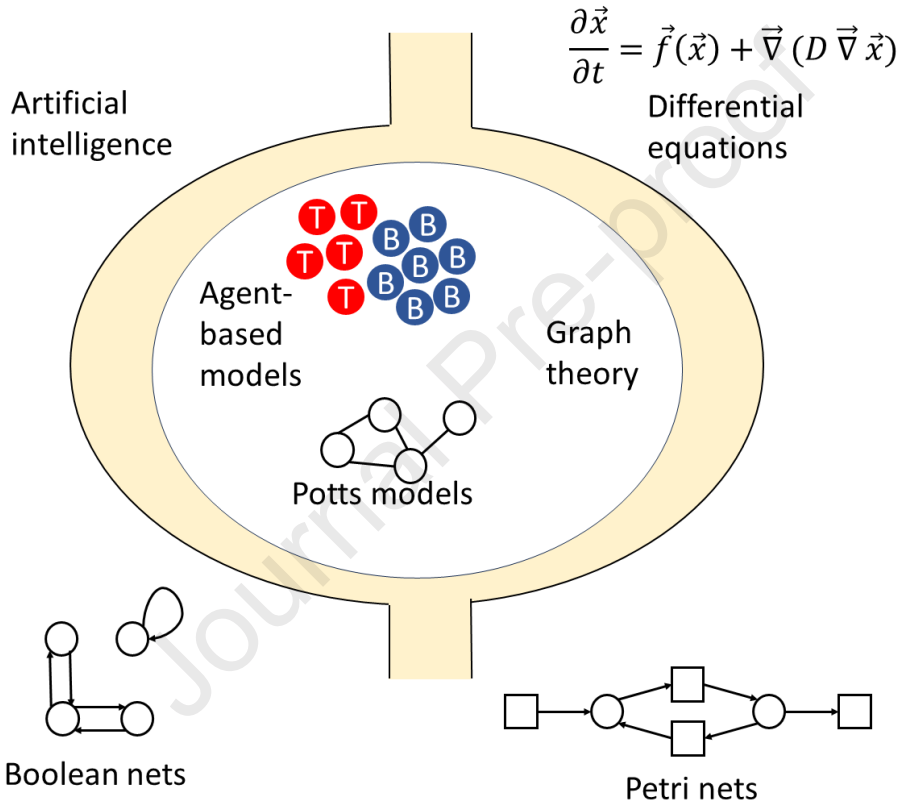

Computational systems biology of cellular processes in the human lymph node

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Abstract

The human immune system is determined by the functionality of the human lymph node. With the use of high-throughput techniques in clinical diagnostics, a large number of data is currently collected. The new data on the spatiotemporal organization of cells offers new possibilities to build a mathematical model of the human lymph node - a *virtual lymph node*. The virtual lymph node can be applied to simulate drug responses and may be used in clinical diagnosis. Here, we review mathematical models of the human lymph node from the viewpoint of cellular processes. Starting with classical methods, such as systems of differential equations, we discuss the values of different levels of abstraction and methods in the range from artificial intelligence techniques formalism

Keywords: lymph node, immune response, modeling, Petri net, agent-based modeling, ordinary differential equation, partial differential equation, artificial intelligence

1. Introduction

The immune system provides the human body with a toolbox of complex defense mechanisms. Even simple immune systems such as in the marine invertebrate tunicate show an intertwined interplay of cellular players that is

yet not fully understood [1]. The evolution of various immune systems has been the topic of comparative immunity studies [2, 3].

Several hundreds of lymph nodes are distributed over the human body. Lymph liquid circulates through the lymph nodes and may transport a foreign substance called antigens, e.g., a COVID-19 virus, AIDS virus, cancer cell, fungus, bacterium, or just a simple virus that may cause a common cold. In the lymph node, densely packed B cells, T cells, plasma cells, and macrophages are responsible for filtering and detecting antigens and triggering an immune response. The immune response has not only to deal with a broad diversity of antigens but to be specific enough to avoid mistakenly targeting and attacking functional parts of the body.

To understand the functionality of the human immune system, we have to consider the main organ, the lymph node, and explore its components and their cellular interplay. In modern pathology, lymph nodes are investigated mainly using imaging techniques. Digitization of tissue sections is part of current workflows in modern pathology. For a more detailed look at the preparation by staining and digitization of lymph node tissue sections, see Appendix A.1. and Appendix A.2. in the supplemental material.

Theoretical modeling is necessary to understand the immune response from a holistic point of view and predict the dynamic behavior under medical treatment. Mathematical models in biology have been applied to answer questions in pharmacology and for treatment procedures of organisms to reduce experiments, in particular, in testing components of applications and the prediction of experimental results. For modern computers, computational tasks have become inexpensive, and numerous user-friendly tools have been developed [4]. Recently, specific methods for precision medicine and digital twins have been developed [5]. Systems biology concepts, e.g., steady states, local stability, and sensitivity, however, are still tractable only for small systems [6–8].

This work is motivated and influenced by our experience in generating experimental 2D, 3D, and 4D data on the human lymph node as well as in modeling the human lymph node by applying the Petri net formalism without the use of kinetic parameters. This review does not intend to give guidance or even a tutorial on how to build a human lymph node model. We further emphasize that we focus on lymph node models of humans and not mice or rats since their physiological behavior significantly differs [9]. This review aims at compiling recently applied and derived modeling techniques to explore the human lymph node. To the best of our knowledge, this is the first review

on that topic. The contribution of the work is the compilation of modeling techniques of the human lymph node published in the last few years. We structure the paper according to the different modeling approaches, first discussing the different levels of abstraction and then the modeling techniques, focusing on models based on ordinary differential equations (ODEs), partial differential equations (PDEs), artificial intelligence techniques, agent-based simulations, and Petri nets in the context of the lymph node.

2. Levels of abstraction

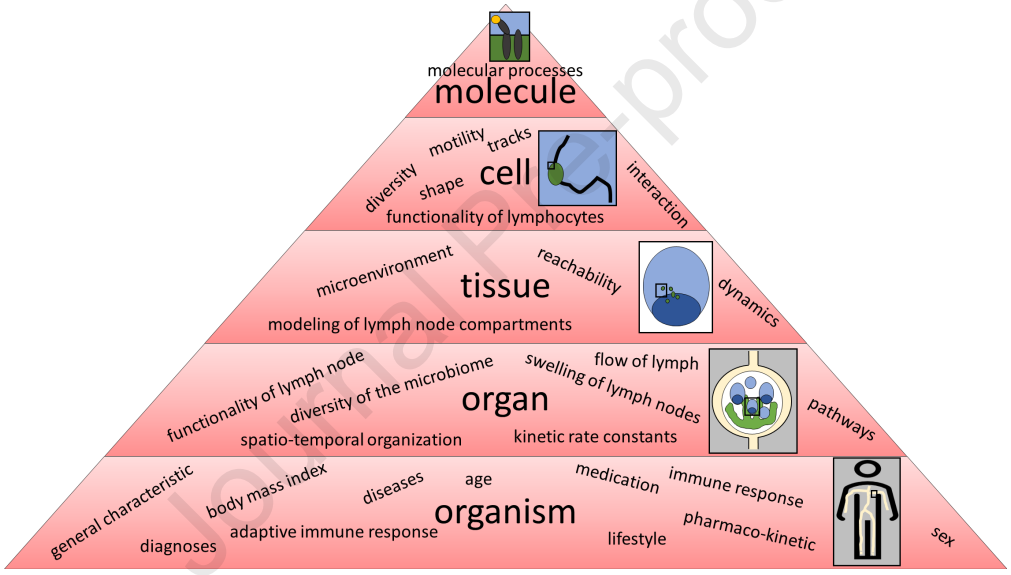


Figure 1: The pyramid of levels of abstraction. We divide the level of abstraction into molecule, cell, tissue, organ, and organism levels. On the top of the pyramid, we find the molecular level with a small graphical pattern that represents a transmembrane protein to which a ligand binds. On the second level, we have the cell with its properties, such as shape and motility, with a lymphocyte as a small figure. The black line indicates a track of the cell. The black box indicates the possible place where a transmembrane protein could be located. The third level describes the tissues and the compartments of the lymph node depicted by a germinal center with light and dark zones. On the fourth level, the organ is addressed with a small figure representing a lymph node with the subcapsular sinus in yellow, the germinal center in blue, and the paracortex in green. On the bottom level, we consider the organism, here, the human body. The small figure represents a patient with his/her lymphatic network, which is not explicitly illustrated.

Theoretical modeling in systems biology has to compromise on the level of abstraction and the choice of aspects the modeler wants to describe. The lack of quantitative data often hampers the simulation of dynamics by ODEs. General characteristics, e.g., the reachability of states may become intractable by complexity issues.

For more than five decades, mathematical modeling has been applied to understand the complex functional behavior of the lymph node, for example, see the review of Siskind and Benacerraf from the year 1969 [10]. For a review of more recent work, we refer to Margaris and Black [11] and Cappuccino *et al.* [12]. The models covered various levels of abstraction, ranging from molecular metabolic and signaling pathways over cell-cell interactions to whole-body models [13–17].

Driven by the data available, we can consider different levels of abstraction in terms of a molecular level, cellular level, tissue level, organ level, and organism level, see Figure 1 Each level of abstraction is associated with its scale of periods and spatial resolution. We summarize the dimensions according to the levels in Table A.2. in the supplementary material.

Molecular level: On top of the pyramid, molecules are located. Typically, the sizes of molecules range from fractions of 0.1 nm, e.g., for a hydrogen atom, to 100 nm, e.g., for an adenovirus. Fast reactions such as the self-ionization of water, may happen on the scale of picoseconds and can be assumed to have reached a steady state. Other biochemical reactions, e.g., protein folding, however, can be much slower and may require milliseconds or several hours. Chemically, the number of molecules is measured in terms of the Avogadro constant, and classical approaches consider the concentrations of molecules by ODEs or PDEs. Diffusion constants in water may range from 0.005 $\mu m^2/s$ for mRNA to 2000 $\mu m^2/s$ for CO_2. Within the lymph node, specific molecules such as chemokines play an essential role in cell-cell communication.

Cellular level: Here, the theoretical modeling describes cells and cellular processes, e.g., replication, movement, cell-cell interaction, maturation, and differentiation. To model a lymph node, we have to know the sizes and numbers of the different cells. Sizes of cells are in the order of 5 μm for B cells to 20 μm for macrophages. The concentrations of cells vary for each cell type and compartment. For example, concentrations in the order of $10^{11} - 10^{12}$ B cells per *liter* have been measured in the germinal center under reactive conditions in lymphadenitis [18]. An exemplary value of 5.8 $\mu m/min$ has been reported for the migration of T follicular helper cells in the germinal

center [19]. The lifespan of cells greatly varies across types and tissues, for example, from $3-5$ *days* for gut epithelia to years for cardiomyocytes [20]. For B cells, mean lifespans of 52 *days* [21] and 76 *days* [22] have been measured. Cells effectively can communicate over a distance of 250 μm within a timescale of $10-30$ minutes [23]. The spatial signaling range is, however, highly dependent on the concrete situation and is often limited to nearby cells only.

Tissue level: The tissue of the healthy lymph node enables the transport of lymph, blood, and cells. The lymph node consists of compartments, e.g., subcapsular sinus, germinal center, and medulla. During the immune reaction, the spatiotemporal organization of the lymph node changes dynamically. The inflow of lymph increases, the lymph node swells, and new germinal centers emerge and grow. Typically, about 60 germinal centers with a median size of about 500 μm are in a lymph node [18]. Due to the dynamic processes in the lymph node, quantitative data exhibit high variations, making the quantitative characterization of a representative compartment, such as a *mean* germinal center, questionable [24, 25].

Organism level: Here, we consider a patient. An individual patient consists of molecules, cells, tissues, and organs but in clinical practice, a patient can not be reduced to its biological functions. Factors such as age, sex, lifestyle, previous illnesses, body mass index, medication, and even mindset are crucial for choosing an appropriate treatment. About 600 lymph nodes in the human body filter the lymph for antigens with a total lymph flow of about $4-5$ *liters per day*. After infection or while exercising, the lymph flow can strongly increase. The recognition of an antigen is the starting point of an immune response, which takes place on the timescale of hours to several days.

A broad range of techniques has been applied to model the lymph node such as systems of ODEs and PDEs [26], agent-based models [27], Potts models [28], graph theory-based models [29], Petri nets [30], and Boolean networks [31]. In the following, we summarize the different modeling techniques in the context of lymph node models without explicitly giving the assignment to the level of abstraction. Because of the desired size of the paper, we will provide a choice of up to now frequently used modeling approaches.

3. Modeling techniques

3.1. Kinetic modeling based on ordinary differential equations

Systems of ODEs usually apply the mass action kinetics and assume a well-mixed, homogeneous concentration of cells. The assumption of homogeneous concentrations is certainly an oversimplification for the lymph node as a whole because there exist different compartments, e.g., subcapsular sinus, germinal centers, light zones, dark zones, T zone, and medulla, which exhibit key functions and different populations of cells. Additionally, cancerous cells may destroy the internal structure of the lymph node, and hence, can cause a lethal breakdown of the immune response [32].

In 1970, Bell developed a system of ODEs of clonal selection and antibody production to predict the constants of simple experimental systems [26, 33]. The mathematical model of Bell describes six species, four types of cells, antigens, and antibodies and discriminates target cells, proliferating cells, plasma cells, and memory cells. The model has been generalized to allow for a multi-group representation of cells and antibody heterogeneity. Appropriate kinetic rate constants have been chosen, and a volume of 0.2 *liters* has been assumed for the immune system. Inside the immune system, simulations have been started with 1000 target cells that have been divided into seventeen groups, each of which with an individual association constant for the antibody (bovine γ-globulin). Antigen has been involved in the models, either as an initial condition or as a prolonged source. Because of the lack of experimental characterization, the number of sites per cell and the response of cells to antigens have to be chosen rather arbitrarily. Nevertheless, many features like an increase in the effective antibody association constant and a faster second response have been modeled in excellent agreement with experiments. However, the model has failed to simulate special effects such as high and low concentration tolerances and threshold doses in secondary responses. Systems of ODEs may reflect a confined view of aspects of a single compartment, for example, to model the dynamics of a single germinal center by Kesmir and De Boer [34]. More theoretically, Kepler and Perelson [35] have applied mathematical techniques of control theory to optimize the mutation rate of the B-cell population dynamics in the lymph node. For further details on the kinetic modeling, see Appendix A.3 in the supplementary material.

3.2. Spatiotemporal modeling based on partial differential equations

Non-homogeneous spatial distributions of cells can be modeled either by the framework of coupled well-mixed reactors or a system of PDEs [36, 37]. Since the individual lymph node is not a rigid organ, the dynamic, complex flow of lymph liquid is yet a challenge for PDEs [38–41]. During an immune response, the velocity of inflow increases, and the lymph node becomes bigger. The swelling of lymph nodes is a typical immune response, indicating an infection. Additionally, germinal centers become initiated, increasing in number and size [18]. PDEs with fixed boundary conditions are not able to reflect such basic aspects of an immune response. Inside the compartments of the lymph node, cells are not present in typical molar concentrations. The size of a compartment can be small, e.g., a germinal center may range from several 10 μm to more than 3 mm [18, 42, 43]. To estimate the number of cells, the Avogadro constant is certainly not an appropriate measure. Additionally , the movement of an individual cell is directed by chemokines and fibers of either fibroblastic reticular cells [44] or follicular dendritic cells [45]. The straight-line movements of cells are interrupted by changes in the direction [46]. On a macroscopic scale, the movement may be approximated by a random Brownian movement and hence, by the diffusion process of a PDE. On the microscopic scale, a PDE fails to reflect the spatial distribution of cells.

3.3. Modeling applying artificial intelligence techniques

Hybrid discrete/continuous models have addressed the shortcomings of systems of ODEs and PDEs, see, for example, Baldazzi *et al.* [47]. The simulation of the movements of cells in the lymph node is, however, still a theoretical challenge as in the cellular Potts model for the interaction and motility of T cells and dendritic cells, see Beltman *et al.* [28]. The motility of cells is important for the timescale of the detection of an antigen and the triggered immune response [48]. Tracks for immunostained cells and machine learning have been applied to define the entities of human cells [49].

The increasing number of clinical data has enabled the application of methods and tools from machine learning. In the field of digital pathology, artificial intelligence has attracted immense interest [50]. The early detection of lymphoma by processing medical imaging with models of artificial intelligence has reached a pooled sensitivity of 87%, for a recent review and meta-analysis, we refer to Bai et al. [51]. Satisfactory diagnostic performance has been obtained in subgroup analyses but the black-box characteristic of

machine learning has raised trust issues for automatic application in diagnostics and clinical decision-making. Since the visual inspection of an image by an experienced pathologist takes only seconds, the high expectations for artificial intelligence models have yet not been realized in the clinical practice. In the future, explainable artificial intelligence [52] may change the situation.

3.4. Modeling with agent-based simulation

The dynamics of cells inside the lymph node has been modeled by numerous groups [53–61]. Special attention has been focused on local dynamics in germinal centers [62–65]. Cells shape their microenvironment of neighboring cells [66–68]. Cancerous cells may exploit a microenvironment of neighboring cells as an immune escape [69]. Note that, evolving therapeutic approaches such as the adaptive T-cell therapy, relies on the redirection of T lymphocytes toward selected tumor-associated antigens [19, 70]. To simulate the complex behavior in a population of interacting cells, agent-based simulation is an attractive framework [27, 56]. The effects of the local microenvironment of the individual cell, cell movement, and spatiotemporal distributions can be explicitly incorporated [27, 56]. Conventionally, agent-based models represent each cell by an object called an agent with individual properties and rules for activation, cell-cell interaction, cell-environment interaction, and movement [27, 71]. Limited computational resources may restrict agent-based simulation of the spatiotemporal organization of cells. Further details on agent-based simulation can also be found in Appendix A.4 in the supplementary material.

3.5. Semi-quantitative modeling with Petri nets

An alternative viewpoint on lymph node models comes from Petri net formalism introduced by Carl Adam Petri in 1962 for the representation and analysis of systems with concurrent processes [72]. The Petri net formalism is well-established in many technical applications, theoretical computer science, and since 1993 in systems biology [73]. In 2020, Pernice *et al.* [74] have referred to the Petri net as a technique that is a "powerful tool for modeling and studying biological systems". Petri nets cover a diverse range of applications for biological systems [75, 76]. Techniques such as the invariant analysis and *in silico* knockout analysis provide valuable methods to partly verify the completeness and correctness of a model [77–82]. Pennisi *et al.* [83] have applied colored Petri nets to the immune system at the cellular level. Moreover, due to the strict separation between the active and the passive

part of the system, modifications to receive hybrid models, Boolean models, or even kinetic models become possible. Signaling during hepatitis C virus infection has been studied by Obaid *et al.* [84]. Other examples are Petri net models of inflammation and oxidative stress [85] and microenvironmental signals on macrophage differentiation [86]. In the last years, several models of signaling pathways at the molecular level, in particular in medical applications, have been developed, for a review, see [76]. Petri nets enable a holistic view of the cellular processes inside the lymph node [30]. For further details on semi-quantitative modeling, see Appendix A.5 in the supplementary material.

4. Summary and Conclusion

Early models have focused on the lymph nodes of animal models such as mice because any experimental verification on humans has been not possible for ethical reasons. Nowadays, automatizing, high throughput, digitization, and data storage produce data from human lymph nodes with rapidly increasing speed and number . Based on our joint work with co-authored pathologists who have been specialists in lymph node research for more than 30 years, we give an overview of ongoing research on a virtual lymph node model.

The literature on the theoretical modeling of lymph nodes is diverse and vast. Here , we cannot give a comprehensive and complete overview. Focusing on the developments in the last years, we considered the modeling concepts according to their different, hierarchical levels of abstraction, see Table A.1 in the supplementary material. We took into account concepts of digital pathology, artificial intelligence, pharmacokinetics, systems biology, network theory, and theoretical computer science to investigate the lymph node. The collection of the necessary data remains the bottleneck in computational modeling, in particular of such complex organs as the human lymph node. Moreover, the data of different quality and quantity have to be combined properly to address all their differences. Therefore, most studies investigate specific research questions in a very narrow range. The review demonstrates not only recent developments but also the strong need for more effort in the field, experimentally as well as computationally.

References

[1] N. Franchi, L. Ballarin, Immunity in protochordates: the tunicate perspective, Frontiers in Immunology 8 (2017) 674.

[2] J. Oriol Sunyer, Evolutionary and functional relationships of B cells from fish and mammals: insights into their novel roles in phagocytosis and presentation of particulate antigen, Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders) 12 (3) (2012) 200–212.

[3] P. R. Rauta, B. Nayak, S. Das, Immune system and immune responses in fish and their role in comparative immunity study: a model for higher organisms, Immunology Letters 148 (1) (2012) 23–33.

[4] J. Ackermann, I. Koch, Quantitative analysis, Modeling in systems biology: the Petri net approach (2011) 153–178.

[5] S. Grimes, M. D. Zarella, F. U. Garcia, D. E. Breen, An agent-based approach to predicting lymph node metastasis status in breast cancer, in: 2021 IEEE International Conference on Bioinformatics and Biomedicine (BIBM), IEEE, 2021, pp. 1315–1319.

[6] J. D. Murray, Mathematical Biology: II: Spatial Models and Biomedical Applications, Vol. 3, Springer, 2003.

[7] P. De Leenheer, S. S. Pilyugin, Immune response to a malaria infection: properties of a mathematical model, Journal of Biological Dynamics 2 (2) (2008) 102–120.

[8] F. F. Chenar, Y. Kyrychko, K. Blyuss, Mathematical model of immune response to hepatitis B, Journal of Theoretical Biology 447 (2018) 98–110.

[9] J. Mestas, C. C. Hughes, Of mice and not men: differences between mouse and human immunology, The Journal of Immunology 172 (5) (2004) 2731–2738.

[10] G. W. Siskind, B. Benacerraf, Cell selection by antigen in the immune response, Advances in Immunology 10 (1969) 1–50.

[11] K. Margaris, R. A. Black, Modelling the lymphatic system: challenges and opportunities, Journal of the Royal Society Interface 9 (69) (2012) 601–612.

[12] A. Cappuccio, P. Tieri, F. Castiglione, Multiscale modelling in immunology: A review, Briefings in Bioinformatics 17 (3) (2016) 408–418.

[13] Z. Liu, M. Y. Gerner, N. Van Panhuys, A. G. Levine, A. Y. Rudensky, R. N. Germain, Immune homeostasis enforced by co-localized effector and regulatory t cells, Nature 528 (7581) (2015) 225–230.

[14] K. Thurley, D. Gerecht, E. Friedmann, T. Höfer, Three-dimensional gradients of cytokine signaling between t cells, PLoS Computational Biology 11 (4) (2015) e1004206.

[15] A. Oyler-Yaniv, J. Oyler-Yaniv, B. M. Whitlock, Z. Liu, R. N. Germain, M. Huse, G. Altan-Bonnet, O. Krichevsky, A tunable diffusion-consumption mechanism of cytokine propagation enables plasticity in cell-to-cell communication in the immune system, Immunity 46 (4) (2017) 609–620.

[16] G. Altan-Bonnet, T. Mora, A. M. Walczak, Quantitative immunology for physicists, Physics Reports 849 (2020) 1–83.

[17] H. S. Wong, K. Park, A. Gola, A. P. Baptista, C. H. Miller, D. Deep, M. Lou, L. F. Boyd, A. Y. Rudensky, P. A. Savage, et al., A local regulatory T cell feedback circuit maintains immune homeostasis by pruning self-activated T cells, Cell 184 (15) (2021) 3981–3997.

[18] C. M. Schemel, P. Wurzel, S. Scharf, H. Schäfer, S. Hartmann, I. Koch, M.-L. Hansmann, Three-dimensional human germinal centers of different sizes in patients diagnosed with lymphadenitis show comparative constant relative volumes of B cells, T cells, follicular dendritic cells, and macrophages, Acta Histochemica 125 (7) (2023) 152075,

∗∗ *The authors measured the sizes of germinal centers and the cellular composition inside them. The numbers are a prerequisite for any quantitative modeling of the lymph node.*

[19] E. Donnadieu, K. B. Reisinger, S. Scharf, Y. Michel, J. Bein, S. Hansen, A. G. Loth, N. Flinner, S. Hartmann, M.-L. Hansmann, Landscape of T

follicular helper cell dynamics in human germinal centers, The Journal of Immunology 205 (5) (2020) 1248–1255.

[20] R. Sender, R. Milo, The distribution of cellular turnover in the human body, Nature Medicine 27 (1) (2021) 45–48.

[21] D. C. Macallan, D. L. Wallace, Y. Zhang, H. Ghattas, B. Asquith, C. de Lara, A. Worth, G. Panayiotakopoulos, G. E. Griffin, D. F. Tough, et al., B-cell kinetics in humans: rapid turnover of peripheral blood memory cells, Blood 105 (9) (2005) 3633–3640.

[22] J. Defoiche, C. Debacq, B. Asquith, Y. Zhang, A. Burny, D. Bron, L. Lagneaux, D. Macallan, L. Willems, Reduction of B cell turnover in chronic lymphocytic leukaemia, British Journal of Haematology 143 (2) (2008) 240–247.

[23] K. Francis, B. O. Palsson, Effective intercellular communication distances are determined by the relative time constants for cyto/chemokine secretion and diffusion, Proceedings of the National Academy of Sciences 94 (23) (1997) 12258–12262.

[24] N. Wittenbrink, A. Klein, A. A. Weiser, J. Schuchhardt, M. Or-Guil, Is there a typical germinal center? a large-scale immunohistological study on the cellular composition of germinal centers during the hapten-carrier–driven primary immune response in mice, The Journal of Immunology 187 (12) (2011) 6185–6196.

[25] A. Martinez-Riano, S. Wang, S. Boeing, S. Minoughan, A. Casal, K. M. Spillane, B. Ludewig, P. Tolar, Long-term retention of antigens in germinal centers is controlled by the spatial organization of the follicular dendritic cell network, Nature Immunology 24 (8) (2023) 1281–1294.

[26] G. I. Bell, Mathematical model of clonal selection and antibody production, Nature 228 (2) (1970) 739–744.

[27] I. Azarov, K. Peskov, G. Helmlinger, Y. Kosinsky, Role of T cell-to-dendritic cell chemoattraction in T cell priming initiation in the lymph node: An agent-based modeling study, Frontiers in Immunology 10 (2019) 1289.

[28] J. B. Beltman, A. F. Marée, R. J. De Boer, Spatial modelling of brief and long interactions between T cells and dendritic cells, Immunology and Cell Biology 85 (4) (2007) 306–314.

[29] S. C. Fischer, G. W. Bassel, P. Kollmannsberger, Tissues as networks of cells: towards generative rules of complex organ development, Journal of the Royal Society Interface 20 (204) (2023) 20230115,

∗∗ *The authors demonstrate how tissue can be translated into spatial networks, such that cells are represented by nodes and intercellular connections by edges. The translation enables the application of network theory to developmental biology.*

[30] S. Scharf, J. Ackermann, L. Bender, P. Wurzel, H. Schäfer, M.-L. Hansmann, I. Koch, Holistic view on the structure of immune response: Petri net model, Biomedicines 11 (2) (2023) 452,

∗∗ *The model applies a well-established mathematical framework and bridges the modeling of the immune response to the reachability problem, a fundamental problem of computer science.*

[31] A. Checcoli, J. G. Pol, A. Naldi, V. Noel, E. Barillot, G. Kroemer, D. Thieffry, L. Calzone, G. Stoll, Dynamical boolean modeling of immunogenic cell death, Frontiers in Physiology 11 (2020) 590479.

[32] M.-L. Hansmann, C. Fellbaum, P. K. Hui, P. Moubayed, Progressive transformation of germinal centers with and without association to Hodgkin's disease, American Journal of Clinical Pathology 93 (2) (1990) 219–226.

[33] G. I. Bell, Mathematical model of clonal selection and antibody production, Journal of Theoretical Biology 29 (2) (1970) 191–232.

[34] C. Keşmir, R. J. De Boer, A mathematical model on germinal center kinetics and termination, The Journal of Immunology 163 (5) (1999) 2463–2469.

[35] T. B. Kepler, A. S. Perelson, Cyclic re-entry of germinal center B cells and the efficiency of affinity maturation, Immunology Today 14 (8) (1993) 412–415.

[36] D. Gammack, S. Ganguli, S. Marino, J. Segovia-Juarez, D. E. Kirschner, Understanding the immune response in tuberculosis using different mathematical models and biological scales, Multiscale Modeling & Simulation 3 (2) (2005) 312–345.

[37] H. Zheng, B. Jin, S. E. Henrickson, A. S. Perelson, U. H. von Andrian, A. K. Chakraborty, How antigen quantity and quality determine T-cell decisions in lymphoid tissue, Molecular and Cellular Biology 28 (12) (2008) 4040–4051.

[38] N. P. Reddy, T. A. Krouskop, P. H. Newell Jr, A computer model of the lymphatic system, Computers in Biology and Medicine 7 (3) (1977) 181–197.

[39] N. Reddy, K. Patel, A mathematical model of flow through the terminal lymphatics, Medical Engineering & Physics 17 (2) (1995) 134–140.

[40] M. Jafarnejad, M. C. Woodruff, D. C. Zawieja, M. C. Carroll, J. Moore Jr, Modeling lymph flow and fluid exchange with blood vessels in lymph nodes, Lymphatic Research and Biology 13 (4) (2015) 234–247.

[41] G. Giantesio, A. Girelli, A. Musesti, A mathematical description of the flow in a spherical lymph node, Bulletin of Mathematical Biology 84 (12) (2022) 142,

∗∗ *The authors derive an analytical solution for pulsatile lymph flow inside a simplified spherical lymph node. The analytical solution allows a better understanding of the fluid behavior inside the lymph node and to validate numerical simulations.*

[42] M. Thomos, P. Wurzel, S. Scharf, I. Koch, M.-L. Hansmann, 3D investigation shows walls and wall-like structures around human germinal centres, probably regulating T-and B-cell entry and exit, PLoS ONE 15 (11) (2020) e0242177.

[43] P. Wurzel, J. Ackermann, H. Schäfer, S. Scharf, M.-L. Hansmann, I. Koch, Detection of follicular regions in actin-stained whole slide images of the human lymph node by shock filter, Biological Chemistry 402 (8) (2021) 991–999,

∗∗ *The authors demonstrate an image recognition technique for the segmentation of a lymph node compartment.*

[44] A. L. Fletcher, S. E. Acton, K. Knoblich, Lymph node fibroblastic reticular cells in health and disease, Nature Reviews Immunology 15 (6) (2015) 350–361.

[45] K. Suzuki, I. Grigorova, T. G. Phan, L. M. Kelly, J. G. Cyster, Visualizing B cell capture of cognate antigen from follicular dendritic cells, Journal of Experimental Medicine 206 (7) (2009) 1485–1493.

[46] A. Kislitsyn, R. Savinkov, M. Novkovic, L. Onder, G. Bocharov, Computational approach to 3D modeling of the lymph node geometry, Computation 3 (2) (2015) 222–234.

[47] V. Baldazzi, P. Paci, M. Bernaschi, F. Castiglione, Modeling lymphocyte homing and encounters in lymph nodes, BMC Bioinformatics 10 (2009) 1–11.

[48] M. Meyer-Hermann, M. T. Figge, K.-M. Toellner, Germinal centres seen through the mathematical eye: B-cell models on the catwalk, Trends in Immunology 30 (4) (2009) 157–164.

[49] P. Wagner, N. Strodthoff, P. Wurzel, A. Marban, S. Scharf, H. Schäfer, P. Seegerer, A. Loth, S. Hartmann, F. Klauschen, K.-R. Müller, W. Samek, M.-L. Hansmann, New definitions of human lymphoid and follicular cell entities in lymphatic tissue by machine learning, Scientific Reports 12 (1) (2022) 18991,

∗∗ The authors demonstrate that a more precise classification of cells is possible by the additional consideration of the three-dimensional movements of cells.

[50] M.-L. Hansmann, F. Klauschen, W. Samek, K.-R. Müller, E. Donnadieu, S. Scharf, S. Hartmann, I. Koch, J. Ackermann, L. Pantanowitz, H. Schäfer, P. Wurzel, Imaging bridges pathology and radiology, Journal of Pathology Informatics 14 (2023) 100298,

∗ The authors emphasize the future aspects of three-dimensional tissue imaging.

[51] A. Bai, M. Si, P. Xue, Y. Qu, Y. Jiang, Artificial intelligence performance in detecting lymphoma from medical imaging: a systematic review and meta-analysis, BMC Medical Informatics and Decision Making 24 (1) (2024) 13.

[52] A. Hedström, L. Weber, D. Krakowczyk, D. Bareeva, F. Motzkus, W. Samek, S. Lapuschkin, M. M.-C. Höhne, Quantus: An explainable AI toolkit for responsible evaluation of neural network explanations and beyond, Journal of Machine Learning Research 24 (34) (2023) 1–11,

∗ *The authors present a toolkit to evaluate the performance of explanations of neural network predictions.*

[53] F. Celada, P. E. Seiden, A computer model of cellular interactions in the immune system, Immunology Today 13 (2) (1992) 56–62.

[54] C. Jacob, J. Litorco, L. Lee, Immunity through swarms: Agent-based simulations of the human immune system, in: International Conference on Artificial Immune Systems, Springer, 2004, pp. 400–412.

[55] V. A. Folcik, G. C. An, C. G. Orosz, The basic immune simulator: an agent-based model to study the interactions between innate and adaptive immunity, Theoretical Biology and Medical Modelling 4 (1) (2007) 1–18.

[56] G. Bogle, P. R. Dunbar, Agent-based simulation of T-cell activation and proliferation within a lymph node, Immunology and Cell Biology 88 (2) (2010) 172–179.

[57] J. J. Linderman, T. Riggs, M. Pande, M. Miller, S. Marino, D. E. Kirschner, Characterizing the dynamics of CD4+ T cell priming within a lymph node, The Journal of Immunology 184 (6) (2010) 2873–2885.

[58] V. A. Folcik, G. Broderick, S. Mohan, B. Block, C. Ekbote, J. Doolittle, M. Khoury, L. Davis, C. B. Marsh, Using an agent-based model to analyze the dynamic communication network of the immune response, Theoretical Biology and Medical Modelling 8 (1) (2011) 1–25.

[59] C. Jacob, V. Sarpe, C. Gingras, R. P. Feyt, Swarm-based simulations for immunobiology: What can agent-based models teach us about the immune system?, Information Processing and Biological Systems (2011) 29–64.

[60] G. Bogle, P. R. Dunbar, On-lattice simulation of T cell motility, chemotaxis, and trafficking in the lymph node paracortex, PloS ONE 7 (9) (2012) e45258.

[61] H. D. Moreau, G. Bogle, P. Bousso, A virtual lymph node model to dissect the requirements for T-cell activation by synapses and kinapses, Immunology and Cell Biology 94 (7) (2016) 680–688.

[62] M. E. Meyer-Hermann, P. K. Maini, D. Iber, An analysis of B cell selection mechanisms in germinal centers, Mathematical Medicine and Biology: a Journal of the IMA 23 (3) (2006) 255–277.

[63] A. Amitai, L. Mesin, G. D. Victora, M. Kardar, A. K. Chakraborty, A population dynamics model for clonal diversity in a germinal center, Frontiers in Microbiology 8 (2017) 1693.

[64] T. Arulraj, S. C. Binder, P. A. Robert, M. Meyer-Hermann, Synchronous germinal center onset impacts the efficiency of antibody responses, Frontiers in Immunology 10 (2019) 2116.

[65] A. K. Garg, T. Mitra, M. Schips, A. Bandyopadhyay, M. Meyer-Hermann, Amount of antigen, T follicular helper cells and affinity of founder cells shape the diversity of germinal center B cells: A computational study, Frontiers in Immunology 14 (2023) 1080853,

∗ *By agent-based simulation of germinal center, the authors demonstrate that, limited antigen availability on follicular dendritic cells expedites a loss of diversity of B cells.*

[66] J. Hannig, H. Schäfer, J. Ackermann, M. Hebel, T. Schäfer, C. Döring, S. Hartmann, M.-L. Hansmann, I. Koch, Bioinformatics analysis of whole slide images reveals significant neighborhood preferences of tumor cells in Hodgkin lymphoma, PLoS Computational Biology 16 (1) (2020) e1007516.

[67] L. Lumer, P. Wurzel, S. Scharf, H. Schäfer, J. Ackermann, I. Koch, M.-L. Hansmann, 3D connectomes of reactive and neoplastic CD30 positive lymphoid cells and surrounding cell types, Acta Histochemica 123 (5) (2021) 151750,

∗∗ *The authors emphasize the importance of three-dimensionality for cell-cell contact recognition.*

[68] K. Merk-Ahmad, J. Bein, S. Scharf, H. Schäfer, T. Bexte, E. Ullrich, A. G. Loth, N. Flinner, T. Senff, O. Schneider, M.-L. Hansmann, M. Piel,

B. Häupl, T. Oellerich, E. Donnadieu, S. Hartmann, The RHOA mutation G17V does not lead to increased migration of human malignant T cells but is associated with matrix remodelling, Cancers 15 (12) (2023) 3226,

∗∗ *The authors track the three-dimensional movement of cells in tissue and measure the influence of a specific mutation on the motility of the cells.*

[69] F. Wein, M. A. Weniger, B. Höing, J. Arnolds, A. Hüttmann, M.-L. Hansmann, S. Hartmann, R. Küppers, Complex immune evasion strategies in classical Hodgkin lymphoma, Cancer Immunology Research 5 (12) (2017) 1122–1132.

[70] E. Donnadieu, L. Dupré, L. G. Pinho, V. Cotta-de Almeida, Surmounting the obstacles that impede effective CAR T cell trafficking to solid tumors, Journal of Leukocyte Biology 108 (4) (2020) 1067–1079.

[71] A. Siokis, P. A. Robert, M. Meyer-Hermann, Agent-based modeling of T cell receptor cooperativity, International Journal of Molecular Sciences 21 (18) (2020) 6473.

[72] C. A. Petri, Kommunikation mit Automaten, Ph.D. thesis, Technische Hochschule Darmstadt (1962).

[73] V. N. Reddy, M. L. Mavrovouniotis, M. N. Liebman, et al., Petri net representations in metabolic pathways., in: ISMB, Vol. 93, 1993, pp. 328–336.

[74] S. Pernice, L. Follia, A. Maglione, M. Pennisi, F. Pappalardo, F. Novelli, M. Clerico, M. Beccuti, F. Cordero, S. Rolla, Computational modeling of the immune response in multiple sclerosis using epimod framework, BMC Bioinformatics 21 (17) (2020) 1–21.

[75] I. Koch, W. Reisig, F. Schreiber, Modeling in Systems Biology: The Petri Net approach, Vol. 16, Springer Science & Business Media, 2010.

[76] I. Koch, B. Büttner, Computational modeling of signal transduction networks without kinetic parameters: Petri net approaches, American Journal of Physiology-Cell Physiology 324 (5) (2023) C1126–C1140,

** *The authors review recent publications on modeling signaling pathways without any knowledge of the kinetic data applying Petri net formalism.*

[77] S. Schuster, D. A. Fell, T. Dandekar, A general definition of metabolic pathways useful for systematic organization and analysis of complex metabolic networks, Nature Biotechnology 18 (3) (2000) 326–332.

[78] A. Sackmann, M. Heiner, I. Koch, Application of Petri net based analysis techniques to signal transduction pathways, BMC Bioinformatics 7 (1) (2006) 1–17.

[79] A. Sackmann, D. Formanowicz, P. Formanowicz, I. Koch, J. Blazewicz, An analysis of the Petri net based model of the human body iron homeostasis process, Computational Biology and Chemistry 31 (1) (2007) 1–10.

[80] S. Grunwald, A. Speer, J. Ackermann, I. Koch, Petri net modelling of gene regulation of the Duchenne muscular dystrophy, BioSystems 92 (2) (2008) 189–205.

[81] L. Amstein, J. Ackermann, J. Scheidel, S. Fulda, I. Dikic, I. Koch, Manatee invariants reveal functional pathways in signaling networks, BMC Systems Biology 11 (1) (2017) 1–11.

[82] J. Hannig, H. Giese, B. Schweizer, L. Amstein, J. Ackermann, I. Koch, isiKnock: In silico knockouts in signaling pathways, Bioinformatics 35 (5) (2019) 892–894.

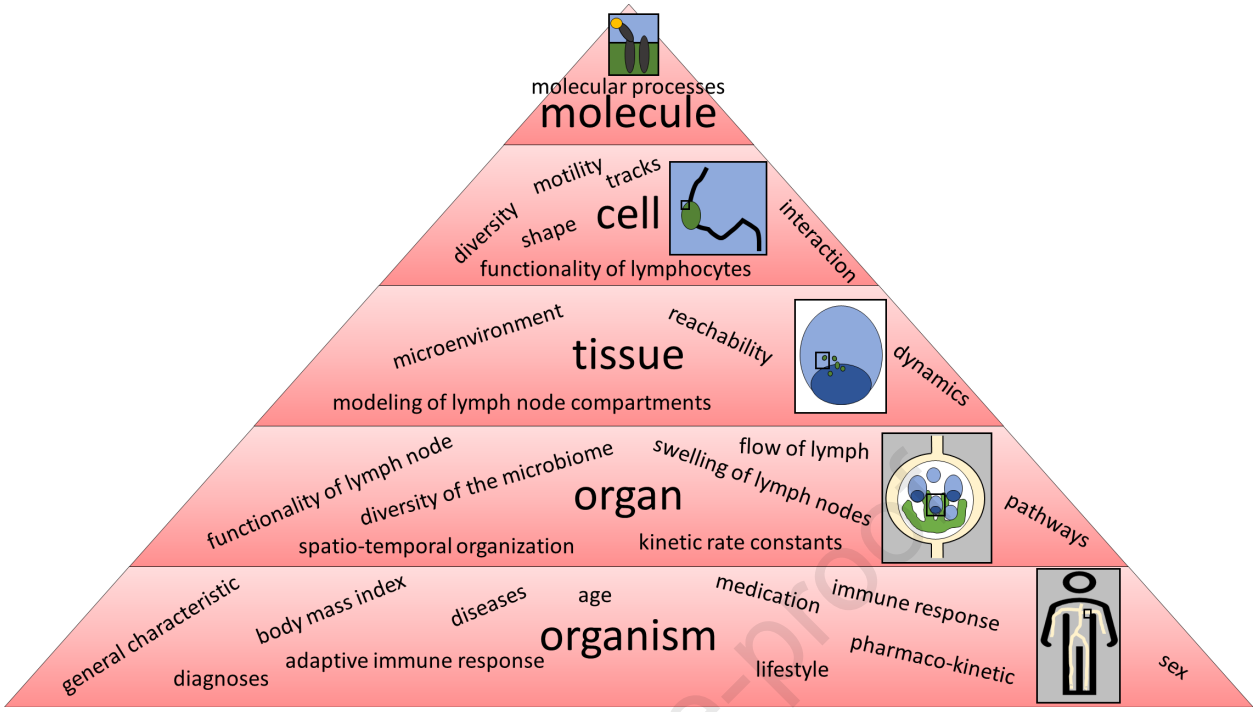
[83] M. Pennisi, S. Cavalieri, S. Motta, F. Pappalardo, A methodological approach for using high-level Petri nets to model the immune system response, BMC Bioinformatics 17 (19) (2016) 91–105.

[84] A. Obaid, A. Naz, A. Ikram, F. M. Awan, A. Raza, J. Ahmad, A. Ali, Model of the adaptive immune response system against HCV infection reveals potential immunomodulatory agents for combination therapy, Scientific Reports 8 (1) (2018) 1–19.

[85] K. Gutowska, D. Formanowicz, P. Formanowicz, Selected aspects of tobacco-induced prothrombotic state, inflammation and oxidative stress:

Modeled and analyzed using Petri nets, Interdisciplinary Sciences: Computational Life Sciences 11 (3) (2019) 373–386.

[86] K. Rżosińska, D. Formanowicz, P. Formanowicz, The study of the influence of micro-environmental signals on macrophage differentiation using a quantitative Petri net based model, Archives of Control Sciences 27 (2) (2017).



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