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REVIEW PAPER

Modeling the T cell immune response: a fascinating challenge

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Abstract The immune system is designed to protect the organism from infection and to repair damaged tissue. An effective response requires recognition of the threat, the appropriate effector mechanism to clear the pathogen and a return to homeostasis with minimal damage to self-tissues. T cells play a central role in orchestrating the immune response at all stages of the response and have been the subject of intense study by both experimental immunologists and modelers. This review examines some of the more critical questions in T cell biology and describes the latest attempts to address those questions using approaches that combine mathematical modeling and experiments.

Keywords T cell · Signaling · Mathematical modeling · Immune regulation

Introduction

The main function of the immune system is to protect the organism from infection with pathogens. This is achieved by an elaborate network of cells and secreted molecules that together coordinate a successful immune response. Three main stages of an immune response can be characterized; (1) recognition i.e. is the perceived threat a danger to the organism?; (2) removal of the pathogen that is achieved via a variety of effector mechanisms and (3) return to homeostasis which involves contraction of expanded immune cell populations and repair of damaged tissues. Each of these stages has generated a great deal of interest from both experimental immunologists and modelers.

Recognition of a pathogen occurs at two main levels. Cells of the innate immune system, such as dendritic cells (DC), macrophages, natural killer (NK) cells possess receptors for pathogen associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS), bacterial DNA, viral RNA and others. These pattern recognition receptors (PRR) include toll-like receptors (TLR) [1, 2], NOD-like receptors (NLR) [3], RIG-I-like receptors (RLR) and C-type lectin receptors (CLR) [4], which are specific for various pathogen-specific structures. An additional stimulus is required to fully activate the innate immune system in the form of damage associated molecular patterns (DAMPs) or alarmins, which are released from dead or dying cells and are indicative of an invasive pathogen. Many DAMPs, including HMGB1, uric acid, ATP, DNA, bind to the same PRR as a PAMP and may initiate the same inflammatory responses [5]. Thus, the innate immune system recognizes a pathogen invasion and detects tissue damage. The second level of recognition is mediated by cells of the adaptive immune system, namely T and B cells, which express clonally restricted receptors that have a high degree of specificity for their target antigens. Each B and T cell expresses a unique receptor that displays a high degree of specificity and sensitivity. As discussed in more detail below the recognition of antigen by T cells, mediated by the T cell receptor (TCR), has been a topic of intense study.

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DCs activated by pathogens in the peripheral tissues migrate to draining lymph nodes (LNs) where they present antigen to specific B and T cells. B cell receptors (BCR) recognize antigens in their natural conformation, whereas the TCR recognizes peptide fragments of the same antigen bound in the groove of major histocompatibility complex (MHC) molecules. Once BCR and TCR encounter their specific antigen, an adaptive immune response is initiated which results in the expansion of specific cells, differentiation into appropriate effectors, and the generation of B and T cell memory [6, 7].

The immune system has developed a wide array of effector mechanisms to deal with specific pathogens, since infection with extracellular bacteria, viruses, parasites such as Helminth worms, etc., pose very different challenges. Thus, T cells have evolved the ability to differentiate into several different subsets whose effector functions are best suited to deal with a specific pathogen threat. T helper (Th)1 cells secrete interferon (IFN)-γ, which is required for optimal macrophage activation, cytotoxic T cell maturation and for infections caused by intracellular organisms such as viruses and certain bacteria. Th17 cells secrete interleukin (IL)-17, which acts to attract neutrophils to the site of an infection with extracellular bacteria. Th2 cells secrete IL-5, IL-4 and IL-13, which activate eosinophils in infections with Helminth worms. How does the naïve T cell determine which type of pathogen is causing an infection? DCs migrating from the site of infection carry this information to the naïve T cell, because the interaction of pathogens with specific PRR on the DC triggers the production of unique sets of cytokines that drive the differentiation of specific Th subsets. For example, IL-12 induced following interaction with several TLRs drives Th1 differentiation, IL-23 produced following activation of other PRRs stimulates Th17 differentiation.

Successful elimination of a pathogen depends on the correct choice of effector response, and there are instances where the wrong choice is made and the infection is not cleared. An example of this is the lepromatous form of *Mycobacterium leprae* infection, which causes leprosy [8]. In these cases an inappropriate Th2 response is generated that fails to control the growth of this intracellular bacterium and there is widespread bacterial dissemination resulting in nerve damage. In contrast infected individuals who generate Th1 responses to this pathogen develop the tuberculous form of the disease, which is characterized by low bacterial burden and a granulomatous reaction that walls off the infection. Damage to nerves still occurs in this form of leprosy but it is caused by the immune response and the bacterial growth is controlled.

The case of tuberculous leprosy highlights the importance of the return to homeostasis. Even when a Th1 response is initiated to respond to *Mycobacterium leprae* infection macrophages are unable to completely clear the infection and a persistent and chronic infection ensues. It is important to prevent over-activation of effector cells and to turn these off when the pathogen has been cleared. This is achieved through immunosuppressive mechanisms, including the generation of both cytokines such as IL-10, IL-27 and TGF-β, and regulatory T (Treg) cells [9–11]. Defects in Treg and IL-10 may lead to complete clearance of a pathogen but, often, with severe immunopathological consequences [12, 13].

Thus, the regulation of the immune response at all of these stages is critical to ensure the elimination of invading pathogens while preventing excessive immune-mediated tissue damage [14]. When these regulatory mechanisms fail disease may result. For example a defect in the ability of immune system to distinguish between an invading and dangerous pathogen and self-tissues can result in autoimmune disease, such as type 1 diabetes or multiple sclerosis. Excessive immune recognition of commensal bacteria in the gut can lead to inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis. Early on we realized the value of mathematical and computational modeling in attempting to understand these complex interactions [15–18]. Experimentalists tend to examine the role of a particular protein or cell in the system by creating model systems in which the protein of interest is either removed from the system, by gene targeting, or over-expressed. This reductionist approach has yielded many important insights but also has limits. For example when the cytokine IL-2 [19], an important growth factor for T cells in vitro, was knocked out in a mouse model, no defects in T cell proliferation in vivo were observed [20]. Rather, the mice developed signs of autoimmunity and excessive T cell activation [21], which was subsequently attributed to the non-redundant role of IL-2 in the development and maintenance of Treg cells [22, 23]. There are many such examples, both in and out of the literature, in which targeted gene deletion in a mouse fails to show the expected phenotype. These findings reveal both a great deal of redundancy in the immune system, such that other factors can replace one that is missing, and also pleiotropy, as demonstrated by the IL-2 example, where previously unknown functions of a protein are revealed. Predicting the effect of such manipulations on phenotype is difficult because it involves the interplay of complex and competing mechanisms, such as feedback loops and competition that resolve in a context-dependent manner [24]. Computational modeling allows us to build representations of the system as a whole, which can be used to test hypotheses and provide predictions that can then be tested experimentally.

There are many other important and interesting questions that are covered in other contributions to this issue, and in this article we are choosing to focus on issues related

to T cell recognition, activation and regulation. These are topics that are pivotal to the immune response, due to the central role that T cells play in orchestrating the immune response and these have motivated a large number of modeling studies. This review will also highlight how technological advances in the study of immune responses are providing new quantitative data that inform computational models and may lead to new insights into T cell dynamics and function.

Big questions in T cell immunology

T cell recognition of antigen has been a subject of intense study for many years and the discovery of MHC restriction [25] led to intense speculation concerning the nature and structure of antigen receptors on T cells. The fact that T cell recognition required the presence of self MHC molecules led to speculations that T cells express two receptors; one for MHC and one for antigen. The description of the elegant structure of MHC molecules [26] clarified the issue when it was revealed that MHC molecules contain a peptide-binding groove into which small peptides derived from self and foreign antigens could bind. In addition it was shown that the well known polymorphic regions of MHC genes were localized to the peptide binding groove [27], thus giving each MHC allele the ability to bind a unique set of peptides. Further structural analysis of the TCR [28, 29] revealed that this structure binds components of both MHC and peptide. Unlike the high affinities with which immunoglobulin binds antigen, TCR have very low measured affinities for the peptide (p)MHC complex [30, 31]. The low affinity of this interaction is in contrast to the high degree of specificity and sensitivity exhibited by the TCR. This raises important questions about how T cell acquire these receptors, what is necessary for T cell activation and how does the signal received from the TCR determine the T cell fate following activation.

How is the T cell repertoire generated and maintained?

T cells develop in the thymus where they acquire different TCRs, through random DNA rearrangements. Interaction with self MHC is required for a cell to survive during the process of positive selection, but cells expressing TCRs that interact too strongly with self pMHC complexes are deleted in negative selection. It is thought that positive and negative selection are governed by different activation thresholds in the developing thymocyte [32]. Thus, the TCR/pMHC affinity required for positive selection is lower than that needed for negative selection. Modeling in this area has focused on many aspects of T cell development [33] including the role of the thymic involution on TCR diversity [34, 35], how signals are integrated by developing thymocytes [36] and the population dynamics of developing T cells [37–39]. Early ordinary differential equation (ODE) models of population dynamics in the thymus used measurements of the proportions of different thymocyte populations to infer the dynamics of thymic selection [40]. These models concluded that most thymocytes fail to pass the positive selection step and predicted that positive selection signaled proliferation of selected thymocytes. More recently a multi-compartment ODE model, which tracks lymphocyte populations in distinct parts of the immune system (i.e., blood, spleen, and LNs), used data from studies in which dividing thymocytes were specifically depleted to calculate kinetic parameters for the flux and steady state levels of relevant thymic populations [41]. Sinclair et al. [39] have taken advantage of detailed experimental data using a transgenic system in which T cell development can be turned on at selected times and thymocyte differentiation can be followed over time. These data suggested that the observed asymmetric differentiation of CD8 versus CD4 T cells could be explained by increased death rate in the CD8 population [39]. Another more recent model [38] used novel experimental data from bim −/− $NUR77^{GFP}$ mice [42]; NUR77 is downstream of TCR signaling and levels of NUR77 expression are directly proportional to the strength of TCR signal whereas the proapoptotic molecule Bim is necessary for negative selection. These data provided estimates for the number of thymocytes undergoing positive and negative selection and thus allowed a more detailed kinetic study of thymocyte differentiation incorporating death rates, proliferation and selection rates [38]. These and other models [33] have developed quantitative estimates of the lifetimes of the individual thymocyte populations and the timing of the selection steps during T cell development. Most of these models assume that TCR engagement depends on the overall affinity of the pMHC/TCR interaction [33], i.e., the total number of TCR that are engaged with pMHC at a given time, but recent data suggest that the duration of the TCR engagement also plays an important role in thymic selection [43–45].

The result of thymic differentiation is the generation of a diverse repertoire of T cells that are restricted to self MHC, lack autoreactivity and maintain enough diversity to withstand pathogen challenge. Another feature of T cell recognition is that it has been estimated that individual TCR may crossreact on a wide array of pMHC complexes with shared structural features [33, 46, 47]. In addition, the influence of so-called coagonist peptides on T cell activation was shown in a recent study, which combined modeling and experiments, to depend on the affinity of the co-receptor CD8 for individual MHC class I molecules [48]. In this context a coagonist peptide is derived from endogenous proteins, is unable to stimulate T cells alone

but enhances the recognition of agonist peptides [49]. Recently it has been observed that virus-specific T cells with a memory phenotype, indicative of prior activation, are found in individuals who had never previously been exposed to that virus [50, 51] suggesting a protective role for this cross-reactivity. These studies were possible because of new technology allowing the isolation of rare antigen-specific T cells in a polyclonal population using soluble pMHC tetramers [52]. As a result of these studies estimates for the frequencies of antigen-specific T cells have been made [53] and these range from 1 in a million to 90 in a million T cells. Recent technical advances in deep sequencing, flow cytometry, spectratyping and analysis of T cell precursor frequencies have allowed for more quantitative assessments of TCR diversity [54]. Spectratyping is a method by which the length of the CDR3 region of TCR α and β chains can be determined and this gives an indication of the diversity of the TCR sequences within a population; diverse T cell populations exhibit a normal distribution of CDR3 lengths whereas a population dominated by a small number of clones may only have one of two CDR3 lengths that dominate [55]. Several statistical approaches have been developed to analyze spectratyping data [56–58] and these have been used to estimate the size of the repertoire [59]. Advances in next generation sequencing allow for detailed sequence analysis of TCR gene diversity at various stages of development and new estimates of diversity are being generated using these data [47]. One challenge with the sequencing data is that each α and β chain is sequenced separately and it is not possible to know how they are paired unless the T cells are first cloned, although novel single cell techniques are now making it possible to pair individual TCR chains [60]. New analysis tools are being developed to handle the large amount of data that is generated from these sequencing platforms [61]. In addition, advances in single cell sequence analysis will allow more accurate assessments of diversity [60, 62], and combined with isolation of T cells with a given specificity [63] a deeper understanding of how the naïve T cell repertoire is generated and how it changes following antigen exposure will be obtained.

Treg cells arise in the thymus following interaction with self pMHC class II complexes and there has been a great deal of interest in analyzing the Treg repertoire. A recent study performed high throughput sequencing of TCR genes from defined human T cell populations [64] and this showed considerable overlap between sequences derived from T cell subsets with different functional properties. A more recent study demonstrated that increased TCR diversity in Treg was necessary for optimal suppressive function [65]. In a commentary to this study Wing and Sakaguchi proposed a simple mathematical model that explained these findings [66]. This model states that the degree of protection from autoimmunity is proportional to the number of Treg cells with specificity for the self-antigen in question. A prediction from this model is that mouse strains, or individuals, which develop autoimmunity have a reduced Treg repertoire diversity. This prediction has been validated in the non-obese diabetic mouse, which spontaneously develops type 1 diabetes [67].

Treg induction following peripheral stimulation of naïve T cells is also critical to determining that nature and strength of the immune response to antigen. Mathematical modeling has recently been used to explore the factors that lead to variability in the level of Treg induction in response to different types of stimulation. We have recently investigated the role of TCR signaling strength in the induction of FoxP3$^+$ Treg via the interaction of multiple signaling pathways, as discussed in more detail below [Miskov-Zivanov, 2013]. Another study combined CFSE labeling and careful quantification of apoptotic cells to determine how differential regulation of both proliferation and cell death can create conditions favorable and unfavorable for Treg induction [68].

How do T cells get activated?

There are two major challenges faced by the immune system in mounting a specific T cell response to a given antigen: (1) the low precursor frequency of T cells specific for a given foreign pMHC; and (2) the small number of antigen-containing pMHC complexes presented by each DC in the draining LN. Understanding how the immune system overcomes each of these challenges has generated a great deal of interest from modelers and experimental immunologists.

It has been known for some time that naïve T cells are constantly circulating between LN and blood [69, 70] and that this circulation involves interactions between T cells and self pMHC necessary for survival [71–73]. Early attempts at quantitating the migratory behavior of lymphocytes used the adoptive transfer of radiolabeled cells followed by enumeration of their location at various time points after injection [74, 75]. Based on such results a partial differential equation (PDE) model was developed [76] that was able to approximate the experimental results. In this model, the blood, spleen, and lymphatic systems were modeled as separate compartments and in addition the spleen and lymphatic compartments were spatially resolved in one dimension to model T cell transport dynamics. Interestingly this model suggested that T cells do not simply enter and exit LN but they also encounter and interact with other cells within the LN [76].

Advances in intra-vital imaging have allowed the direct visualization of T cell DC interactions within a living LN and thus the actual interaction times can now be measured

[77, 78]. A recent study determined the transit times of CD4 and CD8 T cells through lymph nodes using these techniques [79]. Labeled T cells were adoptively transferred, the drug FTY720 was administered to block sphingosine-1-phosphate receptors and sequester lymphocytes in LN and cells were counted in multiple LN [79]. These experiments revealed that CD4 T cells spend less time in LN compared with CD8 T cells (12 h vs 22 h). In addition these studies also showed that the duration of the contact between DC and CD4 T cells depended on the presence of MHC [79], and this has also been shown in an antigen specific system [80]. In this last study it was shown that the overall avidity of the TCR/pMHC interaction was more important than pMHC density in determining contact time [80].

These new and more quantitative data have been used to develop more detailed models of lymphocyte trafficking and activation [81–84]. An ODE-based model [82] was used to model the efficiency with which an antigen-specific T cell migrates through LN and finds the DCs that are presenting the cognate antigen. The results of this model suggest a trade-off between transit times and pMHC density such that detection of a low abundance pMHC is optimal when transit times are slow. Fast transit times favor a more rapid response when the relevant pMHC is in high abundance [82]. Another modeling study using a hybrid two-compartment model reached similar conclusions [81]. In these studies the LN is modeled using a 3D agent based model (ABM) [83] and the blood compartment is modeled using ODEs. The model considers T cell trafficking as well as the generation of primary and secondary immune responses in CD4 and CD8 T cells [81]. This model demonstrated that T cell fate was determined in part by the relative abundance of pMHC such that low abundance favored the development of memory CD4 T cells, whereas intermediate pMHC levels led to CD4 effector T cells. [81]. Interestingly, CD8 T cells required higher levels of pMHC to achieve these fates compared with CD4 T cells.

When a naïve T cell encounters a DC presenting the cognate pMHC complex, a series of signaling events are triggered by the interaction, resulting in activation, proliferation and differentiation of the T cell. T cell activation has fascinated immunologists and modelers for many years since it is unclear how T cells can have such a high degree of specificity and sensitivity when the interaction between TCR and pMHC has an inherently low affinity [85, 86]. Early models proposed that T cells engage in serial triggering [87] along with kinetic proofreading [88–90]. In these models TCR are internalized following engagement with pMHC thereby "counting" the number of interactions (serial triggering) and each interaction stimulates a series of conformational changes in the TCR as it encounters ligands. This provided an explanation for the observed differences in activation profiles between agonist and non-stimulatory ligands [91]. These models associated the degree of T cell activation with the duration of the interaction between T cell and DC and more recent imaging analyses of T cell/DC interactions have leant support to this notion [80, 92].

TCR activation leads to a complex series of early and late signaling events [93]. Many of the early signaling responses follow a digital pattern of response with the number of responding cells in a population increasing over time [86, 94]. This has led to several models based on feedback loops and leading to digital responses necessary for full activation based on ERK [94] or Ras activation [95]. Another important signaling mediator is calcium and a rise in intracellular calcium levels occurs within seconds following TCR stimulation. A recent multi-compartment quantitative model of calcium dynamics investigated the role of the calcium release activated channel (CRAC) in controlling calcium levels, concluding that CRAC played a predominant role in preventing calcium depletion in T cells [96].

These early signaling events are followed by the formation of an immunological synapse (IS), an ordered structure in which TCR and signaling molecules are clustered in the center and they are surrounded by a ring of adhesion molecules, such as LFA-1 [97, 98]. New imaging techniques have allowed for a better understanding of the formation and potential function of the IS [99], although its exact role in T cell activation is still being debated [98]. New advances in imaging and the development of specific tools are further defining the important role that the IS plays in communications between cells of the immune system [98].

Many of the signaling cascades stimulated in T cells are also found in other cells and one good example is the activation of the NFκB pathway, which plays an important role downstream of TCR signaling. This pathway has been extensively studied in multiple systems and has also been the subject of many models [100]. Recently, modeling of this pathway has been used in the development of approaches to identify signal-specific pharmacological targets [101]. In this study the topology of signaling hubs in the NFκB pathway along with dynamical modeling were used to identify pharmacological targets that would inhibit responses to one input signal without disrupting responses to other signals and also to reshape temporal responses to select desirable features and suppress undesirable ones. This approach proved successful in identifying some stimulus-specific inhibitors in the context of the NFκB response to LPS and TNF [101], and could also be applied to development of specific inhibition strategies in T cells. The fact that T cells also contain many of the signaling

pathways that have been widely studied in other cell types suggests that it will be useful to integrate models that have been developed for other systems into modeling of T cell responses.

How the immune response is regulated by the strength of the TCR signal

Signaling via the TCR not only stimulates T cell activation and proliferation but it has become apparent that the differentiation of naïve T cells into specific Th subsets is influenced by the perceived strength of the TCR signal. It has been known for many years that the dose of a stimulating antigen or pathogen has important implications for the nature of the immune response [102, 103]. In these studies it was observed that the development of cell-mediated or humoral immunity was controlled by the dose of the immunizing antigen [102]. This was later found to depend on Th1 and Th2 responses respectively and it was possible to alter the nature of the response by altering the dose and thereby the TCR signal strength [104, 105]. This has important consequences in the context of infection as demonstrated by the Leishmania infection model. Certain mouse strains develop an inappropriate Th2 response to this infection, which proves fatal. It was shown that exposing these mice to a low dose of bacteria would induce a Th1 response that was then able to protect the mice from subsequent infection with the higher and potentially fatal dose [103]. The differentiation of Th cell subsets involves the expression of unique transcription factors that drive the production of the signature cytokines produced by each of the Th subsets. It is known that Th differentiation requires the presence of certain signature cytokines in the milieu: IFN-γ for Th1 cells, IL-4 for Th2 cells, TGF-β for Treg cells and IL-6 and TGF-β for Th17 cells. These cytokines act by inducing transcription factors that augment the production of relevant cytokines and induce expression of specific cytokine receptors that are required for full differentiation and maintenance of the phenotype [106]. Several models have been developed that address Th cell differentiation networks [107–110], although these models did not take TCR signal strength into account. Because of the large number of cytokines, receptors, and signaling components involved in these processes, these models have used more coarse-grained modeling approaches than the studies cited so far. In such models, some or all of the variables are restricted to a small number of possible values, reducing the number of states of the system and simplifying formulation of the model, as we will discuss in more detail below. One cost of such simplifications is that quantitative factors, such as TCR signal strength, are more difficult to incorporate into the model, but, as we also discuss below, it is possible to do so.

We have been interested in this phenomenon in the context of the differentiation of Treg from naïve T cells. We [111] and others [80, 112–114] have shown that Foxp3 expressing Treg are induced when naïve T cell are exposed to low doses of antigen. This has been correlated with signaling via the Akt/mTOR pathway [115, 116] such that the degree of Akt/mTOR signaling is inversely correlated with the induction of Treg [111]. Treg that are expanded following low dose antigen are able to prevent autoimmune diabetes in vivo [112, 117]. We developed a mathematical model, based on Boolean logic, to further understand how TCR signal strength could contribute to Treg expansion and induction [118]. The model was developed through an iterative process of reading the literature, tuning the components and interactions, and comparison with experimental data, which was particularly important for calibrating the time scales of various processes. The components of the signaling pathway are depicted as elements within the model, and each element has only a small number of possible states—either ON or OFF in the case of most variables, or OFF, LOW, and HIGH in the case of a few critical variables, such as TCR and PI3K. Figure 1a presents the overall structure of the model showing model components as nodes and positive and negative influences between these elements as arrows with pointed and flat arrowheads respectively. This diagram is translated into an executable model by constructing update rules for each element. At each point in time, the overall state of the network is represented by the values of each of the variables in the system. The value of each element at the next time step is determined from the current values by the rules, which for each element take the form of logical functions of the influencing elements involving the basic logic operators AND, OR and NOT. For example, Akt, the element circled in red in Fig. 1a, is updated according to the rule

$$\mathrm{AKT}' = \mathrm{PDK1\ and\ MTORC2}$$

which means that the next value of AKT is ON only if both PDK1 and MTORC2 are ON and otherwise the next value is OFF. If the rule used "or" instead of "and", AKT would become ON if either MTORC2 or PDK1 were ON. A "trajectory" corresponding to a single cell containing this network evolving in time is simulated by selecting an initial set of values for the network elements representing the resting state of the cell plus the initial stimulation that is applied in the experiments, e.g., stimulating TCR and CD28. Different methods exist for updating the state of the network through application of the rules [119]. In our work we have chosen an asynchronous stochastic scheme that takes into account stochastic variation among cells [118]. We then compute a large number of individual trajectories and plot the percentage of cells exhibiting a given

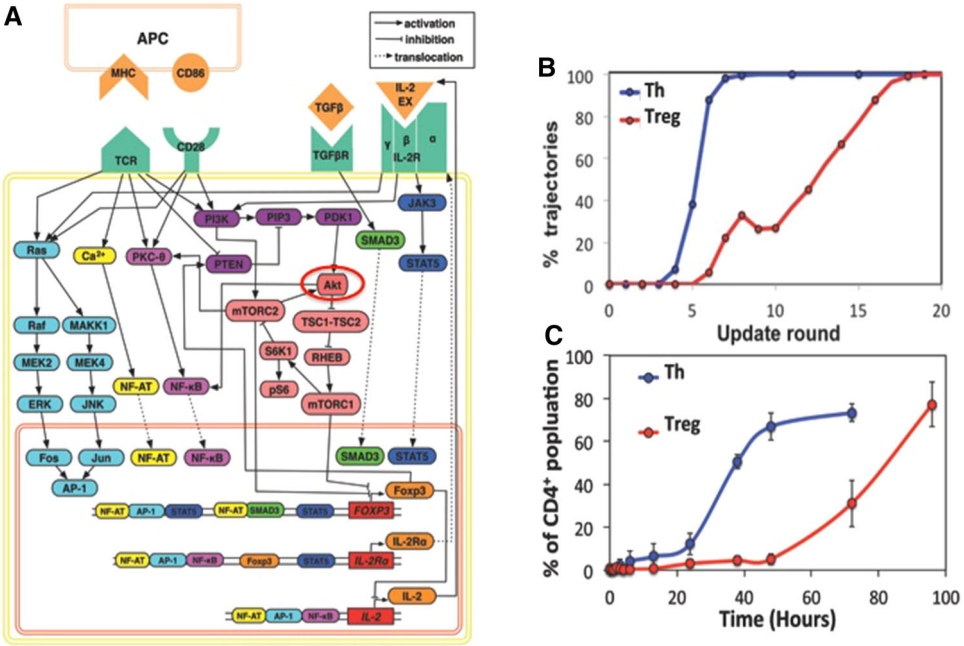

Fig. 1 Computational modeling of T cell differentiation. **a** Signaling network governing differentiation. *Nodes* represent network elements, which include external ligands such as MHC and IL-2, receptors, such as TCR and IL-2R, signaling intermediates such as Ras, Akt, and JAK3, and transcription factors such as AP-1, NF-AT, and FoxP3. *Edges* represent interactions between these elements with *pointed arrowheads* representing activation, *blunt arrowheads* representing inhibition, and *dashed lines* representing translocation of molecules between cellular compartments. The state of a given element in the network, such as Akt (*circled* in *red*) is updated according to the states of its incoming influences—PDK1 and mTORC2 in the case of Akt. **b** Simulated time courses of the percentage of cells destined to become IL-2^+ Th cells (*blue line*) or Foxp3^+ Treg cells (*red line*). In this simulation the TCR signal was interrupted after 6 times steps and the appearance of Th and Treg cells were followed at each time step. Results represent the combined trajectories, from a total of 1,000 simulations, leading to Th (390/1,000) or Treg (419/1,000) outcomes. **c** Corresponding experimental results for percentages of purified CD4+ T cells stimulated on plates coated with anti-CD3 antibody at high (*blue line*) and low (*blue line*) dose in the presence of soluble anti-CD28 antibody. High dose stimulation results in rapid stabilization of the Th (Foxp3^- $CD25^+$) phenotype whereas Treg (Foxp3^+ $CD25^+$) cells take much longer to appear in low dose stimulation. Results shown are mean ± SEM of three similar experiments (Color figure online)

characteristic, e.g., expression of IL2 or FoxP3, as shown in Fig. 1b. These results can be directly compared with experimental results obtained from flow cytometry (Fig. 1c). A major advantage of this kind of modeling is that signaling pathways can be followed from the cell surface to the end result of gene transcription without having to determine the large number of kinetic constants needed in an ODE based model.

The model made several predictions that we were able to verify experimentally [118]. In particular the model predicted that the induction of Treg by adding TGF-β to a high TCR signal strength scenario would not result in reduced Akt/mTOR signaling, which was confirmed in experiments. More significantly, we found that the modeling of low TCR signal strength most accurately reproduced the experimental results when the duration of the TCR engagement was reduced [118]. Interrupting the TCR signal after varying periods of time revealed the plasticity of the activated T cells. Thus, if the TCR signal was allowed to persist throughout the course of the experiment only Th cells were generated, whereas if the signal was interrupted after a period of time a mixed population of cells was generated that included Th, Treg, and also non-activated cells [118]. Another important insight that we gained from the model was that Treg cells take longer to stabilize as a phenotype than Th cells (Fig. 1b). Subsequent experiments confirmed this prediction (Fig. 1c). The model also allowed us to examine the trajectory of cells destined to become Th or Treg and identified the importance of the lipid phosphatase PTEN in determining the ultimate cell fate. Several new avenues of experimental research are being pursued based on these predictions. These results suggest that a population of T cells has the capacity to differentiate down several paths. It seems likely that differences in the behavior of individual cells are related to the observed heterogeneity in the expression levels of critical proteins in the TCR signaling cascade [120].

Treg expansion and differentiation is optimized by low TCR signal strength but other factors also play an important role in Treg biology [121]. These include the cytokines

IL-2, TGF-β, IL-10 and costimulatory pathways such as CD28, PD-1. IL-2 plays a critical role in the development and homeostasis of Treg; this is illustrated by the complete lack of Treg in mice deficient for the signaling chain of the IL-2R [122]. Treg constitutively express the high affinity IL-2R (CD25) whereas conventional Th cells only transiently express this receptor following activation. In addition, Treg do not make IL-2 whereas Th cells are the main source of IL-2 that is used by both Th and Treg to stimulate proliferation and expansion. Models of this interaction between the cells have illustrated how Treg can "steal" IL-2 from neighboring Th cells thereby not only ensuring their own survival but suppressing the proliferation of the Th cells [123]. Defects in IL-2 production have been associated with autoimmune diseases such as type 1 diabetes [124, 125] and this has been correlated with a decrease in Treg [124]. Due to its initial description as a T cell growth factor, IL-2 was also used clinically for the treatment of cancer with the aim of boosting T cell responses which were further enhanced by the addition of complexes of IL-2 with anti-IL-2 [126]. However, it was observed that depending on the type of complex used the result could be either expansion of CD8 T cells or Treg [126]. This phenomenon has been explored in models with the aim of developing the best therapeutic strategies for the use of IL-2 to boost Treg and therefore prevent autoimmunity [127, 128]. A recent model has also suggested that collective decisions by a population of cells are important in determining whether T cells become activated and that IL-2 availability may be one of the mechanisms by which this is achieved [129]. This is a situation where computational modeling can be of great benefit for the optimal design of an immunotherapy because intuition generally fails in systems that can exhibit such complex dynamics. Similarly, modeling has been used to optimize therapeutic intervention with anti-CD3 antibodies [130]; a therapy that involves a short-term course of low dose anti-CD3 antibodies in combination with islet antigens designed to boost antigen-specific Treg and preserve islet function [131, 132]. This model has identified several biomarkers that can be used to predict who may respond to this form of therapy [130].

Modeling approaches and communication between modelers and experimentalists

The field of modeling in immunology has advanced a great deal since the early days and this is in part due to an increasing awareness among immunologists of the need for modeling to increase insights into complex biological processes. In addition, more modelers are becoming familiar with the experimental techniques that are used and there is an increasing number of individuals who are trained in both disciplines [133, 134]. The improvements in technology have also made it possible for detailed quantitative measurements to be made. There is however still a need for closer collaboration and communication. For example modeling TCR activation has involved the use of deterministic ODE-based models that have focused on the earliest steps in T cell activation [94, 135, 136]. The development of rule-based approaches has simplified the development of complex models and has allowed the use of continuous as well as stochastic simulation methods [137]. Because these models require detailed quantitative data that may not be available in many cases, alternative approaches based on logical (aka Boolean) modeling have also been used to model T cell differentiation. Logical models do not require detailed quantitative measurements, but rather allow the development of complex qualitative networks and several have been developed to investigate T cell activation [118, 138, 139] and differentiation [108, 109, 118]. The models of Th cell differentiation focused on the interactions between cytokines, cytokine receptors, signaling molecules and transcription factors in defining a network and identifying scenarios for Th cell differentiation and plasticity [108, 109].

One concern with the present modeling efforts is that few published models are ever revisited or used by other investigators. Different tools and approaches are often used to address similar questions and it can be difficult to compare results from these kinds of models. In addition, the field is in need of more developed systems immunology approaches [140, 141]. These are models that incorporate the spatial and time scales of the whole immune response. These would include molecular and signaling events occurring within the cell, cellular interactions and then effects on the tissue or organism as a whole. Multi-compartment models of antigen presentation [142] and of lymph nodes [81] have been developed. A recent study described a novel methodology that can be used to capture the dynamics of complex biological systems using data from multiple levels of intra and inter-cellular behavior [143]. This method uses algorithmic information theory and the study was able to perform successful analysis of the response of Th and Treg cells to heat shock proteins [143]. Another issue is that experimentalists are often intimidated by the technical aspects of modeling and thus may fail to see the value of the exercise. Several modeling platforms have been developed that allow non-professional modelers to build models and test out ideas in an easy to use platform [144, 145]. These use graphic interfaces that are familiar to immunologists, although at this stage the models are mainly focused on the signaling aspects.

In conclusion, modeling of events in T cell differentiation, activation and function have proved to be extremely valuable in helping to elucidate the role of these important

cells in the immune system. There are now numerous modeling approaches and platforms available and a challenge for the future will be the coordination of these diverse methodologies. Many new therapies are targeting T cell immune functions, such as IL-2 or immune checkpoint blockade in cancer [146], and modeling is likely to play a key role in optimizing these approaches and in identifying new ones

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