Modeling of Naïve Lymphocyte Signaling Pathway

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Abstract: The immune system in general and T cells, in particular, play a critical role in protecting the organism from infection and repairing damaged tissue. T cells are not only key components of the immune system but are also central in mobilizing the adaptive immune responses at all stages of fighting infection. Furthermore, studies have shown that subsets of T helper cells are critical for the activation of antitumor responses. T cells have been intensively studied by both experimental immunologists and modelers. However, none of the research papers represent the complete process that will show the link between antigen-presenting cells, subtypes of T cells, and other signaling pathways. In this paper, we illustrate the first steps of an automation process for quantitative modeling, of the naïve T lymphocytes activation pathway by discrete modeling language using colored Petri nets (CPN). Modeling, simulation, and analyzing T cell activation signaling pathways will improve our understanding of the structure and dynamics of these pathways considerably. Petri nets have been proposed as an effective formalism for Systems Biology and modeling of metabolic pathways.

1 INTRODUCTION

Advances in experimental techniques and biotechnology have revolutionized our understanding of cellular and molecular processes. These techniques have generated vast amounts of empirical data that shed light on the intricate structure and mechanisms of signaling pathways. This wealth of experimental data provides valuable insights into the behavior of cells and molecules under various conditions, allowing scientists to decipher the complexities of biological systems (Mueller SN et. al. 2013). To make sense of this vast amount of experimental data and perform computational analysis on biological systems, it is necessary to represent and encode this data in a way that can be easily processed by computers. This data representation challenge is a well-known problem in the field of bioinformatics and has been extensively studied using various knowledge representation techniques from computer models. One approach that has proven to be particularly useful in modeling molecular and immune signaling pathways is based on a mathematical concept called Petri Nets. Petri Nets, that were initially developed in the early 1960s by Carl Adam Petri and have since been adapted and extended in many directions. They provide a formal framework for representing and analyzing the dynamic behavior of concurrent systems (Petri C.A. 1962) and has subsequently been adapted and extended in many directions (Murta T. 1989). Petri Nets offer a powerful way to model and simulate the intricate interactions and dynamics of molecules and immune signaling pathways. They provide a graphical representation of the system's components and their interactions, allowing researchers to study the behavior of the system over time. This mathematical approach enables the exploration of complex biological processes, such as signal transduction, gene regulation, and immune response, in a computationally tractable manner.

By employing Petri Nets, researchers can capture the essential elements of immune signaling pathway, including molecular species, their states, and the rules governing their interactions and activation. This modeling approach enables the simulation of various conditions and perturbations to better understand the immune system's behavior and predict its response to external stimuli such as pathogens. The utilization of Petri Nets in the modeling of molecular and immune

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signaling pathways has proven to be a valuable tool in bridging the gap between experimental data and computational analysis. It provides a systematic and quantitative framework to study the dynamics and behavior of complex biological systems, aiding in the development of novel therapeutic strategies and the advancement of our understanding of cellular processes (Rzosinska, K. 2017).

1.1 Petri Net

In the realm of systems analysis and modeling, Petri nets have emerged as a powerful mathematical tool for representing, simulating, and analyzing various complex systems. Petri nets are particularly valuable in modeling biological systems, such as T cell activation, where dynamic interactions and events play a crucial role. The fundamental components of a Petri net include circles, known as "places," rectangles, known as "transitions," and directed arcs that connect the places and transitions. Each place represents a state of the system, while transitions represent events or actions that can occur within the system.

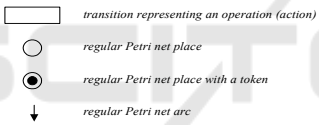

Figure 1: Legend of Petri nets.

The unique graphical representation of Petri nets allows for the depiction of complex systems in a visually intuitive manner. Places are used to represent different states or conditions of the system, while transitions represent the events or actions that can cause a change in the system's state. The arcs connecting the places and transitions represent the flow of tokens, which signify the occurrence or availability of certain resources or conditions.

By utilizing Petri nets, we can model and simulate the behavior of complex biological systems, such as naïve T helper cell activation. In the context of naïve T helper cell activation, Petri nets can capture the intricate interactions between signaling molecules, receptors, and other cellular components. The transitions in the Petri net represent the activation events and the places depict the various states of the T cell during the activation process. One approach of modeling and simulating naïve T helper cell activation is to use a Colored Petri net (Motta S. et. All 2013).

A colored Petri net is a variant of a Petri net that assigns colors or values to the tokens (i.e., the markings) that represent the state of the molecular and biochemical signaling pathways. This allows for a more detailed representation of a system's behavior, as different tokens with different colors can represent different types of molecules, receptors, enzymes, chemical signals, conditions, changes in gene expression, and so on.

Table 1: Petri net terms in the metabolic pathway.

Petri net Terms	Biochemical, and molecular signaling pathway term
Place	State of metabolite, signal, enzyme, gene expression…
Transition	Chemical reaction, metabolic process, gene expression, secretion…
Input arcs	Reagents and substrates of a chemical or metabolic reaction, activation of transcription factor
Output arcs	Products or outputs of a chemical or metabolic reaction, or gene expression…
Initial marking	Initial state of metabolic process e.g. Naïve T cells State of reaction at a giving time
Color	Concentration of molecule, type of chemical, enzyme, molecule

1.2 Colored Petri Net

In Colored Petri nets, sets of places, transitions and arcs are pairwise disjoint: $P \cap T = P \cap A = T \cap A = \emptyset$

- $\boldsymbol{\Sigma}$ is a set of color sets. This set contains all possible molecules, enzymes, transcription factors, signals, operations, and functions used within the color Petri net.
- $\boldsymbol{C}$ is a finite set of color sets (closets). It maps places in P into colors in Σ, and they define the functions.
- $\boldsymbol{N}$ is a node function. It maps A into $(P \times T) \cup (T \times P)$.
- $\boldsymbol{E}$ is an arc expression function. It maps each arc $a \in A$ into the expression e. The input and output types of the arc expressions must correspond to the type of nodes the arc is connected to.
- $\boldsymbol{V}$ is a finite set of variables $\boldsymbol{v} \in \boldsymbol{V}$ of colors $\boldsymbol{c} \in \boldsymbol{C}$. Arc expressions and guards contain variables $\boldsymbol{v} \in \boldsymbol{V}$ of the suitable types.

- ***P*** is a finite set of places {***p1***,…, ***pm***} ∈ ***P***, depicted by ellipses (Fig. 12). Each place ***p*** possesses a color ***c_p*** ∈ ***C*** and a bag ***b_p*** ⊆ ***B*** of tokens of color ***c_p***.
- ***G*** is a guard function. It maps each transition $t \in T$ to a guard expression g. The output of the guard expression should evaluate to a Boolean value (true or false). If false, t cannot be fired.
- ***I*** is an initialization function. It maps each place p into an initialization expression i. The initialization expression must evaluate a multiset of tokens with a color corresponding to the color of the place $C(p)$.

Colored Petri nets offer an enhanced representation that allows for a more detailed and nuanced analysis of complex systems, particularly in the context of molecular and biochemical signaling pathways. Colored Petri nets take the concept of traditional Petri Nets a step further by introducing the notion of colors or values assigned to tokens. Unlike traditional Petri nets, which use uniform tokens, colored Petri nets allow for the assignment of different colors to tokens, enabling the representation of various types of molecules, receptors, enzymes, chemical signals, gene expression changes, and more (Lee D-Y. 2006).

The introduction of colors or values to tokens in colored Petri nets enhances the ability to represent and model the intricate behavior of molecular and biochemical signaling pathways. By assigning specific colors to tokens, researchers can differentiate between different molecular species, their functional states, concentrations, or other relevant properties. This enables a more comprehensive and detailed representation of the system's behavior, taking into account and concentration of the diverse components and their interactions within the biological system. With the capability to differentiate tokens based on colors, colored Petri nets provide a powerful tool for analyzing complex biological systems. Researchers can investigate the dynamics and behavior of molecular and biochemical signaling pathways, observe the effects of different inputs or perturbations, and gain insights into the overall functioning of the system. The use of colored Petri nets in modeling molecular and biochemical signaling pathways offers numerous advantages. It allows for a more precise representation of the system, facilitating the identification of crucial interactions, bottlenecks, feedback loops, and other key characteristics. Additionally, the ability to assign colors to tokens aids in the analysis of specific molecules, signaling cascades, and regulatory mechanisms, contributing to a deeper understanding of complex biological processes (Liu F. 2012).

2 AN OVERVIEW OF IMMUNE SYSTEM

The immune system plays a crucial role in protecting our bodies against pathogens, such as bacteria and viruses. One of the key components of the immune system is the T helper cell, specifically the naive T helper cell. Naive T helper cells are a type of white blood cell that circulates in the bloodstream, constantly surveying for potential threats. Naive T helper cells are "naive" because they have not encountered any specific antigens or foreign substances before. Their activation is a complex process that involves interactions with antigen-presenting cells (APCs) and the recognition of specific antigens (Gullo F. et all 2015).

The process of naive T helper cell activation begins when an APC, such as a dendritic cell or macrophage, encounters a foreign antigen. The APC internalizes the antigen and presents small fragments of it on its surface using a protein called major histocompatibility complex II (MHC II). This MHC II-antigen complex serves as a signal for the naive T helper cell to recognize the antigen. When a naive T helper cell encounters an APC presenting an antigen that matches its specific T cell receptor (TCR), a series of molecular interactions occur. The TCR on the T helper cell binds to the MHC II-antigen complex on the APC, initiating a signaling cascade within the T cell (Paul, W. E et all 2010).

This signaling cascade leads to the activation of the naive T helper cell. The T helper cell undergoes proliferation, or rapid cell division, to produce a population of activated T helper cells specific to the antigen. This clonal expansion ensures a robust immune response to the pathogen. Furthermore, during activation, the naive T helper cell receives additional signals from the APC in the form of co-stimulatory molecules, such as CD28, CD4 and B7. These co-stimulatory signals are necessary for full activation and optimal function of the T helper cell. Once activated, T helper cells differentiate into various subsets, such as Th1, Th2, or Th17 cells. Each subset has specialized functions and produces specific cytokines to regulate different aspects of the immune response. Activated T helper cells play a central role in orchestrating the immune response by secreting cytokines that activate other immune cells, such as B cells, cytotoxic T cells, and macrophages.

They also provide help to B cells for the production of antibodies and help in the recruitment and activation of other immune cells to the site of infection (Daniel B. et all 2002).

In summary, naive T helper cell activation is a crucial step in initiating an effective immune response. It involves the recognition of antigens presented by APCs, signaling between the T cell and APC, clonal expansion of activated T helper cells, and their subsequent differentiation into specific subsets. This activation process ultimately leads to the coordination of the immune response to eliminate pathogens and maintain immune homeostasis in the body (Yates AJ et al 2014).

2.1 T Helper Cell and Antigen Presenting Cell Interaction

T helper cells, also known as CD4+ T cells, play a critical role in the immune response by helping to coordinate and regulate the activities of other immune cells. Studies have shown that the subsets of TH cells may play a critical role in the activation of anti-tumor response either directly by themselves or by stimulating cytotoxic T cell activity (Tay R.E. et all 2020 & Borst J et al 2018).

T helper cells become activated when they recognize foreign antigens presented on the surface of antigen-presenting cells (APCs), such as dendritic cells or macrophages.

The process of T helper cell activation can be divided into several key steps:

1. Antigen presentation: APCs present foreign antigens on their surface, along with molecules called major histocompatibility complex (MHC) molecules. T helper cells recognize these antigens when their T cell receptor (TCR) binds to the antigen-MHC complex
2. Costimulation: In addition to TCR binding, T helper cell activation also requires costimulatory signals provided by APCs. These costimulatory signals are delivered by molecules such as CD80 and CD86, which bind to receptors on the surface of T helper cells.
3. Activation and differentiation: Once a T helper cell has received both TCR and costimulatory signals, it becomes activated and begins to proliferate. Activated T helper cells also differentiate into different subtypes based on the cytokines present in their environment. The two main subtypes of T helper cells are Th1 and Th2 cells, which produce different cytokines and play different roles in the immune response.
4. Effector function: Activated T helper cells can then help to activate other immune cells, such as B cells and cytotoxic T cells, by secreting cytokines that promote their activation and differentiation.

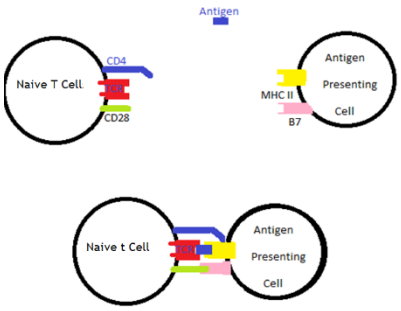

Figure 2: Activation of Naïve T helper cell by Antigen Presenting Cell.

2.2 Petri Net Model of T Cell Activation

The use of modeling approaches in the field of immunology has attracted interest due to an increasing awareness among immunologists of the need for modeling to increase insights into complex biological processes. The behavior of Naive T helper cells can be modeled using a colored Petri net. The components of the model include Naive T helper cells, antigens, antigen-presenting cells (APCs), T cell Receptors (TCR), co-stimulants (CD28, B7), and cytokines. The places represent the states of the system, and the transitions represent the actions that can change the system's state. The places may include "Naive T helper cells," "APCs," "antigens," "activated T cells," "memory T cells," and "cytokines". The transitions may include "antigen presentation," "T cell activation," "cytokine production," and "T cell differentiation". The initial marking specifies the initial state of the system, i.e., the initial distribution of tokens among the places. Initially, the "Naive T helper cells" place will have a token representing the population of Naive T helper cells, while the other places will be empty. The color functions determine which tokens can participate in a firing of a transition, based on their colors. For example, the "antigen presentation" transition may require tokens representing an APC and an antigen to be present in the "APCs" and "antigens" places, respectively, before it can fire.

The model can be simulated to observe the behavior of T-naive cells under different conditions, such as the presence of different antigens or

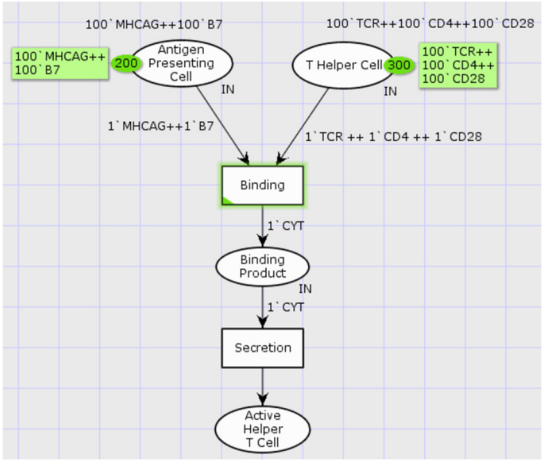

Figure 3: Petri net model of Naïve T helper cell activation by Antigen Presenting Cell. CYT=Cytokine, TCR = T Cell Receptor, MHCAG = MHC protein bound to Antigen. If there is an infection, then Antigen Presenting Cells will take peptide from infectious agent and present it (bind to) Naïve T helper cell's receptor. Co-stimulators such as CD 4, CD28, and B7 which are critical to allow full activation, sustain cell proliferation of Naïve T helper cells also bind. If all the conditions are right, then this binding will result in the release of chemical messenger cytokine. Cytokines in turn will bind to Naïve T helper cells and will activate them. In our experiment we started with an initial concentration of 100 (100 TCR, 100 CD4, 100 CD28…).

variations in the concentration of cytokines. The model can also be analyzed using mathematical techniques to study the properties of the system, such as the threshold antigen concentration required for T-cell activation.

In our colored Petri net model of naive T helper cells, we aimed to simulate the initial state of the system by representing it with a set of tokens. Tokens in Petri nets symbolize the state or condition of the entities being modeled. In this case, we began with 100 tokens, where each token represented a naive T helper cell and an antigen-presenting cell (APC).

The representation of the system with 100 tokens reflects the initial population of naive T helper cells and antigen-presenting cells in the simulated environment. This initial state serves as the starting point for the subsequent events and transitions within the Petri net, simulating the dynamic behavior of the system as it progresses. By employing a colored Petri net approach, we have the flexibility to assign specific colors or values to the tokens representing naive T helper cells and antigen-presenting cells. This color assignment can help differentiate between different subtypes or properties of these cells, providing a more detailed and accurate representation of the biological system.

2.3 T Helper 1, 2 and 17 Activation

T helper (Th) cells are differentiated from one another based on the expression of subset-specific transcription factors (such as T-bet, GATA3, and RORγt), and the secretion of cytokines such as interferons and interleukins. When naïve T helper cells are activated, they differentiate into several sub-types, in this paper we will focus on three major sub-types of Th (Th1, Th2, and Th17). Each of which is specialized for protecting against certain infections.

As mentioned earlier, antigen-presenting cells (APCs), such as dendritic cells, macrophages, or B cells, present antigens to naive T cells in the lymph nodes. In addition to antigen presentation, APCs also provide co-stimulatory signals to activate T cells. This involves the interaction of molecules on the surface of APCs, such as CD80 and CD86, with receptors on the surface of T cells, such as CD28.

Upon binding of APC and naïve T, if the antigen is present, then cytokines are released. Depending on the type of cytokines released, naïve T cells will differentiate into effector cells that can migrate to sites of infection and inflammation, where they release cytokines and directly attack infected or cancerous cells. Depending on the cytokine, naïve T cells could differentiate into either T helper 1 cells, T helper 2, cells, T helper 17 cells, and so on. For example:

- If Naïve T cells are exposed to Interleukin 12 (IL12), then "STAT 4' (Signaling Transducer and Activator of Transcription) is phosphorylated and consequently regulates gene transcription, and Naïve T cells will differentiate to T helper 1 cells.
- If Naïve T cells are exposed to Interleukin 4, then "STAT 6' and "GATA3" transcription factors are phosphorylated and consequently they regulate gene transcription, and Naïve T cells will differentiate into T helper 2 cells.
- If Naïve T cells are exposed to Interleukin 6 then "STAT 3' and "RORyt" transcription factors are phosphorylated and consequently they regulate gene transcription, and Naïve T cells will differentiate into T helper 17 cells.

Overall, the activation of naïve T helper cells is a complex process that involves multiple steps and requires the coordinated interaction of several different cell types and signaling molecules as shown in our Petri net models. Three major subsets of Th cells exist (Th1, Th2, and Th17), and each of these cells is specialized for protecting our body against certain infections.

Differentiated T helper cells in turn release different cytokines for example:

- Th1 cells primarily produce cytokines such as interferon-gamma (IFN-gamma) and interleukin-2 (IL-2), which activate other immune cells such as macrophages and cytotoxic T cells and are associated with protection against intracellular microbes (predominantly viruses) and the onset of anti- or pro-tumorigenic effects (Kaiko GE, et al 2008, and Zhu J et al 2010).
- The 2 cells release cytokines such as IL 4, IL 5, and IL 13, which promote the production of antibodies, which are important for neutralizing extracellular pathogens and eliminating parasites (Walker JA et al 2017).

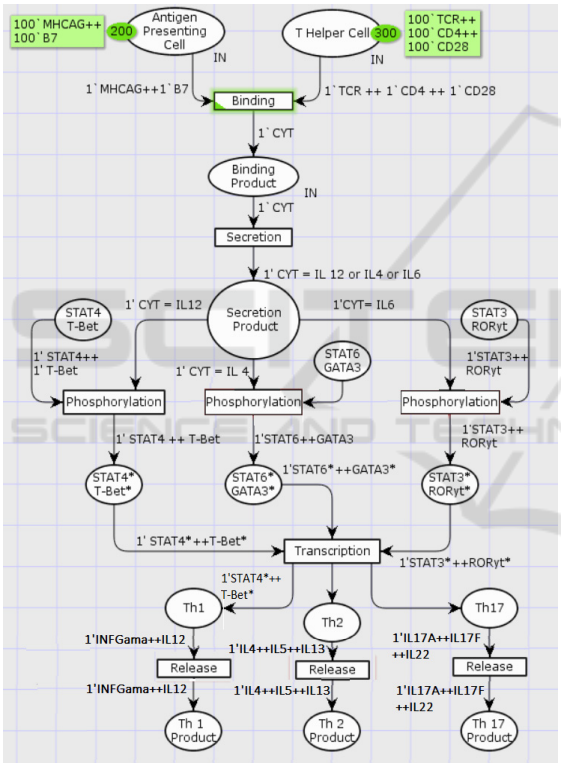

Figure 4: Detailed Petri Net model of naïve T helper cell activation. The first step in activation of Naïve T helper cell is binding of Antigen Presenting Cells to naïve T helper cell. Binding of cells will initiate release cytokine by Antigen Presenting Cells. Depending on the type of cytokine releases (IL 12, IL 6, IL 4…) different genes within naïve T helper cells will be expressed. Expression of these genes will initiate transcription and translation, thus naïve T helper cell will differentiate into active T helper 1, or T helper 2 or T helper 17 cells.

- Th17 cells can migrate to sites of infection and inflammation, where they release cytokines such as IL 22, and IL 17 which fight microbial pathogens and promote inflammation and tissue damage (Kaiko GE, et al 2008, and Zhu J et al 2010).

With a detailed model of naïve T helper cells in the form of a Petri net model, it is easy to analyze and simulate it with the common techniques for analyzing and simulating the behavior of Petri nets. But it should be noted that it is not the final step towards simulation and analysis. Within the overall task of simulation, there are three primary sub-fields: model design, model execution, and model analysis. Models can take many forms including declarative, functional, constraint, spatial, or multi-model. A multi-model is a model containing multiple integrated models each of which represents a level of granularity for the physical system.

The next task, once a model has been developed, is to execute the model on a computing platform. This step involves running the model using appropriate software or tools that can simulate and analyze the behavior of the system described by the model. For this purpose, a computer program can be created to run through the whole process while updating the state and event variables in the mathematical model. In the Petri net model of T cell activation, functional programming languages can be leveraged to represent the various components of the system as functions and define the transitions between states. The places in the Petri net, which represent the different molecular entities and their concentration and quantities, can be encoded as variables or data structures within the functional programming language. The transitions in the Petri net, which signify the events and interactions occurring in T cell activation, can be implemented as functions that take the current state and produce a new state as a result. These functions can encapsulate the complex logic involved in the activation process, including the binding of antigens to T cell receptors, the activation of intracellular signaling pathways, and the subsequent release of cytokines. By utilizing functional programming languages, the Petri net model of T cell activation can be simulated and analyzed efficiently. The declarative and modular nature of functional programming allows for easy composition and abstraction, enabling researchers to focus on specific aspects of the system while maintaining a clear overall representation.

Furthermore, functional programming languages provide a strong foundation for formal verification and rigorous analysis of the Petri net model. The mathematical nature of functional programming aligns well with the underlying principles of Petri

nets, allowing for the application of formal methods to verify properties, detect potential issues, and explore different scenarios.

For ease of understanding, we used the module-programming technique. It means that for each core operation of the naïve T helper cell activation process, there is a separate program module written. For example, module M1 (in Figure 5) programs all operations necessary for Cytokine production after antigen-presenting cell (APC) binds to naïve T helper cell. The language used in this figure is a pseudo-functional language, which can be easily extended into an executable program.

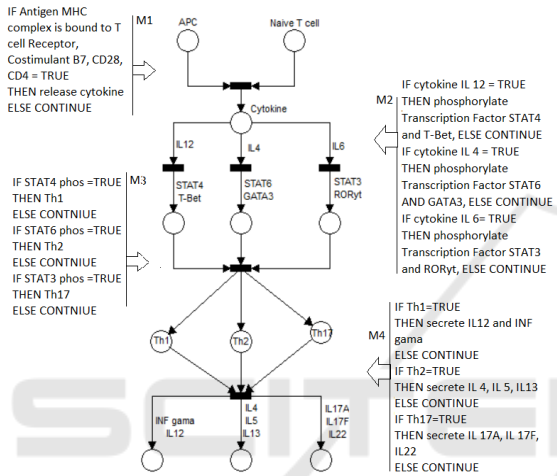

Figure 5: The Petri net model of the T cell activation described by functional programming language.

Finally, to construct an entire program for the naïve T cell activation processes, one should assemble all program modules indicated in Figure 5 into one program. The resulting program is represented below.

```
PROGRAM Naïve T cell activation
BEGIN
    IF cell_state=
       infection:
       THEN CALL M1, M2, M3, M4;
           IF antigen is bound to MHC=
              Cytokine secretion
              THEN CALL M2, M3, M4;
              Phosphorylate
              Transcription Factors
              CONVERSE;
           ELSE CONTINUE;
END
```

3 CONCLUSION

In conclusion, the modeling of naive T helper cells using CPN provides a valuable approach to understanding the complex dynamics and behavior of these cells within the immune system. CPNs offer a formal and graphical representation that captures the interactions, states, and transitions of the T helper cells and their associated signaling pathways. By employing CPN modeling, researchers can simulate and analyze the behavior of naive T helper cells under various conditions, such as different antigen presentations, co-stimulation, or regulatory mechanisms. This enables a deeper understanding of the underlying mechanisms that govern T cell activation, proliferation, differentiation, and cytokine production.

The advantage of using CPNs lies in their ability to handle the dynamic nature of the immune response, capturing both qualitative and quantitative aspects. CPNs can integrate experimental data and help generate hypotheses by simulating the effects of genetic modifications or perturbations in the system. This aids in the exploration of different scenarios and the identification of key factors influencing T cell behavior.

Moreover, CPN modeling facilitates the identification of critical control points or potential interventions for therapeutic purposes. By manipulating the CPN model parameters or introducing virtual interventions, researchers can assess the impact on T cell responses and potentially guide the development of novel immunotherapies or vaccine strategies. However, it is important to acknowledge that CPN modeling of naive T helper cells is a simplification of the complex and dynamic immune system. The accuracy and reliability of the model depends on the quality of the data used to construct it and the assumptions made during the modeling process. Therefore, validation of the model against experimental observations is crucial to ensure its fidelity and usefulness in understanding and predicting T cell behavior.

In summary, CPN modeling of naive T helper cells provides a powerful tool for investigating the intricate processes involved in immune responses. It offers insights into the dynamics of T cell activation and differentiation, helping to unravel the underlying mechanisms and identify potential therapeutic targets and further enhancing our understanding of T cell biology and its implications in health and disease.

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