Review

Effects of immunosuppressants on T-cell dynamics: Understanding from a generic coarse-grained immune network model

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Long-term immunosuppressive therapy is a drug regimen often used to lower aggressive immune responses in various chronic inflammatory diseases. However, such long-term therapy leading to immune suppression may trigger other adverse reactions in the immune system. The rising concern regarding the optimal dose and duration of such treatment has motivated us to understand non-classical immunomodulatory responses induced by various immunosuppressive steroid and secosteroid drugs such as glucocorticoid and vitamin D supplements. The immunomodulatory actions of such immunosuppressants (that govern the adaptive immune response) are often mediated through their characteristic control over CD4+ T-cells involving pro- and anti-inflammatory T-cells. Several early studies attempted to decode temporal and dose-dependent behaviors of such pro- and anti-inflammatory T-cells using the chemical dynamics approach. We first summarize these early works. Then, we develop a minimal coarse-grained kinetic network model to capture the commonality in their immunomodulatory functions. This generic model successfully reproduces the characteristic dynamical features, including the clinical latency period in long-term T-cell dynamics. The temporal behavior of T-cells is found to be sensitive to specific rate parameters and doses of immunosuppressants. The steady-state analysis reflects the transition from an early classified weakly regulated (autoimmune-prone) immune state to a strongly regulated state (immunocompromised state), separated by an intervening state of moderate/balanced regulation. An optimal dose and duration are essential in rescuing balanced immune regulation. This review elucidates how developing a simple generic coarse-grained immune network model may provide immense information that helps diagnose inefficacy in adaptive immune function before and after administering immunosuppressants such as glucocorticoid or vitamin D.

Keywords. Chemical dynamics; glucocorticoid; immune network; T-cell; steroid; vitamin D

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1. Introduction: A brief overview of the human immune system and its components

We continuously encounter many bacteria, viruses, fungi, and other complex organisms during our lifetime. While some are not harmful to us, like the commensal bacteria inhabiting our gut, others are considered pathogenic, leading to severe health ailments. To maintain physiological homeostasis, we have a cellular and humoral system that is able to control and get rid of these foreign organisms from our bodies – the immune system. Distinguishing self from non-self is one of the primary functions of the immune system. The immune system has two interconnected arms, i.e., innate immunity and adaptive immunity. Innate immunity corresponds to a non-specific defense mechanism present in our body at birth, whereas adaptive immunity is acquired immunity that the body develops upon encountering pathogens.

The innate immune system mainly consists of two types of barriers against infection: the anatomical barrier, both physical and chemical, and the cellular immune response. In most cases, the innate immune system provides the first line of defense against foreign pathogens after the physical barriers, i.e., the skin and mucosal epithelia. The chemical barrier includes chemokines and anti-microbial substances. Cells expressing innate immune recognition receptors are macrophages, dendritic cells, mast cells, natural killer (NK) cells, neutrophils, and eosinophils. After recognizing the microbial trespasser in the body, phagocytes are the specialized cells that quickly engulf the microbe through phagocytosis (Perelson 1989; Chaplin 2010; Brodin and Davis 2017; Mayassi *et al*. 2021; Nayak and Roy 2021).

Some innate immunity components have very similar structural blocks across species, not only in mammals and other vertebrates but also in insects and plants. This indicates that innate immunity is well preserved through the course of evolution, presenting an active distinction between self and non-self (Hoffmann *et al*. 1999). Moreover, it has been observed that animals or plants which depend only upon innate immunity have no instance of autoimmunity, allergy, or organ transplant rejection (Janeway *et al*. 2002; Cebula *et al*. 2019).

Adaptive immunity, on the other hand, is a subsystem of the immune system which comprises specialized, systemic cells and processes that help in the elimination of pathogens by preventing their growth. Compared with innate immunity, adaptive immunity is a slow response process. One significant advantage of the adaptive immune system is producing long-term memory cells. These memory cells get quickly activated on the second encounter with the same pathogen, resulting in a quicker and more efficient response against that pathogen. The different lymphocyte populations of the adaptive immune system constitute T-cells and B-cells. T-cells act via cell-mediated responses, and B-cells are responsible for the production of antibodies. The B-cell is produced and undergoes maturation in the bone marrow. B-cells have a significant role in antigen presentation. The diversity of antibodies produced by B-cells is vast, but each B-cell produces only one type of antibody with a unique antigen-binding site. B-cells recognize antigens through the immunoglobulins (Igs), which are cell membrane-bound proteins uniquely expressed by B-cells, also termed B-cell receptors (BCRs). When a B-cell is fully differentiated, it becomes a plasma cell that secretes immunoglobulin with the same antigen specificity as their BCR, called antibodies. Antibodies bind to pathogens or their products. This leads to their phagocytosis by cells of the innate immunity system (Cyster and Allen 2019; Liu *et al*. 2020).

T-cells are a group of lymphocytes produced in the bone marrow, mature in the thymus gland, and play a significant role in immune system response. The interaction of the T-cell receptor (TCR) and CD4 or CD3 as co-receptors with the antigen-MHC II complex, which is presented by antigen presentation cells, causes the antigenic stimulation that triggers the first stage of differentiation of the naïve T-cells. Naïve T-cell proliferation and differentiation into particular effector cells are ultimately activated by a network of downstream signaling pathways induced by combined TCR and CD3 activation (Fouchet and Regoes 2008; Roy *et al*. 2014). Lineage-specific differentiation is influenced by the micro-environment cytokine milieu, antigen concentration, antigen-presenting cell (APC) type, and co-stimulatory molecule levels. Dendritic cells are considered the most significant APC due to their efficient role in the activation process of naïve T-cells (Chaplin 2010).

2. Experimental overview of dynamic behaviors of CD4+ T-cells: Team behavior

CD4+ T-cells play a significant role in the immune responses throughout the host's defense against the pathogen. However, an extension of their critical functions of being helper cells sometimes leads to action against self-cells, leading to autoimmune

disorders and allergies (Jones and Diamond 1995; Smith and Germolec 1999). T-cells that express co-receptor CD4+ are considered helper T-cells. These cells have a crucial role in immune regulation, mainly in the adaptive immune system. Regulatory cells are another class of CD4+ T-cells involved in the immune system's modulation or regulation. In addition to the critical roles as helper cells, CD4+ T-cells may cause some autoimmune diseases and allergies.

Homeostasis maintains a stable internal environment between various physiological variables. Any quantity, either lower or in surplus, causes imbalance. It was earlier believed that the helper T-cells were only limited to two significant subsets: Th1 and Th2 cells. The Th1/Th2 paradigm (Elenkov 2004) is a simplistic yet classical approach to defining the immune response, governed by a balance between Th1 and Th2 cells. However, in the pathogenesis of inflammatory arthritis, it is observed that both Th1 and Th2 cytokines can be pro- or anti-inflammatory in varied circumstances (Elenkov 2004). A new concept about how the disease condition is induced by CD4+ T-cells is summarized in the study by Hirahara and Nakayama (2016). It entails the association between inflammatory diseases and the complexity of the helper T-cell subsets, which include Th1, Th2, Th17, Th9, Th22, T follicular-helper (Tfh) cells, and T-regulatory (Treg) cells. Several such studies articulated that the outcome of this cell fate decision is complex and depends on various parameters like cell–cell interactions and the cellular environment (Nurieva and Chung 2010; Schmidt et al. 2012; Srivastava et al. 2018).

In 1901, Paul Ehrlich observed that in some scenarios, the immune system might attack its own cells (Bordon 2016). In such situations, the immune system attacks the host's self-cells instead of carrying out its classical work of protecting the body against foreign antigens by reacting against them, creating a condition that Paul Ehrlich termed 'horror autotoxicus'. This may result in a clinical syndrome called autoimmunity. He coined the term 'horror autotoxicus' to highlight that autoimmunity contradicts nature's disinclination towards self-injury. In some instances, injury to self-cells or organs is prompted by antibodies, whereas in other cases, T-cells are the chief culprits resulting in fatal autoimmune diseases (Margo and Harman 2016) such as rheumatoid arthritis, multiple sclerosis, and some types of diabetes.

Tolerance or self-tolerance is the mechanism present in our bodies to prevent a self-immune attack. Self-tolerance is a complicated process that involves the elimination of the immune cells that can react against self-antigens, and the active inhibition of the immune response against self-proteins (Margo and Harman 2016; Kaufmann 2019). T-cells that express CD8 are recognized as cytotoxic T-cells or killer T-cells. As the name suggests, they is directly involved in killing the infected cell (Fagnoni et al. 2002; Cowley et al. 2005). CD4+ T-cells can also be classified into different subsets, such as Th1, Th2, Th3, Th17, Th9, Th22, Tfh cells, and Treg cells (Nurieva and Chung 2010; Deenick et al. 2011; Schmidt et al. 2012; Scurr et al. 2014; Golubovskaya and Wu 2016; Srivastava et al. 2018; Chemin et al. 2019). Among these CD4+ T-cells, it has been observed that some act as pro-inflammatory cells (Th1, Th2, Th17, Th9, Th22, and Tfh cells), and others work as anti-inflammatory cells (Th 3 and Treg cells) (Lei et al. 2021; Nayak and Roy 2021).

3. Immunomodulatory effects of immuno-suppressants on CD4+ T-cell population dynamics

As discussed above, CD4+ T-cells have two sub-populations: pro-inflammatory T-cells and anti-inflammatory T-cells. As the name suggests, pro-inflammatory T-cells promote inflammation. In some scenarios, they attack self-cells to cause autoimmune disorders and allergies (Sakaguchi and Sakaguchi 2005; Orihara et al. 2008; Murdoch and Lloyd 2010). In contrast, anti-inflammatory T-cells adopt a downregulatory mechanism acting on the population of pro-inflammatory T-cells. Thus, they can prevent autoimmunity (Grossman et al. 2004; Cao et al. 2007).

Vitamin D and glucocorticoid (GC) are well-known immunosuppressants (Chun et al. 2014). Both are effective in downregulating pro-inflammatory T-cells, i.e., the effector T-cell population, and upregulating anti-inflammatory T-cells, i.e., the regulatory T-cell population. The role of vitamin D in autoimmunity (Cutolo et al. 2011) gained much attention after the discovery of the vitamin D receptor (VDR) and other essential vitamin D-metabolizing enzymes expressed by the immune system cells. The role of vitamin D is discrete both in the case of innate and adaptive immunity (Kongsbak et al. 2013). Several experimental and clinical studies (Bouillon et al. 2021) have shown that the endogenously produced active vitamin D ($1,25(OH)_2D_3$) in macrophages increases the production rate of anti-microbial peptides like cathelicidin and β-defensins, which promote innate immune response. Consequently, the conversion of $25(OH)D_3$ into its functional form, i.e., $1,25(OH)_2D_3$ (active vitamin D) in antigen-presenting cells (APCs), exerts a

beneficial effect on the adaptive immune system. Correale *et al.* (2009) also observed a similar phenomenon. They showed that effector T-cells can convert inactive vitamin 25(OH)D$_3$ into biologically active 1,25(OH)$_2$D$_3$ with the help of the enzyme 1α-hydroxylase, which is present in them (Mora *et al.* 2008; Chun *et al.* 2014).

Experimental studies (Chun *et al.* 2014) based on the immunomodulatory traits of vitamin D showed that autoimmunity is principally guided by the increase in the number of helper T-cells that attack several self-tissues in the body. Some studies (Mora *et al.* 2008; Marcinowska-Suchowierska *et al.* 2018) suggest that vitamin D has an adverse effect on the activation, proliferation, and differentiation of helper T-cells and a promoter effect on regulatory T-cells. It has also been observed that both vitamin D and regulatory T-cells have an inhibitory effect on the conversion of resting APC to active APC (Chun *et al.* 2014; Charoenngam and Holick 2020). With the above notion, vitamin D is expected to have a substantial role in T-cell population dynamics.

Glucocorticoids are also beneficial and cost-effective drugs. They have been in use for more than 60 years. They exert their primary anti-inflammatory and immunosuppressive effects on innate and adaptive immune responses. In several earlier studies, it has been found that glucocorticoids have suppressive effects on eosinophils, basophils, NK cells, monocytes, macrophages, and T-cells, whereas glucocorticoids support growth of regulatory T-cells (Cain and Cidlowski 2017; Timmermans *et al.* 2019). Glucocorticoids cause apoptosis of T-cells and impact T-cell differentiation by regulating the cytokines affecting the differentiation of T-cell subsets. One such case is where GCs inhibit the production of Th2-mediated cytokines. GCs are known to decrease circulating monocytes/macrophages, synthesis of pro-inflammatory cytokines and prostaglandins, and expression of their MHC class II molecules and Fc receptors (Elenkov 2004; Coutinho and Chapman 2011).

Several steroid hormones, including testosterone, and vitamin D are produced and released into the systemic circulation as precursor molecules, mainly in bio-inactive form. These circulating precursors are then converted to end-organ metabolized products (their most bioactive forms) by tissue- and organ-specific enzymes (Bereshchenko *et al.* 2018). In contrast, GCs are produced and released by the adrenal gland in their bioactive forms that can be metabolized by tissue-specific 11-hydroxysteroid dehydrogenase (11-HSD) enzymes. However, the 11-keto metabolites (cortisone and 11-DHC) can diffuse into the cells without restriction. It is reported that lymphocytes can produce bioactive GCs from their physiologically inactive 11-keto (cortisone and 11-DHC) metabolites (Zhang *et al.* 2005). They have reported that 11-HSD1 mRNA and protein are expressed in murine CD4+ and CD8+ lymphocytes. The complete conversion of cortisone (inactive) to cortisol (bioactive) occurs in lymphocytes (Cain and Cidlowski 2017; Weinstein *et al.* 2017). Hence, GCs can also play a vital role in modulating T-cell dynamics.
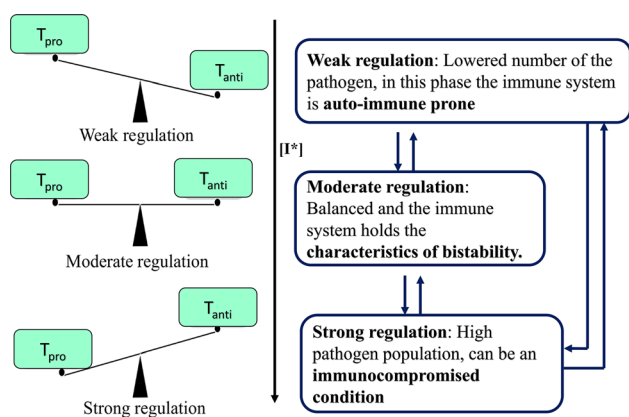
4. Mathematical models capturing T-cell dynamics of the human immune system

Modeling immune networks through an ordinary differential equation (ODE)-based model is inspired by the seminal work of Perelson and co-workers, who developed, among others, immune models of HIV progression (Rong and Perelson 2009; Perelson and Ribeiro 2013). By carefully fitting the models to patient data and inspecting the dynamics of CD4+ T-cells, they designed optimal therapeutic dosing strategies to keep the disease at bay. Such approaches have saved lives and continue to be fruitful, for instance, in the current times, by modeling disease progression in COVID-19 (Perelson and Ke 2021). An immune system is often classified into various regulation regimes: recurrent infection, persistent non-infectious inflammation, and healthy oscillations (Kumar *et al.* 2004). Kumar *et al.* (2004) have shown this in a study through a bifurcation diagram. In the same line of thought, a study by Garcia *et al.* (2020) has pointed out the crucial role of bistability in therapeutic success. Nonlinearity and the emergence of bistability have also been widely studied in the context of physiology and pathology. Through the hysteresis effect, the appearance of bistability in biological systems has been characterized experimentally (Malka *et al.* 2010). In the context of cancer–immune system interplay, one may consider a nonlinear system having mutual inhibitory components (Sahoo *et al.* 2021), and here, the system can even attain multistability (both bistability and tristability) (Lu *et al.* 2013). T-cells are reported to exhibit phenotypic heterogeneity and multistability in an adaptive immune system (Duddu *et al.* 2020).

A specific phenomenon that a model captures is often parameter-dependent. In system-based modeling, bifurcation theory is an efficient method for capturing critical biological interaction-driven phenomena (Kumar *et al.* 2004; Lu *et al.* 2013; Duddu *et al.* 2020).

In the T-cell-mediated immune system context, such theoretical concepts help characterize different immunoregulatory states and their modulation under different environmental situations (Roy *et al.* 2014; Roy and Bagchi 2020; Nayak and Roy 2021). T-cell-mediated immune regulations are often classified into weak, strong, and moderate regulation regimes based on the ratio and relative concentration of pro-inflammatory T-cells and anti-inflammatory T-cells. The characteristics of these categories (Fouchet and Regoes 2008; Roy *et al.* 2014) are depicted in figure 1 and are described below:

1. Weak regulation: The ratio between pro-inflammatory T-cells and anti-inflammatory T-cells is >1. As the population of pro-inflammatory T-cells is high, the immune system is more autoimmune-prone. The pathogenic load under this regulation is low.

2. Moderate regulation: The ratio between the pro-inflammatory T-cells and the anti-inflammatory T-cells is balanced or =1. Interestingly, here the immune system may hold the characteristics of bistability. Moreover, this regime is likely to be sensitive to therapies. Our previous work, including several other studies, has highlighted the importance of bistability and therapeutic sensitivity in a moderate regulation regime (Fouchet and Regoes 2008; Roy and Bagchi 2020; Garcia *et al.* 2020).

3. Strong regulation: The ratio of pro-inflammatory and anti-inflammatory T-cell concentrations is <1. Pathogen load is higher, which can lead to an immunocompromised condition.

5. Immunomodulatory role of vitamin D from a theoretical perspective

The dynamic role of vitamin D in the immune system has been elaborated from an experimental perspective in the above sections. On the other hand, mathematical models in understanding the role of immunosuppressants, especially focusing on Vitamin D, are limited. A few modeling efforts have been made to distinguish the role of serum vitamin D as a metabolite, providing critical new insights into vitamin D and human health (Chun *et al.* 2012). In recent times, Roy *et al.* (2014) have developed a theoretical coarse-grained kinetic network model based on some relevant experimental observations, as shown in figure 2, which depicts the interaction of vitamin D with the adaptive immune system. The details of the immunological processes are described in an event-wise manner in the supplementary material. The study finds that the opposing role of regulatory T-cells and effector T-cells in the regulation of the immune system is dependent on the concentration of vitamin D in the body, along with several known/unknown factors determining the strength of immune regulation and the ultimate fate of a disease.

In an early study, Fouchet and Regoes (2008) also classified the T-cell-mediated immune network into three regulation regions (weak, moderate, and strong), where they investigated the boundaries between any two regions. The model predicted that the role of APC is crucial in the emergence of a restricted bistable region. They did not account for the effects of vitamin D in their model. However, in the presence of vitamin D, expansion of the bistable region signifies the importance of vitamin D in immune regulation and how it prevents over-explosion of effector T-cells, thereby preventing the autoimmune condition (Roy *et al.* 2014).

Figure 1. Classification of T-cell regulation based on theoretical models. The immune system can transit from a weak regulation regime to a moderate regulation regime and then to a strong regulation regime and vice versa depending on the concentration and composition of pro-inflammatory T-cells (T_{pro}) and anti-inflammatory T-cells (T_{anti}) (refer to Roy *et al.* 2014; Roy and Bagchi 2020; Nayak and Roy 2021 for more details).

6. Immunomodulatory role of glucocorticoids from a theoretical perspective

Among others, GCs are well-known immunosuppressants that control T-cell-mediated adaptive immune response similar to vitamin D. Although there are
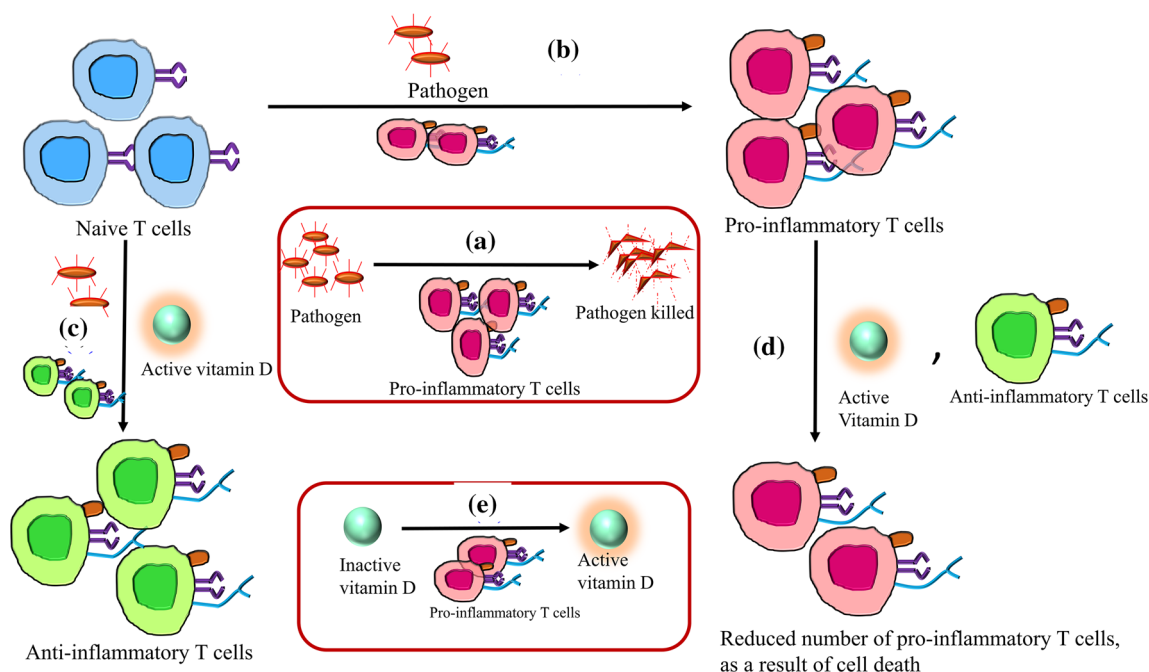
Figure 2. A schematic representation of the adaptive immune responses at a cellular level. This includes the interaction of naïve T-cells, pathogen, pro-inflammatory T-cells, anti-inflammatory T-cells, and vitamin D. It is a derivative and a coarse-grained form of our previous work (Roy *et al.* 2014). In this design, the significant events are the following: (**a**) The primary step after the infection setting is to eliminate the pathogen by pro-inflammatory T-cells. Invasion of pathogens leads to the conversion of naïve T-cells to (**b**) pro-inflammatory T-cells and (**c**) anti-inflammatory T-cells. Vitamin D adds to the maturation of anti-inflammatory T-cells. Moreover, the mature pro-inflammatory T-cells and anti-inflammatory T-cells have a role in inducing naïve T-cells to produce more of themselves. However, controlling the over-explosion of pro-inflammatory T-cells is an exaggerated immune response: vitamin D and anti-inflammatory T-cells cause (**d**) the downregulation of pro-inflammatory T-cells. (**e**) The presence of pro-inflammatory T-cells facilitates the transformation of inactive vitamin D into its active form.

several classes of synthetic glucocorticoids, dexamethasone (Dex) is the most widely used steroid due to its higher binding affinity to GC receptors (GRs) compared with natural cortisol. In addition, it has minimal mineralocorticoid activity. Again, the immunomodulatory functions of GCs are also quite similar to vitamin D. The immunomodulatory actions of GC-mediated adaptive immune response are mainly exerted on the CD4+ T cell. Specifically, those are pro-inflammatory and anti-inflammatory by nature. To better understand the immunomodulatory role of GC, a recent mathematical model by Yakimchuk (2020) used a saturation function with six kinetic rate equations. The saturation function presents a one-time external dose (GC) intake mode. Nayak and Roy (2021) have also developed a kinetic network-based model to understand the direct and indirect effects of GCs on the immune system and anti-inflammatory immune response. In the model of Yakimchuk (2020), the saturation function leads the system to saturate towards a constant dose value. As a result, after a certain concentration, the GC coupling
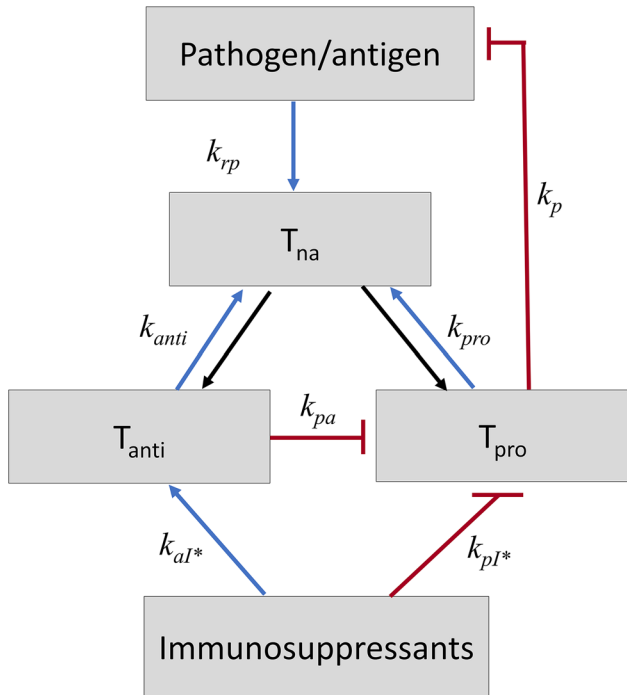
effect is lost. Nayak and Roy (2021), on the other hand, used pharmacokinetic constants accounting for the normal metabolism of GC.

7. A generic coarse-grained kinetic network model for understanding the impact of immuno-suppressants on T-cell regulation

From the above sections, we have seen that the immune system is indeed a complex network where its cells continuously interact and clash with foreign invaders/pathogens to maintain homeostasis. There is a continuous predator/prey-like tussle between the pro-inflammatory T-cells and the pathogen, where the pro-inflammatory T-cells are the predators. On the other end, the pathogen, being the prey, tries to escape from its predator, with assistance from anti-inflammatory T-cells. However, functional balance between pro-inflammatory T-cells and anti-inflammatory T-cells is necessary for maintaining a healthy state. An

immunosuppressant like vitamin D or GC governs that. With this generic view, here we present a generic coarse-grained kinetic network model. It is noteworthy that since the immune system comprises many players, for designing a mathematical model, a coarse-graining approach helps filter essential nodes of a network so as to understand a particular phenomenon or mechanism. Here, the generic model is constructed based on a careful survey of published experimental and theoretical literature, described in the supplementary material and detailed in other references (Roy *et al*. 2014; Roy and Bagchi 2020; Nayak and Roy 2021). A more straightforward, albeit refined, correlation among the various elements of the immune system and their interplay with immunosuppressants is proposed and presented in figure 3, which accounts for the complexities present at a molecular level by coarse-graining them at the cellular level.

Once the developed network appears to be simple and effective, a system of coupled differential equations is used to first model the system without the influence of any immunosuppressants (vitamin D or GC). A detailed method is presented in the supplementary material. The critical elements considered in our coarse-grained model are the following:

1. Pathogen/antigen/self-antigen (P)
2. Naïve T-cell (precursor T-cell) (T_{Na})
3. Pro-inflammatory T-cell (T_{pro})
4. Anti-inflammatory T-cell (T_{anti})

Under any pathogen attack, a healthy immune system always aims to disarm the foreign antigen by enhancing its pro-inflammatory T-cell proliferation and differentiation rate. However, an exceeding number of pro-inflammatory T-cells often fail to differentiate between self-cells and foreign peptides. Moreover, pro-inflammatory T-cells may begin damaging self-tissues. Therefore, a healthy immune system has to operate under a balanced regulation. This signifies that the concentration of pro-inflammatory T-cells must increase to overcome the pathogenic stimulation by resisting its explosive growth efficiently. Herein lies the role of vitamin D and GCs. Their optimum level effectively maintains a balanced immune regulation by inhibiting anti-inflammatory T-cells and downregulating pro-inflammatory T-cells.

8. Dynamic phases of CD4+ T-cell: Emergence of a characteristic lag time or clinical latency

Let us first consider a system free of immunosuppressants in the generic coarse-grained Model-I described in the supplementary material. Here, we have four coupled rate equations. By solving these four coupled differential equations, we find the dynamical behavior of pro-inflammatory T-cells and anti-inflammatory T-cells throughout their time evolution, as depicted in figure 4. A unique dynamical period known as the latent period is identified here. It is the time range within which there is a steady-state-like condition, i.e., the concentration of almost all the cell populations (pro-inflammatory T-cells, anti-inflammatory T-cells, naïve T-cells, and the pathogen) are not changing with time. After that, there is a jump to the real long-term steady-state condition. In this period, the pathogenic load is significantly low, insufficient to cause ailment. It is considered an asymptomatic phase, or clinical latency in clinical terminology. The pro-inflammatory and anti-inflammatory T-cells maintain highly balanced concentrations in this latent period. The pathogen population is too small to cause symptoms. This phase where the pathogen remains latent is called the latent

Figure 3. A generic coarse-grained kinetic network model to decipher the impact of immunosuppressants on T-cell dynamics. This model is the skeletal framework of the schematic representations depicted in figure 2. The black arrows indicate conversion, and the blue and red arrows represent the activation and inhibition processes, respectively. The significance of each network parameter is described in table 1. Additional details are described in supplementary table 1.

Table 1. Definition of representative symbols of rate parameters used in figure 3

Definition	Symbol
1 Rate of pathogen killing by pro-inflammatory T-cells (T_{pro})	k_p
2 Rate of differentiation of naïve T-cells to T_{pro}/T_{anti} cells which is induced by the antigen	k_{tp}
3 Rate of differentiation of naïve T-cells to T_{pro} cells which is induced by the pro-inflammatory T-cell itself (autocatalytic)	k_{pro}
4 Rate of differentiation of naïve T-cell to the pro-inflammatory T-cell which is induced by the pro-inflammatory T-cell itself (autocatalytic)	k_{anti}
5 Rate of inhibition of pro-inflammatory T-cell by anti-inflammatory T-cell	k_{pa}
6 Rate of inhibition of pro-inflammatory T-cell by the active immunosuppressant	k_{pI*}
7 Rate of anti-inflammatory T-cell reactivation by an active immunosuppressant	k_{aI*}
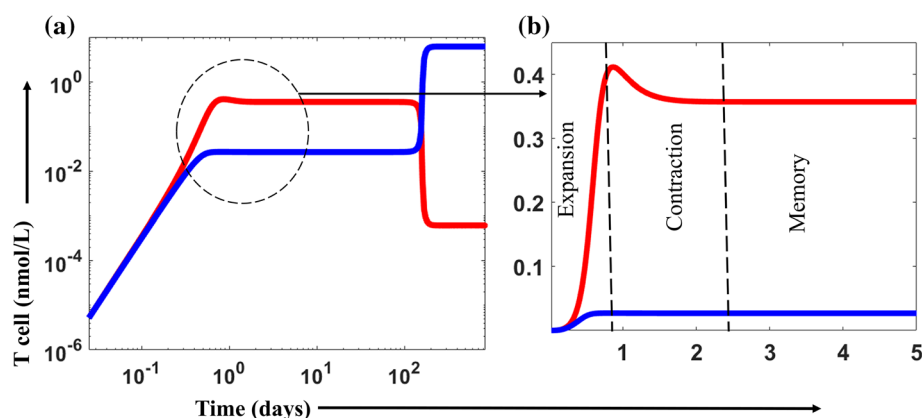
Figure 4. Time evolution of immune response of CD4+ T-cells in the absence of any immunosuppressive drug. (**a**) The log-scale plot represents a regime of the immune phase regulation that contains a latent period (the time range within which there is no change in the concentration of T_{pro} cells and T_{anti} cells). During the post latent period, there is a jump to the steady-state condition. (**b**) Distinctive dynamical phases of immune response mediated by antigen-specific pro-inflammatory T-cells are depicted, where three phases of the immune response (expansion, contraction, and memory) are indicated. Here, the time evolution of pro-inflammatory T-cells and anti-inflammatory T-cells are plotted by solving coupled kinetic equations shown in Model I. We reproduce the T-cell dynamics considering k_{pro}=30, and the other rate values are the same as given in Nayak and Roy (2021). In the plot, red and blue lines represent T_{pro} and T_{anti}, respectively. Note: 1.66×10^{-5} nmol/L corresponds to 1000 T-cells.

period. Finally, after a long time, the system reaches the final long-term steady-state, i.e. a lower number of pro-inflammatory T-cells depicting a scenario in which symptoms resurface in the patient. In the long term, the system reaches a steady state with a higher number of anti-inflammatory T-cells and a lower number of pro-inflammatory T-cells in the post-latent period, depicting the antagonistic nature of pro-inflammatory T-cells and anti-inflammatory T-cells. Such steady-state behavior in T-cell dynamics over a longer time period is shown in figure 4. Our previous study (Nayak and Roy 2021) and many other studies (Mindikoglu *et al.* 2006; Gonzalez and Perrillo 2016) have pointed out that there is a probability of pathogenic reactivation as one of the side effects of long-term immunosuppressant therapy.

The initial phase of pro-inflammatory T-cell time evolution has three sub-phases: expansion, contraction, and memory (figure 4b). After the onset of infection, pro-inflammatory T-cells increase clonally in number. Figure 4b shows some fundamental phenomena during that time. Day 0 to Day 1, i.e., within 24 h, is the time during which infection is set by the pathogen. This peak of T-cell expansion on Day 1 post-infection is a limitation of this model. Adaptive response sets in after a few hours of pathogen incursion (Kaech *et al.* 2002). The pathogen annihilation process starts after recognizing the pathogen, followed by T-cell activation. Soon after the pathogen load decreases, the contraction phase begins. The population of pro-inflammatory T-cells reduces due to apoptosis. After the contraction

phase, the number of pro-inflammatory T-cells reaches its pre-steady state value and is maintained for a significant period, representing the memory phase. Several other studies have also observed similar immune phases (Wang *et al.* 2015; Bertrand 2019).

9. Generic effects of immunosuppressants on CD4+ T-cell population: Immune state transition from weak to moderate to strong regulation

The general immunomodulatory effects of immunosuppressants on T-cell population dynamics can be described by a generic model framework (Model II discussed in the supplementary information). When the system encounters a pathogen of weak to moderate

strength, it immediately switches on the activation process of effector pro-inflammatory T-cells. Subsequently, the upregulation of anti-inflammatory T-cells and pro-inflammatory counterparts plays a crucial role in setting up a competitive dual switch that can trigger either a strong or weakly regulated state (Roy and Bagchi 2020). This competing behavior of anti-inflammatory T-cells and pro-inflammatory T-cells may never end in the presence of a pathogen because our immune system is constantly exposed to some pathogenic load. Thus, it may stay moderately activated by pathogenic stimulation, which we call a moderately regulated state (Roy and Bagchi 2020). As we found earlier, the effects of immunosuppressants become essential when the system falls into a condition where the number of pro-inflammatory T-cells is more than
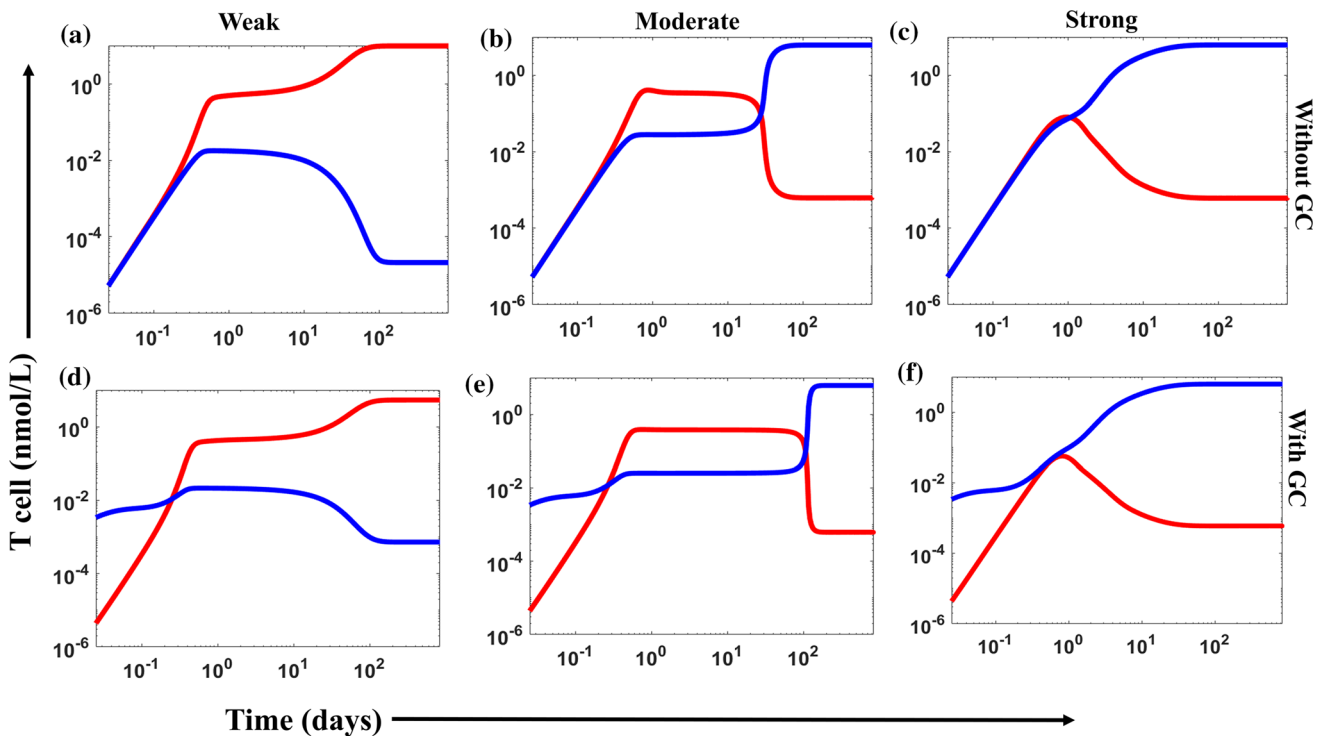
Figure 5. Time evolution of the T-cell population in all three classified regulations regimes, both in the absence and presence of an immunosuppressant, GC. Time evolution of pro-inflammatory T-cells and anti-inflammatory T-cells are plotted by solving coupled kinetic equations using a deterministic approach. These three regulations are obtained by varying a single rate parameter, k_{pro}, an autocatalytic activity of pro-inflammatory T-cells. k_{pro} values are very sensitive towards determining the strong, moderate, and weak regulation of pro-inflammatory T-cells. In the absence of any immunosuppressant (**a**) a weak regulation state appears around $k_{pro} = 50$, (**b**) a moderate regulation appears around $k_{pro} = 30$, and (**c**) a strong regulation appears around $k_{pro} = 10$. In the presence of GC (**d**) a weak regulation state appears around $k_{pro} = 70$, (**e**) a moderate regulation appears around $k_{pro} = 56$, and (**f**) a strong regulation appears again around $k_{pro} = 10$. Apart from k_{pro}, other parameter values are taken from Nayak and Roy (2021). The dose of Dex (synthetic glucocorticoid dexamethasone) is taken to be optimal, which is 38.21 nmol/L. This calculation and figure are reproduced from Nayak and Roy (2021) (Reprinted (calculations/figure) with permission from Sonali Priyadarshini Nayak and Susmita Roy, *Phys. Rev. E* 103, 062401 – Published 3 June 2021 (*https://doi.org/10.1103/PhysRevE.103.062401*). Copyright 2022 by the American Physical Society). In the plot, the red and blue lines represent T_{pro} and T_{anti} respectively. *Note:* 1.66×10^{-5} nmol/L corresponds to 1000 T-cells.
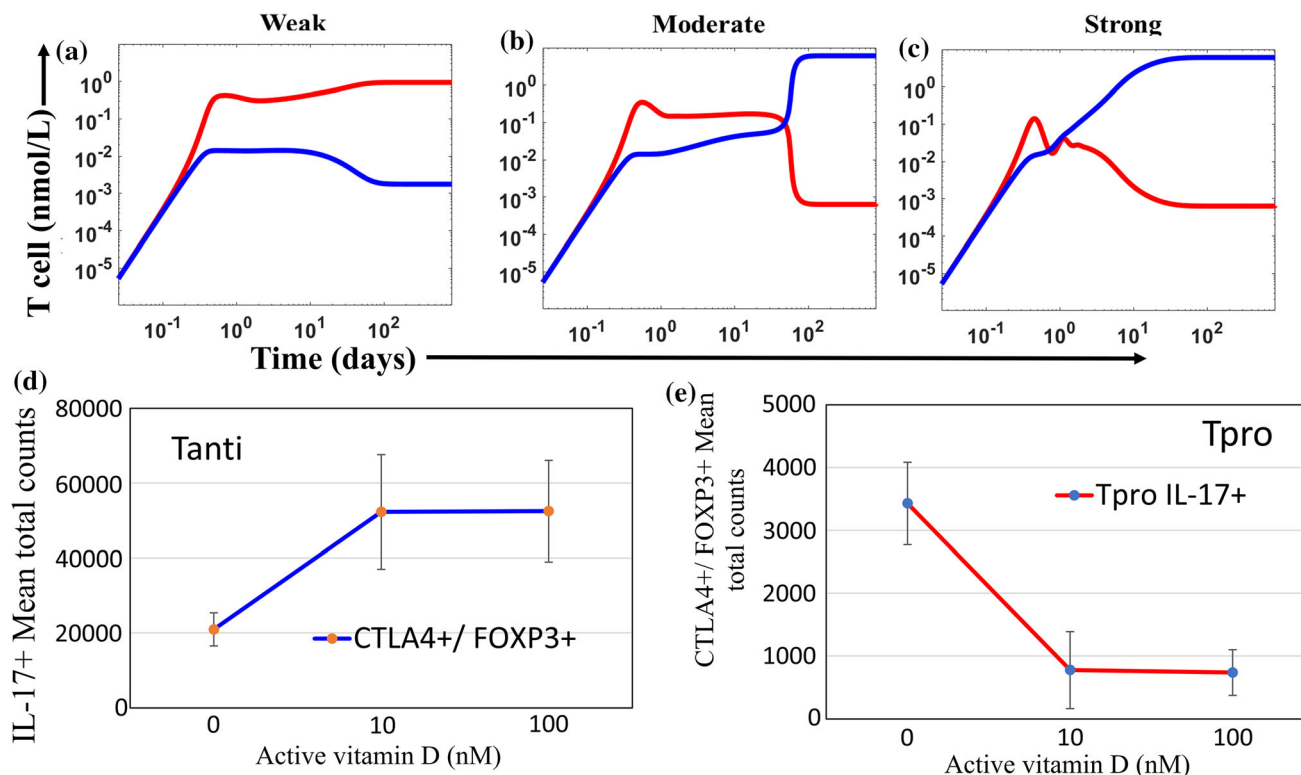
Figure 6. Time evolution of the T-cell population in the presence of a secosteroid vitamin D from a theoretical model, followed by experimental validation (Roy *et al.* 2014). Time evolution of pro-inflammatory T-cells and anti-inflammatory T-cells are plotted by solving coupled kinetic equations from Model II at varying vitamin D concentrations. Strong regulation, moderate regulation, and weak regulation are very sensitive to vitamin D concentration. (**a**) A weak regulation state appears close to vitamin D dose of 10 nM/L. (**b**) Moderate regulation state starts to appear around vitamin D dose of 50 nM/L. (**c**) Strong regulation state appears around vitamin D dose of 900 nM/L, which is an extremely high dose. Other parameter values are taken from Roy *et al.* (2014), apart from variable active vitamin D concentration ([I*]) (Roy *et al.* 2014) to solve Model II. *Note:* 1.66×10^{-5} nmol/L corresponds to 1000 T-cells. (**d–e**) Experimental validation of our model adapted from Jeffery *et al.* (2012). (**d**) An increase in T_{anti} concentration and (e) decrease in T_{pro} concentration with an increase in the concentration of active vitamin D. For review purposes, the data for (**d–e**) are obtained from Jeffery *et al.* (2012). The details regarding the experimental study can be found in Jeffery *et al.* (2012). In the plot, the red and blue lines represent T_{pro} and T_{anti} respectively.

the anti-inflammatory T-cells, transitioning the system into a weakly regulated immune state that is more prone to cause autoimmune disorders. Such a risky state can be shifted to a moderately regulated immune state depending on an optimal dose of immunosuppressant. However, if the dose of immunosuppressant exceeds that optimal level, the system may further shift to another disease-prone immune state classified as a strongly regulated state. Our generic coarse-grained Model II involves those essential and general effects of immunosuppressants that capture the shift of different immune regulation states from strong to moderate to weak regulations, as shown in figure 5d–f.

In an early study, Fouchet and Regoes (2008) analyzed the steady-state values of T-cells in these three regulation regimes. As shown in our earlier work

(Roy and Bagchi 2020), in the absence of vitamin D, a bistable region is found in this generic model, where both strong and weak regulation can coexist. However, we find that in the presence of even a minute vitamin D level, a weakly regulated state can be converted to a strongly regulated state intervened by a moderate regulation regime as the dose intake increases. At a very high dose limit, even when a simulation starts with an initial state of weak regulation, feedback makes this weak regulation stronger. The system mounts the required effective regulation depending on the vitamin D dose variation, as shown in figure 6. The dose-dependent dynamics of the system are captured from a theoretical model in figure 6a–c. From a modeling perspective, vitamin D dose dependence of T-cell population dynamics has been shown by Roy *et al.*

(2014) In this work, in the bistable region, both the variation of T-cell and pathogen levels remain intimately connected and help to determine an optimal vitamin D range (Roy and Bagchi 2020). A similar T-cell variation pattern was obtained experimentally by Jeffery *et al.* (2012), as shown in figure 6d–e. In both cases, with an increase in vitamin D dose level, there is a concomitant increase in anti-inflammatory T-cell concentration and a decrease in pro-inflammatory T-cell concentration. Moreover, similar to the effect of GC, the system reverts to a weakly regulated state at a low vitamin D dose level as the pro-inflammatory T-cells again increase in population size. At the other extreme, when the vitamin D dose is very high, the system reaches a strongly regulated state as the anti-inflammatory T-cells are abundant and suppress the pro-inflammatory T-cell concentration.

10. Summarizing the immunomodulatory role of immunosuppressants and future aspects to comprehend their responses

Beyond their classical role, glucocorticoids (GCs) and vitamin D impact the immune system as immunomodulators. However, as the immune system is a highly complex network, we have generalized a theoretical coarse-grained kinetic model based on a T-cell-mediated interaction network to obtain an insight into the immune regulation exerted by such immunosuppressants. We solved this complex network problem using a chemical dynamics approach. The interaction network dynamically connects different immune components involved in the relevant immune responses. The expressed kinetic design illustrates the time evolution of all critical components, including pro-inflammatory T-cells and anti-inflammatory T-cells. The steady-state analyses of the proposed kinetic equations infer tangled relations between the GC or vitamin D dose and anti-inflammatory T-cells maintained by homeostasis, wherein such immunosuppressants have a significant regulatory role. The absence or presence of a lower concentration of GC (lower than optimum) corresponds to a weak regulatory regime. At the optimal concentration of such immunosuppressants, the system may enable the characteristics of bistability. In this phase, one may observe a unique dynamical phase similar to the clinical latent period (Nayak and Roy 2021). At a concentration higher than optimal, the system moves towards a strong regulation with a decrease in latent time, accounting for the importance of monitoring the appropriate dose of immunosuppressant administered for autoimmune disease or cancer treatment. Hence, it is essential to closely monitor an appropriate dose of an immunosuppressant administrated during the treatment. This helps prevent potential side effects such as compromised immunity or easy pathogen target. At low GC concentration, if any pathogen enters the body, the immune response's nature would be more inflammatory, which can finally lead to an autoimmune condition.

GCs are observed to have a significant role in keeping the immune system in a moderate/bistability regime for a very long time. As shown in figure 4, the immune system has a latent period of very high value at specific concentrations of GCs, which we consider an optimal range. In that phase, the pathogen population is low, and the pro-inflammatory T-cell concentration is also under control. These findings show that at a meager value or in the absence of GC, the latent period is significantly shorter; there is a risk of autoimmune disease due to the uncontrolled growth of pro-inflammatory T-cells. While these phenomena and the optimal range of GC (Dex) are in accordance with the experimental finding (Becker 2013), the dependence of the clinical latency on vitamin D is yet to be explored.

For an immunosuppressant, one may identify one/many sensitive parameters. For instance, take the example of k_{pro}. k_{pro} is the rate at which pro-inflammatory T-cells can induce naive T-cells to produce more of themselves, which is primarily a self-regulatory mechanism. k_{pro} represents how pro-inflammatory T-cells produce more of themselves. The k_{pro} value is not fixed. It may vary from person to person. Moreover, it has been known that the cytokine level does not have a particular value, and it varies over a physiological range (Kim *et al.* 2011; Roy and Bagchi 2020; Nayak and Roy 2021). The range of k_{pro} is dependent on the cytokine level. Thus, to incorporate such effects one may also account for the variability of rate parameters by including a relevant distribution function of rate parameters.

Another essential concern is to address specific responses hosted by various interactions with key immunological components in different physiological conditions. In practice, we investigate the regulation mentioned above and their cross-over by time evolution analysis of each participating element after the pathogen attack to study their long-time steady-state behavior. One may ask, how do we find this co-existence line between any two immune regulation regimes? Clinical and experimental studies also use

some standard values of pro- and anti-inflammatory T-cells; more often, the ratio of these T-cells cells serves as the predictor of the patients' immune profile. However, other related studies or experimental results frequently challenge such standardized ratios or numbers. Often, we ignore the fact that these standard numbers can be personalized and may vary among patients carrying the same disease. Under such circumstances, experimental and theoretical investigations should find suitable parameters to quantify person-specific immune responses. An early study (Roy and Bagchi 2020) accounted for the fluctuations in the number/concentration of active participants along stochastic trajectories. A relevant fluctuating quantity serves as a crucial order parameter capturing a divergent-like behavior that might evoke a characteristic of criticality near the crossover line of immune phases (Roy and Bagchi 2020). The study also addresses the adverse long-term effects of any analogue of vitamin D or other immunosuppressants with their overdose limit in treating autoimmune diseases. The mean-square fluctuation-mediated response parameter is so sensitive that it can efficiently capture the signature of an immune breakdown in the presence of the overdose limit of immunosuppressant by a significant drop in essential fluctuation of pro-inflammatory T-cells. It also demonstrated that the presence of an optimal level of vitamin D rather than its lower concentration limit assists in broadening the tolerance limit. A bistable condition emerges with strong anticorrelated fluctuations between pro- and anti-inflammatory T-cell subsets. The broad fluctuation profile and a significant reduction in the relative fluctuation of effector T-cells in the presence of an immune-modulator like vitamin D suggest that the fluctuation of the active T-cell subset can be more responsive as often found in clinical studies. Hence, these fluctuations can be used as a tool for future diagnostics and therapeutics. We strongly believe that more findings will help shed light and also help explain several clinical results associated with the dose effectiveness of various immunosuppressants used as a critical remedy under emergency conditions.

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References

Becker DE 2013 Basic and clinical pharmacology of glucocorticosteroids. *Anesth. Prog.* **60** 25–32

Bereshchenko O, Bruscoli S and Riccardi C 2018 Glucocorticoids, sex hormones, and immunity. *Front. Immunol.* **9** 1332

Bertrand RL 2019 Lag phase is a dynamic, organized, adaptive, and evolvable period that prepares bacteria for cell division. *J. Bacteriol.* **201** e00697-e718

Bordon Y 2016 The many sides of Paul Ehrlich. *Nat. Immunol.* **17** S6–S6

Bouillon R, Manousaki D, Rosen C, *et al*. 2021 The health effects of vitamin D supplementation: evidence from human studies. *Nat. Rev. Endocrinol.* **18** 96–110

Brodin P and Davis MM 2017 Human immune system variation. *Nat. Rev. Immunol.* **17** 21–29

Cain DW and Cidlowski JA 2017 Immune regulation by glucocorticoids. *Nat Rev. Immunol.* **17** 233–247

Cao X, Cai SF, Fehniger TA, Song J, *et al*. 2007 Granzyme B and perforin are important for regulatory T-cell-mediated suppression of tumor clearance. *Immunity* **27** 635–646

Cebula A, Kuczma M, Szurek E, *et al*. 2019 Dormant pathogenic CD4+ T cells are prevalent in the peripheral repertoire of healthy mice. *Nat. Commun.* **10** 4882

Chaplin DD 2010 Overview of the immune response. *J. Allergy Clin. Immunol.* **125** S3–S23

Charoenngam N and Holick MF 2020 Immunologic Effects of Vitamin D on human health and disease. *Nutrients* **12** 2097

Chemin K, Gerstner C and Malmström V 2019 Effector functions of CD4+ T-cells at the site of local autoimmune inflammation—Lessons from rheumatoid arthritis. *Front. Immunol.* **10** 353

Chun RF, Peercy BE, Adams JS and Hewison M 2012 Vitamin D binding protein and monocyte response to 25-Hydroxyvitamin D and 1,25-Dihydroxyvitamin D: Analysis by mathematical modeling. *PLoS One* **7** e30773

Chun RF, Liu PT, Modlin RL, Adams JS and Hewison M 2014 Impact of vitamin D on immune function: lessons learned from genome-wide analysis. *Front. Physiol.* **5** 151

Correale J, Ysrraelit MC and Gaitán MI 2009 Immunomodulatory effects of Vitamin D in multiple sclerosis. *Brain* **132** 1146–1160

Coutinho AE and Chapman KE 2011 The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Mol. Cell. Endocrinol.* **335** 2–13

Cowley SC, Hamilton E, Frelinger JA, *et al.* 2005 CD4-CD8-T-cells control intracellular bacterial infections both in vitro and in vivo. *J. Exp. Med.* **202** 309–319

Cutolo M, Pizzorni C and Sulli A 2011 Vitamin D endocrine system involvement in autoimmune rheumatic diseases. *Autoimmun. Rev.* **11** 84–87

Cyster JG and Allen CDC 2019 B-cell responses: Cell interaction dynamics and decisions. *Cell* **177** 524–540

Deenick EK, Ma CS, Brink R and Tangye SG 2011 Regulation of T follicular helper cell formation and function by antigen presenting cells. *Curr. Opin. Immunol.* **23** 111–118

Duddu AS, Sahoo S, Hati S, Jhunjhunwala S and Jolly MK 2020 Multi-stability in cellular differentiation enabled by a network of three mutually repressing master regulators. *J. R. Soc. Interface* **17** 20200631

Elenkov IJ 2004 Glucocorticoids and the Th1/Th2 balance. *Ann. N. Acad. Sci.* **1024** 138–1346

Fagnoni FF, *et al.* 2002 T-cell dynamics after high-dose chemotherapy in adults: elucidation of the elusive CD8+ subset reveals multiple homeostatic T-cell compartments with distinct implications for immune competence. *Immunology* **106** 27–37

Fouchet D and Regoes R 2008 A population dynamics analysis of the interaction between adaptive regulatory T-cells and antigen presenting cells. *PloS One* **3** e2306

Garcia V, Bonhoeffer S and Fu F 2020 Cancer-induced immunosuppression can enable effectiveness of immunotherapy through bistability generation: A mathematical and computational examination. *J. Theor. Biol.* **492** 110185

Golubovskaya V and Wu L 2016 Different subsets of T-cells, memory, effector functions, and CAR-T immunotherapy. *Cancers* **8** 36

Gonzalez SA and Perrillo RP 2016 Hepatitis B virus reactivation in the setting of cancer chemotherapy and other immunosuppressive drug therapy. *Clin. Infect. Dis.* **62** S306–S313

Grossman WJ, Verbsky JW, Barchet W, *et al.* 2004 Human T regulatory cells can use the perforin pathway to cause autologous targeT-cell death. *Immunity* **21** 589–601

Hirahara K and Nakayama T 2016 CD4+ T-cell subsets in inflammatory diseases: beyond the T h 1/T h 2 paradigm. *Int. Immunol.* **28** 163–171

Hoffmann JA, Kafatos FC, Janeway CA and Ezekowitz RA 1999 Phylogenetic perspectives in innate immunity. *Science* **284** 1313–1318

Janeway CA Jr and Medzhitov R 2002 Innate immune recognition. *Annu. Rev. Immunol.* **20** 197–216

Jeffery LE, Wood AM, Qureshi OS, *et al.* 2012 Availability of 25-hydroxyvitamin D(3) to APCs controls the balance between regulatory and inflammatory T-cell responses. *J. Immunol.* **189** 5155

Jones DE and Diamond AG 1995 The basis of autoimmunity: an overview. *J. Clin. Endocrinol. Metab.* **9** 1–24

Kaech SM, Wherry EJ and Ahmed R 2002 Effector and memory T-cell differentiation: implications for vaccine development. *Nat. Rev. Immunol.* **2** 251–262

Kaufmann SHE 2019 Immunology's coming of age. *Front. Immunol.* **10** 684

Kim HO, Kim H-S, Youn J-C, Shin E-C and Park S 2011 Serum cytokine profiles in healthy young and elderly population assessed using multiplexed bead-based immunoassays. *J. Transl. Med.* **9** 113

Kongsbak M, Levring T, Geisler C and von Essen M 2013 The vitamin D receptor and T-cell function. *Front. Immunol.* **4** 148

Kumar R, Clermont G, Vodovotz Y and Chow CC 2004 The dynamics of acute inflammation. *J. Theor. Biol.* **230** 145–155

Lei T-Y, Ye Y-Z, Zhu X-Q, *et al.* 2021 The immune response of T-cells and therapeutic targets related to regulating the levels of T helper cells after ischaemic stroke. *J. Neuroinflam.* **18** 25

Liu W, Tolar P, Song W and Kim TJ 2020 Editorial: BCR Signaling and B-cell activation. *Front. Immunol.* **11** 45

Lu M, Jolly MK, Levine H, Onuchic JN and Ben-Jacob E 2013 MicroRNA-based regulation of epithelial–hybrid–mesenchymal fate determination. *Proc. Natl. Acad. Sci. USA* **110** 18144–18149

Malka R, Shochat E and Rom-Kedar V 2010 Bistability and bacterial infections. *PLoS One* **5** e10010

Marcinowska-Suchowierska E, Kupisz-Urbańska M, Łukaszkiewicz J, Płudowski P and Jones G 2018 Vitamin D toxicity–A clinical perspective. *Front. Endocrinol.* **9** 550

Margo CE and Harman LE 2016 Autoimmune disease: Conceptual history and contributions of ocular immunology. *Surv. Ophthalmol.* **61** 680–688

Mayassi T, Barreiro LB, Rossjohn J and Jabri B 2021 A multilayered immune system through the lens of unconventional T-cells. *Nature* **595** 501–510

Mindikoglu AL, Regev A and Schiff ER 2006 Hepatitis B virus reactivation after cytotoxic chemotherapy: The disease and its prevention. *Clin. Gastroenterol. Hepatol.* **4** 1076–1081

Mora JR, Iwata M and von Andrian UH 2008 Vitamin effects on the immune system: vitamins A and D take centre stage. *Nat. Rev. Immunol.* **8** 685–698

Murdoch JR and Lloyd CM 2010 Resolution of allergic airway inflammation and airway hyperreactivity is mediated by IL-17-producing γδ T-cells. *Am. J. Respir. Crit. Care Med.* **182** 464–476

Nayak SP and Roy S 2021 Immune phase transition under steroid treatment. *Phys. Rev. E* **103** 62401

Nurieva RI and Chung Y 2010 Understanding the development and function of T follicular helper cells. *Cell. Mol. Immunol.* **7** 190–197

Orihara K, Nakae S, Pawankar R and Saito H 2008 Role of regulatory and pro-inflammatory T-cell Populations in allergic diseases *World Allergy Organ. J.* **1** 9–14

Perelson AS 1989 Immune network theory. *Immunol. Rev.* **110** 5–36

Perelson AS and Ke R 2021 Mechanistic modeling of SARS-CoV-2 and other infectious diseases and the effects of therapeutics. *Clin. Pharmacol. Ther.* **109** 829–840

Perelson AS and Ribeiro RM 2013 Modeling the within-host dynamics of HIV infection. *BMC Biol.* **11** 96

Rong L and Perelson AS 2009 Modeling latently infected cell activation: viral and latent reservoir persistence, and viral blips in HIV-infected patients on potent therapy. *PLoS Comput. Biol.* **5** e1000533

Roy S and Bagchi B 2020 Fluctuation theory of immune response: A statistical mechanical approach to understand pathogen induced T-cell population dynamics. *J. Chem. Phys.* **153** 45107

Roy S, Shrinivas K and Bagchi B 2014 A stochastic chemical dynamic approach to correlate autoimmunity and optimal Vitamin D range. *PLoS One* **9** e100635

Sahoo S, Nayak SP, Hari K, *et al.* 2021 Immunosuppressive traits of the hybrid epithelial/mesenchymal phenotype. *Front. Immunol* **12** 5347

Sakaguchi S and Sakaguchi N 2005 Regulatory T-cells in immunologic self-tolerance and autoimmune disease. *Int. Rev. Immunol.* **24** 211–226

Schmidt A, Oberle N and Krammer P 2012 Molecular mechanisms of Treg-mediated T-cell suppression *Front. Immunol.* **3** 51

Scurr M, *et al.* 2014 Highly prevalent colorectal cancer-infiltrating LAP^+ $Foxp3^-$ T-cells exhibit more potent immunosuppressive activity than $Foxp3^+$ regulatory T-cells. *Mucosal Immunol.* **7** 428–439

Smith DA and Germolec DR 1999 Introduction to immunology and autoimmunity. *Environ. Health Perspect.* **107** 661–665

Srivastava RK, Dar HY and Mishra PK 2018 Immunoporosis: Immunology of osteoporosis—role of T-cells. *Front. Immunol* **9** 657

Timmermans S, Souffriau J and Libert C 2019 A general introduction to glucocorticoid biology. *Front. Immunol* **10** 1545

Wang L, Fan D, Chen W and Terentjev EM 2015 Bacterial growth, detachment and cell size control on polyethylene terephthalate surfaces. *Sci. Rep.* **5** 15159

Weinstein RS, Hogan EA, Borrelli MJ, *et al.* 2017 The pathophysiological sequence of glucocorticoid-induced osteonecrosis of the femoral head in male. *Mice Endocrinol.* **158** 3817–3831

Yakimchuk K 2020 Mathematical modeling of immune modulation by glucocorticoids. *Bio Syst.* **187** 104066

Zhang TY, Ding X and Daynes RA 2005 The Expression of 11β-Hydroxysteroid Dehydrogenase Type I by lymphocytes provides a novel means for intracrine regulation of glucocorticoid activities. *J. Immunol.* **174** 879

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