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Mathematical Modelling of Allergy and Specific Immunotherapy: Th1-Th2-Treg Interactions

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Abstract

Regulatory T cells (Treg) have recently been identified as playing a central role in the immune response to allergens and during allergen-specific immunotherapy. We have extended our previous mathematical model describing the nonlinear dynamics of Th1-Th2 regulation by including Treg cells and their major cytokines. We hypothesize that immunotherapy mainly acts on the T cell level and that the decisive process can be regarded as a dynamical phenomenon. The model consists of nonlinear differential equations which describe the proliferation and mutual suppression of different T cell subsets. The old version of the model was based upon the Th1-Th2 paradigm and is successful in describing the "Th1-Th2 switch" which was considered to be the decisive event during specific immunotherapy. In recent years, however, the Th1-Th2 paradigm has been questioned and therefore, we have investigated a modified model in order to account for the influence of a regulatory T cell type. We examined the extended model by means of numerical simulations and analytical methods. As the modified model is more complex, we had to develop new methods to portray its characteristics. The concept of stable manifolds of fixed points of a stroboscobic map turned out to be especially important. We found that when including regulatory T cells, our model can describe the events in allergen-specific immunotherapy more accurately. Our results suggest that the decisive effect of immunotherapy, the increased proliferation of Treg and suppression of Th2 cells, crucially depends on the administration of high dose injections in short intervals right before the maintenance phase sets in. Empirical protocols could therefore be improved by optimizing this step of therapy.

Keywords: Nonlinear dynamics, Regulatory T cells, Desensitization

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1. Introduction

T helper cells play a significant role in immune responses to allergenic substances. There are several subtypes of T helper cells which differ in function according to their cytokine profiles. Immunologists distinguish among four major lineages: Th1, Th2, Th17 and T regulatory (Treg) cells (Zhu and Paul, 2009). Among these, mainly specific Th2 cells are responsible for allergic reactions since they activate the production of IgE antibodies which provoke the well known allergic symptoms. According to the "Th1-Th2 paradigm" that has guided immunologists since the late 1980s, the type of immune response depends on which of the two populations prevails in the competition of Th1 and Th2 helper cells. In the case of allergy this entails that there are populations of allergen-specific T helper cells in both allergic and healthy individuals. Yet, in the latter an allergic response is prevented by the predominance of Th1 cells (Romagnani, 1997). The claim that a hygienic childhood environment increases the risk of allergic diseases, which is known as the "hygiene hypothesis", can be explained in this framework as follows: Due to the reduced exposure to bacterial and viral antigens, the Th1 cells are insufficiently stimulated and therefore cannot prevent the Th2 cells from dominating after exposure to allergens (Yazdanbakhsh et al., 2002).

However, doubts have been raised in the past decade about the persuasiveness of this explanation. Some studies show, for instance, that populations with high rates of helminth infections are equally protected from allergic diseases, even though these infections induce strong Th2-mediated immune reactions. On the other hand, a considerable increase in the frequency of type 1 diabetes and other autoimmune diseases, which turn out to be mediated by Th1 cells, has been observed (Wills-Karp et al., 2001). Thus, there seems to be yet another mechanism of regulation which is able to prevent the development of unwanted immune responses in healthy individuals and whose malfunction can lead to either allergic disorder or autoimmunity. An update of the hygiene hypothesis, also known as the "counter-regulation hypothesis" has been suggested.

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According to this modified version, all kinds of infections can possibly prevent the development of allergic disorders by inducing the proliferation of regulatory T cells (Treg) (Murphy et al., 2007; Sakaguchi, 2000).

Different types of regulatory T cells have been identified. Naturally occurring regulatory T cells develop in the thymus and are thought to be mainly involved in the maintenance of immunological self-tolerance (Sakaguchi, 2005). The type of Treg cell which seems to be important in the context of allergic diseases, however, is the so-called *induced* or *adaptive* regulatory T cell (Curotto de Lafaille et al., 2008). Cells of this kind produce cytokines such as IL10 and TGF-β, which can suppress both Th1 and Th2 mediated immune responses, and they differentiate from naive T cells similarly to the other subsets (Battaglia et al., 2006; Taylor et al., 2006).

Allergen-specific immunotherapy (also known as desensitization therapy) consists of repeated injections of allergens or allergen peptides and aims to induce a state of tolerance in the allergic individual. Even though specific immunotherapy has been carried out for more than one century, the underlying mechanisms remain poorly understood. Within the framework of the Th1-Th2 paradigm immunologists assumed that in the course of immunotherapy the Th2 mediated reaction is "switched" to a Th1 dominated response (Murphy et al., 2007). More recent studies indicate, however, that the therapeutic effect is mainly caused by an increase in the population of allergen-specific regulatory T cells (Akdis et al., 1998; Akdis and Akdis, 2007).

In practice, the therapy is performed by starting with very small, innocuous injections which are subsequently increased until a maximum dose is reached. After that, during the *maintenance phase*, this dose is administered once every four weeks over a period of 3-5 years. The small initial doses are thought to mainly induce the desensitization of mast cells and basophils, whereas changes at the T cell level are established over weeks or months (Akdis and Akdis, 2007). Various protocols exist for the initial part of the treatment, which differ in the period of time it takes to reach the maximum dose. In conventional therapies it takes about two months, while in so called "rush protocols" the maintenance dose is reached after only one week (Bousquet et al., 1998).

Under the assumption that the essential therapeutic effect of immunotherapy is due to a change of the T cell equilibrium, and therefore involves only a small number of different cell types, it should be possible to capture the scenario within a mathematical model. Such a model using nonlinear differential equations describing the dynamics of Th1-Th2-Interactions was introduced in Behn et al. (2001) and further investigated in Richter et al. (2002) and Vogel and Behn (2007). On the following pages we will present an extended version of this model that takes into account the influence of a population of allergen-specific regulatory T cells. After discussing the general assumptions of our model and motivating the set of equations that defines it, we will investigate the simple case of periodic injections. By making use of the stroboscopic map, we will already be able to anticipate the qualitative features of realistic therapies. Finally, we will show that we can simulate different therapy protocols provided that the initial conditions are chosen in the right way.

Apart from this work and the models that it is directly based on, we find other attempts in the literature to model T cell interactions by means of population dynamical systems. Mathematical models that describe the dynamics of Th1-Th2 regulation are given in Fishman and Perelson (1994); Fishman and Segel (1996); Fishman and Perelson (1999); Carneiro et al. (1995); Yates et al. (2000); Bergmann et al. (2001); Louzoun et al. (2001); Fenton et al. (2008); Eftimie et al. (2010). Among these, however, only Fishman and Segel (1996) make explicit reference to allergic diseases by formulating a compartment model to describe specific immunotherapy for pollen allergy.

Furthermore, we find a number of models that incorporate regulatory T cells such as León et al. (2000, 2004); Burroughs et al. (2006); Carneiro et al. (2007); Fouchet and Regoes (2008); Bewick et al. (2009); Kim et al. (2010); Alexander and Wahl (2010). Most of these investigate regulatory mechanisms in the context of self-tolerance and autoimmunity. Bewick et al. (2009) introduce a scenario of Treg-Th17 interaction according to which the immune system decides about the type of response based on time dependent information, and they briefly discuss healthy and allergic responses to allergens. In León et al. (2000, 2004) a model describing the dynamics of three types of cells is put forward: antigen presenting, autoreactive, and regulatory. Their approach is particularly interesting as it provides an explanation for the inverse correlation of autoimmunity and infections.

To our knowledge, the present work is the first attempt to provide a mathematical model that includes regulatory T cells in the description of allergen-specific immunotherapy. Moreover, it distinguishes itself from other approaches by focusing on the dynamics of repeated perturbations.

2. The Model

We will first introduce the general assumptions that our modeling approach is based on and discuss the consequences that arise. Afterwards, we will present a concrete instance of the intended kind of model in terms of ordinary differential equations. We believe that the insights that can be gained from our approach hold under rather general conditions and do not depend on the specific form of the equations.

The phenomenon we are confronted with is paradoxical: some individuals show strong allergic reactions on a single allergen encounter, such as an insect sting. However, they can develop tolerance if the allergen is administered repeatedly during specific immunotherapy. Interestingly,

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this beneficial effect has been observed even when no controlled therapy is intended. Such a "natural desensitization" has been observed, for example in beekeepers who are regularly stung (Eich-Wanger and Müller, 1998), or in individuals suffering from mosquito allergy after long-term exposure to mosquito bites (Peng and Simons, 2004).

Each allergen encounter triggers the proliferation of all types of T helper cells that are specific to the particular allergen. The immune system of an allergic patient is initially in a state in which the proliferation of Th2 cells is favored and not under sufficient control by mechanisms of tolerance. In our view, the therapeutic effect of immunotherapy can be explained as a population dynamical phenomenon, that is, as a change in T cell equilibrium caused by the external perturbation that the therapy represents. Our model shows that the concerted action of Th1 and Treg cells can bring about such a change when repeated injections are given. This result follows from the general assumption that by means of their cytokines Th1 and Th2 cells are able to suppress one another, while the Treg cells have a suppressive effect on both Th1 and Th2 cells. During the course of therapy the system is driven to an attractor state that is characterized by low levels of Th2 cells.

The dynamic effect can be explained in abstract terms as follows: initially, when the frequency of activated cells in general is still low, the Treg cells are disadvantaged with respect to the other cell types. Therefore, an allergen injection will induce proliferation mainly of Th2 cells. However, if a second injection is administered before the cell populations have completely died off, the cytokine environment will have changed with respect to the time of the first injection. Th2 proliferation is then alleviated via the suppressive effect of the cytokines produced by Th1 and Treg cells. The Treg cells start their second round of proliferation under improved conditions, as they are not affected by the other cells' cytokines. Repeated injections in short intervals will therefore induce a more and more Treg dominated reaction.

The mentioned effect should be observable as soon as a model embodies the mechanisms of suppression that we have just described, provided that the parameters are chosen in a reasonable way. The modeling approach that we will discuss in the following is supposed to illustrate and explain this in more mathematical detail, and it should be seen as a proposal that is open to further refinement. It consists of a set of nonlinear differential equations describing the temporal behavior of five variables: the concentrations of Th1, Th2 and induced Treg cells (T_1, T_2, T_r respectively), the concentration of naive T helper cells (N), and the concentration of allergen peptides (A) presented by antigen-presenting cells. Figure 1 shows a simplified scheme of the T cell interactions that are incorporated into the model. Following an injection, allergens are taken up by antigen-presenting cells (APC) and presented to naive T helper cells. Upon activation, these naive cells can differentiate into Th1, Th2 or Treg cells. Via their cytokines

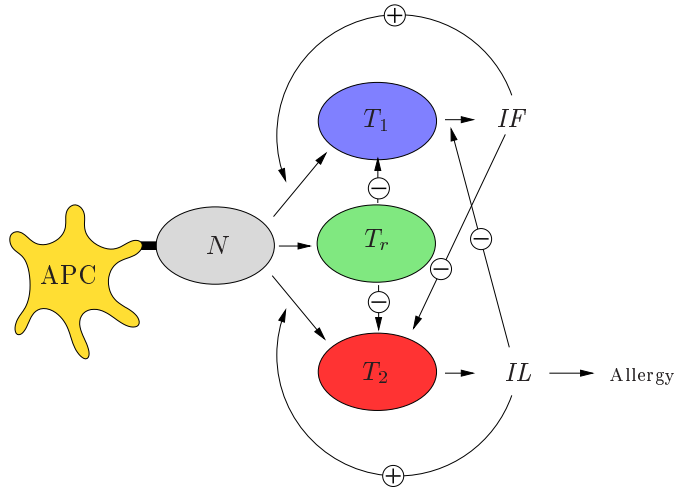

Figure 1: Simplified scheme of T cell interaction in response to allergen encounter. Allergen peptides presented by an antigen presenting cell (APC) activate naive T helper cells (N), which leads to their subsequent differentiation to either Th1, Th2, or Treg cells (T_1, T_2, and T_r respectively). Th1 and Th2 cells suppress each other and support their own proliferation respectively via their cytokines (IF, IL). Treg cells suppress Th1 and Th2 cells. Dominance of Th2 cells leads to allergic reaction.

(IF, IL respectively) activated cells can exert autocrine action on their own population and suppress proliferation of the other. Th1 and Th2 cells suppress each other respectively, whereas Treg cells suppress both other cell types and are themselves not suppressed.

The asymmetric way in which the populations of Th1 and Th2 cells interact is adopted from the previous version of the model (Behn et al., 2001). According to Mosmann and Sad (1996), cytokines produced by Th1 cells have a suppressive effect on Th2 proliferation, whereas cytokines produced by Th2 cells downregulate the cytokine production of Th1 cells. We are aware that it is not clear to what extent this picture remains accurate in the light of new findings, especially about the role of IL10 in immune tolerance (e.g. Couper et al. 2008). However, our general argument does not depend too much on the concrete details of Th1-Th2 cross-suppression.

Our attempt to describe the competition of T cells leads to the following set of equations:

$$\begin{aligned}\dot{N} =& -N + \alpha - NA\left(\frac{T_1}{1+\mu_2 T_2} + c\right), \\ & - \phi NA(T_2 + c) - \chi NA(T_r + c)\end{aligned} \tag{1}$$

$$\dot{T}_1 = -T_1 + \frac{\upsilon NA}{1+\mu_r T_r}\left(\frac{T_1}{1+\mu_2 T_2} + c\right), \tag{2}$$

$$\dot{T}_2 = -T_2 + \phi\frac{\upsilon NA}{1+\mu_r T_r}\left(\frac{T_2 + c}{1+\mu_1 \frac{T_1}{1+\mu_2 T_2}}\right), \tag{3}$$

$$\dot{T}_r = -T_r + \chi \upsilon NA\,(T_r + c), \tag{4}$$

$$\dot{A} = -A(T_1 + T_2 + T_r). \tag{5}$$

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We shall now explain the form of the equations as well as the occurring parameters. A detailed derivation can be found in Appendix A. Examining equations (1)–(3), we find that the specific T cell populations only grow to substantial sizes if allergens are presented. In the absence of such a stimulus most cells die off. All T cells (including the naive cells) are assumed to have the same half-life, and consequently all populations decay at the same rate. The system is already rescaled to dimensionless units, in particular time is measured in units of the half-life of T cells. Naive cells are produced at a constant rate α, whereas the generation of Th1, Th2, and Treg cells is proportional to the concentration of naive cells, the concentration of presented allergen, and to the concentration of their respective cytokines (autocrine stimulation). As the cytokines are degraded fast compared to the half-life of cells, the concentration of cytokines produced by a T cell subpopulation can in good approximation be regarded as proportional to the size of that population itself. For that reason, the cytokines do not explicitly appear in the equations. The parameter c accounts for a small background of cytokines arising from other processes occurring within the immune system. It is assumed to be equal for the three subsets of differentiated helper cells and to be constant over time. Its mathematical role consists in initially driving the system away from the trivial state, where all T cell concentrations are zero. Suppression is modeled by factors of the form $1/(1+x)$ where x stands for the concentration of suppressive cytokines. In order to avoid unnecessarily complex expressions, the small cytokine background has been neglected in these factors. Finally, equation (5) states that the presented allergen decreases proportionally to the total concentration of specific T cells. The main effect on the depletion of allergens is therefore seen in a competition of specific T cells for peptide presented on APCs where naive T cells are neglected due to their comparatively low frequency (Lanzavecchia and Sallusto, 2001). Again, it is not entirely clear to what extent this scenario accurately represents the real situation. For the qualitative results of our approach, however, the only important aspect is that the concentration of presented allergen decreases sufficiently fast.

The phenomenon of T cell memory is not explicitly included in our model. Instead, we indirectly account for effects of memory by not letting the system start from the ground state $(\alpha, 0, 0, 0)$, which would correspond to a system that has not yet been exposed to the specific allergenic substance, but instead from a state with $T_1, T_2, T_r > 0$ that is supposed to reflect the history of previous antigen encounters.

The parameter υ determines how many differentiated T cells arise from one naive cell. The factors ϕ and χ account for differences in the autocrine action of the three subsets. The strength of suppression is regulated by the parameters μ_1, μ_2, and μ_r respectively. In Richter et al. (2002) it was argued that the parameters ϕ, μ_1, and μ_2, which already occur in the old version of the model, have to satisfy the conditions $\phi \gtrsim 1$ and $\mu_1 > \mu_2$. This was based on empirical findings on the dependency of the type of immune response on the allergen dose (Secrist et al., 1995; Ruedl et al., 2000).

We will now try to find restricting conditions for the choice of the newly introduced paramters χ and μ_r. To this end, we turn our attention to the temporal behavior of the ratios T_1/T_r and T_2/T_r. To keep the following calculations simple, we use the approximation $c \approx 0$. It can be shown that the conclusions drawn also hold for the case of small but nonvanishing c.

It follows from (2) and (4) that

$$\frac{d}{dt}\frac{T_1}{T_r} = \upsilon N A \left(\frac{1}{(1+\mu_r T_r)(1+\mu_2 T_2)} - \chi \right) . \tag{6}$$

Setting this expression equal to zero yields

$$T_r = \frac{1}{\mu_r} \left(\frac{1}{\chi(1+\mu_2 T_2)} - 1 \right) . \tag{7}$$

In the same way, for the case of T_2/T_r we find

$$T_r = \frac{1}{\mu_r} \left(\frac{\phi}{\chi \left(1 + \mu_1 \frac{T_1}{1+\mu_2 T_2}\right)} - 1 \right) . \tag{8}$$

Equations (7) and (8) can be satisfied for positive cell concentrations only if we set $\chi < 1$ and $\chi < \phi$. Otherwise, the Treg cells always dominate over the other two subsets, which renders any sensible attempt to simulate allergic reactions impossible. Provided that $\chi < 1 < \phi$, we find that above a threshold given by

$$T_r^{th} = \frac{1}{\mu_r} \left(\frac{\phi}{\chi} - 1 \right) \tag{9}$$

the Treg cells have a higher growth rate than the two other populations. This threshold is independent of the concentrations of Th1 and Th2 cells. If its value is set too high, the Tregs will never be able to compete, which makes a successful therapy impossible. A very low threshold, on the other hand, will make them too dominant. Therefore, the relation given by (9) can lead us to a reasonable choice of μ_r. The freedom that we have in the choice of χ and μ_r leaves room for different possible biological interpretations: the malfunction of the immune system may be due either to a lack of proliferative capacity or of suppressive function of the Treg cells. For numerical simulations, we will always use $\chi = 0.8$ and $\mu_r = 0.25$. From Richter et al. (2002) we adopt the choice of the remaining parameters: $\alpha = 10$, $\upsilon = 8$, $\phi = 1.02$, $\mu_1 = 0.2$, $\mu_2 = 0.1$, and $c = 10^{-4}$.

Equations (1)-(5) constitute an autonomous dynamical system, but this only holds because we have not yet considered how the allergen is taken up by the organism.

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In immunotherapy allergens enter the body via subcutaneous injections. In our model an injection, given at time t, is modeled by changing the concentration of presented allergen peptides instantaneously from $A(t)$ to $A(t) + D$, where $D > 0$ specifies the dose administered. After an injection the three T cell populations expand by several orders of magnitude. However, after a short time (again compared to the half-life of T cells) no allergens are available anymore ($A \approx 0$) and the populations will not grow any longer. It follows from equations (2)-(4) that subsequently their concentrations will drop exponentially. As the half-life is the same for Th1, Th2, and Treg cells, the ratios of concentrations will by then have reached a constant value.

According to Akdis et al. (2004), it is particularly the balance of Th2 and Treg cells that is decisive as to whether there will be an allergic reaction or not. In our model we can directly compare the initial value of the ratio T_2/T_r to the constant value that is reached after an allergen encounter. We will therefore call an immune response to a given dose D *allergic* only if this ratio has increased compared to its initial value, or, mathematically speaking, if

$$\lim_{t\to\infty} \frac{T_2(t)}{T_r(t)} > \frac{T_2^0}{T_r^0}\,. \tag{10}$$

Moreover, to exclude states in which T_2 is small and its relative increase of T_2 is only due to the cytokine background c, we demand that

$$T_2 > T_1, T_r. \tag{11}$$

It may be argued that also the ratio T_1/T_2 should be incorporated in the definition of allergic states. However, it can be shown numerically that our choice is conservative in the sense that it only includes states in which T_2 dominates over T_1 as well.

Starting from the assumption that before the allergen encounter the naive cells are in their stationary state ($N = \alpha$) and that there is no allergen left from previous encounters ($A = 0$), the type of reaction only depends on the initial conditions T_1^0, T_2^0, T_r^0 and on the allergen dose D.

Of course, this description of allergen administration is highly idealized, and it only approximates the case in which allergens are taken up in an injection-like fashion. This may also apply, for example, to insect stings. Allergic reactions to pollen or house dust mite, however, are more complicated as allergens are taken up continuously over time.

3. Fixed Points and Stable Manifolds

The state of our system will be visualized as the position it occupies in the three dimensional space spanned by the concentrations of differentiated T cell subsets. In this space we can define different regions, according to the reaction the system shows upon a particular perturbation. Considering a single allergen encounter, for example, we can decompose the state space into a region of *allergic* and a region of *healthy* response. A different decomposition of the state space, however, is given by the long-term fate of the system upon therapy, that is, upon repeated injections. We will see that different final states exist to which the system can converge during the course of therapy. We will call an initial state *treatable* if repeated injections starting from this state drive the system towards a "healthy" final state, corresponding to a more favorable T cell equilibrium. Successful immunotherapy is possible if the allergic initial state lies in the region of treatable states. This region may change depending on the type of therapy protocol that is used.

Our first step will be to model specific immunotherapy, that is, administration of repeated injections. The mathematically simplest case is that of periodic injections, in other words, administration of the same dose D repeatedly at times $t_0 + n \cdot \tau$, $n = 0, 1, 2, 3, \ldots$ This case is not merely of theoretical interest as it corresponds to the maintenance phase of allergen specific immunotherapy. To investigate it in more detail we will make use of the concept of a *stroboscopic map*. This concept was proposed in Vogel and Behn (2007), and it has proven to be a valuable tool because it considerably reduces the complexity of the mathematical description.

We denote by $\theta(\mathbf{T}^0, \mathbf{A}^0; t)$ the solution of the above system at time t for initial conditions

$$(\mathbf{T}^0, \mathbf{A}^0) = (T_1^0, T_2^0, T_r^0, N^0, A^0)\,, \tag{12}$$

furthermore, let $\theta_{\mathbf{T}}(\mathbf{T}^0, \mathbf{A}^0; t)$ be the projection of this solution on the three-dimensional subspace of T cell concentrations. Elements of this space are vectors of the form $\mathbf{T} = (T_1, T_2, T_r)^T$. The stroboscopic map $\mathbf{S}_{\tau,D}(\mathbf{T})$ for period τ and allergen dose D is then defined as

$$\mathbf{S}_{\tau,D}(\mathbf{T}) = \theta_{\mathbf{T}}(\mathbf{T}, \mathbf{A}_D; \tau)\,, \tag{13}$$

where $\mathbf{A}_D = (\alpha, D)$. Equation (13) maps a vector $\mathbf{T}$ on the state of the system at $t_0 + \tau$ if we start in $\mathbf{T}$ at time t_0 with an injection of dose D. If τ is not too small, then at time $t_0 + \tau$ we can assume $N \approx \alpha$ and $A \approx 0$. Applying the stroboscopic map repeatedly will therefore be a good approximation for describing a periodic therapy.

We will now investigate the long term behavior of the system when such a periodic therapy is applied. As we just concluded, we can attack this by simply looking at repeated applications of the stroboscopic map. If only one single injection is administered, the system will eventually reach the trivial state $(\alpha, 0, 0, 0, 0)$, that is, the activated T helper cells die off. Repeated injections will keep their proliferative activity going, and after each injection the proportions of the different T cell populations may have changed. Under particular initial conditions, however, the perturbations exerted by the injections can create periodic

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orbits in which the concentrations of all cell subpopulations keep oscillating in a uniform fashion, always reaching the same peak values. These orbits correspond to fixed points of the stroboscopic map, that is, to vectors $\mathbf{T}$ that fulfill the equation

$$\mathbf{S}_{\tau,D}(\mathbf{T}) = \mathbf{T}\,. \tag{14}$$

Numerical simulations show that for a given period τ the stroboscopic map has up to three stable fixed points and several unstable fixed points. Considering stroboscopic maps for different periods, we find that their fixed points lie on continuous lines. In figure 2 these are displayed as branches of stable and unstable fixed points. Bifurcations occur at certain critical periods, which means that the number of fixed points may change as τ is changed.

Applying the stroboscopic map $\mathbf{S}_{\tau,D}$ repeatedly will drive the system to one of its stable fixed points. As in general we find three such stable fixed points, there are three possible outcomes for the corresponding periodic therapy. In each of the stable fixed points the concentration of one of the T cell concentrations peaks high while the concentrations of the two others remain much lower. Administration of periodic injections will therefore always result in one cell type eventually dominating the two others. Which one of the three cell types will in the end be successful crucially depends on the initial state that is given by a vector $(T_1^0, T_2^0, T_3^0) \in \mathbb{R}_+^3$. We can therefore subdivide the space of T cell concentrations into three regions, each being the set of all initial vectors leading to the same therapeutic result. These regions are just the domains of attraction of the stable fixed points of the stroboscopic map. The boundaries between the domains of attraction are constituted by the stable manifolds of the unstable fixed points. In addition to showing the branches of fixed points for varying period τ, figure 2 displays these stable manifolds for the specific example of $\tau = 4$.

As we have already mentioned, there is a different possibility of subdividing the state space. It is provided by the definition of allergic responses given in (10). The boundary of the set of allergic states (corresponding to a reference dose) fulfills the condition

$$\lim_{t\to\infty} \frac{T_2(t)}{T_r(t)} = \frac{T_2^0}{T_r^0}\,. \tag{15}$$

Therefore, it is composed of states starting from which a single injection does not alter the long term ratio T_2/T_r. This boundary forms another two-dimensional manifold in the state space to which we will refer as the *separatrix*.

The goal of specific immunotherapy is to drive the system from an initially allergic state to a tolerant state characterized by increased generation of regulatory T cells. In our model this amounts to approaching the Treg dominated stable fixed point of the stroboscopic map. Thus, the initial state must lie in the domain of attraction of this fixed point. The crucial question therefore is: *Are there allergic states in the domain of attraction of the "healthy" fixed point?* In numerical simulations we can show that this is actually the case, provided that the period τ is not too long. In figure 3, the relevant part of the separatrix is shown along with one of the stable manifolds corresponding to $\tau = 1$.

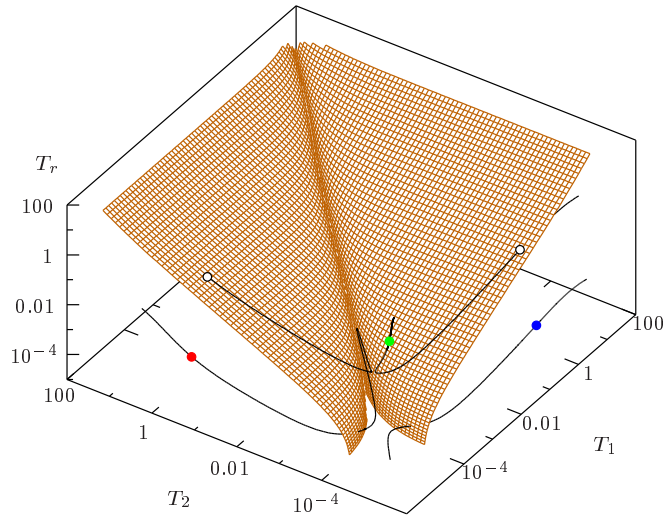

Figure 2: Fixed points and stable manifolds. The set of fixed points of the stroboscopic map (13), for $D = 1$ and varying period τ, consisting of three different branches, is displayed. As an example the stable (●●●) and unstable (○) fixed points corresponding to $\tau = 4$ are shown along with the stable manifolds of the unstable fixed points (brown surfaces). T cell concentrations are represented logarithmically.

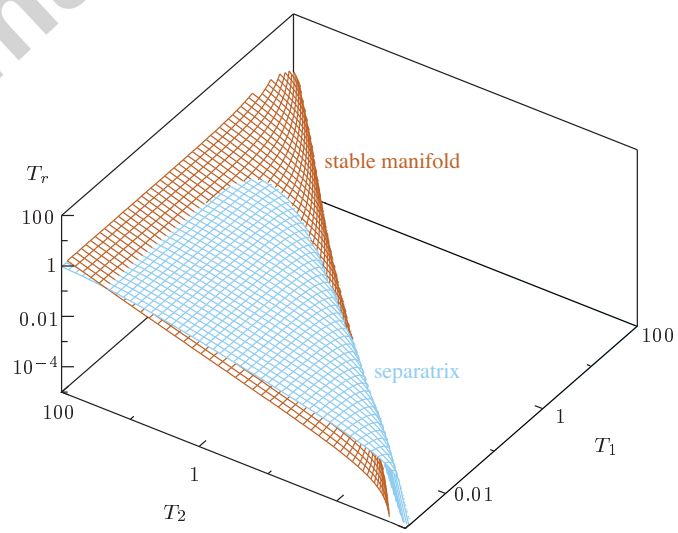

Figure 3: Treatable allergic states. Separatrix (light blue) for $D = 1$ and one of the stable manifolds (brown) corresponding to $\mathbf{S}_{D,\tau}$ with $\tau = 1$. The area between the two surfaces represents the set of allergic states starting from which a successful therapy is possible.

The states that lie *below* the separatrix (i.e. in the allergic region) but *above* the stable manifold (i.e. in the domain of attraction of the healthy fixed point) are the ones that allow for a successful therapy. It turns out that this set of treatable allergic states increases if we reduce

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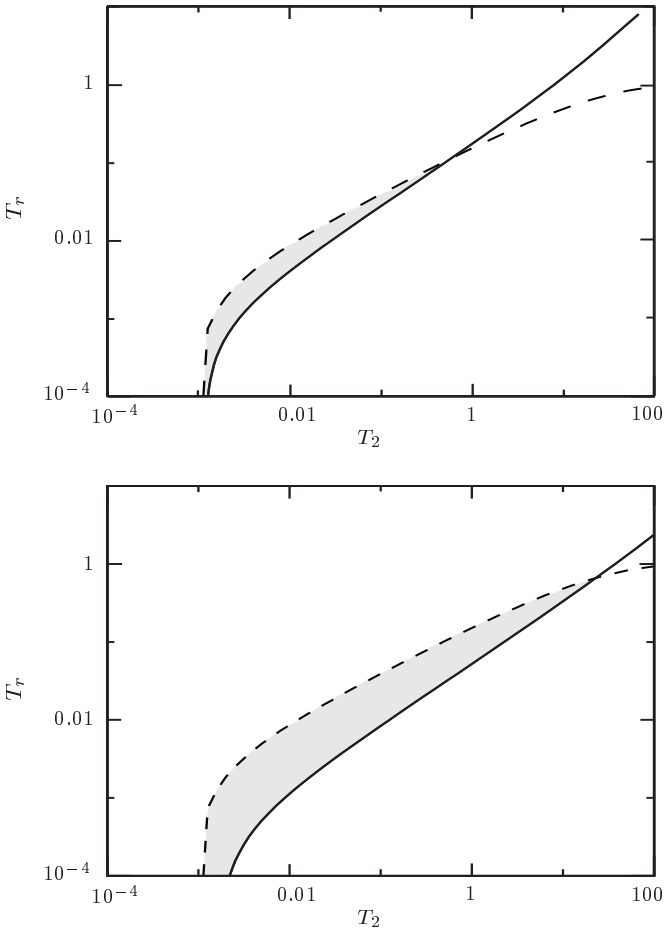

Figure 4: The set of treatable states increases as the period is shortened. Displayed are projections on the T_2-T_r plane for $T_1 = 0.001$. The gray area represents the set of treatable states between separatrix (dashed line) and stable manifold (continuous line) for $\tau = 4$ (top) and $\tau = 1$ (bottom).

the period between injections, as is illustrated in figure 4. This can be explained by recalling what we established earlier about the ratios of cell concentrations. In (9) we had found that above a certain threshold the Treg cells will have the highest growth rate independents of the concentrations of the other two cell types. Therefore, even if in comparison less Treg cells are present, they may be able to catch up. Short intervals and high doses result in high concentrations of specific T cells in general and from this, especially the Treg cells will profit. To avoid misunderstandings: the eventual dominance of the T_r cells is not a direct consequence of our particular choice of parameters. As we have set $\chi < 1 < \phi$, they are even impaired in their proliferative capacity with respect to T_1 and T_2, which is why single injections are able to provoke an allergic reaction in the first place. The therapeutic effect, however, is due to the way in which the suppression among the different populations is modeled.

4. Successful Therapy

So far, we have only considered the simplified case of periodic therapies. However, we have the opportunity to test protocols as they are used in practice by means of numerical simulations. In these protocols both the intervals between injections and the administered dose may vary. Thus, we cannot directly apply the methods we established in the previous section. But the insights we have gained can explain how the therapeutic effect is brought about, even in the case of non-periodic protocols. Most importantly, we had discovered that injections administered in short time intervals will generally favor the population of Treg cells. This also holds if the protocol is not strictly periodic. As figure 2 illustrates, the qualitative structure of the set of fixed points of the stroboscopic map is robust to changes of the period τ, and it can be shown that bifurcations occur only for very large values. Analogous properties hold with respect to the allergen dose D.

In order to be treatable, the allergic state we start from must lie in the domain of attraction of the Treg dominated fixed point corresponding to the interval between the first injections. As figure 4 illustrates, this is more likely the shorter the initial interval is. When the proportion of Treg cells has sufficiently increased, longer intervals between injections are allowed. The most promising strategy according to our model, therefore, starts with a number of injections in short intervals and then continues with a possibly longer period until a stable orbit is reached.

As it turns out, this strategy corresponds to what is done according to empirical therapy schedules. The different protocols that are used in medical practice (e.g. Ruëff et al. 2000) share the feature that right before the maintenance phase sets in, the maximum dose is given several times in short intervals. From our point of view, this represents the decisive step during therapy. It ensures that the system reaches the domain of attraction of the stable fixed point that the therapy is aiming for. It is about at this stage that the Treg cells start overriding the Th2 cells.

Figure 5 shows simulations according to a conventional protocol and according to a rush-protocol. As mentioned in the introduction, all empirical protocols start with a number of very small injections that do not have a significant effect on the T helper cells. The time point $t = 0$ in our simulations therefore corresponds to the first injection that is high enough to induce proliferative activity of the T cell populations in the model. The first dose included in the simulation corresponds to 1/100 (conventional) and 1/250 (rush) of the maintenance dose respectively.

In the conventional protocol the maintenance phase sets in after about two months, in the rush protocol after only a few days. Consequently, the therapeutic effect can be observed much earlier in rush-protocols, which is in accordance with what has been reported in empirical studies (Cox, 2008). In both protocols the same periodic orbit is finally reached since the maintenance phase is identical

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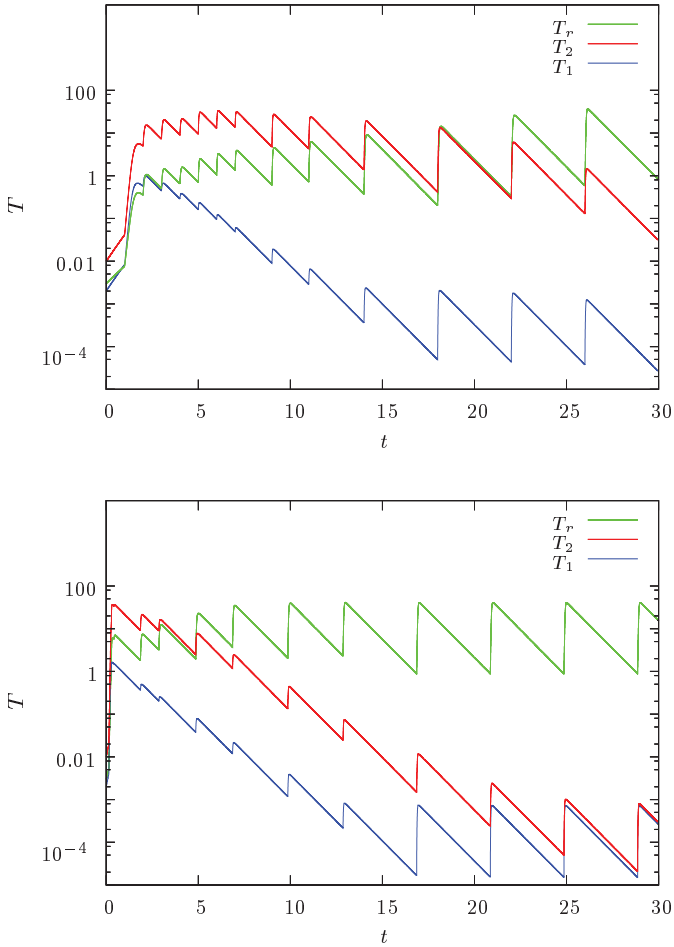

Figure 5: Successful therapy. Development of T cell concentrations according to a conventional protocol (top) and rush-protocol (bottom). Initial concentrations in both cases are given by $\mathbf{T} = (0.002, 0.01, 0.003)^T$. In the conventional therapy the Treg cells start dominating at about $t = 20$, in the rush therapy already at $t = 5$. This roughly corresponds to the time when the maintenance dose is administered for the first time. The therapies are simulated according to protocols found in Ruëff et al. (2000). We assume that $D = 1$ corresponds to the maintenance dose of allergen ($100\mu g$) and that one unit of time corresponds to about one week.

for both therapies. In other words, the final result of successful therapies is always the same, and independent of the protocol.

In our simulations we also find that in the long run the T_1/T_2 ratio increases during therapy (figure 6). In the beginning T_2 is clearly higher than T_1, whereas at the end both cell concentrations have the same order of magnitude. The reason for this is not primarily the suppression of the T_2 population by T_1. Rather, it is a consequence of the suppression by the T_r cells which act in the same way on both other cell types. If the concentrations of T_1 and T_2 are low, as during the later stage of therapy, their mutual suppression becomes negligible. The final ratio lies around 1, as it is mainly determined by the parameter ϕ that can be interpreted as the ratio of proliferative capacities in the absence of suppression. This might provide an alternative explanation for the switch to a more Th1 like immune response that has been observed during specific immunotherapy. The relative increase in Th1 cells is not due to their domination over the Th2 cells, but rather an indirect cause of the suppressive action of the population of Treg cells. Our results therefore support the idea that the cytokine modulation induced by specific immunotherapy should be considered as a return to normal levels, rather than as a "Th1-Th2-switch" (Mamessier et al., 2006).

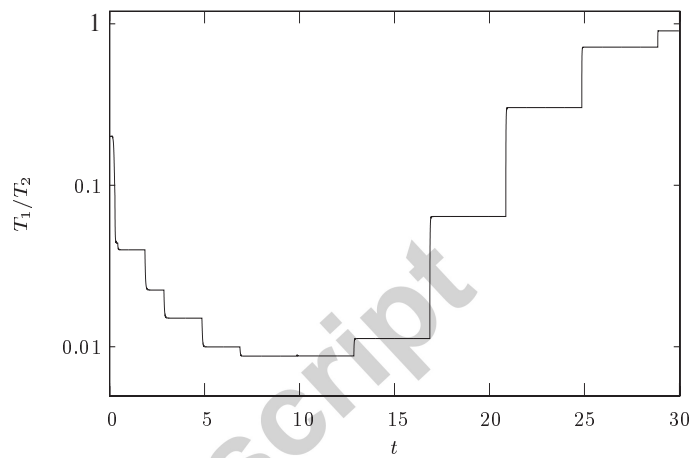

Figure 6: T_1/T_2 increases in the long run. Displayed is the development of the ratio T_1/T_2 during rush-therapy. Protocol and initial conditions are the same as in figure 5. The increase can also be observed during conventional therapy, but it occurs at a much later time.

5. Discussion

Our model is able to describe allergic reactions and the course and outcome of allergen-specific immunotherapy at the T cell level. We have shown that the basic mechanisms in allergic reactions can be explained as a competition between Th2 and Treg cells. As a consequence of our approach, Treg responses are favored by high allergen doses administered in short time intervals. Therefore, the decisive event in immunotherapy is the beginning of the maintenance phase. Protocols in practice could be improved by optimizing this step.

The previous model, as discussed for example in Richter et al. (2002), was able to explain the therapeutic effect of immunotherapy solely in terms of Th1-Th2 interaction. However, successful therapy was only possible for a very small set of initial conditions and depended crucially on the asymmetry in the cross-regulation of Th1 and Th2 cells. In the extended model presented here, the area of treatable states turns out to be of considerable size as can be seen in figure 4. Again, the therapeutic effect can be explained in terms of an asymmetry, but in this case it lies in the different properties of Th2 and Treg cells.

As we have already discussed in the previous section, our model shows robustness with respect to the interval between injections and to the allergen dose. That is, the

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therapy still works as long as the qualitative features of successful protocols are respected. For example, it is not crucial that the maintenance phase is strictly periodic. Our model can, therefore, account for more realistic protocols, where the intervals between injections may often vary by a few days. A different question is, however, what happens if during the maintenance phase an injection is missed completely. If too much time lies between two injections, the system may fall back into the allergic region of the state space. Our results suggest that, in such a case, one may still bring the therapy to a successful end by administering the maximum dose several times in short intervals.

A related issue concerns the behavior of the model after the protocol is finished or if it is interrupted prematurely. As soon as the concentration of presented allergen is practically zero, the activated T cells cease to proliferate and die off exponentially. The concentrations then converge to the ground state of the autonomous system $(\alpha, 0, 0, 0)$. Thus, our model does not account for effects of immunological memory. In an earlier publication, Behn et al. (2001) extended the previous Th1-Th2 model by including populations of memory T cells. They found that the qualitative dynamics of effector T cells are not changed. We therefore believe that the crucial effect of immunotherapy can be explained as a purely dynamical phenomenon without referring to immunological memory. The repeated injections artificially modify the equilibrium of specific T cells over a period of several years and may enable long lasting effects on other levels, such as a decrease in tissue mast cells and eosinophils (Akdis and Akdis, 2007). These effects may vary depending on the type of allergy, which is in accordance with empirical studies that in some individuals show a tendency for sensitivity to return after discontinuation (e.g. Durham et al. 1999). We admit that the scope of our model may appear limited in this respect, but we think that this is preferable to building an overly complex model from which, due to an increased number of parameters, any desired conclusion can be drawn.

The results of our simulations do not imply that all types of allergies can be successfully treated by specific immunotherapy. First of all, certain allergic disorders may not allow for the initial step of mast cell desensitization, which is a necessesary precondition for the administration of higher doses. However, aside from that, our model predicts that if the Th2 dominance in an allergic individual is initially too strong, there will be no prospect of successful therapy at all.

In general, as immunologists themselves have not yet fully understood all the regulatory mechanisms involved in allergic diseases, we are sure that our model will need further refinement. Nevertheless, our investigations already provide general tools to model immune reactions with interacting lymphocytes. Once the immunological picture of T cell regulation is more complete, we will be able to give a more adequate description of the real system.

Further cell types may be incorporated into the model if an important role in allergic diseases can be established for them. This might be the case for the the recently identified Th17 cell (Oboki et al., 2008; Schmidt-Weber et al., 2007). Morover, we have not considered the important influence that antigen presenting cells, especially dendritic cells (DC), may have on the type of immune response (Banchereau and Steinman, 1998; Lanzavecchia and Sallusto, 2001). In particular, there are results suggesting a crucial role for the maturation status of DCs in establishing peripheral tolerance. Immature DCs have been shown to preferentially induce the proliferation of T regulatory like cells, whereas mature DCs tend to be responsible for differentiation of naive T cells towards Th1 or Th2 phenotypes (Jonuleit et al., 2000). The maturation of DCs partly depends on inflammatory stimuli (Cella et al., 1997), which may point to a more important role for the first step of immunotherapy, in which the desensitization of mast cells occurs. Alternatively, regulatory T cells might exert a direct influence on the costimulatory activity of antigen presenting cells or indirectly influence their maturation via the suppression of inflammatory responses (Shevach, 2009). This, however, could be easily incorporated into our model as a further kind of autocrine action of the Treg population. Mathematical models in which the maturation status of DCs has been taken into account explicitly can be found, for example, in Chan et al. (2004) and Fouchet and Regoes (2008). We believe that the heterogeneity of the DC population does not necessarily pose a threat to the general ambition of our approach, but integrating it into the model may be an exciting challenge for further investigation.

The ongoing elucidation of epigenetic mechanisms in molecular biology seems to suggest that differentiated cells are not always irreversibly committed. In particular T helper types show considerable plasticity and heterogeneity (Zhu and Paul, 2009; Wei et al., 2009). Together with the finding that there is a bigger variety of T helper cell phenotypes than previously thought, this puts into question the basic assumption of our model that there are only few and clearly distinct cell types. We are aware that this assumption is a simplification of the complex picture that we are confronted with real situations. However, even if it is sometimes difficult to establish a clear boundary between cell types, the conception of different lineages of T helper cells does not seem meaningless. Van Den Ham and De Boer (2008) give an interesting explanation of phenotypic switches in T cell differentation by explicitely taking into acount the dynamics of master regulators and transcription factors in a mathematical model. The view that cellular phenotypes correspond to stable attractors in high-dimensional state spaces of gene regulation has also been investigated in the work of systems biologists (e.g. Huang et al. 2005) and may provide a theoretical justification of our simplified account. The type of modeling we have adopted here may therefore not only be used to describe the differentiation of progenitor cells, but also to capture events of reprogramming or dedifferentiation, if

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these should turn out to play an important role in the dynamics of T helper cell interactions.

A fundamental problem for immunologists is the origin of allergic disorders. The observations in support of the hygiene hypothesis clearly show that the environment plays a crucial role, but it seems that a genetic predisposition is necessesary for the development of an allergic disease (Galli et al., 2008). The particular setting of parameters and initial conditions in our model may be interpreted as representing the relevant genetic equipment of an allergic individual. The question then arises: Why should this setting correspond to a position in what we had identified to be the set of treatable states? A tentative answer may be given by referring to the existence of a trade-off between two selecting forces, as suggested in León et al. (2004). On the one hand, the immune system has to minimize the risk of harmful overreaction, such as in autoimmunity or allergy. At the same time, there is a need to maximize the reactivity to dangerous pathogens. Therefore, in the context of allergy, the immune system may be poised in a delicate equilibrium between states of tolerance and reactivity. This can explain how perturbations arising from genetic alterations and environmental interactions may trigger the development of allergic disorders. Aside from that, it might provide a reason that, in many cases, they can be treated relatively easily by interfering with the population dynamical equilibrium of T lymphocytes.

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Appendix A. Derivation of the Equations

Originally, the model consists of a set of eight differential equations, the first five of which correspond to equations (1)-(5). We use the hat symbol to indicate that the variables and the time are not rescaled.

$$\begin{aligned}\frac{d\hat{N}}{d\hat{t}} &= -\gamma\hat{N} + \rho - \beta_1\hat{N}\hat{A}(Z_1+\hat{c}) - \beta_2\hat{N}\hat{A}(Z_2+\hat{c}) \\ &\quad - \beta_r\hat{N}\hat{A}(Z_r+\hat{c})\,,\end{aligned} \tag{A.1}$$

$$\frac{d\hat{T}_1}{d\hat{t}} = -\gamma\hat{T}_1 + \upsilon\beta_1\hat{N}\hat{A}\frac{Z_1+\hat{c}}{1+c_rZ_r}\,, \tag{A.2}$$

$$\frac{d\hat{T}_2}{d\hat{t}} = -\gamma\hat{T}_2 + \upsilon\beta_2\hat{N}\hat{A}\frac{Z_2+\hat{c}}{(1+c_1Z_1)(1+c_rZ_r)}\,, \tag{A.3}$$

$$\frac{d\hat{T}_r}{d\hat{t}} = -\gamma\hat{T}_r + \upsilon\beta_r\hat{N}\hat{A}(Z_r+\hat{c})\,, \tag{A.4}$$

$$\frac{d\hat{A}}{d\hat{t}} = -\lambda\hat{A}(\hat{T}_1+\hat{T}_2+\hat{T}_r)\,. \tag{A.5}$$

The parameter γ determines the life span of T cells, which is assumed to be equal for all subtypes. The naive cells, $\hat{N}$, are produced at a rate ρ, and the cells that further differentiate are subtracted from this pool. Autocrine effects on the proliferation of differentiated T cells are mediated by the cytokines Z_1, Z_2 and Z_r respectively. The parameters β_1, β_2, β_r determine the strength of these effects. Aside from that, the Treg cytokines, Z_r, have a suppressive effect on the proliferation of $\hat{T}_1$ and $\hat{T}_2$. The Th1 cytokines, Z_1, on the other hand, have a suppressive effect on the proliferation of $\hat{T}_2$. The parameter $\hat{c}$ stands for a general cytokine background maintained by various other immunological processes.

The three remaining equations describe the cytokine dynamics:

$$\frac{dZ_1}{d\hat{t}} = -\delta Z_1 + \alpha_1\frac{\hat{T}_1}{1+c_2Z_2}\,, \tag{A.6}$$

$$\frac{dZ_2}{d\hat{t}} = -\delta Z_2 + \alpha_2\hat{T}_2\,, \tag{A.7}$$

$$\frac{dZ_r}{d\hat{t}} = -\delta Z_r + \alpha_r\hat{T}_r\,. \tag{A.8}$$

Cytokines are produced at rates α_1, α_2 and α_r by the respective cell population. We assume that all cytokines are degraded at a rate δ. The suppressive effect that Th2 exerts on Th1 is included here as a reduction of the production rate of Z_1 by a factor $1/(1+c_2Z_2)$.

Adiabatic elimination. Assuming that the life span of cytokines is much shorter than that of T cells ($1/\delta \ll 1/\gamma$), their concentrations relax quickly to quasi-stationary states

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given by

$$Z_1 = \frac{\alpha_1}{\delta + c_2\alpha_2\hat{T}_2}\hat{T}_1\,, \tag{A.9}$$

$$Z_2 = \frac{\alpha_2}{\delta}\hat{T}_2\,, \tag{A.10}$$

$$Z_r = \frac{\alpha_r}{\delta}\hat{T}_r\,. \tag{A.11}$$

This allows us to reduce the number of equations by inserting Eqs. (A.9)–(A.11) into (A.1)–(A.4). In order to obtain dimensionless quantities, we measure the time in units of $1/\gamma$ and rescale the variables as

$$\begin{aligned} T_1 &= \frac{\lambda}{\gamma}\hat{T}_1\,, \quad T_2 = \frac{\lambda}{\gamma}\hat{T}_2\,, \quad T_r = \frac{\lambda}{\gamma}\hat{T}_r\,, \\ N &= \frac{\lambda}{\gamma}\hat{N}\,, \quad A = \frac{\alpha_1\beta_1}{\lambda\delta}\hat{A}\,. \end{aligned} \tag{A.12}$$

If we furthermore introduce the new parameters

$$\begin{aligned} \alpha &= \frac{\lambda\rho}{\gamma^2}\,, \quad \phi = \frac{\alpha_2\beta_2}{\alpha_1\beta_1}\,, \quad \chi = \frac{\alpha_r\beta_r}{\alpha_1\beta_1}\,, \\ \mu_1 &= \frac{c_1\alpha_1\gamma}{\delta\lambda}\,, \quad \mu_2 = \frac{c_2\alpha_2\gamma}{\delta\lambda}\,, \quad \mu_r = \frac{c_r\alpha_r\gamma}{\delta\lambda}\,, \end{aligned} \tag{A.13}$$

and adopt the simplifying assumption

$$c = \frac{\delta\lambda}{\alpha_1\gamma}\hat{c} \approx \frac{\delta\lambda}{\alpha_2\gamma}\hat{c} \approx \frac{\delta\lambda}{\alpha_r\gamma}\hat{c}, \tag{A.14}$$

we obtain the system of equations given by Eqs. (1)-(5).

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