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T Helper Regulation: A Theoretical Approach

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Contents

1. Abstract

Helper T (Th) cells are a crucial component of the adaptive immune system and are of fundamental importance in orchestrating the appropriate response to pathogenic challenge. They fall into two broad categories defined by the cytokines each produces. Th1 cells are required for effective immunity to intracellular bacteria, viruses and protozoa whereas Th2 are required for optimal antibody production to T dependent antigens. A great deal of experimental data on the regulation of Th1 and Th2 differentiation have been obtained but many essential features of this complex system are still not understood.

Here we present a mathematical model of Th1/Th2 regulation in presence of a constant antigenic stimulus. We include Fas-mediated activation-induced cell death (AICD) as this process has been identified as an important mechanism for limiting clonal expansion and resolving T cell responses. We see that the strengths of the activation signals for each T helper cell subset, which are dependent on the antigen dose, co-stimulatory signals and the cytokine environment, critically determine the dominant helper subset. In addition we show that the occurence of switches from Th1- to Th2-dominance is based on the antigen dose-dependence of T helper differentiation and can arise from differential susceptibility for AICD of T helper subsets, and asymmetries in the nature of the cross-suppressive cytokine interactions.

In a second step we model interactions between the T helper system and a replicating pathogen and propose a possible default selection mechanism for the appropriate T helper response against a particular pathogen. The decision of a naive T cell to differentiate into Th1 or Th2 is crucial, since it profoundly influences disease outcome. Here we show that the internal behaviour of the T-helper system, which emerges from regulatory mechanisms 'built-in' into the T-helper system, *itself* can usually select the appropriate T-helper response. This phenomenon arises from an initial Th1 bias together with the induction of Th1 $\rightarrow$ Th2 switches when Th1 effectors do not lead to efficient antigen clearance.

For certain dangerous types of pathogens that replicate rapidly or have developed strategies to evade the immune response, however, this default selection mechanism fails and additional stimuli may be necessary. As an additional mechanism for the decision-making process innate immune recognition has been proposed. Mainly *Th1-promoting* APC-derived signals have been experimentally described until now. Here we present an extended version of our model, which suggests that this is due to low fault-tolerance of the T-helper system to incorrect Th1-signals. In the presence of incorrect Th1-stimuli an initial Th1 response is shifted to the correct Th2 dominated response owing to the intrinsic T helper dynamics. By contrast, according to our model there is no fault-tolerance

for incorrect Th2-signals. In fact, Th2-signals are superfluous since the intrinsic T helper dynamics provide an automatic switch to Th2 if Th1 effectors fail to control the pathogen.

Additionally, we discuss the role of feedback where successful pathogen destruction leads to up-regulation of activation of the effective T helper type. As one possibility we examine the role of CpG motifs as indicators for successful pathogen destruction. Differences between instructive and feedback mechanisms are highlighted.

1. Zusammenfassung

T-Helferzellen sind von fundamentaler Bedeutung für das adaptive Immunsystem, da sie die Wahl der Immunantwort als Reaktion auf ein bestimmtes Pathogen in entscheidender Weise beeinflussen. Man unterscheidet zwei Typen von T-Helferzellen abhängig von den von ihnen produzierten chemischen Botenstoffen (Zytokinen). Th1-Zellen werden für eine effektive Immunität gegen intrazelluläre Bakterien, Viren und Protozoen benötigt, wohingegen Th2-Zellen für eine optimale Abwehr interzellulärer Pathogene durch Stimulierung der Antikörperproduktion sorgen. Trotz intensiver experimenteller Forschung auf dem Gebiet der T-Helferdifferenzierung sind essentielle Eigenschaften dieses komplexen Systems noch nicht vollständig verstanden.

Ich möchte hier ein mathematisches Modell der Th1/Th2-Regulierung in Anwesenheit eines konstanten Antigenstimulus präsentieren, welches die wichtigsten Zytokin- und Rezeptor-Interaktionen innerhalb der T-Helfersystems berücksichtigt. Die Dominanz des einen oder anderen T-Helfertyps wird durch Aktivierungssignale beeinflußt, deren Stärke von der Antigendosis, co-stimulierenden Signalen und der Zytokinumgebung abhängig ist. Die Antigendosisabhängigkeit der T-Helferdifferenzierung ist unter anderem eine Folge der unterscheidliche Empfänglichkeit der T-Helferpopulationen für Fas-Rezeptor-induzierte "Selbstmordkommandos" (AICD) und der Asymmetrien in den kreuzweise-hemmenden Zytokininteraktionen. Es stellt sich heraus, daß diese Antigendosisabhängigkeit im Falle ansteigender Antigenkonzentrationen auch zu einem Wechsel von Th1- zu Th2-Dominanz führen kann.

In einem ersten Ausbauschritt wird der konstante Antigenstimulus durch ein sich replizierendes Pathogen ersetzt. Mit Hilfe des so erweiterten Modells wird ein Selektionsmechanismus vorgeschlagen, welcher die Auswahl der für ein bestimmtes Pathogen passenden T-Helferantwort ermöglicht. Diese Auswahl ist kritisch, da sie über Erfolg oder Mißerfolg der Immunantwort entscheidet. Es wird gezeigt, daß das intrinsische Verhalten des T-Helfersystems in vielen Fällen automatisch die richtige Auswahl treffen kann. Entscheidend hierfür ist eine anfängliche Th1-Dominanz – erzeugt durch die Abhängigkeit der Zytokinproduktion von der Anzahl der durchlaufenen Zellteilungen – und ein Umschalten von Th1 nach Th2, wenn eine Th1-dominierte T-Helferantwort nicht zur gewünschten Eliminierung des Pathogens geführt hat.

Für bestimmte gefährliche Arten von Pathogenen, welche sich zu schnell vermehren oder bestimmte Strategien entwickelt haben, der Immunantwort zu entgehen, versagt der vorgeschlagene Auswahlmechanismus und zusätzliche steuernde Signale sind erforderlich. Das angeborene Immunsystem kann pathogen spezifische Muster erkennen und ist daher

in der Lage, in geeigneter Weise in den Differenzierungsprozeß der T-Helferzellen einzugreifen. In einem zweiten Ausbauschritt wird das vorliegende Modell daher um derartige Mechanismen erweitert. Die Analyse dieses Modells zeigt, daß das T-Helfersystem eine Fehlertoleranz gegenüber unpassenden Th1-stimulierenden Signalen aufweist. Für Th2-Signalen hingegen existiert eine solche Fehlertoleranz nicht. Zusammen mit der Tatsache, daß Th2-Signale auf Grund des oben erwähnten automatischen Wechsels von Th1 nach Th2 redundant erscheinen, könnte in jener fehlenden Fehlertoleranz die Ursache dafür liegen, daß bis jetzt experimentell vor allem Th1-Signale identifiziert worden sind.

Abschliessend wird ein Rückkopplungsmechanismus diskutiert, bei dem eine erfolgreiche Immunantwort zur weiteren Stimulierung des entsprechenden T-Helfertyps führt. Ein konkretes Beispiel für einen derartigen Mechanismus stellt die Erkennung von für das Pathogen charakteristischen DNA-Fragmenten (sog. *CpG motifs*) dar, welche die erfolgreiche Zerstörung des Pathogens anzeigen. Anhand dieses Beispiel werden die Unterschiede zwischen der *Instruktion* bestimmter T-Helfertypen durch Signale des angeborenen Immunsystem und der *Rückkopplung* des Ausgangs der Immunantwort auf die Differenzierung der T-Helferzellen herausgestrichen.

2. Preliminary summary

How does the immune system work? What is its strategy? Does it always know what to do? And if that is the case, why? Is there a hierarchy? Who decides?

These are central questions in immunology. To discuss these issues we first have to clarify what the immune system actually has to do. It has to protect the body from all different types of pathogens. Pathogens can be roughly subdivided in two major groups: intracellular and extracellular, which – owing to their location within the body – require different defense strategies. One indeed distinguishes cell-mediated and humoral immune responses often exclusively appropriate for intra- and extracellular pathogens respectively.

If different defense mechanisms exist and have to be selected carefully for a given pathogen the critical question is now: Who decides which counter reaction to choose? And in connection with that: Is there a hierarchy in the immune system? With the discovery of the two different types of T helper cells Th1 and Th2 by Mosmann in 1986 [68] the T helper system has been identified as some sort of arbitration mechanism. By secreting the appropriate set of cytokines Th cells can help to induce an immune response that is appropriate to a given invader. Th1 cells, producing IFN-γ and IL-2, help in the induction of cell-mediated responses. Th2 cells, on the other hand, powerfully trigger humoral responses. As a necessary condition in order to allow appropriate immune responses, the ratio of Th1 and Th2 cells is not predetermined but develops during the immune response.

Now, the above questions have been tracked back to: What regulates the T helper system? Who decides which subset dominates? One approach is to look for a dominant controller that instructs T helper differentiation. It has been proposed that different antigen presenting cells provide different kinds of co-stimulatory signals to T cells, and these different signals can influence the types of cytokines that T helper cells secrete (i.e., the T helper type) [89]. But – and we go again just one step deeper – what activates the different antigen presenting cells?

Signals which activate antigen presenting cells come either directly or indirectly from the innate immune system. The innate system establishes the cytokine environment that determines the initial commitment of Th cells and thus it plays an important part in determining what weapons the adaptive immune system will make. But how does the innate system know?

It has been suggested that evolution has conserved experience and a set of hard-wired recognition receptors has developed, which are able to recognize patterns on certain pathogens. This recognition event leads to the generation of appropriate signals by the innate immune system.

The present work contrasts this top-down approach with a bottom-up approach. We propose that positive and negative feedback experimentally observed within the immune system and in particular in the T helper system builds a self-organizing system. First we study the internal dynamics of the T helper system with a constant antigenic stimulus and analyze the importance of asymmetries in Th1- and Th2 regulation for the system behavior. Using the insights gained a self-regulatory process is suggested as the default-mechanism for the selection of the appropriate T helper type. We combine the described approaches and discuss circumstances under which additional external information provided by the innate immune system is necessary. Furthermore we point out that signals following pattern recognition can well be part of a diffuse feedback instead of being part of a reflexive response.

3. General introduction

The immune system fulfills an essential function in protecting us from the great variety of infectious agents we encounter by means of many different mechanisms. This chapter gives a brief overview of the components of the immune system and their interactions.

3.1. Innate versus adaptive immune response

Defense mechanisms that are present prior to exposure to infectious agents, that are not enhanced by such exposures, and that do not discriminate between most foreign substances, build the arm of the **innate** (also called natural) immunity. The phagocytes of the innate immune system build the first front line against many of the common microorganisms but can not always eliminate them entirely. Additionally, there are many infectious agents such as viruses that can not be recognized by the innate immune system alone. During evolution more potent defense mechanisms have evolved characterized by the following properties:

- They are induced or stimulated by exposure to foreign substances and
- exquisitely specific to distinct agents
- and increase in magnitude and defensive capabilities with each successive exposure (immunological memory).

These mechanism constitute acquired or **adaptive** immunity. Instead of bearing several different receptors, of which one of them recognizes a conserved surface molecule, the components of the adaptive immunity – the lymphocytes – carry only one receptor of a particular specificity. This specificity, which is different from lymphocyte to lymphocyte, is the result of an ingenious genetic mechanism.

3.2. Innate immunity and its link to adaptive immunity

Adaptive and innate immunity, however, do not work independently from each other. The current consensus is that innate and adaptive parts of the immune system take part in a close collaboration.

1. Specific immune responses amplify the protective mechanisms of innate immunity, focus these mechanisms to the sites of antigen entry, and thus improves their ability to eliminate foreign antigens.

2. An important task of the innate immunity is to keep antigenic agents in check until the adaptive immunity after several days starts off.

3. Cells of the innate immunity play an important role in the induction and subsequent regulation of the adaptive immune response. The recognition of an antigen by a lymphocyte is not sufficient to trigger an adaptive immune response and has been shown to cause T lymphocytes to switch to a suppressed state. Co-stimulatory signals from the innate immune system are necessary to activate the adaptive immune system.

4. There is increasing evidence that the innate immune system directs the type of adaptive immune response. Macrophages and neutrophils possess receptors on their surface that recognize universally occurring building blocks on pathogens and bind them. If that happens the phagocytes start to opsonize these pathogens and to release chemical mediators with so-called cytokines (see Sec. 3.6) among them. These chemicals influence the decision whether a humoral or cell-mediated adaptive immune response is initiated.

3.3. The two arms of the adaptive immune response: humoral versus cell-mediated

Specific immune responses are classified roughly into two types, based on the components of the immune system that mediate the response:

- **Humoral immunity** is mediated by molecules (antibodies) in the blood that are responsible for specific recognition and elimination of antigens. These antibodies are produced by B-cells, bind antigens, and block interactions with cells; a process called neutralization. Pure binding, however, is not sufficient to stop replication of bacterias that reproduce extracellularly. In such a case, antibodies additionally have the task to enable phagocytes to pick up the bacterium and destroy it. Wrapping of the pathogen with antibodies is termed opsonization. The third function of antibodies is the activation of the so called complement system, a system of plasma proteins that enhances the bactericidal activity of antibodies in that it complements the effect of antibodies to induce opsonization. The different ways antibodies interact with the antigen are illustrated in Fig. 3.1.

- **Cell-mediated reactions** base on the direct interplay between immune cells and antigen-bearing cells that can be identified by special immune cells called T cells. Antigen fragments are presented to T cells on the major histocompatibility complex

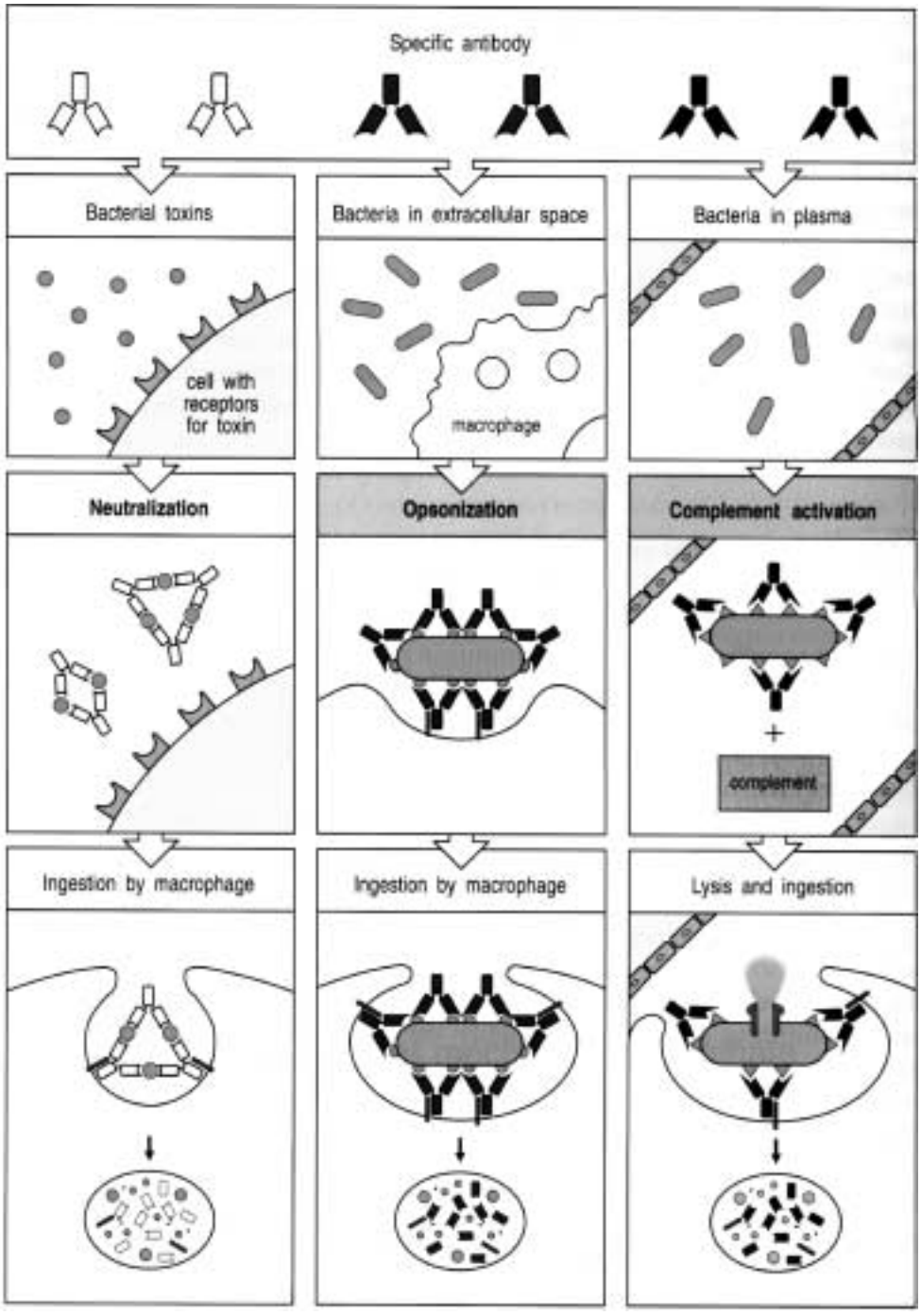


Figure 3.1.: Antibodies from the humoral immune response can participate in three different ways (from [41])

(MHC) molecules. There are two major classes of MHC molecules, called MHC class I and MHC class II. Class-I molecules are found on every cell, while class II molecules occur only on a subset of cells called antigen-presenting cells (APCs).

T cells can be classified into two major groups: cytotoxic CD8+ T cells (CTLs) – also called T killer cells – and helper CD4 T cells (Th).

- **Cytotoxic T cells** can recognize body cells that have been infected by viruses that replicate within the cell by using its synthesis machinery. Antigens of the replicating virus appear at the surface of the cell presented by MHC I complexes, where they can be detected by specific CTLs with the help of their T cell receptor. Since any cell can become virally infected it makes sense that T killer cells see antigen presented on MHC I. The viral infection can then be controlled by killing the infected cell before virus replication has been completed.
- **T helper cells** can be subdivided in T helper cells of type 1 (Th1) and type 2 (Th2). In contrast to T killer cells T helper cells recognize antigen that is presented by antigen presenting cells. These cells take up antigens from their environment, digest them partially, and present some of these antigen fragments bound on MHC II on their surface.

  Here, we concentrate on T helper cells of type 1 the only one being involved in cell-mediated immune responses.

  Some bacteria grow in the vesicles of macrophages (e.g. Mycobacterium tuberculosis). In contrast to other bacterias that invaded a macrophage and that can be destroyed in the lysosomes, these bacterias in the vesicles can only be controled with the help of Th1 cells because vesicles do not merge with the lysosomes. Activated Th1 cells stimulate macrophages which trigger the fusion of the vesicles with the lysosomes and promote other anti-bacterial mechanisms.

Cell-mediated immune reactions are summarized in Fig. 3.2

3.4. The role of T helper cells in adaptive immunity

The name 'T helper cell' already suggest that T helper cells promote immune response effector mechanisms. We have already seen how Th1 participate in the activation of macrophages.

If we think of the immune system as some sort of army against foreign invaders T helper cells play the role of a signals corps which commands the combat troops. Different types of troops are commanded by particular T helper subsets, viz Th1 or Th2. In more biological detail, **Th1** cells activate the whole **cell-mediated** arm of the immune response whereas **Th2** effectors trigger **humoral** responses. In addition to direct effects of Th1 cells on macrophages as demonstrated in the previous section provide Th1 also help for the activation of T killer cells. It has been shown in chronic situations that this additional help

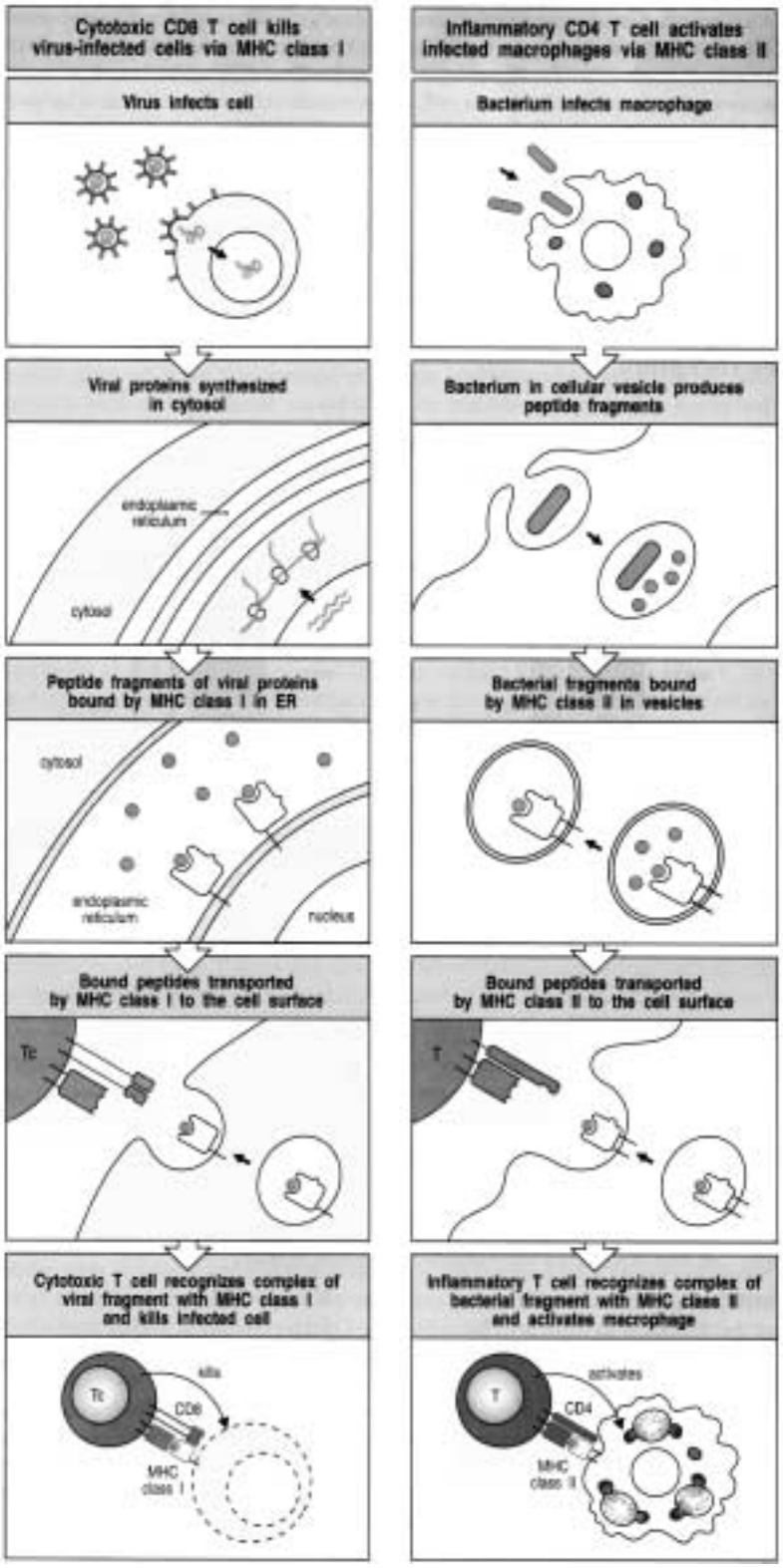


Figure 3.2.: Cell-mediated immune responses are either triggered by cytotoxic CD8+ T cells or directly T helper cells of type 1 (from [41])

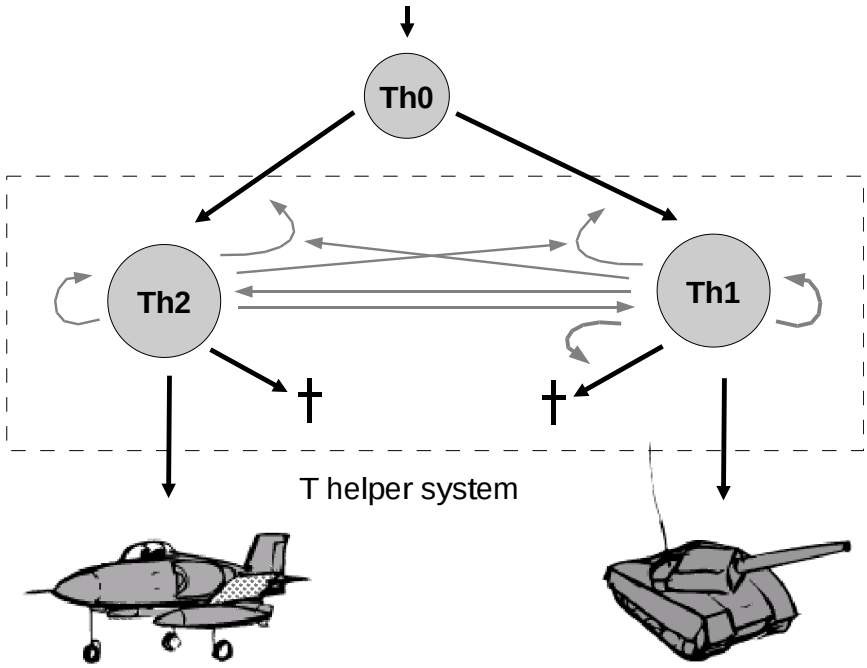


Figure 3.3.: The two T helper subsets lead to different defense mechanisms which are not equally efficient for any enemy

of Th1 cells is even necessary [104]. Whether Th1 support CTL activation via providing growth factors for cytotoxic T cells or via up-regulation of co-stimulatory molecules on APCs that activate CTLs is still not known.

The activation of B cells – the precursor cells of antibody-producing plasma cells – depends generally on the help of Th2 cells. Antigen bound by B-cell receptors is internalized and degraded to peptide fragments. These fragments are presented to Th2 cells on MHC II molecules on the B cell surface. Specific Th2 cells, which recognize the peptide, activate B cells through expression of cell-surface CD40 ligand, a co-stimulatory molecule that binds to CD40 on B cells, and secretion of so-called effector cytokines (also see Sec. 3.6). The role of Th2 cells in humoral immune reactions is illustrated in Fig. 3.4

The decision whether a cell-mediated or humoral immune response is triggered depends on the T helper subset that dominates. If we return to the picture of the immune system as an army there is one major difference that is important to highlight. In contrast to a real army there is no chief commander who would decide which defense strategy to choose dependent on the type of enemy. The two major strategies of the immune system are cell-mediated immune responses versus humoral immune responses. Because of the role of T helper subsets in the induction of these defense mechanisms the question 'Who decides about the appropriate defense strategy?' is reduced to the question 'What decides about Th1- or Th2 dominance and why is this selection appropriate?'. This question is the main issue of the present work.

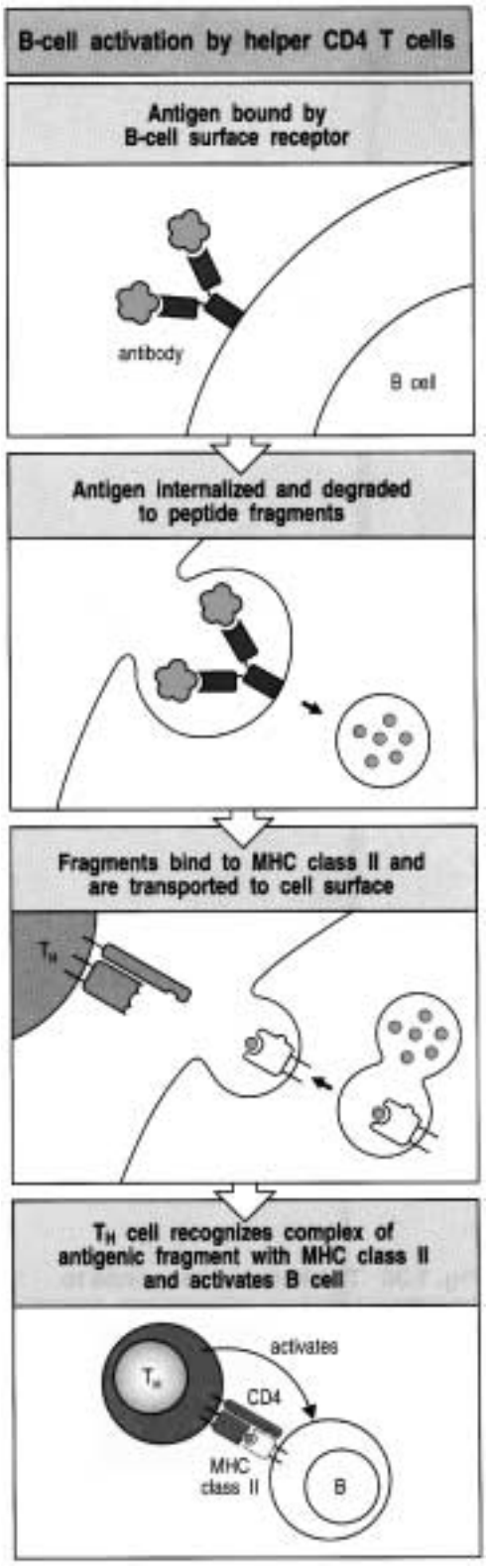


Figure 3.4.: Th2 cells activate B-cells, which differentiate into antibody-producing plasma cells (from [41])

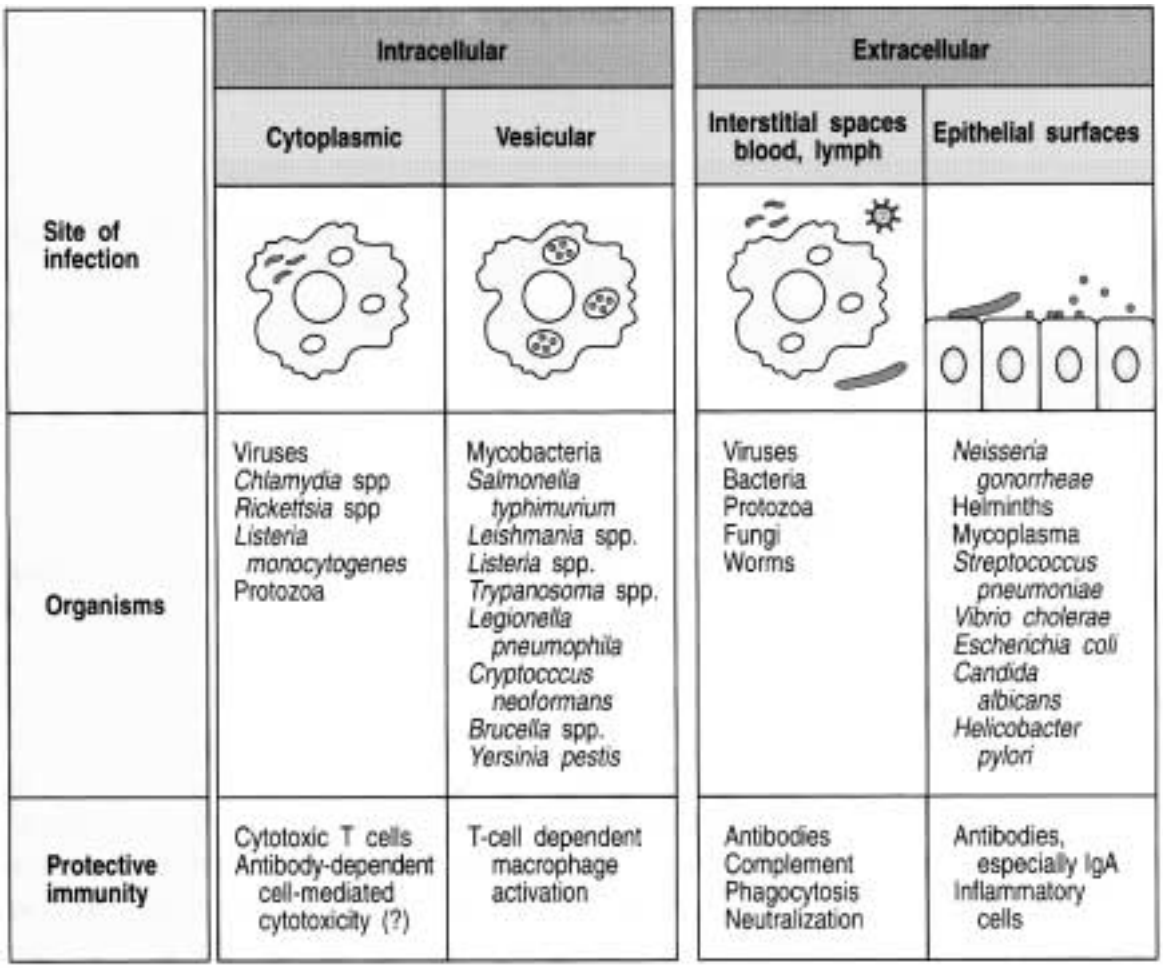


Figure 3.5.: Classification of different pathogens into intracellular or extracellular pathogens

Up to now we did not discuss the question why these two main immune system strategies are not equally efficient in pathogen elimination. The answer is that pathogens can be roughly subdivided into two major groups: Intracellular and extracellular pathogens. Intracellular pathogens have to invade host cells to replicate, and must either be prevented from entering the cell or detected and eliminated from this site. For their elimination cell-mediated immunity is necessary. Many microorganisms, however, replicate in extracellular spaces, either within the body or on the surface of epithelia, and require humoral immune responses for their clearance. Examples of pathogens classified into one of the two groups can be found in Fig. 3.5 from [41].

3.5. The role of cytokines in the induction of cell-mediated and humoral immunity

The only known difference between Th1 and Th2 cells is the production of different so-called effector cytokines – small messenger molecules that induce the activation of cells involved in cell-mediated or humoral immunity. Th1 cells produce interferon-γ, which activates macrophages, and IL-2, which contributes to activation of T killer cells, whereas IL-4 produced by Th2 cells is responsible for activation of B cells. The T helper effector cytokines, however, do not only induce effector functions but also participate in a complex regulatory system, which has been termed the cytokine network.

3.6. The cytokine network

Unlike other organs in the body the immune system is not a single entity. Thus one of its most important features is the process by which the various immune cells communicate with each other. One of the biggest challenges has been to understand its communication pathways and how they regulate immune processes.

Cytokines are molecules – mostly secreted but also sometimes expressed on the cell surface – that convey information from one cell to another. The scope of them is thus dependent on diffusion rates. All cytokines respond to specific receptors expressed on the surface of the target cell, thereby triggering complex intracellular signaling cascades. Most cytokines have multiple and diverse biological functions. Many cytokines are produced by more than one cell type and act on a variety of target cells at different stages. Most cells produce many different cytokines. The resulting system is thus a network of great complexity. The complex pathways between cytokines, their receptors and their generated responses has been termed the "cytokine-network" [20].

Although many details of cytokine interactions have been elucidated and effects of cytokines have been described, nearly nothing is known about the behavior of the network as a whole. Important features within the network are the presence of positive and negative feedback and the non-linearity of the cytokine interactions. Nonlinear interactions can give rise to effects that are even in small systems quite non-intuitive but which can have important biological implications. These properties make the cytokine network too complex to be fully understood by conventional experimental methods. An additional complementary modeling approach using methods of nonlinear dynamics is required, which will be provided for a small but very important subsystem – the T helper system – in the present work.

3.7. Phases of the immune response

T helper cells undergo different phases during their development.

- **Activation:**
  Initiated by antigen recognition T helper cells become activated and differentiate into either T helper cells of type 1 or type 2. The major effector function of differentiated CD4 T helper cells is the secretion of cytokines, which activate effector cells of either the humoral arm or the cell-mediated arm of the immune response but in addition play a role in the regulation of the T helper system (the system consisting of all T helper cells of both types 1 and 2) itself.

- **Proliferation:**
  Activated T cells produce their own growth factors and express surface receptors for these cytokines. Mitotic division of activated T cells – initiated by the growth factors – results in the expansion of clones of cells with the same antigen specificity and thereby augments the immune response to a particular antigen. The binding of T cell growth factors initiates a series of events that culminate in mitotic activity.

- **Death:**
  T cell death can occur either as a result of repeated stimulation in the form of so-called activation induced cell death (AICD) or from cytokine deprivation. Both processes may play important roles in limiting the immune response in its later stage. AICD is a process that causes activated T cells to undergo apoptosis after repeated ligation of CD3/TCR and is triggered by Fas-ligand (FasL) expression on activated T cells when ligation of the Fas receptor on the cell surface activates the apoptotic death pathway.

3.8. Regulatory processes in the T helper system

At any time of their development T helper cells are affected by cytokines. Among other factors such as the nature and dose of the antigen [39] and co-stimulatory molecules expressed by the presenting cell the overall cytokine milieu in which the T cell activation takes place has been shown to most crucially influence T helper differentiation [39]. Furthermore, cross-regulation through inhibitory cytokines produced by the the competing T helper subsets during the development of the response helps to further polarize or modify the proliferating Th1 and Th2 pools. This modulation through cytokine regulation could take place at the time individual cells become committed to one pattern of cytokine expression during antigen presentation, or subsequently by cytokine uptake having the potential to switch the cell into a different pattern of secretion. Evidence for a window of reversibility in the initial phase of a primary response has been reported, but after long-term stimulation individual cells apparently become irreversibly committed to a Th1 or Th2 phenotype [71]. Down-regulation and homeostasis or resolution of T helper responses is also attained by effects of regulatory cytokines.

The main interactions influencing the Th1/Th2-system are summarized in Fig. 3.6, which will be explained in detail when we construct the model. It has become obvious that there is a complex regulatory network of cytokines within the T helper system with

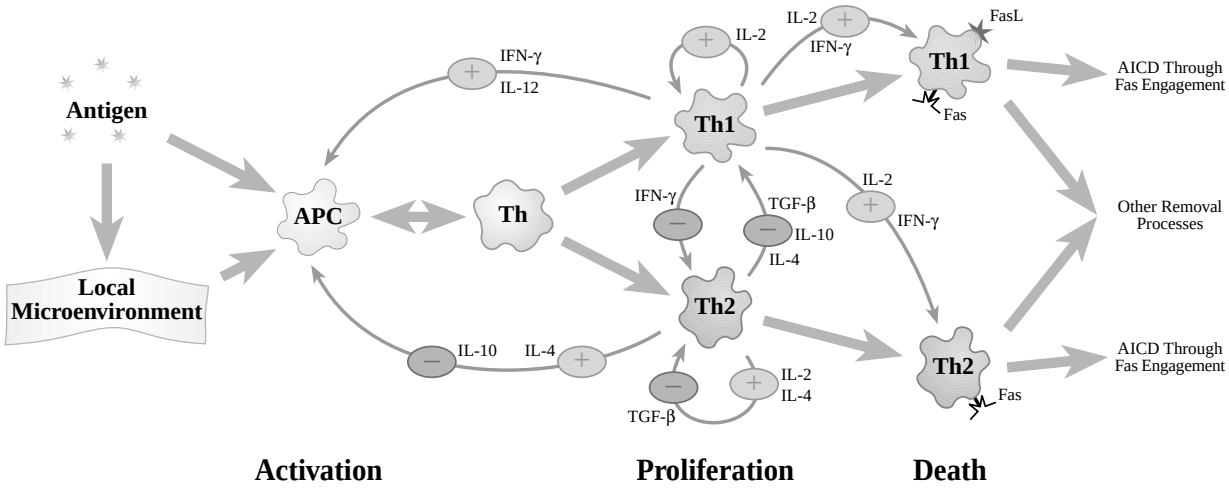


Figure 3.6.: Schematic representation of the interactions governing Th1/Th2 differentiation, proliferation and death. Naive cells stimulated by successful encounters with antigen-presenting cells (APCs) begin to differentiate into Th1-like or Th2-like effectors according to co-stimulatory signals and the ambient cytokine context. They go through several rounds of division before becoming armed effector cells. Previously activated (memory) cells also recirculate, are stimulated and rejoin the proliferating pool. The suppressive (−) or promoting (+) effects of cytokines on cell activity are broadly represented with arrows (for example, IFN-γ has a suppressive effect on Th2 proliferation; IL-10 down-regulates APC function). Activation-induced cell death (AICD) occurs through cell-cell interactions (binding of the Fas surface molecule by FasL induces apoptosis). The effects of cytokines on the production of other cytokines (for example, the inhibition of IFN-γ by IL-4; the up-regulation of IL-2 production by IL-12) are implied.

asymmetries in the regulation of the two T helper subsets Th1 and Th2, which lead to asymmetries in the overall behavior.

Although the classification of the immune response as Th1- or Th2-like is a useful model, individual responses are more likely to be mixed to some extent. Perhaps more interestingly, the nature of the dominant helper subset may change during the normal course of an immune response. For example, Th1 to Th2 switches are observed in the transition from acute to chronic graft-versus-host disease; in Bornea disease; in certain mouse malarial models; and also in the progression from HIV infection to the development of AIDS, although the latter example is perhaps less representative as immune function is significantly disrupted in this case. It has to be highlighted that – up to our knowledge – no without external influences occuring shifts from Th2 to Th1 have been found.

An other asymmetric feature in the behavior of the T helper system is the ability of antigen dose to direct the development of a Th1 or Th2 phenotype from naive T cells. The classical model of Th1/2 cytokine interactions offers no explanation for these Th1 $\rightarrow$ Th2 transitions and the antigen dose-dependence. The standard picture is that the two T helper subsets each produce the factors required for their own differentiation and expansion (a positive feedback loop) and that cytokines produced by one subset inhibit the

activation or proliferation of the other (negative feedback, or cross-regulation). The true picture is however more complex; in particular, this paradigm fails to address the *resolution* of responses, and does not take account of the apparent asymmetries in the system that may help us to understand the Th1/2 decision-making process.

3.9. The mathematical approach: generic features

We have seen in the previous sections that the immune system is more than a collection of independently operating lymphocytes. Many chemical signals are involved in setting up a communication between these cells ('cytokine network') and form a complex system. System behavior in the immune system has been often mathematically described in terms of ordinary coupled nonlinear differential equations as we will do here. For that sake we have to make sure that we can neglect spatial aspects and all the involved components meet at some place in the body and are mixed to a certain extent.

Such a place where all units come together indeed exists: the lymph nodes. Pathogens can invade the body in many different ways and cause infection at any location but pathogens become picked up by phagocytes and brought to the peripheral lymphatic organs – such as the lymph nodes or the spleen. Lymphocytes continuously circulate through the lymphatic tissue where antigen has been transported from any place and where it is kept by specialized cells. Therefore, we approximate that all components of the immune response are well-mixed so that we can describe their population sizes by non-partial but ordinary differential equations.

The general aim of mathematical modeling is to deduce macroscopic properties of the system from the properties and interactions among the elementary components and to select a simple representation of the elementary interactions that give rise to the observed system behavior. Here, we focus on a subsystem of the immune system – the T helper system –, which however is of fundamental importance for orchestrating the performance of the whole immune system. To fully concentrate on the regulatory mechanism within the T helper system and to understand its internal dynamics we restrict ourselves in Chap. 4 to a system with constant antigen stimulation. In Chap. 5 we try to extract the critical asymmetries in Th1/Th2 regulation and describe its importance for the overall behavior. The model of Chap. 4 will be extended in Chap. 6 to a system where we incorporate interference of the T helper-induced immune response with replicating pathogens. The insights in the 'built-in' behavior of the T helper system gained in Chap. 4 will be used to describe a mechanism, which enables the T helper system – via self-regulatory processes that are purely based on the cytokine interactions – to select the – for a particular pathogen – appropriate T helper response. In Chap. 7 we discuss other mechanism for the selection process and contrast two different selection principles: regulation by dominant controller versus self-regulation.

4. Cytokine-modulated T helper regulation

In this chapter we develop a model of the T helper system ('Th1/Th2'-model), which incorporates many of the relevant cytokine interactions involved, including interactions that are both specific and non-specific for Th1 or Th2 branches [102]. These interactions will be explained in detail in the following section. Other authors [21,29–31,54,67,70] have modeled the Th1-Th2 system, with a variety of approaches and areas of emphasis. We return to comparisons of these models and the present one in the discussion. However, the impact of the local microenvironment and the cytokine regulation of cell death mechanisms have not been included before in models of this sort. Our approach allows us to investigate the relative importance of the various stimulatory and regulatory mechanisms at play in effector T cell development, to understand the basis of dynamic helper subset switches and the interplay between activation, proliferation and cell removal processes.

4.1. Components of the model

We characterize a T helper cell response with variables T_1 = [Th1] and T_2 = [Th2], measuring the numbers of committed cells of the two types. Proliferating, effector and memory cells are grouped together. We do not model the dynamics of antigen clearance and have, in principle, a continuous antigenic stimulus. This takes the form of creation terms for both cell types that represent the activation and polarization of naive T lymphocytes by interaction with antigen-presenting cells and other components of the local microenvironment, consistent with recent experimental evidence [46]. In this sense, our approach is complementary to the interpretation by Murphy *et al.* [71] and by Fishman and Perelson [29] that individual T cells become committed to a type 1 or type 2 differentiation pathway on a weighted, probabilistic basis at the presentation stage, and then intercellular cytokine interactions in the proliferating pool modify the Th1- or Th2-dominance at a population level. However, we also include the effect of antigen on the local microenvironment and the cytokine feedback from the proliferating T cell pool, both of which modify the activation state of the antigen presenting cells and influence the probability that a given APC-T cell encounter will lead to Th1 or Th2 differentiation. By not considering the removal of the pathogen, our model addresses the evolution of the immune response during antigen exposure and possible states of chronic infection in which any feedback from success or

failure of effectors has yet to be manifest. In addition to a constant decay term for cell loss and/or terminal maturation into effector cells, we also include a term for activation induced cell death (AICD). Recent experimental evidence indicates that rates of apoptosis increase with repeated antigen stimulation. The rate equations for Th1 and Th2 populations thus take the simple form

$$\text{Rate of change of antigen-specific Th1 cell population} = \text{activation} + \text{proliferation} - \text{death},$$

with a similar equation for the evolution of the Th2 effector population.

4.1.1. Regulation by cytokines

The terms above are modulated by the action of soluble protein messenger molecules (cytokines) that are secreted by the different cells and act by binding to specific surface receptors. In order to reduce the number of parameters, we make the assumption that the majority of important cytokines involved can be classified as either type 1 or type 2 according to whether they are produced predominantly by Th1 or Th2 cells, respectively. These are grouped into variables S_1 and S_2 as generalized cytokine 'signals' produced by each helper subset. In different terms in the model, S_1 may therefore represent the concentration of IFN-γ or IL-2, S_2 may represent IL-4, IL-10, and so on. Clearly there is independent regulation of cytokine production within these groups (for example, IL-12 and IFN-γ are produced by different cell types), but the signals are considered at a population level rather than at that of single cells.

Cytokines play important roles at different stages of the immune response, and indeed are usually multi-functional. For example, with sufficient antigenic stimulus, IFN-γ and IL-2 are important for differentiation and proliferation of Th1 cells whereas later their presence serves to enhance apoptosis of repeatedly stimulated effector cells. In our model, however, we are concerned primarily with continuous antigen stimulation. Representing the time course of cytokine production during the induction and resolution of a response is not our major issue.

Rates of cytokine production, receptor binding, and decay are typically large compared to those of the cell population dynamics. We therefore make a steady state assumption for S_1 and S_2, relating them directly to cell numbers. We incorporate the observation that type 2 cytokines tend to inhibit the production of cytokines by Th1 cells, but type 1 cytokines do not have such a marked suppressive effect on the synthesis of IL-4, IL-10, etc. [13, 90, 91]. This leads to an indirect inhibition of Th1 activation and proliferation by lack of differentiation and growth factors. In contrast direct suppression of Th2 proliferation without effect on Th2 differentiation has been described (see Sec. 4.1.2). This asymmetry is critical for the antigen dose-dependence of T helper differentiation (see Sec. 5.2).

In fact, the rate of binding and removal of any type of cytokine is likely to be highly dependent on the density of cells which express the appropriate receptors for it, and perhaps also on competition between various receptors for common chains. However, in the absence of experimental data to provide estimates of cytokine consumption rates, we make the

simplest possible assumption, viz that diffusion and degradation of cytokines takes place at a rate independent of T cell numbers. The clearance rates of all free cytokines are thus identical and constant.

We employ a parameter k to measure the typical concentration of a cytokine at which its effect becomes significant. More precisely, we represent the inhibitory effects of cytokines with saturating Hill functions of the cytokine signal strength, and k^{-1} is the concentration at half-maximum. Using a single concentration scale for all cytokines assumes that different cytokine receptor densities are comparable and that the various cytokine production rates per cell are approximately the same. As cytokine receptor affinities are typically in the nanomolar range and dose response curves look very similar these are reasonable assumptions and they greatly simplify the analysis. The expressions for the cytokine signals are then

$$S_1 = \frac{\alpha_1 T_1}{1 + k\alpha_2 T_2}, \qquad S_2 = \alpha_2 T_2, \qquad \tilde{S}_1 = \frac{\alpha_1 T_1 + S_0}{1 + k\alpha_2 T_2}, \tag{4.1}$$

where $\tilde{S}_1$ is specifically the effective concentration of IL-2, taking into account the (constant) contribution S_0 from newly-activated Th cells, known as Th0 cells, which produce significant quantities of this cytokine [6] as well as varying quantities of IL-4 and IFN-γ.

4.1.2. T cell activation and differentiation

All CD4+ T cells have a naive phenotype when leaving the thymus and have to be provided with a proper set of signals to differentiate into either Th1 or Th2 effector cells. Here we will discuss the role of various cytokines in the process of T helper differentiation.

We assume that there are distinct activation signals for the type 1 or 2 pathways, and that each is modulated by the local cytokine context. The bare activation strengths represent the combined effect of antigen dose, affinity between antigen and T cell receptor, and the nature of the co-stimulatory signals presented to T lymphocytes by the professional antigen-presenting cells (APCs). Our representation of this process in the model is necessarily simplified, and so we review the essential experimental data here and detail how they are reflected in the model.

Antigen is taken up and processed by antigen presenting cells (dendritic cells, monocytes and B cells) then displayed as small peptide fragments in association with MHC class II on the cell surface. Antigen-specific T helper cells become activated and acquire a Th1 or Th2 phenotype following interactions of sufficient affinity between the T cell receptor and the antigen peptide complex with MHC class II on the APC. The affinity of interaction is enhanced by CD4 binding to non-polymorphic determinants on MHC class II. Before they become fully differentiated after several cycles of cell division they exhibit a transient Th0 phenotype, which is characterized by production of the growth factor IL-2 and, in varying amounts, of the Th1 cytokine IFN-γ and the Th2 cytokine IL-4. T cell activation also depends on co-stimulation through accessory APC receptor-ligand interactions such as CD40/CD40L and B7/CD28 and binding of cytokines, particularly IL-12 secreted by APC [88]. Importantly, it is now recognized that the state of APC activation by antigen

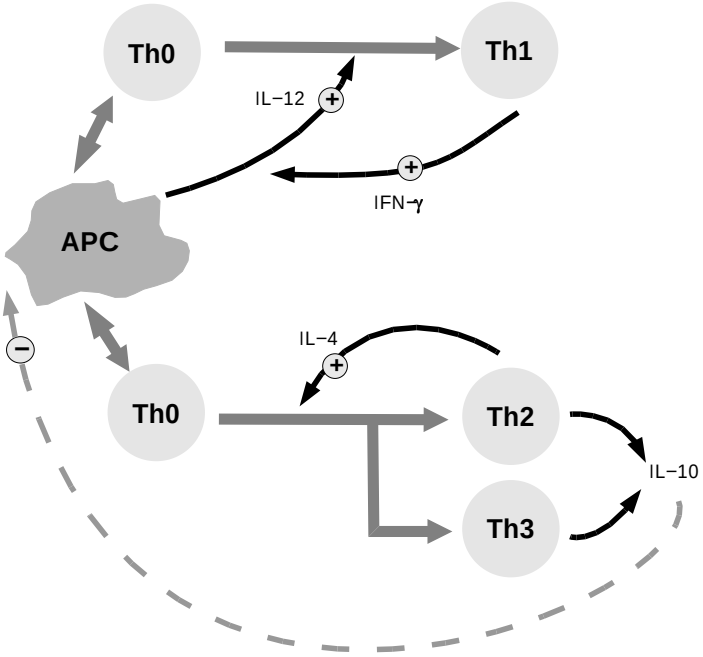


Figure 4.1.: Schematic representation of the interactions governing Th1/Th2 differentiation. Naive cells stimulated by successful encounters with antigen-presenting cells (APCs) begin to differentiate into Th1-like or Th2-like effectors according to co-stimulatory signals and the ambient cytokine context. The suppressive (−) or promoting (+) effects of cytokines on cell activity are represented with arrows (for example, IL-10 down-regulates APC function). Th3 cells are discussed in Sec. 4.1.3.

acting indirectly through the local microenvironment or directly by binding to pattern recognition receptors also have a major impact on Th1 and Th2 differentiation [46] (see also Sec. 7.2). The development into distinct Th1 or Th2 effectors is then determined by the type of antigen, its dose, route of entry, and interactions with APC and local microenvironment, all of which are influenced by the host's genetic background.

The action of cytokines in the local microenvironment has been shown experimentally to be important and is a key factor affecting the Th1/Th2 balance in our model. The cytokines that have most effect on Th activation are IL-4, IL-10, IL-12 and IFN-γ. The interactions, which govern T helper differentiation are illustrated in Fig. 4.1.

IL-12 is produced by dendritic cells and macrophages and plays a crucial role in Th1 development [95]. Its synthesis by APC is increased by IFN-γ secreted by antigen-specific Th1 cells. In turn, IL-12 promotes development of Th1 effectors from naive Th cells by increasing Th0 and Th1 IL-2 receptor (IL-2R) expression and also increasing production of IL-2 and IFN-γ. This implies that Th1 cytokines not only foster promotion of effector functions and proliferation of the Th1 population but additionally provide a positive feedback loop from the already existing Th1 population on the Th1 directed differentiation of the pool of naive T helper cells. We incorporate the interactions between IL-12 and IFN-γ

in an implicit way in our model by an enhancement of the Th1 activation term that is proportional to the strength of the Th1 cytokine signal.

IL-4 plays a similar role for the activation of Th2 lymphocytes. It has been shown that IL-4, which is secreted by activated Th2 cells, not only enables Th2 proliferation but also directs differentiation of naive cells towards the Th2 phenotype [85]. We thus represent the Th2 activation by a term which is proportional to the Th2 signal.

IL-10, produced by activated Th2 cells acts in an suppressive way on the activation of T helper cells. We therefore suggest that it is one of the major factors for limiting pure Th2 populations 4.2.5. It increases the death rate of precursor dendritic cells, inhibits differentiation of monocytes to dendritic cells [3], reduces expression of MHC class II [24], and inhibits IL-12 production by dendritic cells [23] and macrophages.

The activation terms represent rates of successful encounters of naive or recirculating T helper cells with APCs. These rates are proportional to the bare Th1- or Th2-activating capacities of the APCs, which we label ξ_1 and ξ_2, respectively. These parameters in turn could be proportional to the density of loaded MHC class II/peptide complexes on the APC surfaces, and weighted for the types of either pro-type 1 or pro-type 2 co-stimulatory molecules and cytokines expressed by the APCs. The Th1 activation rate is enhanced by IL-12/type I cytokines (i.e., S_1) and reduced by the S_2 signal, corresponding to the down-regulation of APC activity and MHC class II expression by IL-10. Similarly, the Th2 activation rate is equally affected by IL-10 and is boosted by the S_2 signal. In addition, we represent T helper independent activation by γ_1 and γ_2. The ratios γ_1/ξ_1 and γ_2/ξ_2 correspond to the additional contribution to Th1 or Th2 cytokines from external sources as for example components of the innate immune system and/or the local microenvironment including the APC activation state (i.e., not produced by the activated T cells themselves). A detailed discussion of signals from the innate immune system and the role they play will be given in Chap. 7. Thus

$$\text{Th1 activation rate} = \frac{(\gamma_1 + \xi_1 S_1)}{1 + kS_2},$$

$$\text{Th2 activation rate} = \frac{(\gamma_2 + \xi_2 S_2)}{1 + kS_2}.$$

Clearly, the parameters ξ_1 and γ_1 will not always be independent, and similarly for ξ_2 and γ_2. Strong pro-Th1 signals from APCs are most likely to appear in a pro-Th1 cytokine context, and so the relative sizes of γ_1 and γ_2 will generally reflect those of the APC activation signals ξ_1 and ξ_2, respectively. However, in certain circumstances we can manipulate an immune response by external means (for example, using cytokine therapy). We discuss this below.

Co-stimulation affects the differentiation ratios of Th1 and Th2 and may in turn be influenced by the cytokine milieu. However, because data on the roles of co-stimulatory molecules B7.1 and B7.2 on T helper differentiation is quite contradictory, and factors that determine the expression of these co-stimulatory receptors are also not well understood, we neglect these influences in the present model.

In summary, the APC and local microenvironment provide cogent signals to T cells to initiate the choice of differentiation pathway. This is then reinforced or cross-regulated by cytokine interactions among the proliferating T cells, which we now review.

4.1.3. Proliferation

After activation of T lymphocytes the choice of the differentiation pathway is reinforced by proliferation of T helper cells committed to one of the two T helper subsets and by cross-suppression. Proliferation is driven by growth factors secreted by the Th1/Th2 cells.

Role of growth factors

Here we assume that growth factors act in an systemic rather than an autocrine way. IL-2 is produced by activated Th0 and Th1 cells and is a growth factor for both Th1 and Th2. Binding of a sufficient quantity of IL-2 to receptors (IL-2R) on TCR-activated T cells induces the cell to go into cycle. It stimulates the production of IFN-γ by Th1, and prevents activated cells from reverting to a quiescent state. It also plays a complex role in both rescue from and promotion of apoptosis, which we return to in more detail below.

IL-4 is a growth and differentiation factor for Th2 cells that is also produced by them. It acts on Th1 cells to reduce their expression of IL-12R and their production of IL-2 and IFNγ [91]. It is not clear whether IL-4 is also a growth factor for Th1 cells [27,66]. Here we consider it as a growth factor for Th2 but not Th1, both alone and in synergy with IL-2. This allows Th2 cells to respond better to low amounts of IL-2 produced by nearby Th1 or Th0 cells. Alone, IL-4 has approximately a tenth of the potency of IL-2 [19]. Therefore we lie emphasis on the synergy effect between IL-2 and IL-4 when we study Th2 proliferation, which is more in agreement with findings that the presence of IL-2 was essential for IL-4 to act as a growth factor [87].

Other authors [76], however, suggest that there are no synergy effects between IL-2 and IL-4 and that the growth factors promote T cell proliferation through symmetrical pathways indicating a more autocrine role. Because of the opposing views in the literature an alternative role of growth factors will be discussed in Sec. 5.1.

Strictly, division rates are independent of cytokine concentrations: the duration of the cell cycle is of the order of 18 hours. We suggest that growth factors act to maintain cells in cycle (i.e., to increase the number of offspring produced by a given cell) and so postulate exponential growth with a rate constant that is proportional to the relevant growth factor concentration.

Suppression of proliferation

Cytokines not only boost proliferation but also exhibit cross-suppressive or regulatory effects on the competing or secreting T helper cell population.

IFN-γ, a type 1 cytokine, inhibits Th2 proliferation [32] and therefore helps polarize T helper responses.

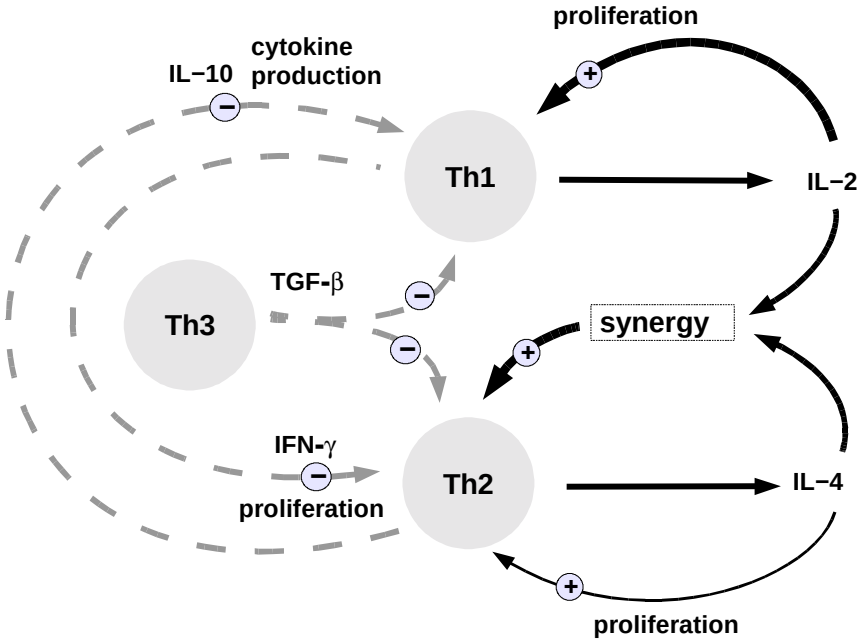


Figure 4.2.: Schematic representation of the interactions governing Th1/Th2 proliferation. Whereas type 1 cytokines directly inhibit Th2 proliferation type 2 cytokines lead to retardation of Th1 proliferation via down-regulation of growth factor production. TGF-β inhibits proliferation.

TGF-β has been reported to play an important role in the homeostasis of immune responses because of its capacity to inhibit IL-2 as well as IL-4 induced proliferation [16,82]. TGF-β is secreted by a new group of regulatory T helper cells, which have been termed Th3 or Tr1 on the basis of their cytokine pattern [52]. Differentiation of Th3 cells has been observed in the presence of IL-10 [35] and IL-4 [40]. Based on the similarity of Th3 and Th2 cytokine secretion patterns and differentiation conditions – for the purposes of our model – we group these two T helper types together, an assumption based on the similarity of their cytokine secretion patterns and differentiation factors [60,84], and take TGF-β as a part of the type 2 signal.

We find that TGF-β is not primarily an anti-inflammatory (anti-Th1) cytokine but in conjunction with IL-10 is a key cytokine for the regulation of Th2 responses. This is entirely consistent with its known ability to suppresses IL-4 production, B-cell maturation and IgG and IgM synthesis. The cytokine interactions that affect T helper proliferation are summarized in Fig. 4.2

In summary, Th1 proliferation is potentiated by IL-2 and inhibited by TGF-β, and is represented by the term

$$\text{Th1 proliferation rate} = \frac{\beta_1 \tilde{S}_1 T_1}{1 + kS_2}.$$

Th2 proliferation is driven by IL-4 alone and in combination with IL-2, and inhibited by

TGF-β and IFN-γ:

$$\text{Th2 proliferation rate} = \beta_2 T_2 \frac{([IL4] + c[IL4][IL2])}{(1 + kS_1)(1 + kS_2)} = T_2 \frac{(\beta_2^{IL4} S_2 + \beta_2^{IL2/4} \tilde{S}_1 S_2)}{(1 + kS_1)(1 + kS_2)},$$

where β_1 and β_2 are proportionality constants and $\beta_2^{IL4}/\beta_1 \simeq 0.1$ [19].

4.1.4. Cell death

T cell death can occur either as a result of repeated activation or from inadequate stimulation and cytokine deprivation. Both may play important roles in limiting the immune response in its later stages [77].

Repeated ligation of the TCR/CD3 complex causes activated T cells to undergo apoptosis [57]. It is thought that this process, called activation-induced cell death (AICD), is triggered by increased Fas-ligand (FasL) expression on activated T cells. This engages the Fas receptor on the cell surface, activating the apoptotic death pathway [45]. This could occur either through secretion of soluble FasL binding to Fas on the same cell (suicide) or through cell-cell contact (fratricide). The increase in FasL expression after repeated reactivation of mature lymphocytes appears to be transient - AICD has been reported to occur only when the cell is in cycle [11], and surface FasL is shed rapidly [1]. AICD rates are enhanced by the presence of IL-2 and IL-15 [51]. These facts suggest that Fas-mediated cell death may be important for limiting the magnitude of an effector T cell response.

The differential importance of AICD for Th1 and Th2 phenotypes is an issue we explore with our model. There is data to suggest that AICD is a suicidal phenomenon [17] largely restricted to Th1 cells [105]. Below, we discuss cases in which the Th1 AICD process is modeled as either a cell-cell or single cell process. Analysis of the susceptibility of T helper cells to activation-induced cell death in response to CD3/TCR ligation has shown that AICD was mainly observed in Th1 clones whereas Th2 were more resistant [97, 105]. Earlier studies have suggested that this is due to higher levels of FasL expression among Th1 cells [36, 78] whereas recent data indicates that these discrepancies also depend on differences in the Fas signal transduction pathway, with PI3'-K as the critical molecule in mediating protection from Fas-induced cell death in activated Th2 cells [98]. In our model, we work under the assumption that Th1 clones express FasL at higher levels than Th0/Th2 cells [36]. Reciprocally, Th2 cells can undergo Fas-induced apoptosis in the presence of activated Th1 cells, but AICD is less frequent among Th2 cells in mixed populations, indicating a lower susceptibility.

IL-2 and apoptosis

It has become clear that IL-2 plays a central role in both proliferation and AICD. It increases the transcription rate and expression levels of FasL, and activated $CD4^+$ T cells in IL-2 gene-inactivated ($IL\text{-}2^{-/-}$) mice show a reduced propensity for AICD, which can be restored by addition of exogenous IL-2 at both the activation and effector stage [79].

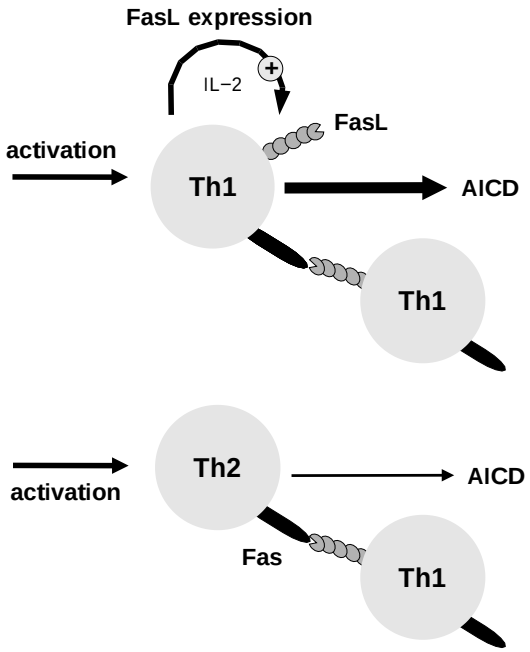


Figure 4.3.: Schematic representation of the interactions governing AICD. Activation leads to up-regulation of Fas expression in Th1 and Th2 cells but to FasL expression only in the Th1 phenotype. Rates of Fas-mediated apoptosis are lower for Th2 cells.

Humans and mice with Fas or FasL gene defects (*lpr* and *gld*) or IL-2 deficiencies exhibit lymphoproliferative disorders [28,75,101], suggesting that while the role of IL-2 as a growth-factor can be filled by other cytokines (e.g. IL-15), its pro-apoptotic effects cannot.

Conversely, IL-2 can also *inhibit* apoptosis [79]. It stimulates the production of anti-apoptotic proteins such as Bcl-2 and removal of IL-2 from activated T cells leads to reduced Bcl-2 expression and apoptosis [2]. This process is commonly referred to as death through cytokine deprivation. We summarize the details just described in Fig. 4.3.

In summary, we model the removal of T helper cells from the effector pool with two terms. One represents IL-2 dependent fratricidal AICD, and the other represents the combined processes of apoptosis through the Bcl-2/Bax pathway, anergy, necrosis, and peripheral diffusion, for which a constant *per capita* rate of removal μ for both Th1 and Th2 is the simplest choice. Note that we model the apoptotic influence of IL-2 through the Fas pathway only. Studies have shown that AICD is the dominant apoptotic mechanism during the development of a response, with death through cytokine deprivation more prominent at the resolution stage [77].

$$\text{Th1 removal rate} = \Delta_1 \tilde{S}_1 T_1^2 + \mu T_1.$$

The form of the Th2 depletion term assumes that only Th1 cells express FasL and Th1-Th2

encounters are required for Th2 AICD:

$$\text{Th2 removal rate} = \Delta_2 \tilde{S}_1 T_1 T_2 + \mu T_2.$$

4.1.5. Model summary

We divide the dynamics of T_1 and T_2 into terms corresponding to activation (creation), proliferation and death. The source or activation term for T_1 is enhanced by the presence of type 1 cytokines (IL-2, IFN-γ/IL-12); similarly for T_2 and S_2 (IL-4). Both activation terms are down-regulated by the type 2 signal (IL-10). Th1 growth is enhanced by S_1 (IL-2) and inhibited by S_2 (TGF-β). Th2 growth is enhanced by both S_1 and S_2 (IL-2 and IL-4), and is suppressed by both S_1 (IFN-γ) and S_2 (TGF-β). We include Fas-induced cell death (up-regulated by IL-2, or $\tilde{S}_1$) in the Th1 population and assume that Th2 cells express sufficient Fas to be susceptible to apoptosis by interaction with FasL-expressing Th1 cells.

The parameters ξ_1, β_1 and Δ_1 are proportionality constants for the rates of activation (creation), clonal expansion and Fas-induced cell death for Th1 cells. Similarly for ξ_2, etc. and Th2 cells. The parameters γ_1 and γ_2 represent the contributions to the activation signals from the local microenvironment.
Gathering the terms detailed above,

$$\frac{\mathrm{d}T_1}{\mathrm{d}t} = \frac{(\gamma_1 + \xi_1 S_1)}{1 + kS_2} + \frac{\beta_1 \tilde{S}_1 T_1}{1 + kS_2} - \Delta_1 \tilde{S}_1 T_1^2 - \mu T_1 \,, \tag{4.2}$$

$$\frac{\mathrm{d}T_2}{\mathrm{d}t} = \frac{(\gamma_2 + \xi_2 S_2)}{1 + kS_2} + T_2 \frac{(\beta_2^{IL4} S_2 + \beta_2^{IL2/4} \tilde{S}_1 S_2)}{(1 + kS_1)(1 + kS_2)} - \Delta_2 \tilde{S}_1 T_1 T_2 - \mu T_2. \tag{4.3}$$

We rescale to the following dimensionless combinations of variables; $x_i = k\alpha_i T_i$, $\tau = \mu t$, $\theta_i = \gamma_i k$, $\chi_0 = kS_0$, $\sigma_i = \xi_i \alpha_i / \mu$, $\delta_i = \Delta_i / \mu k^2 \alpha_i$, $\pi_1 = \beta_1 / \mu k$, $\pi_2 = \beta_2^{IL2/4} / \mu k^2$, $\rho = \beta_2^{IL2} k / \beta_2^{IL2/4}$. Using the expressions (4.1) for the cytokine signals S_1 and S_2, the resulting dynamical equations are

$$\frac{\mathrm{d}x_1}{\mathrm{d}\tau} = \frac{\theta_1}{(1 + x_2)} + \frac{\sigma_1 x_1}{(1 + x_2)^2} + \pi_1 x_1 \frac{x_1 + \chi_0}{(1 + x_2)^2} - \delta_1 \left(\frac{x_1 + \chi_0}{1 + x_2} \right) x_1^2 - x_1, \tag{4.4}$$

$$\frac{\mathrm{d}x_2}{\mathrm{d}\tau} = \frac{\theta_2 + \sigma_2 x_2}{1 + x_2} + \pi_2 \left[\rho + \left(\frac{x_1 + \chi_0}{1 + x_2} \right) \right] \frac{x_2^2}{1 + x_1 + x_2} - \delta_2 \left(\frac{x_1 + \chi_0}{1 + x_2} \right) x_1 x_2 - x_2. \tag{4.5}$$

We have the additional constraint $\rho = \pi_1 / 10\pi_2$. We summarize the biological significance of the parameters in table 4.1.

In the following we would like to address the following issues;

Parameter ($i = 1, 2$)	*Interpretation*
σ_i	Activation strength, weighted for its Th(i)-inducing properties
π_i	Efficacy of growth factors at maintaining activated cells in cycle
δ_i	Susceptibility of Th(i) cells to activation-induced cell death
χ_0	Effective number of IL-2-producing Th0 cells
θ_i/σ_i	Contribution to Th(i) cytokines from other (non-T cell) sources
ρ	Relative efficiency of IL-4 as a growth factor for Th2, compared to that of IL-2 for Th1 cells

Table 4.1.: Biological interpretation of the dimensionless parameters in equations 4.4 and 4.5

- Can we understand the dose-dependence of the response? (Various *in vivo* and *in vitro* studies have shown that Th1 or Th2 responses can depend on antigen dose [39]);
- The role of AICD in T cell homeostasis;
- If AICD is more important for Th1 than for Th2, as we suspect, can we provide a model for Th2 regulation?
- What are the consequences of differential FasL expression and AICD susceptibility for the Th1/Th2 balance?
- The dynamics of Th1 to Th2 switches;
- Can switches of response be induced?

4.2. Analysis

We make some simplifications for the initial round of analysis by setting some parameters to zero. These are reintroduced in the next section, where we find that they modify but do not fundamentally change the conclusions drawn below.

1. Neglect any susceptibility of Th2 cells to Fas-mediated apoptosis ($\delta_2 = 0$). This is in accord with experimental evidence that Th1 cells and not Th2 cells preferentially undergo AICD [105].

2. Ignore the contribution to the IL-2 concentration from Th0 cells ($\chi_0 = 0$).

3. Remove the additional Th1/2 cytokine contributions from other (non- T cell) sources ($\theta_1 = \theta_2 = 0$). Thus the activation signals σ_1 and σ_2 are strongly coupled to cytokine-mediated feedback from the proliferating T cells.

With these simplifications the equations become

$$\frac{\mathrm{d}x_1}{\mathrm{d}\tau} = \sigma_1 \frac{x_1}{(1+x_2)^2} + \pi_1 \frac{x_1^2}{(1+x_2)^2} - \delta_1 \frac{x_1^3}{1+x_2} - x_1, \tag{4.6}$$

$$\frac{\mathrm{d}x_2}{\mathrm{d}\tau} = \sigma_2 \frac{x_2}{1+x_2} + \pi_2 \left(\rho + \frac{x_1}{1+x_2}\right) \frac{x_2^2}{1+x_1+x_2} - x_2. \tag{4.7}$$

A detailed analysis of the nullclines is given in the Appendix. Obviously only states in the positive quadrant have any biological significance. We model the outcome of a particular antigenic challenge by running our model with a specified set of parameters from an initial condition near the origin. In general there are several steady states ($x_1^* \geq 0,\ x_2^* \geq 0$), which we interpret to be the T cell responses to continuous antigenic stimulation.

4.2.1. Primary responses and initial conditions

Importantly, the assumption $\theta_1 = \theta_2 = 0$ turns the origin into a steady state. Without any antigen-specific, pre-committed Th1 or Th2 cells the system remains at the no-response state even in the presence of antigen. With sufficiently large activation signals σ_1 and σ_2, the origin becomes unstable and we encounter problems of sensitivity to initial conditions. The nature of the immune response can be strongly dependent on the composition of a small initial population of predisposed Th1 or Th2 cells that are responsive to the antigen. Although it may be biologically unrealistic, we will first proceed with the analysis using $\theta_1 = \theta_2 = 0$ as this allows us to explore the characteristics of the non-zero steady states analytically. Later, when we introduce non-zero values for θ_1 and θ_2, we see that even though the behavior near the origin is altered (and the response becomes less sensitive to initial conditions), the broad picture of the dynamics of immune responses is essentially unchanged.

A truly naive response, with no assistance from cross-reactive memory T cells or Th0 cells that produce Th1 and Th2 cytokines in small amounts, is represented in our model by trajectories beginning at $(0,0)$. Th0 cells, however, produce IL-4 and IFN-γ – cytokines that direct Th1 and Th2 differentiation, respectively. On the population level this can be interpreted as a small, non zero concentration of Th1 and Th2 cells. This resolves the mathematical bootstrap situation. If we, in addition, consider the fact that cytokine expression of not fully differentiated Th0 cells is cell cycle dependent – only Th1 cytokines are produced during the first rounds of division [10] – we deduce that the initial values for Th1 are slightly higher than for Th2.

4.2.2. Steady states

The system (4.6)-(4.7) exhibits four different fixed points. In particular, we find

- The 'no-response' state $(0,0)$. The corresponding eigenvalues are $\{\sigma_1 - 1, \sigma_2 - 1\}$, which implies that for low stimulation the 'no-response' state is stable. Transcritical bifurcations take place at $\sigma_1 = 1$ and $\sigma_2 = 1$ as unstable, negative Th1 or Th2 fixed points pass through the origin and exchange stability.

- An exclusively-Th2 response $(0, x_2^*)$. If we neglect the contribution to Th2 proliferation from IL-4 alone ($\rho = 0$), then we have $x_2^* = \sigma_2 - 1$, with $\sigma_2 > 1$ necessary for its existence. The eigenvalues are $(1 - \sigma_2)/\sigma_2$ and $(\sigma_1 - \sigma_2^2)/\sigma_2^2$, so $\sigma_2 > \sqrt{\sigma_1}$ is a necessary and sufficient condition for stability. Note that this expression for the magnitude of the pure Th2 state does not involve the proliferation parameters. Therefore this state cannot be considered to represent an immune response. This is clearly a consequence of the dependence of Th2 cells on the Th1 cells for the production of growth factor when $\rho = 0$.

  If we include ρ (that is, if we allow Th2 cells to proliferate in the presence of IL-4 alone) then for $\rho\,\pi_2 \neq 1$ there exists a non-zero pure Th2 response

  $$x_2^* = \frac{\sigma_2 - 1}{1 - \rho\,\pi_2}. \tag{4.8}$$

  We restrict ourselves to the regime $\rho\,\pi_2 < 1$ (as discussed above, we also have the constraint $\rho\,\pi_2 = \pi_1/10$), in which there is a relatively weak proliferative response to IL-4 alone. This constraint guarantees that Th2 responses are always bounded. It also dictates that a Th2 response exists above a threshold level of stimulation $\sigma_2 > 1$ and that its magnitude increases with antigenic stimulus, both of which are biologically reasonable. The pure Th2 response also increases as we increase the IL-4-driven proliferation rate $\rho\,\pi_2$. This steady state has eigenvalues

  $$\frac{(\sigma_2 - 1)(\rho\,\pi_2 - 1)}{\sigma_2 - \rho\,\pi_2} \quad \text{and} \quad -1 + \sigma_1 \left(\frac{1 - \rho\,\pi_2}{\sigma_2 - \rho\,\pi_2}\right)^2.$$

  Since $\rho\pi_2 < 1$, we require $\sigma_2 > 1$ for a Th2 state to exist at all and to keep the eigenvalues bounded. This state is stable if

  $$\sigma_1 < \left(\frac{\sigma_2 - \pi_2\rho}{1 - \rho\,\pi_2}\right)^2. \tag{4.9}$$

  A large efficiency of IL-4 driven proliferation (i.e., large values of $\rho\,\pi_2$) corresponds to an increased stability of the pure Th2 response against pro-Th1-signals, as expected.

- An exclusively-Th1 response $(x_1^*, 0)$, with

  $$x_1^* = \frac{\pi_1 \pm \sqrt{\pi_1^2 + 4\delta_1(\sigma_1 - 1)}}{2\delta_1}.$$

If $\delta_1 \neq 0$ and $\sigma_1 \geq 1$ there exists only one positive non-zero fixed point on the x_1 axis, which is always stable in the x_1 direction and stable in the x_2 direction if $\sigma_2 < 1$. If $\sigma_1 < 1$ then for $\pi_1^2 + 4\delta_1(\sigma_1 - 1) < 0$ there are two positive stationary points, the larger one stable in the x_1 direction and the smaller one unstable. These are created in a saddle node bifurcation in the parameter σ_1 at $\sigma_1 = 1 - \pi_1^2/4\delta_1$. The eigenvalue in the x_2 direction for both values of x_1^* is $\sigma_2 - 1$, and, hence, if $\sigma_2 < 1$ the largest Th1 response is stable. A threshold effect can thus be observed if $\sigma_1 < 1$; for small initial x_1 the system relaxes back to the no-response state $x_1 = 0$, while larger initial x_1 lead to the non-zero stable state x_1^*.

The efficacy of FasL-induced cell-cell killing (δ_1) is crucial for setting the scale of the response. For small δ_1 a Th1 response can be very large. In the limit $\delta_1 \to 0$ (i.e., no Fas/FasL expression on Th1 effectors), the threshold value of x_1 is $(1 - \sigma_1)/\pi_1$, and $x_1^* \to \infty$. Effects of different values for δ_1 on the Th1-nullcline are shown in the Appendix in Fig. A.2.

- A mixed state $M_1 = (x_1^{**}, x_2^{**})$. This exists when activation signals σ_1 and σ_2 are comparable; it is illustrated in Fig. 4.4.

As an aside, we see from the above that we can switch from an ongoing Th1- to a Th2-response by changing the activation parameter σ_2. On the other hand, increasing σ_1 strengthens the Th1 arm of the coexistent-state but does not eliminate the Th2 component. We return to this asymmetry in Sec. 4.2.6.

4.2.3. The effect of AICD for Th2 cells

If we suppose that Th2 cells are susceptible to AICD (that is, $\delta_2 \neq 0$), the exclusively-Th1 response persists and its stability is enhanced. The eigenvalue λ_1 corresponding to the eigenvector in the x_1 direction is unaffected, but the other eigenvalue becomes

$$\lambda_2 = \sigma_2 - 1 - \delta_2 \left(\frac{\pi_1 + \sqrt{\pi_1^2 + 4\delta_1(\sigma_1 - 1)}}{2\delta_1} \right)^2 .$$

Assume $\sigma_2 > 1$ and $\delta_2 = 0$, and so the pure Th1 response is unstable. As we increase δ_2, a transcritical bifurcation takes place at $(x_1^*, 0)$. The pure Th1 state becomes stable and an unstable co-existent state moves out into the positive quadrant. The more susceptible Th2 cells are to AICD, the larger the basin of attraction for the stable Th1 response. This is illustrated in Fig 4.5. The stability of the pure-Th2 state $(0, x_2^*)$ is not affected by δ_2, as Th1 effectors are required for Th2 AICD in our model.

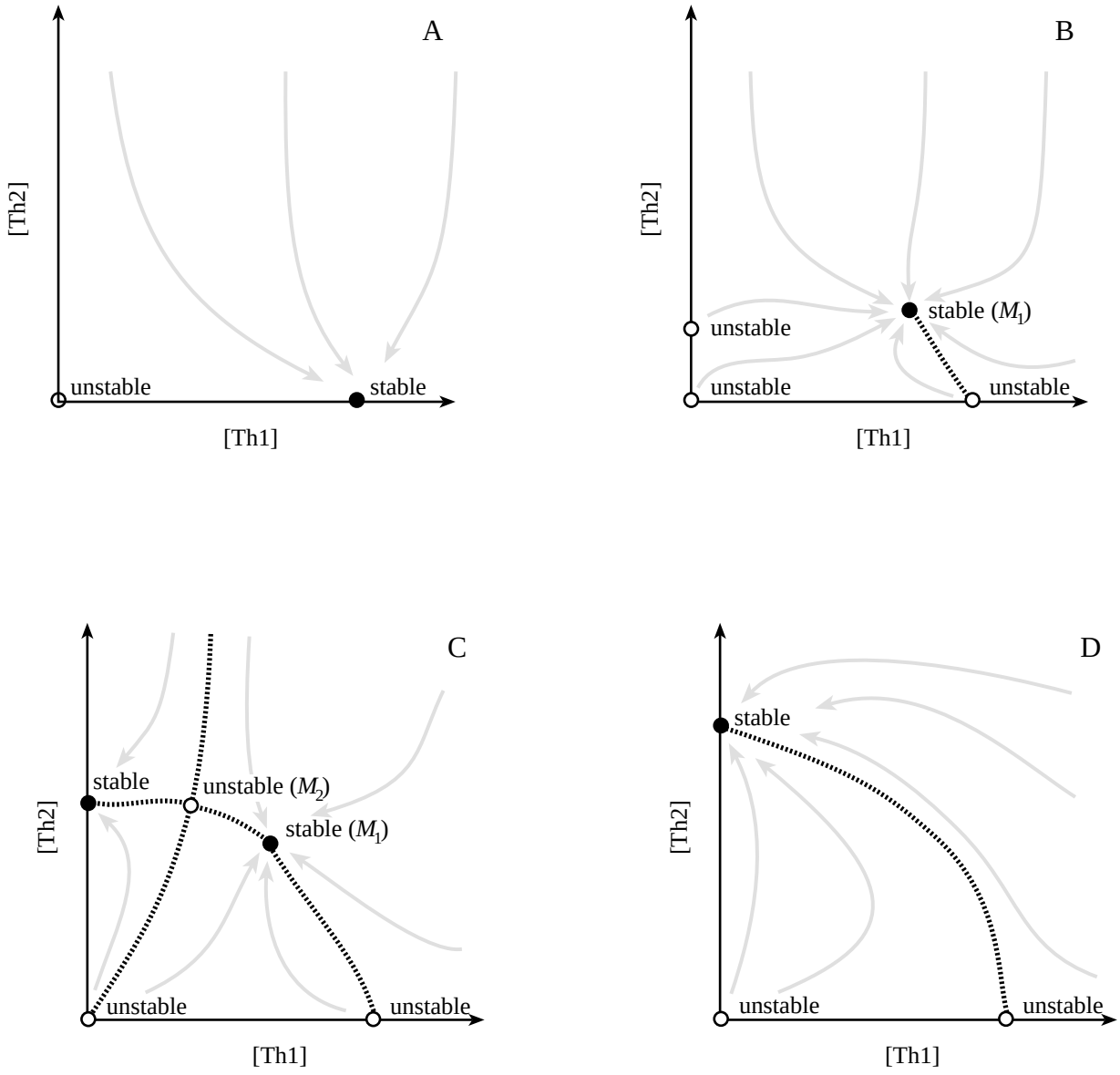


Figure 4.4.: The creation and disappearance of a stable, mixed Th1/2 population with increasing Th2 stimulus (σ_2). In these schematic phase diagrams, open circles represent unstable states and filled circles are stable states. The Th1 activation signal σ_1 is constant (> 1) throughout, ensuring that there is only one non-zero steady state on the Th1 axis. In figure A, $\sigma_2 < 1$ and only the pure Th1 state is stable. As we increase σ_2 through 1, an unstable Th2 state and a stable coexistent state (M_1) appear in the positive quadrant in transcritical bifurcations (B). Increasing σ_2 even further stabilizes the pure Th2 state and an unstable mixed state M_2 appears in another transcritical bifurcation (C). Finally, M_1 and M_2 annihilate in a saddle node and only the Th2 state remains (D).

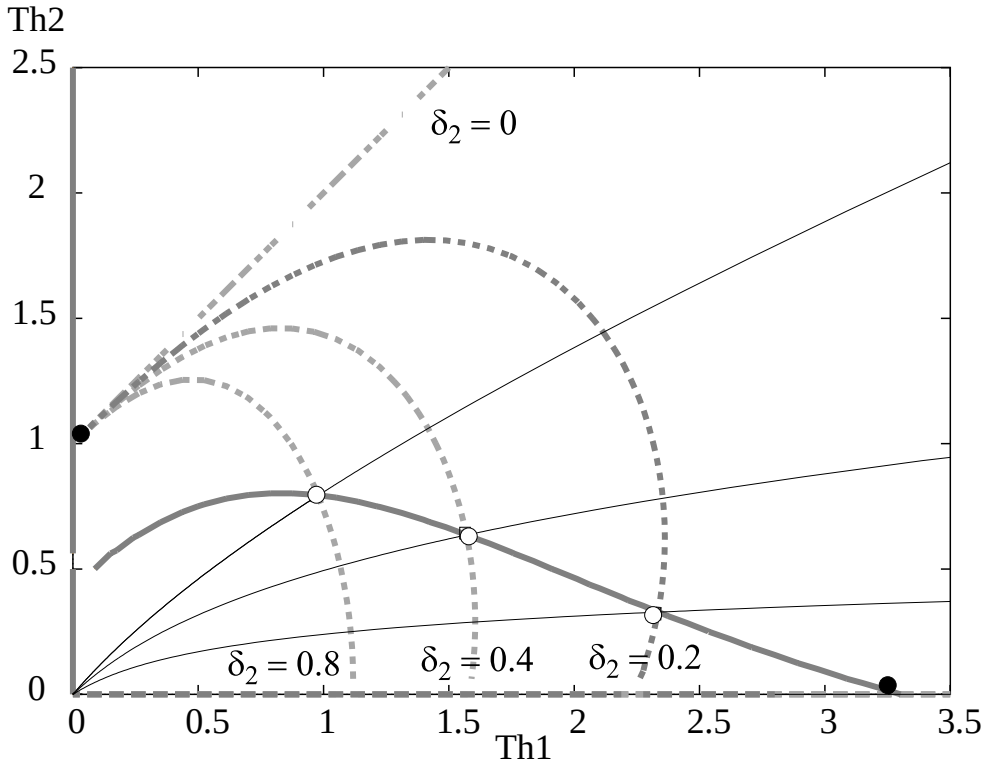


Figure 4.5.: The influence of AICD for Th2 cells on the basins of attraction of the two possible responses of the system. The parameters are $\sigma_1 = \sigma_2 = 2$, $\pi_1 = 3$, $\pi_2 = 2$, $\delta_1 = 1$, $\rho = 0$; δ_2 varies from 0 to 0.8. The Th1 and Th2 nullclines are grey solid and dotted lines, respectively. A stable state (represented by a filled circle) exists at $(x_1^*, 0)$, where $x_1^* \simeq 3.3$; a stable state at $(0, x_2^*)$ (also represented by a filled circle) where $x_2 = 1$ (see Eqn. (4.8)); an unstable state at the origin; and saddles for different values of δ_2 in the positive quadrant, which are marked with open circles. The separatrizes joining the saddle nodes and the origin (represented by thin solid lines) divide the basins of attraction for the pure Th1- and Th2-responses. Here we represent the variation in the position of the saddle nodes with δ_2. At $\delta_2 = 0$ there is no saddle point and the Th1 fixed point is unstable; a bifurcation occurs at $\delta_2 \sim 0.095$, the saddle appears at non-trivial (x_1, x_2) and the Th1 response becomes stable. As we increase the susceptibility of Th2 effectors to AICD, clearly a Th1 response is increasingly favored.

4.2.4. The nature of the Th1 Fas-FasL interaction

The Th1 AICD process may well be autocrine. If this is the dominant mechanism, the relevant death term becomes a single-cell rather than a cell-cell interaction;

$$\delta_1 \left(\frac{x_1}{1+x_2} \right) x_1^2 \longrightarrow \tilde{\delta}_1 \left(\frac{x_1}{1+x_2} \right) x_1$$

where $\tilde{\delta}_1$ is a new parameter, effectively describing a base rate of secretion and binding of soluble FasL. The equation of motion for a pure Th1 population (i.e., with $x_2 = 0$) is then simply $\dot{x}_1 = x_1(\sigma_1 - 1) + x_1^2(\pi_1 - \tilde{\delta}_1)$. In this case the no-response state $x_1 = 0$ is unstable if $\sigma_1 > 1$ and there is only one non-zero steady state, $x_1^* = (\sigma_1 - 1)/(\tilde{\delta}_1 - \pi_1)$. In this simple picture, Th1 cell numbers diverge for $\pi_1 \geq \tilde{\delta}_1$; thus a finite, stable Th1 response only exists if there is sufficient stimulus ($\sigma_1 > 1$) and $\pi_1 < \tilde{\delta}_1$. This would imply, roughly speaking, that the rate of Fas-induced cell death during a response is higher than the rate of cell division. This seems biologically unrealistic. Therefore we continue with the cell-cell model that allows for multiple cell deaths induced by a given Th1 effector expressing FasL.

4.2.5. The influence of TGF-β and IL-10

If we neglect the suppressive influence of TGF-β, our dynamical equations are modified by the removal of a factor of $(1 + x_2)^{-1}$ from the proliferative terms (i.e., those proportional to π_1 and π_2):

$$\frac{\mathrm{d}x_1}{\mathrm{d}\tau} = \sigma_1 \frac{x_1}{(1+x_2)^2} + \pi_1 \frac{x_1^2}{(1+x_2)} - \delta_1 \frac{x_1^3}{1+x_2} - x_1, \tag{4.10}$$

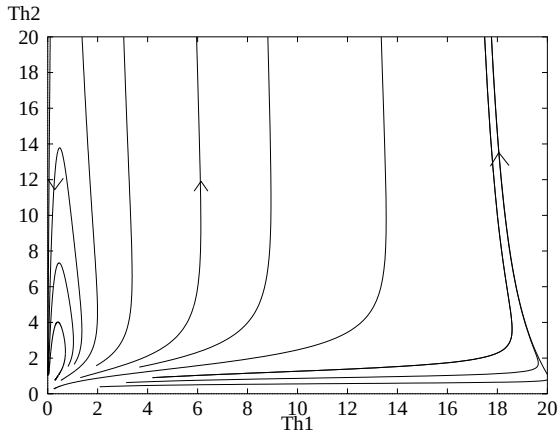
$$\frac{\mathrm{d}x_2}{\mathrm{d}\tau} = \sigma_2 \frac{x_2}{1+x_2} + \pi_2 \frac{[\rho(1+x_2)+x_1]x_2^2}{1+x_1+x_2} - x_2. \tag{4.11}$$

If $\rho > 0$ then the Th2 population diverges for x_2 sufficiently large. However, if we neglect the influence of IL-4 as an autocrine growth factor for Th2 cells (set $\rho = 0$), this divergence is suppressed. This is because removing the term proportional to ρ means that at large x_2 the dominant contribution to Eq. (4.11) is the death term. We then regain the no-proliferation Th2-dominated state $(0, x_2^*)$ where $x_2^* = \sigma_2 - 1$. In this case we observe large, transient Th2 spikes which relax to this non-proliferative Th2 state [see Fig. 4.6(i)]. The suppressive effect of TGF-β has no influence on the magnitude of the exclusively Th1 response, as in our model TGF-β is produced only by Th2/3 cells.

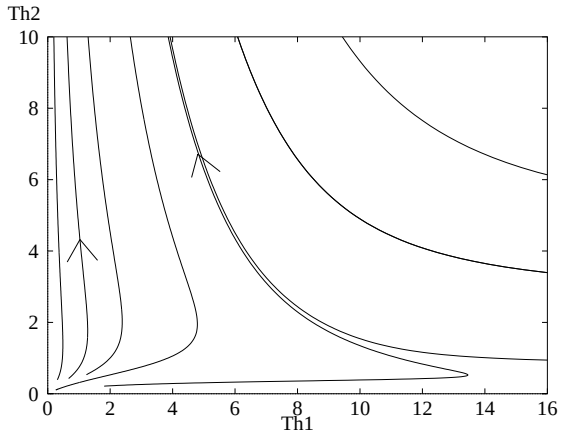
Next we explore the effect of removing the inhibition of T helper cell activation by IL-10. This corresponds to removal of a factor of $(1 + x_2)^{-1}$ from the activation terms (those proportional to σ_1 and σ_2). The dynamical equations Thus take the form

$$\frac{\mathrm{d}x_1}{\mathrm{d}\tau} = \sigma_1 \frac{x_1}{(1+x_2)} + \pi_1 \frac{x_1^2}{(1+x_2)^2} - \delta_1 \frac{x_1^3}{1+x_2} - x_1, \tag{4.12}$$

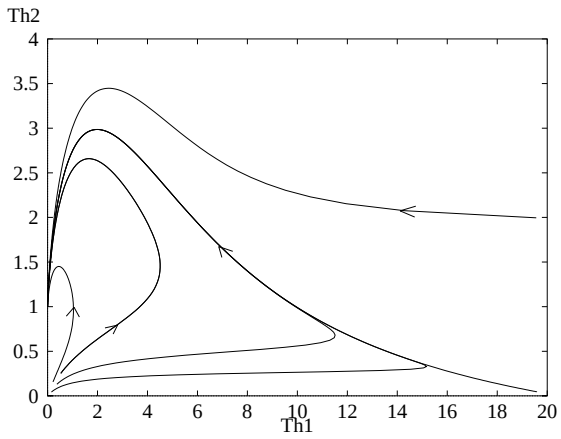
$$\frac{\mathrm{d}x_2}{\mathrm{d}\tau} = \sigma_2 x_2 + \pi_2 \left[\rho + \left(\frac{x_1 + \chi_0}{1+x_2} \right) \right] \frac{x_2^2}{1+x_1+x_2} - x_2. \tag{4.13}$$



(i) without TGF-β suppression



(ii) without IL-10 suppression



(iii) with IL-10 and TGF-β suppression

Figure 4.6.: Homeostatic Th2 regulation. (i) The effect of the absence of TGF-β suppression. The dynamical equations are (4.10) and (4.11) and the parameters are $\sigma_1 = \sigma_2 = 2$, $\pi_1 = \pi_2 = 2$, $\delta_1 = 0.1$, $\delta_2 = 0$, $\rho = 0$. There is a saddle at $(x_1 \simeq 20, x_2 = 0)$ and a stable fixed point at $(x_1 = 0, x_2 = \sigma_2 - 1 = 1)$. Notice the progression Th1 $\rightarrow$ large Th2 response $\rightarrow$ non-proliferative Th2 response. (ii) Removing IL-10 suppression. The stable Th2 state $(x_1 = 0, x_2 = \sigma_2 - 1)$ is removed and we have uncontrolled Th2 responses. (iii) The same parameter set with TGF-β suppression and IL-10 inhibition included [Eqs. (4.6) and (4.7)].

Similar to TGF-β, IL-10 has no influence on the size of Th1 responses as it is not produced by these cells. For all initial conditions with a non-zero Th2 population, we observe an explosion of the Th2 term while the Th1 population vanishes [Fig. 4.6(ii)]. Note the shift from Th1 to Th2 dominance if we start with small Th2 numbers.

In summary we found, that it is the combination of both the suppression of proliferation by TGF-β and the inhibition of activation by IL-10 which ensures that activated Th2 populations sizes remain bounded [Fig. 4.6(iii)]. Experiments indicate the importance of TGF-β in the regulation of Th2 populations. For example, it has been shown that protection against Th2-induced autoimmunity is TGF-β dependent [15]. Further, the function of regulatory T cells, which suppress both Th1 and Th2 immune responses in the mucosa, is dependent on *both* IL-10 and TGF-β [60] and the results from our model are in accord with this observation.

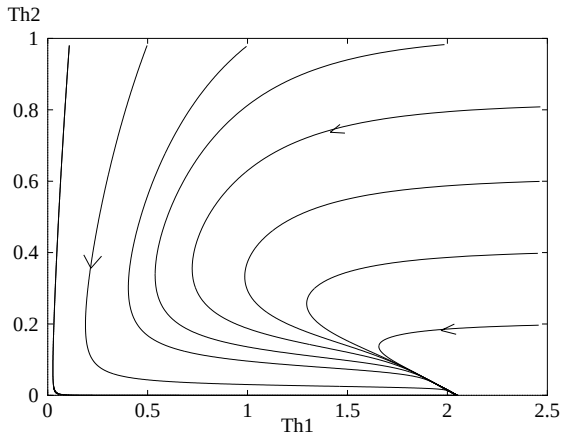
A recent study [56], however, has reported that Fas-deficient mice mount an excessive Th2-driven response to *Trypanosoma cruzi* infection and fail to clear the parasite, whereas successful elimination of the infection occurs through Th1 help and macrophage activation, in combination with up-regulated Fas/FasL expression and high levels of AICD. This suggests that at least some AICD among Th2 cells is normal in wild-type individuals. The most likely scenario is that there is a more complex, differential regulation of the Fas pathway than we have proposed here; nevertheless, we believe that the basic structure of the interactions, with Th1 cells preferentially expressing FasL over Th2, is most in keeping with the experimental observations.

4.2.6. Antigen dose

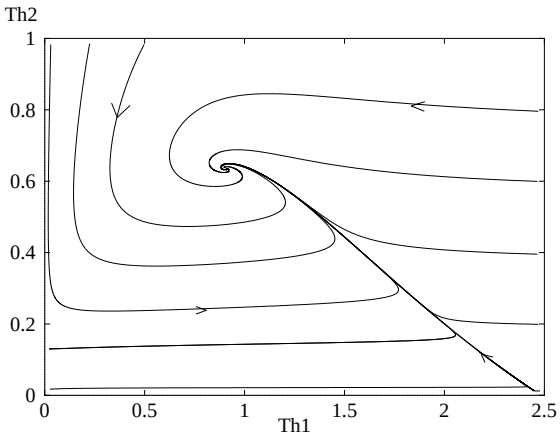
How does a variation of the antigen dose influence the type of the response? The activation efficiency of APCs is dependent on several factors, including the density of MHC-peptide complexes on the surface, which is manipulated by varying the antigen dose, and the interaction strength between the MHC-peptide complex and the T cell receptor. We propose that variation of the concentration of one particular antigen alters the absolute values of the activation parameters σ_1 and σ_2 while preserving their ratio.

Let us assume, for example, that a given antigen is slightly more likely to favor a Th1 than a Th2 response. We set $\sigma_1/\sigma_2 = c > 1$. Changing the antigen dose alters the stimulation strengths σ_1 and σ_2 but keeps their ratio c constant. For low doses of this antigen ($\sigma_2 < 1$), we have stable pure-Th1 states, and with growing activation parameters the Th1 dominance is taken over by an ever-increasing Th2 population. This is illustrated in figure 4.7, where $c = 2$. The above observation is consistent with the experimental observation that low doses favor type 1 responses and intermediate to high doses favor type 2 [64, 73].

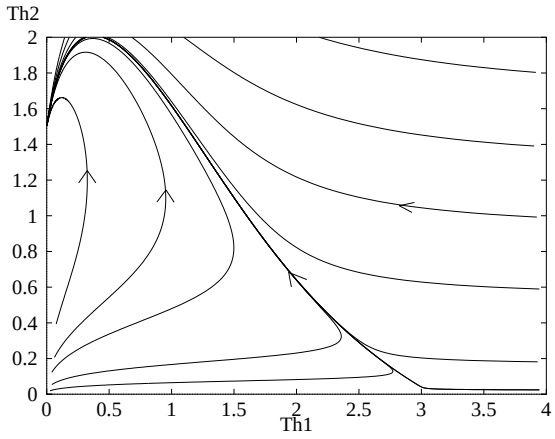
Note, however, that if Th2 cells are sufficient susceptible to Fas-mediated apoptosis ($\delta_2 \gg 0$), the Th1-dominated steady state does not lose its stability and it is then simply the initial conditions which determine whether we end up in a pure type 1 or type 2 state. Over a wide range of parameters, when both Th1 and Th2 cells are susceptible to AICD there exist near-exclusive Th1 and Th2 responses and an unstable mixed-response state [see Fig. 4.8(ii)].



(i) low antigen dose



(ii) medium antigen dose



(iii) high antigen dose

Figure 4.7.: Phase portraits for varying antigen levels, in the absence of Th2 AICD. The parameters are $\sigma_1 = 1.1$, $\sigma_2 = 0.55$, $\pi_1 = \pi_2 = 2$, $\delta_1 = 1$, $\rho = 0.1$, $\delta_2 = \theta_1 = \theta_2 = 0$. (i) Low antigen dose. The only stable fixed point is $(x_1^*, 0)$, where $x_1^* \simeq 2$; no stable Th2-dominated state or coexistence state exists, as $\sigma_2 < 1$. Clearly, low antigen dose favors a Th1 response. (ii) Intermediate antigen dose. We double σ_1 and σ_2 (keeping their ratio constant); other parameters as before. The fixed point $(x_1^*, 0)$ loses its stability in a transcritical bifurcation: with increasing σ_2 a stable coexistence state moves up into the positive quadrant. (iii) High antigen dose. When we again double the activation parameters ($\sigma_1 = 4.4$, $\sigma_2 = 2.2$), the mixed state is lost and the steady state $(0, x_2^*)$ becomes stable. Here high antigen dose leads to Th2 dominance.

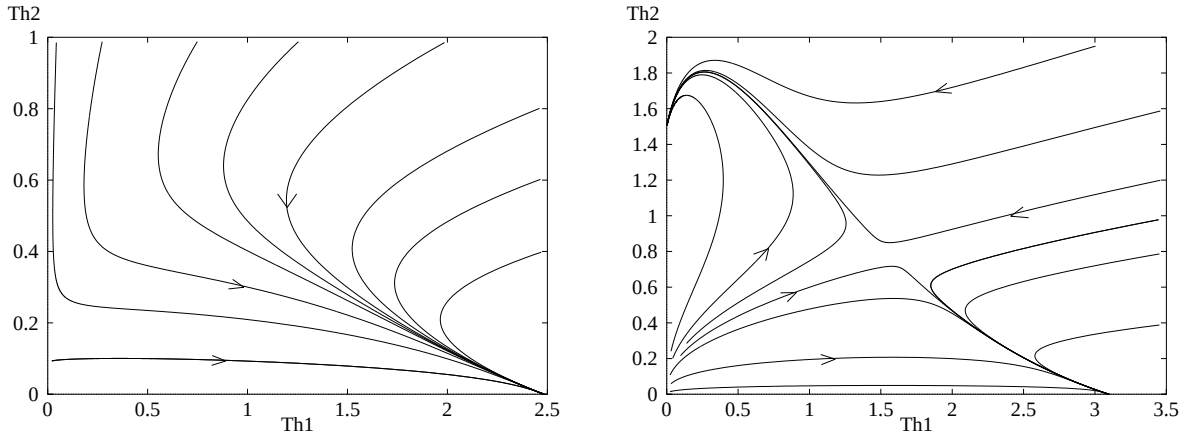


Figure 4.8.: The influence of antigen dose when Th2 cells are susceptible to AICD. We set $\delta_2 = 0.5$, with other parameters as in the previous figure. For low antigen doses, AICD does not alter the system behavior and there is Th1 dominance as in figure 4.7(i). For intermediate antigen concentrations, however, Th1 dominance is still the only steady state (left); at high doses, there are both pure-Th1 and Th2 responses, divided by the stable manifold of the unstable mixed Th1/2 state.

4.2.7. Summary

In this chapter we have developed a model with the following properties.

1. We assume that a non-primed individual mounts a response starting at a state with few antigen-specific T cells;

2. In the absence of Fas-mediated cell death (AICD) for Th2 cells, and for wide ranges of the remaining parameters with $\sigma_2 > 1$, we see one steady state at $(0, x_2^*)$ and dynamics corresponding to an initial Th1 response followed by a chronic (stable) Th2-dominated state. If $\sigma_2 < 1$ there is Th1 dominance with no transient Th2 response;

3. The absence of Fas/FasL interactions leads to uncontrolled inflammatory (type 1) responses. Further, an suicidal AICD process is not sufficient to regulate Th1 cell numbers and a cell-cell interaction is required;

4. The susceptibility of Th2 cells to AICD (parameterized by δ_2) stabilizes the existence of exclusive Th1 responses. For wide ranges of parameters we have stable Th1 and Th2 responses and an unstable coexistent state, with the areas of the basins of attraction governed by the relative sizes of the stimuli σ_1 and σ_2, as expected;

5. TGF-β is essential to avoid explosive expansion of Th2 numbers if any autocrine (IL-4-driven) growth among these cells takes place. However, if Th2 cells require *both* IL-2 and IL-4 for growth ($\rho = 0$), the absence of the suppressive effect of TGF-β leads to a large, transient but bounded Th2 response.

6. IL-10 is an essential component of the control mechanism for Th2 populations.

7. For large regions of parameter space, with Th2 cells less susceptible to AICD than Th1, we see transient Th1-dominance followed by a stable Th2 response.

8. Lower antigen doses tend to favor Th1 responses; higher doses favor Th2.

4.3. Further refinements

To investigate the behavior of the full model we now introduce a Th0 source of IL-2 ($\chi_0 > 0$) and T helper cell-independent activation signals ($\theta_1 > 0$, $\theta_2 > 0$).

4.3.1. IL-2 production by Th0

The effect of IL-2 production by Th0 cells is modeled by the parameter χ_0 in Eqns. (4.4) and (4.5). It represents the number of IL-2-producing Th0 cells. The pure-Th2 response $(0, x_2^*)$, with

$$x_2^* = 1/2 \left(-2 + \sigma_2 + \pi_2\chi_0 + \sqrt{\sigma_2^2 + 2\pi_2\sigma_2\chi_0 + \pi_2\chi_0(\pi_2\chi_0 - 4)} \right),$$

increases monotonically with χ_0. This enhancement is clearly due to the fact that Th2 cells do not synthesize IL-2 themselves, which is a very effective growth factor, and the increase in AICD rates does not affect the population size as Th1 cells are absent. The magnitude of the pure Th1 response is

$$x_1^* = \frac{\pi_1 - \delta_1\,\chi_0 + \sqrt{4\,\delta_1\,(\sigma_1\pi_1 + \chi_0 - 1) + (\pi_1 - \delta_1\,\chi_0)^2}}{2\,\delta_1}.$$

Here the interplay between enhancement of proliferation and AICD is more complex. The overall effect of χ_0 is a slight reduction of the pure Th1 response where we have made the biological realistic assumptions that Th1 undergo AICD, Th1 proliferation is higher than removal, and Th1 activation rates are sufficiently large ($\delta_1 > 0, \pi_1 > 1, \sigma_1\pi_1 > 1$).

4.3.2. T helper-independent activation signals

As described in Sec. 3.2 and 4.1.2, the components of the innate immune system and the local microenvironment may modify APC activation states. We model this by including the terms proportional to θ_1 and θ_2 in Eqns. (4.4) and (4.5). The most obvious effect of θ_1 and θ_2 is that the origin as no longer a fixed point and that the axes are no longer nullclines.

Triggering a Th1 response

Altering the rate of AICD for Th2 cells (δ_2) can give rise to profoundly different types of behavior. Let us look at a situation where the only stable steady state of the system is Th2-dominated, which is a robust feature of the system when Th2 cells are not susceptible to AICD as we have seen before. This allows us to explore how stimuli from the innate immune system help to trigger a Th1 or Th2 reaction.

If Th2 cells do not undergo AICD ($\delta_2 = 0$) then even with a high θ_1 stimulus, the long-term Th2 dominance cannot be switched to Th1. We can see this by examining the Th2 proliferation term, which is then the only route of influence of Th1 cells, since Th2 activation is not affected by Th1 cells;

$$\pi_2 \left[\rho + \left(\frac{x_1 + \chi_0}{1 + x_2} \right) \right] \frac{x_2^2}{1 + x_1 + x_2}.$$

If there is no IL-2 independent proliferation ($\rho = 0$), the Th2 nullcline monotonically increases for $\pi_2 > 1$. This implies that boosting the Th1 activation signal through increased σ_1 or θ_1 also enhances the size of the Th2 population – illustrated in terms of nullclines in Fig. (4.9). This is purely due to the proliferative influence of IL-2, which is counterbalanced at high Th1 concentrations in our model by the anti-proliferative effect of IFN-γ. The overall influence of Th1 cells is a positive, saturating contribution to the Th2 proliferation rate. For small ρ, this picture is essentially unchanged as the proliferation driven by IL-4 alone makes a small contribution to the size of the Th2 population.

Despite this effect on the location of the steady state, θ_1 influences the dynamics by promoting transient Th1 dominance (Fig. 4.10). Even small, external pro-Th1 influences result in large detours towards a Th1-biased response which finally settles into the Th2 state. This assumes of course that antigen clearance has not taken place in the meantime.

If Th2 cells are comparable susceptible to AICD as Th1, then the system exhibits a more intuitive behavior. Effects of Th1-signals θ_1 on the nullclines are illustrated in Fig. 4.11. Small pro-Th1 influences do not lead to noticeable effects, and in theory very high Th1-stimuli lead to global stability of the pure Th1 response. We assume, however, that this is not biologically realistic due to saturation of both cytokine effects and the activating capacity of antigen presenting cells. Thus increasing θ_1 alone (*e.g.* through exogenous IL-12) is never sufficient to induce a permanent switch from a Th2 to a Th1 response (see Sec. 4.3.3)).

Triggering a Th2 response

We start with a parameter set that describes a Th1-dominated system ($\sigma_1 > 1$, $\sigma_2 < 1$, $\pi_1 \sim \pi_2 \sim 2$, $\delta_1 = 1$, $\delta_2 = 0.5$, $\rho = 0.1$, $\theta_1 = \theta_2 = \chi_0 = 0$). Increasing the Th2-stimulus θ_2 leads to coexistence and/or bistability with a pure Th2 response and mixed Th1/Th2 populations and then finally to Th2 dominance, independently of the susceptibility of Th2 cells to AICD. This is illustrated in Fig. 4.12.

The instructive role of innate immunity and its contribution to the decision-making process are still not entirely clear. However, we see from the simple observations above

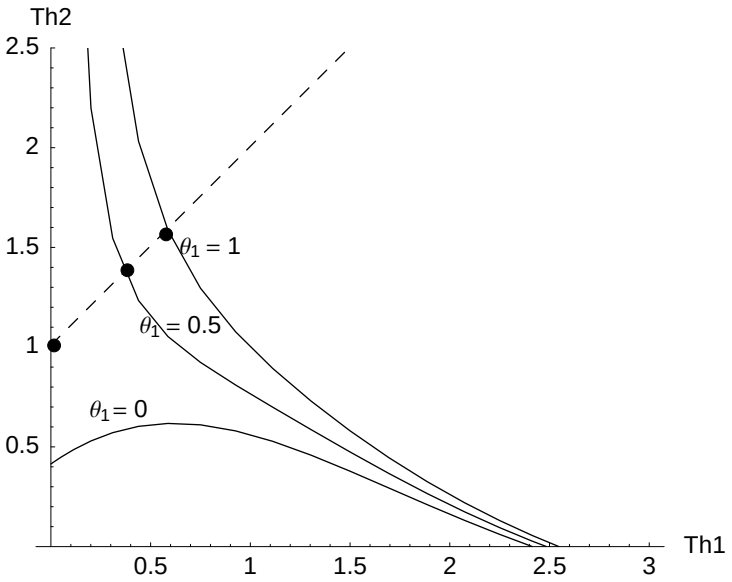


Figure 4.9.: Effects of Th1 signals. The Th1-nullcline (solid line) is shown for various values of a Th1-promoting signal θ_1. The Th2 nullcline is drawn as a dashed line, steady states as filled circles. Increasing the T helper independent activation signal ($\theta_1 > 0$) enhances both the size of the Th1 population and the size of the Th2 population provided that Th2 cells do not undergo AICD ($\delta_2 = 0$). Therefore, boosting Th1 activation does not lead to a shift towards Th1 dominance. Other parameters are $\delta_1 = 1$, $\pi_1 = \pi_2 = 2$, $\sigma_1 = \sigma_2 = 2$.

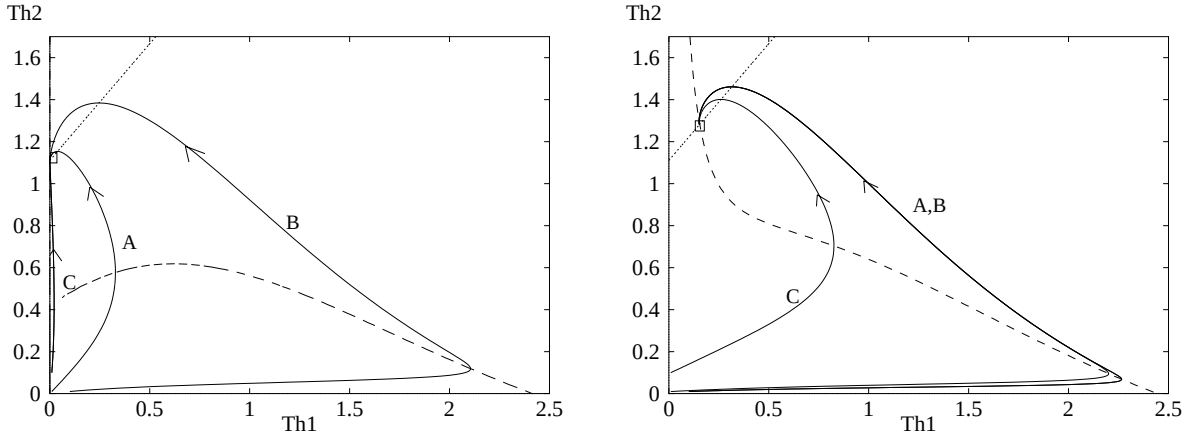


Figure 4.10.: The influence of a Th1 stimulus θ_1 on steady states and orbits. The parameters are $\sigma_1 = \sigma_2 = 2$, $\pi_1 = \pi_2 = 2$, $\delta_1 = 1$, $\delta_2 = 0$, $\rho = 0.1$, $\chi_0 = 0$, $\theta_2 = 0$; θ_1 is set to 0 and 0.2 in the left and right plots, respectively. The dashed and dotted lines correspond to Th1- and Th2-nullclines, respectively. The solid lines are orbits for various initial conditions; A (0.01,0.01), B (0.1, 0.01), C (0.01,0.1). Note that small θ_1-values do not substantially alter the position of the Th2-dominated state but *do* affect the transient Th1 dominance.

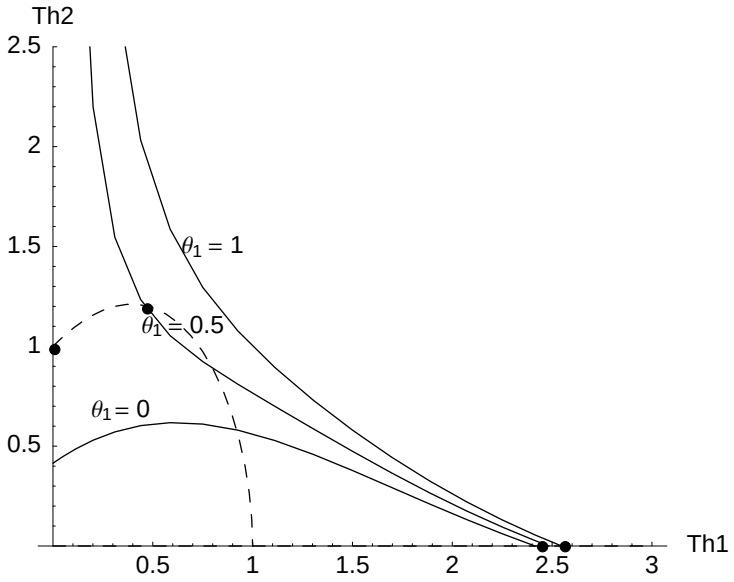


Figure 4.11.: Effects of Th1 signals. The Th1-nullcline (solid line) is shown for various values of a Th1-promoting signal θ_1. The Th2 nullcline is shown as a dashed line, steady states as filled circles. Provided that Th2 cells undergo AICD ($\delta_2 = \delta_1$), an increase of T helper independent signals ($\theta_1 > 0$) leads to a shift from Th2 dominance to a bistability of Th1 dominance and a mixed Th1/Th2 state ($\theta = 0.5$), or to Th1 dominance alone ($\theta_1 = 1$). Other parameters are $\delta_1 = 1$, $\pi_1 = \pi_2 = 2$, $\sigma_1 = \sigma_2 = 2$.

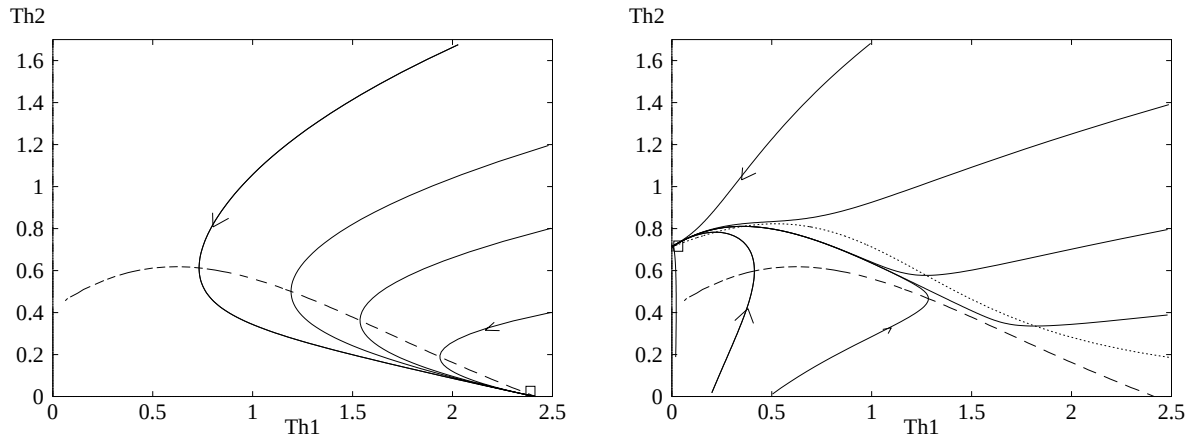


Figure 4.12.: Shifting from Th1- to Th2-dominance by increasing the pro-Th2 co-stimulatory signal θ_2 from 0 (left) to 1 (right). Th1-nullclines are drawn as dashed, Th2-nullclines as dotted lines. The remaining parameters are $\sigma_1 = 2$, $\sigma_2 = 0.8$, $\pi_1 = \pi_2 = 2$, $\delta_1 = 1$, $\delta_2 = 0.5$, $\rho = 0.1$, and $\theta_1 = \theta_2 = \chi_0 = 0$.

that there is a fundamental asymmetry in the Th1-Th2 interactions that manifests itself under chronic stimulus. Short-term changes in the nature of the immune response can be induced by changing θ_1 or θ_2 in an intuitively obvious way.

4.3.3. Treatment with IL-12 to induce a Th2 to Th1 switch

Here we address the question of whether the Th1/Th2 outcome of an immune response can be altered by treatment with cytokines. We begin by supposing that, for example, constant treatment with IL-12 simply changes the Th1-independent activation parameter θ_1 (see Sec. 4.3.2).

Experiments have shown that a treatment with IL-12 alone is not sufficient to shift an established Th2 response to Th1 and that a reduction of the antigen dose is also needed [74]. This is in good agreement with our observations regarding θ_1 in the previous section. Fig. 4.13 shows examples of a simulated cytokine treatment in our model.

Note that the switch from Th2 to Th1 dominance can only be induced when Th2 cells are susceptible to AICD ($\delta_2 \neq 0$). The results of Nabors *et al.* [74] in conjunction with those from our model would indicate that Th2 cells *do* undergo Fas induced cell death to at least some small degree.

4.4. Autoimmunity

Autoimmune disease is simply tissue damage due to immune responses to self-antigens. Examples are diabetes, in which specific T cells attack the pancreatic beta-islet cells responsible for the production of insulin, and multiple sclerosis, in which an immune response is mounted against the myelin sheath of nerves. The majority of serious autoimmune disorders, such as these, are driven by Th1 effectors.

Susceptibility to autoimmune disease is influenced by environmental and genetic factors. For example, it may be caused by genetic defects such as Fas-deficiency, or the presence of certain MHC alleles that are correlated with autoimmune incidence. Autoimmunity seems likely to be caused by the breakdown of regulation mechanisms – such as AICD – leading to failure in eliminating autoimmune cells (for a review see [69]). In terms of our model this would correspond to low AICD rates δ_1 and δ_2. Additional experimental data supports the idea that microbial agents affect the occurrence or course of certain autoimmune diseases due to molecular mimicry (i.e., immunological cross-reactivity between anti-pathogen and anti-self responses) and bystander activation [7]. The concept of bystander activation proposes that pathogens disturb self-tolerance by increasing the visibility or abundance of self-antigens, attracting and potentiating APCs or by perturbing the cytokine balance through inflammation that is associated with infection. All these factors may lead to sufficiently high activation rates of otherwise quiescent, potentially T helper cells specific for self antigens that are present in normal individuals. We would interpret this in the language of our model simply as small activation parameters $\sigma_i < 1$.

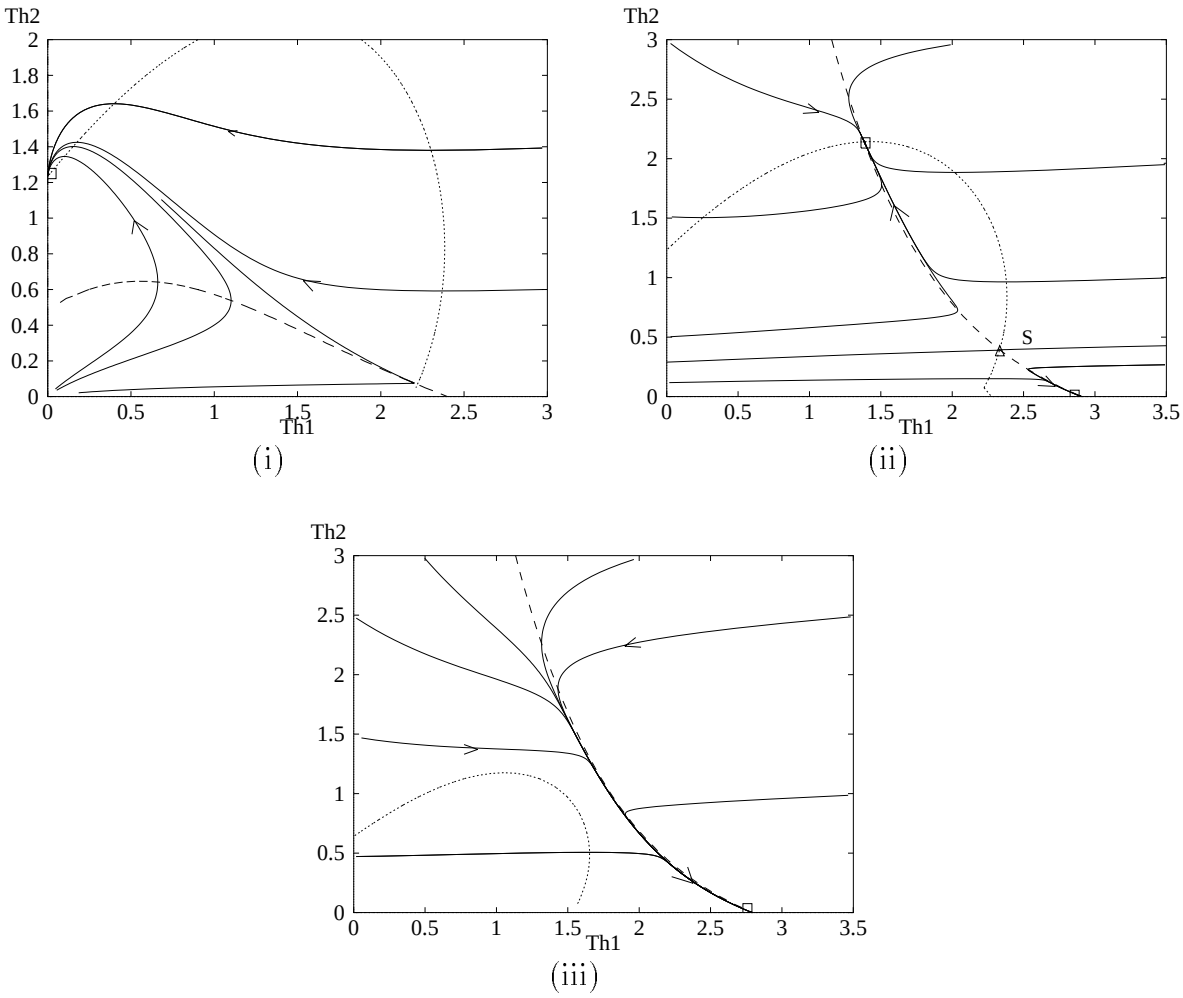


Figure 4.13.: Modification of an immune response with externally applied cytokines. (i) We start with parameters $\sigma_2 = \sigma_1 = 2$, $\pi_1 = \pi_2 = 2$, $\delta_1 = 1$, $\delta_2 = 0.2$, $\rho = 0.1$, $\chi_0 = 0.1$, $\theta_1 = \theta_2 = 0$. Th1-nullclines are drawn as dashed lines, Th2-nullclines as dotted lines. Stable steady states are represented by boxes, saddle nodes by triangles. The only attractor is a pure Th2 response and no exogenous IL-12 is present. (ii) Injecting IL-12 increases the value of the parameter $\theta_1 \to 5$. All other parameters are as before. The stable manifold of the saddle S divides the phase space and a pure Th1 state now exists. Note that starting at the pure Th2 state leads to coexistence of the two phenotypes and not to inversion of the Th1/Th2 ratio. (iii) However, if we additionally decrease the antigen dose ($\sigma_1 = \sigma_2 = 1.5$) all orbits end up in the pure Th1 state.

For activation levels that occur in infection our model predicts that Th2 responses tend to dominate under persistent antigenic stimulus (i.e., in a chronic situation). This is a reasonable choice of the immune system as long-term inflammatory responses are potentially far more damaging to self, and so a Th2 response may well normally be the 'safe' riposte to persistent stimulus. However, as noted above, the majority of serious autoimmune disorders are driven by Th1 effectors. Can we understand how chronic, damaging Th1 responses occur within the terms of our model?

Activation parameters $\sigma_i < 1$ tend to lead to Th1 dominance (section 4.2.6). In the context of autoimmunity T helper independent activation signals do not exist. Self-antigens are not recognized by pattern recognition receptors on APCs and do not induce danger signals, which activate APCs to generate activation signals. In absence of T helper independent activation signals ($\theta_1 = \theta_2 = 0$) and if $\sigma_i < 1$ ($i = 1, 2$), then $(0, 0)$ and an exclusive Th1 response $(x_1^*, 0)$ are the only stable states, with basins of attraction separated by the stable manifold of a second, smaller Th1 state which is a saddle. In this case, disease could be induced by injection of autoimmune Th1 cells, corresponding to initial conditions in the basin of attraction for $(x_1^*, 0)$ rather than the no-response state $(0, 0)$. Induction of autoimmunity in this way is observed in mouse models.

The frequently-observed relapses of autoimmune disorders (in arthritis [38], for example) could conceivably be traced back to oscillatory Th1-Th2 responses, in which harmful Th1 proliferative periods are interspersed with 'safe' Th2 responses to ever-present antigenic stimulus. This failure to make a consistent decision may be linked to the level of antigen stimulus and deficiencies in AICD as demonstrated above. In Sec. 4.5 we will demonstrate that in terms of our model oscillations are possible for parameter values that we suggest to occur in autoimmune situations.

4.4.1. Peptide vaccination

This section attempts to provide an explanation why peptide vaccination, i.e., treatment with peptides of self-antigens, helps to arrest diabetes and other Th1-dominated autoimmune diseases such as EAE, the mouse model of multiple sclerosis.

Type I diabetes is caused by autoimmune T killer cells that attack the insulin-producing β-cells of the islets. These cytotoxic T cells are part of the cell-mediated arm of the immune response promoted by T helper cells of type I. The autoimmune immune response is associated with specific Th1 cell reactivity to a variety of self-antigens including a peptide (p277) of the 60kDa heat shock protein (hsp60), peptides of the glutamic acid decarboxylase (GAD), and insulin [94]. It has been reported that vaccination with the peptides of self-antigens such as p277 [26] , GAD65 [93], or insulin [106] arrested the progression of diabetes. The following observations have been made:

(i) A single administration of peptides could inhibit the cascade of Th1 responses that lead to diabetes.

(ii) A successful treatment of the autoimmune process was associated with the induction of production of IL-4 and IL-10, cytokines of T helper type II, accompanied by a

sharp fall in the Th1 cytokine IFN-γ. Thus, a pathogenic Th1-type response to the peptide such as p277 is replaced by a Th2-type response to p277.

(iii) Interestingly, the Th2 response to peptide therapy does not persist. Instead, a spontaneous decline of the type II response – cytokines IL-4 and IL-10 – some months after the peptide therapy can be observed and thus chronic Th2-type autoimmunity is avoided.

(iv) Resetting the cytokine response to a single epitope – such as p277 – can spread to existing T-cell responses to other auto-antigens involved in diabetes, such as GAD and insulin. This process has been termed determinant spreading.

How can we explain these observations in terms of our model? For the moment – to allow phase plane analysis – we restrict ourselves to a situation with only *one* self-antigen. Recalling the results of Sec. 4.2.6 there is the following intuitive explanation of the success of peptide vaccination. According to Sec. 4.4 diabetes and other Th1-dominated autoimmune responses can be interpreted as immune responses against self with low activation levels. Here, autoimmune reactions would correspond to an exclusive Th1-dominated steady state of the system that exists and is stable under low activation levels. On the other hand, health is represented by the 'non-response' state, which is also stable for small Th1 activation parameters ($\sigma_1 < 1$, see also discussion of the exclusive Th1 steady state). Peptide vaccination increases the concentrations of certain self-antigens and due to the antigen dose dependency (cf. Sec. 4.2.6) the Th1-dominated response against the particular self-antigen is shifted towards Th2-dominance. With decreasing levels of administrated self-antigens due to peripheral diffusion the exclusive Th2 response looses its stability and – as before treatment – the system can end up in the 'non-response' or an exclusive Th1 steady state. In contrast to the situation before treatment the system now – after having reached a Th2-dominated state – starts at an initial condition that lies in the basin of attraction of the healthy 'non-response' state. This leads to the arrest of Th1- and Th2-dominated autoimmunity. This 'gedanken-experiment' – for more details see below – provides an explanation for the experimental observations (i)-(iii).

To analytically investigate the outcome of peptide therapy we incorporate self-antigen levels into the rate equations. (Please note that constant antigen levels in Eq. (4.4) and (4.5) have been directly incorporated into the activation parameters whereas self-antigen levels are treated as separate variables.) The constant parameter p_{self} reflects the level of a certain self-antigen u present in the body whereas p_{vacc} represents the level of self-antigen u added by peptide vaccination. We assume that the concentration of the administrated peptide falls exponentially with rate r. T helper independent activation signals θ_1, θ_2 generated by APCs do not occur in the context of self-antigens. This leads to the equations

$$\frac{\mathrm{d}x_1}{\mathrm{d}\tau} = \sigma_1(p_{\mathrm{self}} + p_{\mathrm{vacc}})\frac{x_1}{(1+x_2)^2} + \pi_1 x_1 \frac{x_1+\chi_0}{(1+x_2)^2} - \delta_1\left(\frac{x_1+\chi_0}{1+x_2}\right)x_1^2 - x_1, \tag{4.14}$$

$$\frac{\mathrm{d}x_2}{\mathrm{d}\tau} = \sigma_2(p_{\mathrm{self}} + p_{\mathrm{vacc}})\left(\frac{x_2}{1+x_2}\right) + \pi_2\left[\rho + \left(\frac{x_1+\chi_0}{1+x_2}\right)\right]\frac{x_2^2}{1+x_1+x_2} - \delta_2\left(\frac{x_1+\chi_0}{1+x_2}\right)x_1x_2 - x_2, \tag{4.15}$$

$$\frac{\mathrm{d}p_{\mathrm{vacc}}}{\mathrm{d}\tau} = -rp_{\mathrm{vacc}}. \tag{4.16}$$

The variation of $p_{\mathrm{vacc}}(t)$ resulting from the vaccination changes the shape of the nullclines and therefore the steady states of the system. This is illustrated in Fig. 4.4.1.

It remains to be shown whether determinant spreading, i.e., resetting the cytokine secretion pattern in response to one epitope spreads to other antigens, can also be explained. To this end we extend Eq. (4.2) and (4.3). For the sake of simplicity we assume that there are only two self-antigens u and w with corresponding specific T helper subsets $T_{u_1}, T_{w_1}, T_{u_2}, T_{w_2}$. Peptide vaccination will be performed with self-antigen u. Because cytokines act systemically Th1 and Th2 cytokine signals can be described as $S_1 = \alpha_1(T_{u_1} + T_{w_1})/(1 + k\alpha_2(T_{u_2} + T_{w_2}))$ and $S_2 = \alpha_2(T_{u_2} + T_{w_2})$. Cytokines produced by naive Th0 and APCs are neglected here. Proliferation and apoptosis parameters β_i and Δ_i are assumed to be equal for both peptides whereas activation parameters ξ_{u_i} and ξ_{w_i} can be unequal due to different affinities for the specific T cell receptors. IL-2 production of Th0 cells is neglected because of its weak impact. The rate equations take the form

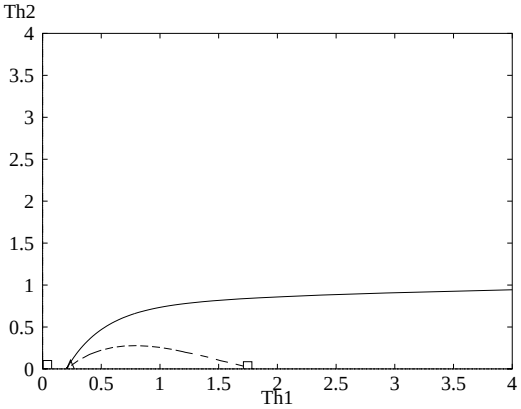
$$\frac{\mathrm{d}T_{u_1}}{\mathrm{d}t} = \frac{(p_{u_{\mathrm{self}}} + p_{u_{\mathrm{vacc}}})\xi_{u_1}S_1}{1+kS_2} + \frac{\beta_1 S_1 T_{u_1}}{1+kS_2} - \Delta_1 S_1 T_{u_1}(T_{u_1} + T_{w_1}) - \mu T_{u_1}, \tag{4.17}$$

$$\frac{\mathrm{d}T_{u_2}}{\mathrm{d}t} = \frac{(p_{u_{\mathrm{self}}} + p_{u_{\mathrm{vacc}}})\xi_{u_2}S_2}{1+kS_2} + T_{u_2}\frac{(\beta_2^{IL4}S_2 + \beta_2^{IL2/4}S_1S_2)}{(1+kS_1)(1+kS_2)} - \Delta_2 S_1(T_{u_1} + T_{w_1})T_{u_2} - \mu T_{u_2}, \tag{4.18}$$

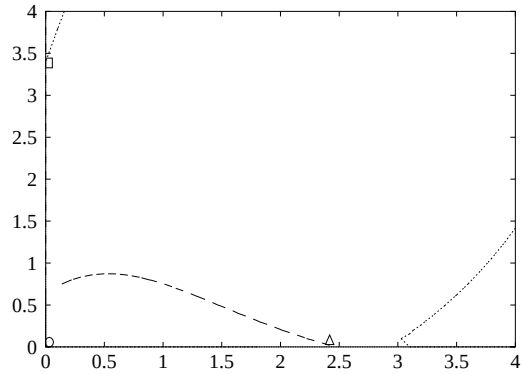
$$\frac{\mathrm{d}T_{w_1}}{\mathrm{d}t} = \frac{p_{v_{\mathrm{self}}}\xi_{w_1}S_1}{1+kS_2} + \frac{\beta_1 S_1 T_{w_1}}{1+kS_2} - \Delta_1 S_1 T_{w_1}(T_{u_1} + T_{w_1}) - \mu T_{w_1}, \tag{4.19}$$

$$\frac{\mathrm{d}T_{w_2}}{\mathrm{d}t} = \frac{p_{v_{\mathrm{self}}}\xi_{w_2}S_2}{1+kS_2} + T_{w_2}\frac{(\beta_2^{IL4}S_2 + \beta_2^{IL2/4}S_1S_2)}{(1+kS_1)(1+kS_2)} - \Delta_2 S_1(T_{u_1} + T_{w_1})T_{w_2} - \mu T_{w_2}. \tag{4.20}$$

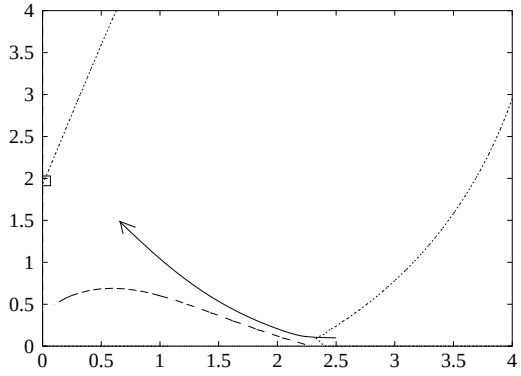
$$\frac{\mathrm{d}p_{u_{\mathrm{vacc}}}}{\mathrm{d}t} = -\varrho p_{u_{\mathrm{vacc}}} \tag{4.21}$$



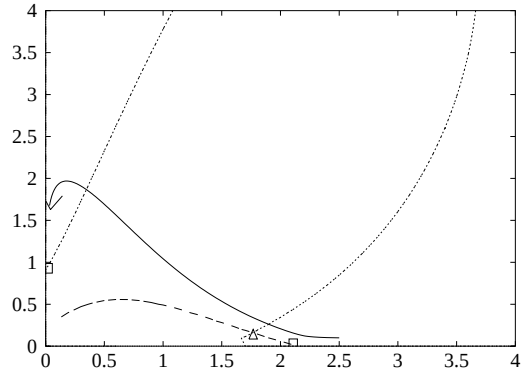
(i) before treatment



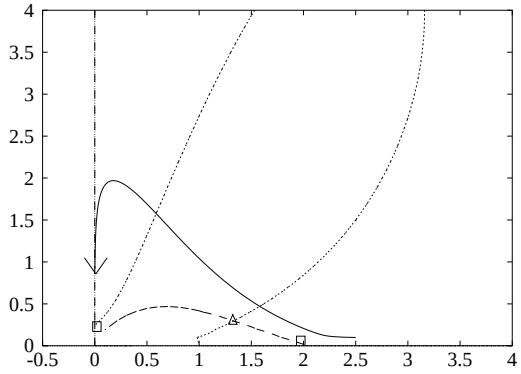
(ii) directly after vaccination (t=0)



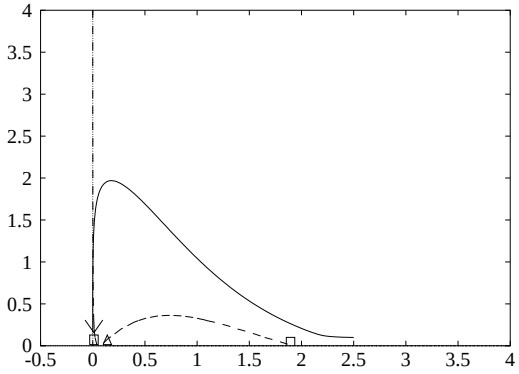
(iii) (t=4)



(iv) (t=8)



(v) (t=12)



(vi) (t=20)

The above expressions for the cytokine signals have been incorporated and the system has been non-dimensionalized with the terms $x_{u_i} = k\alpha_i T_{u_i}$, $x_{w_i} = k\alpha_i T_{w_i}$, $\tau = \mu t$, $\sigma_{u_i} = \xi_{u_i}\alpha_i/\mu$, $\sigma_{w_i} = \xi_{w_i}\alpha_i/\mu$, $\delta_i = \Delta_i/\mu k\alpha_i$, $\pi_1 = \beta_1/\mu k$, $\pi_2 = \beta_2^{IL2/4}/\mu k$, $\rho = \beta_2^{IL2}k/\beta_2^{IL2/4}$, $r = \varrho/\mu$. The resulting dynamical equations are

$$\frac{\mathrm{d}x_{u_1}}{\mathrm{d}\tau} = (p_{u_{\mathrm{self}}} + p_{u_{\mathrm{vacc}}})\sigma_{u_1}\frac{(x_{u_1}+x_{w_1})}{(1+x_{u_2}+x_{w_2})^2} + \pi_1 x_{u_1}\frac{(x_{u_1}+x_{w_1})}{(1+x_{u_2}+x_{w_2})^2} - \delta_1\frac{x_{u_1}}{1+x_{u_2}+x_{w_2}}(x_{u_1}+x_{w_1})^2 - x_{u_1}, \tag{4.22}$$

$$\frac{\mathrm{d}x_{u_2}}{\mathrm{d}\tau} = (p_{u_{\mathrm{self}}} + p_{u_{\mathrm{vacc}}})\sigma_{u_2}\frac{(x_{u_2}+x_{w_2})}{1+x_{u_2}+x_{w_2}} - \delta_2\frac{(x_{u_1}+x_{w_1})^2}{1+x_{u_2}+x_{w_2}}x_{u_2} - x_{u_2} + \pi_2\left[\rho + \left(\frac{x_{u_1}+x_{w_1}}{1+x_{u_2}+x_{w_2}}\right)\right]\frac{x_{u_2}(x_{u_2}+x_{w_2})}{1+x_{u_1}+x_{w_1}+x_{u_2}+x_{w_2}}, \tag{4.23}$$

$$\frac{\mathrm{d}x_{w_1}}{\mathrm{d}\tau} = p_{w_{\mathrm{self}}}\sigma_{w_1}\frac{(x_{u_1}+x_{w_1})}{(1+x_{u_2}+x_{w_2})^2} + \pi_1 x_{w_1}\frac{(x_{u_1}+x_{w_1})}{(1+x_{u_2}+x_{w_2})^2} - \delta_1\frac{x_{w_1}}{1+x_{u_2}+x_{w_2}}(x_{u_1}+x_{w_1})^2 - x_{w_1}, \tag{4.24}$$

Figure 4.4.1 (previous page): Phase portraits before and after peptide vaccination. The parameters are $\sigma_1 = \sigma_2 = 1.5$, $\pi_1 = \pi_2 = 3$, $\delta_1 = 1.5$, $\delta_2 = 0.2$, $\rho = 0.1$, $\chi_0 = 0, p_{\mathrm{vacc}}(0) = 1.5, p_s = 0.3, r = 0.1$. Stable steady states are represented by boxes, saddle nodes by triangles. Th1-nullclines correspond to dashed lines, Th2-nullclines to dotted lines and, orbits or separatrizes to solid lines. (i) before treatment $[p_{\mathrm{vacc}}(t) = 0]$. Low activation rates lead to two stable steady states: a Th1-dominated and a 'non-response' state. Its basins of attraction are divided by the separatrix drawn as solid line. Autoimmune disease corresponds to the exclusive Th1 state, which will also be the initial condition for the system directly after treatment (figure (ii) with added antigen concentration $p_{\mathrm{vacc}}(0) = 1.5$). Administration of self-antigens increases activation rates and leads to an exclusive Th2 steady state as the only possible attractor. Figure (iii) $[p_{\mathrm{vacc}}(4) = 1.01]$. After 4 time units the system starting at the Th1-dominated steady state has moved towards Th2-dominance; Figure (iv) $(p_{\mathrm{vacc}}(8) = 0.67)$. Th2-dominance is reached; Figure (v) $[p_{\mathrm{vacc}}(12) = 0.45]$. With decreasing concentrations of administrated self-antigen the exclusive Th2 state moves towards the 'non-response' state and therewith the system state; Figure (vi) $[p_{\mathrm{vacc}}(20) = 0.20]$. Concentration of the self-antigen has nearly dropped to the starting point. As in (i) the 'non-response' and the exclusive Th1 steady state are the only stable steady states. However, when the exclusive Th2 steady state, in which the system has ended up, looses its stability, the system state lies in the basin of attraction of the 'non-response' state, which leads to disappearance of both Th1- and Th2-dominated autoimmune reactions.

$$\frac{\mathrm{d}x_{w_2}}{\mathrm{d}\tau} = p_{w_{\mathrm{self}}}\sigma_{w_2}\frac{(x_{u_2}+x_{w_2})}{1+x_{u_2}+x_{w_2}} - \delta_2\frac{(x_{u_1}+x_{w_1})^2}{1+x_{u_2}+x_{w_2}}x_{w_2} - x_{w_2} + \pi_2\left[\rho + \left(\frac{x_{u_1}+x_{w_1}}{1+x_{u_2}+x_{w_2}}\right)\right]\frac{x_{w_2}(x_{u_2}+x_{w_2})}{1+x_{u_1}+x_{w_1}+x_{u_2}+x_{w_2}}, \tag{4.25}$$

$$\frac{\mathrm{d}p_{u_{\mathrm{vacc}}}}{\mathrm{d}\tau} = -rp_{u_{\mathrm{vacc}}}. \tag{4.26}$$

Steady states

The model (4.22) - (4.26) exhibits the following steady states.

- An exclusively-Th1 response to self-antigen u (condition $x_{u_1} > 0, x_{u_2} = 0$), which has always the form $(x^*_{u_1}, 0, x^*_{w_1}, 0, 0)$ with

$$x^*_{u_1} = \frac{p_{u_{\mathrm{self}}}\sigma_{u_1}\left(\pi_1 \pm \sqrt{\pi_1^2 + 4\delta_1(p_{u_{\mathrm{self}}}\sigma_{u_1} + p_{w_{\mathrm{self}}}\sigma_{w_1} - 1)}\right)}{2\delta_1(p_{u_{\mathrm{self}}}\sigma_{u_1} + p_{w_{\mathrm{self}}}\sigma_{w_1})}, x^*_{w_1} = \frac{p_{w_{\mathrm{self}}}\sigma_{w_1}}{p_{u_{\mathrm{self}}}\sigma_{u_1}}x^*_{u_1}.$$

- An exclusively-Th2 response to self-antigen u (condition $x_{u_2} > 0, x_{u_1} = 0$), which has always the form $(0, x^*_{u_2}, 0, x^*_{w_2}, 0)$ with

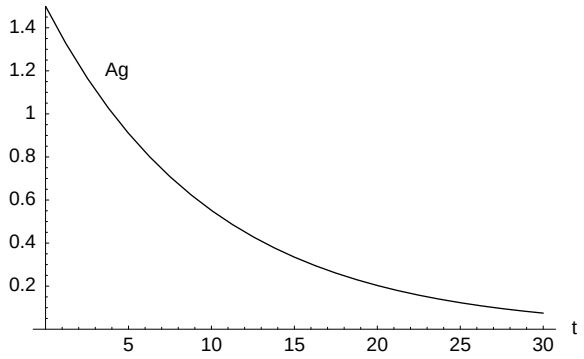
$$x^*_{u_2} = \frac{p_{u_{\mathrm{self}}}\sigma_{u_2}(p_{u_{\mathrm{self}}}\sigma_{u_2} + p_{w_{\mathrm{self}}}\sigma_{w_2} - 1)}{(1-\pi_2\rho)(p_{u_{\mathrm{self}}}\sigma_{u_2} + p_{w_{\mathrm{self}}}\sigma_{w_2})}, x^*_{w_2} = \frac{p_{w_{\mathrm{self}}}\sigma_{w_1}}{p_{u_{\mathrm{self}}}\sigma_{u_1}}x^*_{u_2}.$$

This implies that a T helper response, which is exclusively Th(i) for antigen u will be exclusively Th(i) ($i = 1, 2$) for antigen w as well. If the specific T helper response against antigen u shifts from an exclusive Th1 response to an exclusive Th2 response the same will hold for the T helper response to antigen w. Therefore, we have demonstrated that determinant spreading holds for our model.

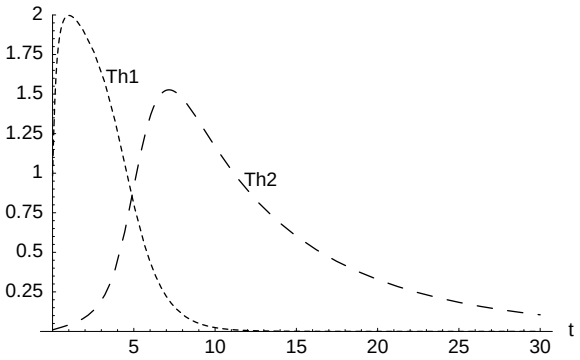
Simulations show that the results presented in Fig. 4.4.1 still hold qualitatively for a situation with two different self-antigens. The development of the T helper concentrations is illustrated in Fig. 4.14.

In summary, all above mentioned experimental observation can be explained in terms of our model. Crucial are the antigen dose dependency of T helper differentiation (in order to allow Th1 $\rightarrow$ Th2 shifts when antigen levels are risen), cytokines that act in a systemic or paracrine manner (in order to allow determinant spreading), and bistability of a 'non-response' state together with Th1-dominance for low activation levels (in order to allow waning autoimmune reaction instead of establishment of a Th2-dominated autoimmune response).

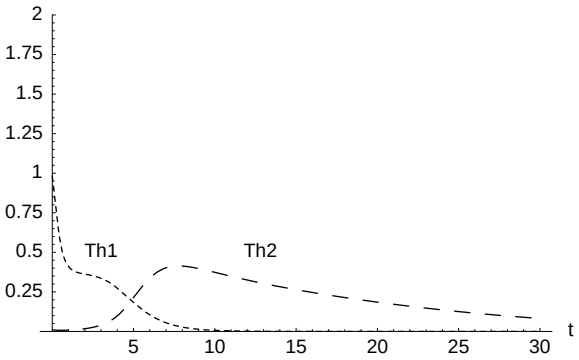
Our model suggests – in agreement with experiments in the case of EAE – that the success of peptide vaccination is not restricted to diabetes but can also be expected for all Th1-dominated autoimmune disorders.



(i) concentration of administrated self-antigen u



(ii) response to self-antigen u



(iii) response to self-antigen w

Figure 4.14.: Time plots of T helper concentrations after peptide vaccination. The parameters are $\sigma_{u_1} = \sigma_{w_1} = \sigma_{u_2} = \sigma_{w_2} = 1.5$, $\pi_1 = \pi_2 = 3$, $\delta_1 = 1.5$, $\delta_2 = 0.2$, $\rho = 0.1$, $\chi_0 = 0, p_s = 0.3, r = 0.1$. Initial condition is the nearly attained exclusively Th1 steady state described above ($x_{u_1} = x_{w_1} \sim 1, x_{u_2} = x_{w_2} \sim 0$), which corresponds to Th1-induced autoimmunity. After treatment with self-antigen u ($p_{u_{\mathrm{vacc}}}(0) = 1.5$) T helper concentrations for both self-antigens u and w shift from Th1 to Th2 dominance. This switch is followed by disappearance of Th2 concentrations specific for both self-antigens.

4.5. Oscillatory behavior

In our model oscillations can only occur under the following conditions

1. low AICD rates for Th1 cells ($\delta_1 \ll 1$) or high Th2 proliferation due to either high Th2 proliferation rates (π_2 large) or availability of growth factors that have not been produced by antigen-specific differentiated Th1 or Th2 cells, that is, by Th0 or other non-specific T helper cells ($\Rightarrow \chi_0 > 0$) in connection with

2. low Th2 activation rates (σ_2 small) and

3. very low susceptibility of Th2 for AICD ($\delta_2 \approx 0$).

Low Th2 activation rates σ_i or low Th1 AICD δ_1 rates could for example occur in autoimmune situations (see Sec. 4.4). Due to bystander activation, which frequently has been brought in connection with autoimmune diseases, additional growth factors could be also available then so that $\chi_0 > 0$. Oscillations between Th1 and Th2 can thus provide an explanation for relapses, which are frequently observed in autoimmunity.

Under the above conditions the following nullcline behavior is observed. If Th2 activation rates are low the non-trivial part of the Th2-nullcline (as discussed in the Appendix A.1) is monotonically decreasing, which leads to a completely different qualitative behavior compared to monotonically increasing nullcline. In order to get intersections with the Th1-nullcline high Th2 proliferation or low AICD rates for Th1 are necessary. If the Th2-nullcline intersects the bell-shaped Th1-nullcline in the monotonically increasing part (to the left of the maximum) oscillations can be found. This will be discussed in more detail below.

To illustrate an example we assume a situation with low Th2 activation rate, regular Th1 activation rate (for example due to a genetically based bias towards Th1 activation) and low AICD rates. Starting from a Th1-dominated state with $\sigma_1 \neq 0$ and $\sigma_2 = 0$, increasing the Th2 stimulus σ_2 drives the system into a mixed state. This state evolves into a stable, globally attracting limit cycle corresponding to oscillatory Th1-Th2 responses. Further increases in the Th2 activation signal lead to a stable Th2-dominated state (Fig. 4.15). The corresponding nullclines and separatrizes are illustrated in Fig. 4.16.

Mathematically, oscillations occur when the Th2-nullcline (g) intersects the bell-shaped Th1-nullcline (f) in its left half. Here, both nullclines are monotonically increasing but the derivative of the Th1 nullcline is smaller than the derivative of the Th2 nullcline ($f' < g'$). Therefore, the determinant as well as the trace of the Jacobian at the fixed point is positive. This implies that the steady state is an unstable spiral or an unstable node. Because of the existence of a confined set (the system is generally bounded) we know because of the Poincaré-Bendixon Theorem that a limit cycle exists. This confined set gets a hole when the steady state on the x_2-axis is stable, which leads to the disappearance of the limit cycle.

Conversely, if we begin with a high Th2 activation rate ($\sigma_2 = 2$) and no Th1 activation ($\sigma_1 = 0$), and increase σ_1, no new fixed points or qualitative changes in behavior appear;

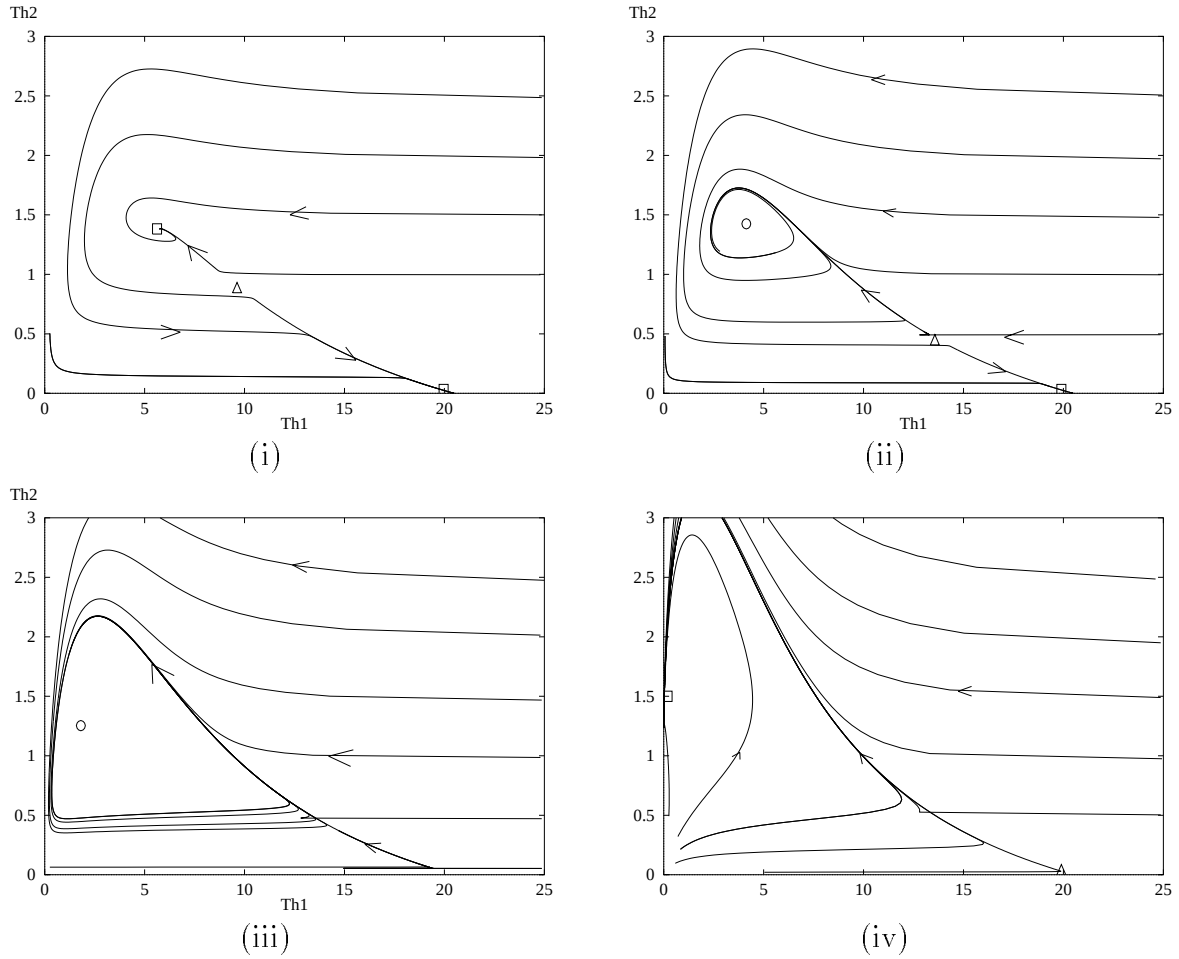


Figure 4.15.: Illustrating the switching from a Th1-dominated state to a Th2-dominated one through an intermediate, oscillatory phase as we change the Th2 stimulus σ_2. In figure (i) the parameters are $\sigma_1 = 2$, $\sigma_2 = 0.35$, $\pi_1 = \pi_2 = 2$, $\delta_1 = 0.1$, $\rho = \chi_0 = 0.1$,$\theta_1 = \theta_2 = 0$, and $\delta_2 = 0$. Stable equilibria are denoted with squares, unstable equilibria with triangles (saddle nodes) or circles (unstable nodes or spirals). Even though a stable coexistent state exists, the basin of attraction for the Th1 response covers the vicinity of the origin. Now we increase the Th2 activation signal to $\sigma_2 = 0.6$ and a Hopf bifurcation at $\sigma_2 \simeq 0.55$ creates a stable limit cycle (ii). Note that the stable Th1 state is about to be annihilated, but its basin of attraction is still dominant for a naive response. We increase the Th2 activation signal further ($\sigma_2 = 1$, figure (iii)), and now the stable limit cycle is the only attractor for the system. This limit cycle decreases in size with increasing σ_2 and vanishes when the steady state created by the intersection of the Th2 nullcline with the trivial part of the Th1 nullcline (x_2-axis) becomes stable at $\sigma_2 > \sqrt{\sigma_1} = 1.41$. A stable, exclusive Th2-dominated state remains (figure (iv), $\sigma_2 = \sigma_1 = 2$). Notice here the Th1→Th2 switches in the absence of AICD for Th2 cells ($\delta_2 = 0$). This scenario is structurally stable to small increases in δ_2, which create a narrow basin of attraction for a stable, pure Th1 response near the Th1 axis.

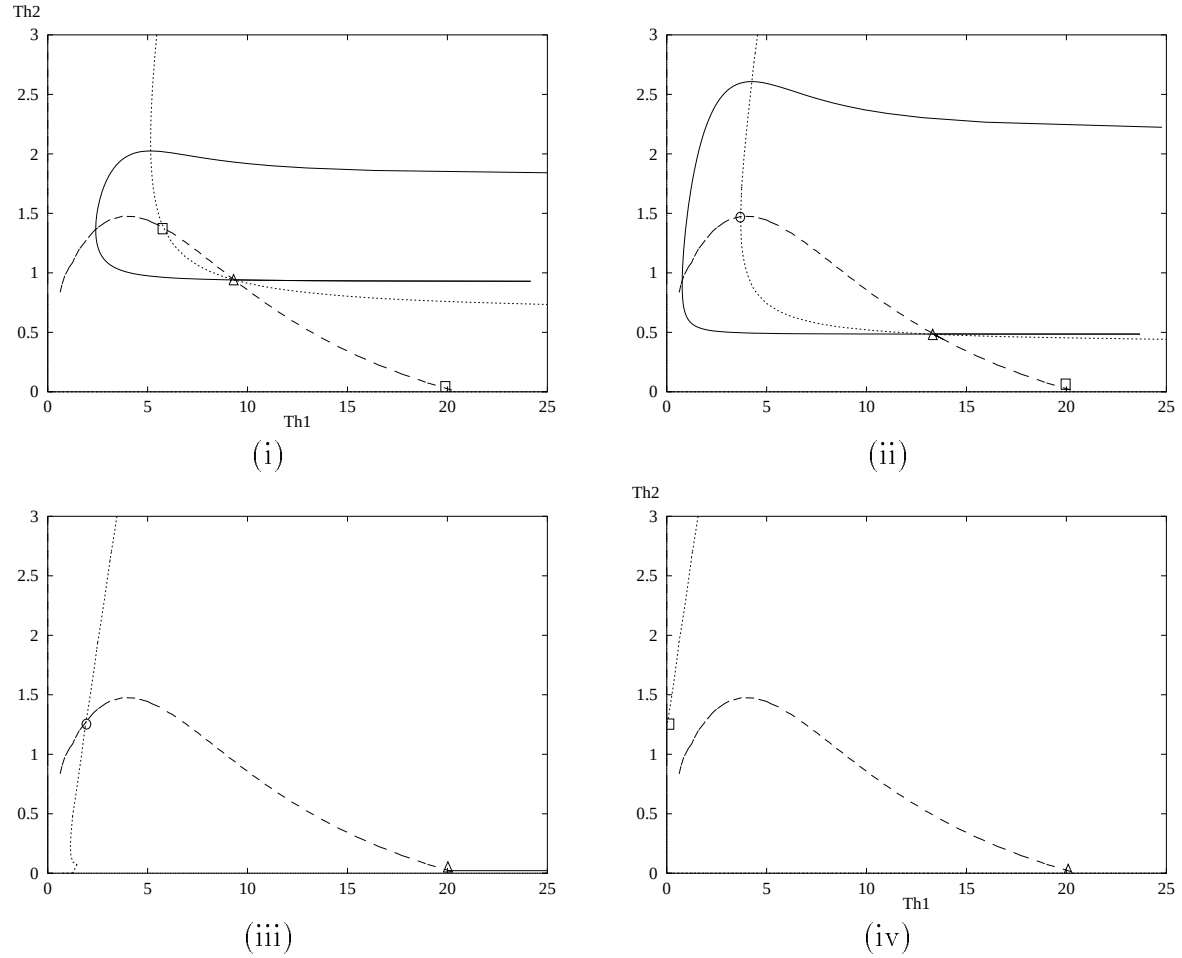


Figure 4.16.: Illustrating the Th1-nullclines (dashed lines), Th2-nullclines (dotted lines), and separatrix (solid line) during the switching from a Th1-dominated state to a Th2-dominated one through an intermediate, oscillatory phase as we change the Th2 stimulus σ_2. Parameters are as in Fig. 4.15. Stable equilibria are marked by squares, unstable equilibria with triangles (saddle nodes) or circles (unstable nodes or spirals). In figure (i) intersections of the Th1 nullcline and the Th2 nullcline create two stable equilibria and an unstable saddle node. Its separatrix divides the state space in basins of attraction for the stable steady states. Increasing the Th2 activation signal to $\sigma_2 = 0.6$ moves the Th2 nullcline left and the stable node representing mixed Th1/Th2 concentrations looses its stability (ii). If we increase the Th2 activation signal further (figure (iii)), the second intersection of the non-trivial part of the Th2 nullcline with the Th1 nullcline disappears; the stable limit cycle around the unstable spiral is the only attractor of the system. The Th2 nullcline moves upwards, the intersection of the non-trivial part of the Th1 nullcline with the Th2 nullcline is lost, and an exclusive Th2 dominated state remains (figure (iv)).

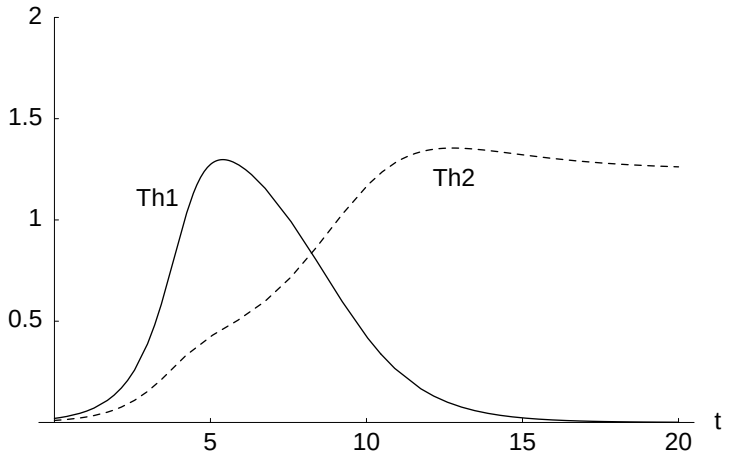


Figure 4.17.: Time plot of a Th1 $\rightarrow$ Th2 shift. Parameters are set to $\sigma_1 = \sigma_2 = \pi_1 = \pi_2 = 2$, $\delta_1 = 1$, $\delta_2 = 0.3$, $\theta_1 = \theta_2 = \chi_0 = 0$, and $\rho = 0.2$. Initial conditions are $x_1(0) = 0.01, x_2(0) = 0.01$.

Th2 responses always dominate, even with a strongly Th1-biased initial condition. The increased Th1-signal strength leads to an increase in the switching time, however. If we were to include the effects of antigen clearance, then quite possibly the infection would be cleared successfully by a type 1 response before the switch. However, the model indicates that – if Th2 cells are not subject to significant AICD (δ_2 is small) – eventually a Th2 response dominates, if the Th1 response fails to resolve the infection.

A common feature of biological systems appears to be the progression from stability through a phase of oscillatory behavior and back to stability with increasing stimulus. This is in contrast to mechanical dynamical systems which tend to progress from stability through oscillatory behavior to chaos provided their dimensionality is high enough. For example, oscillations in levels of the cytokine TNF-α have been observed in the eye following corneal allograft rejection over an intermediate range of antigen concentration [22]. In Fig. 4.15, we see that under conditions that lead to oscillations (as discussed above) low levels of Th2 stimulation lead to Th1 responses; intermediate levels give rise to oscillations in the Th1 and Th2 cell numbers and higher Th2 stimulation stabilizes a Th2 response so that the system is unable to switch cleanly from one stable state to the other as the stimulus is altered.

4.6. Th1 $\rightarrow$ Th2 switches

The only parameter in our model which profoundly alters the qualitative behavior is the susceptibility of Th2 effectors to Fas death, δ_2. If Th2 cells are much less susceptible than Th1 cells ($\delta_2 \ll \delta_1$) and $\sigma_2 > 1$, our model predicts that Th1-Th2 switches are generic features of chronic infections and that dynamic Th2-Th1 switches are not possible. The temporal development of such a shift is illustrated in Fig. 4.17.

This asymmetry is rooted in the interplay between cross-regulation and differences in susceptibility to AICD and Fas/FasL expression; the interactions tend to favor early Th1 dominance, while lack of AICD for Th2 destabilizes the pure Th1 state and drives the system eventually to mount a Th2 response. Work on Th1-Th2 switches *in vitro* has revealed that even polarized, committed Th1 populations from murine lymph nodes contain undifferentiated cells that are capable of becoming either Th1 or Th2 cells [65] and this is in keeping with our findings.

For comparable susceptibility of Th1 and Th2 two polarized Th1- and Th2-dominated steady states exist (see Fig. 4.5). Because of the location of the separatrix between these steady states shifts from Th1-dominance to Th2-dominance are not possible. We may, however, find transient mixed populations before reaching one of the polarized fixed points.

4.7. Summary and Discussion

The true role of Fas and Fas ligand in immune regulation remains to be uncovered but our model demonstrates that with a biologically detailed, simple dynamical model we can reproduce broad features of the immune response. Understanding the interplay of several cooperative and competing biological mechanisms is a task obviously suited to mathematical modeling.

The present model raises both new issues and complements the now significant body of work on Th1/2 dynamics. Previous models differ in their selective emphasis on the various cytokine interactions and the underlying biological assumptions. Fishman and Perelson [31], building on an earlier model of T cell and antigen-presenting cell interactions [30], include distinct Th1 and Th2 activation signals and cross-regulatory interactions in their model. In contrast to their study, we do not include antigen clearance and we consider the effect of feedback from the proliferating effector cells on the APC activation states, as well as a cytokine-regulated death mechanism. Additionally, the roles and extent of influence of IL-2 and IL-4 as growth factors are treated differently – we assume asymmetric roles for the two, and make the further assumption that secreted growth factors are available to the whole proliferating pool rather than just the cells producing them (i.e., systemic rather than autocrine in nature). The structure of the cross-suppressive interactions that they include leads naturally to the instability of mixed populations – only polarized Th1 or Th2 responses are supported in their model. This work is developed further in [29] to account for multi-clonality in a response, with essentially the same robust conclusions derived from principles of competition between clones for activation and cross-suppression of proliferation. Their predictions for the dose-dependence of the response are largely in agreement with those here and in the experimental literature. Muraille and Kaufman [70] focus more (as we do here) on the asymmetries inherent in Th1/2 interactions and mechanisms of switching responses from Th1 to Th2 or *vice versa*, as well as addressing issues of control of T cell clone sizes, although primarily through control of proliferation and activation rather than apoptosis. Morel *et al.* [67] use a model of Th1/2 dynamics to clarify the roles of IL-2 and IL-4 in proliferation and we draw on their conclusions here. Lev Bar-

Or and Segel [54] investigate the roles of different regulatory mechanisms in the T helper system in the context of autoimmune disease; a non-specific, cytokine-mediated suppression and a specific cell-cell interaction mechanism. This takes place through the presentation of T cell receptor peptides rather than through pro-apoptotic surface molecules, however, and so the *structure* of the suppressive interactions they put forward is broadly similar to those presented here, if not their immunological basis. They find that the cells' average sensitivities to Th1- and Th2-derived cytokines play an important role in the decision making process. Carneiro *et al.* [21] also address Th1/2 differentiation without direct treatment of antigen clearance and focus on the early, decision making aspect of a T helper response. They use the concept of an invariant proliferative driving capacity or antigenic 'niche' that is indifferent to the Th1/2 composition of the proliferating lymphocyte pool. They do not address the antigen presentation step in detail, but rather propose that the Th1/2 decision is made primarily by the the dynamics of the T cell populations themselves rather than being directed by cogent choices of co-stimulatory signals offered by APCs. Our results, however, indicate an important role for both these processes in determining the final outcome of a response. Each of the models described above displays features that shed light on various experimental data; but, nevertheless, they differ significantly in their structure and areas of emphasis. All together they give an indication of the complexity of the Th1/2 system.

In summary, we make several distinct points, based jointly on experimental observations and the conclusions from our model.

(i) There are differences between the population control mechanisms of the two major T helper subsets. Homeostatic regulation of Th1 effector populations is dominated by the cytokine-regulated, Fas-mediated AICD mechanism (active control). Th2 regulation appears to be through inhibition of activation and proliferation (passive control).

(ii) Cell-cell killing (fratricide) and not cell suicide appears to be the dominant mechanism for Fas-mediated AICD.

(iii) A critical parameter in our model is the susceptibility of Th2 effectors to AICD (δ_2). Asymmetries in AICD susceptibility lead to non-intuitive system behavior. Comparable susceptibility leads to support of exclusive type 1 or type 2 responses; substantially lower Th2 susceptibility leads to generic Th1 $\rightarrow$ Th2 switches in the absence of antigen clearance.

(iv) Contributory factors to autoimmune diseases may be defective Th1 AICD mechanisms. That in connection with relative absence of AICD among Th2 cells, low Th2 activation rates, and availability of IL-2 produced by Th0 or non-specific differentiated T helper cells can lead to oscillatory Th1/Th2 responses or 'failed decision making'.

(v) Low levels of antigenic stimulation tends to favor Th1 responses, higher doses favor Th2.

(vi) In diabetes and other Th1-dominated autoimmune diseases autoimmune reactions can be arrested by peptide vaccination due to a treatment-induced shift from a far more damaging Th1 to a Th2 response. This shift is a consequence of the antigen dose dependency of T helper dominance (see (v)).

(vii) Signals from the innate immune system can help to bias a response towards one helper subset, or switch the nature of an ongoing response. The long term behavior of the system, however, is again critically dependent on the relative susceptibilities of Th1 and Th2 cells to Fas-mediated cell death.

Much work remains to be done in understanding the decision-making process made at the antigen presentation stage. Our model addresses this process in a necessarily simplified way. A more sophisticated model would treat antigen presentation and differentiation in more detail. However, the model shows how asymmetries in the dynamics of T cell proliferation and regulation, which are subject to cytokine control, can contribute to the final outcome just as significantly as the apparent choice of differentiation pathway made at antigen presentation. In particular Th1 $\rightarrow$ Th2 shifts, a system behavior of our model that was based on particular properties of the regulatory interactions between the two T helper subsets, and which has been frequently observed in chronic infections, can be interpreted in the following way: There is a built-in mechanisms in the T helper system, that enables a shift from an initial Th1-dominated response to a Th2-dominated response when the initial Th1-dominated response could not clear the antigen (that is the case in our model because antigen concentrations stay constant).

The multitude of cytokine interactions in Th1/2 system, many of which seem redundant, may help to make this process of choosing a response more robust. They may have evolved in parallel with viral strategies for subverting the immune system by interference with cytokine signaling or expression of co-stimulatory signals.

Before we further develop these ideas in respect to describing a mechanisms for the decision making process we want to study the role of the asymmetries in Th1 and Th2 regulation in more detail.

5. Alternative models and the role of asymmetries

Mathematical models are not supposed to be a direct picture of reality. Instead, a modeling approach tries to deduce some of the macroscopic properties of the system from interactions among the components. The crucial question is of course, what *are* the elementary interactions that give rise to the observed system behavior? In the T helper system in particular questions related to asymmetries in the overall behavior are of greatest interest. Among these are

- Which T helper type dominates under which circumstances?
- How does antigen dose influence T helper dominance?
- Do shifts from one to the other T helper type occur? Are switches in both directions possible?

Asymmetries in the macroscopic properties arise from asymmetries in the interactions of the elementary components. Asymmetries in the overall behavior of the two T helper subsets can occur due to unequal parameter settings or to unequal regulation principles. The central problem of the present chapter is to understand the role of asymmetries in regulation, as there are

- different effects of Th1/Th2 related growth factors on Th1 and Th2 proliferation,
- asymmetries in cross-suppression,
- and differences between the two T helper subsets with respect to AICD.

In the previous chapter we assumed that there are fundamental differences between the growth factors IL-2 and IL-4 produced by Th1 and Th2 cells. Whereas IL-2 promotes growth of both T helper populations (for Th2 cells in synergy with IL-4), IL-4 enhances proliferation of Th2 cells only. Additionally, IL-2 is – even for IL-4 secreting Th2 cells – a much more potent growth factor for Th2 cells than IL-4. These assumptions makes effective proliferation of Th2 cells highly dependent on the presence of IL-2 and thus to some extent on Th1 cells and therefore could be an explanation for the occurrence of Th1 $\rightarrow$ Th2 shifts and the absence of vice versa switches. The same argumentation leads to an antigen dose dependency as discussed in Sec. 4.2.6. Since the role of growth factors in T helper

proliferation is still not clear and in order to investigate the importance of different effects of IL-2 and IL-4 on the crucial macroscopic properties an alternative model with symmetric effects of the growth factors on Th1 and Th2 cells will be studied in the following.

5.1. An alternative role of growth factors: autocrine versus systemic

T-cell growth can be modeled as depending on the bulk concentration of cytokines in the extracellular medium as we did in the previous model in Chap. 4. In reality, however, cytokine concentrations are not homogenous. In the vicinity of the secreting cell the concentration is highest and drops by diffusion down to a certain bulk concentration with increasing distance. Binding to receptors and subsequent consumption of cytokines further reduces the concentration. Therefore, cytokine concentrations far from the producing cell may well be below effectivity threshold.

In contrast to the previous model ('Th1/Th2'-model, of Chap. 4) we assume here that cytokines act in an autocrine way, i.e., while having an effect on their *producing* cell they do *not* affect other cells. More precisely, we propose that IL-2 and IL-4 are autocrine growth factors for Th1 and Th2 cells, respectively, but they do not influence the proliferation of the competing T helper subset. Both T helper subsets produce their own growth factors. IL-2 is secreted by activated Th1 cells, and binding of a sufficient quantity of IL-2 to receptors on TCR-activated T cells induces these cells to cycle and prevents activated cells from reverting to the resting state. In turn, IL-4 is an autocrine growth and differentiation factor for Th2 cells. Since the local concentration of the growth factors can be deemed to be constant for each secreting cell it can be directly incorporated into the proliferation rate. IL-2 produced by other cells than differentiated Th1 cells ($\chi_0 = 0$) is neglected by the same argument.

Inhibitory effects of cytokines such as IFN-γ and TGF-β are taken into account as in Chap. 4. We do not consider TGF-β – although part of the Th2 cytokine signal S_2 – as acting in an autocrine way in suppressing Th2 proliferation, because TGF-β is not produced by Th2 cells themselves but by Th3 cells. The cytokine interactions that affect T helper proliferation are summarized in Fig. 5.1. Accordingly, we assume

$$\text{Th1 proliferation rate} = \frac{\beta_1 T_1}{1 + kS_2},$$

and

$$\text{Th2 proliferation rate} = \frac{\beta_2 T_2}{(1 + kS_1)(1 + kS_2)}$$

with proportionality constants β_1 and β_2.

Given the autocrine effect of IL-2 as a growth factor we also assume that IL-2 acts in an autocrine way on the expression of FasL on Th1 cells. This leads to the following terms describing activation induced cell death,

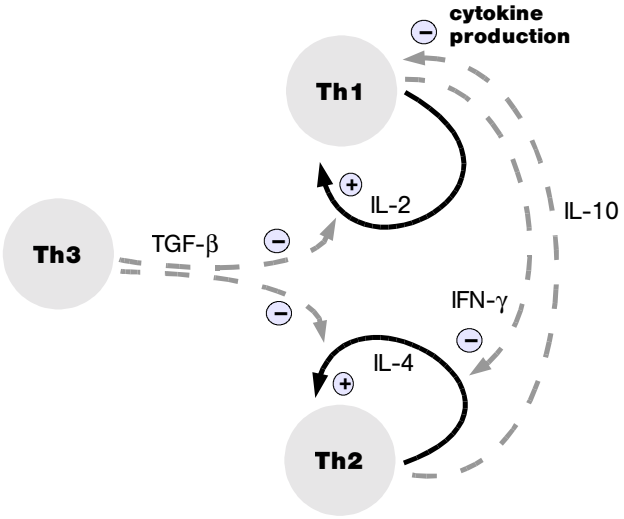


Figure 5.1.: Schematic representation of the interactions governing Th1/2 proliferation when we assume autocrine actions of growth factors. Whereas type 1 cytokines directly inhibit Th2 proliferation type 2 cytokines lead to retardation of Th1 proliferation via down-regulation of growth factor production. TGF-β regulates IL-2 and IL-4 induced proliferation of both T helper subsets.

$$\text{Th1 AICD rate} = \Delta_1 T_1^2$$

and

$$\text{Th2 AICD rate} = \Delta_2 T_1 T_2.$$

After rescaling the equations thus take the form,

$$\frac{\mathrm{d}x_1}{\mathrm{d}\tau} = \frac{\theta_1}{(1+x_2)} + \frac{\sigma_1 x_1}{(1+x_2)^2} + \frac{\pi_1 x_1}{(1+x_2)} - \delta_1 x_1^2 - x_1, \tag{5.1}$$

$$\frac{\mathrm{d}x_2}{\mathrm{d}\tau} = \frac{\theta_2}{(1+x_2)} + \frac{\sigma_2 x_2}{(1+x_2)} + \frac{\pi_2 x_2}{(1+x_1+x_2)} - \delta_2 x_1 x_2 - x_2. \tag{5.2}$$

We refer to these equations as the 'autocrine-Th1/Th2'-model.

5.1.1. Analysis

To fully concentrate on the dynamics of the T helper system without interference from other components of the immune system we first neglect T helper independent activation

($\theta_1 = \theta_2 = 0$). In a first step we assume that there is no AICD for Th2 cells ($\delta_2 = 0$) and discuss its effects later in this section. The following steady states can be found.

- $(x_1, x_2) = (0, 0)$. The eigenvalues of the equilibrium are $(\sigma_1 - \pi_1 - 1, \sigma_2 + \pi_2 - 1)$, implying that for low stimulation and low proliferation this 'no-response' state is stable.

- An exclusively-Th2 response $(0, x_2^*)$, where $x_2^* = \pi_2 + \sigma_2 - 1$ implying $\sigma_2 + \pi_2 > 1$ for existence. Stability additionally requires

$$\sigma_2 + \pi_2 > \frac{1}{2}(\pi_1 + \sqrt{\pi_1^2 + 4\sigma_1}). \tag{5.3}$$

- An exclusively-Th1 response $(x_1^*, 0)$, where $x_1^* = (\pi_1 + \sigma_1 - 1)/\delta_1$. The steady state is stable if

$$(\pi_1 + \sigma_1 - 1)(\sigma_2 - 1) + \delta_1(\pi_2 + \sigma_2 - 1) < 0. \tag{5.4}$$

  Therefore a necessary (not sufficient) condition for stability is that $\pi_2 + \sigma_2 < 1$. This implies that an exclusively-Th1 and an exclusively-Th2 response can never occur for the same set of parameters.

- A mixed state $M_1 = (x_1^{**}, x_2^{**})$. The conditions under which a mixed steady state exists and is stable becomes clear when we look at the different nullcline scenarios.

In contrast to the 'Th1/Th2'-model non-trivial parts (beside the x_2- and x_1-axis) of the Th1- and Th2-nullclines are monotonically decreasing. The derivative of the Th2-nullcline is

$$\frac{1}{2}\left(-1 + \frac{\pi_2 - \sigma_2 + x_1}{\sqrt{\pi_2^2 + 2\pi_2(\sigma_2 - x_1) + (\sigma_2 + x_1)^2}}\right) < 0$$

for all choices of x_1. The same holds for the derivative of the Th1-nullcline

$$-\frac{\delta_1\left[\pi_1^2 + 2(\sigma_1 + \delta_1\sigma_1 x_1) + \pi_1\sqrt{\pi_1^2 + 4(\sigma_1 + \delta_1\sigma_1 x_1)}\right]}{2(1 + \delta_1 x_1)^2\sqrt{\pi_1^2 + 4(\sigma_1 + \delta_1\sigma_1 x_1)}} < 0.$$

Fig. 5.2 illustrates four scenarios that are possible for the 'autocrine Th1/Th2'-model, viz,

- an exclusive Th1-response,
- an exclusive Th2-response,
- bistability of a nearly polarized Th2 helper response with an exclusive Th1 response,
- a mixed T helper response.

Note that bistability of strictly polarized T helper responses is not possible (as discussed in the steady state analysis) and bistability of nearly polarized responses only for limited parameter range. (In that sense our model differs from the competition models presented in [72] page 81). In the next section we will show that a non-zero susceptibility of Th2 for AICD leads to polarization of the T helper responses and allows bistability of pure Th1- and Th2 responses similarly to the 'Th1/Th2'-model.

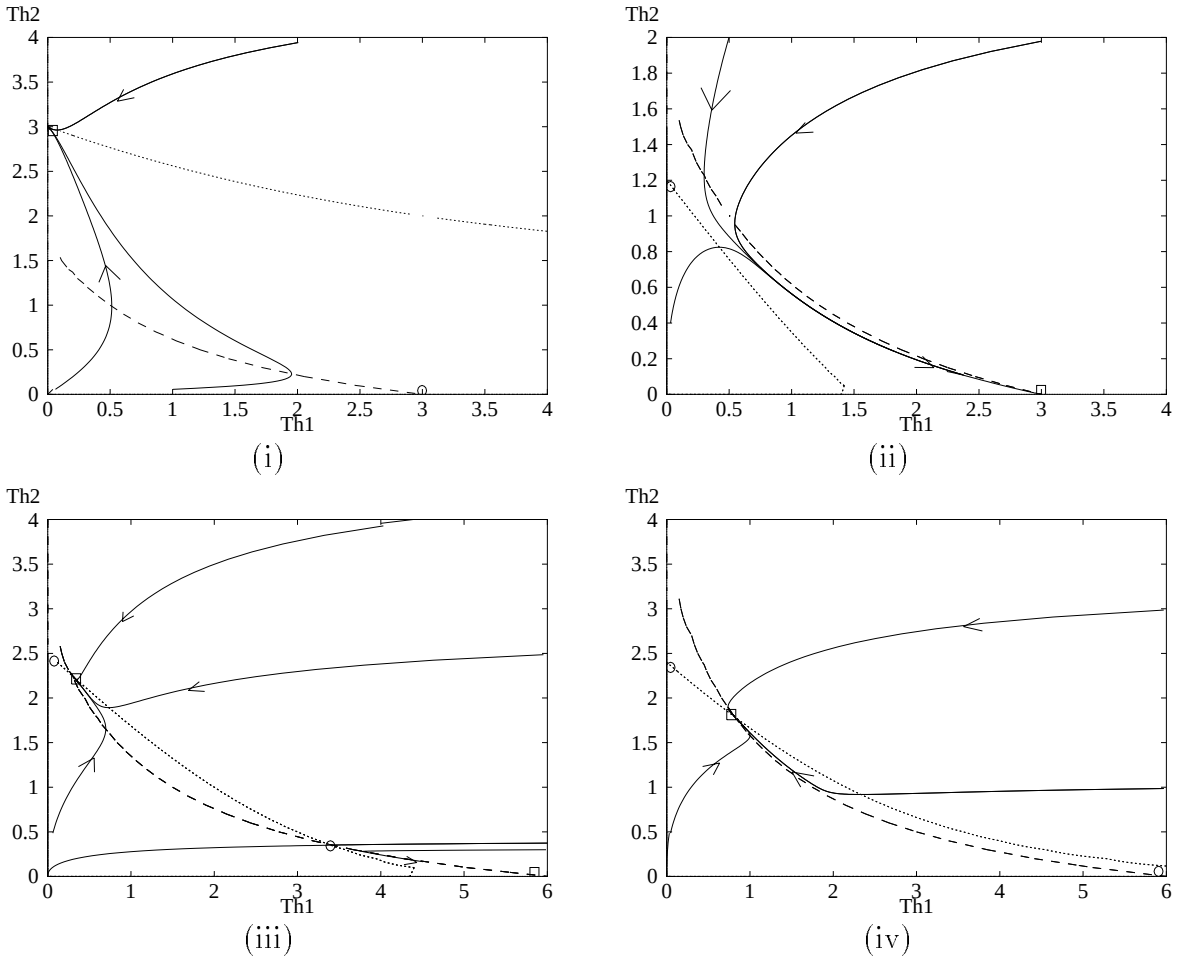


Figure 5.2.: Different scenarios of state space behavior for a Th1/Th2 system with autocrine growth factors. Th1-nullclines are drawn as dashed lines, Th2-nullclines as dotted lines, and orbits as solid lines. Stable steady states are represented by boxes, unstable steady states by circles. In figure (i) with parameters $\sigma_1 = \sigma_2 = \pi_1 = \pi_2 = 2, \delta_1 = 1$ the non-trivial part of the Th2-nullcline is above the non-trivial part of the Th1-nullcline, which leads to an exclusive Th2-response as the only attractor of the system. (ii) For smaller Th2-activation parameter $\sigma_2 = 0.2$ the non-trivial part of the Th1-nullcline is above the Th2-nullcline. The only stable steady state is represented by the intersection of the trivial part of the Th2-nullcline (the x_1-axis) leading to an exclusive Th1-response. (iii) With parameters $\sigma_1 = 4, \sigma_2 = 0.5, \pi_1 = \pi_2 = 3$, and $\delta_1 = 1$ non-trivial parts of the nullclines intersect twice. We have bistability of a nearly exclusive Th2-response with an exclusive Th1-response. (iv) With parameters $\sigma_1 = 3, \sigma_2 = 0.7, \pi_1 = 4, \pi_2 = 2.7$ a mixed T helper response is the only stable steady state.

5.1.2. Similarities and differences between the systemic' and the 'autocrine Th1/Th2'-model

We find the following differences in the system behavior of the 'Th1/Th2'-model with systemic role of growth factors and the 'autocrine-Th1/Th2'-model.

- Oscillations are not possible in the 'autocrine Th1/Th2'-model. In [43] it has been shown that there are no limit cycles around saddle nodes. Limit cycles around fixed points on the axis are also not possible because orbits do not leave the first quadrant. In addition, it has been shown numerically (data not shown) that there are no limit cycles around steady states represented by mixed T helper populations.

- In contrast to the 'Th1/Th2'-model we only find bistability of two nearly polarized Th1 or Th2 responses (cf. Fig. 4.4, which demonstrates bistability of an exclusive Th2 response and a co-existence state and Fig. 4.4.1, which shows bistability of a 'non-response' state together with an exclusive Th1 steady state). As a consequence the reset of a Th1-dominated autoimmune reaction to a 'non-response' state via Th2-dominance following peptide vaccination can not be explained for a system where growth factors are autocrine.

However, the most important features of the system behavior still hold if we assume growth factors to act in an autocrine way.

- For the homeostatic regulation of Th1 and Th2 populations the active and passive control mechanisms described in Sec. 4.2 play the same important role. In absence of AICD the equation of motion for a pure Th1 population (*i.e.*, with x_2=0) is then simply $\dot{x_1} = \sigma_1 x_1 + \pi_1 x_1 - x_1$. If $\sigma_1 + \pi_1 > 1$ the non-response state $x_1 = 0$ is unstable and the T cell numbers diverge. If we neglect inhibition by IL-10 or TGF-β Th2 numbers explode if $\sigma_2 > 0$ or $\pi_2 > 2$, respectively.

- Activation parameters σ_1 and σ_2 critically determine Th1- or Th2-dominance.

- Non-zero susceptibility of Th2 cells for AICD ($\delta_2 > 0$) polarizes the T helper responses and leads to the bistability of exclusive Th1- and Th2-responses, which could not be observed if Th2 were resistant against AICD ($\delta_2 = 0$). The Th2-dominated steady state is not affected by $\delta_2 > 0$, the exclusive Th1 fixed point is stabilized. Its second eigenvalue

$$\lambda_2 = -1 - \frac{\delta_2(\pi_1 + \sigma_1 - 1)}{\delta_1} + \frac{\delta_1 \pi_2}{\delta_1 + \pi_1 + \sigma_1 - 1} + \sigma_2 \tag{5.5}$$

  is decreased by positive δ_2. Conditions for stability of an exclusive Th1 and an exclusive Th2 response are not necessarily contradictory and bistability is possible. The effect of positive AICD rates for Th2 is illustrated in Fig. 5.3.

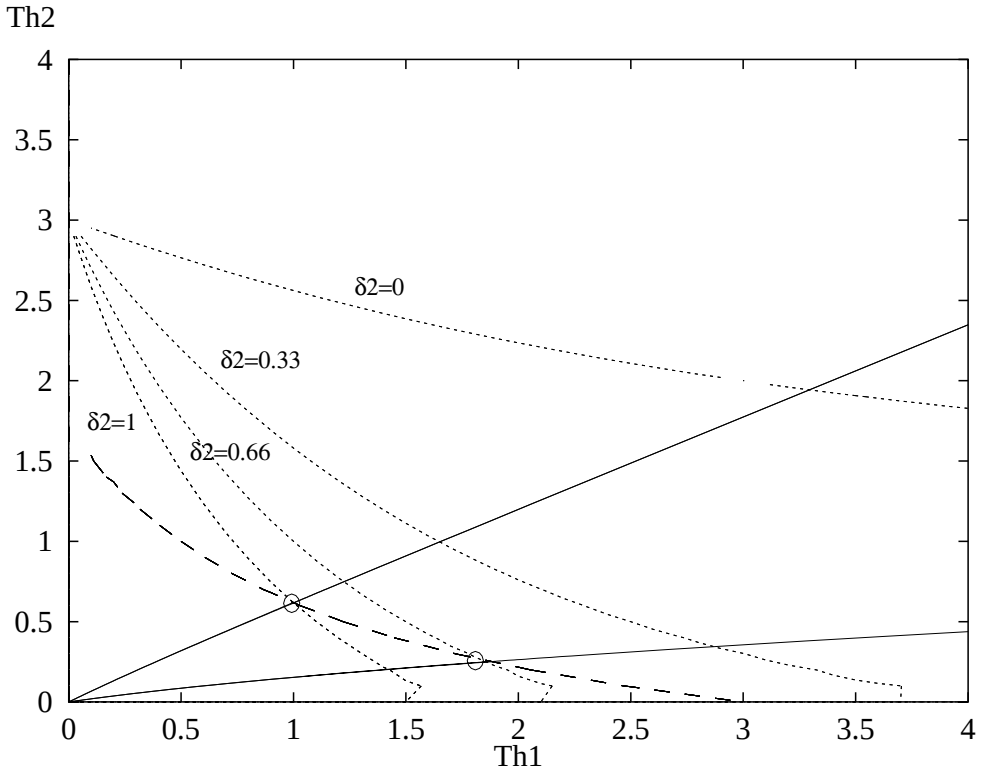


Figure 5.3.: The influence of AICD for Th2 cells in a Th1/Th2 system with autocrine growth factors. Th1- and Th2-nullclines (represented by dashed and dotted lines, respectively) are drawn for various values of $\delta_2 \in 0, 1/3, 2/3, 1$. Growing δ_2 increases the gradient of the Th2-nullcline. When both nullclines intersect a saddle node (represented by a circle) is created. Its stable manifold (represented by solid lines) divides the state space in basins of attraction for polarized Th1- and Th2-responses. Other parameter are set to $\delta_1 = 1, \sigma_1 = 2, \sigma_2 = 0, \pi_1 = 2, \pi_2 = 0$.

- For substantially lower Th2 susceptibility for AICD Th1 $\rightarrow$ Th2 switches are a common feature (Fig. 5.4) but not as distinct as in the 'Th1/Th2'-model, which is a consequence of Th2 proliferation being independent of Th1 growth factors. For initial conditions, which favor Th1, a strong Th1 response is taken over by a Th2 response. If AICD rates of Th1 and Th2 cells are comparable symmetric initial conditions still lead to transient equal increase in Th1 and Th2 concentrations with subsequent switch to Th2 dominance. For initial conditions, however, which are biased towards Th1, no switch can be observed and a Th1-dominated steady state is attained. Note that this asymmetry can arise from the dependence of cytokine production on the cell-cycle, as discussed in Sec. 4.2.1. In that sense high AICD rates of Th2 cells also lead to the disappearance of Th1 $\rightarrow$ Th2 shifts if growth factors act in an autocrine way.

- Antigen dose plays an important role for Th1/Th2 ratios. Low antigen doses lead to Th1-dominance, high antigen levels favor Th2 dominance (Fig. 5.5, see also Sec. 5.2).

- If we consider T helper independent activation signals ($\theta_1 > 0, \theta_2 > 0$) we find similar asymmetries in the effects of Th1- and Th2-inducing signals as in the 'Th1/Th2'-Model. If Th2 cells do not undergo AICD an established Th2 dominance can not be shifted towards Th1 dominance (illustrated in Fig. 5.6 (i)). The reason is as in the case of systemic growth factors that Th2 activation is not affected by θ_1. If Th2 are susceptible for AICD, however, Th1-inducing signals can induce a switch as illustrated in Fig. 5.6 (ii). Increasing the T helper independent Th2-stimulus (θ_2) leads to co-existence and finally to Th2-dominance (Data not shown).

The fact that alternative roles of growth factors have no influence on the important macroscopic properties rises the question which of the asymmetries in regulation of T helper subsets are the *critical* ones in order to obtain the asymmetries in the overall behavior.

5.2. What are the important asymmetries in the T helper regulation?

In the previous section we have demonstrated that not all of the asymmetries in T helper regulation are crucial in order to obtain the essential asymmetries in the behavior of the T helper system. We have already seen that different roles of growth-factors do not qualitatively affect the overall behavior. Throughout this section the model with autocrine growth factors (Sec. 5.1) will therefore be used.

5.2.1. The impact of FasL expression on Th2 cells

What is the role of the asymmetries in activation induced cell death? We have seen that AICD is mainly *induced* by Th1 cells and that mainly Th1 cells are affected by AICD.

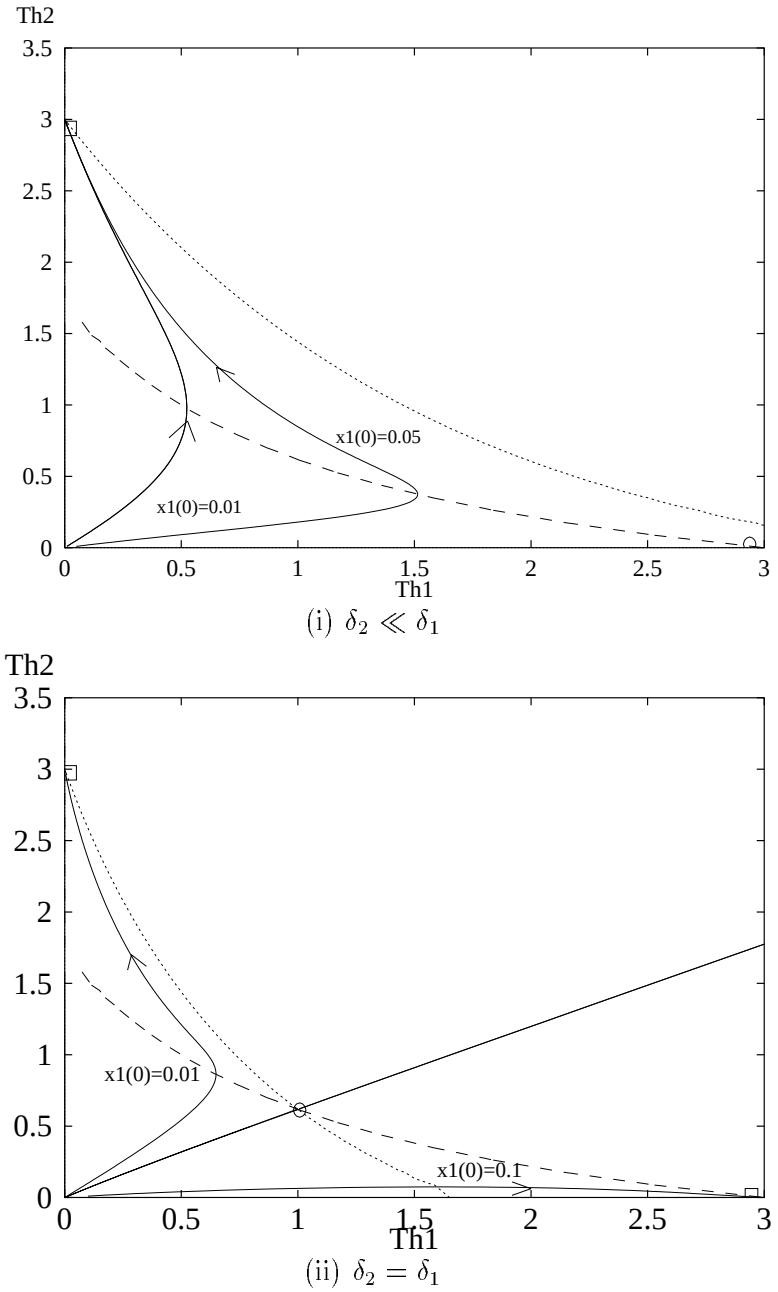


Figure 5.4.: Illustrating Th1 $\rightarrow$ Th2 shifts for a Th1/Th2 system with growth factors acting in an autocrine way. Orbits are represented by solid lines, Th1-nullclines by dashed lines, and Th2 nullclines by dotted lines. (i) If Th2 cells undergo AICD to a much lower extent as Th1 cells ($\delta_2 = 0.4$) then symmetric initial conditions $[x_1(0) = 0.01, x_2(0) = 0.01]$ lead to equally growing T helper concentrations until the Th2 response takes over and the exclusive Th2 steady state (represented by a box) is reached. In case of a Th1 bias in the initial conditions $[x_1(0) = 0.1, x_2(0) = 0.01]$ a strong Th1 response evolves that is later switched towards Th2. (ii) If apoptosis rates of Th1 cells and Th2 are comparable ($\delta_2 = 1$) symmetric initial conditions lead to a similar shift as described for (i). For asymmetric initial conditions $[x_1(0) = 0.1, x_2(0) = 0.01]$, however, an exclusively-Th1 steady state is reached. Parameter are set to $\delta_1 = 1, \sigma_1 = 2, \sigma_2 = 2, \pi_1 = 2, \pi_2 = 2$.

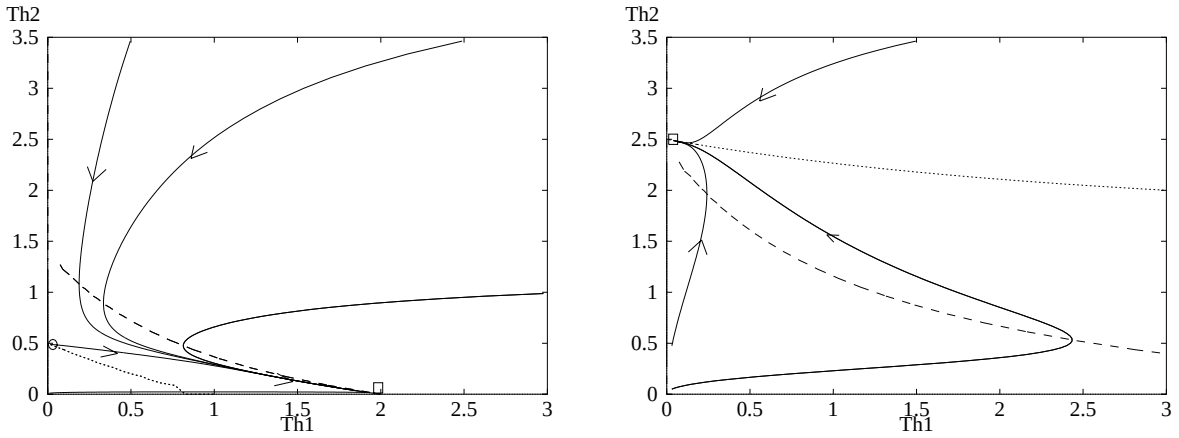


Figure 5.5.: Illustrating antigen dose dependency for a Th1/Th2 system with autocrine growth factors. Low antigen levels – represented by small activation parameters $\sigma_1 = 1, \sigma_2 = 0.5$ – lead to Th1 dominance (left figure) whereas higher antigen concentrations ($\sigma_1 = 5, \sigma_2 = 2.5$) favor Th2 dominance. Orbits are represented by solid lines, Th1-nullclines by dashed lines, and Th2-nullclines by dotted lines. Boxes indicate stable steady states, circles unstable steady states. Other parameter are set to $\delta_1 = 1, \delta_2 = 0, \pi_1 = 2, \pi_2 = 1$.

The reasons are different FasL expression rates and susceptibilities for the Fas-mediated apoptotic signal of the two T helper subsets. In this section we assume that FasL is expressed not only by Th1 cells but also to a certain amount by Th2 cells. The rate of Th2 FasL expression relative to the expression on Th1 cells is represented by the parameter a that is incorporated into the rate equations in the following way.

$$\frac{\mathrm{d}x_1}{\mathrm{d}\tau} = \frac{\sigma_1 x_1}{(1+x_2)^2} + \frac{\pi_1 x_1}{(1+x_2)} - \delta_1 x_1 (x_1 + a x_2) - x_1, \tag{5.6}$$

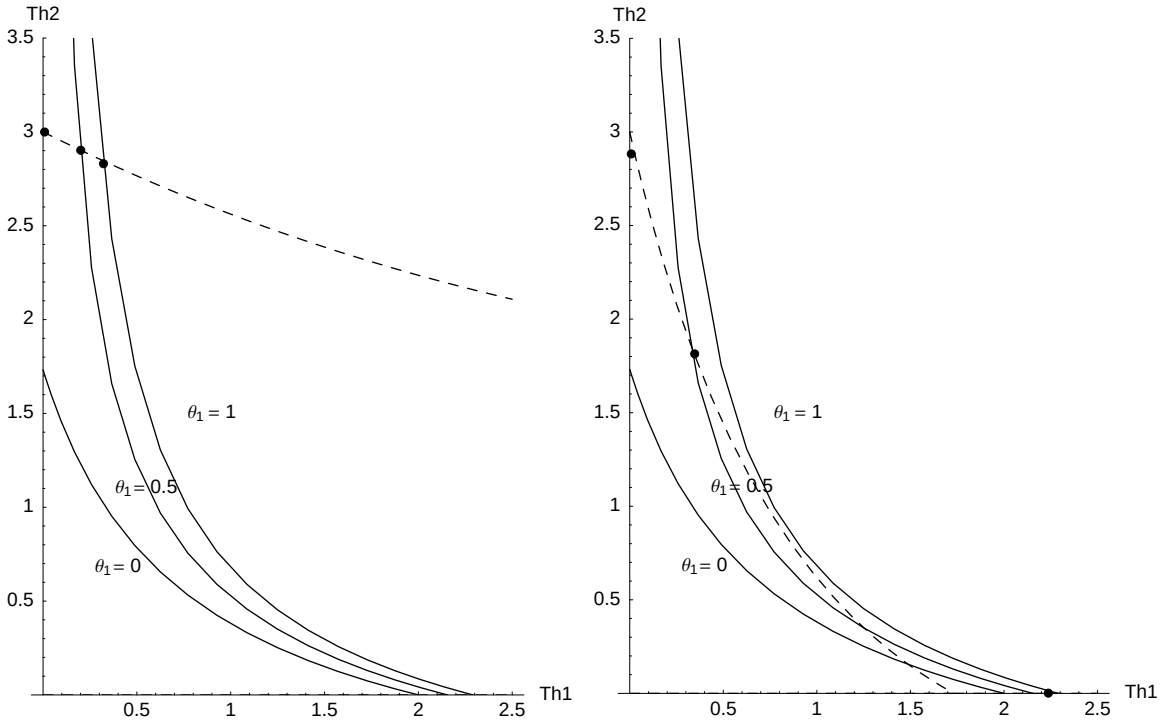
$$\frac{\mathrm{d}x_2}{\mathrm{d}\tau} = \frac{\sigma_2 x_2}{(1+x_2)} + \frac{\pi_2 x_2}{(1+x_1+x_2)} - \delta_2 x_2 (x_1 + a x_2) - x_2. \tag{5.7}$$

Analysis

The effects of $a > 0$ on location and stability of the steady states are the following.

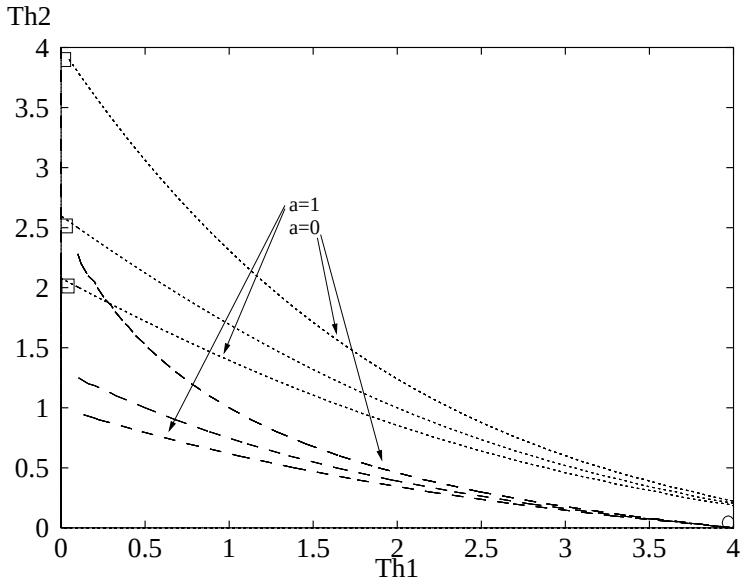
- Interestingly, the location and stability of the exclusive Th1 steady state is unaffected.
- The concentration of Th2 cells, however, is diminished by non-zero FasL expression of Th2 cells. This is illustrated in Fig. 5.7.

Note that Th1 $\rightarrow$ Th2 switches still can be observed. FasL expression by Th2 cells only affects the absolute Th2-value of the attracting steady state but an initial Th1-dominance,

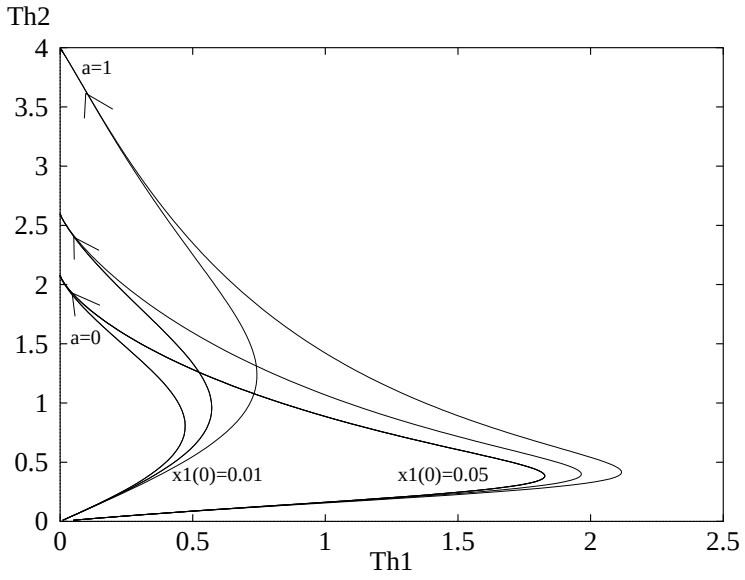


(i) $\delta_2 = 0$ (ii) $\delta_2 \sim \delta_1$

Figure 5.6.: Effects of – not T helper derived – Th1 signals in a Th1/Th2 system with autocrine growth factors. The Th1-nullcline (solid line) is shown for various values of a Th1-promoting signal θ_1. The Th2 nullcline are drawn as dashed lines, steady states as filled circles. (i) If Th2 cells do not undergo AICD ($\delta_2 = 0$) increasing T helper independent activation signal ($\theta_1 > 0$) leads to a weak decrease in the Th2 population and a weak increase in the Th1 population but does not induce a Th1 $\rightarrow$ Th2 shift. (ii) If Th2 cells do undergo AICD ($\delta_2 = 1$) increasing T helper independent signals ($\theta_1 > 0$) lead to a shift from Th2 dominance to Th1 dominance ($\theta_1 = 2$). Other parameters are $\delta_1 = 1.5$, $\pi_1 = \pi_2 = 2$, $\sigma_1 = \sigma_2 = 2$, $\theta_2 = 0$.



(i)



(ii)

Figure 5.7.: The impact of FasL expression on Th2 cells. Nullclines (i) and orbits (ii) for various relative FasL expression rates on Th2 cells $a \in \{0, 0.5, 1\}$ are shown. Th1-nullclines are represented by dashed lines, Th2-nullclines by dotted lines, and orbits by solid lines. Stable steady states are marked by boxes, unstable steady states by circles. Figure (i) shows that if the ratio a of the FasL expression rate is increased then both Th1- and Th2-nullclines start at lower Th2-values. This affects the location of the exclusive Th2 steady state. The location of the exclusive Th1 steady state (which is represented by the intersection of the non-trivial part of the Th1-nullcline with the x_1-axis), however, is unchanged. In figure (ii) orbits starting from $(0.01, 0.01)$ and $(0.05, 0.01)$ are shown. The parameter a does not affect the occurrence of Th1 $\rightarrow$ Th2 shifts but the final Th2-value (represented by the exclusive Th2 steady state) of the response. Other parameter are set to $\sigma_1 = \sigma_2 = 2, \delta_1 = 1, \delta_2 = 0.3, \pi_1 = 3, \pi_2 = 3$.

which later is taken over by Th2-dominance, can be observed for any possible value of a between zero and one (equivalent to equal FasL expression rates on Th1 and Th2 cells). In addition, it has be proved (similar to Fig. 5.5, data not shown) that the final outcome depends on the activation rates, i.e. low activation levels favor Th1 whereas higher activation rates lead to Th2-dominance. Since activation levels are correlated with antigen levels we observe the common antigen dose-dependence. We therefore conclude that the system behavior is not sensitive to changes in FasL expression rates of Th2 cells.

5.2.2. The role of the susceptibility of Th2 for AICD

In this section we concentrate on different susceptibilities of T helper subsets for AICD. We claim that this asymmetry is crucial for the antigen dose-dependence of Th1/Th2 ratio where low antigen levels lead to Th1 dominance whereas high antigen levels favor Th2 dominance. Mathematically, we can formulate this correlation in the following way. Increasing antigen levels correspond to higher activation parameters σ_1 and σ_2 while keeping their ratio $c, d > 0$ ($\sigma_1 = c\,\sigma_2$ and $\sigma_2 = d\,\sigma_1$ with $d = 1/c$) constant. We can find the described antigen dose dependency if $\exists \Omega_1, \Omega_2 > 0$ with

$$\text{(i)}\sigma_2 > \Omega_2 \Leftrightarrow \text{ the exclusive-Th2 steady state } (0, x_2^*) \text{ is stable,} \tag{5.8}$$

$$\text{(ii)}\sigma_1 < \Omega_1 \Leftrightarrow \text{ the exclusive-Th1 steady state } (x_1^*, 0) \text{ is stable.} \tag{5.9}$$

Let us first assume that Th2 are totally resistant to AICD ($\delta_2 = 0$). For the 'autocrine-Th1/Th2'-model from Sec. 5.1 we find that the exclusive Th1 steady state $(x_1^*, 0)$ is stable if the corresponding eigenvalues are negative, i.e., if

$$A\sigma_1^2 + B\sigma_1 + C < 0$$

with $A = d > 0$, $B = d\pi_1 - d - 1 + \delta_1 d$ and $C = 1 - \pi_1 - \delta_1 - \delta_1\pi_2 < 0$, cf. Eq. (5.4). Defining $U_\pm = (-B \pm \sqrt{B^2 - 4A\,C})/2A$ the following cases can be discerned.

(i) $U_\pm \in \mathbb{C} \setminus \mathbb{R}$, which is the case if $B^2 - 4A\,C < 0 \Leftrightarrow \pi_1 < \frac{1}{d}[-1 + d(1 - \delta_1 + 2\sqrt{d\delta_1\pi_2})]$ so that $A\sigma_1^2 + B\sigma_1 + C > 0$ for all $\sigma_1 \in \mathbb{R}$ and $(x_1^*, 0)$ is unstable whatever σ_1.

(ii) $U_- < 0, U_+ > 0$. This happens if $-4A\,C > 0 \Leftrightarrow \pi_1 < 1 + \delta_1(\pi_2 - 1)$ so that $(x_1^*, 0)$ is stable for all $\sigma_1 < U_+ =: \Omega_1$ and unstable if $\sigma_1 > U_+$.

(iii) $U_\pm > 0$, which is the case if $B^2 - 4A\,C > 0, -4A\,C < 0$ and $-B > 0$ (or equivalently $\pi_1 < 1 - \delta_1 + 1/d$) so that $(x_1^*, 0)$ is stable for $U_- < \sigma_1 < U_+ =: \Omega_1$. In this case $(x_1^*, 0)$ gets unstable both for very small and for very large activation parameters.

(iv) $U_\pm < 0$. In this case $(x_1^*, 0)$ is always unstable for all positive σ_1.

Summarizing, we can say that for wide parameter ranges an upper bound $\Omega_1 > 0$ for σ_1 exists so that the pure Th1 steady state $(0, x_1^*)$ is stable if $\sigma_1 < \Omega_1$. This fulfills condition (5.9).

If, however, δ_2 is too large then this upper boundary does not exists any longer. For $\delta_2 > 0$ we found that $(0, x_2^*)$ is stable if the real part of the eigenvalue (5.5) with $\sigma_2 = d\sigma_1$ is negative, i.e., $A\sigma_1^2 + B\sigma_1 + C < 0$ with $A = d\delta_1 - \delta_2$, $B = -\delta_1 - d\delta_1 + d\delta_1^2 + 2\delta_2 - \delta_1\delta_2 + d\delta_1\pi_1 - 2\delta_2\pi_1$, and $C = \delta_1 - \delta_1^2 - \delta_2 + \delta_1\delta_2 - \delta_1\pi_1 + 2\delta_2\pi_1 - \delta_1\delta_2\pi_1 - \delta_2\pi_1^2 - \delta_1^2\pi_2$. Note that here $A < 0$ if $\delta_2 > d\delta_1$. In this case $(x_1^*, 0)$ is stable if $\sigma_1 < U_-$ or $\sigma_1 > U_+$. For all of the four possible cases

(i) $U_\pm \in \mathbb{C} \setminus \mathbb{R}$,

(ii) $U_- < 0, U_+ > 0$,

(iii) $U_\pm > 0$,

(iv) $U_\pm < 0$,

there is *no* upper boundary for the stability of $(x_1^*, 0)$. We therefore suggest that different susceptibilities are necessary in order to ensure that high antigen dose leads to Th2-dominance. The condition (5.9) is not fulfilled.

The role of different susceptibility of Th1 and Th2 cells for AICD in the context of Th1 $\rightarrow$ Th2 shifts has already been discussed for the 'Th1/Th2'-model in Sec. 4.6 and for the 'autocrine-Th1/Th2'-model in Sec. 5.1.2. We found that the switching behavior is lost for similar susceptibilities ($\delta_1 \approx \delta_2$). The reason is that higher δ_2 values stabilize the exclusive Th1 steady state and orbits with strong initial Th1-bias (as it is the case for Th1 $\rightarrow$ Th2 shifts) tend to end up in that fixed point. We conclude that the different rates to undergo Fas-mediated apoptosis are essential in order to obtain Th1 $\rightarrow$ Th2 switches.

5.2.3. Asymmetries in cross-suppression

What do we mean exactly by cross-suppression? The Th1 cytokine IFN-γ influences the Th2 population by inhibiting its proliferation. IL-10, a component of the Th2-cytokine signal S_2, however, suppresses Th1-cytokine production. This leads to an indirect inhibition of Th1-proliferation and Th1-activation via inhibition of the positive Th2-cytokine derived feedback. Additionally, IL-10 directly suppresses T helper (Th1 and Th2) activation by down-regulating MHC expression.

We propose that asymmetries related to cross-suppression are – in addition to different susceptibilities for AICD – necessary for the antigen dose dependency. In particular, these asymmetries are responsible that the condition 5.8 is fulfilled. In a first step we will show that there is a lower bound Ω_2 for σ_2 so that the exclusive Th2 steady state $(0, x_2^*)$ is stable if $\sigma_2 > \Omega_2$. In a second step we will study several modified models with various implications of cross-suppressions for their ability to react to different antigen doses in the described way.

The exclusive Th2 steady state $(0, x_2^*)$ is stable if the eigenvalue of Eq. (5.3) with $\sigma_1 = c\sigma_2$ is negative, i.e., if $A\sigma_2^2 + \sigma_2 B + C < 0$ with $A = -1$, $B = c - 2\pi_2 + \pi_1$ and $C = \pi_2(\pi_1 - \pi_2)$. Note that different values for δ_2 do not affect the stability of this fixed

point. We define $U_\pm := 1/2(B \pm \sqrt{B^2 - 4A\,C})$. If we set $\Omega_2 := U_+$ then the desired boundary for $(0, x_2^*)$ stability has been found. In summary, the following cases can occur.

(i) $U_\pm \in \mathbb{C} \setminus \mathbb{R}$. This happens if $\pi_2 > (4 + \pi_1)^2/4c$ so that $(0, x_2^*)$ is stable for all values of σ_2.

(ii) $U_- < 0, U_+ > 0$, which occurs if $\pi_2 < \pi_1$ so that $(0, x_2^*)$ is stable if $\sigma_2 > U_+ > 0$ and unstable else.

(iii) $U_\pm > 0$, which is that case if $\pi_2 < (4 + \pi_1)^2/4c$, $\pi_2 > \pi_1$, and $\pi_2 < (\pi_1 + c)/2$. This leads to stability of $(0, x_2^*)$ if $\sigma_2 > U_+$ and $\sigma_2 < U_-$ and instability if $\sigma_2 < U_-$ or $\sigma_2 > U_+$.

(iv) $U_\pm < 0$, which occurs if $\pi_2 < (4 + \pi_1)^2/4c$, $\pi_2 > \pi_1$, and $\pi_2 > (\pi_1 + c)/2$. This leads to stability of $(0, x_2^*)$ for all values of σ_2.

The simultaneous existence of boundary values Ω_1 for the stability of $(x_1^*, 0)$ and Ω_2 for the stability of $(0, x_2^*)$ as in case (ii) is guaranteed for $\delta_1 > 1/(\pi_2 - 1)$. In this case low antigen doses lead to Th1-dominance and high doses to Th2-dominance. Note that in case (iii) – dependent on the initial conditions – very low antigen doses can lead to Th2-dominance, whereas intermediate doses – dependent on the stability conditions for $(x_1^*, 0)$ – favor Th1-dominance, and high doses result again in Th2-dominance. Such a behavior has been observed experimentally by Hosken et al. [39] but no theoretical explanation has been available so far. The present model, however, provides a natural explanation for this observation, which is illustrated in Fig. 5.8. Note that Th2-dominance is always preceded by a strong Th1-dominance – a feature that not explicitely has been described. Therefore, further experiments are required to decide whether our explanation for the experimental results of Hosken et al. is correct.

In order to fully understand the impact of the asymmetries in the cross-suppression on the behavior of the system let us analyze several alternative models. In each example we drop one of the asymmetries and discuss the resulting behavior.

(i) Assume that IL-10 does not inhibit Th1-cytokine production but instead explicitely inhibits both Th1-proliferation and Th1 activation. The corresponding rate equations are

$$\begin{aligned} \dot{x_1} &= \sigma_1 x_1/(1 + x_2) + \pi_1 x_1/(1 + x_2) - \delta_1 x_1^2 - x_1, \\ \dot{x_2} &= \sigma_2 x_2/(1 + x_2) + \pi_2 x_2/[(1 + x_1)(1 + x_2)] - \delta_2 x_1 x_2 - x_2, \end{aligned}$$

(ii) IL-10 does not explicitely inhibit Th1-activation but inhibits Th1-cytokine production with rate equation of the from

$$\begin{aligned} \dot{x_1} &= \sigma_1 x_1/(1 + x_2) + \pi_1 x_1/(1 + x_2) - \delta_1 x_1^2 - x_1, \\ \dot{x_2} &= \sigma_2 x_2/(1 + x_2) + \pi_2 x_2/(1 + x_1 + x_2) - \delta_2 x_1 x_2 - x_2, \end{aligned}$$

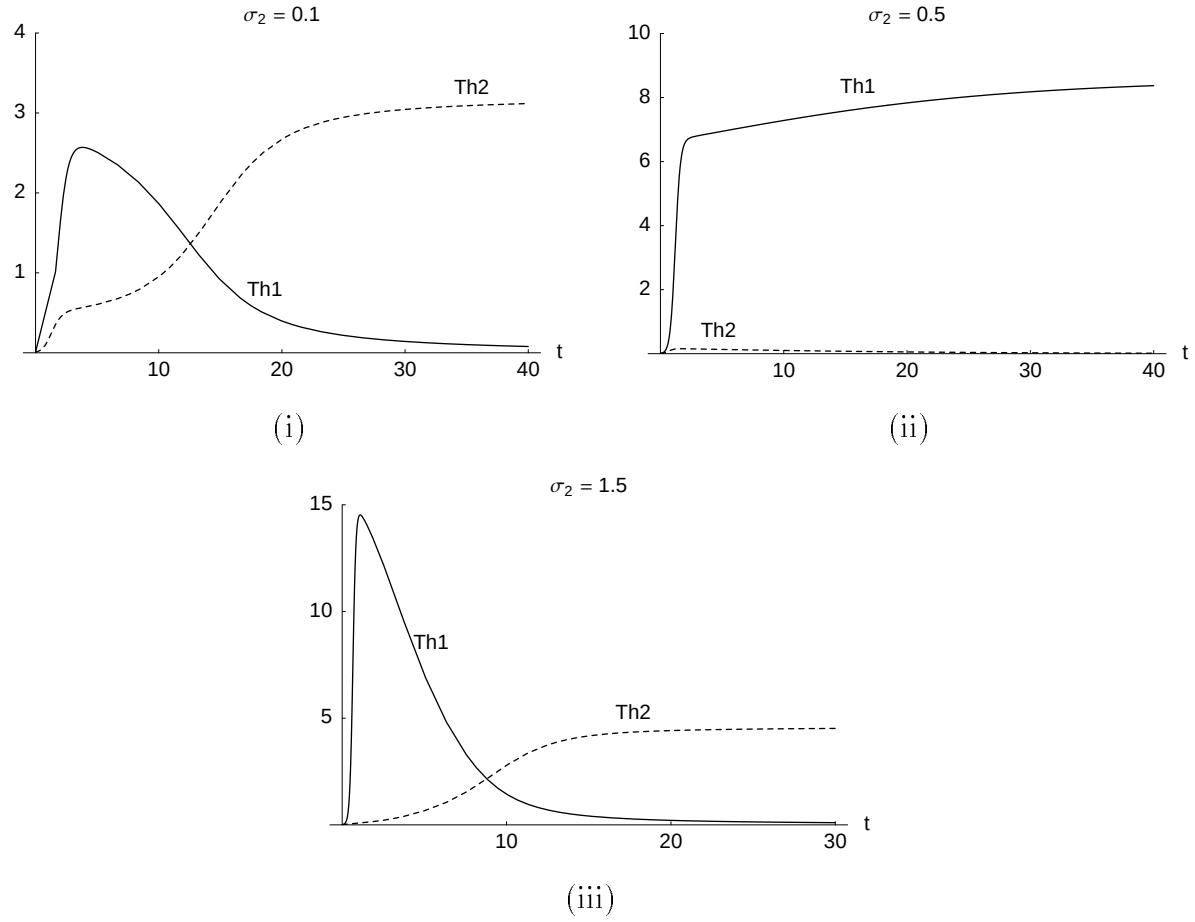


Figure 5.8.: Illustrating Th2 $\rightarrow$ Th1 $\rightarrow$ Th2 antigen dose dependency for increasing antigen levels. We show time plots of T helper concentrations for various activation parameters σ_2 and $\sigma_1 = c\sigma_2$, which correspond different to antigen levels. In figure (i) and (iii) low and high activation levels lead to Th2-dominance. In contrast intermediate activation levels lead to Th1-dominance [figure (ii)]. Other parameters are set to $c = 6, \delta_1 = 0.7, \delta_2 = 0, \pi_1 = 4, \pi_2 = 4.1$.

(iii) IL-10 only inhibits Th1-proliferation with corresponding rate equation

$$\begin{aligned} \dot{x_1} &= \sigma_1 x_1 + \pi_1 x_1/(1+x_2) - \delta_1 x_1^2 - x_1, \\ \dot{x_2} &= \sigma_2 x_2/(1+x_2) + \pi_2 x_2/[(1+x_1)(1+x_2)] - \delta_2 x_1 x_2 - x_2. \end{aligned}$$

All these modifications do not affect the location and the stability of the exclusive Th1 steady state $(x_1^*, 0)$. The conditions for the stability of the pure Th2 steady state $(0, x_2^*)$, however, are the following.

(i) $\sigma_2 > (\pi_1 - \pi_2)/(1-c)$, which is positive if either ($\pi_1 > \pi_2$ and $c < 1$) or ($\pi_2 > \pi_1$ and $c > 1$). That means that the desired lower boundary $\Omega_2 := (\pi_1 - \pi_2)/(1-c)$ exists.

(ii) Same as in (i).

(iii) $c\sigma_2^2 + \sigma_2\pi_2(c-1) + (\pi_1 - \pi_2) < 0$. This is equivalent to $U_- < \sigma_2 < U_+$ for $U_\pm = -B \pm \sqrt{B^2 - 4AC}/(2A)$ with $A = c, B = \pi_2(c-1), C = \pi_1 - \pi_2$. If $U_+ < 0$ or $U_+ \in \mathbb{C}\backslash\mathbb{R}$ then $(0, x_2^*)$ is never stable. Otherwise $(0, x_2^*)$ gets *unstable* with increasing σ_2. This is in contradiction to condition (5.8) that states that high activation rates (equivalent to high antigen doses) should favor Th2.

Above we have studied the local stability properties analytically, globally we find by simulations (Data not shown) that indeed case (iii) does not lead to antigen dose dependency whereas cases (i) and (ii) do. We propose that the crucial asymmetry for antigen dose dependency is the ability of Th2 cytokines to suppress (directly or indirectly via inhibition of positive feedback of Th1-cytokines) Th1 activation but not vice versa. Since activation becomes more important for high antigen concentrations (which leads to higher activation parameters) this inhibition gains in importance and causes the antigen dose-dependence.

To understand the impact of asymmetric cross-regulation on Th1 $\rightarrow$ Th2 shifts analytic investigation and simulations have been done. Necessary (but not sufficient) for Th1 $\rightarrow$ Th2 shifts is the stability of an exclusively-Th2 or at least Th2-dominated steady state. For the three cases above we find the following stability conditions for the exclusively Th2 steady state $(x_1, x_2) = (0, \sigma_2 + \pi_2 - 1)$.

(i) $\sigma_2 + \pi_2 > \sigma_1 + \pi_1$,

(ii) Same conditions as in (i),

(iii) $(\sigma_1 - 1) + \pi_1/(\pi_2 + \sigma_2) < 0$.

In case (iii) the exclusively Th2 steady state is never stable if Th1 activation levels are sufficiently high ($\sigma_1 > 1$). Instead a coexistence state has become the attractor of the system [see Fig. 5.9(i)]. One could argue that in case (iii) unequal activation or proliferation parameters ($\sigma_2 > \sigma_1, \pi_2 > \pi_1$) would lead to a coexistence state that is Th2-dominated and that Th1 $\rightarrow$ Th2 shifts of nearly polarized – not exclusive – T helper responses could be possible. In this case, however, high initial Th2 values are favored rather then high

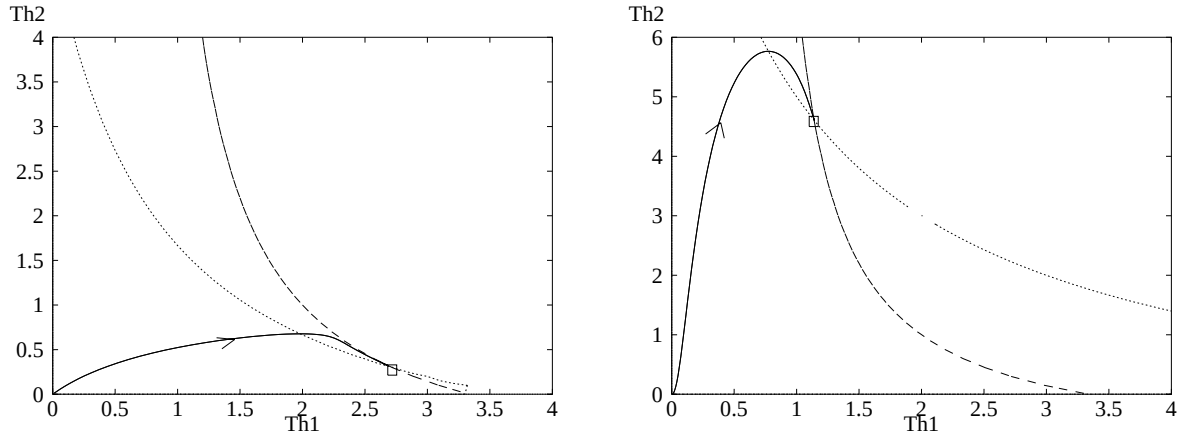


Figure 5.9.: Illustrating the disappearance of Th1 $\rightarrow$ Th2 shifts if asymmetries in cross-regulation are varied. If IL-10 does *not* inhibit Th1 activation Th1 $\rightarrow$ Th2 shifts do not occur. Equal activation and proliferation parameters ($\sigma_1 = \sigma_2 = 2, \pi_1 = \pi_2 = 4$) lead to a Th1-dominated coexistence of Th1 and Th2 [figure (i)], higher Th2-related parameters ($\sigma_2 = 6, \pi_2 = 6$) result in Th2-dominated coexistence of Th1 and Th2 [figure (ii)]. Starting from initial values (0.01, 0.01) Th2-values dominate rather then Th1-values. Th1-nullclines are drawn as dashed lines, Th2-nullclines as dotted lines and orbits as solid lines. Stable steady states are represented by boxes. Other parameter are set to $\sigma_1 = \sigma_2 = 2, \delta_1 = 1.5, \delta_2 = 0.5, \pi_1 = \pi_2 = 4$.

Th1-values. This is illustrated in Fig. 5.9(ii). We therefore propose that for case (iii) Th1 $\rightarrow$ Th2 switches can not be observed whereas cases (i)-(ii) allow switches (confirmed by simulations, data not shown).

We therefore conclude that antigen dose dependency (Th1 $\rightarrow$ Th2 with increasing antigen dose) and Th1 $\rightarrow$ Th2 switches occur under similar conditions.

5.3. Summary and Discussion

In this section we analyzed the asymmetries in the elementary interactions between the system components that give rise to the essential asymmetric features of macroscopic behavior of the T helper system. The crucial factors for the occurrence of antigen dose dependency (Th1 $\rightarrow$ Th2 when antigen dose rises) and Th1 $\rightarrow$ switches during the ongoing immune response are

(i) the suppression of Th1 activation by Th2-related cytokines but not vice versa and

(ii) different susceptibilities for AICD of the two T helper subsets.

The natural question is, what is the relation between the antigen dose dependency and Th1 $\rightarrow$ Th2 shifts? It seems plausible that Th1 $\rightarrow$ Th2 shifts are a direct consequence of the antigen dose dependency. Low initial antigen levels lead to Th1 dominance but as soon

as the antigen concentration rises Th2 dominance is induced and a Th1 $\rightarrow$ Th2 shift can be observed. Note, however, that switches could also be observed if antigen concentrations are constant (Sec. 4.6). Thus, rising antigen concentration are not necessary for a switch to be induced. We propose that the regulatory mechanisms that lead to the antigen dose dependency and to Th1 $\rightarrow$ Th2 switches are the same and that both phenomena are closely related.

How do the results of the present chapter compare with results from other models of T helper subsets? Fishman and Perelson [30] or Behn [5] have also described antigen dose dependency. They find – in agreement with our results – that low antigen dose leads to Th1 dominance whereas higher antigen levels favor Th2 dominance. In both cases the only asymmetry in Th1 and Th2 regulation lies in the fact that Th1-cytokines inhibit Th2 proliferation whereas Th2-cytokines suppress production of Th1-cytokines, which leads to an inhibition of Th1 proliferation and activation. Fishman and Perelson, in addition, analyzed different roles of growth factors (autocrine versus systemic). Their findings that the overall behavior is unaffected by these different choices is in agreement with our results.

Muraille et al. [70] and Lev Bar-Or [53] describe the occurrence of Th1 $\rightarrow$ Th2 switches. In Muraille's model the crucial asymmetry is that Th2 cells inhibit Th1 activation whereas Th2 cells inhibit Th2 proliferation. Lev Bar-Or suggests that Th1 $\rightarrow$ Th2 shifts occur if the macrophage presentation ability has a larger positive sensitivity to Th1 cytokines than Th2 cytokines. In other words T helper activation rates are more negatively affected by Th2 cytokines than by Th1 cytokines. As a consequence Th2 cytokines – on average – inhibit Th1 activation whereas Th1 cytokines do not inhibit Th2 activation.

We conclude that in – to our knowledge – all models that described antigen dose dependency or Th1 $\rightarrow$ Th2 shifts, these features are based on an asymmetric inhibition of activation of the two T helper subsets on the competing type. In our model, in addition, different roles of AICD for the two phenotypes are necessary.

6. How an appropriate T helper response can be made

The aim of this chapter is to present an intrinsic mechanisms of the T helper system for the selection of the appropriate T helper response [9]. This mechanism is based on the ability of the T helper system to switch from an unsuccessful Th1 to an eventually more successful Th2 response.

6.1. Introduction

Studies of *in vivo* immune responses have described infectious agents with a predisposition to induce either cell-mediated or humoral forms of immunity. Defense against intracellular pathogens tends to be associated with Th1 dominance and resultant cellular cytolytic activity whereas resistance to extracellular infectious agents is most often dominated by Th2 effectors, which lead to the production of high levels of antigen-specific immunoglobulins. It therefore seems that the immune system is able to *select* the appropriate immune response, which is triggered by one of the T helper types. A great number of publications, both experimental and theoretical, elucidate the influence of a large number of different factors on the Th1/Th2 balance. The principal novelty of this approach here is its attempt to deal with the central problem of to what extent all these factors provide balances that are *appropriate* to the variety of different challenges that the immune system faces.

Concerning the question of how the immune system selects the appropriate T helper type that efficiently destroys a particular pathogen several possible mechanisms have been proposed:

- Pattern recognition by the innate immune system [61]
- "Diffuse feedback" [86]
- 'Voting' of memory cells [12]

For all these mechanisms additional information on the pathogen provided by other immune system components in form of signals that promote one T helper type is necessary. Here we want to show by means of a mathematical model that the T helper system *itself* can – under certain circumstances – select the most efficient immune response. For this process

no additional external information is required because it is based only on the relevant cytokine interactions and on Fas-mediated apoptotic signals.

This chapter is organized as follows. Sec. 6.2 will review the relevant biological background. We analyze a simple mathematical model in Sec. 6.3 in order to understand the internal behavior of the T helper system in reaction to varying antigen levels. In Sec. 6.3.1 we argue for a role of the cell-cycle dependence of the T helper cytokine production in giving an initial Th1-bias. The relevant steady states and their properties are presented in Sec. 6.3.2 and the impact of unequal Th1/Th2 activation and proliferation parameters are analyzed in Sec. 6.3.3. We study the necessity of Th1-biases in Sec. 6.4.1 and discuss the role of antigen dose-dependence of the Th1/Th2 ratio (Sec. 6.4.2). Furthermore, in Sec. 6.4.3, we investigate when infections can become chronic. Finally, we present a mechanism for the selection of the appropriate T helper response in Sec. 6.4.4 and discuss the important effects of differences in susceptibility for activation induced cell death between Th1 and Th2 cells (Sec. 6.4.5).

6.2. Components of the simplified mathematical model

In the previous chapter we presented a model of cytokine-modulated regulation of helper T cell populations under a constant antigenic stimulus. This model did not consider pathogen removal and growth but concentrated instead on the evolution of the T helper response under antigen exposure, owing to cytokine and Fas/FasL interactions.

A novel feature of the model presented here is that we explicitly include antigen clearance and growth. Moreover, following the ideas of the 'autocrine-Th1/Th2'-model of Sec. 5.1, we assume that IL-2 and IL-4 induce proliferation and that IL-2 induces up-regulation of FasL on Th1 in an autocrine way. As in the previous chapter we describe the rate equations for T helper populations in the form

$$\begin{aligned}&\text{Rate of change of antigen-specific Th1/Th2 cell population}\\ &= \text{differentiation} + \text{proliferation} - \text{death}.\end{aligned}$$

Additionally we directly model pathogen dynamics and its interference with the T helper system by the rate equation

$$\begin{aligned}&\text{Rate of change of pathogen concentration}\\ &= \text{replication} - \text{loss induced by Th1-effectors} - \text{loss induced by Th2 effectors}.\end{aligned}$$

6.2.1. T cell activation and differentiation

The antigen concentration P now directly affects activation rates. The more antigen is available the more gets presented on antigen presenting cells to the T cells and the more T helper cells become activated.

Thus,

$$\text{Th1 activation rate} = \frac{\xi_1 S_1 P}{1 + kS_2}, \text{Th2 activation rate} = \frac{\xi_2 S_2 P}{1 + kS_2}.$$

6.2.2. Proliferation

In the previous chapter we have modeled T helper regulation with two different roles of growth factors: systemic in Sec. 4.1 and autocrine in Sec. 5.1. We found that the most important features of the qualitative behavior – such as Th1 $\rightarrow$ Th2 shifts and antigen dose-dependence of T helper differentiation – are not affected by different roles of IL-2 and IL-4 and that the crucial asymmetries within Th1/Th2 regulation lie in the differences in the cross-suppression and susceptibility for Fas-mediated apoptotic signals. Thus, we analyze the simpler model of autocrine utilization, which leads to the equations

$$\text{Th1 proliferation rate} = \frac{\beta_1 T_1}{1 + kS_2}$$

and

$$\text{Th2 proliferation rate} = \frac{\beta_2 T_2}{(1 + kS_1)(1 + kS_2)}.$$

6.2.3. Death

In the previous chapter we investigated consequences of the differential importance of AICD for Th1 and Th2 phenotypes in a system with continuous antigen stimulation. We found that some Th2 susceptibility for AICD is necessary for stabilizing the two polarized arms of the T helper response but, at the same time, a substantially lower susceptibility of Th2 cells compared to Th1 cells is crucial for the occurrence of Th1 $\rightarrow$ Th2 shifts. As in Chap. 4 we assume here that FasL expression occurs purely in the Th1 phenotype. In accordance to Sec. 5.1 we neglect effects of IL-2, which has been shown to up-regulate FasL expression, in the terms for AICD because of the autocrine nature of effects of IL-2.

Based on the previous discussion we model the removal of T helper cells from the effector pool by means of two terms.

$$\text{Th1 removal rate} = \Delta_1 T_1^2 + \mu T_1$$

and

$$\text{Th2 removal rate} = \Delta_2 T_1 T_2 + \mu T_2.$$

6.2.4. Pathogen rate equations

We assume exponential growth for the pathogen with replication rate r. Pathogen clearance is modeled by the simplest possible choice $\omega_i T_i P$, $(i = 1, 2)$, with pathogen destruction efficiency of Th1- and Th2-induced effector cells measured by the parameters ω_1 and ω_2.

6.2.5. Model summary

The preceding discussion yields the following equations:

$$\frac{\mathrm{d}T_1}{\mathrm{d}t} = \frac{\xi_1 P S_1}{1+kS_2} + \frac{\beta_1 T_1}{1+kS_2} - \Delta_1 T_1^2 - \mu T_1, \tag{6.1}$$

$$\frac{\mathrm{d}T_2}{\mathrm{d}t} = \frac{\xi_2 P S_2}{1+kS_2} + \frac{\beta_2 T_2}{(1+kS_1)(1+kS_2)} - \Delta_2 T_1 T_2 - \mu T_2, \tag{6.2}$$

$$\frac{\mathrm{d}P}{\mathrm{d}t} = \varrho P - \omega_1 T_1 P - \omega_2 T_2 P. \tag{6.3}$$

We rescale to the following dimensionless combinations of variables; $x_i = k\alpha_i T_i$, $p = rP$, $\tau = \mu t$ giving rise to the following dimensionless parameters

$$\sigma_i = \xi_i \alpha_i / \mu r, \quad \delta_i = \Delta_i / \mu k \alpha_i, \quad \pi_i = \beta_i / \mu, \quad \nu_i = \omega_i / \alpha_i k \mu, \quad \text{and} \quad r = \varrho/\mu.$$

With the expressions (4.1) for the cytokine signals S_1 and S_2, the resulting dynamical equations are

$$\frac{\mathrm{d}x_1}{\mathrm{d}\tau} = \frac{\sigma_1 x_1 p}{(1+x_2)^2} + \frac{\pi_1 x_1}{(1+x_2)} - \delta_1 x_1^2 - x_1, \tag{6.4}$$

$$\frac{\mathrm{d}x_2}{\mathrm{d}\tau} = \frac{\sigma_2 x_2 p}{(1+x_2)} + \frac{\pi_2 x_2}{(1+x_1+x_2)} - \delta_2 x_1 x_2 - x_2, \tag{6.5}$$

$$\frac{\mathrm{d}p}{\mathrm{d}\tau} = p(r - \nu_1 x_1 - \nu_2 x_2). \tag{6.6}$$

The biological significance of the parameters is summarized in Table 6.1.

6.3. Analysis

In our analysis we will address the following issues;

- Can the T helper system *itself* choose the most appropriate T helper type?
- What are the important parameters for the decision-making process?
- Under which conditions does this mechanism work? What is the role of AICD?
- Is there any role for apparent artefacts such as antigen dose-dependence of T helper differentiation and cell-cycle dependence of cytokine production?
- Are there any default responses? If so, what are they?
- Under which conditions do infections become chronic? Is it equally likely for Th1- or Th2-dominated situations to become chronic?

Parameter ($i = 1, 2$)	*Interpretation*
σ_i	Activation strength, weighted for its Th(i)-inducing properties
π_i	Efficiency of growth factors at maintaining activated cells in cycle
δ_i	Susceptibility of Th(i) cells to activation-induced cell death
r	Growth rate of pathogens
ν_i	Pathogen elimination efficiency of Th(i)-induced effectors

Table 6.1.: Biological interpretation of the dimensionless parameters in equations (6.4) - (6.6).

6.3.1. Initial conditions

What are the initial conditions for our model?

If the adaptive immune system is challenged with a particular pathogen it has not seen before then antigen peptides are presented to not-yet-differentiated Th0 cells, which produce both Th1 and Th2 cytokines in small amounts. On the population level this can be interpreted as small, non-zero initial values for the Th1 and Th2 populations. This resolves the mathematical bootstrap situation where – in the absence of stimuli from other immune system components – new T helper cells of both phenotypes can only be generated in the presence of Th1/Th2 signals, which in turn rely on already existing T helper populations. In our integrations we therefore consider the initial conditions for a truly naive response, with no assistance from cross-reactive memory T cells, as represented by trajectories beginning at low initial values for Th1 and Th2.

Effector cytokine expression has been shown to be cell-cycle dependent. IFN-γ expression increases in frequency with successive cell cycles, while IL-4 secretion does not start before three cell divisions are completed [10]. These relationships were consistent regardless of time-dependent variation in the distribution of Th1 and Th2. Additionally it has been shown that IFN-γ mRNA is induced within 6 hr in activated, IL-12 primed cells, while IL-4 mRNA is induced only after 48 hr, even when cells are cultivated in the presence of rIL-4 [50]. Therefore cell cycle provides fundamental order to the differentiation of Th cells. A naive cell can become a Th1 cell with increasing likelihood after each successive cell cycle, but can only become a Th2 cell when eight siblings have been born. Because of the cell cycle dependence of cytokine patterns we assume that initial values for Th1 are higher than those for Th2, which gives the system an initial Th1-bias. In Sec. 6.4.1 we will argue for the *need* for a Th1-bias.

6.3.2. Steady states and their stability

To keep the number of free parameters small, because of lack of data, and to extract a system behavior independent of preferences for one of the T helper types, we assume that proliferation and activation rates of Th1 and Th2 cells are equal: $\sigma_1 = \sigma_2 = \sigma$, $\pi_1 = \pi_2 = \pi$. Implications of unequal parameters will be discussed in Appendix B.

We are only interested in steady states in the positive quadrant. We analyze the conditions for existence and stability of those fixed points, which will be represented by a vector $\Omega = (x_1, x_2, p)$.

- naive state: $\Omega_{\text{naiv}} = (0, 0, 0)$, with eigenvalues $\{\pi - 1, \pi - 1, r\}$. This steady state is never stable, since we assume that the proliferation rate is high enough to counterbalance cell death, i.e., $\pi > 1$.

- 'cure:Th2': $\Omega_{\text{cure:Th2}} = (0, \pi - 1, 0)$. Eigenvalues for this fixed point are $\{0, -1 + 1/\pi, r + \nu_2(1-\pi)\}$. It only becomes stable if Th2-induced effectors are efficient enough in pathogen destruction and elimination, i.e., if $\nu_2 > r/(\pi - 1) > 0$. Otherwise antigen is persistent and infection becomes chronic. The smaller the replication rate r for the pathogen the larger is the set of initial conditions that lead to antigen clearance.

- 'chronic:Th2':

$$\Omega_{\text{chronic:Th2}} = (0, \frac{r}{\nu_2}, \frac{r}{\sigma\nu_2} + \frac{1-\pi}{\sigma})$$

  with eigenvalues

$$\lambda_1 = \frac{-r + \nu_2(\pi - 1)}{(r + \nu_2)^2}, \lambda_{2,3} = \frac{-r \mp \sqrt{r(r - 4(r + \nu_2)(r + \nu_2(1 - \pi)))}}{2(r + \nu_2)}.$$

  The condition $r + \nu_2(1 - \pi) > 0$ that $\Omega_{\text{chronic:Th2}}$ has to be in the first quadrant implies that – if the steady state exists – all eigenvalues do have negative real part. The more destructive Th2 effectors (larger ν_2), the less Th2 effectors and antigen are involved in the chronic interactions. Increasing ν_2 exchanges stability between a Th2-dominated chronic infection and a successful Th2 response, leading to cure.

- 'cure:Th1': $\Omega_{\text{cure:Th1}} = (\frac{(\pi-1)}{\delta_1}, 0, 0)$. Study of the eigenvalues

$$\{1 - \pi, -1 + \frac{\delta_1\pi}{(-1 + \delta_1 + \pi)} - \frac{\delta_2(\pi - 1)}{\delta_1}, r + \frac{\nu_1(1 - \pi)}{\delta_1}\}$$

  shows that the efficiency of Th1-induced effectors determines whether antigen is cleared by Th1 effectors or remains persistent in a chronic situation. If $\nu_1 > \delta_1 r/(\pi - 1)$ and $\delta_2 > \delta_1(\delta_1 - 1)/(\delta_1 + \pi - 1)$ then this steady state is stable. If $\nu_1 < \delta_1 r/(\pi - 1)$ the system ends up in Th1- or Th2-dominated chronic situations or antigen is cleared by Th2 effectors, depending on other parameters such as ν_2 and on the initial conditions.

- 'chronic:Th1':

$$\Omega_{\text{chronic:Th1}} = (\frac{r}{\nu_1}, 0, \frac{\delta_1 r}{\sigma\nu_1} + \frac{1-\pi}{\sigma}).$$

A necessary condition for existence of this steady state is $\nu_1 < \delta_1 r/(\pi - 1)$; this is the case if the first eigenvalue of 'cure:Th1' is positive, which is equivalent to a situation where 'cure:Th1' is unstable. The last two eigenvalues

$$\lambda_{2,3} = \frac{-\delta_1 r \mp \sqrt{{\delta_1}^2 r^2 - 4r\nu_1(\delta_1 r + \nu_1(\pi - 1))}}{2\nu_1}$$

do always have negative real part if the existence condition is fulfilled. The first eigenvalue

$$\lambda_1 = \frac{(\delta_1 - \delta_2)r}{\nu_1} - \frac{\pi r}{(r + \nu_1)}$$

becomes negative if $\nu_1 > r(\delta_1 - \delta_2)/(\pi - \delta_1 + \delta_2)$. The two conditions for stability and existence can only be fulfilled simultaneously if $\delta_2 > \delta_1(\delta_1 - 1)(\delta_1 + \pi - 1))$. For this choice of δ_2 there exists a parameter window – illustrated in Fig. 6.2 – where Th1-chronic is stable; the width of this window is dependent on the rate with which Th2 cells undergo AICD. Note that AICD for Th2 only influences the stability of both types of Th1-dominated steady states 'cure:Th1' and 'chronic:Th1'. We will come back to this issue in Sec. 6.4.5.

- 'cure:Th1/Th2':

$$\begin{aligned}\Omega_{\text{cure:Th1/Th2}} \quad = \quad & (-\frac{\delta_1 + \delta_2 - \sqrt{\delta_1 - \delta_2}\sqrt{\delta_1 - \delta_2 + 4\delta_1\delta_2\pi}}{2\delta_1\delta_2}, \\ & \frac{\delta_1(1 - 2\delta_1 - 2\delta_2) - \delta_2 + \sqrt{\delta_1 - \delta_2}\sqrt{\delta_1 - \delta_2 + 4\delta_1\delta_2\pi}}{2\delta_1(\delta_1 - \delta_2)}, 0).\end{aligned}$$

We have analyzed the stability of this steady state numerically for a wide range of parameters. The result is that it can not be stable and positive at the same time, because conditions for existence and stability are incommensurable. This is illustrated for typical parameters in Fig. 6.1.

- 'chronic:Th1/Th2': Steady states with co-existence of Th1 and Th2 under persistent antigen stimulation can not be calculated analytically. We instead have investigated large parameter ranges numerically and find that for low Fas-mediated apoptosis rates chronic situations are polarized in their T helper response – after having reached the steady state and in the absence of APC-derived stimuli.

The conditions for existence and stability of the relevant steady states are summarized in Table 6.2.

Steady state	*Existence*	*Stability*
naive state	exists always	$\pi < 1, r < 0$
'cure:Th2'	$\pi > 1$	$\nu_2 > r/(\pi - 1)$
'chronic:Th2'	$\nu_2 < r/(\pi - 1)$	stable when existent
'cure:Th1'	$\pi > 1$	$\nu_1 > \frac{\delta_1 r}{\pi - 1}, \delta_2 > \frac{\delta_1(\delta_1 - 1)}{\delta_1 + \pi - 1}$
'chronic:Th1'	$\nu_1 < \frac{\delta_1 r}{\pi - 1}$	$\nu_1 > \frac{r(\delta_1 - \delta_2)}{\pi - \delta_1 + \delta_2} \Rightarrow \delta_2 > \frac{\delta_1(\delta_1 - 1)}{\delta_1 + \pi - 1}$
'chronic:Th1/Th2'	not existent if δ_2 small enough	not stable if δ_2 small enough

Table 6.2.: Steady states and conditions for their existence and stability. As noted in the text, we assume that $\pi > 1$.

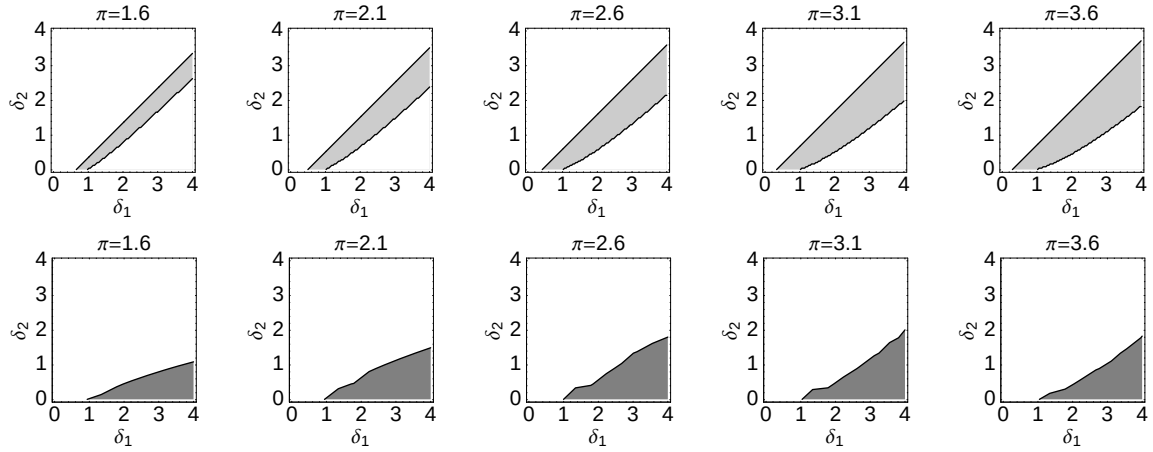


Figure 6.1.: Existence and stability of the 'cure:Th1/Th2' steady state. For different values of the proliferation rate π we plot areas of stability (dark gray) and areas where the 'cure:Th1/Th2' steady state is in the positive quadrant (light gray) on the AICD parameter plane. These two conditions are not fulfilled simultaneously, implying that a steady state with co-existing populations of Th1 and Th2 after antigen clearance can not be reached. The remaining parameters are $\sigma = 2$, $r = 1$, $\nu_1 = 2$, and $\nu_2 = 0.01$, which reflect scenarios with the largest areas of existence and stability for the parameter ranges that have been studied.

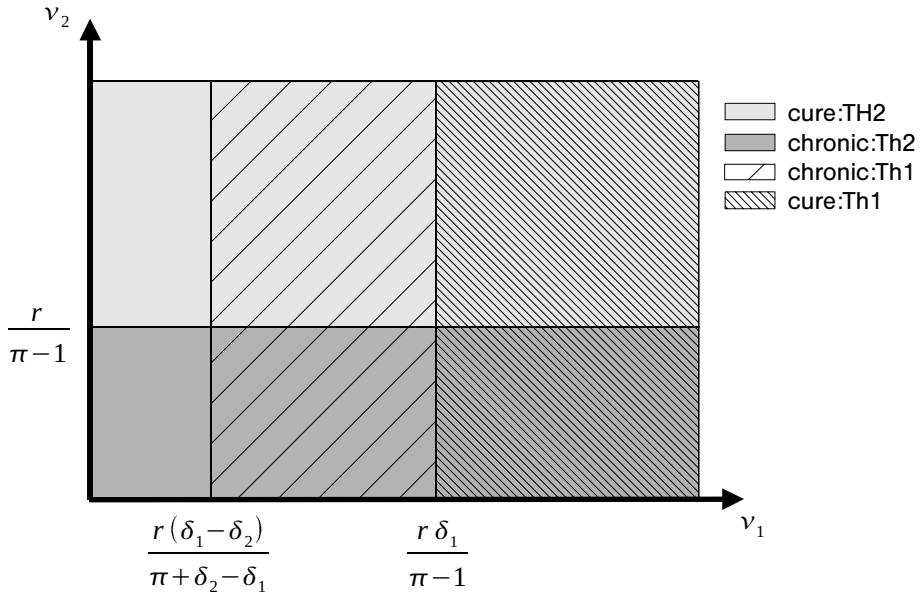


Figure 6.2.: Existence and stability are marked by different shadings in the two-dimensional pathogen-elimination parameter plane (ν_1, ν_2) for $\delta_1 > 1$, $\pi > 1$, and $\delta_1 > \delta_2 > \delta_1(\delta_1 - 1)/(\delta_1 + \pi - 1)$.

The main features of the steady state analysis are summarized in Fig. 6.2, a diagram of the 2-dimensional pathogen-elimination parameter space (ν_1, ν_2). From this analysis we reach the following conclusions.

- If only one T helper type leads to pathogen destruction ($\nu_1 \approx 0, \nu_2 > 0$ or $\nu_2 \approx 0, \nu_1 > 0$) – as is reasonable to assume for pathogens that are purely extra- or intracellular – we find bistability either with a successful Th1 response and a chronic Th2-dominated situation or vice versa. This means that initial conditions decide whether pathogen clearance or chronic disease is obtained.

- Although T helper responses will be polarized towards one of the phenotypes after reaching the steady states we find a transient co-existence of both. Orbits that reach the pure Th2 steady state often exhibit long 'detours'. An initial Th1 dominance is later taken over by Th2 dominance. This is illustrated in Fig. 6.3.

- The rate with which Th2 cells undergo AICD does not change the location and local stability of both Th2-dominated steady states but decreases the real parts of the eigenvalues corresponding to the Th1-dominated steady states and in that sense stabilizes them.

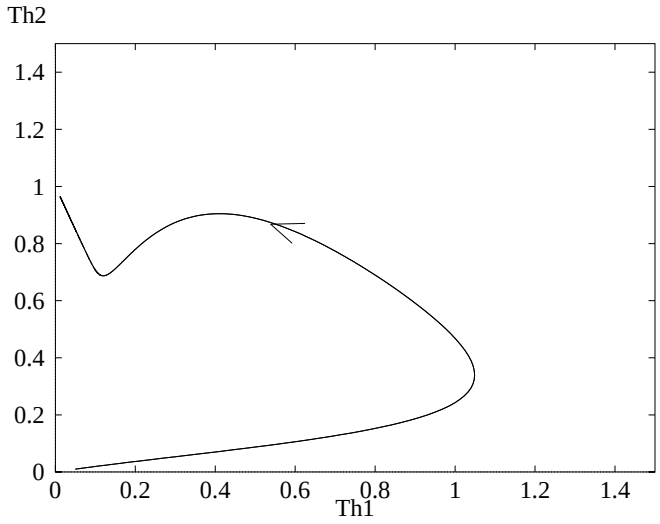


Figure 6.3.: Transient co-existence of Th1/Th2 populations. The plot shows a trajectory in the Th1/Th2 state space with the initial condition $x_1(0) = 0.05, x_2(0) = 0.01, p(0) = 0.1$. Transient Th1 dominance and co-existence is followed by Th2 dominance. The asymmetry in the initial conditions is a consequence of the cell-cycle dependency of cytokine production. Parameters are $\sigma = 2, \pi = 2, \delta_1 = 2, \delta_2 = 1, r = 1, \nu_1 = 0.1, \nu_2 = 2$. Here and in several figures below, for ease in interpretation we label curves Th1 and Th2, instead of x_1 and x_2.

6.3.3. Unequal proliferation and activation parameter

In our first model for the sake of simplicity we set activation and proliferation parameters of both T helper subsets to the same values. Here we summarize consequences of unequal parameter settings. A detailed analysis of the steady states and their stability for unequal parameter settings of activation and proliferation parameters can be found in the Appendix.

- Higher activation rates of one T helper type generally stabilize 'chronic' steady states dominated by the corresponding T helper subset, and destabilize chronic situations dominated by the competing T helper type. As a consequence increasing σ_1 and σ_2 respectively enlarge and reduce the size of the parameter window for stability of 'chronic:Th1'. This is illustrated in Fig. 6.4.

- Higher efficiency of Th2-growth factors stabilizes Th2-dominated steady states and destabilizes Th1-dominated steady states and vice versa. With increasing π_2, stability of 'chronic:Th1' is lost. Increasing π_1, however, decreases the lower boundary of the 'chronic:Th1' stability parameter window, which is given by the stability condition, but also decreases the upper boundary given by the existence condition. Effects on the size of the parameter window are illustrated in Fig. 6.5.

- The overall behavior of the system is not affected by choices of activation and proliferation parameters.

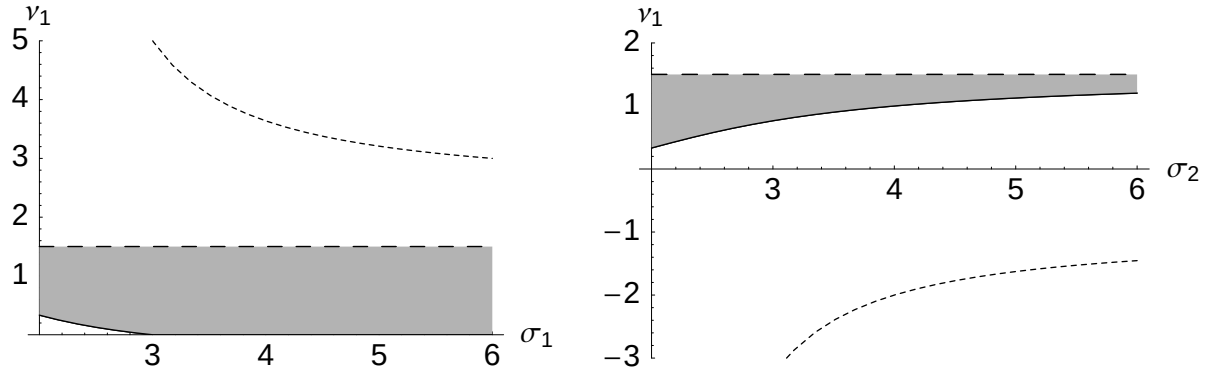


Figure 6.4.: Upper and lower boundaries of the parameter-window where 'chronic:Th1' is stable plotted as a function of the activation parameters $\sigma_{1,2}$. Dashed lines represent the condition for existence, and dotted (solid) lines the condition for stability represented by B_1 (B_2), cf. in Appendix B. For parameters that lie in the area between the solid and dashed line, 'chronic:Th1' is stable (shaded area). Left figure: $\sigma_1 > \sigma_2$. Increasing Th1-activation strength leads to extension of the parameter-window where 'chronic:Th1' is stable. For Th1-activation strengths above a certain threshold the lower boundary is lost. Right figure: Higher Th2-activation parameters reduce the size of the parameter-window. The remaining parameters are $\sigma_i = 2$, $\pi = 2$, $\delta_1 = 1.5$, $\delta_2 = 0.5$, and $r = 1$.

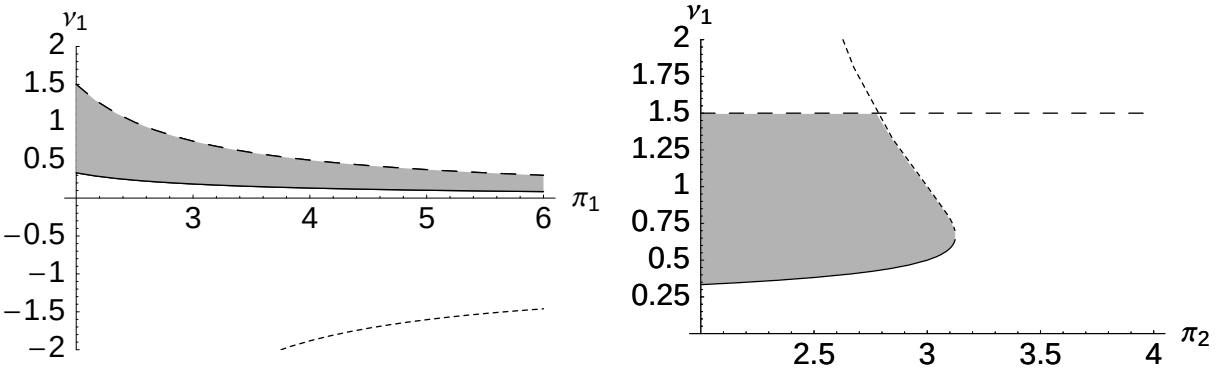


Figure 6.5.: Upper and lower boundaries of the parameter-window where 'chronic:Th1' is stable plotted as a function of the proliferation parameters $\pi_{1,2}$. Dashed lines represent the condition for existence, and dotted (solid) lines the condition for stability represented by B_1 (B_2), cf. Appendix B. For parameters that lie in the shaded area 'chronic:Th1' is stable. Left figure: $\pi_1 > \pi_2$. Increasing Th1-proliferation strength leads to extension of the lower boundary of the parameter-window where 'chronic:Th1' is stable. Right figure: Higher Th2-activation parameters reduce the size of the parameter-window. For values of π_2 above a certain threshold the stability of 'chronic:Th1' is lost. The remaining parameters are $\sigma_i = 2$, $\pi = 2$, $\delta_1 = 1.5$, $\delta_2 = 0.5$, and $r = 1$.

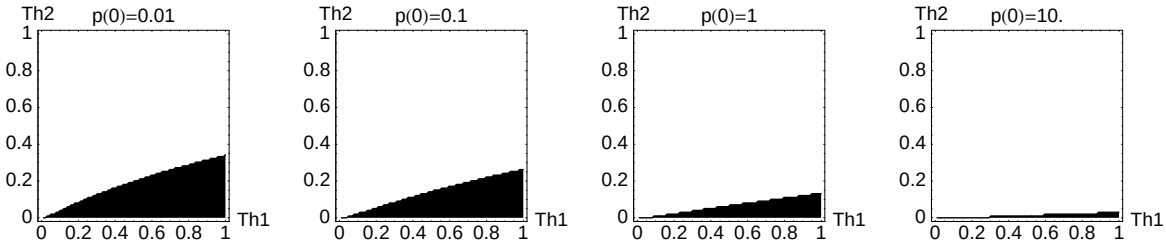


Figure 6.6.: Illustrating the dependence on a Th1 bias in order to obtain Th1-dominance. For different values of initial antigen values, basins of attraction of the two stable steady states 'cure:Th1' (black area) and 'chronic:Th2' (white area) are plotted. For equal initial conditions for the two T helper subsets 'cure:Th1' – the appropriate T helper response for this setting of ν_1 and ν_2 – is never reached. If Th1 is favored initially then for a sufficiently weak antigenic challenge a successful Th1 response can be attained. Parameters are $\sigma = 2$, $\pi = 2$, $\nu_1 = 2$, $\nu_2 = 0.1$, $\delta_1 = 1.5$, $\delta_2 = 0.5$ and $r = 1$.

6.4. Interpretation

6.4.1. Obtaining a Th1-dominated response

In order to obtain a Th1-dominated immune response in our model the presence of a Th1 bias is essential. This is particularly important if a Th1 response is the desired one. In Fig. 6.6 we show basins of attraction for a parameter setting where – depending on the initial conditions – the system can end up in a successful Th1-dominated immune response or in a chronic Th2-dominated disease state. We find that symmetrical initial conditions or symmetrical parameters for the two T helper subsets will always lead to the latter case. The important fact that initial antigen dose also influences the basins of attraction will be discussed in Sec. 6.4.2.

According to our model there are several alternatives for a Th1-bias. Theoretically, a Th1 bias can arise due to unequal initial concentrations (also see Sec. 6.3.1), higher efficiency of Th1 growth factors, higher Th1 activation or Th1-promoting APC-derived signals. If IL-2 were a more efficient inducer of Th1 proliferation than IL-4 is as an inducer of Th2 proliferation, leading to different proliferation parameters $\pi_1 > \pi_2$, we find that a Th2-dominated state could never be attained. We therefore conclude that discrepancies in proliferation should be neglected as a possible source for the Th1-bias – for a well-working immune system must retain the option of a Th2 response. Which of the other possibilities might be relevant in nature, and under which circumstances, has still to be investigated. In summary, a Th1 bias is needed to attain Th1 dominance if this is the desired immune response. This bias, however, also increases the risk of chronic Th1 infections.

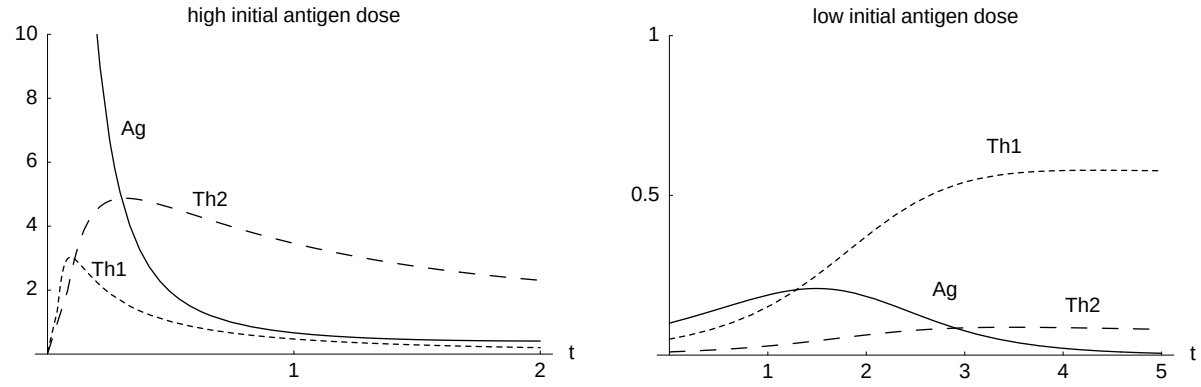


Figure 6.7.: Illustrating antigen dose dependency. Dotted, dashed and continuous lines represent Th1,Th2, and antigen concentration, respectively. Left figure: The Th1 bias promotes Th1 but high antigen doses ($p(0) = 50$) induce a rapid shift to Th2-dominance. Right figure: At low initial antigen levels ($p(0) = 0.1$) Th1 eliminates that antigen and the initial Th1 bias is maintained. The remaining parameters are $\sigma = 2$, $\pi = 2$, $\delta_1 = 1.5$, $\delta_2 = 0.5$, $\nu_1 = 4, \nu_2 = 0.1$, and $r = 1$. Cell cycle dependence of cytokine production leads to unequal initial T helper concentration $x_1(0) = 0.05, x_2(0) = 0.01$

6.4.2. Influence of the initial antigen concentration

In agreement with the results of Sec. 4.2.6 according to our current model increasing initial antigen levels promote the development of Th2 dominance whereas lower antigen doses favor Th1. This is illustrated for an effective Th1-dominated situation in Fig. 6.6. If Th2 cells not Th1 cells, are effective in attacking the pathogen, the Th1-bias leads to a short transient Th1-dominance, which – as Fig. 6.7 shows – is rapidly taken over by Th2 cells because of the high antigen levels.

The antigen dose-dependence presented above is a consequence of asymmetries in cross-regulation and of different susceptibilities of the two T helper subsets for AICD but is independent of the efficiency of the two T helper subpopulations to remove the pathogen. Crucial for this effect is that IL-10 secreted by Th2 lymphocytes inhibits cytokine-secretion of Th1 cells, which results in an indirect inhibition of proliferation and activation, whereas Th1 cytokines only inhibit Th2 proliferation. The inhibition of activation becomes more important with increasing intrinsic activation strength.

At first glance the fact that antigen dose *alone* can alter the Th1/Th2 ratio seems to make no sense for a selecting the correct T helper response against a particular pathogen. When we look closer we find that a built-in self-organizing mechanism for a decision-making process is based on the occurrence of Th1 $\rightarrow$ Th2 switches if antigen concentrations increase sufficiently. Asymmetries in regulation that cause switches also cause antigen dose-dependence. In a situation where the initial Th1 response is ineffective and thus antigen concentrations reach high levels – a situation which favors Th2 dominance – switches from Th1 to a more appropriate Th2 response are even accelerated. However, if pathogens replicate very rapidly then antigen concentrations can be reached that are sufficiently high

to induce a Th1 $\rightarrow$ Th2 switch even if Th1-induced effectors are more efficient in pathogen destruction than Th2. In such, dangerous, situations it would appear that additional Th1-promoting signals from other immune system components such as the innate immune system may be necessary in order to reinforce the Th1 response.

As already pointed out for the 'Th1/Th2'-model in Sec. 4.2.6 antigen dose has indeed experimentally been shown to influence the class of immune response, but the direction of this influence is controversial. Some authors [4, 14, 25, 64, 83] have found that low doses of antigen result in Th1 cells producing IFN-γ and undetectable levels of IL-4, whereas increasing the dose leads to disappearance of Th1 and development of IL-4 producing Th2 cells. Our findings support this relationship. In addition, Menon and Bretscher [64] investigated time courses of cytokine production and found that at high antigen doses initially Th1 cytokines dominate but that the cytokine pattern switches to Th2, which correlates with our findings presented in Fig. 6.7. However, there is a report [39] that at extremely low doses of antigen, IL-4 production is favored, indicating Th2 dominance. Similarly, recent findings [34, 80] suggest that Th2 predominates at lower levels of initial signaling, whereas high doses of high affinity peptides lead to selectively IFN-γ-secreting Th1 helper cells. With our present model we can not explain these latter findings. The defect may lie in our simplified representation of T helper cell - APC interactions. A more elaborate model might well need to incorporate the influences of co-stimulatory signals, APC-derived signals, time courses of cytokine production and spatial aspects in the antigen presentation. And of course the different experimental findings may well be caused by subtle differences in the experimental setup.

6.4.3. When does an infection become chronic?

An infection will become chronic if the immune response induced by both T helper subsets is effective enough to limit pathogen growth but not sufficient to clear antigen completely. In terms of the parameter space in Fig. 6.2 this situation corresponds to $\nu_1 < r\delta_1/(\pi - 1)$ and $\nu_2 < r/(\pi - 1)$. With increasing pathogen replication rate or decreasing T helper proliferation rate the likelihood of an infection to become chronic increases. Note that we did not consider different life cycles or latency of pathogens, which may cause a different relation between the risk for chronic infections and pathogen replication rate.

If we distinguish between Th1- and Th2-dominated chronic situations we find the following differences as a consequence of the parameter window discussed in Fig. 6.2. The probability for Th2-dominated chronic situations continually increases with decreasing efficiency of Th2-induced effectors. With decreasing success of Th1-induced effectors the risk of Th1-dominated chronicity increases. However, if the immune response triggered by Th1 cells destroys pathogen sufficiently inefficient, according to our model infection will never end up in a chronic Th1-dominated infection. A similar situation occurs when Th2 proliferation is sufficiently more rapid than Th1 proliferation. In that case stability of 'chronic:Th1' is lost (see right graph of Fig. 6.5). Higher efficiency of Th1 growth factors, however, do not lead to instability of 'chronic:Th2'.

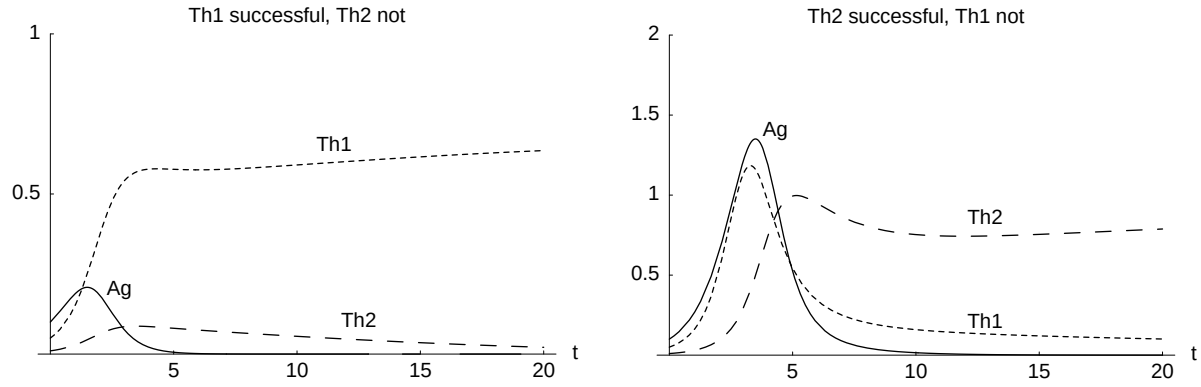


Figure 6.8.: Self-organized selection of the appropriate T helper response. Dotted, dashed and continuous lines represent Th1, Th2, and antigen concentration, respectively. Left figure: Th1-responsive antigen is successfully cleared by Th1 ($\nu_1 = 2, \nu_2 = 0.01$). Right figure: For a Th2-responsive antigen ($\nu_1 = 0.01$) a Th1 $\rightarrow$ Th2 switch is induced by early antigen proliferation; then the humoral response ($\nu_2 = 2$) leads to pathogen destruction. The remaining parameters are $\sigma = 2$, $\pi = 2$, $\delta_1 = 1.5$, $\delta_2 = 0.5$, and $r = 1$. The initial concentrations for T helper cells are asymmetric, viz $x_1(0) = 0.05, x_2(0) = 0.01$.

6.4.4. Choice of the appropriate T helper response

We now examine more carefully the important finding that in many circumstances the T helper system itself can select the appropriate T helper response that leads to successful antigen clearance. In Fig. 6.8 we show the temporal development of T helper and antigen concentrations for different types of pathogens (typically intracellular or extracellular) where different types of T helper responses are required. In both cases the system selects the efficient T helper type and clears the antigen. The strategy is

1. Try Th1 first,

2. induce a Th1 $\rightarrow$ Th2 switch if Th1 has not been successful.

For fast replicating intracellular pathogens the above strategy fails. Because pathogens can not be cleared sufficiently fast a Th1 $\rightarrow$ Th2 shift is induced *before* antigen has been eliminated (see Fig. 6.9). In that case further reinforcement of the appropriate T helper response is necessary.

6.4.5. Role of the susceptibility for Fas-mediated AICD

In Chap. 4 we have argued that AICD is important for homeostasis of T helper populations of type 1. Homeostasis of Th2 cells, however, is attained by regulatory cytokines. Asking the question why these differences of the role of AICD may have evolved, we have proposed that differences concerning the susceptibility of Th1 and Th2 cells for AICD are necessary to obtain Th1 $\rightarrow$ Th2 shifts (see Sec. 5.1.2). We now show that for our model the important

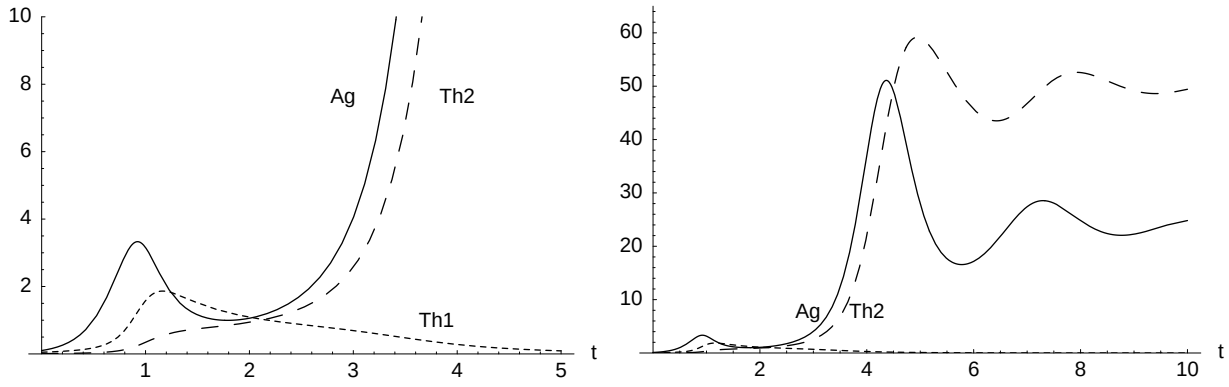


Figure 6.9.: Failure of the default strategy for fast replicating pathogens. Time plots: dotted, dashed and continuous lines represent Th1, Th2, and antigen concentration, respectively. The correct Th1-dominated response is switched into a Th2-dominated response (left figure) because antigen can not be eliminated sufficiently rapidly, owing to its high rate of replication. The antigen and Th2 concentrations reach high levels before the system settles into a chronic Th2-dominated state (right figure). The remaining parameters are $\sigma = 2$, $\pi = 3$, $\delta_1 = 1.5$, $\delta_2 = 0.5$, $\nu_1 = 4$, $\nu_2 = 0.1$, and $r = 5$. The initial concentrations for T helper cells are asymmetric $(x_1(0) = 0.05, x_2(0) = 0.01)$.

findings of Sec. 6.4.4 rely on the asymmetry in susceptibility to AICD of Th1 and Th2 lymphocytes.

Let us first assume that Th2 cells are totally resistant to AICD, i.e., $\delta_2 = 0$. Only the two Th1-dominated steady states are affected by δ_2. As a necessary condition for stability for these steady states we found $\delta_2 > \delta_1(\delta_1 - 1)/(\delta_1 + \pi - 1)$. Because of the importance of AICD in T cell homeostasis we assume that removal by Fas-mediated AICD is more important than loss by other mechanisms; this means that $\delta_1 > 1$, which implies that stability requires δ_2 is sufficiently large. Therefore sufficiently high susceptibility of Th2 for AICD is essential for the accessibility of Th1-dominated immune responses. If δ_2 is too small then bistability of 'cure:Th1' and 'chronic:Th2' or 'chronic:Th1' and 'cure:Th2' is lost (see Fig. 6.2). This phenomenon is illustrated in the right graph of Fig. 6.10.

On the other hand, comparable susceptibilities for the Fas-mediated apoptotic signal of Th1 and Th2 cells ($\delta_1 \approx \delta_2$) lead to the loss of Th1 $\rightarrow$ Th2 switches (as demonstrated in Fig. 6.11), which is crucial for the ability to select the appropriate T helper response. High δ_2 values strongly stabilize both Th1-dominated steady states and extend their basins of attraction. In a case where purely Th2-induced effectors would lead to pathogen destruction this favors the development of chronic Th1-dominated T helper responses with very high antigen load at the peaks. Additionally, as illustrated in the right graph of Fig. 6.10, the parameter window in the (ν_1, ν_2)-space where 'chronic:Th1' is stable is expanded leading to bistability of 'cure:Th2' and 'chronic:Th1' even for very low efficiency of Th1-induced effectors ($\nu_1 \approx 0$). That increases the risk of chronic Th1-dominated situations.

In the case of inefficient Th1 effectors ($\nu_1 \approx 0$) we also find that elevating the suscep-

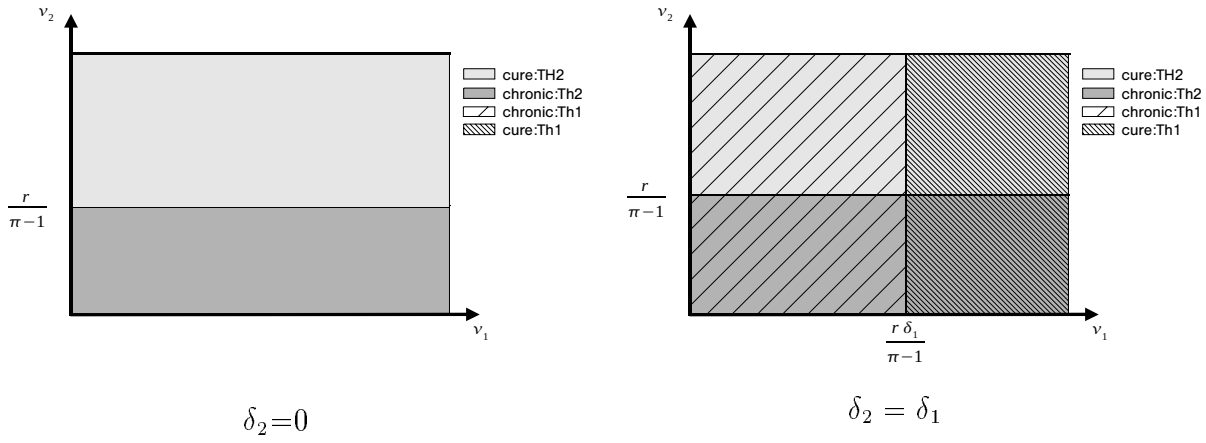


Figure 6.10.: Stability diagram for the relevant steady states in the pathogen-elimination parameter space (ν_1, ν_2). For AICD-resistant Th2 cells, i.e., $\delta_2 = 0$ (left) the stability of the Th1-dominated steady states is lost. For high values of δ_2 (right) the risk of chronic Th1-dominated infections is increased.

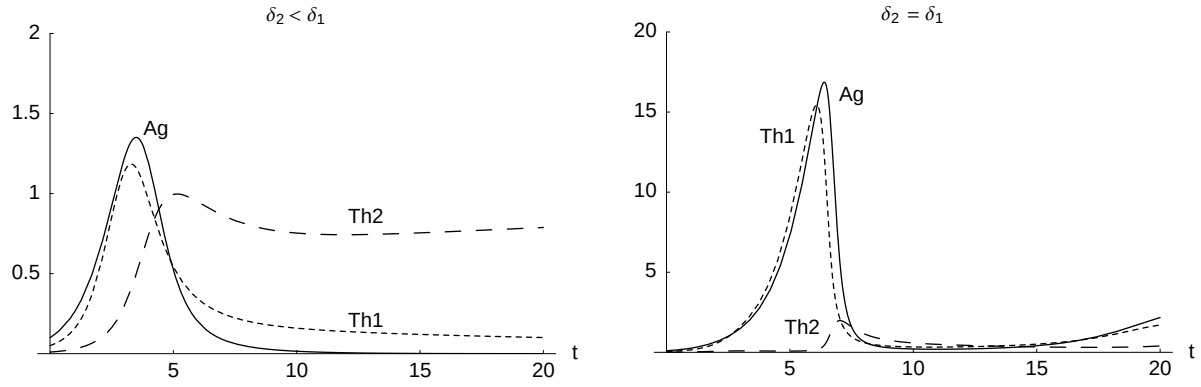


Figure 6.11.: Time plots showing presence and absence of a Th1 $\rightarrow$ Th2 switch, depending on the resistance of Th2 cells to AICD. Dotted, dashed and continuous lines represent Th1,Th2, and antigen concentration, respectively. Left figure: If Th2 are more resistant to AICD than Th1 ($\delta_2 = \delta_1/3$) then a Th1 $\rightarrow$ Th2 switch is induced if Th1 is not effective; after this switch the humoral response leads to pathogen destruction. Right figure: Comparable susceptibility of Th1 and Th2 to AICD ($\delta_2 = \delta_1$). Antigen and Th1 concentrations reach very high values before Th2 effectors diminish the antigen load. The remaining parameters are $\nu_1 = 0.01$, $\nu_2 = 2$, $\sigma = 2$, $\pi = 2$, $\delta_1 = 1.5$, and $r = 1$. The initial concentrations for T helper cells are unequal: $x_1(0) = 0.05, x_2(0) = 0.01$.

tibility of Th2 for Fas-mediated apoptosis not only increases the risk of Th1-dominated chronic diseases but also that of chronic infections with co-existing T helper populations. Steady states with both T helper subsets can not be analyzed analytically. Therefore, we show results of numerical calculations in the bifurcation diagram of Fig. 6.12. Increasing δ_2 beyond a certain threshold stabilizes the steady state that represents chronic disease with co-existing T helper populations. For values of δ_2 beyond the Hopf-bifurcation the 'chronic:Th1/Th2' becomes unstable again and the system ends up in the 'chronic:Th1' steady state.

The basic assumption that susceptibility of Th2 lymphocytes for AICD is less than that of Th1 cells but greater than zero is supported by experiment. It has been shown that Th2 cells are not entirely resistant to AICD but the death rate by AICD is remarkably lower than for Th1 cells [98]. According to our model a well-balanced susceptibility of Th2 for AICD leads to the selection of the appropriate T helper response while minimizing the risk of chronic Th1-dominated diseases. We therefore suggest that evolutionary pressure has optimized the Fas-mediated AICD rate of Th2 cells in order to minimize damage to the host. Additionally, we predict that chronic infections are more likely to be Th2-dominated than Th1- dominated. Autoimmune diseases, however, have been excluded from this statement because they are influenced by other factors that we did not consider here.

6.4.6. Phase space requirements

Our model represents our best attempt to capture the essential biology of Th1/Th2 interaction. But the major results of Sec. 6.4.4 remain valid for other models that include the essential phase space requirements, which are as follows. If the pathogen is best handled by Th2 cells, i.e., if $\nu_2 > \nu_1$, then the governing system of equations should have a single stable steady state, 'cure:Th2'. If the pathogen is best handled by Th1 cells, the system should have two stable steady states, 'cure:Th1' and 'chronic:Th2'. If x_1 is initially rather greater than x_2 and $p(0)$ is not too large then the initial point should be in the domain of attraction of 'cure:Th1'. However if $p(0)$ exceeds a certain critical value then the initial point is in the domain of attraction of 'chronic:Th2'.

6.5. Summary and Discussion

The importance of the balance between Th1 and Th2 cell subsets and their influence on the development of different immune responses has been well established. However, the influences that guide the initial activation of one particular T helper type are not clearly understood. A number of potentially important elements have been proposed, among them the overall cytokine milieu, the presence of co-stimulatory molecules, the dose of antigen, the nature of the early antigen-presenting cell, and influences of components of the innate immune system arising from pattern recognition of infectious agents. One approach to Th1/Th2 selection is to search for some sort of dominant controller that is responsible for the decision-making process. Yet it has proved difficult to single out a single dominant

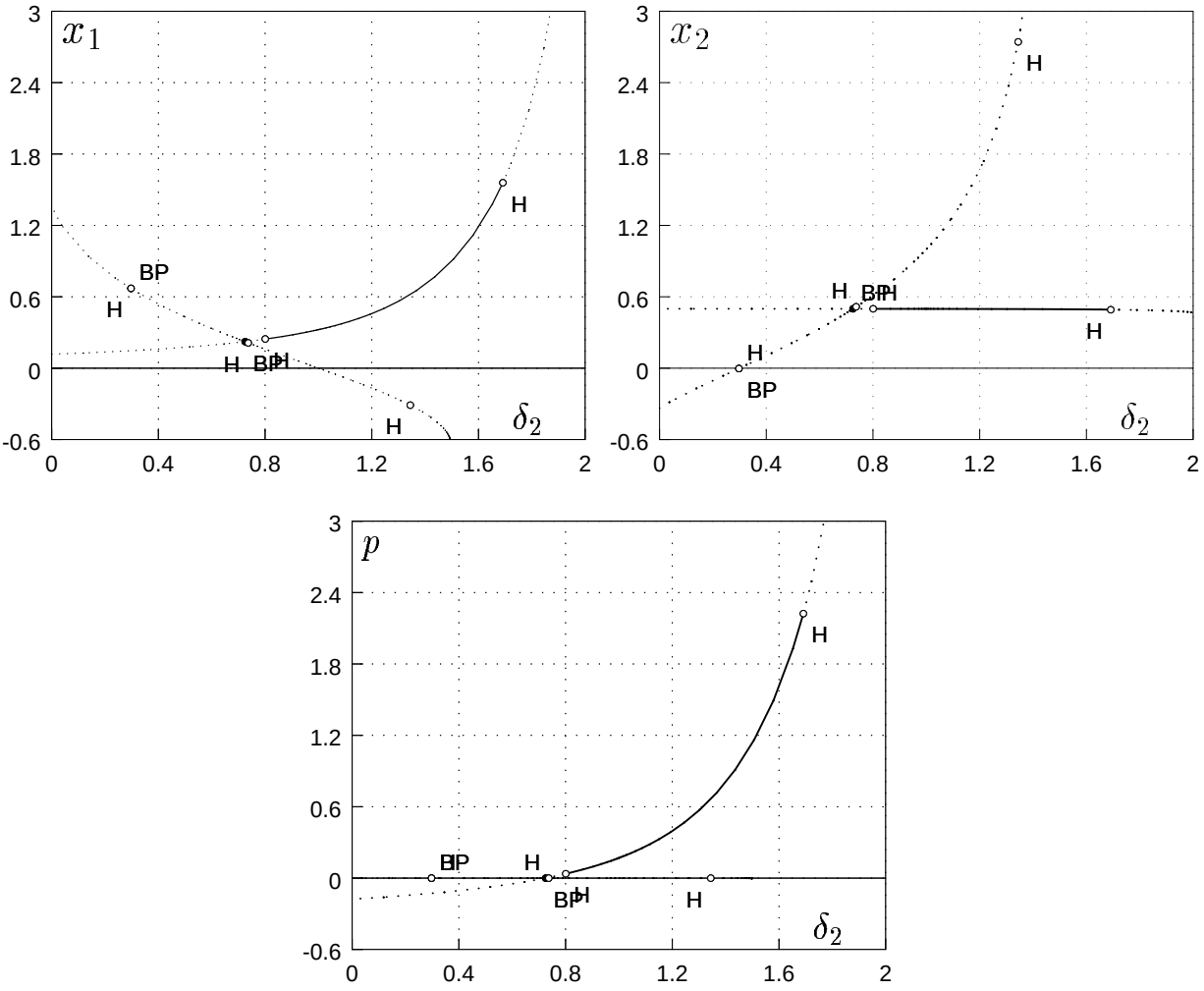


Figure 6.12.: Bifurcation diagram with δ_2 as bifurcation parameter (horizontal axis). Increasing values of δ_2 stabilize the steady state that represents chronic disease with co-existing T helper population. Left, middle, and right figures show the corresponding Th1, Th2 and pathogen concentrations, respectively. Stable steady states are shown as thick lines, unstable steady states as dotted lines. Hopf-bifurcation points and branching points are represented by H and BP. The other parameters are $\sigma = 2, \pi = 2, \delta_1 = 1.5, \nu_1 = 0.1, \nu_2 = 2$.

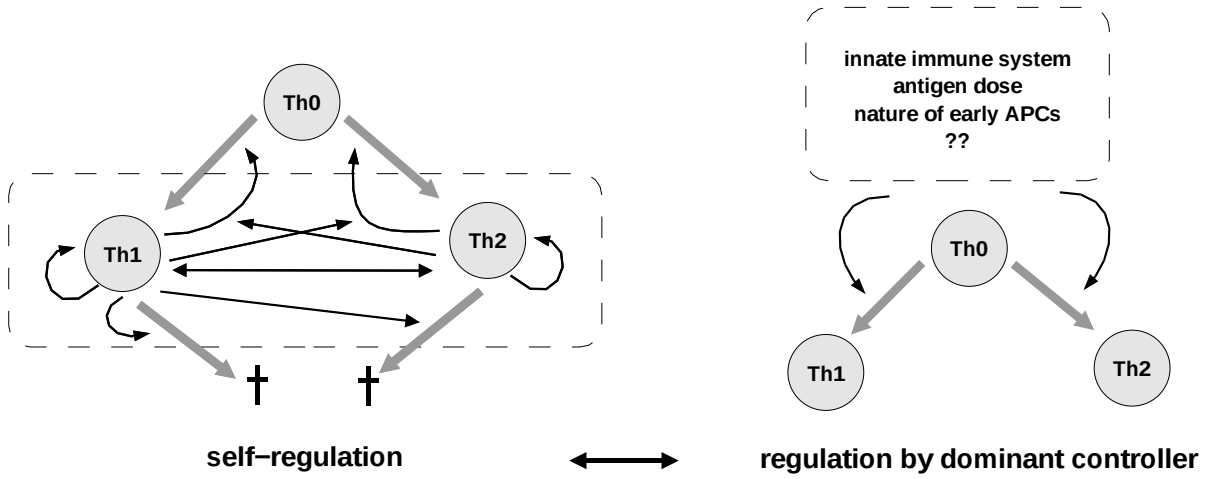


Figure 6.13.: Contraposition of different regulation concepts

factor, since all the different factors interact. Accordingly, we view the T helper cells as a self-organizing system that is able to make the decision itself due to regulatory mechanisms from within the system that influence the activation and differentiation step (see Fig. 6.13).

In Chap. 4 we presented a model of cytokine-modulated regulation of helper T cell populations under a constant antigenic stimulus. This model did not consider pathogen removal and growth but concentrated instead on the evolution of the T helper response under antigen exposure controlled by cytokine and Fas/FasL interactions. We concluded from this model that the dominant T helper subset of an immune response with persistent antigen is determined by the strengths of activation signals, which are correlated with the antigen concentration. Low antigen levels leads to Th1 dominance whereas higher levels favor Th2 dominance. This is due to asymmetries in the cross-regulation of the two T helper subsets. Moreover, switches from Th1- to Th2-dominance are a common feature for wide parameter ranges and arise from unequal Fas-meditated apoptosis for the two T helper subsets and from asymmetries in the nature of cross-suppression. These conclusions still hold if we assumed growth factors to act in an autocrine rather then systemic way (see Sec. 5.1).

In the context of how the immune system can select the appropriate T helper response we interpreted these results in the following way:

- Failure in antigen clearance is a sign of an insufficient T helper response.
- Shifts from Th1 to Th2 predominance under these circumstances could mean that there are mechanisms within the T helper system that somehow allow a switch to a different type of T helper response if the one triggered first did not lead to pathogen elimination.

Here, we present a simple mathematical model, which explicitly (albeit with considerable approximation) incorporates most of the known regulatory cytokine interactions within

the T helper system and the interplay between the immune system – initiated by T helper subsets – and pathogens. Here is a summary of our conclusions.

(i) Cytokine interactions within the T helper system itself provide a built-in self-organizing mechanism for the selection of the appropriate T helper response. The selection is appropriate for many circumstances but not all (see (iii)).

(ii) The immune system's default response to pathogen is a primary Th1 response followed by a Th1 $\rightarrow$ Th2 switch in case of a failure of the Th1 response.

(iii) For fast replicating Th1-susceptible pathogens this built-in mechanism fails to destroy the pathogen and additional stimuli provided by other immune system components are necessary in order to induce an effective immune response.

(iv) Crucial for the function of the selection process is antigen dose-dependence of the T helper ratio (high antigen levels promote a Th1 $\rightarrow$ Th2 switch) and an initial Th1 bias, which stems from cell-cycle dependence of cytokine production. Therefore, we show here that these dependences, which could be considered as artefacts of the system without functional significance, play important roles for the function of the T helper system.

(v) The rate with which Th2 undergo AICD must be suitably balanced to enable this selection mechanism and to reduce the risk of chronic Th1-dominated disease.

(vi) As a consequence of (v) infections are more likely to become Th2-dominated than Th1-dominated. This may be part of effects of evolutionary pressure to minimize damage to the host.

Other authors have also modeled the response of the T helper system to pathogens [5, 21,29,31,70]. To our knowledge, there is no detailed study on implications of the regulatory mechanisms within the T helper system on the decision making process. Behn et al. [5] incorporate cross-regulation of T helper subsets and feedback of cytokines of the existing T helper pool on differentiation of naive T helper cells, as we do here, but neglect different efficiencies of T helper subsets in pathogen clearance and AICD. This article provides a mechanism for hyposensitization that is based on antigen dose-dependence of Th1/Th2 ratios, similar to our findings. Fishman and Perelson [30] concentrate on consequences of asymmetries in cross-regulation. Their predictions concerning antigen dose-dependence of the immune response are in agreement with those presented in Sec. 6.4.2, which is based on similar implementations of the cross-regulation. In [29] Fishman and Perelson focus on on T helper differentiation upon interaction with antigen-bearing accessory cells and account for multi-clonality. They find that T helper responses are polarized towards one phenotype, Th1 or Th2, – owing to autocatalytic Th1/Th2 cross-suppressive processes, similar to our findings – and one receptor specificity due to competition for re-stimulation of different clones. Carneiro et al. [21] find that the key feature underlying the regulation of Th differentiation pathways is the population dynamics of the lymphocytes themselves.

According to their model the regulation of T helper differentiation is not driven by an APC-dominated selection process – where certain types of antigen are preferentially taken up and presented by particular types of APCs, which activate a certain type of lymphocyte subset. They point to the alternative that the major decisional events in the immune system are determined by its own intrinsic dynamics but they do not provide a concrete mechanisms how correct decisions are made. Muraille et al. [70] consider cytokine interactions that are similar to those analyzed here but neglect AICD. They also find Th1 $\rightarrow$ Th2 shifts and an association between chronicity and Th2 responses. They argue that chronicity and Th2 responses have been linked together evolutionarily because helminth infections, where protective immunity depends upon Th2 responses, tend to be long and chronic. In agreement with our results they propose that persistence of the pathogen can represent a positive signal for the induction of a Th2 response. Our results, however, indicate an important role of differences of T helper subsets in AICD and provide a mechanism for the selection of appropriate and protective T helper responses for both intercellular and intracellular pathogens.

In this chapter we have restricted ourselves to events that influence the decision making process within the T helper system only. As pointed out the proposed mechanisms may be suitable and lead to appropriate T helper responses for most pathogens and could therefore be seen as the default decision making process. However, other external influences – such as signals from the innate immune system, the nature of early antigen presenting cells etc. – are likely to play greater or lesser roles in different circumstances. For cases of fast replicating Th1-sensitive pathogens (as we have seen) or pathogens that have found other ways to evade the immune response additional mechanisms such as pattern recognition and pathogen destruction feedback might be necessary. These issues will be the subject of the next chapter.

7. The role of the innate immune system in the decision making process

Apart from the intrinsic T helper dynamics other factors provided by the innate immune system after "immune recognition" – also termed "pattern recognition" – (for introduction and review see [62, 63]) have been proposed to direct T helper differentiation. This chapter deals with the question to what extent these factors drive the selection of a particular T helper response [8] and under which circumstances they are necessary.

7.1. Introduction

In Chap. 6 we presented a model of the T helper system in response to pathogens where the appropriate T helper response is selected by a self-regulatory process within the T helper system. This process was purely based on the intrinsic cytokine-modulated T helper dynamics without influences from outside the T helper system. Regulatory processes induced a shift from an initial Th1-dominated response to Th2 dominance when Th1-induced effectors cannot eliminate pathogen. Th1 response failure is indicated by rising antigen concentrations. We have proposed that this selection process is the default mechanism, which works in most cases. However, the dependence of the Th1/Th2 decision on only one criterion – such as antigen concentration – makes the system highly susceptible for pathogen interference. Additionally, in certain situations reinforcement of the effective T helper response may be beneficial to accelerate pathogen elimination that minimizes damage to the host. It therefore makes sense that the decision as to which effector mechanisms is required should be based on a complex matrix of interlocking interactions. This gives us a sufficient reason to study the role of external influences – outside of the T helper system – on the decision making process.

This chapter is organized as follows: First we discuss the biological background of APC-derived influences on T helper regulation owing to immune recognition in Sec. 7.2 and present a mathematical model that incorporates APC-derived signals in Sec. 7.3. Differences in their effects and necessities between Th1- and Th2-promoting signals derived from antigen presenting cells (APCs) due to pattern recognition are highlighted in Sec. 7.3.1 and Sec. 7.3.2. In Sec. 7.4 we discuss circumstances under which the default selection mechanism based on regulatory interactions in the T helper system may fail and in which cases the innate immune system is needed. The process of detecting the pathogen destruction is explained in Sec. 7.5 and a detailed example discussed in Sec. 7.5.1.

7.2. On the role of pattern recognition

The T helper system is not only regulated by signals generated within the T helper system but in addition is influenced by other immune system components. With the discovery of the ability of the innate immunity to recognize conserved molecular patterns, patterns that are absent from the host organism and characteristic for microorganisms [42], a link between innate and adaptive immunity has been found. It has been proposed that this link allows advantage to be taken of experience gained during evolution of how to cope with particular pathogens. This is accomplished by the ability of the innate immune system components after pattern recognition to provide signals in the form of cytokines that (among other actions) direct T helper differentiation. Thus the innate immune system plays an instructive role in influencing the Th1/Th2 ratio and therefore in the decision making process.

The pathogen-associated molecular patterns seem to be represented by structures essential for the microorganism and therefore unsuppressable by actively-mutating pathogens. The receptors of the innate immune system are relatively limited in their diversity and unable to make fine distinctions between closely related structures. Nevertheless, they can recognize certain patterns shared by groups of pathogens. Among these are the following

- lipopolysaccharide (LPS), a part of the cell walls of gram-negative bacteria,
- immunostimulatory DNA: CpG motifs (see also Sec. 7.5.1),
- double-stranded RNA of viruses,
- mannans, a component of yeast cell walls, among others.

Signals induced by pattern recognition can be grouped into three categories:

1. inflammatory cytokines including IL-1, TNF, IL-6,
2. co-stimulators of T-cell activation including B7.1 and B7.2
3. effector cytokines including IL-12 and IFN-γ.

In contrast to earlier findings, recent results indicate that the cytokines IL-1, IL-6 and TNF of the first group do not direct T helper differentiation. Thus, in a study [44] on previously activated Th1 and Th2 effectors it could not be confirmed – as found elsewhere for Th1 and Th2 clones – that differentiation towards the Th2 phenotype is increased by inflammatory cytokines. Instead, these cytokines mainly enhance naive T cell responses and augment proliferation. Differentiated T helper cells are only moderately responsive to all three cytokines.

Although it is suspected that co-stimulatory molecules may direct T helper differentiation [58, 92] interpretation of the results is complex. We therefore neglect inflammatory cytokines and co-stimulation as possible signals for generation of a dominance of one of the two T helper subsets.

Effector cytokines such as IL-12 and IFN-γ, however, promote Th1 responses, mainly by up-regulating of Th1 differentiation. In contrast, Th2-promoting signals derived from the innate immune system have been identified to much lower extent. Romagnani et al. [81] and Yoshimoto et al. [103] described the capacity of microbial structures characteristic of helminthic parasites to stimulate IL-4 secretion but pattern recognition receptors that skew towards a type 2 immune response have – to our knowledge – not yet been identified.

7.3. Incorporation of signals based on pattern recognition

To study the influence of the innate immune system on the Th1/Th2 decision making process we restrict ourselves to cases wherein biassing signals only act on the differentiation step. Therefore – for example – influences of APC-derived IFN-γ on Th2 proliferation are neglected. Stimuli from the innate immune system are incorporated into the rate equations (6.4)-(6.6) for T helper and pathogen concentrations as T helper independent activation signals similar to Sec. 4.3.2. In particular, the terms

$$\mathrm{Th1-stimulus} = \frac{\gamma_1 p}{1+kS_2}, \tag{7.1}$$

$$\mathrm{Th2-stimulus} = \frac{\gamma_2 p}{1+kS_2}, \tag{7.2}$$

represent the contributions of signals derived from components of the innate immune system to Th1 or Th2 differentiation in the absence of further cytokine modulation from the developing T helper cell populations. The factor $1+kS_2$ occurs because, as for activation induced by T helper-derived signals, the Th2 related cytokine TGF-β inhibits activation by suppressing MHC-II expression and therefore antigen presentation and IL-12 production by APCs. The parameters γ_1 and γ_2 represent the overall strengths of the stimulatory effects. After making a quasi steady state assumption for the cytokine-signals S_2 (as in 4.1) and introducing $\theta_i = \gamma_i k$, we find that the new equations generalize to

$$\frac{\mathrm{d}x_1}{\mathrm{d}\tau} = \frac{\theta_1 p}{(1+x_2)} + \frac{\sigma_1 x_1 p}{(1+x_2)^2} + \frac{\pi_1 x_1}{(1+x_2)} - \delta_1 x_1^2 - x_1, \tag{7.3}$$

$$\frac{\mathrm{d}x_2}{\mathrm{d}\tau} = \frac{\theta_2 p}{(1+x_2)} + \frac{\sigma_2 x_2 p}{(1+x_2)} + \frac{\pi_2 x_2}{(1+x_1+x_2)} - \delta_2 x_1 x_2 - x_2 \tag{7.4}$$

$$\frac{\mathrm{d}p}{\mathrm{d}\tau} = p(r - \nu_1 x_1 - \nu_2 x_2). \tag{7.5}$$

We will now analyze how stimuli from innate immunity affect the stability and location of the steady states of the extended system (7.3)-(7.5) in comparison to the steady states of

system (6.4)-(6.6) where external influences have been neglected. Furthermore we discuss consequences of asymmetries in the action of external Th1 or Th2 promoting signals.

7.3.1. Influences of Th1 promoting stimuli

First, we study the consequences of Th1-promoting signals ($\theta_1 > 0, \theta_2 = 0$). We find that only steady states that represent chronic situations are affected.

- 'chronic:Th2'. This steady state becomes a 'chronic:Th1/Th2´ state with mixed T helper populations. Its stability can not be calculated analytically but will be analyzed numerically later on.

- 'chronic:Th1'

$$\Omega_{\text{chronic:Th1}} = \left(\frac{r}{\nu_1}, 0, \frac{r(r\delta_1 + \nu_1(1-\pi))}{\nu_1(r\sigma + \theta_1\nu_1)} \right).$$

  The higher the Th1-stimulus the lower is the pathogen concentration, weighted with ν_1, of the chronic Th1 dominated steady state. The relevant eigenvalue for stability is the first one,

$$-1 - \frac{\delta_2 r}{\nu_1} + \frac{\pi\nu_1}{r+\nu_1} + \frac{r\sigma(r\delta_1 - \nu_1 - \pi\nu_1)}{\nu_1(r\sigma + \theta_1\nu_1)}.$$

  The last two eigenvalues are always negative if the steady state is in the positive quadrant.

We pointed out in Sec. 6.3.2 that – if the susceptibility of Th2 for AICD is significantly lower than that for Th1 – for a sufficiently low pathogen destruction efficiency of a Th1-induced immune response ($\nu_1 < r(\delta_1 - \delta_2)/(\pi + \delta_2 - \delta_1)$) the only stable fixed points of the system are Th2 dominated steady states. For this case a successful Th2 dominated T helper response is triggered if Th2-induced effector cells are sufficiently effective in pathogen destruction. Moreover, although APC-derived Th1 promoting signals stabilize the chronic Th1 dominated steady state we still find that 'chronic:Th1' is unstable. This happens because the effect of Th1-signals, θ_1, is weighted by ν_1. Mathematically, we find for the limit of the the first eigenvalue

$$\lim_{\nu_1 \to 0} \left[-1 - \frac{\delta_2 r}{\nu_1} + \frac{\pi\nu_1}{r+\nu_1} + \frac{r\sigma(r\delta_1 - \nu_1 - \pi\nu_1)}{\nu_1(r\sigma + \theta_1\nu_1)} \right] = \infty$$

if $\delta_2 < \delta_1$. This results in the instability of 'chronic:Th1' if Th1 responses are sufficiently ineffective even in the case where incorrect Th1-stimuli have been given. We interpret this outcome as providing fault-tolerance of the T helper system against incorrect Th1-signals from the innate immune system.

For intermediate values of the pathogen elimination efficiency of Th1-induced effectors ($\nu_1 > r(\delta_1 - \delta_2)/(\pi + \delta_2 - \delta_1)$ and $\nu_1 < r\delta_1/(\pi - 1)$) 'chronic:Th1' becomes stable and

fault-tolerance independent of the initial conditions is lost. The domain of attraction for this steady state grows with increasing ν_1.

The fault-tolerance, however, only holds for sufficiently small incorrect stimuli. For θ_1 sufficiently high the steady state, which represents a chronic situation with co-existing T helper populations 'chronic:Th1/Th2', can also can become stable, depending on θ_1 and ν_1. In Fig. 7.1 we show that 'chronic:Th1/Th2' does not exist for small Th1-stimuli; increasing the Th1-stimulation leads via a saddle-node bifurcation to the creation of a stable and an unstable steady state with nonzero antigen concentration and co-existing T helper populations.

The dependence of fault tolerance on the rates with which Th2 cells undergo AICD is illustrated in Fig. 7.2. Here we show that if there is higher susceptibility of Th2 for Fas-mediated apoptosis then *incorrect* Th1-signals from the innate immune system have more dangerous consequences for the system. Only if δ_2 is significantly lower than δ_1 a successful Th2 response will be triggered although an inappropriate Th1-signal from the innate immune system promotes chronic Th1 dominated disease.

Correct detection of intracellular pathogens, however, strengthens the likelihood of appropriate Th1 responses. The effect of a *correct* Th1-signal ($\theta_1 > 0$ and ν_1 sufficiently large) is shown in Fig. 7.3 where we plot the basins of attraction of the steady states that correspond to the induction of successful Th1 response or to Th2 dominated chronic disease. Increasing the strength of the Th1-promoting signal not only promotes global stability of Th1 but also increases the speed with which Th1 responses are triggered (Data not shown).

7.3.2. Influences of Th2 promoting stimuli

The position and stability of steady states is shifted by Th2-promoting ($\theta_1 = 0, \theta_2 > 0$) signals as follows.

- 'chronic:Th2':
$$\Omega_{\text{chronic:Th2}} = \left(0, \frac{1}{\nu_2}, \frac{r[r + \nu_2(1-\pi)]}{\nu_2(r\sigma - \theta_2\nu_2)}\right).$$
The last two eigenvalues are always negative because of the existence condition; the first eigenvalue
$$\frac{[\nu_2(\pi - 1) - r)(\sigma r^2 + \theta_2 r\nu_2(1+\nu_2)]}{(r+\nu_2)(r\sigma + \theta_2\nu_2)}$$
is negative if $\nu_2 < \frac{r}{\pi - 1}$, which is the same stability condition as without Th2-stimulus.

- 'chronic:Th1': becomes a steady state with mixed T helper populations 'chronic:Th1/Th2´; stability can not be calculated analytically;

In the previous section we showed that even in the case where Th1-signals promote the incorrect T helper response, this bias can be overridden later by the internal T helper dynamics leading to Th2 dominance and successful pathogen elimination. In contrast,

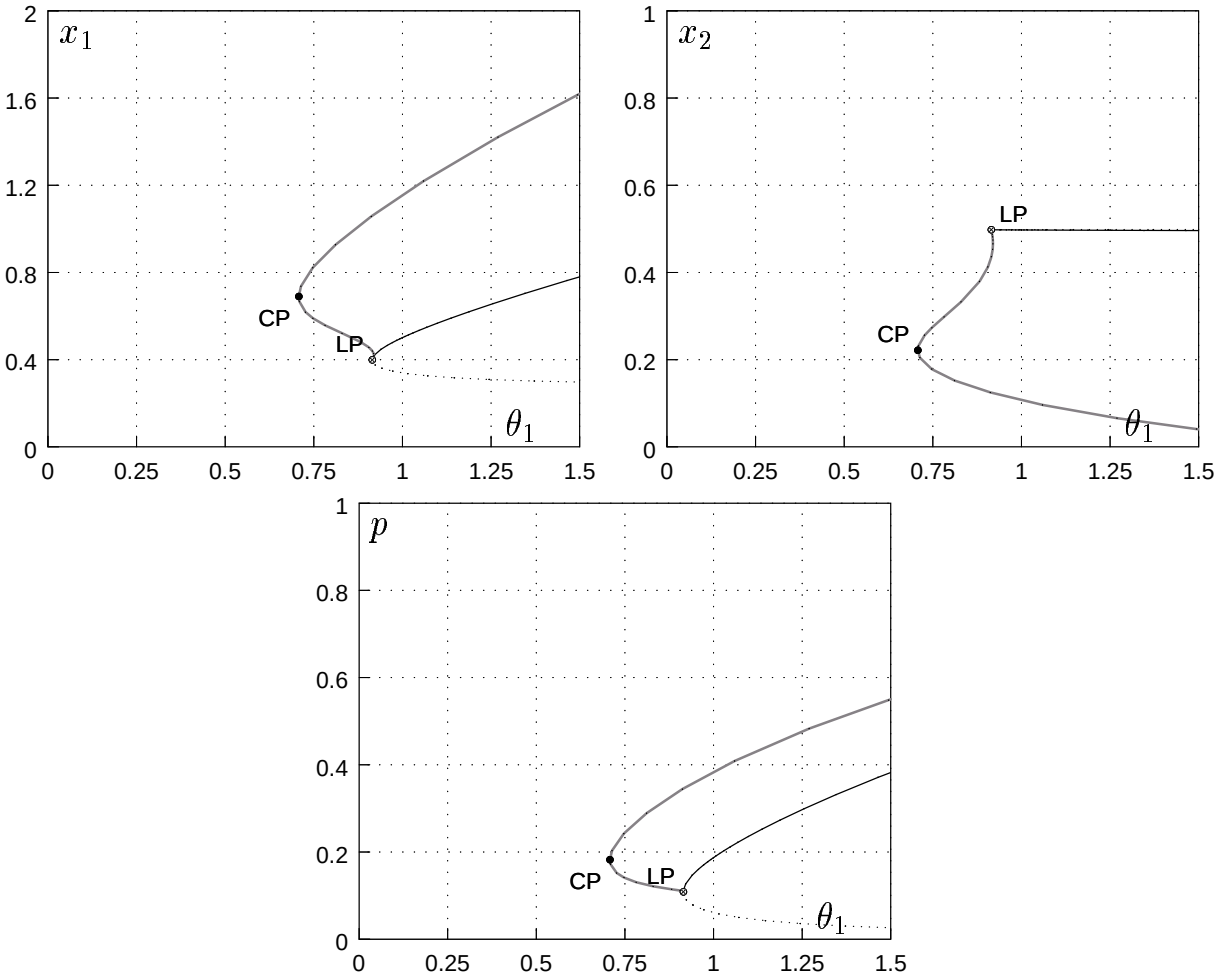


Figure 7.1.: Bifurcation diagram with θ_1 as bifurcation parameter. Sufficiently high Th1-stimuli θ_1 stabilize the 'chronic:Th1/Th2' steady state. For Th1, Th2 and pathogen stable steady states are shown as thick lines, unstable steady states as dotted lines. Increasing θ_1 leads to a saddle-node bifurcation; a stable steady state is created, which represents a chronic state with co-existing T helper populations. Grey lines represent development of a saddle-node bifurcation point, labeled with LP, depending on increasing the parameter ν_1. CP represents cusp points. The other parameters are $\sigma = 2, \pi = 2, \delta_1 = 1.5, \delta_2 = 0.5, \nu_1 = 0.1, \nu_2 = 2, r = 1$, and $\theta_2 = 0$.

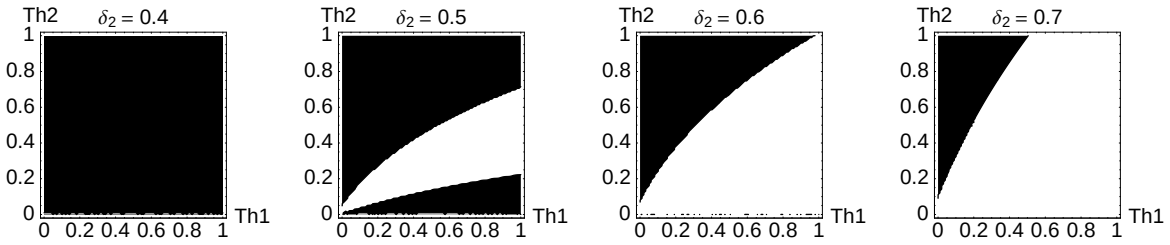


Figure 7.2.: Fault-tolerance for incorrect Th1-stimuli in dependence of the susceptibility of Th2 to AICD δ_2. For different values of δ_2 basins of attraction of the two stable steady states 'cure:Th2' (black area) and 'chronic:Th1/Th2' (white area) are plotted. With decreasing δ_2 the basins of attraction for 'chronic:Th1/Th2' become smaller. Other parameters: $\sigma = 2$, $\pi = 2$, $\nu_1 = 0.1$, $\nu_2 = 2$, $\delta_1 = 1.5$, $r = 1$, $\theta_1 = 1$, $\theta_2 = 0$ and $p(0) = 0.01$.

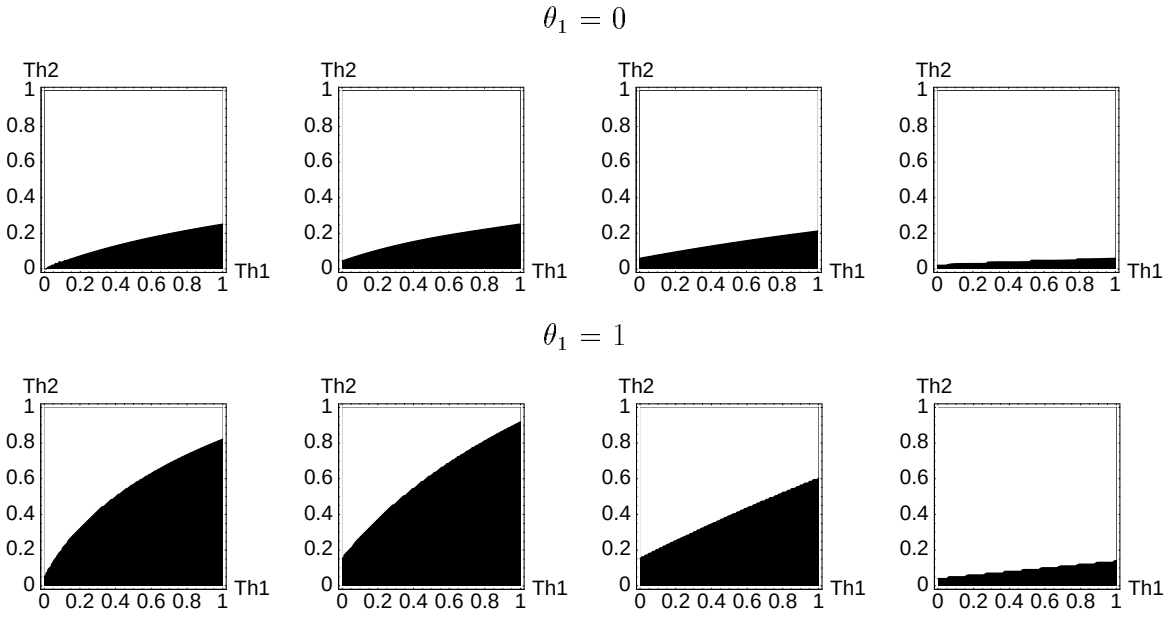


Figure 7.3.: Effect of correct Th1-signals θ_1 on the basins of attraction of the two stable steady states 'cure:Th1' (black) and 'chronic:Th2' (white). The initial antigen concentrations from left to right are $p(0) = 0.01$, $p(0) = 0.1$, $p(0) = 1$, and $p(0) = 10$. Other parameters are set to $\sigma = 2$, $\pi = 2$, $\nu_1 = 2$, $\nu_2 = 0.01$, $\delta_1 = 1.5$, $\delta_2 = 0.5$, and $r = 1$.

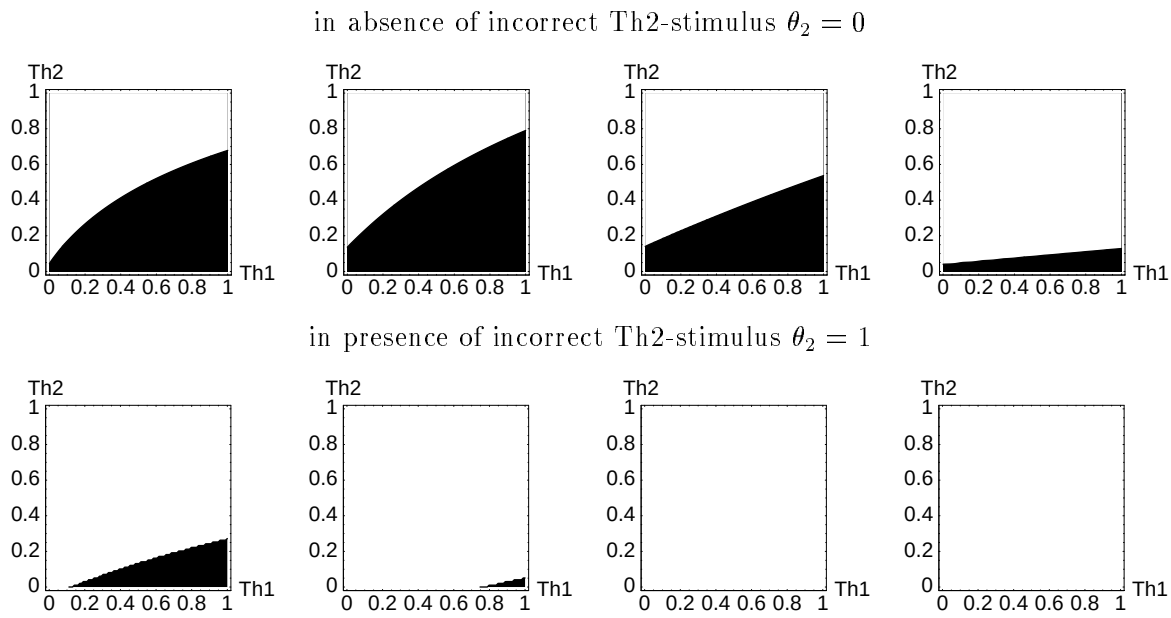


Figure 7.4.: Basins of attraction of the steady states 'cure:Th1' (black area) and 'chronic:Th1/Th2' (white area) in absence and presence of an incorrect Th2-stimulus. The graphs are drawn for a case where Th1 is much more efficient than Th2 in eliminating pathogen ($\nu_1 = 2, \nu_2 = 0.01$). Incorrect Th2-stimuli enlarge the stability of 'chronic:Th1/Th2' and can even override Th1-stimuli. Other parameters are set to $\sigma = 2$, $\pi = 2$, $\delta_1 = 1.5$, $\delta_2 = 0.5$, $r = 1$, and $\theta_1 = 1$. Initial antigen concentrations, from left to right, are $p(0) = 0.01$, $p(0) = 0.1$, $p(0) = 1$, $p(0) = 10$.

according to our model there is no way founded in the cytokine interactions of the T helper system to override incorrect Th2-signals from the innate immune system. In situations where Th1 is the 'correct' T-helper subset Th2-signals promote Th2-dominated immune responses and override even correct Th1-signals. This leads to chronic disease with co-existent T helper populations; cf. Fig. 7.4.

As we have mentioned before, numerous signals promoting Th1 responses that arise from pattern recognition have been found, but Th2 signals have been described to a much lower extent. This might be due to fundamental asymmetries in the effects of Th1 and Th2 promoting signals. Based on our results here, we propose an alternative explanation for the relative absence of Th2-signals. We suggest that Th2-biases are not required to obtain a Th2-dominated T helper response. For cases where purely Th2-induced effectors ($\nu_1 \approx 0$) are efficient in pathogen destruction the correct Th2-dominated immune response is the only possible one. For δ_2 sufficiently small (Th2 weakly subject to AICD) 'chronic:Th1', which could compete with 'cure:Th2', is unstable.

7.4. When do we need the innate immune system?

If the T helper system itself can usually select the appropriate T helper response, as proposed in Sec. 6, we can ask for which circumstances additional information provided by the innate immune system is still necessary or helpful.

As pointed out in Sec. 6.4.1 a Th1-bias is necessary in order to enable Th1-dominated immune responses if desired. This bias could be constant, in the form of a higher Th1-activation-driving capacity of Th1-cytokines and cell cycle dependence of cytokine production, or the bias could be temporary, provided by signals of the innate immune system after pattern recognition. Although it has been suggested that molecular patterns consist of indispensable components of the pathogen [61], full reliance on APC-derived signals would make the immune system highly dependent on complete and gap-less recognition of pathogens. Therefore we suggest that the default Th1-bias does not stem from the innate immune system. However, there might be situations where built-in Th1 biases are too weak to induce Th1-dominated responses and sufficient Th1-promoting signals are *only* provided by the innate immune system. In that case a Th2-dominated response would be the default response triggered in the absence of further information. This view corresponds with a position presented by Gause et al. [33] where it was suggested that type 2 responses develop as a default pathway when signals that promote a type 1 response are absent.

Under which circumstances are constant Th1-biases not strong enough? Our model suggests that for pathogens that require a humoral immune response for their elimination additional stimuli are *not* necessary for the selection of a beneficial Th2 response. Additionally, also beneficial Th1 responses can be selected in absence of pattern recognition of slow replicating pathogens. For fast replicating pathogen, however, antigen levels may not be controlled sufficiently rapidly by Th1 effectors. Increasing antigen levels are misinterpreted as failure of the Th1-dominated immune response and lead to a Th1 $\rightarrow$ Th2 switch. The subsequent Th2-dominated response can not clear the intracellular pathogen and the situation settles at very high Th2 and pathogen concentrations, which may lead to death of the host. In such situations additional reinforcement of the beneficial immune response is necessary in order to control the pathogen. In Fig. 7.5 we illustrate that Th1-stimuli of the innate immune system can provide sufficient support for the appropriate Th1 response. Note, however, that additional Th1-signals also enlarge the domains of attraction for 'chronic:Th1'. If the Th1-stimulus is very strong ($\theta_1 \rightarrow \infty$) and the replication rate of a certain Th1-sensitive pathogen is high enough then the 'chronic:Th1' state becomes stable $[-1 - \delta_2 r/\nu_1 + \pi\nu_1/(r + \nu_1) < 0]$. In that case our model suggests that other mechanisms have to be activated in order to ensure that the appropriate T helper response is selected.

In the case of fast replicating intercellular pathogens, however, fast growing antigen levels even lead to a faster induction of the beneficial Th2 response. Therefore, Th2-signals are not required in this situation. In general, additional information accelerates the selection process, which may be necessary for pathogen elimination and a speed up of pathogen clearance.

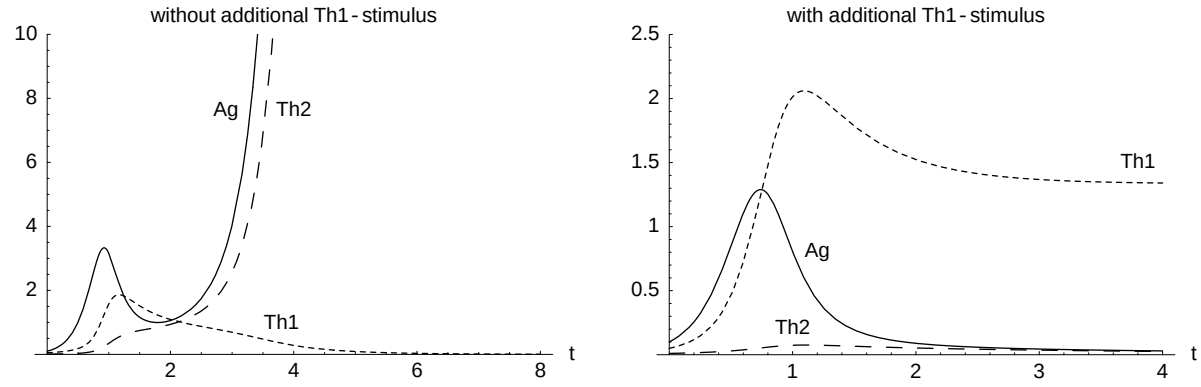


Figure 7.5.: Additional reinforcement of the beneficial Th1-response is necessary in order to eliminate fast replicating intracellular pathogens. Time plots: dotted, dashed and continuous lines represent Th1, Th2, and antigen concentration, respectively. Left figure: Without reinforcement of the beneficial Th1-response ($\theta_1 = 0$) high antigen levels result in a Th1 $\rightarrow$ Th2 shift, which leads to persistence of high pathogen levels. The system ends up in a steady state with high antigen and Th2 concentrations (out of scale). Right figure: Additional Th1-stimuli ($\theta_1 = 1$) avoid the shift and lead to pathogen clearance. The remaining parameters are $\sigma = 2$, $\pi = 3$, $\delta_1 = 1.5$, $\delta_2 = 0.5$, $\nu_1 = 4$, $\nu_2 = 0.1$, and $r = 5$. The initial concentrations for T helper cells are $x_1(0) = 0.05, x_2(0) = 0.01$.

7.5. Pathogen destruction feedback

Until now we have concentrated on the view that signals generated by pattern recognition events are purely part of some sort of reflexive response, which occurs when special stimuli (in this case pattern recognition events) have been generated. This leads to an immune response that is rather inflexible. Possible situations that in this case would lead to wrongly directed T helper responses are

- pathogens that require different T helper responses during their life stages due to, e.g., latency periods,
- pathogens that have evolved strategies to generate the *wrong* T helper response (for example a Th2-sensitive pathogen that can produce Th1-promoting signals),
- or pathogens where a combination of both T helper responses leads to greatest success.

We now examine an alternative view of events following perception of parts of pathogens. Instead of inducing a reflexive immune response, recognition of parts of dead, destroyed pathogens could be part of a feedback-regulation [86]. Here, if certain types or combinations of effector components are responsible for pathogen destruction and elimination then parts of the deleted pathogenic agents that signal their destruction (termed "scalps" [86]) will lead to an up-regulation of the victorious components of the immune system and therefore reinforce the success of the immune response ('pathogen destruction feedback'). This

reinforcing feedback could in general act at several levels of the immune response. To illustrate that this concept is not just an abstract idea but may well be implemented we consider DNA fragments as indicators of pathogen destruction.

7.5.1. On the role of CpG motifs in pathogen destruction feedback

Examples of a "scalp" that *can* be recognized by receptors of the innate immune system and that are characteristic for certain pathogens are particular parts of prokaryotic DNA termed CpG motifs. Several experimental groups have demonstrated that CpG dinucleotides are ubiquitous in almost all bacteria, fungi, and large eukaryotic viruses [18,47], but absent in vertebrate DNA.

By using pattern recognition receptors, macrophages recognize non-self DNA through CpG motifs and react with a strong Th1-inducing innate response – similar to pattern recognition events discussed in Sec. 7.2 – owing to the secretion of pro-inflammatory cytokines, such as TNF-α, IL-1, IL-6, IL-12 (for reviews see [96, 99]). Major effects on dendritic cells are up-regulation of MHC II presentation and of co-stimulatory molecules such as B7.2 and the production of large amounts of IL-12, TNF-α, IL-6 and, to a lesser extent, IL-10. CpG appears to switch the isotype pattern to a Th1-profile [55] as antigen-specific IgG2a become dominant. This observation has been reinforced by the finding that immunostimulation by CpG-ODN (synthetic oligodeoxynucleotides containing CpG dinucleotides), which mimics the immunostimulatory qualities of bacterial DNA, not only prevents the development of Th2 responses but also inverts already established Th2 polarization toward a Th1 response [107].

If the innate immune system components react to CpG by providing a Th1 bias why should recognition of special DNA fragments that occur in *equal* amounts in intracellular and extracellular pathogens lead to a selection of an appropriate T helper response? Or in a different formulation: Why do extracellular pathogens that carry CpG *not* induce an innate reaction to its CpG? Recent data indicates that CpG receptors are intracellular [49] and that CpG DNA can stimulate cells only if it has been internalized [59], or if the extracellular concentrations has reached a very high level. Therefore it has been suggested that immune recognition of CpG has evolved as a defense mechanism against intracellular bacteria, viruses and retro-viruses [48].

We propose that CpG can be seen – in contrast to LPS, which is a 'classical' pattern recognition molecule – as a component that signals pathogen destruction and selectively up-regulates differentiation of effective Th1 cells. To that end let us now concentrate on the question under which circumstances high CpG concentrations can be detected *inside* a macrophage. One obvious possibility is that CpG-displaying pathogens have invaded the macrophage. This is the case for intracellular pathogens such as those causing tuberculosis and leprosy, which grow in the phagolysosomes or if macrophages have internalized the infectious agents. This detection inside a macrophage occurs only under certain circumstances or for a particular set of pathogens. A much broader class of intracellular pathogens bearing CpG motifs can be detected if we incorporate cytotoxic T cell (CTL) responses and the generation of apoptotic bodies.

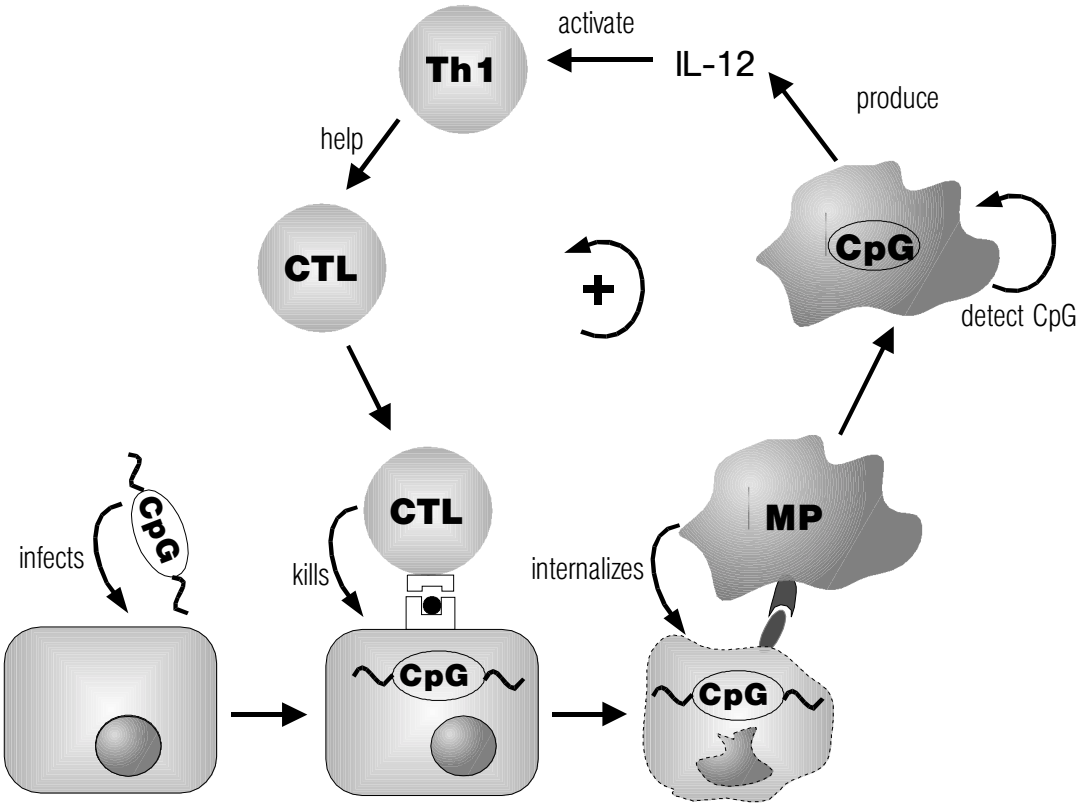


Figure 7.6.: Schematic representation of the interactions governing CpG induced pathogen destruction feedback

When an intracellular pathogen bearing immunostimulatory DNA sequences infects *other* cells than macrophages and replicates there then these sequences can *only* can be detected in sufficient amounts by the corresponding receptors inside a macrophage when this pathogen has been destroyed by appropriate components of an immune response and phagocytized by macrophages, which ingest dead material. In this picture DNA fragments can indeed be seen as some sort of 'scalp' that signals pathogen destruction. Activation of cytotoxic T lymphocytes (CTLs), which kill infected host-material, is reinforced by T helper cells of type 1. For chronic viral infections it even has been shown that CTLs need Th1 help [104]. Therefore, the induction of Th1 promoting signals by CpG can be interpreted as reinforcement of the appropriate immune response through pathogen destruction feedback. This occurs via promotion of activation of this particular T helper type that has triggered the efficient immune response. A sketch of the mechanism described above is shown in Fig. 7.6.

In the present context we emphasize the following differences between LPS seen as a prototypical pattern recognition molecule and CpG DNA. Although having similar effects on the immune system we propose that not only the molecular mechanisms differ [48] but

also that these two molecules play different roles. Whereas the effects of LPS, which is part of the outer cell walls of bacterias, can be interpreted as a reflexive reaction to infectious danger, the CpG-mediated response can be explained as a feedback that reinforces an effective type of attack on a class of pathogens.

7.5.2. Pathogen destruction feedback in the model

We have seen how pathogen destruction feedback leads to up-regulation of activation of the successful T helper type. Therefore, although they are different mechanisms from point of view of regulation, 'classical' pattern recognition and pathogen destruction feedback influence Th1/Th2 ratios in the same fashion. Activation terms in our model generated by PDF look exactly like activation generated by innate immune recognition where the activation parameters θ_i are replaced by products $\nu_i f_i$. The new parameters f_i describe the strength of the feedback. The results obtained in Sec. 7.3 also hold for this effects for PDF with the differences that

- only correct signals are – by definition – induced via pathogen destruction feedback,
- the strength of the signal is now dependent on the level of pathogen destruction and the pathogen destruction feedback strength.

We summarize, that whereas pattern recognition acts in a reflexive, instructive way, unable to respond to changes, pathogen destruction feedback modifies the immune response dependent on present needs. This may in particular be important for pathogens – such as viruses with a complex life cycle – that require effector mechanism induced by both T helper subsets for successful elimination. The differences between instructive pattern recognition and pathogen destruction feedback are demonstrated in Fig. 7.7.

7.6. Summary and Discussion

In Chap. 6 we presented a new model of the T helper system and its interactions with infectious agents. Our model (schematically) incorporates most of the known cytokine-interactions within the T helper system but neglects other influences from outside the T helper system. The major feature of the model is that it describes a self-organizing process for the selection of the appropriate T helper response as an implementation of the strategy: Try Th1 first and shift to Th2 if Th1 fails. The crucial point here is that rising pathogen concentrations are interpreted as a failure of the Th1-dominated response. We proposed that this process is a default mechanism which works in most cases. However, if the development of antigen concentrations during an immune response would be the *only* criterion responsible for the Th1-Th2 decision, it is likely that microorganisms, e.g. intracellular pathogens with rapid evolutionary rates, could escape. Furthermore, pathogen concentrations can increase although Th1-induced effectors are the best choice for this particular pathogen. This feigns a failure of the Th1-dominated immune response and causes a switch towards Th2.

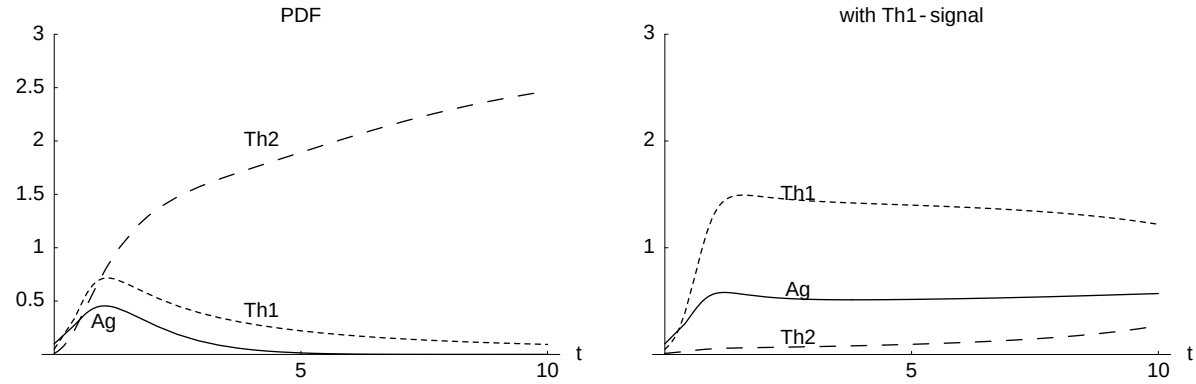


Figure 7.7.: Timeplots illustrating pathogen destruction feedback (PDF) versus 'classical' pattern recognition for situations where both T helper subsets induce an effective immune response against pathogen. Left figure: Pathogen destruction feedback – with feedback strengths $f_1 = f_2 = 1$ and no additional Th1- or Th2-stimuli $\theta_1 = theta_2 = 0$ – up-regulates both T helper subsets and leads to rapid antigen elimination with low antigen load. Right figure: Up-regulation of only the Th1 response by APC-derived signals – $\theta_1 = 2, \theta_2 = 0$ – leads to a chronic situation. The remaining parameters are $\nu_1 = 2, \nu_2 = 2$, $\sigma = 2$, $\pi = 4$, $\delta_1 = 3$, $\delta_2 = 1$, and $r = 3$. The initial concentrations for T helper cells are $x_1(0) = 0.05, x_2(0) = 0.01$.

In the present chapter we discuss how the innate immune system can provide additional stimuli for the decision making process. We first concentrate on the 'classical' view that innate immune system components recognize patterns of an invading pathogen, which give information on the immune responses required to eliminate the pathogen. In the wake of pattern recognition specific signals are generated that promote activation and differentiation of the appropriate T helper subset.

In summary, we find the following properties of instructive APC-derived signals.

(i) Correct APC-derived signals owing to pattern recognition of the innate immune system reinforce the differentiation of the appropriate type of T helper subset.

(ii) These additional stimuli may be particularly important for fast replicating Th1-sensitive pathogens.

(iii) Lower susceptibility for AICD of Th2 cells than of Th1 cells leads to fault-tolerance for incorrect Th1-promoting signals. In such a case the ineffective Th1-response is rapidly taken over by a Th2-dominated response due to the intrinsic T helper dynamics.

(iv) Incorrect Th2-stimuli, however, generate inefficient Th2-responses leading to chronic disease.

(v) The relative absence of APC-derived Th2-signals may be a consequence of evolutionary pressure to minimize the risk of a chronic disease.

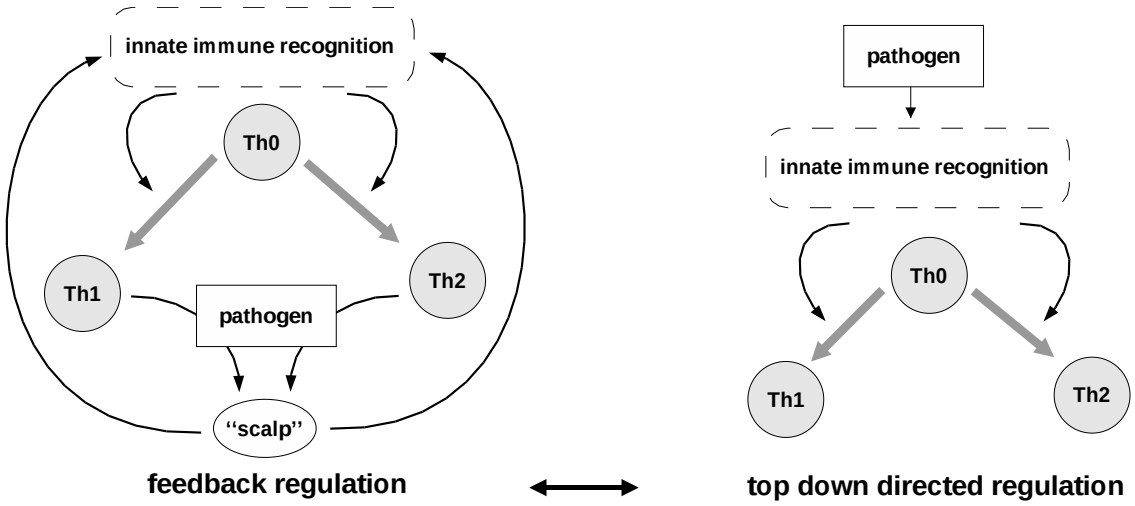


Figure 7.8.: Contraposition of different regulation concepts

(vi) Evolutionary pressure thus selects organisms that do not rely on Th2-signals derived by pattern recognition.

(vii) This is confirmed by the observation that Th2-signals are not *necessary* for the generation of Th2 dominance.

The view of a reflexively instructed T helper response owing to signals provided by the innate immune system is contrasted with an alternative role of pattern recognition. In a process called pathogen destruction feedback "scalps" (parts of the pathogen that signal its destruction) are recognized so as to up-regulate the differentiation of the appropriate T helper subset that is responsible for the destruction.

Although both 'classical pattern recognition' and pathogen destruction feedback finally lead to an up-regulation of the differentiation of the appropriate T helper type (Fig. 7.8) there are fundamental differences in the mechanisms. 'Classical' pattern recognition leads to instructed regulation of the T helper system. A dominant controller (the innate immune system) 'knows' the appropriate defense strategy and directs the Th1 or Th2 dominance accordingly. This knowledge has been evolutionary selected. The strength of the APC-derived signal depends on the antigen concentration. This 'top down' approach is complemented by the 'bottom up' mechanism of the pathogen destruction feedback. Here, positive feedback – owing to present experience instead of learned knowledge and in its strength dependent on the efficiency of the pathogen destruction – on the successful T helper type leads to a self-regulation of the T helper system. Signals of the pathogen destruction feedback *selectively* up-regulate that T helper type that at this stage of the infection leads to the most effective elimination of the pathogen. This results in a flexible rather than a reflexive response, which may be particularly important for pathogens with different life stages.

In summary, we propose that there is a whole set of different mechanisms for the

selection of the appropriate T helper type including

- self-organization of the T helper system owing to the intrinsic dynamics of the T helper system,
- directed regulation via pattern recognition,
- pathogen destruction feedback.

It is sensible that the decision making process that selects the required effector mechanisms relies on a complex matrix of interlocking mechanisms, which are selectively important for different situations. This makes the immune system more resistant against pathogen interference.

More work has to be done concerning the interactions between pathogens and the immune response – in terms of a co-evolution of immune system and pathogens under constant selective pressure – and how this influences the decision making process. It has been proposed that the interaction between microorganisms and host cells is not a linear chain of events in that sense that pathogens induce the host to synthesize cytokines which warn and protect the host. Instead, microorganisms exert a more fundamental control over the cytokine network [37]. For example, viruses have developed their own defense strategies to counteract the broad variety of effector mechanisms that otherwise would inhibit viral propagation in that they express molecules termed 'virokins' that mimic or modulate key immune regulators (for a review see [37, 100]). It is also tempting to speculate that the latency of viruses has evolved as a means to distract the decision making process from finding the appropriate T helper response.

A. State space analysis of the 'Th1/Th2'-model

A.1. Nullclines

Understanding the shape of the nullclines in the two dimensional 'Th1/Th2'-model allows us to draw conclusions on the steady states of the system, which correspond to the intersections of the nullclines, and their stability. We restrict ourselves to the analysis of the simplified system where we set the production of IL-2 by Th0 ($\chi_0 = 0$), IL-4 dependent Th2 proliferation ($\rho = 0$) and T helper independent activation signals ($\theta_1 = \theta_2 = 0$) to zero.

A.1.1. The Th1-nullcline

The Th1-nullcline is given by the solution of the equation

$$\frac{\mathrm{d}x_1}{\mathrm{d}\tau} = 0 \Leftrightarrow \sigma_1 \frac{x_1}{(1+x_2)^2} + \pi_1 \frac{x_1^2}{(1+x_2)} - \delta_1 \frac{x_1^3}{1+x_2} - x_1 = 0$$

Obviously, the x_2-axis is part of the Th1-nullcline. Non-trivial parts of the Th1-Nullcline have the form

$$x_2 = \frac{1}{2}\left(-2 - \delta_1 x_1^2 \mp \sqrt{4\sigma_1 + 4\pi x_1 + \delta_1^2 x_1^4}\right).$$

The first part with the negative square root is always negative and therefore not relevant. The second part has a single maximum at $\pi_1/2\delta_1$. In Fig. A.1 typical shapes of the Th1-nullcline are shown for various values of σ_1. Fas-induced cell death for Th1 cells δ_1 is responsible for the decline of the Th1-nullcline for higher x_1 values. Increasing death rate for Th1 δ_1 diminishes total Th1 numbers as illustrated in Fig. A.2.

A.1.2. The Th2-nullcline

The Th2-nullcline is given the by the solution of

$$\frac{\mathrm{d}x_2}{\mathrm{d}\tau} = 0 \Leftrightarrow \sigma_2 \frac{x_2}{1+x_2} + \pi_2 \frac{x_1 x_2^2}{(1+x_2)(1+x_1+x_2)} - \delta_2 \frac{x_1^2 x_2}{1+x_2} - x_2 = 0$$

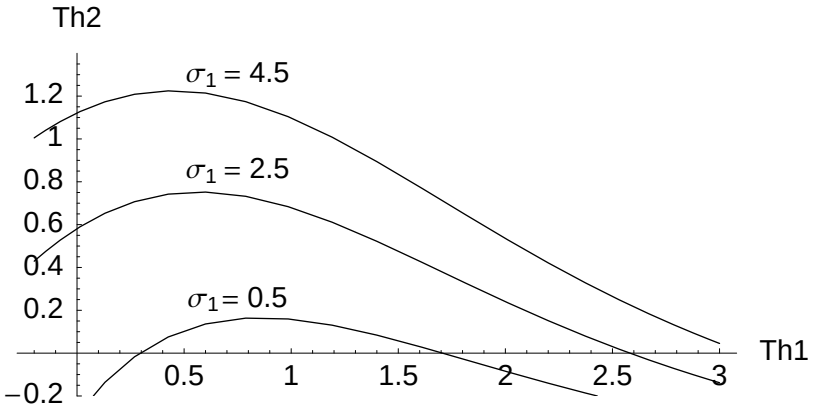


Figure A.1.: The Th1-nullcline of the 'Th1/Th2'-model for various values of σ_1. Other parameters are $\delta_1 = 1$, $\pi_1 = 2$.

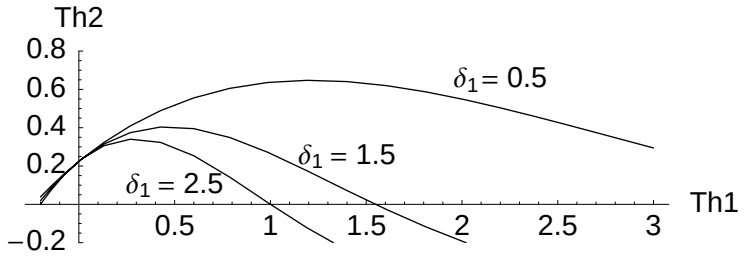


Figure A.2.: The Th1-nullcline of the 'Th1/Th2'-model for various values of δ_1. Other parameters are $\sigma_1 = 1.5$, $\pi_1 = 2$.

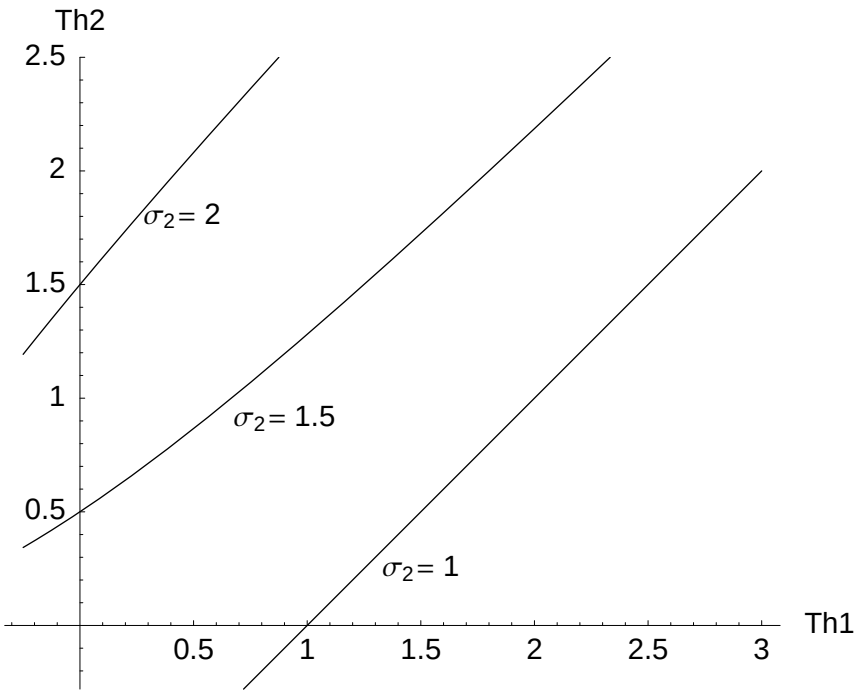


Figure A.3.: The Th2-nullcline for varying values of σ_2. Other parameters are $\pi_2 = 2$.

Let us first assume that there is zero susceptibility of Th2 for antigen induced cell death ($\delta_2 = 0$). The x_1-axis is part of the Th2-nullcline. Non-trivial parts of the Th2-nullcline in the positive quadrant are either

(i) monotonically increasing for $\sigma_2 > 1$ as illustrated in Fig. A.3.

(ii) or monotonically decreasing for $\sigma_2 < 1$ as illustrated in Fig. A.4. Increasing π_2 moves the graph down to the left. We expect intersections with the Th1-nullcline only for high Th2-proliferation (π_2 or ρ large) or for very low rates of AICD for Th1 (δ_1 small).

If, however, there is non-zero susceptibility of Th2 for AICD the shape of the Th2-nullcline changes dramatically.

(i) Even for low values of δ_2 the Th2-nullcline is squeezed as illustrated in Fig. 4.5.

(ii) For $\sigma_2 < 1$ there is no non-trivial part (apart from the Th1-axis) of the Th2-nullcline in the first quadrant. The only intersection of the Th1- and the Th2 nullcline is the intersection on the x_1 axis.

We assume that $\sigma_2 < 1$ [case (ii)] occurs mainly in autoimmune situations (see Sec. 4.4) whereas for situations of infections $\sigma_2 > 1$. System behavior for the case $\sigma_2 < 1$ is discussed in Sec. 4.5 whereas we otherwise assume that $\sigma_2 > 1$ so that the Th2-nullcline is monotonically increasing.

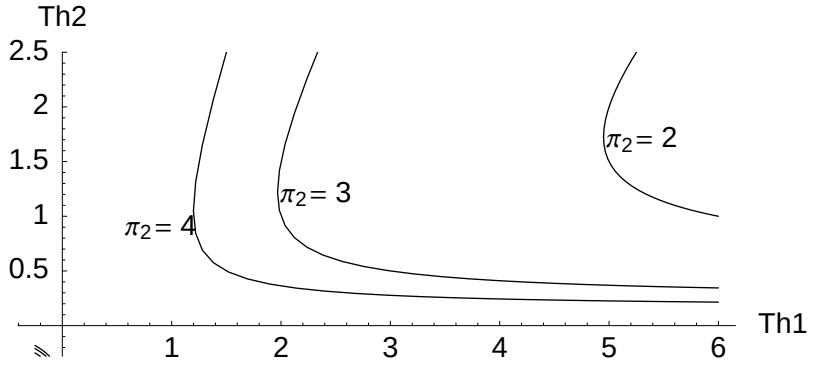


Figure A.4.: The Th2-nullcline is shown for low values of $\sigma_2 = 0.5$. With increasing Th2 proliferation parameter π_2 the nullcline is moving downwards to the right.

A.1.3. Orbits

Orbits can not leave the positive quadrant because $\mathrm{d}x_1/\mathrm{d}\tau = 0$ on the x_2-axis and $\mathrm{d}x_2/\mathrm{d}\tau = 0$ on the x_1-axis. The system is generally bounded.

B. Role of various parameters in the intrinsic decision making process

B.1. Asymmetric activation parameters

We find that the location and stability of Th1- or Th2-dominated 'cure' steady states is unaffected by asymmetric activation rates ($\sigma_1 \neq \sigma_2$), as is the stability but not the location of the 'chronic' steady states. Higher activation rates of one T helper type generally stabilize 'chronic' steady states that are dominated by the corresponding T helper subset and destabilize chronic situations dominated by the competing T helper type. This becomes clear if we look at the relevant eigenvalues

$$\lambda_1 = -1 + \frac{\pi\nu_2}{r+\nu_2} + \frac{\sigma_1}{\sigma_2}\frac{\nu_2[r+\nu_2(1-\pi)]}{(r+\nu_2)^2}$$

for 'chronic:Th2' and

$$\lambda_1 = -1 - \frac{\delta_2 r}{\nu_1} - \frac{\pi\nu_1}{r+\nu_1} + \frac{\sigma_2}{\sigma_1}\left(1-\pi+\frac{\delta_1 r}{\nu_1}\right)$$

for 'chronic:Th1'. The parameter regions in the (ν_1, ν_2)-space (pathogen-elimination efficiency), where 'chronic' steady states are stable, are the following.

- If $\sigma_1 > \sigma_2$ then 'chronic:Th2' is stable if $\nu_2 < r/(\pi-1)$ and $\nu_2 < r/(\sigma_1/\sigma_2 - 1)$, or if $\nu_2 > r/(\pi-1)$ and $\nu_2 > r/(\sigma_1/\sigma_2 - 1)$. If $\sigma_1/\sigma_2 > \pi$ conditions for the first case are even more restrictive than the conditions for equal activation parameters. The latter case can be never fulfilled because of the conditions for existence. If $\sigma_1 < \sigma_2$ then 'chronic:Th2' is stable if $\nu_2 < r/(\pi-1)$ and $\nu_2 > 0$ as in the case $\sigma_1 = \sigma_2$.

- Different activation strengths affect 'chronic:Th1' in the following way. We define

$$B_{1;2} = \frac{[r(1-\delta_2)\sigma_1 + (\pi-1-\delta_1)\sigma_2]}{2(\pi-1)(\sigma_2-\sigma_1)} \pm\sqrt{r[(1-\delta_2)\sigma_1 + (\pi-1-\delta_1)\sigma_2]^2 + 4(\pi-1)r^2(\sigma_2-\sigma_1)(\delta_2\sigma_1 - \delta_1\sigma_2)}.$$

  Then 'chronic:Th1' becomes stable if

1. for $\sigma_1 > \sigma_2$: $min(B_1, B_2) < \nu_1 < max(B_1, B_2)$
2. for $\sigma_1 < \sigma_2$: $\nu_1 < min(B_1, B_2)$ or $\nu_1 > max(B_1, B_2)$.

In Fig. 6.4 we illustrate that the activation parameters affect the lower boundary of the parameter window where 'chronic:Th1' becomes stable. Higher Th2-activation strengths reduce the risk of chronic Th1-dominated situations, whereas higher Th1-activation parameters increase it.

B.2. Asymmetric proliferation parameters

Asymmetric proliferation parameters ($\pi_1 \neq \pi_2$) lead to the following changes in the stability of the steady states.

- 'cure:Th2' becomes unstable when Th1-proliferation is higher than Th2-proliferation because of the eigenvalue $\lambda_2 = -1 + \pi_1/\pi_2$. This occurs via a transcritical bifurcation with the steady state 'Th1/2-chronic', which represents a chronic situation with co-existing T helper populations.

- 'cure:Th1', however, is stabilized by higher Th1-proliferation. The corresponding eigenvalue has the form

$$\lambda_2 = -1 - \frac{(\pi_1 - 1)\delta_2}{\delta_1} + \frac{\delta_1 \pi_2}{\pi_1 - 1 + \delta_1}.$$

- In contrast, 'chronic:Th2', with relevant eigenvalue

$$-1 + \frac{\pi_1 \nu_1}{r + \nu_2} + \frac{\nu_2(r + \nu_2 - \pi_2 \nu_2)}{r + \nu_2},$$

is destabilized by higher Th1-proliferation.

- For 'chronic:Th1' the relevant eigenvalue for the stability analysis has the form

$$-\pi_1 + \pi_2 \nu_1 + \frac{r(\delta_1 - \delta_2)}{\nu_1}.$$

Higher Th1-proliferation and Th2 proliferation increase and decrease stability of this fixed point, respectively. Effects of different proliferation parameters on boundary conditions for ν_1 on the stability are illustrated in Fig. 6.5. We define

$$B_{1,2} = \frac{r[\delta_1 - \delta_2 - \pi_1 \pm \sqrt{\delta_1^2 + \delta_2^2 - 2\delta_2\pi_1 + \pi_1^2 + 4\delta_2\pi_2 - 2\delta_1(\delta_2 - \pi_1 + 2\pi_2)}]}{2(\pi_1 - \pi_2)}.$$

The steady state 'chronic:Th1' becomes stable for values of ν_1 between B_1 and B_2 if $\pi_2 > \pi_1$ and for ν_1 greater than the maximum of both B_1 and B_2 otherwise. We find that increasing π_2 diminishes the ν_1-parameter-window. For values of ν_1 close to zero and close to $r\delta_1/(\pi_1 - 1)$ 'chronic:Th1' is unstable.

Abbreviations

AICD activation induced cell death

APC antigen presenting cell

CTL cytotoxic T lymphocyte

FasL Fas-ligand

HSP heat shock protein

IL interleukin

IFN interferon

MHC major histocompatibility complex

TCR T cell receptor

Th1 T helper type 1

Th2 T helper type 2

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