Doctoral Thesis

Competition and coexistence of T lymphocytes

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**Competition and Coexistence of T Lymphocytes**

A dissertation submitted to the
SWISS FEDERAL INSTITUTE OF TECHNOLOGY
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presented by

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Summary

Pathogens are masters of disguise, and frequently escape recognition by the immune response. Therefore broad immune responses, directed at many epitopes of the pathogen, are thought to improve control of infection. There is evidence that competition between immune cells of different epitope specificity reduces the breadth of the immune response. This thesis focuses on T-cell immune responses, studying mechanisms by which T-cells specific for different epitopes of a pathogen might compete with each other and how broad T-cell responses may nevertheless be maintained. Chapter 1 introduces the scientific context of the questions posed in this thesis. This includes an outline of the sequence of events occurring between pathogen infection and pathogen specific T-cell immune response and a review of the current literature on T-cell competition.

In Chapter 2, I develop and analyse a mathematical model in which T-cells compete for access to Antigen Presenting Cells (APCs). For simplicity, APCs are assumed to have only one T-cell interaction site. Thus, these APCs are a shared resource for T-cells specific for different epitopes, and competition leads to loss of the subdominant T-cell population. I then introduce a mechanism called epitope down-modulation, and show how it can generate resource differentiation amongst APCs. This resource differentiation facilitates the coexistence of the immunodominant and subdominant T-cell populations.

In Chapter 3, I investigate T-cell competition in an individual-based model, and relax a simplifying assumption of the mathematical model of Chapter 2, by modelling APCs as cells with multiple T-cell interaction sites. When APCs have multiple T-cell interaction sites, the epitope expression level of the presented epitopes is a crucial parameter in determining whether or not T-cells of different epitope specificity compete with each other. Competition between T-cells of different epitope specificity only occurs at high epitope expression levels, because then access to the APC sites, rather than to the specific epitope, is limiting. At low epitope expression levels, i.e. when epitope is limiting, competition occurs only between T-cells of the same specificity.

Finally, Chapter 4 describes a mouse experiment with which I tested our model prediction that competition between T-cells of distinct epitope specificity occurs mainly at high epitope expression levels. Every second day, mice were injected with APCs, loaded with two epitopes at either high or low epitope expression level. When the responses were measured after eight days, the difference in size of the two epitope-specific T-cell populations was larger at high than at low epitope expression levels. Hence, the experimental results support the theoretical predictions of the model.

The results presented in this thesis extend our understanding of mechanisms that influence the diversity of T-cell responses against pathogens. The results suggest that low epitope expression, and epitope down-modulation augment this diversity, but the finite size of APCs limits it. These insights into the biological factors that affect T-cell response diversity may help in the development of T-cell based vaccines.
Zusammenfassung


Die Resultate dieser Dissertation erweitern unser Verständniss über die Mechanismen
Chapter 1

Introduction

1.1 Infectious diseases and T-cell responses

The vertebrate immune system fights infections with both innate and adaptive recognition mechanisms. The adaptive immune system is evolutionary younger than the innate one and evolved at some point in the transition of cyclostomes (e.g. Lamprey) to cartilaginous fish (e.g. shark) (Kasahara et al., 2004). The innate immune system consists of many protective mechanisms, including anti-microbial peptides, cytokines and chemokines, that recognise evolutionarily conserved pathogen cues and respond very rapidly upon infection (reviewed in Janeway and Medzhitov (2002); Tosi (2005)). The adaptive immune system (made up of mainly T and B-cells), can recognise a much greater diversity of antigens, but its response to infection is much slower than that of the innate immune system. The innate and adaptive arms of the vertebrate immune system act in a tightly interwoven manner (see reviews by Dempsey et al. (2003); Tosi (2005)).

This thesis focuses on mechanisms of T-cell competition. Hence I start this introduction by briefly summarising basic aspects of the generation of the T-cell repertoire, the processing and presentation pathway of T-cell epitopes and T-cell dynamics in acute and chronic infection, and finish by giving an overview of current experimental data on T-cell competition.

1.1.1 The T-cell repertoire

T lymphocytes are derived from multi-potential haemopoetic stem cells in the bone marrow\(^1\). During their maturation in the thymus, T-cells start to express CD4 and CD8 co-receptors and obtain a semi-random T-cell receptor (TCR) by a process that involves rearrangement of the \(\alpha\)- and \(\beta\)-chain coding gene stretches of the TCR\(^2\) (Sangster et al., 1986) and subsequent nucleotide additions (Candeias et al., 1996). Antigen recognition occurs via interactions between the TCR and major histocompatibility molecules (MHC) on Antigen Presenting Cells (APCs). MHC molecules display epitopes, \(i.e.\) short peptides, of both self and foreign origin. The interactions between TCRs and MHC:epitope complexes play a crucial role in T-cell maturation. As soon as T-cells express their TCR and the CD4 and CD8 co-receptor molecules, they go through positive selection, during

\(^{1}\)For a detailed review on repertoire selection, see \(e.g.\) (von Boehmer, 2004).

\(^{2}\)Apart from \(\alpha\beta\) T-cells, that constitute approximately 80% of the T-cell repertoire, the T-cell repertoire also contains \(\gamma\delta\) T-cells.
which only those T-cells survive that receive sufficient signalling via their TCRs. This ensures a minimal affinity of the TCR to the MHC:epitope complexes presented on APCs in the thymus. During positive selection, the CD4-CD8 double positive T-cells furthermore become committed to the CD4 or CD8 lineage by down-regulation of one of the two co-receptors. To what extent this signal commitment is determined stochastically or by the signals the T-cell obtains during positive selection is unclear (Basson and Zamoyska, 2000). Either way, the lineage commitment to the CD4 or CD8 co-receptor is associated with a functional specialisation to so-called ‘helper T-cells’ or ‘killer T-cells’ respectively.

The second selection mechanism, called negative selection, tests T-cells for tolerance to self-peptide:MHC conjugates presented in the thymus. Remarkably, thymic epithelial cells, one of the main antigen presenting cells responsible for negative selection, seem to be specialised to promiscuously express proteins, such that thymic selection can also tolerise T-cells to tissue specific peptides (i.e. peptides from proteins whose expression is restricted to cells of specific tissues, e.g. liver or pancreas) (Klein and Kyewski, 2000; Derbinski et al., 2005; Derbinski and Kyewski, 2005).

A hallmark of the T-cell repertoire is its diversity in terms of different TCR specificities, estimated at $10^7$ and $10^{11}$ TCR clones (Arstila et al., 1999; Kesmir et al., 2000). This great diversity is due to the semi-random nature of the generation of TCRs mentioned above (Nikolich-Zugich et al., 2004). Expansion of specific T-cell populations after infection with pathogens may skew this diversity temporarily, but over the long-term, the diversity of the T-cell repertoire seems to be homeostatically regulated (Freitas and Rocha, 2000; Mahajan et al., 2005; Almeida et al., 2005). The mechanisms that allow the maintenance of T-cell repertoire diversity are thought to include competition for aspecific and specific factors, such as cytokines and self-peptide:MHC conjugates, respectively (Mahajan et al., 2005). In the following, I briefly describe the pathway by which peptides reach the surface of APCs, and the estimated diversity of the presented self peptides.

### 1.1.2 Antigen processing and presentation

All nucleated cells present MHC Class I molecules on their surface and are ‘scanned’ by T-cells for presentation of foreign ligands. CD8+ ‘killer’ T-cells recognise their ligands in conjunction with MHC Class I, and CD4+ ‘helper’ T-cells in conjunction with Class II molecules. Here, I describe the pathway of presentation of MHC Class I restricted epitopes only, since this thesis focuses on killer T-cells. The degradation of all intracellular proteins (e.g. pathogen derived proteins, misfolded proteins) starts with cleavage by the proteasome, which is a protease. Proteasomal products of a length of 8 - 16 amino acids can be transported by the transporter associated protein (TAP) into the endoplasmatic reticulum (ER) (Heemels and Ploegh, 1995; Yewdell and Bennink, 1999). In the ER, peptides are shortened by the amino-peptidase ERAAP until they fit an MHC molecule (for MHC Class I molecules, this is at a length of eight to ten amino acids), or until they are destroyed (Serwold et al., 2002; Rammensee, 2002). Those ‘fitters’ that have the right binding motif for one of the MHC Class I molecules of the individual\(^3\) (Rammensee et al., 1999), are loaded onto MHC, and transported in Golgi vesicles to the cell

\(^3\)For MHC Class I molecules, the epitope-binding groove is formed by an α-chain. Humans have three MHC Class I α-genes. Hence, individuals who are heterozygous on the Class I α-chain loci have six different MHC Class I molecules.
surface (see Fig. 1.1) (for a detailed description of the Class I processing and presentation pathway, I refer to Yewdell et al. (2003)). Only approximately one percent of peptides of the right length can bind to any particular MHC molecule. The diversity of the presented self epitopes of the human genome has been estimated to be approximately $10^5 - 5 \times 10^6$ (Müller and Bonhoeffer, 2003; Mahajan et al., 2005). This diversity of presented self-epitopes would be the specific resource involved in the maintenance of the homeostasis of the naïve T-cell repertoire diversity mentioned in section 1.1.1.

When a host is infected with a pathogen, foreign epitopes will be presented on APCs alongside self-epitopes. MHC Class I molecules are mainly loaded with self-epitopes and epitopes from intracellular bacteria and viruses. Extracellular pathogen epitopes may, however, also be displayed on class I molecules by a mechanism referred to as cross-presentation, by which extra-cellular proteins are fed into the class I processing and presentation pathway (Melief, 2003; Groothuis and Neefjes, 2005).

![Figure 1.1: Simplified representation of the Class I antigen processing and presentation pathway. Cytosolic proteins are cleaved by the cellular proteasome machineries. Cleaved peptides of 8 up to 16 amino acid length can be transported by the Transporter Associated Proteins (TAP) into the Endoplasmatic Reticulum (ER). In the ER, peptides are loaded onto MHC Class I molecules, which are then transported in Golgi vesicles to the cell surface. These presented MHC:epitope complexes can subsequently be recognised by 'patrolling' Cytotoxic T-cells (CTLs).](image)

There is a continuous, rapid turn-over of MHC:epitope complexes presented on the surface of a cell. The expression of self- or foreign MHC:epitope complexes derived from intracellular proteins is replenished by the cellular processing and presentation machinery. In contrast, epitopes that are loaded exogenously onto Dendritic Cells (DCs, an important type of APCs), are lost from the cell surface quite rapidly. In experiments, exogenously peptide-loaded DCs are frequently used as immunising agent. The in vivo stimulatory effect of such exogenously peptide-loaded DCs is maintained for at most one to two days (Ludewig et al., 2001). The rate of turn-over of MHC:epitope complexes
can be estimated from the natural rate of production of MHC:epitope complexes and the number of complexes displayed per APC. The endogenous production of MHC:epitope complexes yields slightly less than $10^4$ complexes per hour (Yewdell et al., 2003), and the total number of MHC:epitope complexes per APC is approximately $10^5 - 10^6$ (Engelhard, 1994; Delaney et al., 1998). Hence, the average duration of presentation of an MHC:epitope complex is around ten hours.

Most epitopes are expressed on APCs at approximately $10^2$ to $10^3$ copies, but copy numbers as low as one and as high as $10^4$ have also been observed (Stevanovic and Schild, 1999; Crotzer et al., 2000; Engelhard et al., 2002; Herbergs et al., 2003a,b; Basler et al., 2004). In Chapter 3 and 4, I investigate the importance of the per APC expression level of epitopes on T-cell coexistence with a simulation model and a mouse experiment, respectively.

### 1.1.3 T-cell dynamics

When a T-cell contacts an APC presenting the T-cell's specific ligand, many molecules aggregate at the interface of the T-cell and the APC. The semi-stable structure at the contact site is referred to as the immunological synapse (Monks et al., 1998; Grakoui et al., 1999; Krummel et al., 2000; Faroudi et al., 2002; Kropshofer et al., 2002; Jensen, 2002; Vogt et al., 2002). The movement of MHC:epitope complexes, TCRs, co-stimulatory receptors and adhesive molecules into the T-cell:APC contact area is rapid, and the immunological synapse is formed approximately two to ten minutes after the initial, specific contact (Wülffing et al., 2002; Krummel and Davis, 2002; Tkivitaria-Fuller et al., 2003; Purbhoo et al., 2004). It has been debated whether the formation of an immunological synapse is essential or simply helpful for T-cell activation. The latter view relates to the serial engagement model, which postulates that T-cells become activated from signals obtained by scanning multiple APCs, rather than from one long-lasting interaction with an APC (Gunzer et al., 2000; Rachmilewitz and Lanzavecchia, 2002). A recent review by Friedl et al. (2005), discusses contact modes between T-cells and APCs and their influence on the outcome of the T-cell:APC interaction.

Another interesting feature of the specific interaction between T-cells and APCs was described a few years ago. Epitope loaded APCs that were injected into mice were found to reduce their expression levels much more when specific T-cells were co-injected. Possible consequences for T-cell competition of this epitope down-modulation (Kedl et al., 2002) is the focus of Chapter 2 of this thesis.

When a naïve T-cell becomes activated by its interaction with one or several APCs, it goes through five to ten rounds of proliferation (Wong and Pamer, 2001; Van Stipdonk et al., 2001; Kaech and Ahmed, 2001; Krogsgaard et al., 2003), without the need for re-exposure to the antigen, a phenomenon called programmed proliferation (Mercado et al., 2000). Previously, it was assumed that a T-cell needs an antigenic stimulus for each round of proliferation. Wodarz and Thomsen (2005) have recently investigated mathematically what the effects of programmed versus non-programmed proliferation might be on the efficiency of the immune response. They found that multiple rounds of programmed division generate an over-shoot in the immune response, which allows for more efficient
Virus infects DCs and other target cells.

Infected DCs migrate to lymphoid tissue.

Activated T cells migrate into periphery and start to proliferate.

Activated T cells migrate into infected tissue.

T cells and infected DCs migrate to lymphoid tissue.

Activated T cells migrate into the tissue.

Infection has already been cleared.

T cells die or mature into memory T cells.
**Acute infections**

T-cell dynamics in acute infections typically consist of an expansion, a contraction and a memory phase (Kaech et al., 2002). When a host organism encounters an infectious agent for the first time, the dynamics of the infectious agent is far more rapid than that of the immune response against it. Thus, the infectious load usually peaks before the immune response (Murali-Krishna et al., 1998; Wong and Pamer, 2003). Because of these different dynamics, the ratio of T-cells to infected APCs can change during the infection. In the first phase of infection this ratio is expected to be low. Antigen levels will be abundant because of the fast expansion dynamics of the infectious agent, and specific T-cells populations will still be very low because they expand from small naïve precursor frequencies of 10^-50 cells per specificity (Zinkernagel, 1996; Casrouge et al., 2000; Blattman et al., 2002). In a later phase of the infection, when most infected cells have already been cleared by the immune response, but there are large expanded populations of T-cells, the ratio of T-cells to infected APCs is expected to be high. After clearance of the pathogen, the T-cell immune response contracts by apoptosis of most effector T-cells, save those that differentiate to memory T-cells (see Fig. 1.2). Most memory T-cell populations seem to be able to survive without exposure to their specific antigen for many years. The response of memory T-cells upon secondary infection of the same pathogen is very rapid (for reviews on T-cell memory see for example (Kaech et al., 2002; Geginat et al., 2003; Antia et al., 2005; Miller et al., 2005a).

**Chronic infections**

When a pathogen infection is not successfully cleared, T-cell responses do not follow the same pathway of contraction and differentiation to memory cells as in acute infections. CD8 T-cell dynamics in chronic infections follow more complex patterns and
differ depending on the infection (Klenerman and Hill, 2005). One difference is that the maintenance of effector T-cells in chronic infections becomes antigen dependent (Wherry et al., 2004). For example, in Human Immunodeficiency Virus (HIV) or Hepatitis C Virus (HCV) infection, T-cell responses decrease when viral loads are suppressed by anti-viral therapy, and rebound upon cessation of therapy (Oxenius et al., 2002; Lauer et al., 2005). Further, chronic stimulation can lead to full or partial functional impairment (Appay et al., 2002b,a; Fuller and Zajac, 2003; Wherry et al., 2003) or to physical exhaustion of T-cells (Moskophidis et al., 1993; Probst et al., 2003b; Robinson, 2003; Zhou et al., 2004), and to changes in the size hierarchy of the epitope-specific responses involved (van der Most et al., 2003; Probst et al., 2003b; Lichterfeld et al., 2005).

1.1.4 T-cell response diversity

Diversity of T-cell responses against pathogens is observed on two levels. First, there is diversity in the pathogen epitopes that are targeted by the response, and this diversity is often maintained over a prolonged time. In HIV infection, for example, epitope-specific responses have been measured longitudinally over several years (Oxenius et al., 2002; Yu et al., 2002). Second, each epitope-specific response consists of many T-cell receptor (TCR) clone specificities, that are often maintained from primary infection into secondary or even chronic infection (Bitmansour et al., 2001; Douek et al., 2002; Cohen et al., 2002; Turner et al., 2003; Karrer et al., 2003).

However, it is also known that the diversity of the response is not nearly as high as the diversity of pathogen epitopes that are displayed on APCs (Yewdell and Bennink, 1999; Yewdell and Del Val, 2004) and loss of clonal diversity over time has been observed as well (see section 1.2.2). T-cell competition might be one of the factors that limits T-cell response diversity, and is described in detail in section 1.2.

1.1.5 Immune escape

The number of pathogen epitopes targeted is an important property of a T-cell response in terms of coping with immune evasion strategies of the infecting agent. One immune evasion strategy is viral immune escape, where epitope-specific T-cell responses are strongly decreased in size, or even lost, due to mutations in or around the epitope gene. These mutations can interfere with processing and presentation of an epitope, or recognition of the epitope by its specific T-cell. Immune escape mutations occur in most viruses that cause chronic infections, such as HIV, SIV or HCV (Phillips et al., 1991; Price et al., 1997; Borrow et al., 1997; Harcourt et al., 1998; Klenerman et al., 2002b; Leslie et al., 2004; Yokomaku et al., 2004; Bowen and Walker, 2005)

When viral strains with immune escape mutations are transmitted to MHC-mismatched hosts (i.e. hosts that do not carry the restricting MHC type for the specific T-cell response that selected this particular immune escape mutation), they usually revert back to the wild-type sequence (Kent et al., 2005; Barouch et al., 2005; Bowen and Walker, 2005). This reversion indicates that immune escape mutations bear a fitness cost for the virus. If immune escape is costly to the pathogen, broad T-cell responses, directed at many epitopes, should be more protective for the host than narrow ones.

One of the mechanisms that is thought to limit the breadth of T-cell responses upon infection is competition between T-cells of different epitope specificity. In the following,
I introduce some background ecological theory on resource competition, and present the experimental data for resource competition between T-cells.

1.2 T-cell competition

1.2.1 Ecological theory of competitive exclusion

Classical theoretical ecology models predict that the diversity of species that can coexist in a habitat is limited by the number of limiting resources. When two species compete for a single, limiting resource, the species that exploits the limiting resource more efficiently is predicted to out-compete the other species (Yodzis, 1989; De Boer and Perelson, 1994). Simple ecological population dynamics models further predict that multiple species can coexist when the competition between individuals of the same species (within-species competition) is stronger than competition between individuals of different species (between-species competition). The balance of within- and between-species competition is dictated by the amount of overlap that species have in their resource utilisation pattern (also called niche, see Fig. 1.3). Competition between species that share a limiting resource can be reduced by resource differentiation, which increases within-species competition compared to that between-species, and thus ‘pulls’ niches further apart (see Fig. 1.3 B and C).

\[
\text{A} \quad \text{within} = \text{between} \quad \text{B} \quad \text{within} > \text{between} \quad \text{C} \quad \text{within} >> \text{between}
\]

![Figure 1.3: ‘Niche’ overlap determines the strength of between-specificity competition. The niches of two species are represented by red and black circles, which indicate resource usage in a multi-dimensional space of limiting resources. The between-species competition is given by the amount of overlap of the two circles. If the circles overlap completely, the two species have the same requirements of limiting resources, and within-species competition equals between-species competition (A). Such conditions are expected to lead to competitive exclusion of the sub-dominant species. By pulling the niches apart (B and C), competition between-species is relaxed.](image)

Competition between T-cells is thought to affect many aspects of the immune system, such as the diversity of the naïve T-cell repertoire (see section 1.1.1), the clonality of epitope-specific T-cell responses, and the immunodominance patterns within T-cell populations specific for different epitopes of a pathogen. In the following, I will briefly review the experimental evidence for within and between-specificity competition.
1.2.2 Competition between T-cells of the same epitope specificity

Since competition between T-cells is only observed when the ratio of T-cells to antigen is high (Grufman et al., 1999; Roy-Proulx et al., 2001; Lanzavecchia, 2002), T-cell-competition experiments are usually designed to set such conditions. This is typically done with adoptive transfer of donor T-cells specific for a particular epitope into a normal or TCR-transgenic mice, and subsequent immunisation of these mice with the appropriate epitopes. In TCR-transgenic mice, all T-cells express the same T-cell receptor, and hence the precursor frequencies of T-cells for the specific epitope are orders of magnitudes higher than in normal mice.

Within-specificity competition can be observed in adoptive transfer experiments by tracking the expansion of host and donor T-cells specific for the epitope used in the immunisation. First, expansion of donor T-cells specific for a particular epitope is far poorer when transferred into host animals that are TCR transgenic for the same epitope, than when transferred into non-transgenic hosts (Smith et al., 2000). Second, adoptively transferred cells reduce the expansion of host T-cells of the same epitope specificity (Smith et al., 2000; Moses et al., 2003; Kedl et al., 2003; Probst et al., 2003b). Third, several studies observed preferential selection of high affinity TCR clones into memory responses, which suggests that T-cell clones specific for the same epitope might out-compete each other (McHeyzer-Williams et al., 1999; Savage et al., 1999). However, other studies have reported similar clonal diversities in primary and secondary or memory responses (Bitmansour et al., 2001; Douek et al., 2002; Cohen et al., 2002; Turner et al., 2003; Karrer et al., 2003).

1.2.3 Competition between T-cells of different epitope specificity

Between-specificity competition5 is thought to be one of the mechanisms that affect the immunodominance pattern of multiple epitope-specific T-cell populations in an infection. The suggested limiting resource that T-cells of different epitope specificity might compete for is access to APCs. Between-specificity competition has been observed experimentally, and can be broken both by presenting different epitopes on separate APCs, or increasing the frequency of APCs (Wolpert et al., 1998; Grufman et al., 1999; Kedl et al., 2000; Roy-Proulx et al., 2001). In the first case, epitopes that are usually displayed mixed on the APC surface, and thus might be seen as a single resource, are artificially split into two separate resources. In the second case, the resource, namely APC surface, is artificially made non-limiting by adding excessive numbers of APCs.

Other evidence for between-specificity competition include data showing compensation: After knocking out, or reducing the size of an immunodominant T-cell response, the size of subdominant responses was increased (Probst et al., 2003b; Chen et al., 2004; Andreansky et al., 2005). Compensation effects were typically seen in secondary challenges, which is when the ratio of T-cells to antigen is higher than in the primary response. However, compensation is not always observed. For example, in a study by Vijh et al. (1999), the response to sub-dominant epitopes was unaltered by the removal of one or two dominant epitopes from the bacterial pathogen.

Between-specificity competition is only one of the many factors that can influence the

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5In many publications, competition between T-cells of different epitope specificity is referred to as "immunodomination."
size hierarchy of the epitope-specific T-cell populations against a pathogen (Chen et al., 2000). Differences in precursor frequency (Choi et al., 2002), and in epitope expression levels (Gallimore et al., 1998b; Tourdot and Gould, 2002; Basler et al., 2004) have also been observed to contribute to immunodominance.

Both within- and between-specificity competition can occur by direct or indirect interference. Competition for access to the APC, or for access to specific MHC:epitope complexes on APCs would be types of direct competition, where the competing T-cells hinder each other physically in accessing their resource. Additionally, the killer function of effector T-cells can increase competition. Competition by clearance of the antigen is a form of indirect competition. For example, when memory T-cells, primed in a previous infection, cross-react with one or some epitopes of a new infection, they are likely to clear the infection before new naïve precursors can be recruited into the response. It is often hard to distinguish direct and indirect competition mechanisms. In Chapter 4, in which we study mechanisms for T-cell competition in a mouse experiment, we measured the rates with which epitope-loaded DCs were killed in the different treatment groups to check whether the observed competition effect might be due to indirect interference between T-cells.

1.2.4 Current model for T-cell competition

Given the current body of experimental evidence, the following model of T-cell competition has been suggested (Lanzavecchia, 2002; Kedl et al., 2003): T-cells do not compete when the ratio of T-cells to antigen is low (see Fig. 1.4 A), i.e. early in infection (see also Fig. 1.2). When the ratio of T-cells to antigen is high, i.e. later in the response, or upon secondary challenge, T-cells will start to compete with each other. Depending on whether epitopes are expressed on separate (Fig. 1.4 B) or on shared APCs (Fig. 1.4 C), competition is only within-specificity or both within- and between-specificity, respectively.

T-cell competition between T-cells of the same specificity has been observed consistently in T-cell competition experiments, but competition between T-cells of different specificity only in some. Also, the strength of within-specificity competition seems to be stronger than that of between-specificity competition.

What mechanisms could induce such a difference between within- and between-specificity competition? Recently, Kedl et al. (2002) reported observations that suggested that the interaction between T-cells and APC can lead to a reduction of the density of their cognate MHC:epitope complex on the APC. This epitope-specific resource down-modulation mechanism has been suggested to explain why within-specificity competition seems more prominent (Kedl et al., 2002; Lanzavecchia, 2002) (see Fig. 1.4). In chapter 2, I explore the effect of epitope down-modulation on T-cell competition in a mathematical model, and in chapter 3, I suggest a second mechanism that can explain stronger within- than between-specificity competition.

1.3 Thesis outline

This thesis deals with fundamental questions of T-cell competition. Our modelling approaches for studying T-cell competition build on the ecological theory of resource competition between species with (partly) overlapping niches.
Figure 1.4: Schematic representation of the current model for T-cell competition (Lanzavecchia, 2002; Kedl et al., 2003). The thickness of the arrows indicate the strength of competition between T-cells of the same or of different specificity. A: If T-cells are present in low numbers, and antigen is abundant, T-cells will not compete with each other. At a high ratio of T-cells to APCs, T-cells can compete with each other. When epitopes are presented on separate APCs, only T-cells of the same specificity compete (B), and when epitopes are on shared APCs, both T-cells of the same and of different specificity can compete with each other (C). In the latter case, competition is believed to be for access to APCs. The balance between within and between-specificity competition can be tipped towards within-specificity competition by epitope down-modulation.

In Chapter 2, I assume that T-cells of different epitope specificity compete for access to APCs, and thus have fully overlapping niches, as in Fig. 1.3 A and Fig. 1.4 C. I extend simple mathematical models of T-cell competition to study the effect of epitope down-modulation on competition between T-cells of the same and of different epitope specificity.

To be able to understand the mechanisms that drive competition between T-cell populations, the niche that these populations occupy must be defined. The central theme of Chapter 3 is just that, i.e. defining the resource limitations of T-cells. The methodology used in Chapter 3, is an individual-based computer simulation model, which allows us to incorporate more details of the immune system than the mathematical models used in Chapter 2.
In Chapter 4, I test experimentally whether our model of resource limitation from Chapter 3 is supported by a mouse model.

Finally, in Chapter 5, I update the current model of T-cell competition with the results obtained from this thesis.
Chapter 2

Epitope down-modulation as a mechanism for the coexistence of competing T-cells

Almut Scherer and Sebastian Bonhoeffer


2.1 Abstract

Efficient immune responses against pathogens are frequently characterised by the simultaneous targeting of multiple epitopes. However, it remains unclear how the targeting of multiple epitopes is maintained in the face of competition for antigenic stimulation. Here we investigate this question by using mathematical models of the population dynamics of a viral pathogen, antigen presentation sites and T-cells. We first show that direct competition for access to antigen presenting sites and indirect competition through killing of the pathogen select for dominance of the T-cell response with the highest affinity for its epitope. We then incorporate in our model that epitopes can become down-modulated following interaction with epitope-specific T-cells. We demonstrate that epitope down-modulation leads to differentiation of epitope presentation on antigen presenting sites. This differentiation promotes the coexistence of multiple epitope-specific responses. Hence, we propose that the functional relevance of epitope down-modulation may be to enable the persistence of a broad immune response despite competition for antigenic stimulation.

2.2 Introduction

From an engineer's perspective an immune response should be designed to recognise and control a pathogen on the basis of several epitopes (antigenic determinants). Indeed, a striking characteristic of T-cell responses is the multitude of epitopes that are targeted si-
multaneously. Broad responses are believed to be particularly important in the control of rapidly evolving pathogens, that can escape immune recognition by acquiring mutations in epitopes or epitope flanking regions (Phillips et al., 1991; Goulder et al., 1997; Draenert et al., 2004). A broad response might be more robust to immune escape because the wider the array of epitopes recognised, the smaller the benefit that the pathogen acquires from immune escape of any single epitope-specific response.

From an ecologist’s point of view, however, it is puzzling how a diverse immune response can be maintained. In essence, clonal immune cell populations require their cognate antigen much like a food resource, since recognition of that antigen is required as a stimulus for proliferation. Moreover, in analogy to a food resource, the immune cells deplete their resource by killing the pathogen. As a consequence we expect immune cells to be in competition for antigens. This competition can either be direct, through physical impairment of access to antigens, or indirect, via reduction of the pathogen load by the immune responses. Since the pathogen population essentially serves as a single resource for all immune cells that recognise epitopes derived from this pathogen, the question arises how a diverse immune response can be maintained in the face of competition for antigens. The classical view in ecology in this context is the competitive exclusion principle, which states that the number of coexisting species is limited by the number of shared limiting resources (Gause, 1934).

Several lines of evidence suggest that T-cells do indeed compete over antigen resources. First, some T-cell clones have the ability to expand in the absence of antigenic stimuli when transferred into lymphopenic mice, or transgenic mice of different clonotype, but not upon transfer into mice of the same clonotype (Moses et al., 2003; Troy and Shen, 2003; Smith et al., 2000). Such ‘homeostatic’ proliferation is thought to depend on survival signals that T-cells obtain through interaction with self epitopes presented on antigen presenting cells (APCs). Competition for survival signals may regulate the expansion of the different clonal populations of T-cells (Moses et al., 2003; Troy and Shen, 2003), although other mechanisms of homeostatic proliferation based on ‘fratricide’ have been proposed (Callard et al., 2003). Second, when large numbers of transgenic epitope specific T-cells are transferred into infection naive mice, proliferation of host T-cells upon infection is impaired. Proliferation is impaired in particular in those T-cell clones that have the same epitope specificity as the transferred T-cells, and to a lesser extent also in T-cell clones specific for other epitopes of the pathogen (Kedl et al., 2000, 2002). This implies that host T-cells and transferred T-cells compete for the antigen resource. Third, the affinity of an epitope-specific response tends to increase with the duration of antigen exposure, indicating a competitive advantage of high affinity T-cell clones (Busch and Pamer, 1999; McHeyzer-Williams et al., 1999; Mikszta et al., 1999; Savage et al., 1999; Sylfka and Whitton, 2001; Lawson et al., 2001a,b). Fourth, competition is thought to influence the size hierarchy of multiple epitope-specific responses against pathogens (Yewdell and Bennink, 1999; Grufman et al., 1999; Chen et al., 2000), since differences in the size of epitope-specific responses can not be fully attributed to differences in epitope processing and presentation.

The experimental evidence for competition and the expected theoretical consequence (competitive exclusion) are in marked contrast to the observation that the immune response in chronic infections, such as Human Immunodeficiency Virus (HIV) or Lympohocytic Choriomeningitis Virus (LCMV) are frequently characterized by a broad, persistent response to multiple epitopes (Yu et al., 2002; Oxenius et al., 2002; Wherry
et al., 2003)). Here, we use mathematical models to address the paradox of coexistence of diverse T-cell responses in face of competition for a single pathogen resource. We highlight mechanisms leading to competition and investigate possible biological scenarios that may lead to coexistence. In particular we address down-modulation of epitopes on APCs as a potential mechanism to allow stable coexistence of multiple epitope-specific T-cell responses.

2.3 The models

2.3.1 Modelling competition

In experimental studies, competition between multiple epitope-specific responses is only observed when epitopes are presented on the same APC, provided T-cell densities are high (Kedl et al. (2000) and Fig. 2.1, compare panels A and B). When epitopes are expressed on separate APCs, only T-cells of the same epitope specificity hinder each others expansion (Kedl et al. (2000) and Fig. 2.1 C). Thus, T-cells seem to compete for access to APCs that present their cognate epitopes, whenever the ratio of T-cells to APCs is high. This mechanism is often referred to as competition by 'crowding' of T-cells on APCs. Bousso and Robey (2003) observed that up to 20 T-cells interact with one APC simultaneously, and that the number of T-cells interacting with an APC increased with decreasing availability of APCs.

When T-cells compete for access to the APC, the T-cell clone with the highest avidity for its epitope is expected to have a competitive advantage, since the activation and proliferation of T-cells depends on their avidity for their cognate epitope (Andersen et al., 2001; Hesse et al., 2001). Avidity is a composite measure that depends on the intrinsic affinity between TCR and MHC:epitope complex, the density of TCR and MHC molecules, and on a host of co-receptors and co-stimulatory molecules involved in T-cell activation (Anderton and Wraith, 2002). We investigate the effect of direct competition with the following population dynamical model. Assume a population of virus infected cells, V, that grows with a maximal rate r in a density dependent manner and has a carrying capacity of CC. Virus infected cells are cleared with clearance rate c by effector T-cells E1 and E2, that have respective avidities r1 and r2 for antigen presenting sites on

![Figure 2.1: At low ratios of effector cells to APCs (A), there is little direct competition between T-cells. At higher ratios the direct competition through physical impairment increases (B). Intra-clonal competition describes competition between cells of the same clone (light grey box) and between-specificity competition describes competition between cells from clones specific for different epitopes (double lined box). Resource differentiation, e.g. by expressing different epitopes on separate APCs, increases within-specificity competition, thus facilitating the coexistence of two T-cell populations (C).](image-url)
APCs that express both epitopes \( (A_1^1) \). Cells of type \( E_1 \) have a higher functional avidity for their cognate epitope than cells of type \( E_2 \), \( i.e. r_1 > r_2 \). We assume that unloaded APCs are in excess. Hence, the influx of 'new' epitope expressing APC sites equals the number of infected cells times a proportionality constant \( \sigma \). Antigen presenting sites and T-cells have death rates \( \delta_A \) and \( \delta_E \) respectively. The decay rate of APC sites in our model represents loss due to death of the APCs and due to a background turn-over of MHC:epitope complexes on APCs. Thus, we have the following set of equations:

\[
\begin{align*}
\frac{dV}{dt} &= rV(1 - V/CC) - cVE_1 - cVE_2 \\
\frac{dA_1}{dt} &= \sigma V - \delta_A A_1 \\
\frac{dE_1}{dt} &= r_1 A_1 F_1(E_1, E_2) - \delta_E E_1 \\
\frac{dE_2}{dt} &= r_2 A_1 F_2(E_1, E_2) - \delta_E E_2
\end{align*}
\]

To reduce the number of parameters of the model, we assume that both effector T-cells have the same clearing rate, \( c \), and the same half saturation constant, \( K \). These assumptions do not affect our results qualitatively. Direct competition between T-cells for accessing APCs can be modelled by using a saturated proliferation function, \( i.e. F_1(E_1, E_2) = E_1/(1/K + E_1 + E_2) \) and \( F_2(E_1, E_2) = E_2/(1/K + E_1 + E_2) \), where \( K \) describes the binding and dissociation kinetics of T-cells \( E_1 \) and \( E_2 \) with APC sites presenting their cognate epitope (see Appendix 2.6.1). From \( \frac{dE_1}{dt} = 0 \) it follows that \( r_1 A_1/(1/K + E_1 + E_2) = \delta_E \). Since \( r_1 > r_2 \), we have \( r_2 A_1/(1/K + E_1 + E_2) < \delta_E \) which implies that \( E_2 \) decreases because its death rate exceeds its growth rate. Hence, direct competition leads to competitive exclusion.

The activation of T-cells depends on their functional avidity for their cognate epitope, and T-cell clones with a high avidity for their cognate epitope become activated at lower pathogen levels (Andersen et al., 2001; Hesse et al., 2001). Indirect competition occurs because T-cells reduce the pathogen density to levels that are sufficient to sustain only the population of high avidity effector T-cells. When T-cells do not interact directly we can describe T-cell activation with a linear function, \( i.e. F_1 = E_1 \) and \( F_2 = E_2 \). By setting \( \frac{dE_1}{dt} = 0 \), we find the number of antigen presenting sites at which the net growth rate of \( E_1 \) is zero, \( A_1^1 = \delta_A / r_1 \). Since \( r_1 > r_2 \), the growth rate of \( E_2 \) is negative at this density of antigen presenting sites, and cells of type \( E_1 \) and \( E_2 \) can not coexist. Hence, also indirect competition leads to competitive exclusion.

Direct and indirect competition act equivalently between T-cell clones specific for the same and for different epitopes, as long as epitopes are distributed randomly on the surface of APCs. Competition between T-cell clones of the same epitope specificity leads to avidity maturation of the epitope-specific population (De Boer and Perelson, 1994), while competition between T-cell clones of disparate specificity leads to competitive exclusion.

### 2.3.2 Modelling epitope down-modulation

One way to prevent competitive exclusion and to allow for coexistence is resource differentiation. Indeed, when epitopes are presented on different APCs, direct between-specificity competition is eliminated because the shared resource of mixed epitopes
is split into two separate resources (Kedl et al. (2000); Palmowski et al. (2002) and Fig. 2.1 C). Ecological models on resource differentiation and species diversity predict that species can coexist even with considerable resource overlap (MacArthur and Levins, 1964). Resource differentiation enables coexistence of competing populations by increasing within-specificity competition relative to between-specificity competition. Hence, mechanisms that provide some degree of antigenic resource differentiation might be able to explain the large diversity of epitope-specific responses in chronic infection.

One potential mechanism that could lead to antigenic resource differentiation is epitope down-modulation. The first data suggesting the existence of epitope down-modulation were purely phenomenological. Using in vitro studies with transgenic T-cells, Manca (1992) observed that APCs that had just served to stimulate T-cells specific for a certain epitope were no longer capable of eliciting a proliferative response from naïve T-cells of the same specificity. In contrast, these APCs were still able to provide proliferation stimuli to T-cells of different epitope specificity. Based on these findings, Manca (1992) proposed a model in which specific T-cells induced internalisation of their cognate MHC:epitope complexes. More recently, it was observed that, upon contact between APCs and T-cells, some co-receptors and MHC:epitope complexes were transferred from APCs to T-cells (Huang et al., 1999; Hwang et al., 2000; Hudrisier et al., 2001; Hudrisier and Bongrand, 2002). Kedl et al. (2002) performed an elegant study that yields insight into the dynamics of epitope down-modulation. They tracked the density of a particular MHC:epitope complex during co-culture with T-cells specific for that epitope, and showed that the rate of loss of the MHC:epitope complex critically depended on the presence of T-cells specific for that epitope. In summary, the data suggest that the interaction between specific T-cells and their cognate epitope can lead to a decrease in the density of this epitope on the APC. It is not fully clear whether this is due to internalisation of MHC:epitope complexes by the APCs, or to the ‘ripping’ of MHC:epitope complexes by the interacting epitope-specific T-cells, or both. However, since both processes lead to a reduced epitope presentation we refer to this process in the following as epitope down-modulation.

In what follows, we study epitope down-modulation as a potential mechanism for differentiation of the antigen resource, and investigate under what circumstances this mechanism can explain coexistence of multiple epitope-specific T-cell responses in chronic diseases. To this end we derive equations that include direct competition for access to APCs, indirect competition through clearance of infected cells and epitope down-modulation as a consequence of interaction with epitope-specific effector T-cells. For simplicity, we model a system in which two epitopes of a pathogen are presented on APCs, as illustrated in Fig. 2.2. We subdivide the antigen presenting sites into four classes: those expressing both epitopes (A\textsuperscript{11}), those expressing only one of the two epitopes (A\textsuperscript{10}, A\textsuperscript{01}), and those expressing none of the two epitopes (A\textsuperscript{00}). We describe interactions between T-cells and APC sites rather than APCs, because multiple T-cells can simultaneously interact with a single APC (Bousso and Robey, 2003). Here we interpret APC sites as independent entities that do not interchange epitopes amongst each other. This is a conservative assumption, since allowing diffusion of epitopes between sites would lead to a cell wide down-modulation of epitopes on the APC and would therefore have a stronger effect. We define the down-modulation rate $\gamma$ as the fraction of interactions between T-cells and APC sites that lead to epitope down-modulation per unit of time. For example, interactions between effector T-cells specific for epitope 1 ($E_1$) and
Figure 2.2: The model describes the dynamics of virus infected cells, viral epitopes presented on APC sites, the different subsets of APC sites and effector T-cells specific for the presented epitopes. 

**A:** Epitopes of virus infected cells are processed and presented on the surface of antigen presenting cells. Effector T-cells proliferate dependent on their resource, that is, APC sites that express both epitopes or ones that express only their cognate epitope. T-cells have an average life-span of $1/\delta_T$ days and clear virus infected cells with rate $c$. 

**B:** The APC sites that only present one of the viral epitopes are generated by T-cell interaction dependent epitope down-modulation. Down-modulated epitopes can be re-expressed with a rate $u$, giving rise to sites that present both epitopes. Finally, APC sites have a natural turn-over rate, $\delta_A$. 

Equations (2.5) - (2.11) describe these processes in terms of ordinary differential equations and are derived in detail in Appendix 2.6.1.
APC sites $A^{11}$ can lead to down-modulated sites $A^{01}$, and subsequent encounters with T-cells specific for epitope 2 ($E_2$) can lead to fully down-modulated APC sites $A^{00}$. When sites are not interacting with a T-cell, epitopes can be re-expressed with a rate $u$. These biological assumptions can be translated into the following set of ordinary differential equations (derived in detail in Appendix 2.6.1).

$$\frac{dV}{dt} = rV(1 - \nu/CC) - cV(E_1 + E_2)$$  \hspace{2cm} (2.5)

$$\frac{dA^{11}}{dt} = \sigma V - \frac{\gamma A^{11}E_1 + E_2}{1/K + E_1 + E_2} + \frac{uA^{10}}{1 + KE_1} + \frac{uA^{01}}{1 + KE_2} + uA^{00} - \delta A^{11}$$  \hspace{2cm} (2.6)

$$\frac{dA^{10}}{dt} = \frac{\gamma A^{11}E_2}{1/K + E_1 + E_2} - \frac{\gamma A^{10}E_1}{1/K + E_1} - \frac{uA^{10}}{1 + KE_1} - \delta A^{10}$$  \hspace{2cm} (2.7)

$$\frac{dA^{01}}{dt} = \frac{\gamma A^{11}E_1}{1/K + E_1 + E_2} - \frac{\gamma A^{01}E_2}{1/K + E_2} - \frac{uA^{01}}{1 + KE_2} - \delta A^{01}$$  \hspace{2cm} (2.8)

$$\frac{dA^{00}}{dt} = \frac{\gamma A^{10}E_1}{1/K + E_1} + \frac{\gamma A^{01}E_2}{1/K + E_2} - uA^{00} - \delta A^{00}$$  \hspace{2cm} (2.9)

$$\frac{dE_1}{dt} = \frac{r_1 E_1 A^{11}}{1/K + E_1 + E_2} + \frac{r_1 E_1 A^{10}}{1/K + E_1} - \delta E_1$$  \hspace{2cm} (2.10)

$$\frac{dE_2}{dt} = \frac{r_2 E_2 A^{11}}{1/K + E_1 + E_2} + \frac{r_2 E_2 A^{01}}{1/K + E_2} - \delta E_2$$  \hspace{2cm} (2.11)

Little is known on quantitative aspects regarding the rate and duration of epitope down-modulation. Using the data from Kedl et al. (2002), we estimate that the net epitope down-modulation rate is around 0.2 day$^{-1}$ \hspace{2cm} (Appendix 2.6.2). Here, we vary $\gamma$ over a wide range of values, while keeping the up-regulation rate $u$ constant. (Note, that if down-modulation is due to epitope ripping rather than epitope internalisation, this would be represented by $u = 0$. However, setting $u = 0$ simply increases the net down-modulation rate, but does not affect our results qualitatively.)

**2.4 Results**

**2.4.1 Effect of epitope down-modulation on resource differentiation and coexistence**

To investigate whether resource-differentiation caused by epitope down-modulation is a potent mechanism for the coexistence of multiple epitope-specific T-cell responses, we plot the equilibrium densities of APC sites expressing either both epitopes or one epitope and the relative densities of two epitope-specific T-cell responses at equilibrium (Fig. 2.3). With increasing down-modulation rate $\gamma$, the shared resource $A^{11}$ gradually becomes differentiated into sites expressing solely epitope 1 or 2. Interestingly, the ratio $E_2/E_1$ increases rapidly with increasing $\gamma$. For large down-modulation rates the relative T-cell density of the competing T-cell populations, $E_2/E_1$, approaches the ratio of their respective T-cell:epitope avidities, $r_2/r_1$. Note that we assume that the rate of down-modulation does not depend on the avidity between the specific effector T-cell and the MHC:epitope complex (i.e., $\gamma_1 = \gamma_2 = \gamma$). For the question of whether epitope
down-modulation allows a low avidity T-cell to coexist with a high avidity T-cell, this is a conservative assumption because avidity dependent down-modulation preferentially benefits the lower avidity T-cell. Assuming that the difference in down-modulation between the two T-cell population equals their difference in avidity (i.e. $\gamma_2/\gamma_1 = r_2/r_1$), coexistence approaches one rather than $r_2/r_1$ for $\gamma \to \infty$ (data not shown).

![Figure 2.3: With increasing epitope down-modulation rate $\gamma$, more APC sites become down-modulated and express either epitope 1 or 2 (lines labelled 'Fraction A10' and 'Fraction A01'), rather than both (line labelled 'Fraction A1'). 'Fraction A* is defined as $A^*/(A11 + A10 + A01)$. The resource differentiation caused by the epitope down-modulation allows for coexistence of multiple epitope-specific T-cell responses (thick line labelled $E_2/E_1$). Parameter-settings: $\sigma = 0.05 \text{ day}^{-1}$, $r = 10^{6} \text{ day}^{-1}$, $CC = 10^{6} \text{ cells}^{-1}$, $c = 10^{-4} \text{ day}^{-1} \text{ cell}^{-1}$, $r_1 = 0.01 \text{ day}^{-1}$, $r_2 = 0.007 \text{ day}^{-1}$, $K = 0.02 \text{ cell}^{-1}$, $\delta_E = \delta_A = 0.1 \text{ day}^{-1}$ and $u = 0.5 \text{ day}^{-1}$.

2.4.2 Threshold of differentiation for coexistence

When effector cells specific for epitope 1 expand, they down-modulate epitope 1 on $A^{11}$ sites and transform these sites into $A^{01}$ sites. These $A^{01}$ sites can then only activate $E_2$ cells. We address next whether any arbitrarily low number of $A^{01}$ sites will allow $E_2$ cells to coexist with $E_1$ cells or whether there is a threshold level of $A^{01}$ sites required for coexistence of $E_1$ and $E_2$ cells.

To answer this question we record at what epitope down-modulation rate $E_2$ cells are able to invade the equilibrium in which only $E_1$ cells are present. This threshold level of epitope down-modulation needed for coexistence is referred to as $\theta_\gamma$, and the number of $A^{01}$ sites present at $\theta_\gamma$ as $\theta_{A01}$. Fig. 2.4 shows that there is a threshold $\theta_\gamma > 0$ for coexistence of $E_1$ and $E_2$, and that this threshold critically depends on the avidity difference between $E_1$ and $E_2$. The larger the avidity difference between $E_1$ and $E_2$, the more differentiated sites $A^{01}$ are needed to allow for coexistence.

Indirect competition is included in our model via viral clearance by effector T-cells. We investigate the relative importance of direct versus indirect competition by comparing the full model, which includes both kinds of competition, to a model without indirect competition (where $c = 0$). Omitting indirect competition decreases the threshold down-modulation rate, $\theta_\gamma$, while leaving the threshold level of $A^{01}$ sites, $\theta_{A01}$, unaffected. This is intuitively reasonable, because, for a given difference in avidity between the competing T-cell populations, the determining factor for coexistence is the number of $A^{01}$ sites. Setting viral clearance to zero, the number of infected cells is higher, and hence the
critical number of differentiated antigen presenting sites needed for invasion of the lower avidity T-cell population is achieved at lower down-modulation rates than in the model with viral clearance.

Increasing the number of infected cells by blocking indirect competition facilitates the invasion of the lower avidity T-cell. It should be noted though that the impact of indirect competition on coexistence is only evident for values of $\gamma$ near $\theta_\gamma$. For larger values of $\gamma$ the results of the models with and without indirect competition are very similar, since coexistence is then primarily shaped by the increased direct within-specificity competition arising from the differentiation of antigen presenting sites.

### 2.4.3 Costs and benefits of epitope down-modulation

We have argued above that a broad immune response is superior to a narrow one, because the recognition of multiple epitopes confers better control against pathogens that attempt to escape the immune response. However, since epitope down-modulation lowers the amount of antigen displayed on APCs, maintenance of T-cell response diversity by epitope down-modulation might be costly for the infected host. Fig. 2.5 A and B show the detrimental effect of epitope down-modulation on the number of infected cells and on the total immune response size respectively. For small avidity differences between the immunodominant and sub-dominant T-cell populations, the impact of epitope down-modulation is limited. For intermediate and high avidity differences there is an initial phase during which $\gamma < \theta_\gamma$, and the number of infected cells increase linearly and the total immune response size decreases linearly with increasing epitope down-modulation rate. For $\gamma > \theta_\gamma$, the cost of epitope down-modulation is larger, because the low avidity T-cells that coexist with the high avidity T-cells proliferate less and hence contribute less to viral clearance. In summary, epitope down-modulation improves the quality of the T-cell immune response by allowing it to be diverse, but reduces its size by lowering the amount of displayed pathogen antigens. The size-reducing effect of epitope down-modulation need not be detrimental for the efficiency of the immune response, however,
since size and efficiency of immune response do not always correlate (Oxenius et al., 2002). Other factors than size might be more important for the efficiency of the immune response. For example, in HIV-infection, there is data suggesting that T-cell responses directed against epitopes that are expressed early in the viral replication cycle are more efficient in clearing virus infected cells than ones directed against late epitopes (Osterhaus et al., 1999). Given the current lack of data, it is difficult to compare the benefit of a diverse immune response with the cost of a smaller total immune response.

Figure 2.5: Epitope down-modulation reduces the amount of presented antigen. Therefore, the total immune response size decreases (A), and the viral load increases (B) for increasing down-modulation rates $\gamma$. Shown are runs for three differences in avidity, $r_1 = 0.01$ and $r_2 = 0.007, 0.001$ and 0.0001. Other parameter settings are as in Fig. 2.3.

2.5 Discussion

Identifying the mechanisms responsible for the maintenance of a broad immune response against several epitopes in the face of competition for antigenic stimulation represents a challenge to immunologist and ecologists. We have addressed this question specifically in the context of a T-cell immune response to a viral infection. There is ample evidence for the role of competition between T-cells. This competition can either occur directly through physical impairment of access to antigen on APCs or indirectly through the reduction of antigen levels by killing of infected cells. Using mathematical models of T-cell competition we showed that in either case competition leads to competitive exclusion. However, since long-term coexistence rather than competitive exclusion seems to be the default situation observed in T-cell immunology, this calls for an explanation.

One possible mechanism leading to the maintenance of broad immune response is epitope down-modulation. APCs that have recently interacted with a T-cell specific for a given epitope present a reduced density of this epitope. Thus these APCs can no longer stimulate T-cells with the same specificity, but can still stimulate T cells with other specificities. Based on numerical simulations of a model that incorporates epitope down-modulation, we showed that this mechanism leads to resource differentiation.
Resource differentiation aids coexistence by increasing the within-specificity competition relative to the between-specificity competition. While down-modulation may offer the benefit of maintaining a broad immune immune response, it also involves a potential cost in terms of an increased virus load. Our simulations suggest that, as long as the differences in avidity of the coexisting T-cell populations are not too large, the increase of virally infected cells due to epitope down-modulation is limited, even for very high epitope down-modulation rates. Note, that down-modulation may explain the coexistence of clones that recognise different epitopes, but not of clones that recognise the same epitope.

While it is appealing to hypothesise that the function of epitope down-modulation is to maintain a broad immune response, we need to evaluate alternative hypotheses to explain coexistence of multiple epitope-specific responses. First, for acute infections one might argue that the competition over the limited duration of an infection is not strong enough to select against diversity in the immune response. A similar argument can be made for chronic infections: if T cells are maintained in the periphery in a more or less antigen independent manner, competition for antigen would be irrelevant. However, there are several observations that suggest that the level of T-cells in chronic infections depends on the pathogen load. For example, during interruptions of drug therapy of HIV-1 infection, the virus load rises, and HIV epitope-specific T-cells become significantly more abundant (Oxenius et al., 2002). Also, in EBV infection, T-cell responses tend to contract strongly when going from high levels of virus load during primary infection to low levels of virus load during latent infection (Callan et al., 1998). A second alternative explanation for the maintenance of a broad immune response would be that the effector T-cell diversity is maintained though a continuous replenishment of new naive T-cells, that, following emigration from the thymus, become activated, proliferate and assume effector function. However, the estimates of thymic output are far from sufficient to explain the maintenance of effector T-cell populations of the order of magnitude as observed in chronic infections (see Appendix 2.6.3). A third solution was proposed by Davenport et al. (2002). Since the number of cell divisions a single cell can make is limited, clonal senescence and clonal succession would offer a dynamic solution for the coexistence of multiple epitope-specific immune responses. Although the theory is appealing, it is difficult to reconcile with the long term persistence of some epitope-specific T-cell populations observed in chronic infections (Yu et al., 2002).

Several hypotheses for the relevance of epitope down-modulation have been described in a recent review by Hudrisier and Bongrand (2002). These include (i) the termination of the APC:T-cell contact in order to free the APC after the T-cell has obtained sufficient stimuli, (ii) the regulation of the immune response size to prevent immune-pathology from unrestricted immune responses and (iii) affinity maturation of the epitope-specific responses. We suggest that overcoming T-cell competition and enabling the persistence of a broad immune response may be another function of epitope down-modulation. The rates of down-modulation observed in the experiment of Kedl et al. (2002) are sufficient to explain coexistence of multiple epitope-specific T-cell responses by epitope down-modulation. A testable prediction from our model is that between-specificity competition should increase if down-modulation is blocked. Although experiments have been performed to test the effect of blocking down-modulation on within-specificity competition (Hayball et al., 2004), to our knowledge no such studies have yet been performed for between-specificity competition.
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2.6 Appendix

2.6.1 Appendix: T-cell Activation functions with multiple resources

Following De Boer and Perelson (1995) we derive functions describing T-cell activation after the interaction between an epitope-specific T-cell and an APC site displaying its cognate epitope, and incorporate epitope down-modulation into our derivation. We assume that a fraction $\gamma$ of all interactions between T-cells and antigen presenting sites that lead to activation of the T-cell simultaneously lead to down-modulation of the antigen presenting site.

We first describe T-cell activation and proliferation and down-modulation of antigen presenting sites in terms of reaction kinetics:

$$E_1 + A_f^{11} \xrightarrow{k_b} C_1^{11} \xrightarrow{k_d} (n_1 + 1)E_1 + \gamma A_f^{01} + (1 - \gamma)A_f^{11} \quad (2.12)$$

$$E_2 + A_f^{11} \xrightarrow{k_p} C_2^{11} \xrightarrow{k_d} (n_2 + 1)E_2 + \gamma A_f^{10} + (1 - \gamma)A_f^{11} \quad (2.13)$$

$$E_1 + A_f^{10} \xrightarrow{k_b} C_1^{10} \xrightarrow{k_d} (n_1 + 1)E_1 + \gamma A_f^{00} + (1 - \gamma)A_f^{10} \quad (2.14)$$

$$E_2 + A_f^{01} \xrightarrow{k_p} C_2^{01} \xrightarrow{k_d} (n_2 + 1)E_2 + \gamma A_f^{00} + (1 - \gamma)A_f^{01} \quad (2.15)$$

$$A_f^{00} \xrightarrow{u} A_f^{11}, A_f^{01} \xrightarrow{u} A_f^{11}, \text{ and } A_f^{01} \xrightarrow{u} A_f^{11} \quad (2.16)$$

Here, $k_b$ is the rate with which a T-cell ($E_i$) and a free APC ($A_f^{i}$) site form a conjugate ($C_i^{i}$), $k_d$ is the rate with which such a conjugate dissociates again (without leading to proliferation) and $k_d$ is the rate with which the T-cell in the conjugate becomes activated and proliferates into $n_i$ daughter cells. Free down-modulated sites can become up-modulated again with a rate $u$.

From this reaction scheme we can derive the differential equations for the variables in this system.
By making a quasi-steady-state (QSS) assumption for the conjugates (by setting $\frac{dC_i}{dt} = 0$ and solving for $C_i$), we can write for the conjugates: $C_{11} = K_1 E_1 A_{f1}$, $C_{21} = K_1 E_2 A_{f1}$, $C_{10} = K_1 E_1 A_{f0}$ and $C_{20} = K_2 E_2 A_{f0}$, where $K_i = k_i^P/(k_i^P + k_i^P)$. Further, the QSS assumption for $C_i$ implies:

\[
\begin{align*}
\frac{dE_1}{dt} &= -k_1^P E_1 A_{f1} + (k_1^P + n_1 k_1^P) C_{11} - k_1^P E_1 A_{f0} + (k_1^P + n_1 k_1^P) C_{10} \\
\frac{dE_2}{dt} &= -k_2^P E_2 A_{f1} + (k_2^P + n_2 k_2^P) C_{21} - k_2^P E_2 A_{f0} + (k_2^P + n_2 k_2^P) C_{20} \\
\frac{dC_{11}}{dt} &= k_1^P E_1 A_{f1} - (k_1^P + k_1^P) C_{11} \\
\frac{dC_{10}}{dt} &= k_2^P E_2 A_{f1} - (k_2^P + k_2^P) C_{11} \\
\frac{dC_{20}}{dt} &= k_2^P E_2 A_{f0} - (k_2^P + k_2^P) C_{20} \\
\frac{dA_{f1}}{dt} &= -k_1^P E_1 A_{f1} - k_2^P E_2 A_{f1} + k_1^P C_{11} + k_2^P C_{21} + (1 - \gamma) k_1^P C_{11} + (1 - \gamma) k_2^P C_{21} + u(A_{f0} + A_{f1} + A_{f1}) \\
\frac{dA_{f0}}{dt} &= -k_1^P E_1 A_{f0} + k_1^P C_{10} + \gamma k_2^P C_{21} + (1 - \gamma) k_2^P C_{21} - uA_{f0} \\
\frac{dA_{f1}}{dt} &= -k_2^P E_2 A_{f1} + k_2^P C_{21} + \gamma k_1^P C_{11} + (1 - \gamma) k_1^P C_{11} - uA_{f1} \\
\frac{dA_{f0}}{dt} &= \gamma k_1^P C_{10} + \gamma k_2^P C_{20} - uA_{f0}
\end{align*}
\]

Next, we write conservation equations for the different subsets of epitope presenting sites, and substitute the conjugates, $C_i$'s, by their QSS expressions.
\[ A^{11} = A_{f}^{11} + C_{1}^{11} + C_{2}^{11} \rightarrow A_{f}^{11} = \frac{A^{11}}{1 + K_{1}E_{1} + K_{2}E_{2}} \] (2.33)

\[ A^{10} = A_{f}^{10} + C_{1}^{10} \rightarrow A_{f}^{10} = \frac{A^{10}}{1 + K_{1}E_{1}} \] (2.34)

\[ A^{01} = A_{f}^{01} + C_{2}^{01} \rightarrow A_{f}^{01} = \frac{A^{01}}{1 + K_{2}E_{2}} \] (2.35)

Substituting the expressions for \( A_{f}^{i} \)'s into Equations 2.17 to 2.26, we obtain equations for the dynamics of effector T-cell populations and APC sites. Since \( A^{11} = A_{f}^{11} + C_{1}^{11} + C_{2}^{11} \), and since \( dC_{i}/dt = 0 \) (QSS), \( dA^{11}/dt = dA_{f}^{11}/dt \).

\[ \frac{dE_{1}}{dt} = \frac{n_{1}k^{p}K_{1}E_{1}A^{11}}{1 + K_{1}E_{1} + K_{2}E_{2}} + \frac{n_{1}k^{p}K_{1}E_{1}A^{10}}{1 + K_{1}E_{1}} \] (2.36)

\[ \frac{dE_{2}}{dt} = \frac{n_{2}k^{p}K_{2}E_{2}A^{11}}{1 + K_{1}E_{1} + K_{2}E_{2}} + \frac{n_{2}k^{p}K_{2}E_{2}A^{01}}{1 + K_{2}E_{2}} \] (2.37)

\[ \frac{dA^{11}}{dt} = -\frac{\gamma k^{p}K_{1}E_{1}A^{11}}{1 + K_{1}E_{1} + K_{2}E_{2}} - \frac{\gamma k^{p}K_{1}E_{1}A^{10}}{1 + K_{1}E_{1}} + \frac{uA^{10}}{1 + K_{1}E_{1} + K_{2}E_{2}} + \frac{uA^{01}}{1 + K_{2}E_{2}} \] (2.38)

\[ \frac{dA^{10}}{dt} = \frac{\gamma k^{p}K_{2}E_{2}A^{11}}{1 + K_{1}E_{1} + K_{2}E_{2}} - \frac{\gamma k^{p}K_{1}E_{1}A^{10}}{1 + K_{1}E_{1}} - \frac{uA^{10}}{1 + K_{1}E_{1}} \] (2.39)

\[ \frac{dA^{01}}{dt} = \frac{\gamma k^{p}K_{1}E_{1}A^{11}}{1 + K_{1}E_{1} + K_{2}E_{2}} - \frac{\gamma k^{p}K_{2}E_{2}A^{01}}{1 + K_{2}E_{2}} - \frac{uA^{01}}{1 + K_{2}E_{2}} \] (2.40)

\[ \frac{dA^{00}}{dt} = \frac{\gamma k^{p}K_{1}E_{1}A^{10}}{1 + K_{1}E_{1} + K_{2}E_{2}} + \frac{\gamma k^{p}K_{2}E_{2}A^{01}}{1 + K_{2}E_{2}} - uA^{00} \] (2.41)

These equations form the backbone of our model. Additionally, our model encompasses:

- Terms describing death of T-cells and APC sites (\( \delta_{E}E_{i} \) and \( \delta_{A}A^{j} \) respectively)

- A term describing the generation of new APC sites derived from infected cells (\( \sigma V \))

- An ordinary differential equation describing the dynamics of infected cells (\( dV/dt \)).

Further, we make the following simplifying assumptions:

- We set \( \gamma = \gamma k^{p} = \gamma k^{p} \), which implies \( k^{p} = k^{p} \)

- We replace \( n_{1}k^{p} \) and \( n_{2}k^{p} \) in the effector T-cell equations by \( r_{1} \) and \( r_{2} \) respectively. Since \( k^{p} = k^{p} \) and \( r_{1} > r_{2} \), \( n_{1} \) must be larger than \( n_{2} \). We also set \( K = K_{1} = K_{2} \). Together, this implies that the high and low avidity T-cell populations only differ in their maximum proliferation per APC site.
2.6.2 Appendix: Quantitative estimate of the epitope down-modulation rate

Kedl et al. (2002) followed the dynamics of loss of OVA-peptide on APCs. The APCs were pulsed with OVA-peptide and then transferred into mice, either with or without transfer of T-cells specific for the OVA-peptide (OT1 T-cells). The density of OVA-peptide was measured at multiple time-points with an OVA-specific antibody.

From the control experiment, in which only OVA-coated APCs were transferred, we can estimate the background decay rate of OVA-staining, \( k \). When OT1 T-cells (denoted by \( E_1 \)) are transferred together with the APCs (denoted by \( A^{10} \)) we expect an additional decay of OVA-staining due to the effect of epitope down-modulation. By comparing the rates of exponential decay of the experiments with and without transfer of OT1 T-cells, we can get a rough estimate of the epitope down-modulation rate, \( \gamma \). Assuming that the rate of epitope down-modulation saturates at high densities of T-cells we write for the kinetics of \( A^{10} \)

\[
\frac{dA^{10}}{dt} = -\frac{\gamma A^{10} E_1}{h+E_1} - kA^{10}
\]  

(2.42)

where \( h \) is the number of effector T-cells at which the per APC site proliferation is half-maximal. If \( h < E_1, E_1/(h+E_1) \approx 1 \). Hence, we approximate the above equation as

\[
\frac{dA^{10}}{dt} = -\gamma A^{10} - kA^{10}
\]  

(2.43)

In our epitope down-modulation model, the assumption \( h < E_1 \) holds, since \( h = 1/K = 50 \) and \( E_1 \) is approximately \( 2 \times 10^4 \) cells in equilibrium. In the experiment of Kedl et al. (2002), the number of transferred T-cells is very large, so we expect that the assumption of being in the saturated situation is justified.

Hence, we can estimate the epitope down-modulation rate \( \gamma \) from these experiments as

\[
\gamma = s_{\text{exp}} - k
\]  

(2.44)

where \( s_{\text{exp}} \) is the slope of decay on a log-scale observed in the experiment with transfer of OT1 T-cells, and \( k \) the slope of decay in the control experiment. Estimating the slopes from Figure 4b of Kedl et al. (2002) we find: \( \gamma = 0.37 \text{ day}^{-1} - 0.17 \text{ day}^{-1} = 0.2 \text{ day}^{-1} \).

2.6.3 Appendix: Effector diversity reflects naïve diversity of pathogen specific T-cells

In this simple model we describe a T-cell immune response, which is solely maintained by production of naïve cells (denoted by \( N \)). We assume a daily inflow of \( v \) naïve T-cells from the thymus. Naïve cells become activated with rate \( \alpha \), and then proliferate \( p \) rounds as effector cells (denoted by \( E \)). Effector cells die with rate \( \delta_E \).

\[
\frac{dN}{dt} = v - \alpha N
\]  

(2.45)

\[
\frac{dE}{dt} = 2^p\alpha N - \delta_E E
\]  

(2.46)
The equilibrium solution of this system yields:

\[ \tilde{E} = \frac{2^p v}{\delta_E} \]  

(2.47)

The thymic influx is approximately $10^5$ cells per day (Clark et al., 1999). The precursor frequency of any epitope specificity in the naïve repertoire has been estimated to be $10^{-5}$ (Blattman et al., 2002). Hence the daily influx of naïve cells per epitope specificity is set to one cell.

Epitope-specific effector T-cell populations frequently reach densities of 0.1 to 10% of the total CD8+ T-cell repertoire (e.g. (Papagno et al., 2002)). The total CD8+ T-cell repertoire is around $10^{10}$ cells (Arstila et al., 1999). Hence, effector T-cell populations specific for one epitope can attain densities of $10^7$ or higher.

Using expression 2.47, we find that, for an average effector T-cell life-span of 10 days, the number of rounds of proliferation of an activated naïve cell ($p$) needs to be as high as 19 in order to maintain an effector T-cell population of $10^7$ cells. However, the number of rounds of proliferation of an activated T-cell is thought to be around 8-10 (Van Stipdonk et al., 2001; Kaech and Ahmed, 2001). Taking a $p$ of 10, the effector T-cell life span $\delta_E$ would have to be of order of 10000 days to explain effector T-cell population sizes of $10^7$ cells.

These calculations suggest that feed-back loops, where effector cells home to lymphoid tissue and become re-activated by antigen are necessary to maintain of effector T-cell populations in chronic infection. Such feed-back loops create a situation in which large numbers of pathogen specific T-cells compete for a limiting resource of antigen presented on APCs.
Chapter 3

Competition and coexistence of T-cell responses – a stochastic model

Almut Scherer, Marcel Salathé and Sebastian Bonhoeffer

3.1 Abstract

Both theoretical predictions and experimental findings suggest that T-cell populations can compete with each other. There is some debate on whether T-cells compete for aspecific stimuli, such as access to the surface on antigen presenting cells (APCs) or for specific stimuli, such as their cognate epitope ligand. We have developed an individual-based computer simulation model to study T-cell competition. Our model shows that the expression level of foreign epitopes per APC crucially determines whether T-cell competition is mainly for specific or aspecific stimuli. Under low epitope expression, competition is mainly for the specific epitope stimuli, and hence, different epitope-specific T-cell populations coexist readily. However, if epitope expression levels are high, aspecific competition becomes more important. Such between-specificity competition can lead to competitive exclusion between different epitope-specific T-cell populations. Epitope down-modulation, introduced in the previous chapter, can improve coexistence of multiple T-cell specificities under high epitope expression levels. Summarising, our model allows us to delineate the circumstances that facilitate coexistence of T-cells of different epitope specificity. Understanding mechanisms of T-cell coexistence has important practical implications for immune therapies that require a broad immune response.

3.2 Introduction

Competition amongst living organisms is all around. Individuals of the same or different species can compete with each other if they share limiting resources. Such within- and between-species competition allows the success of only those species that are particularly reproductively fit in the given environmental conditions. A well studied example of the potential effects of such competition is the often devastating effect of introduced 'invasive' species on native species (see e.g. (Tilman, 1982; Callaway and Aschehoug, 2000;
Suding et al., 2004)). Next to resource competition, other factors, such as introduction of new diseases by invasive species, can also contribute to the apparent competition between native and invasive species.

T-cell biology is no exception when it comes to competition. When a pathogen infects a vertebrate host, it is displayed as epitopes (eight to ten amino acid long protein fragments) to the T-cells of the host immune system. T-cells specific for any of the displayed epitopes can become activated, then proliferate rapidly and clear pathogen-infected cells. Most of these effector T-cells will die rapidly after their expansion, unless they are re-stimulated by remaining foreign antigen. In this setting, when a large number of T-cells depend on a relatively small number of antigenic stimuli for further expansion, competition between T-cells of the same (within-specificity competition) and of different epitope specificity (between-specificity competition) is expected to affect the T-cell dynamics. Indeed, in T-cell competition experiments, competitive effects only occurred when the ratio of T-cells to antigen was artificially increased (Gruftman et al., 1999; Roy-Proulx et al., 2001). In the introduction to Chapter 2 and in the thesis introduction (section 1.2) we described experimental evidence for the existence of between and within-specificity T-cell competition. Despite of the fact that different epitope-specific T-cell populations depend on the same pathogen for their expansion, coexistence of multiple epitope-specific T-cell populations seems to be the rule rather than the exception, in both acute (e.g. van der Most et al. (1998); Altfeld et al. (2002); Lauer et al. (2005)) and chronic infections (e.g. Yu et al. (2002); Klenerman et al. (2002a); Oxenius et al. (2002); Yu et al. (2005)). In this chapter we use a stochastic computer simulation model to investigate mechanisms that might explain this coexistence.

Most previous modelling approaches that describe the main aspects of T-cell activation and proliferation are in form of ordinary differential equation (ODE) models, in which the interactions between T-cells and infectious agents are modelled in a predator-prey like manner (De Boer and Perelson, 1995). These models do not yield equilibrium coexistence of multiple epitope-specific T-cell populations, unless the strength of competition between T-cells of the same epitope specificity is increased, e.g. by density dependent T-cell death (Korthals-Altes et al., 2003), or T-cell expansion and contraction dynamics are modelled heuristically (fitted function) (Antia et al., 2003). The benefit of using ODE models lies in their simplicity, which allows some results to be derived analytically. However, some important immunological features can not easily be incorporated into ODE models. For example, the resource of the T-cells, i.e. the sites on antigen presenting cells (APCs) that express the cognate foreign epitopes, are usually treated as independent, homogeneous entities. For example, De Boer and Perelson (De Boer and Perelson, 1994, 1995) described the T-cell resource as independent APC sites that are either free or engaged in an interaction with a T-cell. These sites were assumed to present sufficient amounts of MHC:epitope complexes for each of the competing T-cell populations. Hence, competition took place on an aspecific level, that is, for access to the epitope presenting APC sites, resulting in competitive exclusion of the lower affinity T-cell population.

In the previous chapter, we built on these models, and developed an ODE model in which the T-cell resource was also modelled as independent APC sites. The main extension to the model of De Boer and Perelson (1995) was that a fraction of the APC sites that activate a T-cell undergo a reduction in the epitope expression of the specificity of the interacting T-cell. This so-called epitope down-modulation leads to resource differ-
entiation, which allows immunodominant and subdominant T-cells to coexist. In reality, the T-cell's resource, i.e. the interaction 'sites' on APCs, are not independent entities. Up to 14 T-cells have been observed to interact with one APC simultaneously (Bousso and Robey, 2003). Since molecules can move rapidly in the cell membrane (Smith et al., 1999; Wülfling et al., 2002; Kasson et al., 2005), T-cell:APC interactions on one part of the APC might well influence the state of other APC sites on the same APC.

In this chapter, we develop an individual-based model (IBM) describing two T-cell populations and APCs with multiple T-cell interaction sites, to study potential mechanisms for coexistence of multiple epitope-specific T-cell populations. Our main finding is that, at low per APC epitope expression levels, T-cell coexistence is possible even in the absence of epitope down-modulation. At low epitope expression levels, competition is specific, namely for access to the specific epitope ligand, and therefore T-cells of different epitope specificity do not interfere with each other's expansion. However, at high epitope expression levels, competition becomes aspecific, namely for access to the APC sites, and a resource differentiation creating mechanism, such as epitope down-modulation, is again necessary to allow for coexistence of multiple epitope-specific T-cell populations. Further, we analyze the effect of several immunological parameters on T-cell coexistence, namely: i) the number of APC sites, ii) the number of rounds of programmed proliferation an activated T-cell goes through and iii) variability in epitope expression levels on APCs. Finally, we extend our investigation on the effect of epitope expression levels on T-cell response diversity from two to many epitopes.

3.3 The Model

Our simulation describes the dynamics of virally infected cells, antigen presenting cells (APCs) and T-cell populations specific for different epitopes of the virus. For simplicity, we consider the case of two viral epitopes and two T-cell populations specific for these epitopes. In the discussion of this chapter, we discuss how our results translate to situations with n epitope-specific T-cell populations. In the following, we first give a general introduction in individual-based models (IBMs), and then describe the 'players' in the model and their respective properties in detail.

3.3.1 Individual Based Models

Contrary to ODE models, which describe the dynamics of entire populations, IBMs describe the actions and interactions of individuals. The population dynamics in IBMs are derived directly from the processes that occur with the individuals that make up the population.

In Grimm and Railsback (2005), four criteria for individual-based models are listed: (1) aspects of the complexity of the individual's life cycle should be reflected in the model, (2) the dynamics of the resources used by individuals should be explicitly represented, (3) the size of a population should be represented in real or integer numbers and (4) variability among individuals of the same age class should be considered.

In the following, we give examples of how each of these criteria is incorporated in our model. Important aspects of the T-cell life-cycle are incorporated in our model, such as the programmed proliferation of T-cells upon activation by their cognate antigen
Figure 3.1: The basic features of the model; The production of APCs that present the two foreign viral epitopes (displayed as hexagonal shapes) depends on the number of virally infected cells (displayed as ellipses with star). Epitopes are displayed in black and white circles, where one black dot represents 25 copies of the epitope. T-cells (displayed as rod shaped cells) can form a conjugate with sites on APCs that display sufficient copies (i.e. 50) of their cognate epitope. Upon dissociation of the conjugate, T-cells can become activated, upon which they go through five rounds of programmed proliferation (only three rounds displayed). After proliferation, T-cells clear virally infected cells, and, after on average of five days, they start scanning APCs again in search of remaining antigenic stimulation. Mortality of T-cells and APCs is not depicted in this schematic representation, but is described in the model section 3.3.

(criterion one, see section 3.3.5). The T-cell resources in our models are explicitly modelled, but in a strongly simplified way. Importantly, there is a feed-back of T-cells on their resource, by the clearance of virally infected cells (criterion 2, see section 3.3.3). In our model, populations are nothing more than the set of the individuals that they encompass, and hence the population sizes are indeed represented by integer numbers (criterion three). Variability amongst individuals of the same age class is rather limited in our model. The fourth criterion is therefore only partly met in our model. All T-cells act according to the same rules (‘methods’), and depend on the same, often stochastic, parameters. However, in the last section of the results, where we investigate the influence of variability of epitope expression levels on APCs on T-cell coexistence, variation between APCs is introduced. Also, depending on current and/or previous interactions with T-cells, APCs differ amongst each other in terms of epitope availability and availability of space to accommodate T-cells (see section 3.3.4).

3.3.2 Time-step size and duration of simulations

Each virally infected cell, APC and T-cell in our model is initialised with a certain set of specific properties. In each time-step, a certain set of events (for example, T-cell activation or death) can take place. The length of a time-step is set to 1/50th of a day. This
way, each event in the simulation occurs with only a small likelihood per time-step, and hence the order in which methods are 'called' in the simulation is irrelevant. All simulations last 400 days (20,000 time-steps). For most parameter settings, the simulations equilibrate within that timespan. The equilibria in our simulations are either stable steady states or stable oscillations, or, in some cases, slow transients. In either case, simulations are stopped after 400 days.

3.3.3 Dynamics of virally infected cells

We set the probability of proliferation of T-cells and the rate of clearance of infected cells by T-cells such that the virus is not eradicated by the T-cell response, but causes a chronic infection. Viral replication is assumed to be density dependent, i.e. in absence of a T-cell response, the population of virus-infected cells grows to its carrying capacity, which is set to $10^5$. For the sake of simplicity, we do not explicitly model free viral particles and target cells, but only virally infected cells.

Virally infected cells are cleared by T-cells at a probability given by the product of the rate of clearance of infected cells, which is set to $10^{-5}$, and the number of effector T-cells (see sections 3.3.5). Although we refer to the pathogen as a viral pathogen throughout this chapter, the model can be applied to any pathogen that is displayed on MHC Class I molecules on APCs, including intracellular bacteria or cross-presented extra-cellular pathogens (see e.g. reviews by Wong and Pamer (2003) and Brode and Macary (2004)). We chose not to include the T-helper cell populations in our model, because this adds an unnecessary layer of complexity and because T-cell competition experiments that use epitope-loaded, mature DCs as antigen are also conducted in absence of T-helper cells.

3.3.4 APCs

The biology of epitope presentation on MHC Class I molecules in infected APCs is a complex process. In our model, we implement antigen presentation heuristically. We assume that the number of infected APCs is proportional to the number of virally infected cells, and set the number of new epitope presenting APCs per day to be the product of the infected cells and a constant, $\sigma$.

The APCs in our model have multiple T-cell binding sites, and the default number of T-cell binding sites per APC is set to six. Recent imaging studies have observed one to 14 T-cells interacting simultaneously with one dendritic cell (DC), which is an important type of APC (Bousso and Robey, 2003; Gunzer et al., 2004). In section 3.4.1 the number of T-cell binding sites per APC is varied to study its influence on coexistence of T-cells.

In our model, we assume that a T-cell needs to interact with 50 copies of its specific epitope:MHC complex to form a conjugate with an APC, and that each APC expresses 300 copies of each of the two epitopes. Throughout this chapter, the per APC epitope expression level is varied and indicated at each simulation result. Quantitative data on epitope expression levels on APCs in the literature suggest values ranging from one to 10,000 copies per epitope (Stevanovic and Schild, 1999; Crotzer et al., 2000; Engelhard et al., 2002; Herberts et al., 2003a,b; Basler et al., 2004). The minimal number of epitope ligands needed to activate a T-cell is very low, probably less than 10 copies (Irvine et al., 2002; Purbhoo et al., 2004). For our model studies, only the ratio of the epitope expression level per APC and the number of epitope copies required to activate a T-cell...
is relevant. This ratio needs to be larger than or equal to one to allow one or more T-cells of a particular specificity to interact and potentially become activated by an APC. In our model, epitopes are assumed to move freely on the surface of APCs, and, when a T-cell forms a conjugate with an APC site, the amount of epitope the T-cell needs to become activated is allocated to this site. For simplicity, the two foreign epitopes modelled here are assumed to be presented in equal densities on the APC. Effects of unequal presentation densities are discussed in the discussion.

**Epitope down-modulation**

When a T-cell has become activated by the interaction with its cognate epitope (see section 3.3.5), these epitopes are subsequently down-modulated. Down-modulated epitope is re-expressed again at a rate of 50 ligands per day. Up-regulation continues until the maximum epitope expression level is reached (i.e. the level with which new APCs are initialised). Note that epitope down-modulation is not per default used in the simulations.

**APC death**

In vitro data on the survival of DCs suggests that they live on average for two to six days (Hou and Van Parijs, 2004; Moran et al., 2005). In our model, we set the average lifespan of APCs to five days (the per day probability of death of an APC is set to 0.2). When an APC dies, the T-cells that are conjugated to it dissociate without becoming activated. We have not included killing of APCs by T-cells, since including APC-killing by T-cells does not alter the results qualitatively (data not shown).

**3.3.5 T-cells**

Naïve T-cell precursor frequencies have been estimated to be approximately one in \(10^5\) cells, that is, 10 to 50 precursor cells per specificity per mouse (Zinkernagel, 1996; Casrouge et al., 2000; Blattman et al., 2002). At the start of a simulation, we seed small populations of two types of epitope-specific T-cells (50 cells each) into the model immune system. The two T-cell specificities can be distinguished by their T-cell receptor specificity (set to 0 or 1), and only differ from each other in terms of their probability of proliferation upon dissociation from a conjugate with an APC site. In our model, the probability of proliferation is a composite parameter that describes the intensity of signals a T-cell receives while it is in conjugation with an APC site. This signal strength is shaped by, among other things, the TCR density on the T-cell membrane, the affinity of a TCR to its cognate epitope, and the density of the cognate epitopes on the APC surface (the latter is set equal for both epitopes). For simplicity, we will refer to T-cells with a high probability of proliferation as immunodominant or high-affinity T-cells and to those with a low probability of proliferation as subdominant or low-affinity T-cells. T-cells occur in the following states: As effector T-cells, conjugated with an APC site, or proliferating.

**‘Scanning’ and conjugate formation**

In the model, there are two kinds of effector T-cells: ‘Scanning’ effectors and ‘pure’ effectors. A scanning effector T-cell scans 50 randomly picked APCs with free binding
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Default in model</th>
<th>Experimental value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virally infected cells</td>
<td></td>
<td>10^5 cells</td>
<td></td>
</tr>
<tr>
<td>Carrying capacity</td>
<td>0.5 day^-1</td>
<td>0.5 day^-1</td>
<td></td>
</tr>
<tr>
<td>Intrinsic growth rate</td>
<td>10^-5 day^-1</td>
<td>10^-5 day^-1</td>
<td></td>
</tr>
<tr>
<td>Per T-cell viral clearance rate</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Minimum antigen level</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Curving capacity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virally infected cells</td>
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</tbody>
</table>

sites for antigenic stimuli per day and, upon success, it engages in a conjugate with the binding site. Note that we assume that T-cells only scan APCs with one or more free binding sites. This is because APCs that are fully covered with T-cells are no longer accessible to T-cells. Most data on T-cell and DC interaction rates count the number of T-cells that scan one DC per hour when antigen is absent. These estimates range from 500-5000 per hour (Gunzer et al., 2004; Stoll et al., 2002; Bousso and Robey, 2003; Miller et al., 2004). For a T-cell precursor frequency of one in $10^5$ cells, this would mean that a DC encounters a T-cell specific for a particular epitope once every 20 - 200 hours (i.e. approximately one to ten per day). For the situation when a specific antigen is present, and specific T-cell levels are far higher than precursor levels, the contact rate is naturally much higher, motivating us to set the number of APCs that a T-cell scans per day to 50. In equilibrium, APCs with free binding sites are very rare. If all APCs are full, scanning of APCs by T-cells stops until some sites become free again. Once a T-cell has conjugated with a site on an APC, it remains bound to the APC site for an average of one day. Upon dissociation from the conjugate, the T-cell proliferates according to its probability of proliferation (also referred to as "T-cell affinity").

Experimental data on the dynamics of the interactions between APCs and T-cells suggests that T-cells first engage in multiple, short encounters, then in longer interactions that last up to 30 minutes (Mempel et al., 2004). For our model results, both kinds of interactions are qualitatively equal. What counts is the total interaction time, during which a T-cell occupies space and / or epitopes on an APC. Hence we have condensed the multiple conjugate interactions into one long-lasting interaction.

**Proliferation**

T-cells become activated by interacting with an APC site that presents its specific epitope. The probability that a T-cell starts to proliferate upon dissociation of the T-cell:APC conjugate is varied in the simulations, but usually set to values around 0.1. Several years ago, our understanding of T-cell proliferation was drastically changed by data showing that T-cells, after an initial lag-phase of approximately one day, go through five to ten rounds of proliferation without the need for re-exposure to the antigen (Wong and Pamer, 2001; Van Stipdonk et al., 2001; Kaech and Ahmed, 2001; Krosgaard et al., 2003). Before, it was assumed that T-cells require antigen contact for each round of expansion. This antigen-independent sequence of several rounds of division upon activation is referred to as programmed proliferation (Mercado et al., 2000). In our model, activation of the T-cell upon dissociation from the APC immediately leads to the first of $n$ rounds of programmed proliferation. The subsequent rounds of proliferation are more rapid, and occur with a rate of three per day. In our model, the default number of rounds of programmed proliferation of an activated T-cell is set to five. In section 3.4.3 we vary the number of rounds of programmed proliferation and analyse the effect of the number of rounds of proliferation on coexistence of T-cells.

**Effector function**

After $n$ rounds, proliferation stops and the T-cells become 'pure' effector cells. Effector T-cells clear virally infected cells, and do not scan APCs for further antigenic stimulation. T-cells remain in the pure effector state for an average of five days and subsequently start
scanning APCs again, in search for antigenic stimuli. Scanning T-cells continue to clear virally infected cells until they engage into a new conjugate with an epitope presenting T-cell binding site on an APC.

**T-cell mortality**

Model-assisted data analysis has estimated mortality rates for T-cells between 0.05 and 0.2 per day (Macallan *et al.*, 2003; Ganusov *et al.*, 2005). In our model, we set the rate of death of T-cells to 0.2 per day. We further assume that T-cells do not die during programmed proliferation.

### 3.3.6 Parameter values

The parameter values used in our model simulations are, where possible, based on experimental values (see Table 3.1). Since it is our objective to study under what circumstances T-cell populations of different epitope specificity are in competition with each other, we look at longer-term interactions of T-cell populations and their resource, and hence evaluate equilibrium results of chronic infections. When T-cells are stimulated very efficiently by the antigen, or if they have a very high viral killing rate, they can rapidly clear infections. In our model, efficient stimulation of T-cells can arise from, for example, high probabilities of proliferation of T-cells, a high number of rounds of proliferation upon activation or very efficient antigen presentation by infected APCs (*i.e.* high \( \sigma \)).

### 3.4 Results

#### 3.4.1 Epitope down-modulation in a stochastic simulation model – from one to multiple APC sites

In the previous chapter, we found that epitope down-modulation can allow for coexistence of two competing T-cell populations, and that this coexistence increases with an increasing rate of epitope down-modulation, \( \gamma \). The parameter \( \gamma \) represents the fraction of productive APC:T-cell interactions (*i.e.* those that result in T-cell proliferation) that lead to epitope down-modulation. In the individual-based model presented in this chapter, we take a similar approach. Here, productive dissociation of an APC:T-cell conjugate leads to epitope down-modulation of 50 ligands (*i.e.* all those MHC:epitope complexes with which the T-cell interacts in the conjugate), with a probability set by the probability of epitope down-modulation.

To check whether the basic results obtained with the ODE model in chapter 2 can be reproduced in the individual-based simulation, we ran simulations in which APCs have only one T-cell interaction site, and both viral epitopes are expressed at sufficient levels to activate their T-cell specificity, *i.e.* at 50 copies each. This setting is most similar to the ODE model with independent APC sites in chapter 2. In Fig. 3.2A, the dynamics of T-cells and virally infected cells in the absence of epitope down-modulation is shown. The immunodominant and subdominant T-cells only differ in terms of the probability with
Figure 3.2: Dynamics of T-cells and virally infected cells in absence (A) and presence (B) of epitope down-modulation. The probability of epitope down-modulation is set to one, i.e. all productive APC:T-cell conjugates lead to epitope down-modulation of the epitope engaged in the conjugate. APCs have one T-cell interaction site, and present 50 copies of both the dominant and subdominant epitope specificity. The black line represents the virally infected cells, the red line the immunodominant T-cell population and the blue line the subdominant one. The affinities of the sub- and immunodominant T-cell specificities were set to 0.21 and 0.3 respectively. All other parameter settings are listed in table 3.1.

which conjugation with a suitable APC leads to activation and proliferation (0.21 and 0.3 respectively). At equilibrium, the immunodominant T-cell population out-competes the subdominant one. Fig. 3.2 B displays the dynamics of T-cell and virally infected cells with epitope down-modulation, where all productive APC:T-cell conjugate dissociation events lead to epitope down-modulation. In line with the results of the ODE model (Chapter 2), this epitope down-modulation allows the T-cell populations to coexist.

To more closely examine the relationship between epitope down-modulation and T-cell coexistence, the equilibrium T-cell coexistence, defined as the ratio of the subdominant and the immunodominant T-cell population size after 400 days, was monitored for increasing rates of epitope down-modulation (Fig. 3.3, black line). Fig. 3.3 again shows that, without down-modulation, the subdominant T-cell is out-competed, and coexistence equals zero. With increasing rates of epitope down-modulation, coexistence approaches the relative probability of proliferation of the subdominant versus the immunodominant T-cell populations (black dashed line). This coexistence results from the resource differentiation that epitope down-modulation brings about. This resource differentiation is reflected in the decreasing fraction of APCs that display sufficient copies of both epitopes (full red line), and the increasing fraction of APCs that display sufficient copies of either the subdominant (red dashed line) or the immunodominant (red dotted line).

In the ODE model in chapter 2, we assumed that T-cell binding sites on APCs are independent entities. This simplification allowed the derivation of simple equations for the population dynamics of APC sites and T-cells. Here we relax this assumption, because recent in vivo imaging methods have shown that multiple T-cells can and do interact with
Figure 3.3: Increasing epitope down-modulation leads to increased resource differentiation and hence to increased T-cells coexistence, coexistence being defined as the relative population size of subdominant versus immunodominant T-cells. For each probability of epitope down-modulation, the mean ± standard deviation of three replicate runs are shown. APCs have one T-cell interaction site, and present 50 copies of both the dominant and subdominant epitope specificity. The dashed line represents the relative affinity of the subdominant versus the immunodominant T-cell, which are set to 0.21 and 0.3 respectively. All other parameter settings are listed in table 3.1.

a single APC simultaneously (Bousso and Robey, 2003; Gunzer et al., 2004). To see whether having multiple, interdependent T-cell binding sites per APC has an effect on T-cell coexistence, we increase the number of T-cell binding sites per APC. However, in doing so, one also needs to consider increasing the epitope expression level. In the one site per APC simulations, we had 50 copies of each epitope, i.e. each epitope specificity was presented in sufficient amounts to activate a T-cell of the appropriate specificity. The equivalent epitope expression would be 100 copies of each epitope for a two-site APC, and 300 copies of each epitope for a six-site APC. To study the effect of multiple, linked APC sites, we plot T-cell coexistence versus the per APC expression levels of each epitope, either without (Fig. 3.4 A) or with (Fig. 3.4 B) epitope down-modulation. Both epitope specificities are expressed at equal levels on newly generated APCs (but can become skewed later on by epitope down-modulation).

The turquoise line in Fig. 3.4 A, which represents simulations with APCs with one T-cell binding site, shows that without epitope down-modulation, the immunodominant T-cell population out-competes the subdominant one, no matter how high epitope expression levels are. Interestingly, when APCs have two T-cell binding sites (i.e. they can simultaneously allow two T-cells to interact, blue line), the subdominant and immunodominant T-cell population coexist readily at epitope expression levels of 50. For 100 copies per specificity, we again observe competitive exclusion. For four and six sites per APC (red and black line respectively), T-cell coexistence remains high for epitope expression levels up to 100 and 150 copies of each specificity respectively, and then drops
Figure 3.4: T-cell coexistence depends on the epitope expression level and the number of T-cell interaction sites per APC. Panel A is without and panel B with epitope down-modulation. Shown are the mean ± standard deviation of three replicate runs for each epitope expression level. Simulations for APCs with one (turquoise line), two (blue line), four (red lines) and six (black lines) are shown. The T-cell affinities are kept at 0.21 versus 0.3 for simulations with one, two and four sites. For simulations with six sites, they are lowered to 0.07 versus 0.1, because at higher affinities, the virus is cleared by the T-cell response. All other parameter settings are listed in table 3.1.

In panel B of the same figure, the simulations are run with epitope down-modulation. The turquoise line for one site per APC starts off with a coexistence of near 0.55 (as in Fig. 3.3, for a probability of epitope down-modulation of one), but, with increasing epitope expression levels, the coexistence rapidly drops towards zero. This is because epitope down-modulation improves coexistence by creating resource differentiation. In other words, epitope down-modulation creates APCs on which some or all sites are only suitable for only one of the two T-cell specificities. However, at higher epitope expression levels per APC it requires stronger epitope down-modulation on APCs to provide this resource differentiation. At very high epitope expression levels, APCs no longer become sufficiently differentiated to allow the subdominant T-cell specificity to coexist with the immunodominant one. Also for more than one T-cell binding site per APC, we see that coexistence is enhanced by adding epitope down-modulation, but that the effect of epitope down-modulation wanes at high epitope expression levels, where coexistence again approaches zero.

Taking a vertical cross-section in either of the two panels, one can see that for increasing number of T-cell interaction sites per APC at a given epitope expression level, the strength of competition for access to the APC decreases. For example, in Fig. 3.4 A, APCs with epitope expression levels of 150 copies of each ligand and only two T-cell binding sites (blue line), have more than sufficient epitope to activate T-cells of each epitope specificity at each site. Hence access to the T-cell binding sites on APCs, rather than specific epitope, is limiting, which results in competitive exclusion. However, when there are four or six sites per APC (red and black line respectively), there is not enough epitope...
to accommodate T-cells of each specificity on each APC site. Hence, in this situation, competition is for the specific epitope rather than for access to T-cell binding sites on the APC, which results in coexistence of the two T-cell populations.

One final interesting point in Fig. 3.4 B is that the effect of epitope down-modulation on T-cell coexistence depends on the absolute T-cell affinities. This is demonstrated by the fact that the lines of simulations with four and six T-cell binding sites per APC (red and black lines respectively) cross each other (around epitope expression levels of 300 copies). All simulations were run with probabilities of proliferation of 0.21 and 0.3 for the sub- and immunodominant T-cell specificities respectively, save for the simulations with six APC sites, in which they were lowered to 0.07 and 0.1. The reason for lowering the T-cell affinities is that when APCs can activate so many T-cells simultaneously, the T-cell response expands to a level such that it clears the virally infected cells. As mentioned in section 3.3.6 of the model description, some parameters need to be adjusted such that infections become chronic, to enable us to look at T-cell coexistence in a non-trivial equilibrium rather than a transient. Interestingly, although coexistence in absence of epitope down-modulation does not depend on the absolute affinities of T-cells (the lines of two, four and six T-cell binding sites are very similar, only shifted to the right), coexistence in presence of epitope down-modulation does. Epitope down-modulation occurs at each productive dissociation of an APC:T-cell conjugate, which is given by the T-cell affinity. Therefore, the four-site simulation with high T-cell affinities has a higher net rate of epitope down-modulation than the six-site simulation with lower T-cell affinities.

In the following section, we focus on the mechanisms that allow for T-cell coexistence at low epitope expression levels. Furthermore, we ask how epitope expression levels affect T-cell population sizes and the number of virally infected cells.

### 3.4.2 Less is more? T-cell coexistence by low epitope expression levels

In Fig. 3.5 A, we re-plot T-cell coexistence with (bold line) and without (thin line) epitope down-modulation in one panel (same as the two black lines from Fig. 3.4 A and B). Viewing along the horizontal axis of Fig. 3.5 A, we distinguish three qualitatively different competition regimes, in which competition is (i) only specific, (ii) both specific and aspecific and (iii) only aspecific. In the following, we discuss in more detail how epitope expression levels shape each of these competition regimes, using schematic visualisations of each regime (Fig. 3.6). For regime (i), that is, for epitope expression levels up to 150 copies per specificity, the specific epitope, rather than access to T-cell binding sites on APCs, is limiting (top row of Fig. 3.6). Although epitopes are presented in a mixed fashion on the APC membrane when they are first expressed, simultaneous interactions with multiple T-cells can bring about a degree of spatial segregation between the two different epitopes. For example, if three T-cells specific for the immunodominant epitope have formed conjugates with an APC that expresses 150 copies of each epitope, the remaining three sites will solely present the subdominant epitope. Hence, under such limiting epitope expression levels, T-cells of different epitope specificity do not compete with each other directly, and the level of T-cell coexistence (i.e. the ratio of subdominant to immunodominant population size) is given by their relative T-cell affinities.

For regime (ii), that is, for epitope expression levels ranging from 150 to 300 copies of each specificity, some APC sites only express enough of one epitope specificity, while
Figure 3.5: The effect of the per APC epitope expression level on T-cell coexistence (A), T-cell numbers (B) and the number of virally infected cells (C). Thin lines are without epitope down-modulation, bold lines with. Shown are the mean ± standard deviation of three replicate simulations. A: Coexistence of T-cells depends on the per APC epitope expression level. The black dashed line indicates the ratio of epitope affinities of the subdominant and the immunodominant T-cell specificity. B: Increasing epitope expression levels affect the total (black), the immunodominant (red) and the subdominant (blue) T-cell population sizes. In parallel to the increase in the total number of T-cells, the number of virally infected cells decreases (C). T-cell affinities are set to 0.07 and 0.1 for the sub- and immunodominant T-cell specificity respectively. All other parameter settings are listed in table 3.1.

others express enough of both specificities (middle row of Fig. 3.6). This means that there is both shared and un-shared resource for the two T-cell populations. The more APC sites express sufficient epitope of both specificities, the higher the competition between T-cells of different epitope specificity, and consequently, the lower the coexistence of the two T-cell specificities.

For epitope expression levels above 300 copies of each specificity (regime (iii)), all
Figure 3.6: Schematic representation of the three qualitatively different competition regimes. From top to bottom, the three rows represent schematic examples of epitope expression levels in which competition is solely specific, both specific and aspecific or solely aspecific (regions (i), (ii), and (iii) in Fig. 3.5 A respectively). The larger, six-sided cells represent APCs, and the rod-shaped cells represent T-cells. Each black or white filled circle on an APC represents 25 copies of the black or the white epitope specificity respectively. Upon interaction with 50 copies of its specific epitope, T-cells can become activated. Activated T-cells are indicated with a star.

T-cell binding site of the APCs present sufficient epitope of each specificity for T-cells to form a conjugates (bottom row of Fig. 3.6). At such high epitope expression levels, the limiting factor is no longer epitope, but rather access to T-cell binding sites on APCs. In this situation, both T-cell specificities depend on the same resource, i.e. competition within and between T-cell specificities is equivalent, and the immunodominant T-cell specificity out-competes the subdominant one because it replicates faster. Therefore, T-cell coexistence does not occur at high epitope expression levels.

Summarising, low levels of epitope expression enhance coexistence of T-cells (Fig. 3.5 A). However, this enhanced coexistence is accompanied by a decrease in total T-cell numbers (Fig. 3.5 B), and hence an increase in the number of virally infected cells (Fig. 3.5 C). In the simulations in Fig. 3.5 C, the number of virally infected cells is almost double at low (e.g., 50 copies of each specificity) than at high epitope expression levels (greater than 150 copies of each specificity). The ‘optimal’ epitope expression level would be one at which there is a single batch (of 50 epitope copies) of epitope per T-cell binding site. At this level, T-cell coexistence is maximal, and the number of virally infected cells is as low as for higher epitope expression levels. The detrimental effect of low epitope expression levels only arises at expression levels at which not all APC sites are ‘filled’ with epitope.

In panels A, B and C of Fig. 3.5, the relative affinity of the subdominant versus the
immunodominant T-cell specificity was 0.7. We next investigated whether the range of affinities of T-cells that can coexist is limited to very similar affinities or if T-cells of quite distinct affinities can coexist. In Fig. 3.7, T-cell coexistence is plotted over a wide range of relative affinities of the sub- versus the immunodominant specificity. The black lines represent simulations with high epitope expression levels (300 copies of each epitope), the thin line being without, and the bold line being with epitope down-modulation. Without epitope down-modulation, we again observe competitive exclusion of the subdominant T-cell population for at a high epitope expression level. Only when the subdominant T-cell population has a similar affinity as the immunodominant one is there coexistence. This is because the dynamics of competitive exclusion are so slow that the equilibrium is not reached within 400 days, i.e. the coexistence observed for high relative affinities is transient. With epitope down-modulation (bold black line), coexistence is much improved, but still lies far below the coexistence observed at low epitope expression levels (red line). Since resource differentiation is complete at this low epitope expression level (150 copies of each epitope), coexistence equals the relative affinity of the subdominant versus the immunodominant T-cell. Therefore, the slope of T-cell coexistence versus relative T-cell affinity at low epitope expression levels equals one.

At very low affinity of the subdominant T-cell specificity, coexistence is lost, independent of the epitope expression level. This effect arises because the subdominant T-cell population does not receive a sufficient stimulus for proliferation. In mathematical terms, this implies that the basic reproductive rate of the subdominant T-cell population is below one. In epidemiology, the basic reproductive rate, R₀, is defined as the number of secondary infections caused by one infected individual introduced into a fully susceptible
host population (Anderson and May, 1991). In analogy, we define the basic reproductive rate of a T-cell, \( R_b \), as the number of offspring it produces during its lifetime in resource unlimited and competition free conditions. This \( R_b \) is proportional to the probability of proliferation and the number of offspring produced during programmed proliferation.

### 3.4.3 T-cell coexistence does not hinge on the number of rounds of programmed proliferation

In the simulation results presented so far, an activated T-cell undergoes five rounds of programmed proliferation. In the following, we will refer to the number of rounds of programmed proliferation as the T-cell’s proliferative capacity, and ask whether this proliferative capacity might influence T-cell coexistence. This question is topical, because it has been suggested that, at least in acute infections, programmed proliferation explains the lack of competition between responses to different epitopes of a pathogen (Antia et al., 2005). In the following we look at the effect of varying proliferative capacities on T-cell coexistence, first in chronic infections and second in acute infections that are resolved by the immune response.

**Chronic Infections**

Competition between T-cells of different epitope specificity has been observed less frequently than competition between T-cells of the same epitope specificity. As mentioned before, it has been argued that programmed proliferation explains why T-cells of different epitope specificity do not compete with each other (Antia et al., 2005). Although programmed proliferation is incorporated in our model, the immunodominant T-cell populations out-compete the subdominant ones at high epitope expression levels. However, it is possible that the observed relationship between epitope expression levels and T-cell coexistence might be sensitive to the proliferative capacity of T-cells, and that the range of epitope expression levels at which T-cells coexist can be widened by increasing the proliferative capacities of T-cells.

To address this question, we ran additional simulations in which T-cells go through four or six rounds of programmed proliferation upon activation. First, we look at the potential effect of the proliferative capacity while keeping the basic reproductive rate, \( R_b \), of the T-cell populations fixed. This can be done by altering the probability of proliferation relative to the difference in proliferative capacities of five rounds versus \( n \) rounds of proliferation. For example, a T-cell with a proliferative capacity of five produces \( 2^5 - 1 \) offspring cells, and one with a proliferative capacity of four only \( 2^4 - 1 \). Hence the probabilities of proliferation of the sub- and immunodominant specificities are multiplied by \( \frac{31}{15} \), when proliferative capacity is reduced from five to four. When increasing the proliferative capacity from five to six, T-cell affinities are multiplied by \( \frac{31}{63} \). Secondly, we look at the potential effect of the proliferative capacity of T-cells on coexistence given a fixed probability of proliferation. In this case, the basic reproductive rate increases with increasing proliferative capacity.

When keeping the basic reproductive rate of T-cells fixed, T-cell coexistence in absence of epitope down-modulation does not depend on the T-cell’s proliferative capacity (Fig. 3.8 A). However, T-cell coexistence in presence of epitope down-modulation seems to depend strongly on the number of rounds of proliferation (Fig. 3.8 C). Coexistence
Figure 3.8: T-cell coexistence for varying proliferative capacities of four (red lines), five (black lines) and six (red lines). A and B are without and C and D with epitope down-modulation. In the simulations with five rounds of programmed proliferation, the probability of proliferation of the subdominant and immunodominant T-cell specificities are set to 0.07 and 0.1, respectively. A: To keep the same basic reproductive rate as in the simulations with five rounds of division upon activation, the affinities are adjusted in the simulations with four and six rounds of programmed proliferation (see main text in section 3.4.3). At four rounds of proliferation, affinities are set to 0.1446 versus 0.2067 and at six rounds to 0.034 versus 0.04921. B: The proliferative capacity is increased and decreased by one round of division while keeping the T-cell affinities at 0.07 versus 0.1 for the sub- and immunodominant T-cell specificities. Other parameters are as given in Table 3.1.

with epitope down-modulation is higher at lower proliferative capacities because we increased the T-cell affinities, in order to maintain the same reproductive rate. Hence, more T-cells have to become activated to yield the same number of offspring, and therefore,
more epitope down-modulation events occur, yielding more resource differentiation and better coexistence. This dependence of the effect of epitope down-modulation on the absolute T-cell affinities already was already observed in Fig. 3.4.

The effect of the proliferative capacity on T-cell coexistence in presence of epitope down-modulation is indeed a side-effect of changing the T-cell affinities. When the T-cell affinities are kept fixed, and $R_b$ free, changes in the proliferative capacities do not affect coexistence (Fig. 3.8 B and D).

**Acute infections**

In the previous section, we showed that the proliferative capacity of T-cells does not affect the dependence of T-cell coexistence on epitope expression levels in chronic infections (i.e. at equilibrium after 400 days). Here, we ask whether the same holds for acute infections, that are cleared rapidly by the immune response. One might argue that particularly in acute infection, competition between T-cells might not influence T-cell dynamics, since few naïve T-cells are activated under conditions when antigen is very abundant, and then go through several rounds of antigen-independent expansion. To study whether differences in epitope expression levels also affect T-cell dynamics in acute infection, we ran simulations of T-cells with a proliferative capacity of seven rounds, rather than the default five rounds used in most simulations so far. At this high proliferative capacity, the infection is cleared by the immune response at day 20 (Fig. 3.9). At high epitope expression levels, the subdominant response expands very poorly, reaches a peak of approximately 12,000 cells, and is out-competed by the immunodominant T-cell response by day 40 (Fig. 3.9 A). At low epitope expression levels, both epitope-specific T-cell responses expand according to their relative affinity to their ligand, and the ratio of the subdominant and the immunodominant T-cell response size equals the ratio of their affinities (i.e. 0.7) at the peak of the immune response at day 25 (Fig. 3.9 B).

Summarising, our model results show that programmed proliferation does not prevent T-cells from competing with each other, neither in acute, nor in chronic infection.

### 3.4.4 The effect of variability in epitope expression levels on T-cell coexistence

In our model, the copy number at which epitopes are presented is exactly the same on each newly produced APC. In reality, there will be variability in the epitope expression levels between APCs. Variability will arise, for example due to stochasticity in the steps of the epitope processing and presentation pathway, or due to differences between different types of APCs. Such variability could add to resource differentiation, by some APCs per chance displaying less of one epitope than of the other. We implement natural variability in epitope expression levels by drawing the epitope expression level of each epitope of a newly produced APC from either a Poisson (Fig. 3.10 A) or from Normal distributions with different standard deviations. For the Normal distributions, negative epitope expression levels are set to zero (Fig. 3.10).

Variability in epitope expression levels extends the range of epitope expression levels at which T-cell populations coexist, and the size of the effect depends on the width of the distribution that epitope expression levels were drawn from. The coexistence-curve is shifted to the right by approximately 50 epitopes for the Poisson distributions
High epitope expression level

Low epitope expression level

Figure 3.9: Dynamics of T-cells and virally infected cells for high (300 copies of each epitope, panel A) and low (150 copies of each epitope, panel B) epitope expression levels. The proliferative capacity of T-cells was set to seven rounds of proliferation (rather than the default value of five used in most of the previous results). The affinities of the sub- (blue lines) and immunodominant T-cell specificities (red lines) were set to 0.07 and 0.1 respectively. All other parameter settings are listed in table 3.1.

(Fig. 3.10 C), and far more so for the normal distributions. For example, in Fig. 3.10 D, at epitope expression levels of 300 copies of each specificity, coexistence is still approximately half-maximal for a standard deviation of 100 copies (blue line in Fig. 3.10), whereas without variability of epitope expression levels, the subdominant T-cell population is already out-competed at such high epitope expression levels (black line). Variability in epitope expression levels improves T-cell coexistence because, although the over- and under-expression of both epitopes is symmetric, its effect is not. If epitope expression levels are already high, over-expression of one or both epitopes on some APCs will not affect coexistence. However, under-expression of one or both epitopes will generate some resource differentiation, and thus allows sub-dominant T-cells to coexist at higher mean epitope expression levels.

Summarising, variability in epitope expression levels simply extends the range of epitope expression levels at which T-cells can coexist. Otherwise, our results are robust to variability in epitope expression levels.

3.4.5 Scaling it up: Is there a maximum to the number of epitope-specific T-cell populations that can coexist?

Up to this point we have studied the question of competition and coexistence of multiple epitope-specific T-cell responses in the most simple setting of two epitopes and two T-cell populations. In this section we investigate T-cell coexistence in a model with many epitopes, and ask whether there is a limit to the number of T-cell populations that can coexist in equilibrium. To address this question, we simulate our model, in which APCs have six T-cell binding sites, with six and ten presented epitopes (Fig. 3.11 A and B re-
Figure 3.10: Distributions used for modelling variability in epitope expression levels (A and B) and the resulting coexistence plots (C and D). Panel A, shows examples of Poisson distributions used in the simulations with variable epitope expression levels. Shown are distributions with means of 50 (full line), 150 (dashed line) and 300 epitopes (dotted line). The standard deviations in Poisson distributions equals the square root of the mean. Panel C shows the resulting coexistence plot, which is slightly shifted to the right for Poisson distributed epitope expression levels (red line) compared to simulations without variability in the epitope expression level (black line). In Panel B, examples of Normal distributions used for drawing random epitope expression levels values from are plotted. Normal distributions with a mean epitope expression level of 150 copies and standard deviations of 0% (black line), 10% (red line), 25% (turquoise line) and 50% of the mean (blue line) are shown. The normal distributions were truncated at zero, i.e. negative random numbers were set to zero. Panel D shows the resulting coexistence plots. The affinities of the sub- and immunodominant T-cell specificities were set to 0.07 and 0.1 respectively. All other parameter settings are listed in table 3.1.

spectively). When epitopes are expressed at exactly the T-cell sensitivity for epitope (i.e. 50 copies), six out of six and nine out of ten presented epitopes are targeted. If epitope
expression levels are increased above the T-cell sensitivity (i.e. for epitope expression levels of 100 copies and up), epitope-specific responses are lost sequentially, in order of their affinity ranking. Interestingly, for this range of epitope expression levels, the diversity profiles of T-cell responses generated with six-site-APCs presenting six or ten epitopes are indistinguishable. Similar results were obtained with APCs with ten T-cell binding sites. The profiles of T-cell response diversity of APCs presenting 10 and 17 epitopes were equivalent for all but the epitope expression level of 50 copies per epitope per APC. At expression levels equal to the T-cell sensitivity, all of the ten presented epitopes (Fig. 3.11 C) or 15 out of 17 of the presented epitopes (Fig. 3.11 D) were targeted.

These data suggest that, when the total number of epitope types presented on an APC is larger than the available space in terms of T-cell binding sites, some epitopes will not elicit an immune response, even if epitopes are expressed at very low levels. Still, when epitope presentation equals the T-cell sensitivity, set in our model to 50 copies of epitope per APC, more T-cell specificities can coexist than the number of T-cell binding sites on the APC. This is because, when APCs present only sufficient of each epitope to stimulate one T-cell of the corresponding specificity, the within-specificity competition is much stronger than the between-specificity competition.

Another point that can be observed from Fig. 3.11 is that when many rather than just two epitopes are presented on APCs, the cost for low epitope presentation levels in terms of reduced total immune response size and higher viral load, as observed in Fig. 3.5, vanishes. This implies that in infections, where typically many epitopes are presented, the optimal epitope expression level in terms of T-cell response diversity equals the T-cell sensitivity.

3.5 Discussion

In this chapter, we developed an individual-based stochastic simulation model, in which APCs have multiple T-cell interaction sites, to study T-cell competition. With this model, we addressed the following two questions: (1) Under what conditions do T-cells specific for different epitopes of a pathogen compete with each other and (2) why don't all immunogenic epitopes elicit a measurable T-cell response? For the first question, the model predicts that, when epitope expression levels are low, competition is for the specific epitope, and hence immunodominant and subdominant T-cells of different epitope specificities coexist readily. However, at high epitope expression levels T-cells compete for access to the APC, which is a shared resource. This competition can severely reduce the expansion of the subdominant T-cell specificity, or even lead to the extinction of the subdominant T-cell specificity (i.e. competitive exclusion). The dependence of T-cell coexistence on epitope expression levels holds for wide ranges of T-cell affinities, and for a wide range of proliferative capacities of T-cells (i.e. the number of rounds of division in programmed proliferation). The second question, why only so few of all presented pathogen epitopes are targeted upon infection, has also been debated elsewhere (Yewdell and Bennink, 1999; Yewdell and Del Val, 2004). The theoretical maximum breadth of T-cell immune responses in our model, set by the available number of T-cell binding sites on APCs and the T-cell sensitivity to epitopes in terms of the copy number required for activation, might explain why only a subset of the presented epitopes elicit measurable T-cell immune responses.
Figure 3.11: T-cell diversity decreases with increasing per APC epitope expression level. APCs with six T-cell binding sites were simulated with 6 or 10 presented epitopes (panel A and B, respectively), and APCs with ten T-cell binding sites with 10 and 17 epitopes (panel C and D, respectively). Per bar, the different colours indicate the size of the different epitope-specific T-cell populations. In panel C and D, some colours appear twice per bar, but indicate different responses. For increasing epitope expression levels, T-cell diversity drops from its maximum, which is at epitope expression levels equal to the epitope sensitivity of the T-cells (i.e. 50 copies of epitope), down to one for high epitope expression levels. Diversity reaches one when each epitope is expressed at sufficient levels to activate a T-cell of the corresponding epitope specificity at each site of the APC, which is at epitope expression levels of 300 and 500 for APCs with six and ten T-cell binding sites respectively. T-cell affinities of epitopes presented on APCs with six sites were as follows: For six epitopes [0.05, 0.06, 0.07, 0.08, 0.09, 0.1] and for ten epitopes [0.03, 0.035, 0.04, 0.045, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1]. On APCs with 10 sites and 10 epitopes, affinities ranged from 0.0375 to 0.06 and on APCs with 10 sites and 17 epitopes, affinities ranged from 0.02 to 0.06. Subsequently ranked T-cell specificities always differed by 0.0025 in terms of their affinities. All other parameter settings are listed in table 3.1.
Our model is not the first model of multiple coexisting epitope-specific responses, but it is the first in which this coexistence emerges from mechanistic aspects of the interactions between the T-cells and the antigen. In a paper on diverse HIV-specific T-cell immune responses, an extra term of competition between T-cells of the same epitope specificity allowed multiple epitope-specific T-cell responses to coexist (Korthals-Altes et al., 2003). The mechanistic basis for the assumed higher intensity of within- than of between-specificity competition suggested by Korthals-Altes et al. (2003), was that epitope-specific T-cell populations might expand locally in tissue, and hence interact more with each other than with cells of other specificities. Another study modelled the expansion dynamics heuristically, by feeding observed expansion and contraction dynamics into the model (Antia et al., 2003). Also, Chao et al. (2004)'s model of acute infection assumes that T-cells of different epitope specificity do not compete with each other, because a small number of precursors is stimulated by a high dose of antigen.

Based on the observed T-cell expansion capacities, we have argued in this chapter, that, even in acute infection, the situation in which many T-cells compete for limited antigen does occur (section 3.4.3 and Fig. 3.9).

Broad immune responses are thought to be important for the protection of infected hosts, in face of rapidly evolving pathogens that can accumulate mutations that interfere with presentation of the epitope or recognition of the epitope by the specific T-cells. Loss of an immune response due to such mutations is called immune escape, and is thought to be a central problem of the immune system's fight against HIV (see e.g. (Price et al., 1997; Phillips et al., 1991; Borrow et al., 1997; Harcourt et al., 1998; Leslie et al., 2004; Yokomaku et al., 2004)). Our model suggests that the breadth of T-cell immune responses is maximal when epitope expression levels are close to the T-cell sensitivity. Data on the expression level revealed copy numbers between one and 10,000 per cell, although most measurements yield copy numbers between 10 and 1000 (Van Bleek and Nathenson, 1990; Falk et al., 1991; Tsomides et al., 1991; Christinck et al., 1991; Wallny et al., 1992; Huczko et al., 1993; Delaney et al., 1998; Stevanovic and Schild, 1999; Crotzer et al., 2000; Engelhard et al., 2002; Herberts et al., 2003a,b; Basler et al., 2004). The epitope expression levels of epitopes are likely to differ between different pathogens, depending on the genome size of the pathogen and on the absolute amount of pathogen protein that is produced in infected cells. Pathogens with small genomes, but high transcription rates in infected cells, might yield relatively high epitope expression levels, while pathogens with large genomes, but low transcription rates might yield relatively low epitope expression levels. However, the relationship between mRNA and expressed peptide is complex. For example, when comparing normal cells and tumor cells, only 50-60% of over-expressed mRNAs were also over-expressed in terms of the peptide presented on the cell (Weinzierl, 2006). The antigen sensitivity has been estimated to lie around 50 and 400 for CD4 (Harding and Unanue, 1990; Demetz et al., 1990; Kimachi et al., 1997; Reay et al., 2000) and between 1 and 50 for CD8 positive T-cells (Christinck et al., 1991; Brower et al., 1994; Sykulev et al., 1996; Irvine et al., 2002). The experimental data of epitope expression levels and T-cell sensitivities suggest that epitopes tend to be expressed at substantially higher levels than the minimal copy number needed to activate one T-cell of a particular specificity on an APC. Our model suggests that under such circumstances, not all of the epitopes expressed on an APC will also elicit a T-cell response. Our model further suggests that epitope down-modulation, a mechanism by which the interaction of a specific T-cell with its cognate epitope reduces the expression level of this epitope
(Kedl et al., 2002), can partially compensate for the negative effect of epitope expression levels on T-cell diversity (see Chapter 2 and Fig. 3.4).

The results shown in this chapter are based on simulations in which epitope expression levels of the two epitopes are equal. Skewed epitope expression in favour of the low affinity T-cell specificity can compensate partially or fully for the affinity disadvantage of the subdominant T-cell response. On the other hand, a subdominant T-cell specificity will be out-competed more easily if the cognate epitope of the immunodominant T-cell specificity is over-expressed (data not shown).

The main result from our modelling study, that competition between T-cells of different epitope specificity is only expected to occur at high per APC epitope expression levels, is very intuitive. Still, there is currently no direct experimental evidence to supports this idea. In the following chapter, we present an in vivo T-cell expansion experiment in mice, that was designed to test our theoretical result experimentally.

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Chapter 4

Less is more – Epitope expression levels and T-cell coexistence in mice

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4.1 Abstract

A central question in this thesis is whether T-cells of different epitope specificity compete with each other for access to Antigen Presenting Cells (APCs). In the previous chapter, we developed an individual-based computer simulation model to shed light on the mechanisms underlying T-cell competition. The central result of this modelling study was that the expression level of foreign epitopes per APC is a crucial factor in T-cell competition. The model predicts that, under low epitope expression, competition is on the level of the specific epitope. Therefore, competition between T-cells of the same epitope specificity (within-specificity competition) is strong, but competition between T-cells of different epitope specificity (between-specificity competition) is weak. Hence, multiple epitope-specific T-cell populations can coexist. At high epitope expression levels, competition is predicted to be for access to APCs. Under such circumstances, between-specificity competition is stronger, and immunodominance more pronounced. We here test our theoretical prediction, that epitope expression levels on APCs affect competition, in a mouse experiment. Our results show a significant effect of epitope expression level on the degree of coexistence of two T-cell responses.
CD8+ T lymphocyte responses play a central role in the clearance of pathogen infections. The T-cell response against a pathogen typically consists of multiple sub-populations, specific for different epitopes of the pathogen. These epitope-specific T-cell populations often differ considerably of their size. The reasons for the different expansion efficiency of different epitope-specific populations include factors such as T-cell receptor (TCR) affinity to the MHC:epitope ligand, differences in timing of the expression of the MHC:epitope ligand, and the level at which the ligand is expressed on the surface of antigen presenting cells (APCs) (Chen et al., 2000; Sewell et al., 2002; Yewdell and Del Val, 2004). A further factor that has been proposed to influence the relative sizes T-cell populations specific for different epitopes of a pathogen, is competition between T-cells. Competition between T-cells has been observed when the ratio of T-cells to antigen is high (Grufman et al., 1999; Roy-Proulx et al., 2001), and could either occur within the epitope-specific T-cell population (within-specificity competition), or between different epitope-specific T-cell populations (between-specificity competition). Whereas the experimental evidence for, and the existence of, within-specificity competition is well accepted, the issue of between-specificity competition is much more controversial (Vijh et al., 1999; Chen et al., 2000; Kedl et al., 2000; Probst et al., 2002; Palmowski et al., 2002; Kedl et al., 2003).

Adoptive transfer experiments are a frequently used approach to study competition between T-cells specific for different epitopes of the same pathogen. T-cell numbers specific for a defined epitope are artificially increased in a recipient mouse by adoptive transfer from a donor mouse. Following the adoptive transfer, the mouse is either infected with a pathogen or immunised with epitope-loaded Dendritic Cells (DCs). In either case, the immunising agent includes the epitope of the previously transferred T-cell specificity, and the effect of the transferred T-cells on the host immune response is measured. Using this approach, Kedl et al. (2000) found evidence for interference between transferred cells and both host cells of the same (within-specificity competition) and of different epitope specificity (between-specificity competition). In contrast, Probst et al. (2002), using a very similar approach, only observed within-specificity competition. Further, Vijh et al. (1999) studied the interactions between different epitope-specific T-cell populations by knocking out one or several epitopes of the bacteria Listeria monocytogenes. Interestingly, the elimination of dominant epitopes did not increase the T-cell responses to subdominant epitopes. However, a study by Probst et al. (2003a) found such compensation between sub- and immunodominant responses. In this study, reduced expansion of two dominant responses by tolerisation resulted in increased expansion of a subdominant response. Also, reduction of the immunogenicity of an immunodominant epitope by introduction of a point mutation in the epitope gene, and the resulting reduced expansion of the immunodominant T-cell response, led to increased expansion of the subdominant responses (Tebo et al., 2005).

In the present study, we address how epitope expression levels affect the competition between T-cells of different epitope specificity. Our experimental design tests the predictions of the individual-based computer model presented in the previous chapter. The central prediction of this model is that competition between T-cells specific for different epitopes should increase with increasing epitope expression levels (Fig. 4.2). Under conditions of low epitope expression, competition is predicted to be on the level of the
specific epitope type. When competition is for the specific epitope, competition between T-cells of the same epitope specificity (within-epitope competition) is expected to be strong and competition between T-cells of different epitope specificity (between-epitope competition) weak (see Fig. 4.1 A). Under high epitope expression levels, the specific epitope ceases to be limiting, and competition is predicted to shift to the level of access to DCs (see Fig. 4.1 B). Under such circumstances, between-specificity competition is strong and may lead to more pronounced immunodominance. Between-specificity competition, also referred to as crowding competition, requires the ratio of T-cells to APCs to be high. Observations from in vivo imaging studies show that, at low per DC epitope expression levels, more T-cells interact simultaneously with one DC than at high epitope expression levels (Bousso and Robey, 2003). This suggests that competition for access to the DC may indeed be more relevant at high than at low epitope expression levels.

**Figure 4.1:** The levels at which epitopes are expressed on APCs may influence whether or not T-cells of different epitope specificity compete with each other. In panel A, an APC expressing two epitopes of a pathogen at low expression levels is illustrated. Due to the sparsity of epitope, the T-cells that interact with the APC do not block epitope that could otherwise be seen by other T-cells. Therefore, in this situation, only T-cells of the same specificity can hinder each other in accessing the shared resource, namely the specific epitope type. In panel B, an APC with high epitope expression levels is illustrated. In this case, some of the expressed epitope can not be accessed by T-cells, due to the fact that the APC is 'crowded'. In this second scenario, T-cells both compete within and between specificities.

### 4.3 Material and Methods

**Mice**

C57BL/6 mice were obtained from the Institut für Labortierkunde (University of Zürich), and were kept under specific pathogen free (SPF) conditions in the University Hospital Zürich (Biologisches Zentrallabor). Experiments were carried out in accordance with the Swiss Federal and Cantonal law on animal protection. Within experiments, mice were age- and sex-matched.
**Figure 4.2:** Our experiment is based on theoretical predictions derived from the individual-based model developed in Chapter 3. The graph on the left schematically represents the main prediction of this model. Shown is the dependence of the immunodominance, that is, the ratio of the T-cell population size of the immunodominant specificity over the subdominant one, on the per DC epitope expression level. Our model predicts that T-cells coexist well at low epitope expression levels, because competition is for the specific epitope. This means that at low epitope expression levels, T-cells only compete with cells of the same specificity (i.e. red T-cells only compete with red ones and blue T-cells only with blue ones). The model predicts that immunodominance becomes more pronounced at higher epitope expression levels. The increased immunodominance at high epitope expression levels is due to competition shifting away from the specific epitope towards an aspecific factor, namely access to the DCs. Hence, at high epitope expression levels, T-cells additionally compete across specificities (i.e. red T-cells also compete with blue ones). In our control experiment, in which the two epitopes are presented on separate DCs, the possibility of competition between T-cells of different epitope specificity is prevented. The table on the right displays our prediction for the experiment. Based on the results of our theoretical model, we expect stronger immunodominance when epitopes are presented together on DCs and epitope expression levels are high.

**Peptides**

Lymphocytic choriomeningitis virus (LCMV)-derived peptides GP34-41 (AVYNFATC, H-2K^b^) and NP396-404 (FQPQNGQFI, H-2D^b^) were synthesised by NeoMPS (Strasbourg, France) in immunograde quality.

**Generation of Bone Marrow-derived DC (bmDC)**

Bone marrow was flushed from femurs and tibias of C57BL/6 mice and subsequently depleted of erythrocytes with ammonium chloride. Cells were cultured at 10^6/ml in RPMI supplemented with 10% FCS and antibiotics and 10 ng/ml of recombinant murine granulocyte-macrophage colony-stimulating factor (GM-SCF containing supernatant from cell line X63-GMSCF, kindly provided by David Gray, Edinburgh, UK). Every second day, 50% of the supernatant was removed and replenished with fresh medium containing GM-SCF. From day eight on, non-adherent cells were collected and further purified over metrizamide (14.5% in RPMI 1640 containing 5% FCS) (Sigma) to remove cell debris and high-density cells. The resulting bmDC populations showed a high purity, with 80 to 90% of the cells showing the distinct stellate DC morphology and
CD11c expression.

Immunisation of mice

Purified C57BL/6 bmDCs were re-suspended in 5 ml of medium containing the peptides GP34-41, NP396-404 or both at a concentration of $10^{-5}$ M (high) or $10^{-9}$ M (low). After incubation for 60 min at 37 °C on a rocking platform, cells were washed with balanced salt solution (BSS) and re-suspended at $2.5 \times 10^6$/ml in BSS. 0.2 ml ($5 \times 10^5$) was i.v. injected into naïve age- and sex-matched C57BL/6 mice. Using this protocol, mice received peptide-loaded DC on days two, four and six after the initial immunisation.

In vivo cytotoxicity assay

On day nine after the initial immunisation, the in vivo cytotoxicity of the GP34-41 and the NP396-404-specific T-cell populations were measured. Splenocytes from naïve C57BL/6 mice were loaded with the GP34-41 peptide, the NP396-404 peptide or both at a concentration of $10^{-5}$ M (high) or $10^{-9}$ M (low) as described above. These were subsequently labelled with 3 μM or 0.3 μM of the fluorescent dye CFSE (5- and 6-carboxyfluorescein diacetate succinimidyl ester, Molecular Probes, Eugene, Oregon) in BSS containing the respective peptides in the respective concentrations. Cells were incubated with CFSE for 10 min at 37 °C. Subsequently, FCS was added to a final concentration of 5%, and the cells were washed once. Cell viability after CFSE labelling was 95%, as determined by trypan blue exclusion. 5 x 10^6 CFSE-labelled, peptide-loaded splenocytes were injected i.v. into age- and sex-matched immunised mice. To determine 0% lysis (i.e. the splenocyte recovery in absence of killing by CTL), cells were injected into age- and sex-matched naïve C57BL/6 mice. Mice were bled 16 h after transfer of cells and the elimination of CFSE-labelled cells was quantified by FACS after gating on live cells.

Staining with Tetrameric MHC Class I-Peptide Complexes

Tetrameric peptide-MHC complexes containing chemically biotinylated H-2D^b or H-2K^b murine class I heavy chains, human β2-microglobulin, the relevant peptide and phycoerythrin-labelled streptavidin were generated as described previously (Altman et al., 1996; Gallimore et al., 1998a). Approximately 10^6 cells were stained with 0.5-1 μg of Tetramer in 25 μl FACS buffer (PBS + 2% FCS + 0.03% NaN3 + 20 mM EDTA) at 37 °C for 10 minutes. Subsequently, 1 μl of anti-CD8α FITC (clone 53-6.7) was added in 25 μl FACS buffer and staining was performed for 20 minutes at 4 °C. Cells were washed two times, erythrocytes were lysed, and cells were fixed (FACS lysing solution, Becton Dickinson, Mountain View, CA) and analysed by flow cytometry (FACS Calibur, Becton Dickinson, Mountain View, CA) using FlowJo Analysis Software (BD Biosciences, Mountain View, CA).

Statistical methods

Statistical analyses were performed in R Version 1.01 (http://www.R-project.org).
Figure 4.3: The experimental set-up of the competition experiment. To test for an effect of epitope expression level on T-cell coexistence, DCs were loaded with mixtures of the gp34 and the np396 epitopes of high ($10^{-5}$) and low ($10^{-9}$) molarity ('Together'). As a control treatment, the two epitopes were loaded onto separate DCs, again with high and low epitope levels ('Separate'). Per treatment group, six B6 mice were injected with $5 \times 10^5$ DCs loaded with the two LCMV epitopes on day zero, two, four and six. On day eight, T-cells specific for the gp34 and the np396 epitope were measured by Tetramer staining with FACS analysis. To measure in vivo T-cell effector function, mice were injected with splenocytes loaded with epitopes according to the respective treatment group, on day nine. This time, $5 \times 10^6$ splenocytes, labelled with different concentrations of CFSE, were injected. 16 hours later, mice were bled and splenocyte recovery was measured with FACS analysis (see Materials and Methods, section 4.3 for further details).
Results

In order to test our theoretical prediction that epitope expression levels affect T-cell coexistence, we set up a competition experiment in C57BL/6 mice (B6 mice). Our prediction is that T-cell responses expand independently of each other when epitope expression levels are low, but that they compete with each other for access to APCs when epitope expression levels are high. To test this, we measured the in vivo expansion of two T-cell populations upon antigenic stimulation by DCs loaded with high or low amounts of the Lymphocytic Choriomeningitis Virus (LCMV) glycoprotein epitope gp34-41Kb (gp34) and the nucleoprotein epitope np396-404Db (np396).

DCs were loaded with a mixture of the gp34 and np396 epitopes of high (10^{-5} Mol) or low (10^{-9} Mol) epitope concentration in the ‘Together’ treatment group (in our pilot experiments (described in Appendix, section 4.6.1), we observed that an epitope concentration of 10^{-9} sufficed to stimulate similar T-cell expansion as with the high epitope concentration). 5 \times 10^5 DCs were transferred every second day from day zero up to day six (i.e. four transfers in total). T-cell responses were measured by Tetramer staining on day eight. As a control treatment, in which the possibility of competition for access to the DCs is excluded, the two epitopes were loaded onto separate DCs (‘Separate’ treatment group). In these groups, 5 \times 10^5 DCs loaded with gp34 plus 5 \times 10^3 DCs loaded with np396 in either high or low epitope concentrations were transferred into the mice (see Fig 4.3). An example of the raw FACS data of the ‘Together-Low’ and ‘Together-High’ treatment are given in Fig. 4.5.

When the gp34 and the np396 epitopes were loaded onto separate DCs, they reached mean levels of 2 and 1 %Tet+ of CD8+ cells respectively, irrespective of the epitope expression levels (Fig. 4.4 A, note the logarithmic scale in the plot). This indicates that the epitope expression levels obtained on DCs with a 10^{-9} M solution are high enough to trigger both gp34 and np396-specific responses. When epitopes were loaded together onto DCs, at low epitope expression levels, similar population sizes as in the separate treatment groups were obtained in five out of six cases. However, one mouse had a larger np396 response of 13.6%. This data-point pulls the mean of the together-low treatment group up to 3.2%. However, when this mouse is excluded, the group means of the gp34 and the np396 responses are similar in the ‘Together-low’ treatment group as in the ‘Separate’ treatment groups, namely 2.2 and 1.1 %Tet+ of CD8+ respectively. In the treatment group in which epitopes were loaded together onto DCs, with high epitope expression levels, the np396 response was approximately half (mean of 0.6%Tet+ of CD8+) and the gp34 response less than double (mean of 3.7%Tet+ of CD8+) in size compared to all other treatment groups.

The prediction of our theoretical model, presented in Chapter 3, that we wished to test in this experiment, is that the relative immune response size of two epitope-specific T-cell populations depends on the epitope expression level (Fig. 4.2). To investigate this prediction in the mouse experiment data, we plot the immunodominance, that is, the rel-
Figure 4.5: T-cell population sizes of gp34 and np396 specific T-cell responses measured by Tetramer staining. Panel A shows the results of the treatment groups in which the gp34 and the np396 epitopes were loaded onto separate DCs and panel B those in which the two epitopes were loaded together onto the DCs. 'Low' epitope expression levels are when DCs were loaded with $10^{-9}$ M solution of epitopes, and 'High' with $10^{-5}$ M. Per mouse, the set of T-cell response sizes against gp34 and np396 is represented in an individual colour, and connected with a coloured line to improve readability. The fat, dotted lines indicate the group means, and the thin dotted line in the together-low group indicates the group mean when the data of the mouse labelled in turquoise is excluded.

Theative immune response size of the gp34 T-cells compared to the np396 specific T-cells (Fig. 4.6). One of the data-points in the ‘Together-High’ treatment group showed an exceptionally high immunodominance of 21. This data-point was taken as an outlier and removed from the data\(^1\). Since the effect that we are testing in the data is that immunodominance is higher at high than at low epitope expression level treatments, taking an outlier with extremely high immunodominance out of the ‘Together-High’ treatment group is conservative. We performed a two-way ANOVA with the main effects ‘DC’ (Separate versus Together) and ‘Epitope expression level’ (Low versus High). Epitope expression level had a significant effect on the immunodominance ($p = 0.002$). This effect was dependent on the expression of the two epitopes on the same DCs, as reflected in the significant interaction between the DC treatment (Separate versus Together) and the epitope expression level ($p = 0.02$).

The analysis with the full data-set (i.e. including the outlier in the ‘Together-High’ treatment group with immunodominance of 21) yielded similar significances for the main effect of epitope expression level and the interaction between the DC treatment and the epitope expression level. However, in this case the immunodominance data had to be transformed by a power of 0.2 prior to the statistical analysis to warrant normal distribution of deviations (data not shown). Non-parametric statistics (Wilcoxon test) support

\(^1\)The standardised residual of this outlier deviated more than 2 standard deviations from the fitted value and its Cook's distance value was to 0.8, implying that the data-point influenced the data-set over-proportionally. After excluding this data-point, the immunodominance data did not deviate significantly from a normal distribution (Kolmogorov-Smirnov test, $p = 0.52$).
Figure 4.6: We use the relative T-cell response size \%Tet34 over \%Tet396 as a measure for immunodominance. Displayed are the data points of the individual mice (black) and the mean ± standard deviation for each treatment group (red). One outlier with an extremely high immunodominance of 21 was removed from the 'Together-High' group. On average over both DC treatment groups, there is a significant effect of epitope expression level on T-cell immunodominance (main effect of epitope expression level treatment, \( p = 0.002 \)). Most importantly, the effect of epitope expression level on immunodominance is different between the 'Separate' and the 'Together' groups (significant interaction term between DC treatment and epitope expression level treatment, \( p = 0.02 \)).

The results reported here are supported by our pilot studies (see Appendix, section 4.6.1).
4.5 Discussion

The question we addressed in this study is whether competition between T-cells of different epitope specificity depends on the level at which epitopes are expressed on APCs. Our working hypothesis was that T-cell competition for access to APCs occurs only when the ratio of T-cells to antigen is high and the per APC epitope expression levels are high. At low epitope expression levels, we predicted competition for the specific epitope (within-specificity competition, see Fig. 4.1 and 4.2). To test this model prediction, we designed a mouse experiment, in which mice were immunised with DCs loaded with high or low concentrations of the LCMV-derived gp34 and np396 epitopes. We find that immunodominance is more pronounced at high than at low epitope expression levels. This supports our prediction concerning the effect of epitope expression levels on T-cell competition.

Data showing immunodominance in T-cell responses is substantial, as are the identified factors influencing these size hierarchies (Deng et al., 1997; Pion et al., 1999; Yewdell and Bennink, 1999; Chen et al., 2000; Belz et al., 2000; Wherry et al., 2003; Probst et al., 2003b; Cho et al., 2003; Crowe et al., 2003; Chen et al., 2004). One of these factors is competition between T-cells of different epitope specificity. The relevance of this factor has been debated, since experiments on T-cell competition have yielded contrasting results (Grufman et al., 1999; Kedl et al., 2000; Probst et al., 2003a; Vijh et al., 1999; Probst et al., 2002). The recent discovery of the partly antigen-independent nature of T-cell expansion, or programmed proliferation (Van Stipdonk et al., 2001; Kaech and Ahmed, 2001), was offered as an explanation for the experimental results that show a lack of competition between T-cell responses of different epitope specificity (Antia et al., 2005). As the initial naive frequencies are usually low compared to the number of relevant APCs, different epitope-specific T-cell populations could expand independently of each other, if T-cells can expand to large numbers without need for re-stimulation. The setting of many T-cells depending on limited antigen signals, which is a prerequisite for T-cell competition to take place (Grufman et al., 1999; Roy-Proulx et al., 2001), might never occur. However, the four to five log expansions of T-cell populations starting out with small precursor frequency of around 10 to 50 cells (Zinkernagel, 1996; Casrouge et al., 2000; Blattman et al., 2002) to epitope-specific T-cell populations of around $10^7$ cells (Murali-Krishna et al., 1998; Miller et al., 2005b) would require proliferative capacities of 14 to 17 rounds of division. This is higher than the five to ten rounds of division typically observed (Van Stipdonk et al., 2001; Kaech and Ahmed, 2001; Krogsgaard et al., 2003), and see also Appendix 2.6.3).

The proposed limiting resource for which T-cells of different epitope specificity compete is their access to APCs. For the mechanism of between-specificity competition to work, T-cells must occupy a part of the APC for a significant time. Recently, T-cells have been observed to interact with epitope-loaded APCs for time-periods in the order of hours (Bousso and Robey, 2003; Mempel et al., 2004). During this time, a so-called immunological synapse can be formed at the interface between T-cell and APC (Krummel and Davis, 2002; Burroughs and Wulfing, 2002). However, other groups have suggested that T-cells collect signals from short, dynamic interactions with multiple APCs (Friedl and Gunzer, 2001). The current view on this topic is that signal acquisition is a mix of both serial dynamic encounters and longer-lasting interactions with APCs (Mempel et al., 2004; Gunzer et al., 2004).
The experimental system used in the life-microscopy study by Bousso and Robey (2003) bears similarity to the ours, and yields valuable insight into the interaction dynamics of T-cells and APCs in this system. Bousso and Robey (2003) used DCs loaded with the LCMV-derived gp33 epitope, that were transferred into B6 mice together with T-cells specific for this epitope. Their results support that (i) T-cell proliferation is not impaired at the low epitope concentration of 1 nM used in our experiment, (ii) clustering on and in some cases saturation of the DC surface occurs at the number of DCs transferred in our experiment (5 × 10^5) and (iii) that clustering is affected by the epitope expression level on DCs, although no images for the 1nM concentration, used in our experiment, were published.

An important difference of the system used by Bousso and Robey (2003) to ours is that both epitope loaded DCs (±5×10^5-2×10^6 cells) and transgenic T-cells (±5-10×10^6 cells), were adoptively transferred. Thus a high ratio of T-cells to DCs is achieved at the onset of the experiment. In our experiment, T-cells are only expected to outnumber the antigen-loaded DCs during the later stage of the experiment, when the epitope-specific T-cell populations have already expanded. With our set-up, in which we injected 5 × 10^5 DCs at two-day intervals, approximately 10^4 DCs are expected to reach the lymphoid tissue. The T-cell populations measured in blood at day eight were around 2% of CD8+ T-cells, which, given an estimated 5 × 10^7 T-cells per mouse (Bachmann et al., 1994), would amount to approximately 5 × 10^5 cells. Hence, the ratio of T-cells to DCs is likely to become as high as 50 in our experiment.

We propose that the effect of epitope expression levels on the coexistence of T-cell responses is due to competition for access to DCs. In ecology, such competition is also referred to as direct resource competition. Alternatively, indirect resource competition, which is also called exploitation competition in ecological literature (Yodzis, 1989), could lead to a similar result. By indirect resource competition, ecologists mean competition by one competitor exploiting and clearing the resource faster than the other competitor. This competition is independent of direct physical interactions of the competitors at the resource. Indirect competition, by rapid clearance of the virally infected cells, has been proposed as a mechanism involved in setting the T-cell response hierarchies in a study of LCMV infection (Weidt et al., 1998). In our experiment, indirect competition could act as follows. The immunodominant gp34 specific T-cell population, which may be present at higher naive precursor frequencies than the sub-dominant np396 specific population, will have an expansion advantage after the first round of DC transfer (see epitope-specific responses specific for gp34 and np396 in pilot experiment 2 after 1x DC transfer, Appendix 4.6.1, Fig. 4.10 A). At subsequent transfers, gp34 specific T-cells might clear DCs rapidly and thereby hinder the np396 specific T-cells from becoming activated. Indeed, when specific T-cells are around, epitope-loaded DCs are cleared before they can activate further T-cells (Hermans et al., 2000). To address the possibility that DC killing might be lower at low epitope expression, we tested the in vivo killing capacities of the expanded T-cell populations. Killing was equally efficient in mice immunised with DCs loaded with high and low epitope expression levels. Therefore we would argue that indirect resource competition does not seem to be the driving force of the observed effect of epitope expression levels on T-cell competition.

Our experimental results suggest that immunodominance through between-specificity competition occurs when expression levels per APC are high. We would therefore speculate that, in the studies in which no evidence for competition between T-cell populations
specific for different epitopes was found (i.e. those by Vijh et al. (1999) and Probst et al. (2002)), expression levels of epitopes were low. Observed ligand densities range from $10^2$ to $10^4$ copies (reviewed in (Stevanovic and Schild, 1999)), and are discussed in more detail in the discussion of Chapter 3.

In acute infection with the Armstrong LCMV strain, the np396 epitope usually expands to larger population sizes than the gp34 response (Murali-Krishna et al., 1998; Tebo et al., 2005). This dominance of np- over gp-specific responses was shown to correlate with differences in timing of epitope expression (Probst et al., 2003b). The fact that gp34-specific T-cell population dominates the np396-specific response in our experiment, in which there is no difference in timing of the expression of the epitopes, might be due to higher precursor frequencies of gp34-specific T-cells than of those with np396 specificity.

Summarising, we propose a competition model in which T-cell populations expand and contract unhindered by between-specificity competition, when epitope expression levels are low (see Fig. 4.7 A). When epitope expression levels are high, T-cells will be affected little during the initial priming phase, because APCs outnumber the T-cells (i.e. at the first and maybe second transfer of DCs). During the later DC transfers, expanded T-cell populations will compete for access to DCs. This competition for access to the APCs is proposed to lead to a more pronounced immunodominance, as observed in our experiment (see Fig. 4.7 B). Contrary to most competition experiments (Kedl et al., 2000; Probst et al., 2002), we did not use adoptive transfer to artificially increase the ratio of T-cells to antigen. We observed competition between T-cells of different epitope specificity in the setting of normal precursor frequencies.

Acknowledgments

We are grateful to Lucy Crooks and Ewalt Scherer for statistical advice.
Figure 4.7: Proposed model of T-cell dynamics in the mouse experiment described in the current chapter. DCs were transferred every second day, and their frequency is displayed as grey filled areas in the graphs. The T-cell densities are represented in coloured lines. Below each schematic representation of DCs and interacting T-cells, a list of potential competitive interactions and their expected strength is depicted. A thin dashed line in the competition table indicates weak or absent competition, and a thick line indicates medium to strong competition. A: In the initial phase of the experiment (during the first one or two transfers of DCs), T-cells do not compete with each other because the ratio of T-cells to APCs is low. When DCs are loaded with low epitope levels, competition over antigen during the later DC transfers is restricted to T-cells of the same specificity. B: At high epitope expression levels, between-specificty competition can result in a more pronounced immunodominance, due to competition for access to the DCs during the later phase of the experiment (e.g. third and fourth DC transfer).
4.6 Appendix

4.6.1 Pilot experiments 1 and 2

The first pilot experiment was conducted with the following treatment factors: (i) with adoptive transfer versus without adoptive transfer, (ii) one time versus four times DC transfer, and (iii) high versus low epitope expression level.

In the first experiment, the expansion of all T-cell populations was rather limited after only one transfer of DCs, and the differences between the transferred and the host gp33 response were rather small. After four rounds of DC transfer, the host T-cell response against the gp33 epitope was severely reduced in size in presence of the adoptively transferred gp33-specific T-cells (left half of Fig. 4.9 B). This is a signal of within-specificity competition, that is typically observed in adoptive transfer experiments. In the mice that received four transfers of epitope loaded DCs, both in those who did and those who did not receive an adoptive transfer of 318 T-cells at day -1, the np396 response is larger in the 'Low' than in the 'High' epitope expression level treatment. This might suggest that the np396 response is suppressed by the gp33 specific T-cells (either of host or of transferred origin) at high epitope expression levels, but not at low epitope expression levels.

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>Experiment 2</th>
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<tbody>
<tr>
<td><strong>DCs</strong></td>
<td><strong>DCs</strong></td>
</tr>
<tr>
<td>1x</td>
<td>4x</td>
</tr>
<tr>
<td>No transfer</td>
<td>2</td>
</tr>
<tr>
<td>Transfer of 318 T-cells</td>
<td>2</td>
</tr>
<tr>
<td><strong>Epitope expression level</strong></td>
<td><strong>Low</strong></td>
</tr>
<tr>
<td><strong>adoptive transfer</strong></td>
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<tr>
<td>Low</td>
<td>2</td>
</tr>
<tr>
<td>High</td>
<td>2</td>
</tr>
</tbody>
</table>

**Figure 4.8:** Experimental set-up of the pilot experiments. **Left:** Experiment 1: The effect of epitope expression level was tested in groups of mice that were immunised with DCs once ('DCs 1x', at day zero) or four times ('DCs 4x', at day zero, two, four and six), either with or without adoptive transfer of \(1 \times 10^6\) 318 T-cells at day -1. 318 T-cells are specific for the epitope gp33-41. Each treatment group contained two replicate mice. **Right:** Experiment 2: Experiment 1 was repeated, this time without the adoptive transfer treatment, and taking three replicate mice per treatment group. DCs were, in both experiments, loaded with \(10^{-9}\) ('Low') or \(10^{-5}\) ('High') molar mixtures of the gp33 and the np396 epitopes. At day eight, epitope-specific T-cell responses were measured with Tetramer staining. In experiment 1, only the gp33 and np396 specific responses were measured while in experiment 2, the gp34 specific response was measured in addition to the gp33 and the np396 response. In both experiments, each DC transfer contained \(1 \times 10^6\) DCs, loaded according to the respective treatment group.

In the second experiment, no adoptive transfer was performed before immunisation of the mice with epitope loaded DCs. In this experiment, the response against the gp34-41 epitope, which is included in the gp33-41 epitope was measured, in addition to the gp33 and the np396 response (the gp34 specific T-cell response is also present in the first experiment, but but measured).

The result from experiment 1, that the np396-specific response was lower in the high than in the low treatment, was not found in experiment 2.
Figure 4.9: Experiment 1: T-cell population sizes of np396 ('h396') specific T-cell responses and gp33 specific T-cell responses of host ('h33') and donor origin ('d33'), measured by Tetramer staining. Panel A shows the results of the treatment groups in which mice were immunised with epitope-loaded DCs only at day zero, and panel B those in which mice were immunised at day zero, two, four and six. 'Low' epitope expression levels are when DCs were loaded with $10^{-9}$ M solution of epitopes, and 'High' with $10^{-5}$ M. Per mouse, the set of T-cell response sizes against gp33 and np396 is represented in an individual colour. The fat lines indicate the group means.

Figure 4.10: Experiment 2: T-cell population sizes of gp33 ('33'), gp34 ('34') and np396 ('396') specific T-cell responses measured by Tetramer staining. All other experimental methods and data labelling as in Fig. 4.9.
Chapter 5

General Conclusions

This thesis has addressed the following questions:

In chapter 2 we asked: What is the effect of epitope down-modulation on the co-existence of T-cells that compete for access to APCs? The results of our mathematical model confirm experimental findings on epitope down-modulation (Kedl et al., 2002), namely that epitope down-modulation shifts competition towards within-specificity competition (see Fig. 5.1). Kedl et al. (2002) proposed that this increased within-specificity competition might explain why the clonal diversity of epitope-specific T-cell responses sometimes decreases over time. We additionally suggest that epitope down-modulation facilitates coexistence of T-cell responses specific for different epitopes of a pathogen that compete for access to APCs.

In chapter 3 we asked: Under what conditions do T-cells compete for access to APCs? Our individual-based model revealed a simple factor that influences the level of T-cell competition, namely the copy number at which epitopes are expressed on APCs. When epitopes are expressed in limiting copy numbers, T-cells compete at the level of the specific epitope, and within-specificity competition is stronger than between-specificity competition (see Fig. 5.1 C). However, when epitopes are not limiting, T-cells compete for access to APCs. When a general resource like access to the APC is limiting, within- and between-specificity competition are equivalent (see Fig. 5.1 D).

In chapter 4, we asked: Is competition between T-cells of different epitope specificity indeed stronger at high than at low epitope expression levels in a mouse model? With our mouse experiment, we confirmed that immunodominance is more pronounced when epitope expression levels are high.

Together, our theoretical studies and mouse experiment suggest that T-cell competition between T-cells of different epitope specificity might only limit diversity if one or some of the pathogen epitopes are expressed at high copy numbers. When epitopes are expressed at low copy numbers, the diversity of the T-cell immune response can be large, but not infinite, because the size of APCs sets a maximum to the breadth of a T-cell response (see Fig. 3.11).
Low ratio of T-cells over APCs

Epitopes on separate APCs

only within specificity competition

High ratio of T-cells over APCs

Epitopes together on APCs

Epitope expression low

within & between specificity competition

Epitope expression high

Epitope down-modulation

Stronger within specificity competition

A

B

C

D

Figure 5.1: Based on the current body of experimental data, and on the results of this thesis, we here describe an extended model for T-cell competition model presented in the introduction of this thesis. The thickness of the arrows between T-cells of the same or of different specificity indicate the strength of competition between them. 

A: If T-cells are present in low numbers, and antigen is abundant, T-cells will not compete with each other. At a high ratio of T-cells to APCs, T-cells can compete with each other. 

B: When epitopes are presented on separate APCs, only T-cells of the same specificity compete. When epitopes are on shared APCs, T-cell competition depends on the epitope expression level. 

C: When epitopes are presented at low epitope expression levels, competition is for the specific epitope, and hence only T-cells of the same epitope specificities compete with each other. At high epitope expression levels, competition is for access to APCs, and hence both T-cells of the same and of different epitope specificity can compete with each other (D). 

Especially at high epitope expression levels, epitope down-modulation might be important to tip the balance of competition more towards within-specificity competition.
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