In: T-Cell Vaccination
Eds: Jingwu Zhang and Irun R. Cohen, pp. 139-158

ISBN 978-1-60021-178-4

Chapter 9

THE ROLE OF TH1/TH2 PHENOTYPES IN T CELL VACCINATION: INSIGHTS FROM A MATHEMATICAL MODEL

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Abstract

We give a historical overview of the diverse theoretical models that have been developed to better understand the role of anti-idiotypic regulation in T cell vaccination (TCV). More recently the importance of the Th1/Th2 phenotype of the T cells involved in TCV became apparent. To understand the combined role of anti-idiotypic regulation and Th1/Th2 phenotype differentiation in TCV we have developed a new phenomenological model. The model consists of four differential equations, one for each cell type involved: Th1 autoreactive cells, Th2 autoreactive cells, Th1 anti-idiotypic cells and Th2 anti-idiotypic cells. To incorporate the interactions between these cell types we used data from experimental autoimmune encephalomyelitis (EAE), the mouse model of multiple sclerosis. In accordance with experiments, our phenomenological model shows that: (i) challenging the autoreactive cells causes transient EAE followed by protection against re-challenges, (ii) after TCV with the Fr3 peptide of the T cell receptor (TCR) of the Th1 autoreactive cells, challenging the autoreactive cells does not lead to disease, and (iii) after nasal instillation of the TCR Fr3 peptide, challenging the autoreactive cells leads to exacerbated disease. In addition, the model gives an explanation for the phenotypic shift from a Th1 autoreactive response towards a Th2 autoreactive response seen after TCV, and it shows that a Th2 response to MBP has the potential to regulate EAE. By reproducing the experiments on TCV in EAE, the model provides a transparent framework pinpointing the interactions and mechanisms that result in the complex behaviour observed in TCV experiments.

1. Introduction

T cell vaccination (TCV) is the process by which the injection of the very cells that cause autoimmune diseases can provide protection against autoimmunity. In order to avoid the induction of autoimmunity, autoreactive cells are either injected at very low concentrations, or are attenuated before injection. It has been shown for several autoimmune diseases, including experimental autoimmune encephalomyelitis (EAE) (1), adjuvant arthritis (2) and insulin-dependent diabetes mellitus (3) that this controlled administration of autoreactive cells can change the state of the immune system from susceptibility to a state in which the animal is protected against induction of the autoimmune disease. The key players in TCV are self antigens, autoreactive T cells and anti-idiotypic T cells which recognize peptides from the T cell receptor (TCR) of the autoreactive T cells.

The coupling of interactions between these key players can give rise to counterintuitive behaviour, which may be difficult to understand by intuitive reasoning alone. Several mathematical and bioinformatic models have been developed to help interpret the ever growing body of empirical data on TCV. The beauty of these models lies in their simplicity; the largest contribution to our understanding of the role of anti-idiotypic regulation in protection against autoimmunity has come from relatively simple models that have generated transparency in the growing body of experimental results on TCV.

In the late 1990s it became clear that besides the presence of anti-idiotypic T cells, the Th1/Th2 phenotype of the T cells involved, plays an important role in the induction of protection against disease (4). This chapter first gives a historical overview of the diverse theoretical models that have been developed to understand the role of anti-idiotypic cells in TCV. Subsequently, a new phenomenological model is introduced incorporating both anti-idiotypic interactions and the influence of Th1/Th2 switches to study their roles in TCV. To build the model we used data from EAE, the mouse model of multiple sclerosis (MS) that shares several of its pathological and immune dysfunctions. Our phenomenological model, including only four cell types, reproduces all experiments on TCV in EAE. It thereby provides a framework pinpointing the interactions and mechanisms that result in the complex behaviour observed in TCV experiments.

2. Experimental findings

It is still not completely understood how MS develops in human individuals, and which regulatory mechanisms fail to maintain immune tolerance. In animal models of MS, MBP-reactive T cells of the Th1 phenotype have been shown to be encephalitogenic, while the presence of MBP-reactive T cells with a Th2 phenotype has been found to protect against EAE (5; 6). Experiments in BL10.PL mice indicated that the pathological T cells involved in EAE have a limited clonal diversity (7). During the course of disease there is a dominant T cell clone reactive to the MBP Ac1-9 peptide, which expands in the lymphoid organs and infiltrates the CNS tissue (7) (see Figure 1A). This T cell clone, which uses the $V\beta 8.2 - J\beta 2.7$ TCR gene segments and has a Th1 phenotype, is lost from these tissues during the spontaneous recovery phase, while many expansions of sub-dominantly Th2 MBP-reactive cells remain (7). During the recovery phase there is an expansion of anti-idiotypic T cells which are reactive to the $V\beta 8.2$ epitope, B5 aa 76-101 (referred to as

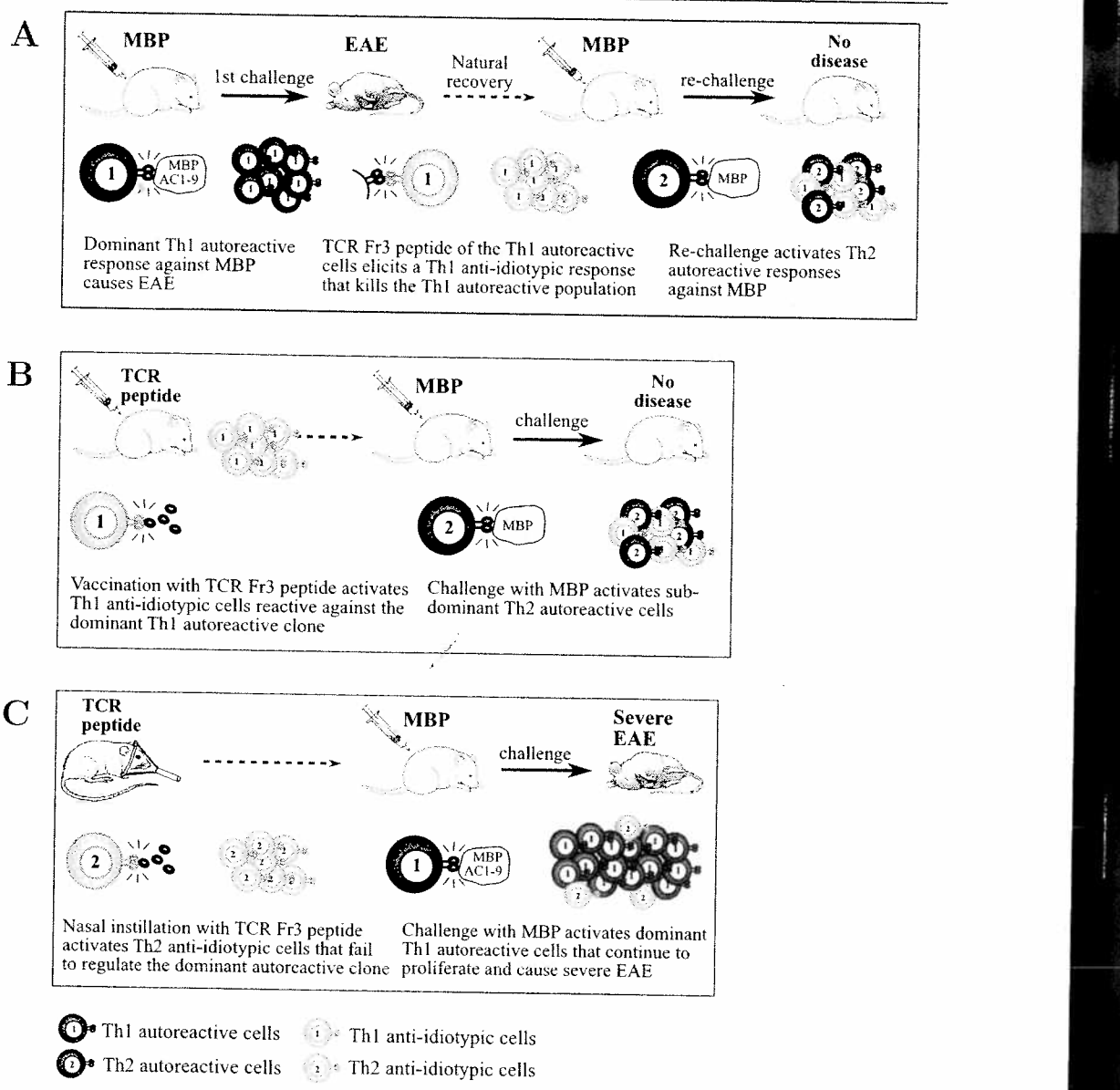

Figure 1. **A**: Challenging BL10.PL mice with MBP activates a dominant Th1 clone reactive against MBP peptide Ac1-9, and causes transient disease. Concomitant to recovery there is an expansion of Th1 anti-idiotypic cells reactive to the *V*β8.2 TCR peptide B5 aa 76-101 (also referred to as the TCR Fr3 peptide) of the dominant autoreactive clone (4). Re-challenge with MBP is not associated with disease and causes expansion of sub-dominant Th2 autoreactive cells. **B**: TCV with the TCR Fr3 peptide activates a Th1 anti-idiotypic clone. MBP challenges after TCV lead to the expansion of sub-dominant Th2 autoreactive clones and are not associated with disease (4). **C**: Nasal instillation of the TCR peptide Fr3 activates a Th2 anti-idiotypic clone. Challenge with MBP activates the dominant Th1 autoreactive clone and leads to severe EAE (4).

the TCR Fr3 peptide) of the TCR of the dominant MBP-reactive T cell clone (4). After recovery, mice are naturally protected against rechallenge with MBP.

The earliest TCV experiments have shown that autoimmunity can be avoided in naive animals by artificial induction of anti-idiotypic cells, either via their direct injection, or by priming them through TCV with attenuated or low doses of MBP-reactive cells, or by vaccination with the TCR Fr3 peptide of the dominant MBP-reactive clone (Figure 1B). More recently, it has been shown that the Th1/Th2 phenotype of the anti-idiotypic cells is important for the success of the induced anti-idiotypic regulation (4; 8; 9). It was demonstrated that only anti-idiotypic cells of the Th1 phenotype provide protection to EAE (9). When anti-idiotypic cells were primed by nasal instillation of the TCR Fr3 peptide, which is known to deviate responses into a Th2 direction (10), no protection from EAE was observed (Figure 1C). Instead this kind of priming led to the contraction of a more chronic and severe form of disease (4). An important difference between Th1 anti-idiotypic and Th2 anti-idiotypic cells is that the anti-idiotypic cells with a Th1 phenotype can recruit $CD8^+$ anti-idiotypic cells, whereas anti-idiotypic cells with a Th2 phenotype cannot. The $CD8^+$ anti-idiotypic cells have been shown to deplete activated $CD4^+$ T cells of only the Th1 phenotype that have a $V\beta 8$ gene segment in their TCR (11). This explains why private expansions of predominantly Th2 MBP-reactive cells persist after recovery from EAE, despite the presence of anti-idiotypic T cells (9; 11). In summary, the Th1/Th2 phenotype of both anti-idiotypic cells and MBP-reactive cells plays a crucial role in the regulation of EAE, but the precise mechanisms by which TCV occurs remain elusive.

3. Historical overview of models for TCV

3.1. Automaton models

The first theoretical study of T cell vaccination was the work of Atlan, Weisbuch, and Cohen (12; 13). This work described several models defining interactions between antigen-specific helper T cells, antigen-specific effector T cells, anti-idiotypic suppressor T cells, anti-idiotypic helper T cells, and antigen-specific suppressor T cells. Ignoring the sizes of these different T cell populations, it was assumed that each T cell population is either "active" (1) or "in-active" (0). By keeping a fixed order of the different T cell populations, the state of the immune system was subsequently described by a vector, in which e.g. "11000" corresponds to a state of autoimmunity in which antigen-specific helper cells and effector cells are active. The model consisted of a series of transition rules between the different states of the immune system.

TCV experiments were modelled by introducing effectors in the normal state "00000" and by applying the transition rules of the model until a stable state of the system was attained. A model like this would account for TCV if the introduction of antigen-specific effector T cells can push the system to a state in which the antigen-specific effector cells are suppressed. It was shown that autoimmunity could never be a stable steady state in this model. Disease was therefore assumed to be due to an inadequate network connection. Cohen & Atlan (12) proposed that TCV might prevent autoimmunity by strengthening the connections in the network.

Our first criticism on this work is that the model is rather loosely connected to the data. It is based upon quite a large number of cell types and it is not obvious which of the five cell types corresponds to which cell type in TCV experiments. Secondly, autoimmune disease

can also be interpreted as a "slow transient" leading to a state of vaccination, and need not be due to an inadequate network connection. A slow transient, however, is ill-defined in an automaton model.

3.2. Reverse engineering

Segel et al. (14; 15) used a very different approach by modelling the phenomenology of TCV, rather than the immunological processes underlying TCV, a method called "reverse engineering." The idea behind this approach is to investigate which set of mathematical models can describe a set of immunological phenomena. These models can subsequently serve as a first step in developing more realistic, data-based models.

The first step was to identify a set of stable steady states that suffices to describe all phenomena related to TCV. Segel et al. (14; 15) defined a normal state in which the number of autoreactive T cells is small, a disease state in which the number of autoreactive T cells is large, and a vaccinated state in which the number of anti-idiotypic T cells is large. They subsequently constructed different mathematical models that could account for these three stable steady states. Secondly the set of initial conditions that led to each of the three stable steady states was defined. Experiments have demonstrated that the effect of the injection of autoreactive cells into a healthy individual is dependent on the dose of the injected cells: the injection of autoreactive cells should take the immune system across a first "critical boundary" beyond which it approaches the vaccinated state, while the injection of even more autoreactive cells would take the system across a second boundary beyond which it approaches the disease state. Based on such constraints, Segel et al. (14; 15) developed a set of models that can account for TCV. Although this reverse engineering approach is quite unusual, it has helped to identify the different types of models that possibly provide insights into TCV, and has suggested novel experiments. The model suggested that inoculating too large a dose of autoreactive cells in an animal suffering from autoimmunity could cure the animal without leading to vaccination (14), and has thereby allowed us to "think the unthinkable" (15).

3.3. Idiotypic network models

Interactions in idiotypic networks have extensively been studied for B cells (16; 17; 18; 19), and were traditionally based on the assumption that idiotypic interactions are symmetric, i.e. that a cell stimulates or inhibits its anti-idiotypic partner as strongly as the anti-idiotypic partner stimulates or inhibits the specific cell. TCV has briefly been investigated in an anti-idiotypic network model (20). However, the assumption of symmetric interactions does not hold for T cells, because a T cell that recognizes the TCR of another T cell via its presentation on MHC molecules, is unlikely to be recognized by the latter T cell.

Borghans et al. (21) developed a mathematical model of EAE that did not assume symmetric interactions between autoreactive and anti-idiotypic T cells. The model showed how complex features of the disease and induction of immunity by TCV could be explained by a few relatively simple interactions between autoreactive and anti-idiotypic T cells. The model was defined in terms of differential equations describing the rate of change of these two T cell populations. The core of the model consisted of two feedback loops: a positive feedback loop between autoreactive T cells and the expression of self antigen, and a

negative feedback loop between autoreactive T cells and anti-idiotypic T cells. The negative feedback loop was based on the experimental observation that anti-idiotypic T cells proliferate in response to autoreactive T cells, while autoreactive T cells are inhibited by anti-idiotypic T cells. The positive feedback loop was based on the assumption that activated autoreactive T cells produce cytokines that enhance the presentation of self antigen (22). Hence autoreactive cells stimulate their own proliferation, leading to clonal expansion and autoimmunity.

The model has two stable steady states, a passive state and an active state of self-tolerance, denoted as the naive state and the vaccinated state, respectively. In the naive state both the autoreactive T cell clone and the anti-idiotypic T cell clone are non-activated, while in the vaccinated state autoreactive T cells are actively suppressed by anti-idiotypic T cells. Disease was defined as a transient of very high autoreactive T cell numbers which could be reached by increasing the number of autoreactive T cells in the naive state beyond a certain threshold. If the increase was sufficiently large (e.g. due to the presence of a self-mimicking antigen), the autoreactive T cells would continue to proliferate and clonally expand until the anti-idiotypic T cell clone would expand as well and suppress the autoreactive T cell clone.

Vaccination in the model corresponded to a switch from the naive state to the vaccinated state without going through this transient of disease. The model predicted qualitatively different results for vaccination with a low-dose of autoreactive T cells, leading to long lasting protection, and vaccination with attenuated autoreactive T cells or anti-idiotypic T cells, both leading to a temporary increase in anti-idiotypic T cell numbers resulting in dose-dependent transient protection from disease.

4. New TCV model including Th1/Th2 differentiation

None of the above models took into account the more recent finding that the Th1/Th2 phenotype of both autoreactive and anti-idiotypic T cells plays an important role in the outcome of TCV. We therefore developed a new model to combine the experimental findings on Th1/Th2 regulation in EAE with the interactions between autoreactive and anti-idiotypic T cells that were described in the previous model (21). In the previous model (21), T cell clones required sustained antigen presentation to remain at an elevated population size. Instead, we modeled a state of immunological memory giving distinct primary and secondary immune responses without the need for sustained exposure to antigen to maintain the memory cells. Below we first describe the phenomenological basis of the new model, then show how we extended the model with differentiation of naive T cells into a Th1 or Th2 phenotype, and finally illustrate how regulation of autoreactive T cells by anti-idiotypic T cells was incorporated to study TCV.

4.1. General T cell model

Our general T cell model combines naive, memory, and effector cells of one particular T cell clone into a single variable T. Changes in the number of cells of this clone are described by:

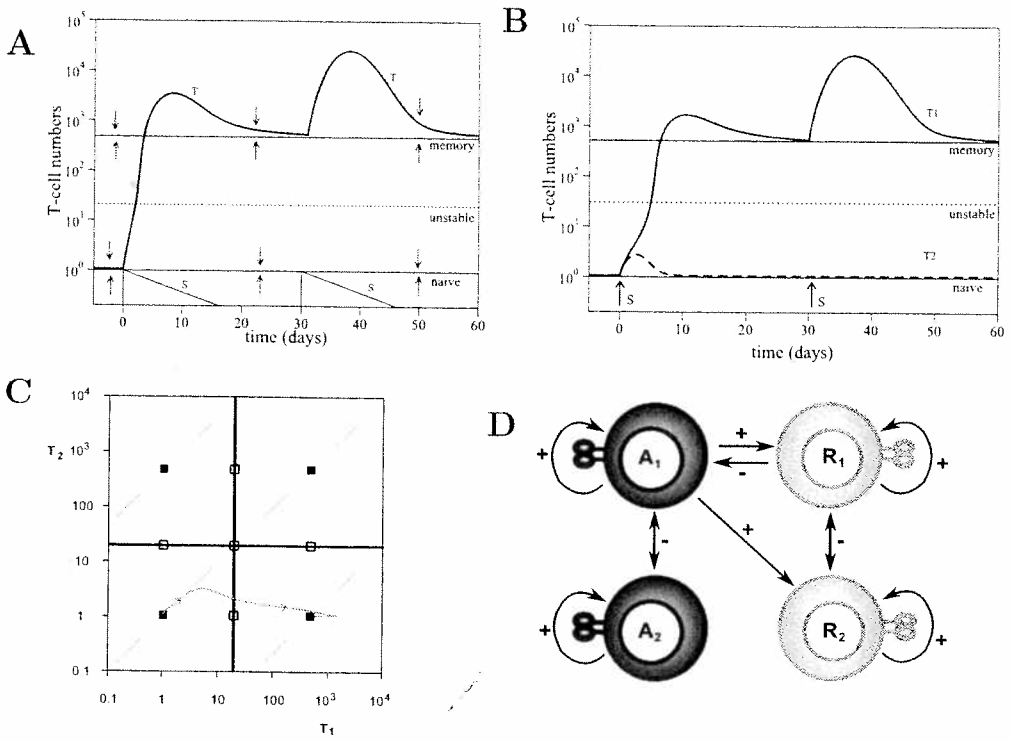

Figure 2. Basis of the new Th1/Th2 model for TCV. A & B: Primary and secondary responses in the general T cell model (A) and the phenomenological Th1/Th2 model (B). Steady states are depicted by horizontal lines; solid lines denote the stable naive and memory states and the dotted line denotes the unstable state. The unstable steady state forms the boundary (separatrix) between the basins of attraction of the stable steady states. Immune responses after primary stimulation at day 0 (S=1), and after re-stimulation at day 30 (S=1) are shown by the thick curves. In (A) arrows indicate the attraction towards the stable steady states and antigen stimulation is denoted by the thin curves. In (B) antigen stimulation is denoted by the arrows and the thick solid curve denotes the Th1 cells and the dashed thick curve denotes the Th2 cells. C: The number of Th1 cells (T1) plotted against the number of Th2 cells (T2) for the Th1/Th2 phenomenological model. Stable and unstable steady states are shown as solid and open squares, respectively. Arrows indicate the attraction towards the stable steady states and bold lines represent the separatrices between the basins of attraction of the steady states. The grey curve depicts the trajectory the model makes after primary stimulation ($S = 1$). D: Schematic representation of the interactions between the T cells of the new TCV model including Th1/Th2 differentiation. A_1= MBP-reactive Th1 cells, A_2= MBP-reactive Th2 cells, R_1= anti-idiotypic Th1 cells and R_2= anti-idiotypic Th2 cells. Parameters: $i = 1$ cell/day, $p = 2$ per day, $m = 500$ cells/day, $h = 100$ cells, $d = 1$ per day, $f = 0.9$, $r = 0.1$, $S(t) = e^{-rt}$.

$$\frac{dT}{dt} = \text{influx} + \text{antigen-dependent proliferation} + \text{memory renewal} - \text{cell death} \quad (1a)$$

$$\frac{dT}{dt} = i + p \cdot T \cdot S + m \cdot \frac{T^2}{h^2 + T^2} - d \cdot T \quad (1b)$$

Here i is a small daily production of naive T cells by the thymus, and possibly naive T cell renewal. Cells die naturally at a rate d per day. Foreign antigenic stimulation, S, which may

vary between zero and one, decays exponentially at rate r per day, i.e. $S(t) = e^{-rt}$, reflecting the rate at which the foreign antigen is cleared. Antigen-dependent T cell proliferation occurs at maximal rate p per day when antigen stimulation is maximal, i.e. $S = 1$. If the T cell population size becomes large enough, the threshold for memory T cell induction is breached. At a clonal population size of $T = h$, memory renewal division proceeds at half its maximal rate of m cells per day.

When parameters are set properly, this equation allows for maximally three steady states. The first steady state is the state in which the T cell clone is naive and there is no T cell memory because it has not yet encountered (enough) antigen ($T \ll h$). Because the contribution of the proliferation term and the memory renewal term can be neglected in this state, the number of T cells in the naive steady state is approximately $T = i/d$ cells. When a naive T cell clone is temporarily exposed to antigen with the appropriate co-stimulatory signals, antigen-dependent proliferation sets in and initiates clonal expansion. As a result, the population size breaches the threshold for memory induction. When memory sets in, another steady state can be reached. Once the antigen has been cleared, and the T cell clone is sufficiently large, the model can be simplified into $\mathrm{d}T/\mathrm{d}t = m \cdot \frac{T^2}{h^2+T^2} - d \cdot T$ because the small production i by the thymus has a negligible contribution compared to memory T cell renewal divisions. Calculating the steady states of this equation shows that there is a large stable steady state of $T \simeq m/d - \frac{d \cdot h^2}{m}$ cells and a smaller unstable steady state of $T \simeq \frac{d \cdot h^2}{m}$ cells. The above described total of three steady states are obtained if the threshold for memory T cell induction is substantially higher than the naive clonal population size, i.e. if $h \gg i/d$.

To illustrate that the above model correctly describes naive and memory responses to antigen, we simulated the response of a T cell clone to repeated antigenic stimulation (Figure 2A). When a naive T cell clone was challenged with antigen at day 0, the T cell clone expanded and breached the threshold for the induction of memory T cells. A primary immune response was initiated reaching a peak of $\pm$ 4000 cells at approximately seven days post-stimulation. When the stimulus decreased, memory renewal divisions took over and maintained the T cell clone at approximately 500 cells. When this T cell clone was rechallenged with the same antigen at day 30, the secondary immune response reached a peak of $\pm$ 20000 T cells and eventually returned to the same memory state.

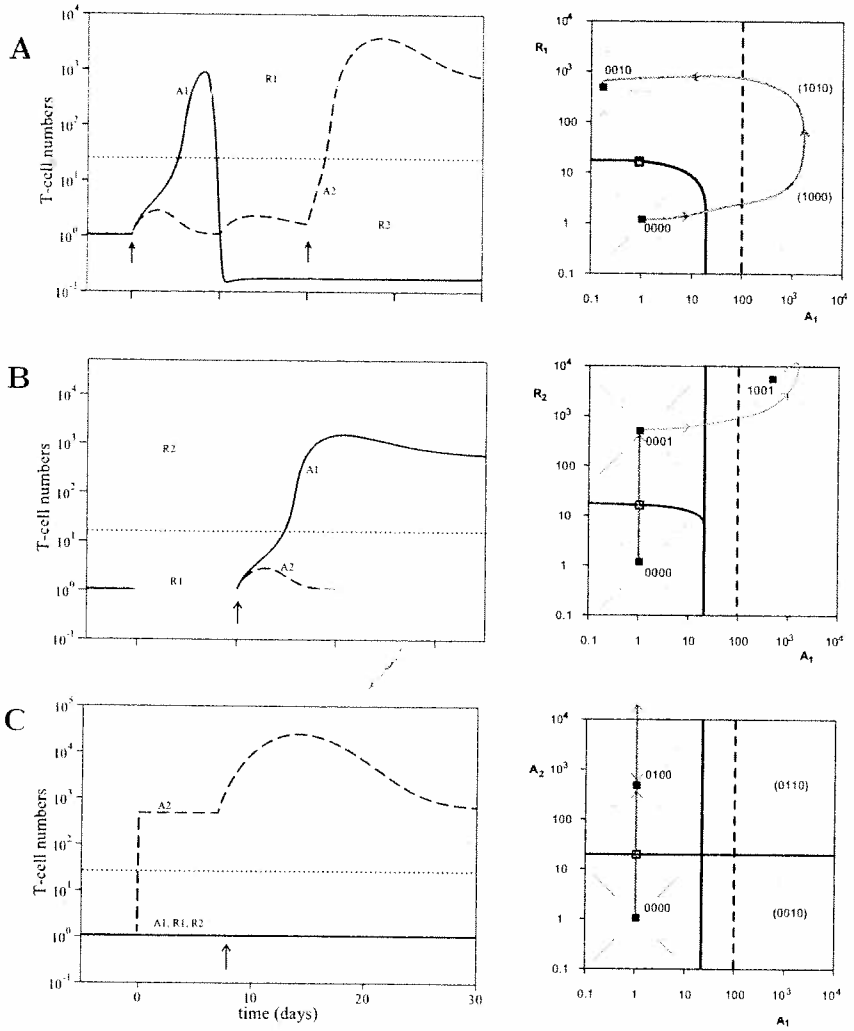

Figure 3. Simulation in the new model with Th1/Th2 differentiation and anti-idiotypic regulation. The left hand panels depict time plots, the right hand panels depict phase diagrams. In the time plots stimulation of the autoreactive cells is depicted by the small arrows and the threshold for memory by the dotted horizontal line. In the phase diagrams stable and unstable steady states are depicted by solid and open squares respectively. Thick solid lines depict the separatrices between the stable steady states and the arrows indicate the direction of the attraction. The region in which the Th1 autoreactive population is large is interpreted as "disease" and reached when the system crosses the thick dashed line. Trajectories the model makes through the diagrams after stimulation ($S = 1$) are depicted by the grey curves. A: The effect of primary stimulation and secondary challenge with MBP. Right: Only primary stimulation is shown. B: Simulation of nasal instillation with the TCR Fr3 peptide and subsequent challenge with MBP on day 10. Nasal instillation activates Th2 anti-idiotypic cells and was simulated by setting the Th2 anti-idiotypic cells at their memory state at t=0. C: Protection by phenotype. The system was stimulated on day 7, in the presence of Th2 autoreactive memory cells to see if they are protective. Parameters: $i = 1$ cell/day, $p = 2$ per day, $f = 0.9$, $m = 500$ cells/day, $h = 100$ cells, $d = 1$ per day, $r = 0.1$ per day, $c = 0.1$, $h_R = 10$ cells, $k = 0.01$ per day.

4.2. A Phenomenological Th1/Th2 model

Model description

The balance between Th1 and Th2 responses has been modeled before, among others by Fishman et al. (23), Louzoun et al. (24) and Yates et al. (25). These earlier models were quite detailed, in that they were based on the specific influences of various cytokines. In order to understand the effect of the Th1/Th2 switch on TCV, we prefered a simpler caricature model for the Th1/Th2 balance; this allowed us to focus on the effect of T cell phenotypes in a complex multi-cellular regulatory system, rather than on the precise Th1/Th2 molecular machinery.

Differentiation of a naive T cell towards a Th1 or Th2 phenotype is dependent on its cytokine environment. Since T-helper cells secrete cytokines that enhance differentiation into their own phenotype and inhibit differentiation into the opposite phenotype, we made T cell differentiation dependent on the fraction of Th1 and Th2 cells present. To keep track of the number of Th1 and Th2 cells, the general T cell model was applied to Th1 and Th2 cells separately:

$$\frac{dT_1}{dt} = i + p \cdot T_1 \cdot S \cdot v + m \cdot \frac{T_1^2}{h^2 + T_1^2} - d \cdot T_1 \tag{2a}$$

$$\frac{dT_2}{dt} = i + p \cdot T_2 \cdot S \cdot (1 - v) + m \cdot \frac{T_2^2}{h^2 + T_2^2} - d \cdot T_2 \tag{2b}$$

Here, T_1 and T_2 represent the number of T cells with a Th1 or Th2 phenotype, respectively, and v ($0 < v < 1$) is a function representing the influence of the Th1 and Th2 cells on the differentiation of naive cells into Th1 cells:

$$v = \frac{T_1}{T_1 + f \cdot T_2} \tag{3}$$

Because the influence of cytokines is most important during the onset of the initial immune response, when the activated T cell clone is maturing into one particular phenotype, the function v only influences antigen-dependent proliferation. We assumed that daughter cells arising by memory renewal take over the same phenotype as their progenitors. Indeed, it has been shown that memory T cells of either the Th1 or Th2 phenotype, are much less likely to switch to the opposite phenotype than naive T cells, when exposed to changes in the cytokine environment (26; 27). The parameter f was used to favour differentiation into either of the two phenotypes. Because the dominant T cell response in mice with MBP-induced EAE is typically of the Th1 phenotype (5) we set $f = 0.9$, thereby introducing a small bias in favour of Th1 differentiation (i.e. at equal Th1 and Th2 densities, $v = 0.53$). Every clone alone has its own independent memory-renewal term that will maintain a population of memory cells once the threshold for memory induction is breached.

Steady state description

Since the v-function is the only difference between the the general T cell model and the equations of the phenomenological Th1/Th2 model, each phenotype equation has the same

two stable steady states as the general T cell model: the naive state and the memory state. We will represent the different steady states by assigning '0' to naive clones and '1' to memory clones, in the order '$T_1 T_2$'. Thus, the naive state is denoted by '00', the Th1-dominated memory state by '10', the Th2-dominated memory state by '01', and the state with both memory phenotypes by '11'. In our model the latter state cannot be attained through antigenic stimulation, and is only reached if memory Th1 and Th2 cells are artificially brought together.

Like before, we simulated the response to repeated stimulation with antigen, now following both the Th1 and the Th2 cells. After antigen stimulation at day 0 ($S = 1$), at first both clones expanded, but since the model favours differentiation into the Th1 phenotype, the Th2 clone was rapidly inhibited by the more dominant Th1 response. Stimulation was strong enough to drive the Th1 population over the threshold for memory induction, so that a stable Th1-dominated response (10) was maintained in the absence of stimulation (Figure 2B). The same behaviour can be depicted by plotting the number of Th1 autoreactive cells against the number of Th2 autoreactive cells (Figure 2C). Antigenic stimulation pulled the system out of the basin of attraction of the naive steady state, into the basin of attraction of the Th1 dominated memory state 10. In this 10 memory state the function v approximated one, making it impossible to obtain a Th2 response by re-stimulation with antigen as shown in Figure 2B where the system was re-stimulated at day 30.

4.3. TCV model with anti-idiotypic regulation and Th1/Th2 differentiation

We used the phenomenological Th1/Th2 model described above to extend the model for anti-idiotypic regulation in EAE (21). The model consists of four $CD4^+$ T cell clones, a Th1 autoreactive clone (A_1), a Th2 autoreactive clone (A_2), a Th1 anti-idiotypic clone (R_1) and a Th2 anti-idiotypic clone (R_2). The Th1 autoreactive clone, A_1, is the dominant $V\beta 8.2 - J\beta 2.7$ T cell clone, which induces a response against the immuno-dominant MBP peptide Ac1-9 in BL10.PL mice. The Th2 autoreactive cells are stimulated by other, subdominant, MBP epitopes, and have a different T cell receptor (7). In contrast, both Th1 anti-idiotypic cells and Th2 anti-idiotypic cells are of the same clonal origin and reactive to the same peptide, the dominant TCR peptide Fr3 of the Th1 autoreactive T cells. As a consequence, only the Th1 autoreactive cells are down-regulated by anti-idiotypic cells. Since only Th1 anti-idiotypic cells can activate $CD8^+$ cytotoxic T cells, they are the ones that have an inhibitory effect on the autoreactive Th1 cells while the Th2 anti-idiotypic cells do not have any direct influence on the autoreactive Th1 clone (7).

Because both Th1 autoreactive cells and Th2 autoreactive cells are activated by MBP on probably the same APCs, the cytokine environment in which both clones are activated is expected to be very similar. We therefore assumed that upon activation, Th1 autoreactive cells and Th2 autoreactive cells exert an inhibitory effect on each other as described in the phenomenological Th1/Th2 model. The same was done for the inhibitory effect between the Th1 anti-idiotypic cells and Th2 anti-idiotypic cells which are both activated by the same peptide. The above mentioned interactions between the autoreactive Th1 and Th2 clones and the anti-idiotypic Th1 and Th2 clones are represented schematically in Figure 2D, and are mathematically described by the following differential equations:

$$\frac{dA_1}{dt} = i + p \cdot A_1 \cdot S \cdot v_A + m \cdot \frac{A_1^2}{h^2 + A_1^2} - d \cdot A_1 - k \cdot A_1 \cdot R_1 \quad (4a)$$

$$\frac{dA_2}{dt} = i + p \cdot A_2 \cdot S \cdot (1 - v_A) + m \cdot \frac{A_2^2}{h^2 + A_2^2} - d \cdot A_2 \quad (4b)$$

$$\frac{dR_1}{dt} = i + p \cdot R_1 \cdot S_R \cdot v_R + m \cdot \frac{R_1^2}{h^2 + R_1^2} - d \cdot R_1 \quad (4c)$$

$$\frac{dR_2}{dt} = i + p \cdot R_2 \cdot S_R \cdot (1 - v_R) + m \cdot \frac{R_2^2}{h^2 + R_2^2} - d \cdot R_2 \quad (4d)$$

where

$$v_A = \frac{A_1}{A_1 + f \cdot A_2} \quad (5)$$

and

$$v_R = \frac{R_1}{R_1 + f \cdot R_2} \quad (6)$$

Note that anti-idiotypic $CD8^+$ cells are not modelled explicitly; instead, their recruitment is assumed to be proportional to the number of Th1 anti-idiotypic cells (A_1). Therefore the anti-idiotypic suppression term is $-k \cdot A_1 \cdot R_1$, with a suppression rate k per R_1 cell per day.

To model the antigenic stimulation of the anti-idiotypic cells we adopted the stimulation function proposed by Borghans et al. (21), which saturates as a function of the number of Th1 autoreactive cells, and which involves competition for antigen binding between anti-idiotypic cells:

$$S_R = \frac{A_1}{A_1 + c \cdot (R_1 + R_2) + h_R} \quad (7)$$

Here, c determines the strength of the competition, and prevents unrealistically high levels of anti-idiotypic cells during sustained antigen stimulation. In the absence of competition ($c(R_1 + R_2) = 0$), half-maximal stimulation is reached at $A_1 = h_R$ Th1 autoreactive cells. The parameter h_R thus determines the sensitivity of the anti-idiotypic cells for activation by the Th1 autoreactive cells. There is no direct interaction between the Th2 autoreactive cells and any of the anti-idiotypic cells (see Figure 2D). In fact, the only influence the Th2 autoreactive population exerts on the system is its inhibitory effect on the differentiation of autoreactive cells into Th1 cells.

Steady state description

Just like in the general T cell model, every clone can only be in either of two stable steady states, the stable naive or the stable memory state. Without interactions between the clones this yields a maximum of $2^4 = 16$ stable steady states. Again, steady states are represented by assigning '0' to naive clones and '1' to memory clones; 'x' is used to denote that a clone can be either naive or memory, and the states of the clones are given in the order '$A_1A_2R_1R_2$'.

In the presence of interactions between the clones there are fewer stable steady states. We chose h_R high enough to ensure that a naive Th1 autoreactive cell population (A_1) would not cause expansion of the anti-idiotypic cells ($h_R \gg i/d$) and small enough to allow the anti-idiotypic cell population to expand beyond the memory threshold when the Th1 autoreactive cells approach their memory state ($h_R \ll m/d - \frac{d \cdot h^2}{m}$). By doing so, the 1x00 states no longer existed, because the memory Th1 autoreactive cells would stimulate the anti-idiotypic cells to expand.

The suppression parameter was set such that once the Th1 anti-idiotypic cells (R_1) have reached their memory state, their influence would be strong enough to keep the Th1 autoreactive cells (A_1) at a very low level. Consequently, the 1x1x states were also no longer steady states of the model, because the anti-idiotypic suppression ensures that memory Th1 autoreactive cells and memory Th1 anti-idiotypic cells cannot exist together.

The above parameter setting removed six steady states, i.e., $2\times$ (1x00) + $4\times$ (1x1x), from the model leaving 10 stable steady states (see Table 1).

4.4. Results

Antigen-induced EAE and re-challenge

We simulated antigen-induced EAE (experiment in Figure 1A) by starting the system in the naive steady state (0000) and setting $S = 1$ at day 0, which stimulates both autoreactive clones (Figure 3A). Immediately after the antigenic stimulation, clonal expansion of both autoreactive clones started at maximal proliferation rate p. Due to the imposed Th1 bias ($f = 0.9$), the autoreactive response differentiated into a Th1 phenotype, and within a few days the Th2 autoreactive cells fell back to their naive level (Figure 3A). Stimulated by the expansion of the Th1 autoreactive cells both anti-idiotypic populations started to expand. Just like the autoreactive response, the anti-idiotypic response differentiated into a Th1 phenotype within a few days, and the Th2 anti-idiotypic (R_2) cells started to fall back to their naive level. Subsequently, the expanding Th1 anti-idiotypic (R_1) cells started to suppress the Th1 autoreactive (A_1) cells. By the time the Th1 anti-idiotypic population approached its memory level, the Th1 autoreactive population had decreased dramatically from $\pm$ 1000 cells until below the naive level. Deprived of antigenic stimulation due to this drop in the number of Th1 autoreactive cells, the Th1 anti-idiotypic population started to fall back to its memory level. This stable steady state 0010 was subsequently maintained by memory renewal divisions of the Th1 anti-idiotypic cells. The same behaviour was observed when the number of Th1 autoreactive cells (R_1) was plotted against the number of Th1 anti-idiotypic cells (Figure 3B). Antigenic stimulation pulled the system out of the basin of attraction of the naive steady state, through the region of disease and then towards steady state 0010. Stimulating the autoreactive Th1 cells with antigen thus led to a temporal increase in the number of autoreactive cells, which got cured by the induction of anti-idiotypic Th1 cells, in line with the experimental observations in mice (Figure 1A).

Since most animals that have contracted EAE are subsequently resistant to the disease, we tested what happens if the autoreactive cells in the 0010 steady state are re-stimulated by antigen at day 20 (Figure 3A). The Th2 autoreactive cells could expand in response to the stimulation, because they were not inhibited by Th1 autoreactive cells, which were still suppressed by the Th1 anti-idiotypic clone. The stimulation brought the Th2 autoreactive

cells, which are not suppressed by anti-idiotypic cells, over the threshold for memory induction. However, the presence of Th2 autoreactive cells failed to influence the other T cell populations, and once the antigenic stimulation had faded, the system approached the steady state with Th2 autoreactive and Th1 anti-idiotypic memory cells (0110, Figure 3A day 40). Thus re-challenge did not lead to an expansion of disease-causing Th1 autoreactive cells (Figure 3A), in accordance with the experimental results (Figure 1A). Follow-up stimulations of the autoreactive cells caused the Th2 autoreactive memory cells to expand temporarily, as long as the stimulation lasted, but the system always returned to the same steady state (0110, not shown).

T cell vaccination with TCR Fr3 peptide

TCV with the TCR Fr3 peptide of the autoreactive cells, activates Th1 anti-idiotypic T cells (R1). We therefore simulated TCV in a Th1 context by initializing the Th1 anti-idiotypic cells at their memory level, i.e. by initiating the system at steady state 0010. When the autoreactive cells in the system were subsequently stimulated ($S = 1$), only the Th2 autoreactive cells responded to the stimulation and after the antigen was cleared the system approached state 0110 (similar to Figure 3A, day 20). Thus, in accordance with the experimental findings (Figure 1), TCV in a Th1 context did not cause autoimmune disease.

Nasal instillation with TCR Fr3 peptide

We also simulated nasal instillation with TCR Fr3 peptide, by setting the Th2 anti-idiotypic cells at their memory level, i.e. by starting the system in state 0001 (Figure 3B). When the autoreactive cells were subsequently stimulated with antigen on day 10 ($S = 1$), the autoreactive response rapidly differentiated into a Th1 phenotype. Stimulated by the expansion of the Th1 autoreactive cells, the Th2 anti-idiotypic memory population started to expand suppressing the activation of the Th1 anti-idiotypic cells. When the antigen was cleared, the Th1 autoreactive population fell back to its memory level. The Th1 autoreactive memory population continued to stimulate the Th2 anti-idiotypic population, leading the system to state 1001. The same behaviour can be depicted by plotting the number of Th1 autoreactive cells against the number of Th2 anti-idiotypic cells (Figure 3D). Antigenic stimulation in state 0001, pulled the system out of the basin of attraction of the 0001 state, into the disease region, towards steady state 1001.

Antigen challenge after TCV by nasal instillation thus led the system to a state of permanent autoimmunity in the presence of Th2 anti-idiotypic cells (Figure 3B). This is in line with the experimental observation that mice that are challenged with antigen after TCV through nasal instillation get severe EAE and do not recover (Figure 1C). Re-stimulation of the autoreactive cells in this state led to an even further, temporary, expansion of the Th1 autoreactive population followed by a temporary re-expansion of the Th2 anti-idiotypic population. After the antigen had been cleared the system returned to the 1001 state (not shown).

Protection by Th2 autoreactive cells

From experiments on EAE in mice it became clear that Th2 responses to MBP do not cause disease (7; 28), and can even be protective (7; 8). To study the influence of Th2 autoreactive cells in our model, the system was initiated in the state 0100, in which only autoreactive Th2 memory cells are present. When both autoreactive T cell clones were subsequently stimulated by antigen, only the Th2 autoreactive T cell clone expanded, because of the memory Th2 cells already present (Figure 3C). The autoreactive Th1 cells failed to respond because they were suppressed by the autoreactive Th2 memory cells. Both anti-idiotypic clones R_1 and R_2 remained in their naive state, because the Th2 autoreactive cells failed to stimulate them. The same behaviour was depicted by plotting the number of Th1 autoreactive cells (A1) against the number of Th2 autoreactive cells (A2) (Figure 3D). The system was pushed out of state 0100 by the antigenic stimulation but could not leave the basin of attraction of state 0100, and was therefore pulled back to state 0100 after the stimulation faded away. These simulations suggest that 0100 is an alternative "vaccinated" state in the absence of anti-idiotypic regulation, in which Th2 autoreactive cells avoid the outgrowth of Th1 autoreactive cells.

Summary of the results

The above in silico experiments show that the effect of stimulating autoreactive cells depends crucially on the initial state of the system. To summarize all possible effects, we have stimulated the autoreactive T cells in all stable steady states, and categorized them according to the result of the stimulation (Table 1). The stable steady states of this four-dimensional model can be categorized as follows: 'Naive/Vulnerable' (0000, 0001), 'Vaccinated' (0100, 0010, 0110, 0101, 0011, 0111) and 'Disease' (1001, 1101). In the vaccinated states the system is protected from disease since stimulation does not lead the system through the disease region. The vulnerable states do not lie within the disease region, but stimulation starting from such a state will bring the system to a state in the disease region. Within the logic framework of Table 1 we depicted the activation or depletion of the four different T cell populations during these stimulations without drawing a full time plot (like was done in the early automaton models (12; 13)).

5. Discussion

Over the last years the use of models that attempt to provide a full description of the immune system has gained considerable popularity (eg. IMMSIM (29; 30) and Simmune (31)). Such general models attempt to include all of the detailed immunological knowledge that is available. There is no chance, however, that any model will ever be complete, no matter how much detail is incorporated, and a great disadvantage of this complex modelling approach is that it hampers the interpretation of the results, and thereby often fails to deliver insights into the experimental findings. We are convinced that the beauty of more conceptual models lies in their simplicity and transparency, which truly helps to improve our insights into complex systems like the immune system (32). In that respect we fully agree with the previously made statement: "All models are wrong, but some are useful" (33), and although many

Table 1. Behavior of the four-dimensional model in a logical framework. A: Stable steady states approached when the system is initiated in either of the 16 steady states of the model. The stable steady states are shown in boldface. The arrows indicate the states passed when converging from each of the possible steady states to the stable steady states. B: The response of the model to antigenic stimulation of the MBP-reactive cells, $S(t) = e^{-rt}$, starting from the ten stable steady states. Stimulation is indicated by $\Rightarrow$.

A	**All steady states**	**B**	**Naive/Vulnerable**
	0000		$0000 \Rightarrow 1000 \rightarrow 1010 \rightarrow 0010$
	0001		$0001 \Rightarrow 1001$
			Vaccinated
	$1000 \rightarrow 1010 \rightarrow$ **0010**		$0010 \Rightarrow 0110$
	$1011 \rightarrow$ **0011**		$0011 \Rightarrow 0111$
	0100		$0100 \Rightarrow 0100$
	0101		$0101 \Rightarrow 0101$
	$1100 \rightarrow 1110 \rightarrow$ **0110**		$0110 \Rightarrow 0110$
	$1111 \rightarrow$ **0111**		$0111 \Rightarrow 0111$
			Disease
	1001		$1001 \Rightarrow 1001$
	1101		$1101 \Rightarrow 1101$

models "certainly do not capture the reality in full, and no model does ... they capture certain aspects and give a general direction on how to understand the issue better (34)."

Despite the relative simplicity of our mathematical model for the role of Th1/Th2 phenotypes in TCV, all of the in silico experiments are in good agreement with the experimental observations on TCV. The model accounts for i. transient disease after a first encounter with an MBP-mimicking antigen, ii. protection from EAE after TCV in a Th1-mode, iii. exacerbation of disease after TCV in a Th2 context and, iv. the shift from a Th1-dominated MBP-reactive response to a Th2-dominated response after TCV. All of these phenomena are apparently a natural consequence of the interactions between the different T cell populations involved.

The fact that anti-idiotypic cells could shift the phenotype of autoreactive cells from a Th1 to a Th2 phenotype following TCV was initially conceived with surprise (35). Our modelling results show, however, how such a Th1/Th2 shift of autoreactive cells results from the interactions between anti-idiotypic cells and Th1 and Th2 autoreactive cells, without the need for any direct influence of anti-idiotypic cells on the phenotype of autoreactive cells. The shift arises naturally because Th1 anti-idiotypic cells only suppress the Th1 autoreactive cells, and thus allow the Th2 autoreactive cells to proliferate in response to MBP stimulation.

Our previous model (21) already showed the general features of TCV in EAE by modeling anti-idiotypic regulation alone. There are, however, several important differences between the current and the previous model. In the new model autoreactive T cells can be

maintained by memory renewal, while in the previous model they could only be maintained by the positive feedback between autoreactive cells and the presentation of their self peptide. As a result, the previous model showed a qualitative difference between vaccination with attenuated autoreactive cells, and vaccination with a low dose of live autoreactive T cells (21). Vaccination with attenuated autoreactive cells could only give rise to transient protection from disease, because it failed to trigger the positive feedback loop between autoreactive cells and self antigen, while low-dose vaccination with live autoreactive cells could give rise to long-lasting protection from disease. In the new model both methods of vaccination give rise to long-lasting protection, because Th1 anti-idiotypic cells can be maintained by memory renewal.

Another characteristic of the previous model was that it could explain relapsing-remitting disease by changing only one parameter of the model (21). Relapsing-remitting disease characterized by sporadic attacks (relapses) followed by a period of partial or total recovery (remission) is commonly seen in MS patients. In the current model we have not yet found a parameter setting that could give rise to such relapses, which does not exclude the possibility that a more extensive parameter search would have revealed this kind of dynamic behaviour. Alternatively, relapses may be due to other mechanisms that are not included in the models, such as the emergence of new autoreactive clones. Indeed, in clinical trials of TCV in MS patients, new T cell responses against MBP with similar functional properties but different clonal origins compared to the cells that were used for TCV have repeatedly been observed a few years after TCV (36). Such novel autoreactive responses against MBP have, however, never been observed after TCV in mice. A possible explanation for this discrepancy is that most experiments on TCV in mice have been performed on H-2^u strains of BL10.PL mice. These mice have a T cell repertoire of which 60% expresses the $V\beta 8$ chain (personal communication V. Kumar). When Th1 anti-idiotypic cells become activated they can down-regulate all activated Th1 cells expressing this TCR chain, which should leave the mice with only 40% of their repertoire.

Summarizing, simple mathematical models have contributed significantly to our understanding of the role of anti-idiotypic regulation in TCV. By also including Th1/Th2 phenotype switches, the current TCV model helps to structure TCV experiments by showing how the large variety of experimental observations on TCV naturally results from a few relatively simple interactions between Th1 and Th2 autoreactive and anti-idiotypic cells.

Very recently, a novel population of nonintestinal CD8alphaalpha+TCRalphabeta+ regulatory T cells (Treg) has been shown to be capable of controlling the activated V β8.2+ CD4+ T cells that mediate EAE. Adoptive transfer or in vivo activation of these CD8+ Treg cells was shown to prevent the induction of EAE (37; 38). Extension of the here proposed model with this class of CD8+ Treg cells may shed light on the relative contribution of anti-idiotypic CD4+ T cells and these CD8+ Treg cells in TCV experiments.

Acknowledgements

The authors would like to thank Vipin Kumar and Eli Sercarz for extensive discussions on the topic of TCV, and are grateful to Vipin Kumar for critically reading the manuscript.

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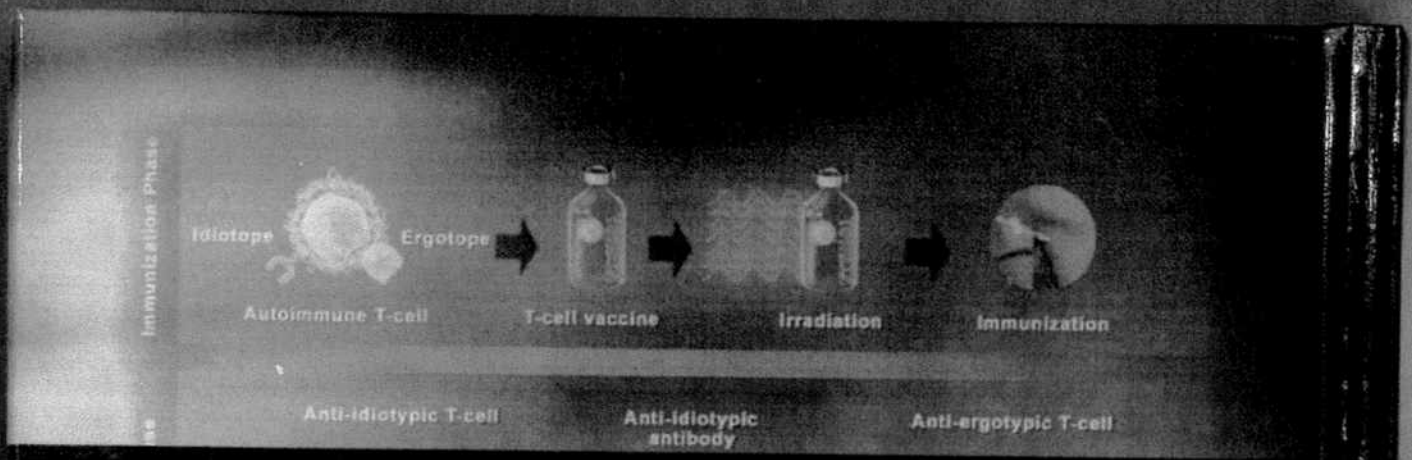

T-CELL VACCINATION

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www.novapublishers.com

Nova Biomedical

ISBN-13: 978-1-60456-178-4
ISBN-10: 1-60456-178-5

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