T Cell Vaccination in Experimental Autoimmune Encephalomyelitis: A Mathematical Model

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T Cell Vaccination in Experimental Autoimmune Encephalomyelitis: A Mathematical Model

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T cell vaccination (TCV) is a method to induce resistance to autoimmune diseases by priming the immune system with autoreactive T cells. This priming evokes an anti-idiotypic regulatory T cell response to the receptors on the autoreactive T cells. Hence resistance is induced. To prevent the inoculated autoreactive cells from inducing autoimmunity, cells are given in a subpathogenic dose or in an attenuated form. We developed a mathematical model to study how the interactions between autoreactive T cells, self epitopes, and regulatory cells can explain TCV. The model is based on detailed data on experimental autoimmune encephalomyelitis, but can be generalized to other autoimmune diseases. We show that all of the phenomena collectively described as TCV occur quite naturally in systems where autoreactive T cells can be controlled by anti-idiotypic regulatory T cells. The essential assumption that we make is that TCV generally involves self epitopes for which T cell tolerance is incomplete. The model predicts a qualitative difference between the two vaccination methods: vaccination with normal autoreactive cells should give rise to a steady state of long lasting protection, whereas vaccination with attenuated cells should only confer transient resistance. Moreover, the model shows how autoimmune relapses can occur naturally without the involvement of T cells arising due to determinant spreading. The Journal of Immunology, 1998, 161: 1087–1093.

Paradoxically, many autoimmune diseases can be prevented or ameliorated by priming the immune system with autoreactive T cells. This priming evokes a regulatory T cell response to the receptors on the autoreactive T cells, which induces resistance to autoimmunity. To prevent the autoreactive cells from inducing autoimmunity, they are given in a subpathogenic dose (1, 2) or in an attenuated form (3, 4). This vaccination method, termed T cell vaccination (TCV),3 has been successful against several autoimmune diseases, including experimental autoimmune encephalomyelitis (EAE) (3), adjuvant arthritis (5), autoimmune thyroiditis (6) and insulin-dependent diabetes mellitus (7).

In many autoimmune models, the regulatory cells responsible for resistance against autoimmunity are anti-idiotypic T cells (8–13). These cells, which recognize epitopes of the TCR of the autoreactive cells, can, for example, be detected in mice recovering from EAE (12, 13). Transfer experiments have demonstrated that CD4+ and CD8+ anti-idiotypic T cells cooperate to down-regulate the autoreactive response. Based on these observations, a regulatory circuitry for the control of EAE has been proposed (12, 13).

Here we studied whether and how TCV can be explained in terms of the proposed interactions between autoreactive and anti-idiotypic cells. To this end we develop a mathematical model for the cell circuitry involved in EAE (12, 13). Simplification of our model clarifies that the phenomena described as TCV occur quite naturally in systems where autoreactive T cells can indeed be controlled by regulatory T cells. The essential assumption upon which our results are based is that TCV involves T cells reactive to self epitopes for which T cell tolerance is incomplete (e.g., T cells reactive to subdominant self determinants), and that these T cells are present in the mature peripheral repertoire.

Modeling a T Cell Regulatory Circuitry

We devised a mathematical model for a previously published regulatory T cell circuitry involved in EAE (13) (see Fig. 1). This autoimmune disease, resembling human multiple sclerosis, can be induced in mice by giving myelin basic protein (MBP) or activated MBP-specific T cells. It has been shown that mice recovering from EAE harbor T cells that are specific for peptides from the TCR of an autoreactive clone. Such an anti-idiotypic T cell response seems normal physiological response, because it is also evoked if disease is induced by giving MBP. Cloned anti-idiotypic T cells were shown to be CD4+ and to recognize the framework region 3 peptide of the autoreactive TCR Vβ8.2-chain in the context of MHC II. Because mouse T cells generally do not express MHC II molecules, it was proposed that APCs, for example macrophages or B cells, pick up the Vβ8.2-chain peptide and present it to the CD4+ cells in the context of class II MHC molecules (A+). On adoptive transfer, the cloned anti-idiotypic cells were shown to inhibit autoreactive responses and to protect mice from MBP-induced EAE (12). CD8+ cells also appeared to play a role in the induction of resistance. When CD8+ T cells in the recipient mouse were deleted by anti-CD8 mAb treatment, the CD4+ cells were unable to confer resistance (12, 14). Therefore, it was concluded that the CD4+ cells exert their regulatory effect by recruiting anti-idiotypic CD8+ cells down-regulating the autoreactive response (12).

For our model, we consider three T cell clones: an autoreactive clone A, a CD4+ regulatory clone R+, and a CD8+ regulatory clone R+. The dynamics of each clone is described by a differential equation of the form:

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3 Abbreviations used in this paper: TCV, T cell vaccination; EAE, experimental autoimmune encephalomyelitis; MBP, myelin basic protein; LCMV, lymphocytic choriomeningitis virus.
\[
\frac{dN}{dt} = \text{influx} + \text{proliferation} - \text{inhibition} - \text{death}.
\]

All clone sizes (N) increase due to an influx of naive T cells from the thymus and by T cell proliferation. The autoreactive cells proliferate in response to presented self peptides, whereas both regulatory clones proliferate in response to TCR peptides of the autoreactive cells. Because only little is known about the presentation of TCR peptides on MHC molecules, we do not explicitly model the dynamics of MHC-peptide complexes. Instead we assume that this presentation occurs on such a fast time scale compared to the T cell dynamics, that the proliferation of both regulatory clones can be approximated to be proportional to the number of autoreactive cells. All clones decrease in size due to natural cell death. The autoreactive clone is inhibited by the CD8\(^+\) regulatory cells; the inhibition term is absent in the equations of the regulatory cells. The full model is described in Appendix 1.

The equations of the CD4\(^+\) and the CD8\(^+\) regulatory populations given in Appendix 1 are very similar. The only difference pertains to the help the CD8\(^+\) population receives from the CD4\(^+\) regulatory population. Because we want to obtain basic, fundamental insights into the working of TCV, we simplify the model by lumping both regulatory populations into one regulatory population R (see Appendix 2). Such a simplification also facilitates the analysis of the model. In fact, our simplification amounts to assuming that the proliferation of CD8\(^+\) cells is never limited by help from the CD4\(^+\) regulatory cells.

**Tolerance and Autoimmunity**

In a previous study (15), we demonstrated that TCV can be accounted for in a mathematical model if two assumptions are made. First, we assumed that TCV involves T cells reactive to self epitopes for which tolerance induction is incomplete. Usual processes of self tolerance, such as clonal deletion, active regulation, or anergy induction, might not take place for these self epitopes because of inadequate presentation. Indeed, it has been shown that tolerance induction involves only immunodominant, and not subdominant or cryptic, epitopes (16, 17). Thus some autoreactive clones would remain immunologically ignorant, lacking both tolerance induction and appropriate T cell activation (18). Such a state of ignorance has indeed been found in double-transgenic clones would remain immunologically ignorant, lacking both tolerance induction and appropriate T cell activation. Indeed, the expression of MHC II molecules on target cells has been demonstrated for many organ-specific autoimmune diseases (18, 24). Moreover, clinical diabetes could be induced in transgenic mice by aberrant expression of MBP molecules or IFN-\(\gamma\) on pancreatic \(\beta\) cells (25).

This is incorporated in our model as a positive feedback between the autoreactive cells and the presented self epitopes. Thus, the number of presented self peptides increases with the number of autoreactive cells (see Appendix 1).

**Results**

The simplified model (Appendix 2) can be schematized by two coupled feedback loops (see Fig. 2): a negative loop between the regulatory cells R and the autoreactive cells A, and a positive loop between the autoreactive cells and the presented self peptides S\(_A\). Because both feedback loops are coupled, it is hard to predict

\[\text{MBP} \rightarrow \text{A} \rightarrow \text{S}_A \rightarrow \text{A} \rightarrow \text{S}_A \rightarrow \text{A} \rightarrow \ldots\]

\[\text{MBP} \rightarrow \text{R} - \text{S}_R \rightarrow \text{R} - \text{S}_R \rightarrow \text{R} - \text{S}_R \rightarrow \text{R} - \text{S}_R \rightarrow \ldots\]

\[\text{MBP} \rightarrow \text{A} \rightarrow \text{S}_A \rightarrow \text{A} \rightarrow \text{S}_A \rightarrow \text{A} \rightarrow \ldots\]

\[\text{MBP} \rightarrow \text{R} - \text{S}_R \rightarrow \text{R} - \text{S}_R \rightarrow \text{R} - \text{S}_R \rightarrow \text{R} - \text{S}_R \rightarrow \ldots\]
The dynamics of the differential equations can be studied using time plots, i.e., by plotting the numbers of autoreactive and regulatory cells against time. To study the general behavior of the model, however, it is much more informative to plot the numbers of autoreactive and regulatory cells against each other, as one is used to in FACS analysis. Such a plot is called a “state space.” In a state space one can mark the attractors or stable steady states of the model, i.e., the numbers of autoreactive and regulatory cells to which the system is attracted. For the parameters chosen, the system described in Appendix 2 has two stable steady states, denoted by the black squares in Figure 3. One of these states is the “normal” state of incomplete tolerance, in which both the autoreactive and the regulatory clone are small. In this state, denoted by N in the left-hand corner of Figure 3, both feedback loops are nonfunctional. In the other steady state, the autoreactive cells are actively controlled by the regulatory cells. We interpret this state as the vaccinated state (V); the individual is healthy and resistant to autoimmune disease.

Because the system has only two stable steady states, the injection of cells into a normal individual will either lead to vaccination or to a return to the normal state. To visualize which steady state will eventually be attained, we have drawn the separatrix of the system (see the heavy line in Fig. 3), which separates all states leading to the vaccinated state from those leading to the normal state. Although the system will always end up healthy (i.e., in the normal state N or in the vaccinated state V), the number of autoreactive cells can temporarily become very large. These transient high numbers of autoreactive cells are interpreted as autoimmunity (see the shaded region in Fig. 3). In our model the intensity of autoimmunity is proportional to the number of autoreactive cells. (High numbers of regulatory cells only shorten the duration of the autoimmune disease.) In many animal models it is indeed observed that autoimmunity vanishes spontaneously, leaving the animal resistant to subsequent attempts to induce disease (26, 27).

Evoking autoimmunity
EAE can be evoked in susceptible animals by introducing MBP or activated autoreactive T cells. Because both methods ultimately amount to increasing the number of autoreactive cells in the recipient, we model the induction of autoimmunity by introducing autoreactive cells into the naïve state. Figure 4a shows that this indeed evokes an autoimmune response. Initially (see time point a in Fig. 4a) the autoreactive cells respond vigorously, as they initiate their positive feedback loop, and reach the high levels that we interpret as autoimmune disease. During the second phase of the response (see time point b in Fig. 4a), however, the regulatory cells effectively control the autoreactive cells. The autoimmune disease vanishes and the system approaches the vaccinated state (see time point c in Fig. 4a). In this state the immune system is protected against autoimmunity; the number of regulatory cells is so high that a previously pathogenic dose of autoreactive T cells can no longer induce autoimmunity (Fig. 4b).

Vaccinating with normal autoreactive T cells
To protect animals against autoimmunity without inducing disease, one would have to attain the vaccinated state by giving a low dose of autoreactive cells. Figure 3 shows that an injection of autoreactive cells can only lead to a switch to the vaccinated state if the injected dose is large enough to cross the separatrix. Too small a dose of autoreactive cells failed to initiate both feedback loops. Giving a dose of autoreactive cells that is small but sufficient (Fig. 4c), we observe that the vaccinated state is approached while no autoimmune disease is induced. The proliferation of autoreactive cells is so slow that the regulatory cells can keep up with them and control the autoreactive cells from the start.
Vaccinating with attenuated autoreactive T cells

TCV has also been achieved with large doses of attenuated autoreactive cells (3, 4). Because attenuation blocks cell division, the ultimate effect of an injection of attenuated autoreactive cells is a stimulation of the regulatory cells. This will obviously lead to protection against disease, because it is a way to stimulate the regulatory feedback loop without stimulating the disease-causing positive feedback loop (see Fig. 2). According to the separatrix of Figure 3, however, it should be impossible to attain the vaccinated state by introducing attenuated cells. Stimulating the regulatory cells only, one can never cross the separatrix, because the vaccinated state requires that the positive feedback loop between the autoreactive cells and the presented self epitopes is initialized. Thus as soon as the attenuated cells have disappeared, the regulatory population will gradually decrease due to normal turnover. We conclude that, according to the model, long-term protection against autoimmune disease (see the horizontal line in Fig. 4d), the latter cells proliferate less vigorously and approach the vaccinated state without reaching the high numbers required for autoimmunity (Fig. 4d).

In summary, the model predicts a qualitative difference between vaccination with normal and with attenuated autoreactive cells. A low dose of normal autoreactive cells can lead to a switch to the vaccinated steady state. Therefore it is an all-or-nothing phenomenon that gives rise to long-lasting protection. Inoculation with attenuated cells, in contrast, can only confer transient protection. Because resistance reduces with time, the latter form of vaccination should be dose dependent.

Relapsing disease

Although animals often spontaneously recover from autoimmunity, many human autoimmune diseases are characterized by relapses. It has been suggested that such relapses are due to the stimulation of newly recruited T cells reactive to spreading determinants (28, 29). In our model, relapses can also occur in the absence of determinant spreading. The vaccinated state of our model need not be a stable steady state; instead, for particular parameter combinations, it can be unstable (denoted by the open square in Fig. 5 left) and be surrounded by an attracting limit cycle.
corresponding to oscillatory behavior. For the current parameter setting such oscillations are observed if the inhibitory effect of the regulatory cells $i$ is increased or the saturation constant for stimulation of regulatory cells $k_i$ is decreased. The system will then oscillate around the vaccinated state. If the oscillations are sufficiently large, the autoreactive cells repeatedly pass through the region of disease, which would be observed as a relapsing disease (Fig. 5). Recent experiments showing that relapses in EAE do not require spreading determinants, but can be driven by T cells reactive to the initial dominant determinant of MBP (30), support this mechanism for relapsing disease.

According to the model, TCV should fail to provide protection against such a relapsing disease. If a large oscillation surrounds the vaccinated state, there is no state of protection the system can switch to. The only possibility of curing such a relapsing autoimmune disease would be to induce a switch back to the normal state. Because this would require breaking the positive feedback loop of autoreactive cell-induced Ag presentation, this is probably too difficult. Moreover, if the cause of the autoimmune disease is still present, one would expect autoimmunity to recur.

**For which self Ags do we expect TCV?**

We have studied TCV for self epitopes for which self tolerance is incomplete. The presentation of such self peptides strongly depends on the stimulation by autoreactive cells. The presentation of other self peptides, which are generally visible to the immune system (e.g., dominant epitopes), need not depend on the presence of autoreactive cells. In our model such self peptides could be characterized by a low value of $k_A$ (see Appendix I). If $k_A$ is low there is no normal state $N$ of the system, because the self epitopes always trigger the autoreactive cells. The only stable state that is left is the regulated state $V$. Indeed, inhibition or depletion of certain T cell subsets can lead to autoimmunity (31, 32), suggesting that regulatory cells were down-regulating the autoreactive T cells. Thus, TCV is inducing a switch to an active form of tolerance, which the immune system itself failed to attain due to the poor presentation of the self epitopes.

**Discussion**

Using a simple mathematical model we have analyzed whether and how the interactions between autoreactive cells, self peptides, and anti-idiotypic regulatory cells can explain the phenomena described as TCV. In contrast with more phenomenological models for TCV (see Ref. 33), we have based our model on the experimental data on EAE. However, the model’s simplicity allows one to generalize the results to other autoimmune diseases as well. Our analysis suggests that TCV is a natural phenomenon when autoreactive cells can be controlled by anti-idiotypic regulatory cells. Vaccination in our model relies on the stimulation of anti-idiotypic regulatory T cells by giving either attenuated autoreactive cells or a dose of normal autoreactive cells that is too small to induce disease.

The results of the model hinge upon the assumption that TCV involves T cells reactive to self peptides for which tolerance is incomplete. Obviously low affinity autoreactive clones may escape from tolerance induction. High affinity autoreactive clones, on the other hand, may remain in a state of immunological ignorance due to the poor presentation of their specific self epitopes. The autoreactive cells might, however, be subject to some kind of tolerance induction. For example, the number of autoreactive cells in the normal state could be reduced due to negative selection. In our model this would correspond to a lower influx $m_A$ which would not affect the qualitative results of our model. Some experiments have suggested that interactions between autoreactive cells and regulatory cells prior to vaccination are essential for a positive outcome of TCV (34, 35). Our interpretation of these data is that autoreactive cells could be responsible for the positive selection of regulatory cells in the thymus. We have studied the effect of such a positive selection by modeling the source of regulatory cells as a function of the autoreactive cells. We found no qualitative change of the results as long as the number of regulatory cells in the normal state remained too low to actively control the autoreactive cells.

There is increasing evidence that infectious agents play an important role in the initiation of autoimmune responses (36–38). Dominant epitopes on infectious agents might cause autoimmunity by inducing a cross-reactive immune response against self epitopes (39, 40). Alternatively, tissue damage and up-regulation of MHC expression induced by infectious agents might induce an immune response to epitopes that were not well displayed previously (18, 28). These data confirm our notion that autoreactive cells that have remained immunologically ignorant can initiate their positive feedback loop in the context of an infection and hence cause autoimmunity.

Based on the data on EAE, we have analyzed TCV for systems where autoreactive cells are controlled by anti-idiotypic cells. It
can easily be seen that the model results do not change qualitatively if this regulation is not anti-idiotypic, but rather occurs at the level of the Ag. If the regulatory cells were to be Ag-specific rather than anti-idiotypic, their stimulation function $S_p$ (see Equation 1e) would remain similar. The autoreactive cells indirectly stimulate Ag-specific regulatory cells because they up-regulate the presentation of self peptides. Thus, the results of our model also hold for systems where autoimmune control is Ag-specific. An example would be the regulation of autoimmunity by T-helper type switches (41).

Our model predicts a qualitative difference between vaccination with normal and vaccination with attenuated autoreactive cells. Because vaccination with normal autoreactive cells leads to a switch to the vaccinated steady state, this type of vaccination gives rise to life-long protection. Vaccination with attenuated autoreactive cells or with recombinant single chain TCR proteins (42), on the contrary, should only confer transient and dose-dependent protection. This is a strong prediction that can be tested experimentally.

The relatively new method of vaccinating with DNA has recently also been used in TCV (43). Waismann et al. (43) used DNA encoding a TCR V-region to induce resistance against EAE upon intramuscular injection. For yet unknown reasons vaccination with DNA encoding foreign peptides is known to induce long-term immunity, which is probably due to the stability of episomal DNA in slowly dividing muscle cells (44). Such a long-lasting stimulation of the regulatory cells might indeed keep the regulatory response at a high level and thus protect against autoimmunity.

### Appendix 1. The Full Model

The full model for the regulatory circuitry as presented in Figure 1 incorporates three T cell clones: an autoreactive clone A, a CD4+ regulatory clone $R_1$, and a CD8+ regulatory clone $R_2$. The dynamics of these three clones are modeled by the following differential equations:

\[
\begin{align*}
\frac{dA}{dt} &= m_A + pA S_A - iAR_A - dA - \epsilon A^2, \\
\frac{dR_1}{dt} &= m_{R_1} + pR_1 S_R - dR_1 - \epsilon R_1^2, \\
\frac{dR_2}{dt} &= m_{R_2} + pR_2 S_H - dR_2 - \epsilon R_2^2,
\end{align*}
\]

where the number of presented self epitopes ($S_i$) and the number of presented autoreactive TCRs ($S_A$) are given by:

\[
S_A = \frac{A}{k_A + A} \quad \text{and} \quad S_R = \frac{A}{k_R + A}.
\]

The influxes of autoreactive and regulatory cells from the thymus are represented by $m_A$ and $m_{R_i}$, respectively. The maximum proliferation rate of all T cells is $p$. Autoreactive cells proliferate in response to presented self peptides ($S_A$) whereas both regulatory clones are stimulated by presented autoreactive TCRs ($S_A$). Because the presentation of self peptides is assumed to be reinforced by activated autoreactive cells, the presented self epitopes $S_A$ are modeled as a saturation function of the number of autoreactive cells. The stimulatory effect of the autoreactive cells on the regulatory cells is also modeled by a saturation function ($S_R$). Previously, we have used more complicated proliferation functions (15). Here we aim for maximum clarity by taking simple saturation functions. For proliferation, CD8+ cells require both their specific ligand and T cell help from CD4+ cells. T cell help can be modeled by another saturation function $H$, e.g., $H = H/(k_H + H)$. Autoreactive cells are inhibited by CD8+ regulatory cells at rate $i$. If clone sizes are small, cells die naturally at rate $d$. For large clone sizes, cells undergo an extra concentration-dependent cell death (the terms $i A^2$, which is supposed due to competition. Note that because so many parameters are unknown, time and all parameters have been scaled into arbitrary units.

### Appendix 2. The Simplified Model

To obtain basic insights into TCV, we simplify the full model described in Appendix 1 by lumping the CD4+ and CD8+ regulatory cells into one regulatory population $R$. The simplified model becomes:

\[
\frac{dA}{dt} = m_A + pAS_A - iAR - dA - \epsilon A^2, \\
\frac{dR}{dt} = m_R + pRS_R - dR - \epsilon R^2,
\]

where $S_A$ and $S_R$ are as defined by Equations 1d and 1e.

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