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How Instruction and Feedback Can Select the Appropriate T Helper Response

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The decision of the immune system to trigger immune responses that are, respectively, induced by Th1 or Th2 effectors is a critical one, because it profoundly influences disease outcome. We have recently constructed a mathematical model of Th1–Th2-pathogen interactions that shows that the major decisional events can often be successfully determined by the intrinsic behaviour of the T helper system itself. For certain dangerous types of pathogens, however, which replicate rapidly or have developed strategies to evade the immune response, additional stimuli may be necessary.

As a possible mechanism for the decision-making process innate immune recognition has been proposed. Here we present an enlarged version of our model, which incorporates signals created from the innate immune system after pathogen recognition. The model analysis suggests that there is fault-tolerance of the T helper system to incorrect Th1 signals. In the presence of incorrect Th1 stimuli an initial Th1 response is shifted to the correct Th2-dominated response owing to the intrinsic T helper dynamics. By contrast, according to our model there is no fault-tolerance for incorrect Th2 signals. In fact, if timing is unimportant then Th2 signals are superfluous since the intrinsic T helper dynamics provide an automatic switch to Th2 if Th1 effectors fail to control the pathogen. Th2 signals may, however, be required to accelerate the onset of the Th2 response.

Additionally, we discuss the role of feedback where successful pathogen destruction leads to up-regulation of activation of the effective T helper type. As one pos-

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sibility we examine the role of CpG motifs as indicators for successful pathogen destruction. Differences between instructive and feedback mechanisms are highlighted.

1. INTRODUCTION

Host organisms are infected by different types of pathogens, which may require different means of elimination by the immune system. Therefore, a number of distinct effector mechanisms have evolved and these are not equally efficient in the clearance of any particular pathogen. How does the immune system decide which effector mechanisms to induce against a given pathogen?

Important activators of effector mechanisms are T helper cells, which have been divided into two groups, Th1 and Th2, on the basis of their cytokine secretion pattern. To a first approximation Th1 and Th2 cells favour the cell-mediated and the humoral arms of the immune response, respectively. What regulates the T helper system and at which stage?

T lymphocyte subsets develop from a common precursor (Th0) into differentiated Th1 or Th2 cells. The development depends on signals that can be provided by the T helper system itself or by other immune system components such as macrophages and other parts of the innate immune system. To what extent do these factors provide balances that are *appropriate* to the variety of different challenges that the immune system faces and what finally leads to selection of a particular T helper response?

In a companion paper (Bergmann *et al*., 2001) we presented a new model of the T helper system in response to pathogens where the appropriate T helper response was selected by a self-regulatory process within the T helper system. This process was purely based on the intrinsic cytokine-modulated T helper dynamics without influence from outside the T helper system. Regulatory processes induced a shift from an initial Th1-dominated response to Th2 dominance when Th1-induced effectors could not eliminate pathogen. T helper type 1 response failure was indicated when antigen concentrations rose to sufficiently high values. We have proposed that this selection process is the default mechanism, which in principle works well except in the case of fast-growing Th1-sensitive pathogens. Moreover the dependence of the Th1/Th2 decision on one criterion—such as antigen concentration—makes the system highly susceptible to pathogen interference. Additionally, in certain situations reinforcement of effective T helper response may be beneficial to accelerate pathogen elimination that minimizes damage to the host. It therefore makes sense that the decision as to which effector mechanism is required should be based on a complex matrix of interlocking interactions. This gives us ample reason to study the role of external influences—outside of the T helper system—on the decision making process.

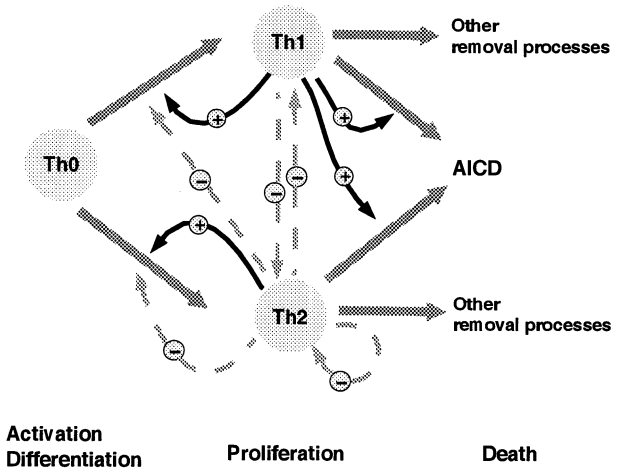

Figure 1. Schematic representation of the interactions governing the T helper system. (AICD = antigen induced cell death.)

Our paper is organized as follows: in Section 2 we review the regulatory interactions within the T helper system. We discuss the biological background of pattern recognition in Section 3 and present a mathematical model that incorporates APC-derived signals in Section 3.1. Highlighted in Sections 3.2 and 3.3 are differences in their effects and necessities between Th1- and Th2-promoting signals derived from antigen presenting cells (APCs) due to pattern recognition. The process of detection of pathogen destruction is explained in Section 4 and a detailed example discussed in Section 4.1.

2. Default Regulation within the T Helper System: Try Th1—if it Does not Work, Try Th2

In a recent paper (Bergmann *et al*., 2001) we presented a detailed description of the interactions within the T helper system, and we formulated equations for system dynamics in response to pathogen. However, we neglected influences of the innate immune system. Here, we briefly review the features of our earlier model that are essential for the present analysis.

At all stages T helper subsets are regulated by signals generated by the corresponding or competing T helper type. The cells communicate via production of messenger molecules—called cytokines—or via receptor expression. A schematic qualitative representation of the main interactions is presented in Fig. 1. Noteworthy is the presence of activation-induced cell death (AICD), a process that causes T helper cells (Fas-expressing Th1 and Th2 cells) to undergo apoptotic death after repeated ligation of their Fas-receptor. The corresponding ligand Fas-L is mainly expressed on Th1 cells.

Table 1. Biological interpretation of the dimensionless parameters in equations (1) and (2).

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

The basic variables in the model immune system are the concentration of available antigen and the sizes of T helper clones. We lump cytokines into two types, depending on whether they are primarily expressed by Th1 or Th2. With the aid of quasi-steady state assumptions we can express cytokine concentrations in terms of Th1 and Th2 concentrations. The state of the system is thus described by the following differential equations, which are presented in dimensionless form. For further discussion of how these equations arise we refer the reader to Bergmann *et al*. (2001) and Yates *et al*. (2000). For example, Th1 activation is directly inhibited by the Th2-related cytokine IL-10, and increased by the Th1-related cytokine IL-12. The secretion of IFN-γ, which is an inducer of IL-12 production, is also inhibited by IL-10. That gives rise to a Th1-activation term $\sigma_1 x_1 p/(1+x_2)^2$. In contrast, the Th2-activation term takes the form $\sigma_2 x_2 p/(1 + x_2)$ because Th1 cytokines do not affect Th2 activation and only IL-10 is suppressing Th2 activation.

$$\frac{dx_1}{d\tau} = \underbrace{\frac{\sigma_1 x_1 p}{(1+x_2)^2}}_{\text{activation}} + \underbrace{\frac{\pi_1 x_1}{(1+x_2)}}_{\text{proliferation}} - \underbrace{\delta_1 x_1^2}_{\text{AICD}} - x_1, \tag{1}$$

$$\frac{dx_2}{d\tau} = \underbrace{\frac{\sigma_2 x_2 p}{(1+x_2)}}_{\text{activation}} + \underbrace{\frac{\pi_2 x_2}{(1+x_1+x_2)}}_{\text{proliferation}} - \underbrace{\delta_2 x_1 x_2}_{\text{AICD}} - x_2, \tag{2}$$

$$\frac{dp}{d\tau} = p(r - \nu_1 x_1 - \nu_2 x_2). \tag{3}$$

Here x_1, x_2 and p embody non-dimensionalized concentrations of Th1, Th2 and pathogen. A state in the state space is represented by a vector $\Omega = (x_1, x_2, p)$. The biological significance of the parameters is summarized in Table 1.

The important steady states of the system are:

- the naive state, with all concentrations zero;
- 'cure:Th2', with elimination of the pathogen and dominance of Th2 cells;

Table 2. Steady states and conditions for their existence and stability.

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

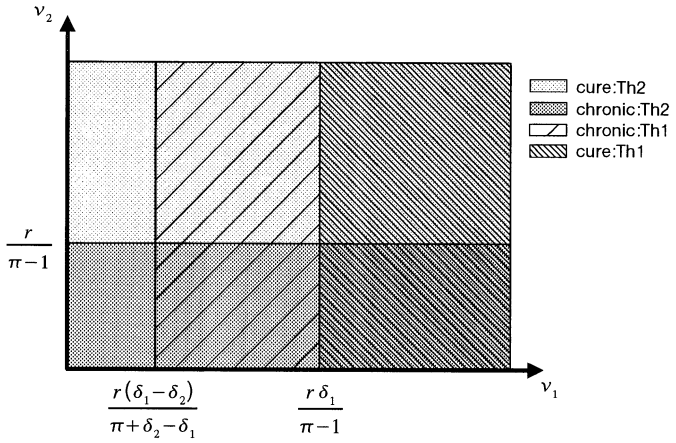

Figure 2. In the two-dimensional pathogen-elimination parameter plane, existence and stability of steady states is described under the conditions $\delta_1 > 1$, $\pi > 1$, and $\delta_1 > \delta_2 > \frac{\delta_1(\delta_1 - 1)}{\delta_1 + \pi - 1}$. Whereas low efficiency of Th2-induced effectors (ν_2 small) always causes a certain risk for Th2-dominated chronic diseases, we find a parameter window for the stability of Th1-dominated chronic situations. Chronic Th1-dominated situations for very low efficiency of Th1-induced effectors are not possible if the susceptibility of Th2 cells to undergo AICD is less than for Th1 cells.

- 'chronic:Th2', with persistence of pathogen and a Th2-dominated T helper response;
- 'cure:Th1', with elimination of the pathogen and dominance of Th1 cells;
- 'chronic:Th1', with persistence of pathogen and a Th1-dominated T helper response;
- 'chronic:Th1/Th2', with antigen persistence and co-existence of both T helper subsets.

[By Th(i) dominance ($i = 1, 2$) at steady state, we mean Th(i) > 0; Th(j) $= 0$, $i \neq j$.] The existence and stability conditions for these steady states are summarized in Table 2. The main features of the steady state analysis are summarized in Fig. 2, a diagram of the two-dimensional pathogen-elimination parameter space (ν_1, ν_2).

We summarize the observations of Bergmann *et al*. (2001) that are most relevant in our ensuing discussion:

- if mainly Th1-induced effector cells are successful in pathogen destruction then—provided that there is an initial Th1 bias—the system ends up in the steady state where pathogen has been successfully cleared by Th1. For some initial conditions, notably high concentration of pathogens that are mainly susceptible to Th1 and not Th2, it is also possible to end up in a chronic situation with dominance of Th2 cells;
- provided that only Th2-induced effectors lead to pathogen elimination whereas Th1-induced effectors do not supply any contribution to pathogen destruction then for any possible initial condition the system will reach a steady state where pathogen has been cleared by a Th2-dominated immune response;
- the size of the parameter window for the stability of 'chronic:Th1' is dependent on the susceptibility of Th2 cells for AICD (δ_2). The lower boundary is given by $\nu_1 > r(\delta_1 - \delta_2)/(\pi + \delta_2 - \delta_1)$, which is positive for $\delta_1 > \delta_2$ and π sufficiently large.

3. On the Role of Pattern Recognition

The T helper system is not only regulated by signals generated within the T helper system but, in addition, is influenced by other immune system components. With the discovery of the ability of the innate immune system to recognize conserved pathogenic molecular patterns, patterns that are absent from the host organism and characteristic for microorganisms (Janeway, 1992), a link between innate and adaptive immunity has been found. It has been proposed that this link allows advantage to be taken of experience gained during evolution of how to cope with particular pathogens. This is accomplished by the ability of the innate immune system components after pattern recognition to provide signals in the form of cytokines that (among other actions) direct T helper differentiation. Thus the innate immune system plays an instructive role in influencing the Th1/Th2 ratio and, therefore, in the decision making process.

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

- lipopolysaccharide (LPS), a part of the cell walls of gram-negative bacteria;
- immunostimulatory DNA: CpG motifs (see also Section 4.1);
- double-stranded RNA of viruses;
- mannans, a component of yeast cell walls, among others.

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

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

In contrast to earlier findings, recent results indicate that the cytokines IL-1, IL-6 and TNF of the first group do not direct T helper differentiation. Thus, in a study on previously activated Th1 and Th2 effectors (Joseph *et al*., 1998) it could not be confirmed—as found elsewhere for Th1 and Th2 clones—that differentiation towards the Th2 phenotype is increased by inflammatory cytokines. Instead, these cytokines mainly enhance naive T cell responses and augment proliferation. Differentiated T helper cells are only moderately responsive to all three cytokines.

Although it is suspected that co-stimulatory molecules may direct T helper differentiation (Thompson, 1995; Manickasingham *et al*., 1998), interpretation of the results is complex. We therefore neglect both inflammatory cytokines and co-stimulation as possible signals for generation of a dominance of one of the two T helper subsets.

Effector cytokines such as IL-12 and IFN-γ, however, promote Th1 responses, mainly by up-regulating Th1 differentiation. In contrast, Th2-promoting signals derived from the innate immune system have been identified to a much lower extent. Romagnani (1992) and Yoshimoto *et al*. (1995) described the capacity of microbial structures characteristic of helminthic parasites to stimulate IL-4 secretion but pattern recognition receptors that skew towards a type 2 immune response have—to our knowledge—not yet been identified.

3.1. ***Incorporation of pattern recognition signals into the model.*** We now modify our model in order to study the influence of the innate immune system on the Th1/Th2 decision making process. We restrict ourselves to cases wherein biasing signals only act on the differentiation step (Fig. 1, left). Stimuli from the innate immune system are incorporated into T helper independent activation signals. In particular, the terms

$$\text{Th1} - \text{stimulus} = \frac{\gamma_1 p}{1 + kS_2}, \tag{4}$$

$$\text{Th2} - \text{stimulus} = \frac{\gamma_2 p}{1 + kS_2}, \tag{5}$$

represent the contributions of signals derived from components of the innate immune system to Th1 or Th2 differentiation in the absence of further cytokine modulation from the developing T helper cell populations. Th1 differentiation occurs when antigen is presented to naive Th0 cells in the presence of IL-12. Since macrophages produce IL-12 due to pathogen recognition when internalizing and presenting the pathogen we assume the activation term to be proportional to antigen concentration. The factor $1 + kS_2$ occurs because, as for activation induced

by T helper-derived signals, the Th2 related cytokine TGF-β inhibits activation by suppressing MHC-II expression, thereby suppressing antigen presentation and IL-12 production by APCs. The parameters γ_1 and γ_2 represent the over-all strengths of the stimulatory effects. After making a quasi-steady state assumption for the cytokine signals S_2 and introducing $\theta_i = \gamma_i k$, we find that equations (1)–(3) generalize to

$$\frac{dx_1}{d\tau} = \frac{\theta_1 p}{(1+x_2)} + \frac{\sigma_1 x_1 p}{(1+x_2)^2} + \frac{\pi_1 x_1}{(1+x_2)} - \delta_1 x_1^2 - x_1, \tag{6}$$

$$\frac{dx_2}{d\tau} = \frac{\theta_2 p}{(1+x_2)} + \frac{\sigma_2 x_2 p}{(1+x_2)} + \frac{\pi_2 x_2}{(1+x_1+x_2)} - \delta_2 x_1 x_2 - x_2, \tag{7}$$

$$\frac{dp}{d\tau} = p(r - \nu_1 x_1 - \nu_2 x_2). \tag{8}$$

We will now analyse how stimuli that stem from innate immunity affect the stability and location of the steady states. Furthermore we discuss consequences of asymmetries in the action of external Th1- or Th2-promoting signals.

3.2. ***Influences of Th1-promoting stimuli.*** First, we study the consequences of Th1-promoting signals ($\theta_1 > 0, \theta_2 = 0$). We find that the position and the local stability properties of only those steady states that represent chronic situations are affected:

- 'chronic:Th2'. Its position moves in the Th1 direction; stability cannot be calculated analytically but will be analysed numerically later on;
- 'chronic:Th1'

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

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

$$\lambda_1 \equiv -1 - \frac{\delta_2 r}{\nu_1} + \frac{\pi \nu_1}{r + \nu_1} + \frac{r\sigma(r\delta_1 - \nu_1 - \pi\nu_1)}{\nu_1(r\sigma + \theta_1\nu_1)}.$$

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

We pointed out in Bergmann *et al*. (2001) that—if the susceptibility of Th2 for AICD is significantly lower than that for Th1—for a sufficiently low pathogen destruction efficiency of a Th1-induced immune response ($\nu_1 < r(\delta_1 - \delta_2)/(\pi + \delta_2 - \delta_1)$)—the only stable fixed points of the system are Th2-dominated steady

states. For this case a successful ($p = 0$) Th2-dominated T helper response is triggered if Th2-induced effector cells are sufficiently effective in pathogen destruction. Moreover, although APC-derived Th1-promoting signals provide a stabilizing influence on the chronic Th1-dominated steady state nonetheless 'chronic:Th1' still remains unstable. This happens because the effect of Th1 signals, θ_1, is weighted by ν_1. Mathematically, we find for the limit of the relevant eigenvalue, if $\delta_1 > \delta_2$, then $\lambda_1 \to \infty$ as $\nu_1 \to 0$. This results in the instability of 'chronic:Th1' if Th1 responses are sufficiently ineffective. Instead, cure by Th2 effectors is attained. For intermediate values of the pathogen elimination efficiency of Th1-induced effectors—($\nu_1 > r(\delta_1 - \delta_2)/(\pi + \delta_2 - \delta_1)$ and $\nu_1 < r\delta_1/(\pi - 1)$)—'chronic:Th1' becomes stable and the domain of attraction for this steady state grows with increasing ν_1.

To interpret these results we introduce the concept of *fault-tolerance to incorrect stimuli*. By incorrect stimuli we mean the 'instruction' by the system to promote Th1 responses when Th2 responses are appropriate and vice versa. Presumably the major source of incorrect stimuli is pathogen evolution to evade immune attack. We ascribe the model's switch to Th2 responses when Th1 responses are sufficiently ineffective as providing fault-tolerance of the T helper system against incorrect Th1 signals from the innate immune system. The fault-tolerance, however, only holds for sufficiently small incorrect stimuli. For a sufficiently high value of the innate Th1 stimulus θ_1 the steady state 'chronic:Th1/Th2', which represents a chronic situation with co-existing T helper populations, can also become stable, depending on θ_1 and ν_1. In Fig. 3 we show that 'chronic:Th1/Th2' does not exist for small Th1 stimuli; increasing the Th1 stimulation leads (via a saddle-node bifurcation) to the creation of a stable (and an unstable) steady state with non-zero antigen concentration and co-existing T helper populations.

The dependence of fault tolerance on the rates with which Th2 cells undergo AICD is illustrated in Fig. 4. Here we show that if there is higher susceptibility of Th2 for Fas-mediated apoptosis then *incorrect* Th1 signals from the innate immune system have more dangerous consequences for the system. Only if δ_2 is significantly lower than δ_1 will a successful Th2 response be triggered even though an incorrect Th1 signal from the innate immune system tends to promote chronic Th1 dominated disease.

Correct detection of intracellular pathogens, however, strengthens the likelihood of appropriate Th1 responses. The effect of a *correct* Th1 signal ($\theta_1 > 0$ and ν_1 sufficiently large) is shown in Fig. 5 where we plot the basins of attraction of the steady states that correspond to the induction of successful Th1 response or to Th2-dominated chronic disease. Increasing the strength of the Th1-promoting signal not only promotes global stability of Th1 but also increases the speed with which Th1 responses are triggered (data not shown).

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

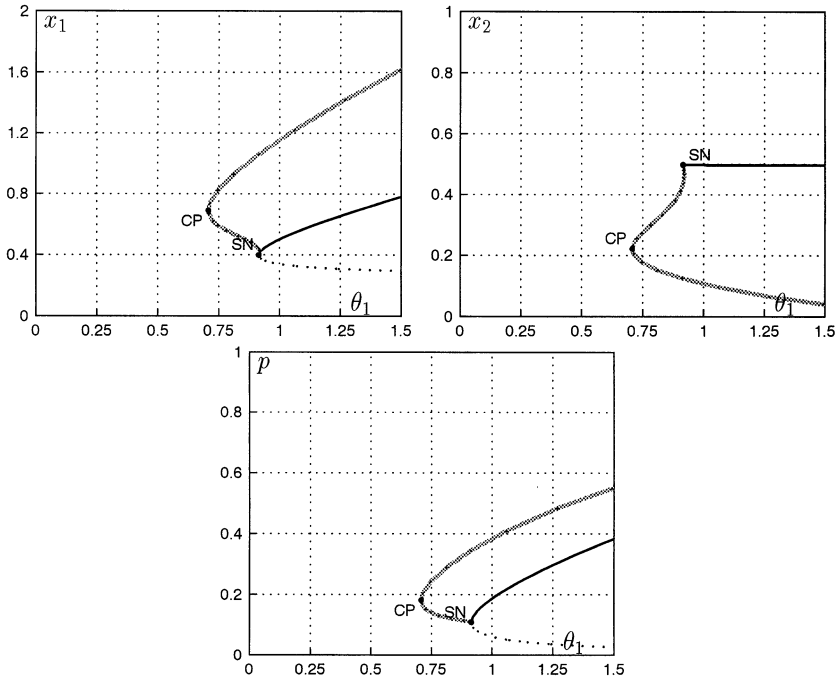

Figure 3. Bifurcation diagram with the Th1 stimulus θ_1 as bifurcation parameter. Sufficiently high Th1-stimulus strength θ_1 stabilizes the 'chronic:Th1/Th2' steady state. For Th1, Th2 and pathogen stable steady states are shown as thick black lines, unstable steady states as dotted lines. Increasing θ_1 leads to a saddle-node bifurcation labelled with SN; a stable steady state is created, which represents a chronic state with co-existing T helper populations. Grey lines represent the development of this saddle-node bifurcation point depending on increasing the parameter ν_1. CP represents cusp points. The other parameters are $\sigma = 2$, $\pi = 2$, $\delta_1 = 1.5$, $\delta_2 = 0.5$, $\nu_1 = 0.1$, $\nu_2 = 2$, $r = 1$, and $\theta_2 = 0$.

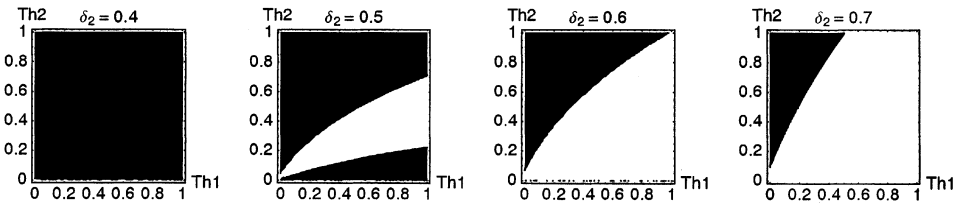

Figure 4. Fault-tolerance for incorrect Th1 stimuli: dependence on δ_2, the susceptibility of Th2 to AICD. For different values of δ_2 basins of attraction of the two stable steady states 'cure:Th2' (black area) and 'chronic:Th1/Th2' (white area) are plotted. With decreasing δ_2 the basins of attraction for 'chronic:Th1/Th2' become markedly smaller. Other parameters: $\sigma = 2$, $\pi = 2$, $\nu_1 = 0.1$, $\nu_2 = 2$, $\delta_1 = 1.5$, $r = 1$, $\theta_1 = 1$, $\theta_2 = 0$ and $p(0) = 0.01$. Here and in several other figures, for ease in interpretation we label axes or curves Th1 and Th2, instead of x_1 and x_2.

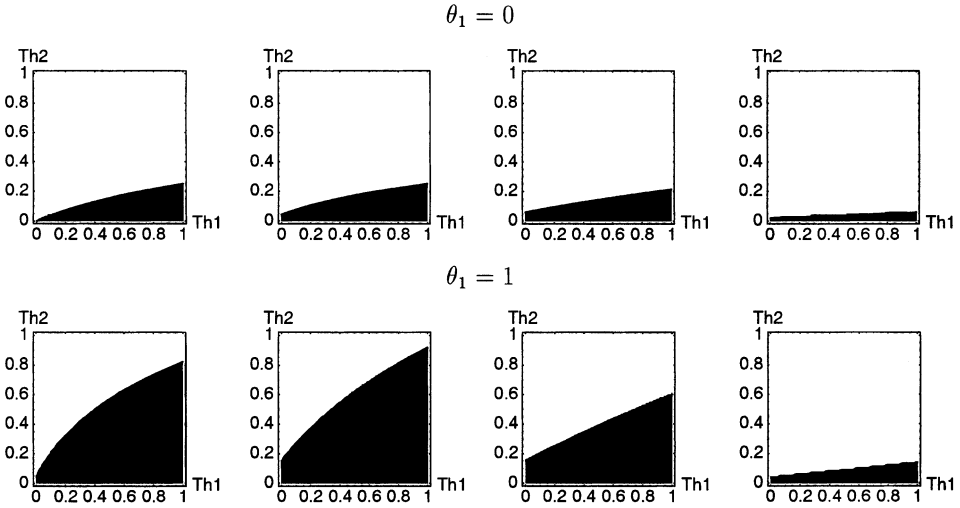

Figure 5. Effect of correct Th1 signals θ_1 on the basins of attraction of the two stable steady states 'cure:Th1' (black) and 'chronic:Th2' (white). The initial antigen concentrations from left to right are $p(0) = 0.01$, $p(0) = 0.1$, $p(0) = 1$, and $p(0) = 10$. Other parameters are set to $\sigma = 2$, $\pi = 2$, $\nu_1 = 2$, $\nu_2 = 0.01$, $\delta_1 = 1.5$, $\delta_2 = 0.5$, and $r = 1$.

- 'chronic:Th2':

$$\Omega_{\text{chronic:Th2}} = \left(0, \frac{1}{\nu_2}, \frac{r[r + \nu_2(1-\pi)]}{\nu_2(r\sigma - \theta_2\nu_2)}\right).$$

  The last two eigenvalues are always negative because of the existence condition; the first eigenvalue

$$\frac{[\nu_2(\pi - 1) - r)(\sigma r^2 + \theta_2 r\nu_2(1 + \nu_2)]}{(r + \nu_2)(r\sigma + \theta_2\nu_2)}$$

is negative if $\nu_2 < \frac{r}{\pi - 1}$, which is the same stability condition as without Th2 stimulus;

- 'chronic:Th1': position moves in the Th2 direction; stability cannot be calculated analytically.

In the previous section we showed that even in the case where Th1 signals promote the incorrect T helper response, this bias can be overridden later by the internal T helper dynamics, leading to Th2 dominance and successful pathogen elimination. In contrast, according to our model there is no way based on the cytokine interactions of the T helper system that can override incorrect Th2 signals from the innate immune system. In situations where Th1 is the 'correct' T helper subset Th2 signals promote Th2-dominated immune responses and override even correct Th1 signals. This leads to chronic disease with co-existent T helper populations (cf. Fig. 6).

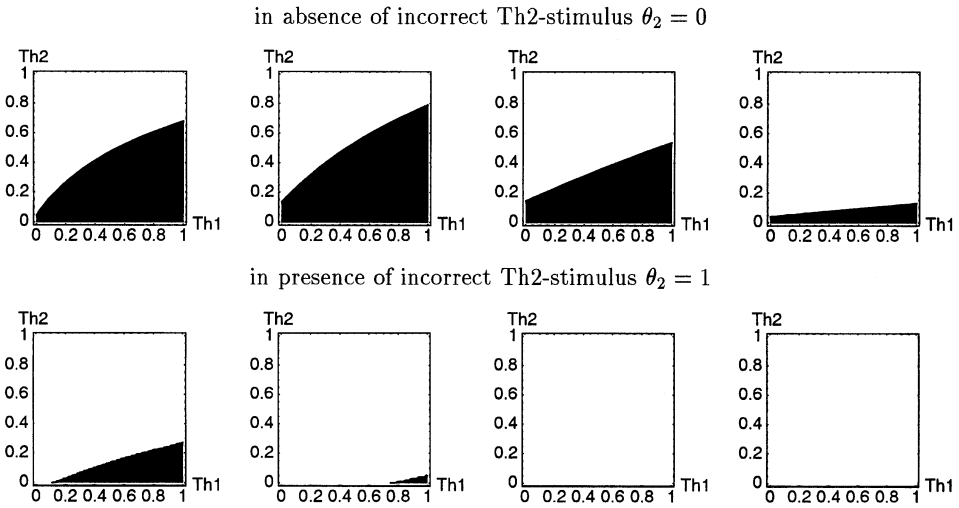

Figure 6. Basins of attraction of the steady states 'cure:Th1' (black area) and 'chronic:Th1/Th2' (white area) for increasing antigen concentrations, in the absence and in the presence of an incorrect Th2 stimulus. The graphs are drawn for a case where Th1 is much more efficient than Th2 in eliminating pathogen ($\nu_1 = 2$, $\nu_2 = 0.01$). Incorrect Th2 stimuli enlarge the stability of 'chronic:Th1/Th2' and can even override Th1 stimuli. Other parameters are set to $\sigma = 2$, $\pi = 2$, $\delta_1 = 1.5$, $\delta_2 = 0.5$, $r = 1$, and $\theta_1 = 1$. Initial antigen concentrations, from left to right, are $p(0) = 0.01$, $p(0) = 0.1$, $p(0) = 1$, $p(0) = 10$.

4. PATHOGEN DESTRUCTION FEEDBACK

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

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

We now examine an alternative view of events following perception of parts of pathogens. In addition to inducing a reflexive immune response, recognition of parts of dead, destroyed pathogens could be part of a feedback-regulation process (Segel and Lev Bar-Or, 1999). Here, if certain types or combinations of effector components are responsible for pathogen destruction and elimination then intracellular components of the deleted pathogenic agents that signal their destruction

[termed 'scalps' (Segel and Lev Bar-Or, 1999)] can lead to an up-regulation of the efficacious components of the immune system and, therefore, reinforce the success of the immune response ('pathogen destruction feedback'). To illustrate how this concept could be implemented we consider DNA fragments as indicators of pathogen destruction.

4.1. ***On the role of CpG motifs in pathogen destruction feedback.*** A molecule can be regarded as a 'scalp' if it is an intracellular molecule that is characteristic for certain pathogens, if it can be recognized by receptors of the innate immune system, and if its recognition modifies the immune response. Good examples of scalp molecules are parts of prokaryotic DNA termed CpG motifs.

Several experimental groups have demonstrated that CpG dinucleotides are ubiquitous in almost all bacteria, fungi, and large eukaryotic viruses (Burge *et al*., 1992; Karlin *et al*., 1994), but absent in vertebrate DNA. By using pattern recognition receptors, macrophages recognize non-self DNA through CpG motifs and react with a strong Th1-including response—similar to pattern recognition events discussed in Section 3—owing to the secretion of pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, and IL-12 [for reviews see Van Uden and Raz (1999), and Wagner (1999)]. Major effects on dendritic cells are up-regulation of MHC II presentation and of co-stimulatory molecules such as B7.2 and the production of large amounts of IL-12, TNF-α, IL-6 and, to a lesser extent, IL-10. CpG appears to switch the isotype pattern to a Th1 profile (Lipford *et al*., 1998), as antigen-specific IgG2a becomes dominant. This observation has been reinforced by the finding that immunostimulation by CpG-ODN (synthetic oligodeoxynucleotides containing CpG dinucleotides), which mimics the immunostimulatory qualities of bacterial DNA, not only prevents the development of Th2 responses but also inverts already established Th2 polarization towards a Th1 response (Zimmerman *et al*., 1998).

If the innate immune system components react to CpG by providing a Th1 bias why should recognition of special DNA fragments that occur *equally* in intracellular and extracellular pathogens lead to a selection of an appropriate T helper response? Or in a different formulation: why do extracellular pathogens that carry CpG *not* induce an innate reaction to their CpG? Recent data indicates that CpG receptors are intracellular (Krieg *et al*., 1995) and that CpG DNA can stimulate cells only if it has been internalized (Manzel and Macfarlane, 1999), or if the extracellular concentrations have reached a very high level. Therefore it has been suggested that immune recognition of CpG has evolved as a defence mechanism against intracellular bacteria, viruses and retro-viruses (Krieg, 2000).

We propose that CpG can be seen as a component that signals intracellular pathogen destruction and selectively up-regulates differentiation of effective Th1 cells. To that end let us now concentrate on the question under which circumstances high CpG concentrations can be detected *inside* a macrophage. One obvious possibility is that CpG-displaying pathogens have invaded the macrophage. This is

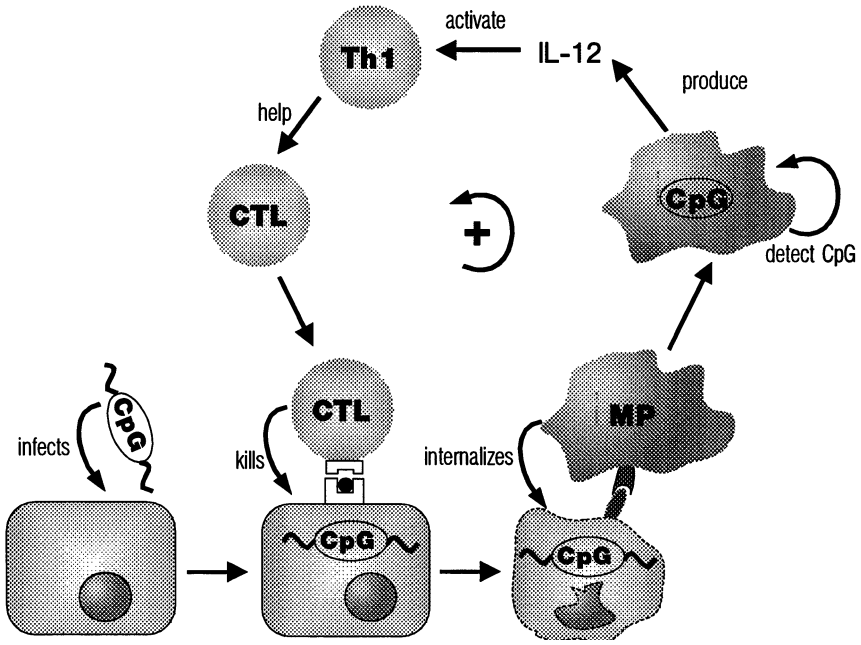

Figure 7. Schematic representation of the interactions governing CpG induced pathogen destruction feedback. The process starts at lower left, when a pathogen bearing CpG infects a host cell. MP = macrophage.

the case for intracellular pathogens, such as those causing tuberculosis and leprosy, which grow in the phagolysosomes, or if macrophages have internalized the infectious agents. When an intracellular pathogen bearing immunostimulatory DNA sequences infects *other* cells than macrophages and replicates there, then these sequences can *only* be detected in sufficient amounts by the corresponding receptors inside a macrophage when this pathogen has been destroyed by appropriate components of an immune response and phagocytized by macrophages. This indeed occurs when cytotoxic T lymphocytes (CTL) induce apoptosis of the pathogen-afflicted cells and the resulting apoptotic bodies are ingested by the macrophages, using their special scavenger receptors (Savill *et al.*, 1993).

In this picture immunostimulatory DNA fragments can indeed be seen as some sort of 'scalp' that signals pathogen destruction. Activation of CTLs is reinforced by T helper cells of type 1; in particular this has been shown for chronic viral infections (Zajac *et al.*, 1998). Therefore, the observed induction of Th1-promoting signals by CpG (see earlier) can be interpreted as reinforcement of the appropriate immune response through pathogen destruction feedback. This occurs via promotion of activation of this particular T helper type that has triggered the efficient immune response. See Fig. 7.

In the present context we emphasize the following differences between LPS seen as a prototypical pattern recognition molecule and CpG DNA. Although they have

similar effects on the immune system we propose that not only do the molecular mechanisms differ (Krieg, 2000) but also that these two molecules play different roles. The effects of LPS, which is part of the outer cell walls of bacteria, can be interpreted as reflexive reactions to the probability of infectious danger (Matzinger, 1994). By contrast, the response mediated by the intracellular CpG entities can be interpreted as a feedback that reinforces an effective type of attack on a class of pathogens.

4.2. ***Pathogen destruction feedback (PDF) in the model.*** By definition pathogen destruction feedback is up-regulation of successful effector types by components that signal pathogen destruction. In the context of Fig. 7 elimination of pathogens by Th1 effectors up-regulates activation of new Th1 cells. According to our model equation (3) the rate at which pathogen is eliminated by Th1 effectors is equal to the product of the pathogen concentration p, the Th1 concentration x_1 and the pathogen elimination efficiency of Th1 effectors ν_1. Therefore PDF-induced Th1 activation levels should be proportional to $\nu_1 p x_1$. More precisely, we take the PDF related Th1 activation terms as

$$\frac{f_1 \nu_1 x_1 p}{1 + x_2} \tag{9}$$

where the parameter f_1 reflects the feedback strength. The term $1 + x_2$ comes from the inhibiting capacity of Th2 cytokines on T helper activation (see Section 3.1).

4.3. ***Analysis.*** The location or local stability properties of only those steady states that represent chronic situations are affected by the addition of the term (9) to equation (6) for Th1;

- positive 'chronic:Th2' is stable if $\nu_1 f_1 < r\sigma/(r + \nu_2)$. That implies that high efficiency of Th1 effectors together with non-zero feedback strength destabilize 'chronic:Th2';
- 'chronic:Th1' is stabilized by pathogen destruction feedback ($f_1 \nu_1 > 0$). Since this steady state only exists for small values of ν_1 this effect will only be of relevance for high feedback strength f_1.

As illustrated in Figs. 8 and 9 in the case of a Th1-sensitive pathogen that is trying to circumvent the immune response by generating incorrect Th2 signals, PDF appears to be considerably more resistant against pathogen interference than instructive pattern recognition.

5. Summary and Discussion

This paper deals with the central question: what factors contribute to an appropriate T helper response to infection?

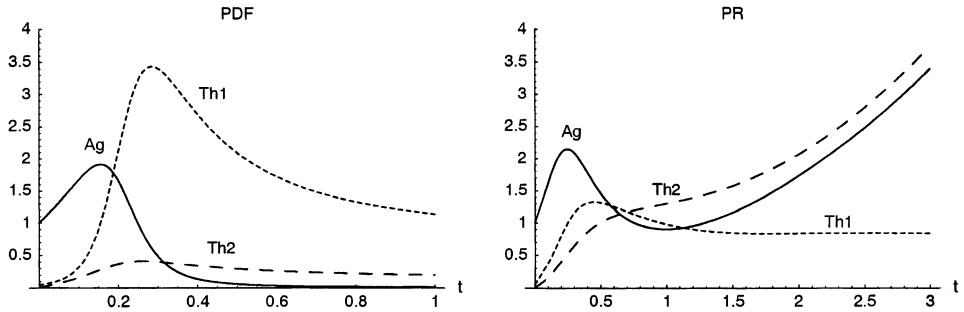

Figure 8. Time-plots for situations where the pathogen tries to circumvent elimination by generating incorrect Th2 signals ($\theta = 1$). Left figure: pathogen destruction feedback—with feedback strengths $f_1 = 2$, no additional Th1 stimulus $\theta_1 = 0$—sufficiently up-regulates the Th1 subset and leads to rapid antigen elimination. Right figure: 'classical' pattern recognition—$f_1 = 0, \theta_1 = 2$—cannot overcome the incorrect Th2 signals; a chronic situation is ultimately attained. Initial values are $x_1(0) = 0.05, x_2(0) = 0.01$, $p(0) = 1$. The remaining parameters are $\nu_1 = 7$, $\nu_2 = 0.1$, $\sigma = 2$, $\pi = 3$, $\delta_1 = 2$, $\delta_2 = 0.7$, and $r = 6$.

In a companion paper (Bergmann *et al.*, 2001) we presented a new model of the T helper system in response to infectious agents. This model (schematically) incorporates most of the known cytokine interactions within the T helper system but neglects other influences from outside the T helper system. The major feature of the model is that it describes a self-organizing process for the selection of the appropriate T helper response as an implementation of the strategy: try Th1 first and shift to Th2 if Th1 fails. The crucial point here is that pathogen concentrations that have risen to a sufficiently high value are interpreted as a failure of the Th1-dominated response. We proposed that this process is a default mechanism that works in most cases. However, if the development of antigen concentrations during an immune response were the *only* criterion responsible for the Th1–Th2 decision, it is likely that some microorganisms could escape. Indeed, the concentrations of fast growing pathogens can increase for a very considerable time although Th1-induced effectors are the best choice for this particular pathogen and would eventually eliminate it if there were no shift to Th2. This increase feigns a failure of the Th1-dominated immune response and causes an inappropriate switch towards Th2.

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

In the model of Bergmann *et al.* (2001) a Th1 bias is necessary in order to enable Th1-dominated immune responses. This bias could be intrinsic, in the form of a

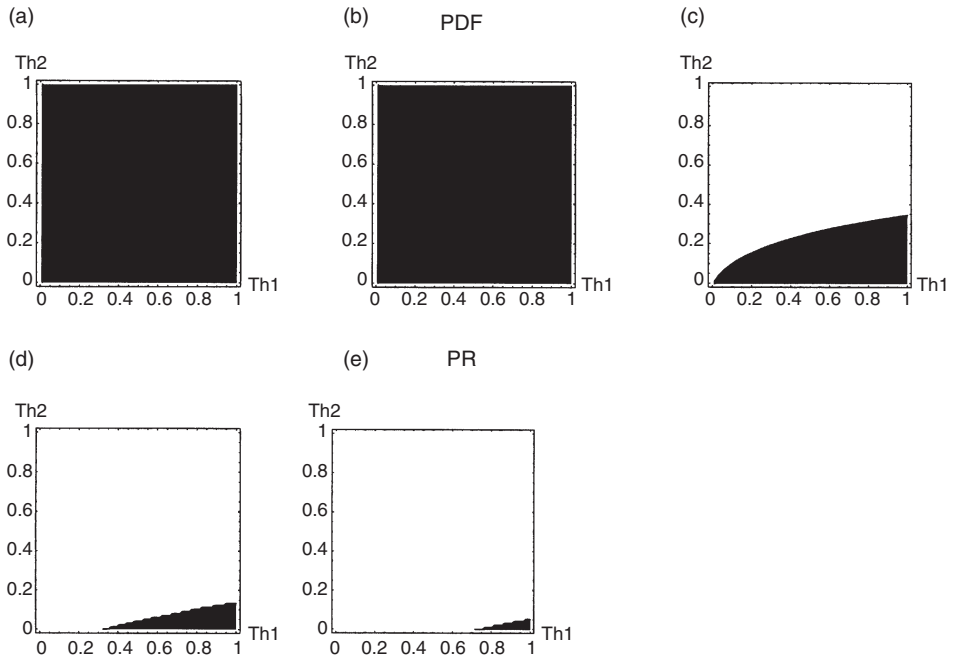

Figure 9. Contrasting pure pathogen destruction feedback (PDF) to Th1 ($f_1 > 0, \theta_1 = 0$) vs classical pattern recognition (PR) ($f_1 = 0, \theta_1 > 0$). Basins of attraction of the steady states 'cure:Th1' ('successful response'—black area) and chronic situations ('unsuccessful response'—white area) for situations where the pathogen tries to circumvent elimination by generating incorrect Th2 signals ($\theta_2 = 1$). In the left and middle columns, respectively, [(a), (d) and (b), (e)] initial antigen doses p are $p(0) = 0.01$, $p(0) = 0.01$. (a) and (b): PDF—with feedback strength $f_1 = 2$ and no additional Th1 stimulus ($\theta_1 = 0$)—leads to exclusive stability of 'cure:Th1'. This appears because stability of 'chronic:Th2' is diminished by positive $\nu_1 f_1$. (c) with initial antigen dose $p(0) = 0.01$ demonstrates that reducing the feedback strength to $f_1 = 1.3$ diminishes the basin of attraction of 'cure:Th1' because another stable steady state 'chronic:Th1/Th2' emerges. (d) and (e): 'Classical' pattern recognition (PR)— $f_1 = 0, \theta_1 = 2$ leads to bi-stability of 'cure:Th1' and 'chronic:Th1/Th2' with comparatively small basins of attraction for the 'cure:Th1' steady state. This general behaviour is essentially unchanged if Th1 stimuli increase (data not shown). The remaining parameters are $\nu_1 = 7$, $\nu_2 = 0.1$, $\sigma = 2$, $\pi = 3$, $\delta_1 = 2$, $\delta_2 = 0.7$, and $r = 6$.

higher Th1-activation-driving capacity of Th1 cytokines and cell-cycle dependence of cytokine production, and there can be an additional temporary bias, provided by signals of the innate immune system after pattern recognition.

For fast replicating pathogens the intrinsic Th1 bias may be too weak and antigen levels may not be controlled sufficiently rapidly by Th1 effectors. This problem is exemplified in the model of Bergmann *et al*. (2001) wherein increasing antigen levels are misinterpreted as failure of the Th1-dominated immune response, leading to a Th1→Th2 switch. The subsequent Th2-dominated response cannot clear the intracellular pathogen and the situation settles at very high Th2 and pathogen

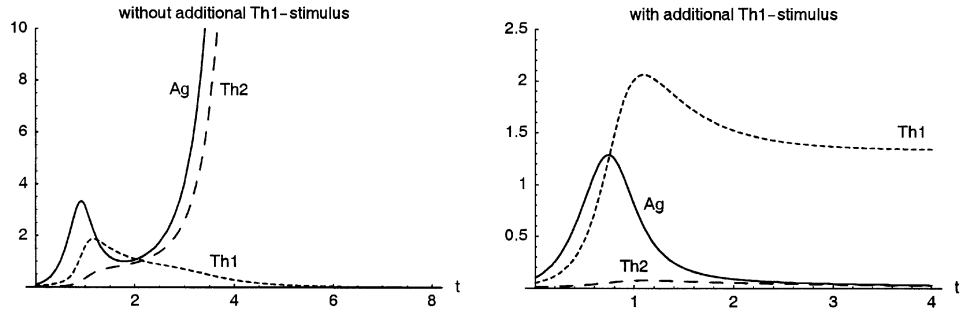

Figure 10. Time plots showing a situation wherein additional reinforcement of the beneficial Th1 response is necessary in order to eliminate fast replicating intracellular pathogens. Left figure: Without reinforcement of the beneficial Th1 response ($\theta_1 = 0$) high antigen levels result in a Th1→Th2 shift, which leads to persistence of high pathogen levels. The system ends up in a steady state with high antigen and Th2 concentrations (out of scale). Right figure: Additional Th1 stimuli ($\theta_1 = 1$) avoid the shift and lead to pathogen clearance. The remaining parameters are $\sigma = 2$, $\pi = 3$, $\delta_1 = 1.5$, $\delta_2 = 0.5$, $\nu_1 = 4$, $\nu_2 = 0.1$, $\theta_2 = 0$, and $r = 5$. The initial concentrations for T helper cells are $x_1(0) = 0.05$, $x_2(0) = 0.01$.

concentrations, which may lead to death of the host. An additional measure may be to reinforce the beneficial Th1-dominated immune response in order to control the pathogen. In Fig. 10 we illustrate that Th1 stimuli of the innate immune system derived from classical pattern recognition can support the appropriate Th1 response. Similar behaviour can be observed if the Th1-reinforcing signals stem from PDF (data not shown). However, this support is limited since it cannot overcome insufficient efficacy of Th1 cells in pathogen elimination ν_1 in combination with high replication rates r. Additional Th1 signals—no matter whether they come from classical pattern recognition or from PDF—do not affect the local stability properties of 'cure:Th1'. Therefore very high replication rates of a certain Th1-sensitive pathogen result in instability of 'cure:Th1' [$\nu_1 < \delta_1 r/(\pi - 1)$].

How does the immune system deal with such situations? One way is to directly suppress pathogen replication in situations where this is particularly necessary. Thus, the Th1 cytokine IFN-γ and inflammatory cytokines such as IFN-α, IFN-β are potent inhibitors of virus replication [for reviews see Biron (1998), and Guidotti and Chisari (1996, 1999)], which could help to avoid failure of Th1-dominated immune responses against Th1-sensitive pathogens. Moreover, since pathogen populations have an interest in their own spread and spread might be only possible if the host survives it is reasonable to assume that pathogen–host co-evolution has sorted out too virulent pathogens. The Myxoma virus in European rabbits is one of the best documented examples of host–virus co-evolution. When introduced into wild European rabbit populations in Australia, Europe and Great Britain, the virus was initially highly lethal, killing 99% of the infected rabbits. Later attenuated virus strains emerged which allowed the survival of moderately resistant rabbits (Best and Kerr, 2000).

In contrast to fast replicating Th1-sensitive pathogens our model suggests that for pathogens additional stimuli are *not* necessary for the selection of a beneficial *Th2 response* since a switch from Th1 to Th2 is automatically induced when antigen concentrations rise to a sufficiently high value. Therefore, fast growing antigen levels lead to an even faster induction of the beneficial Th2 response. Nevertheless, additional Th2 signals accelerate the onset of the Th2 response, which may well be important for certain pathogens.

Let us now summarize the other findings derived from our model:

(i) correct APC-derived signals that arise from pattern recognition of the innate immune system reinforce the differentiation of the appropriate type of T helper subset;
(ii) lower susceptibility for AICD of Th2 cells compared to Th1 cells leads to fault-tolerance for incorrect Th1-promoting signals. In such a case the ineffective Th1 response is rapidly taken over by a Th2-dominated response, owing to the intrinsic T helper dynamics;
(iii) incorrect Th2 stimuli, however, generate inefficient Th2 responses leading to chronic disease.

Of course our conclusions are based on the particular model we have derived. An example of a change in that model that produces different results is incorporation of feedback in such a way that growth factors act in an autocrine way and are not directly incorporated into the proliferation rates π_1 and π_2. With this change, the proliferation terms in equations (1) and (2) are proportional to x_1^2 and x_2^2 instead of x_1 and x_2. Now cure states are no longer stable. However stability is retained if a further feature is included in the model, and IL-2 production of Th0 cells and synergy of IL-2 and IL-4 is taken into account [see Yates *et al*. (2000) for details concerning the proliferation terms]. In the face of a highly complex system like the immune system, one never can know whether or not new qualitative results will hold up if one's present and necessarily incomplete model is one day expanded so as to be 'essentially complete'. One hopes nonetheless that the new results are sufficiently stimulating to lead to further productive ideas concerning the nature of immune response and/or to the motivation of new experiments.

We emphasize the contrast between the view of a purely reflexively instructed T helper response owing to signals provided by the innate immune system with the alternative that there is an additional role of pattern recognition. In the alternative studied here, called pathogen destruction feedback, 'scalps' (parts of the pathogen that signal its destruction) are recognized so as to up-regulate the differentiation of the appropriate T helper subset that is responsible for the destruction.

Although both 'classical pattern recognition' and pathogen destruction feedback lead to up-regulation of the differentiation of the appropriate T helper type (Fig. 11) there are fundamental differences in the mechanisms. 'Classical' pattern recognition via APC leads to instructed regulation of the T helper system. A dominant controller (the innate immune system) 'knows' the appropriate defence strategy

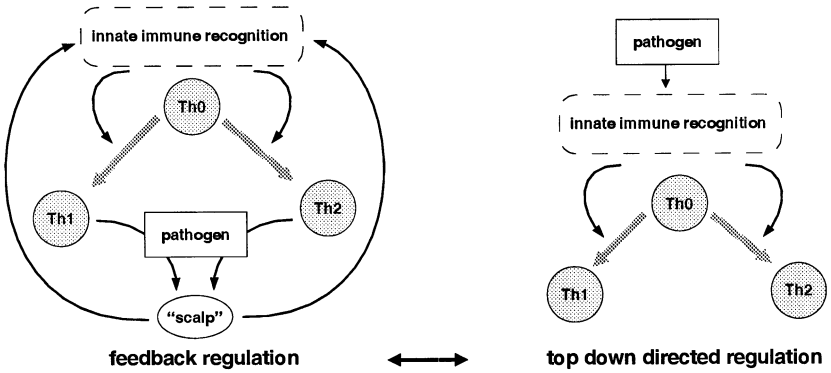

Figure 11. Contraposition of different regulation concepts.

and directs the Th1 or Th2 dominance accordingly. This knowledge has been evolutionary selected. The strength of the APC-derived signal depends on the antigen concentration. This 'top down' approach is complemented by the 'bottom up' mechanism of pathogen destruction feedback. Positive feedback on the successful T helper type—which is based on present 'on line' 'real time' experience and whose strength depends on the efficiency of pathogen destruction—leads to a self-regulation of the T helper system. Signals of pathogen destruction feedback *selectively* up-regulate that T helper type that at each stage of the infection leads to a more effective elimination of the pathogen. This results in a flexible rather than a reflexive response.

We thus propose that there is a whole set of different mechanisms for the selection of the appropriate T helper type including:

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

Doubtless there are other mechanisms. For example pathogen destruction feedback has been suggested to be part of a more general distributed feedback process where aspects of immune response in addition to pathogen destruction are taken into account (Segel and Lev Bar-Or, 1999). It is sensible that the decision making process that selects the required effector mechanisms relies on a complex matrix of interlocking mechanisms, which are selectively important for different situations.

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REFERENCES

Bergmann, C., J. L. van Hemmen and L. A. Segel (2001). Th1 or Th2: How an appropriate T helper response can be made. *Bull. Math. Biol.* **63**, 405–430.

Best, S. M. and P. J. Kerr (2000). Coevolution of host and virus: the pathogenesis of virulent and attenuated strains of myxoma virus in resistant and susceptible European rabbits. *Virology* **267**, 36–48.

Biron, C. A. (1998). Role of early cytokines, including alpha and beta interferons (IFN-α/β), in innate and adaptive immune responses to viral infections. *Semin. Immunol.* **10**, 383–390.

Burge, C., A. M. Campbell and S. Karlin (1992). Over- and under-representation of short oligonucleotides in DNA sequences. *Proc. Natl. Acad. Sci. USA* **89**, 1358–1362.

Guidotti, L. G. and F. V. Chisari (1996). To kill or to cure: options in host defense against viral infection. *Curr. Opin. Immunol.* **8**, 478–483.

Guidotti, L. G. and F. V. Chisari (1999). Cytokine-induced viral purging—role in viral pathogenesis. *Curr. Opin. Microbiol.* **2**, 388–391.

Janeway, C. A. Jr. (1992). The immune system evolved to discriminate infectious nonself from noninfectious self. *Immunol. Today* **13**, 11–16.

Joseph, S. B., K. T. Miner and M. Croft (1998). Augumentation of naive, Th1 and Th2 effector CD4 responses by IL-6, IL-1 and TNF. *Eur. J. Immunol.* **28**, 277–289.

Karlin, S., W. Doerfler and L. R. Cardon (1994). Why is CpG suppressed in the genomes of virtually all small eukaryotic viruses but not in those of large eukaryotic viruses? *J. Virol.* **68**, 2889–2897.

Krieg, A. M. (2000). The role of CpG motifs in innate immunity. *Curr. Opin. Immunol.* **12**, 35–43.

Krieg, A. M., A-K. Yi, S. Matson, T. J. Waldschmidt, G. A. Bishop, R. Teasdale, G. A. Koretzky and D. M. Klinman (1995). CpG motifs in bacterial DNA trigger direct B-cell activation. *Nature* **374**, 546–549.

Lipford, G. B., K. Heeg and H. Wagner (1998). Bacterial DNA as immune cell activator. *Trends Microbiol.* **6**, 496–500.

Manickasingham, S. P., S. M. Anderton, C. Burkhart and D. C. Wraith (1998). Qualitative and quantitative effects of CD28/B7-mediated costimulation on naive T cells in vitro. *J. Immunol.* **161**, 3827–3835.

Manzel, L. and D. E. Macfarlane (1999). Lack of immune stimulation by immobilized CpG-oligodeoxynucleotide. *Antisense Nucleic Acid Drug Dev.* **9**, 459–464.

Matzinger, P. (1994). Tolerance, danger and the extended family. *Annu. Rev. Immunol.* **12**, 991–1045.

Romagnani, S. (1992). Induction of Th1 and Th2 responses: a key role for the 'natural' immune response. *Immunol. Today* **13**, 379–381.

Savill, J., V. Fadok, P. Henson and C. Haslett (1993). Phagocyte recognition of cells undergoing apoptosis. *Immunology Today* **14**, 131–136.

Segel, L. A. and R. Lev Bar-Or (1999). On the role of feedback in promoting conflicting goals of the adaptive immune system. *J. Immunol.* **163**, 1324–1349.

Thompson, C. B. (1995). Distinct roles for the costimulatory ligands B7-1 and B7-2 in the T helper differentiation? *Cell* **81**, 979–982.

Van Uden, J. and E. Raz (1999). Immunostimulatory DNA and applications to allergic disease. *J. Allergy Clin. Immunol.* **104**, 902–910.

Wagner, H. (1999). Bacterial CpG DNA activates immune cells to signal infectious danger. *Adv. Immunol.* **73**, 329–368.

Yates, A., C. Bergmann, J. L. van Hemmen, J. Stark and R. Callard (2000). Cytokine-modulated regulation of helper T cell populations. *J. Theor. Biol.* **206**, 539–560.

Yoshimoto, T., A. Bendelac, C. Watson, J. Hu-Li and W. E. Paul (1995). Role of NK1.1+ cells in a Th2 response and in immunoglobulin E production. *Science* **270**, 1845–1848.

Zajac, A. J., K. Murali-Krishna, J. N. Blattmann and R. Ahmed (1998). Therapeutic vaccination against chronic viral infection: the importance of cooperation between CD4+ and CD8+ T cells. *Curr. Opin. Immunol.* **10**, 444–449.

Zimmerman, S., O. Egeter, S. Hausmann, G. B. Lipford, M. Röcken, H. Wagner and K. Heeg (1998). CpG oligonucleotides trigger protective and curative Th1 responses in lethal murine leishmaniasis. *J. Immunol.* **160**, 3627–3630.

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