J. theor. Biol. (2000) **206,** 539–560
doi:10.1006/jtbi.2000.2147, available online at http://www.idealibrary.com on IDEAL®

Cytokine-modulated Regulation of Helper T Cell Populations

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(*Received on* 11 *January* 2000, *Accepted in revised form on* 17 *July* 2000)

Helper T (Th) cells are a crucial component of the adaptive immune system and are of fundamental importance in orchestrating the appropriate response to pathogenic challenge. They fall into two broad categories defined by the cytokines each produces. Th1 cells produce interferon-γ and are required for effective immunity to intracellular bacteria, viruses and protozoa whereas Th2 produce IL-4 and are required for optimal antibody production to T-dependent antigens. A great deal of experimental data on the regulation of Th1 and Th2 differentiation have been obtained but many essential features of this complex system are still not understood. Here we present a mathematical model of Th1/Th2 differentiation and cross regulation. We model Fas-mediated activation-induced cell death (AICD) as this process has been identified as an important mechanism for limiting clonal expansion and resolving T cell responses. We conclude that Th2 susceptibility to AICD is important for stabilizing the two polarized arms of the T helper response, and that cell–cell killing, not suicide, is the dominant mechanism for Fas-mediated death of Th1 effectors. We find that the combination of the anti-proliferative effect of the cytokine TGF-β and the inhibiting influence of IL-10 on T cell activation are crucial controls for Th2 populations. We see that the strengths of the activation signals for each T helper cell subset, which are dependent on the antigen dose, co-stimulatory signals and the cytokine environment, critically determine the dominant helper subset. Switches from Th1- to Th2-dominance may be important in chronic infection and we show that this phenomenon can arise from differential AICD susceptibility of T helper subsets, and asymmetries in the nature of the cross-suppressive cytokine interactions. Our model suggests that in some senses a predominantly type 2 reaction may well be the "default" pathway for an antigen-specific immune response, due to these asymmetries.

1. Introduction

T lymphocytes form a major arm of the adaptive immune system. They include $CD8^+$ cytotoxic T cells whose main role is to kill virally infected cells, and $CD4^+$ T helper cells. T helper cells can be subdivided into two subpopulations defined broadly by the cytokines they produce. Th1 cells secrete interferon-γ (IFN-γ), TNF-α and IL-2 whereas Th2 secrete IL-4, IL-13 and IL10 (for a review, see Mosmann & Sad, 1996; Romagnani, 1997). Functionally, Th1 lymphocytes are associated with inflammation and cell-mediated responses, whereas Th2 cells provide help to B cells and so are linked with antibody production.

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0022–5193/00/200539 + 22 $35.00/0

Infections that can be cleared by type 1 responses include mycobacterial infections such as tuberculosis and *Leishmania major*, and viruses such as influenza; Th1 cells are also associated with autoimmune diseases and graft rejection. Th2 responses, on the other hand, give protection against helminths, certain viral infections (e.g. measles) and are strongly associated with allergy.

Th1 and Th2 cells do not arise from distinct lymphocyte lineages but develop from a common precursor (Seder & Paul, 1994) and differentiate according to the nature and dose of the antigen (Hosken *et al*., 1995), co-stimulatory molecules expressed by the presenting cell, and the cytokine milieu in which T cell activation takes place. Furthermore, cross-regulation through cytokine production during the development of the response helps to further polarize or modify the proliferating Th1 and Th2 pools. This modulation through cytokine regulation could take place at the time individual cells become committed to one pattern of cytokine expression during antigen presentation, or subsequently by cytokine uptake having the potential to switch the cell into a different pattern of secretion. Evidence for a window of reversibility in the initial phase of a primary response has been reported, but after long-term stimulation individual cells apparently become irreversibly committed to a Th1 or Th2 phenotype (Murphy *et al*., 1996). The main interactions influencing Th1 and Th2 differentiation are summarized in Fig. 1.

Although the classification of T helper cells as Th1- or Th2-like is a useful model, individual responses are more likely to be mixed to some extent. Perhaps more interestingly, the nature of the dominant helper subset may change during the normal course of an immune response. For example, Th1 to Th2 switches are observed in the transition from acute to chronic graft-vs.-host disease; in Bornea disease; in certain mouse malaria models; and also in the progression from HIV infection to the development of AIDS, although the latter example is perhaps less representative as immune function is significantly disrupted in this case.

The classical model of Th1/2 cytokine interactions offers no explanation for these transitions. The standard picture is that the two T helper subsets each produces the factors required for their own differentiation and expansion (a positive feedback loop) and that cytokines produced by one subset inhibit the activation or proliferation of the other (negative feedback, or

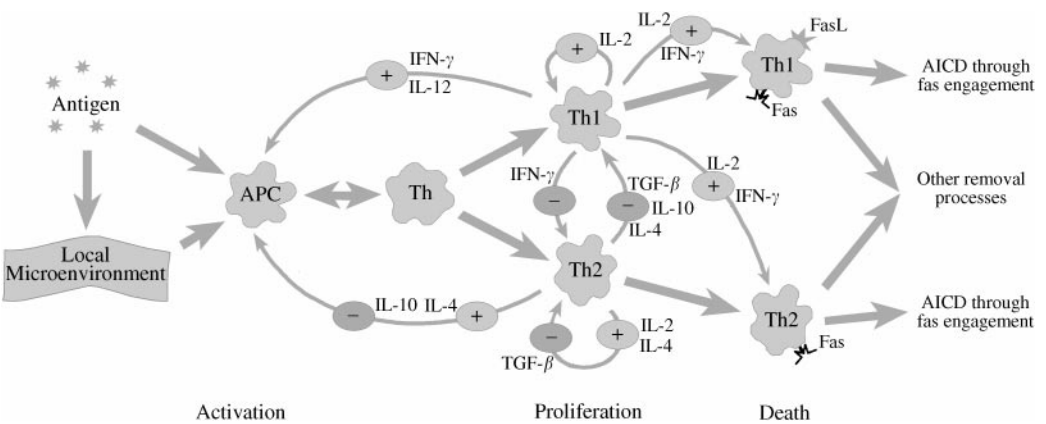

FIG. 1. Schematic representation of the interactions governing Th1/2 differentiation, proliferation and death. Naive cells stimulated by successful encounters with antigen-presenting cells (APCs) begin to differentiate into Th1-like or Th2-like effectors according to co-stimulatory signals and the ambient cytokine context. They go through several rounds of division before becoming armed effector cells. Previously activated (memory) cells also recirculate, are stimulated and rejoin the proliferating pool. The suppressive (−) or promoting (+) effects of cytokines on cell activity are broadly represented with arrows (for example, IFN-γ has a suppressive effect on Th2 proliferation; IL-10 downregulates APC function). Activation-induced cell death (AICD) occurs through cell–cell interactions (binding of the Fas surface molecule by FasL induces apoptosis). The effects of cytokines on the production of other cytokines (for example, the inhibition of IFN-γ by IL-4; the upregulation of IL-2 production by IL-12) are implied.

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

In this paper, we model many of the relevant cytokine interactions involved, including interactions that are both specific and non-specific for Th1 or Th2 branches. Other authors. (Morel *et al*., 1992; Fishman & Perelson, 1993, 1994, 1999; Carneiro *et al*., 1995; Muraille *et al*., 1995; Lev Bar-or & Segel, 1998) have modelled the Th1–Th2 system, with a variety of approaches and areas of emphasis. We return to comparisons of these models and the one presented here in the discussion. However, the roles of the local microenvironment and cytokine regulation of cell death mechanisms have not been included before in models of this sort. Our approach allows us to investigate the relative importance of the various stimulatory and regulatory mechanisms at play in effector T cell development, to understand the basis of dynamic helper subset switches and the interplay between the activation, proliferation and cell removal processes.

2. Components of the Model

We characterize a T helper cell response with variables $T_1 = [\text{Th1}]$ and $T_2 = [\text{Th2}]$, measuring the numbers of committed cells of the two types. Proliferating, effector and memory cells are grouped together. We do not model the dynamics of antigen clearance and have, in principle, a continuous antigenic stimulus. This takes the form of creation terms for both cell types that represent the activation and polarization of naive T lymphocytes by interaction with antigen-presenting cells and other components of the local microenvironment, consistent with recent experimental evidence (Kalinsky *et al*., 1999). In this sense, our approach is complementary to the interpretation by Murphy *et al*., (1996) and by Fishman and Perelson (1999) that individual T cells become committed to a type 1 or 2 differentiation pathway on a weighted, probabilistic basis at the presentation stage, and then intercellular cytokine interactions in the proliferating pool modify the Th1 or Th2 dominance at a population level. However, we also include the effect of antigen on the local microenvironment and cytokine feedback from the proliferating T cell pool, both of which modify the activation state of the antigen-presenting cells and influence the probability that a given APC-T cell encounter will lead to Th1 or Th2 differentiation. By not considering the removal of the pathogen, our model addresses the evolution of the immune response during antigen exposure and possibly states of chronic infection in which any feedback from success or failure of effectors has yet to be manifest. In addition to a constant decay term for cell loss and/or terminal maturation into effector cells, we also include a term for activation-induced cell death (AICD) based on recent experimental evidence that rates of apoptosis increase with repeated antigen stimulation. The rate equations for Th1 and Th2 populations then take the simple form

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

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

2.1. REGULATION BY CYTOKINES

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

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

Rates of cytokine production, receptor binding and decay are typically large compared to those of the cell population dynamics and we therefore make a steady-state assumption for S_1 and S_2, relating them directly to cell numbers. We incorporate the observation that type 2 cytokines tend to inhibit the production of cytokines by Th1 cells, but type 1 cytokines do not have such a marked suppressive effect on the synthesis of IL-4, IL-10, etc. In fact, the rate of binding and removal of any one cytokine is likely to be highly dependent on the density of cells which express the appropriate receptors for it, and perhaps also on competition between various receptors for common chains. However, in the absence of experimental data to provide estimates of cytokine consumption rates, we make the simplest assumption and assume that diffusion and degradation of cytokines takes place at a rate independent of T cell numbers. The clearance rates of all free cytokines are therefore assumed to be the same, and constant.

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

The expressions for the cytokine signals are then

$$S_1 = \frac{\alpha_1 T_1}{1 + k\alpha_2 T_2}, \quad S_2 = \alpha_2 T_2,$$

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

where $\tilde{S}_1$ is specifically the effective concentration of IL-2, taking into account the (constant) contribution S_0 from newly activated Th cells, known as Th0 cells, which produce significant quantities of this cytokine (Bendelac & Schwartz, 1991) as well as varying quantities of IL-4 and IFN-γ.

2.2. T CELL ACTIVATION

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

Antigen is taken up and processed by antigen-presenting cells (dendritic cells, monocytes and B cells) then displayed as small peptide fragments in association with MHC class II on the cell surface. Antigen-specific T helper cells become activated and acquire a Th1 or Th2 phenotype following interactions of sufficient affinity between the T cell receptor and the antigen peptide complex with MHC class II on the APC. The affinity of interaction is enhanced by CD4 binding to non-polymorphic determinants on MHC class II. T cell activation also depends on co-stimulation through accessory APC receptor–ligand interactions such as CD40/CD40L and B7/CD28 and binding of cytokines, particularly IL-12 secreted by APC (Sousa *et al.*, 1997). Importantly, it is now recognized that the state of APC activation by antigen acting indirectly through the local microenvironment or directly by binding to pattern recognition receptors also has a major impact on Th1 and Th2

differentiation (Kalińsky *et al*., 1999). The development into distinct Th1 or Th2 effectors is then determined by the type of antigen, its dose, route of entry and interactions with APC and local microenvironment, all of which are influenced by the host's genetic background.

The action of cytokines in the local microenvironment has been shown experimentally to be important and is a key factor affecting the Th1/Th2 balance in our model. The cytokines that have most effect on Th activation are IL-4, IL-10, IL-12 and IFN-γ. IL-12 is produced by dendritic cells and macrophages and plays a crucial role in Th1 development (Trinchieri, 1995). Its synthesis by APC is increased by IFN-γ secreted by Th1 cells. In turn, IL-12 promotes development of Th1 effectors from naive Th cells by increasing Th0 and Th1 IL-2R expression and production of IL-2 and IFN-γ. This positive feedback loop incorporating IL-12 and IFN-γ promotes Th1 differentiation/proliferation and is represented in a simplified way in our model by an enhancement of the Th1 activation term that is proportional to the strength of the Th1 cytokine signal. The effect of IL-12 is therefore implicit. IL-10 is an immunosuppressive cytokine produced by activated Th2 cells. It increases the death rate of precursor dendritic cells, inhibits the differentiation of monocytes to dendritic cells (Allavena *et al*., 1998) reduces the expression of MHC class II (de Waal Malefyt *et al*., 1991) and inhibits IL-12 production by DCs (de Smedt *et al*., 1997) and macrophages. It has also been reported to inhibit the production of Th1 cytokines (Taga & Tosato, 1992).

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

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

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

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

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

2.3. PROLIFERATION

The Th1 and Th2 proliferation rates are simple functions of the concentrations of the growth factors IL-2 and IL-4, as well as of IFN-γ and TGF-β. Strictly, division rates are independent of cytokine concentrations: the duration of the cell cycle is of the order of 18 h. We suggest that growth factors act to maintain cells in cycle (i.e. to increase the number of offspring produced by a given cell) and so postulate exponential growth with a rate constant that is proportional to the relevant growth factor concentration.

IL-2 is produced by activated Th0 and Th1 cells and is a growth factor for both Th1 and Th2. Binding of a sufficient quantity of IL-2 to receptors (IL-2R) on TCR-activated T cells induces the cell to go into cycle. It stimulates the production of IFN-γ by Th1, and prevents activated cells

from reverting to a quiescent state. It also plays a complex role in both rescue from and promotion of apoptosis, which we return to in more detail below.

IL-4 is an autocrine growth and differentiation factor for Th2 cells. It acts on Th1 cells to reduce their expression of IL-12R and their production of IL-2 and IFNγ (Tanaka *et al*., 1993). It is not clear whether IL-4 is also a growth factor for Th1 cells (Fernandez-Botran *et al*., 1988; Morel *et al*., 1996). Here we consider it as a growth factor for Th2 but not Th1, both alone and in synergy with IL-2., This allows Th2 cells to respond better to low amounts of IL-2 produced by nearby Th1 or Th0 cells. Alone, IL-4 has approximately one-tenth of the potency of IL-2 (Burke *et al*., 1997).

Whereas T cells stimulated in the presence of IL-12 and IL-4 differentiate into Th1 or Th2, respectively, addition of IL-10 leads to inhibition of the immune response (Groux *et al*., 1997) through an apparently distinct (Th3) subset that produces large amounts of IL-5, IL-10 and TGF-β, but little IL-2 or IL-4 and has a poor proliferative response to IL-2. Others have suggested that Th3 differentiation occurs in the presence of IL-4 (Inobe *et al*., 1998) and that these cells produce IL-10, TGF-β and IL-4 (Letterio & Roberts, 1998). It has also been shown that both TGF-β and IL-10 must be neutralized to reverse the effect of regulatory Th3 cell clones indicating that both cytokines have roles in immunosuppression (Groux *et al*., 1997).

In our model we group Th2 and Th3 cells together an assumption based on the similarity of their cytokine secretion patterns and differentiation factors (Seder *et al*., 1998; Mason & Powrie, 1998). TGF-β produced by Th3 inhibits IL-2-dependent T cell proliferation, which largely accounts for its anti-inflammatory activity (Seder *et al*., 1998). It blocks the signalling pathway of the IL-2 receptor (Bright *et al*., 1997), which may also account for its pro-apoptotic effects as signalling through IL-2R can promote the production of anti-apoptotic factors—see Section 2.4. TGF-β has also been shown to inhibit T cell proliferation by preventing G_1 to S-phase progression stimulated by IL-2 or IL-4 (Ruegemer *et al*., 1990). We find that TGF-β is not primarily an anti-inflammatory (anti-Th1) cytokine but in conjunction with IL-10 is a key cytokine for the regulation of Th2 responses. This is entirely consistent with its known ability to suppress IL-4 production, B cell maturation and IgG and IgM synthesis.

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

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

Th2 proliferation is driven by IL-4 alone and in combination with IL-2, and inhibited by TGF-β and IFN-γ:

$$\text{Th2 proliferation rate}$$

$$= \beta_2 T_2 \frac{([\text{IL4}] + c[\text{IL4}][\text{IL2}])}{(1 + kS_1)(1 + kS_2)}$$

$$= T_2 \frac{(\beta_2^{IL4} S_2 + \beta_2^{IL2/4} \tilde{S}_1 S_2)}{(1 + kS_1)(1 + kS_2)},$$

where β_1 and β_2 are proportionality constants and $\beta_2^{IL4}/\beta_1 \simeq 0.1$ [24].

2.4. CELL DEATH

T cell death can occur either as a result of repeated activation or from inadequate stimulation and cytokine deprivation. Both may play important roles in limiting the immune response in its later stages (Orteu *et al*., 1998).

Repeated ligation of the TCR/CD3 complex causes activated T cells to undergo apoptosis (Lynch *et al*., 1995). It is thought that this process, called activation-induced cell death (AICD), is triggered by increased Fas-ligand (FasL) expression on activated T cells. This engages the Fas receptor on the cell surface, activating the apoptotic death pathway (Ju *et al*., 1995). This could occur either through secretion of soluble FasL binding to Fas on the same cell (suicide) or through cell–cell contact (fratricide). The increase in FasL expression after repeated reactivation of mature lymphocytes appears to be transient—AICD has been reported to occur only when the cell is in cycle (Boehme & Lenardo, 1993), and surface FasL is shed rapidly (Akbar, pers. comm.). AICD rates are enhanced by the

presence of IL-2 and IL-15 (Lenardo, 1991). These facts suggest that Fas-mediated cell death may be important for limiting the magnitude of an effector T cell response.

The differential importance of AICD for Th1 and Th2 phenotypes is an issue we explore with our model. There are data to suggest that AICD is a suicidal phenomenon (Brunner *et al*., 1995) largely restricted to Th1 cells (Zhang *et al*., 1997). In our model, we work under the assumption that Th1 clones express FasL at higher levels than Th0/Th2 cells (Hahn *et al*., 1995). Reciprocally, Th2 cells can undergo Fas-induced apoptosis in the presence of activated Th1 cells, but AICD is less frequent among Th2 cells in mixed populations, indicating a lower susceptibility (Ramsdell *et al*., 1994). Below, we discuss cases in which the Th1 AICD process is modelled as either a cell–cell or single-cell process.

2.4.1. *IL-2 and apoptosis*

It has become clear that IL-2 plays a central role in both proliferation and AICD. It increases that transcription rate and expression levels of FasL, and activated $CD4^+$ T cells in IL-2 gene-inactivated ($IL\text{-}2^{-/-}$) mice show a reduced propensity for AICD, which can be restored by addition of exogenous IL-2 at both the activation and effector stage (Refaeli *et al*., 1998). Humans and mice with Fas or FasL gene defects (*lpr* and *gld*) or IL-2 deficiencies exhibit lymphoproliferative disorders (Wanatabe-Fukunaga *et al*., 1992; Nagata & Suda, 1995; Fisher *et al*., 1995), suggesting that while the role of IL-2 as a growth factor can be filled by other cytokines (e.g. IL-15), its pro-apoptotic effects cannot.

Conversely, IL-2 can also *inhibit* apoptosis (Rafaeli *et al*., 1998). It stimulates the production of anti-apoptotic proteins such as Bcl-2 and the removal of IL-2 from activated T cells leads to reduced Bcl-2 expression and apoptosis (Akbar *et al*., 1993). This process is commonly referred to as death through cytokine deprivation.

In summary, we model the removal of T helper cells from the effector pool with two terms. One represents IL-2-dependent fratricidal AICD, and the other represents the combined processes of apoptosis through the Bcl-2/Bax pathway, anergy, necrosis, and peripheral diffusion, for which a constant *per capita* rate of removal μ for both Th1 and Th2 is the simplest choice. Note that we model the apoptotic influence of IL-2 through the Fas pathway only. Studies have shown that AICD is the dominant apoptotic mechanism during the development of a response, with death through cytokine deprivation being more prominent at the resolution stage (Orteu *et al*., 1998).

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

The form of the Th2 depletion term assumes that only Th1 cells express FasL and Th1–Th2 encounters are required for Th2 AICD:

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

2.5. MODEL SUMMARY

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

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

Gathering the terms detailed above,

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

$$\frac{dT_2}{dt} = \frac{\xi_2(\gamma_2 + S_2)}{1 + kS_2} + T_2 \frac{(\beta_2^{IL4} S_2 + \beta_2^{IL2} \tilde{S}_1 S_2)}{(1 + kS_1)(1 + kS_2)} - \Delta_2 \tilde{S}_1 T_1 T_2 - \mu T_2, \quad (3)$$

We rescale to the following dimensionless combinations of variables; $x_i = k\alpha_i T_i$, $\tau = \mu t$, $\theta_i = \gamma_i k$, $\chi_0 = kS_0$, $\sigma_i = \xi_i \alpha_i / \mu$, $\delta_i = \Delta_i / \mu k^2 \alpha_1$, $\pi_1 = \beta_1 / \mu k$, $\pi_2 = \beta_2^{IL2/4} / \mu k^2$, $\rho = \beta_2^{IL2} k / \beta_2^{IL2/4}$. Using expressions (1) for the cytokine signals S_1 and S_2, the resulting dynamical equations are

$$\frac{dx_1}{d\tau} = \sigma_1 \frac{\theta_1(1 + x_2) + x_1}{(1 + x_2)^2} + \pi_1 x_1 \frac{x_1 + \chi_0}{(1 + x_2)^2} - \delta_1 \left(\frac{x_1 + \chi_0}{1 + x_2} \right) x_1^2 - x_1, \quad (4)$$

$$\frac{dx_2}{d\tau} = \sigma_2 \left(\frac{\theta_2 + x_2}{1 + x_2} \right) + \pi_2 \left[\rho + \left(\frac{x_1 + \chi_0}{1 + x_2} \right) \right] \times \frac{x_2^2}{1 + x_1 + x_2} - \delta_2 \left(\frac{x_1 + \chi_0}{1 + x_2} \right) x_1 x_2 - x_2. \quad (5)$$

We have the additional constraint $\rho = \pi_1 / 10\pi_2$. We summarize the biological significance of the parameters in Table 1.

We would like to address the following issues.

- Can we understand the dose dependence of the response? [Various *in vivo* and *in vitro* studies have shown that Th1 or Th2 responses can depend on antigen dose (Hosken *et al.*, 1995)]
- The role of AICD in T cell homeostasis.
- If AICD is more important for Th1 than for Th2, as we suspect, can we provide a model for Th2 regulation?
- What are the consequences of differential FasL expression and AICD susceptibility for the Th1/Th2 balance?
- The dynamics of Th1 to Th2 switches.
- Can switches of response be induced?

3. Analysis

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

1. Neglect any susceptibility of Th2 cells to Fas-mediated apoptosis ($\delta_2 = 0$). This is in keeping with experimental evidence that Th1 cells and not Th2 cells preferentially undergo AICD (Zhang *et al.*, 1997).
2. Ignore the contribution to the IL-2 concentration from Th0 cells ($\chi_0 = 0$).
3. Remove the additional Th1/2 cytokine contributions from other (non-T cell) sources ($\theta_1 = \theta_2 = 0$). Thus, the activation signals σ_1 and σ_2 are strongly coupled to cytokine-mediated feedback from the proliferating T cells.

The equations become

$$\frac{dx_1}{d\tau} = \sigma_1 \frac{x_1}{(1 + x_2)^2} + \pi_1 \frac{x_1^2}{(1 + x_2)^2} - \delta_1 \frac{x_1^3}{1 + x_2} - x_1, \quad (6)$$

TABLE 1
Biological interpretation of the dimensionless parameters in eqns (4) *and* (5)

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

$$\frac{dx_2}{d\tau} = \sigma_2 \frac{x_2}{1+x_2} + \pi_2 \left(\rho + \frac{x_1}{1+x_2}\right) \times \frac{x_2^2}{1+x_1+x_2} - x_2. \quad (7)$$

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

Primary responses and initial conditions. A truly naive response, with no assistance from cross-reactive memory T cells, is represented in our model by trajectories beginning at (0, 0). Importantly, the assumption $\theta_1 = \theta_2 = 0$ makes the origin a steady state. When precisely zero antigen-specific, pre-committed Th1 or Th2 cells are available, we remain at the no-response state even in the presence of antigen. With sufficiently large activation signals σ_1 and σ_2, the origin is unstable and we encounter the problem of sensitivity to initial conditions. The nature of the immune response can be strongly dependent on the composition of a small initial population of predisposed Th1 or Th2 cells that are responsive to the antigen. Although it is biologically unrealistic, we will first proceed with the analysis using $\theta_1 = \theta_2 = 0$ as this allows us to explore analytically the characteristics of the non-zero steady states. Later, when we introduce non-zero values for θ_1 and θ_2, we see that even though the behaviour near the origin is altered (and the response becomes less sensitive to initial conditions), our broad picture of the dynamics of immune responses is essentially unchanged.

Steady states

- (0, 0). The eigenvalues of the equilibrium are $(\sigma_1 - 1, \sigma_2 - 1)$, implying that for low stimulation this "no-response" state is stable. Transcritical bifurcations take place at $\sigma_1 = 1$ and $\sigma_2 = 1$ as unstable, negative Th1 or Th2 fixed points pass through the origin and exchange stability with it.
- An exclusively Th2 response $(0, x_2^*)$. If we neglect the contribution to Th2 proliferation from IL-4 alone ($\rho = 0$), then we have $x_2^* = \sigma_2 - 1$, with $\sigma_2 > 1$ necessary for its existence. The eigenvalues are $(1 - \sigma_2)/\sigma_2$ and $(\sigma_1 - \sigma_2^2)/\sigma_2^2$, so $\sigma_2 > \sqrt{\sigma_1}$ is a necessary and sufficient condition for stability. Note that this expression for the magnitude of the pure Th2 state does not involve the proliferation parameters, and so this cannot really be considered to represent an immune response. This is clearly a consequence of the dependence of Th2 cells on the Th1 cells for the production of growth factor when $\rho = 0$.

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

$$x_2^* = \frac{\sigma_2 - 1}{1 - \rho\pi_2}. \quad (8)$$

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

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

Since $\rho\pi_2 < 1$, we require $\sigma_2 > 1$ for a Th2 state to exist at all and so the eigenvalues never diverge. This state is stable if

$$\sigma_1 < \left(\frac{\sigma_2 - \pi_2\rho}{1 - \rho\pi_2}\right)^2. \quad (9)$$

As expected, the more efficient IL-4-driven proliferation is (i.e. the larger we make $\rho\pi_2$), the

more stable the pure Th2 response is against pro-Th1 signals.

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

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

so if $\delta_1 \neq 0$ and $\sigma_1 \geqslant 1$ there exists only one positive non-zero fixed point on the x_1-axis, always stable in the x_1 direction and stable in the x_2 direction if $\sigma_2 < 1$. If $\sigma_1 < 1$ then for $\pi_1^2 + 4\delta_1\ (\sigma_1 - 1) < 0$ there are two positive stationary points, the larger one stable in the x_1 direction and the smaller one unstable. These are created in a saddle node bifurcation in the parameter σ_1 at $\sigma_1 = 1 - \pi_1^2/4\delta_1$. The eigenvalue in the x_2 direction for both values of x_1^* is $\sigma_2 - 1$, and so if $\sigma_2 < 1$ the largest Th1 response is stable.

The efficacy of FasL-induced cell–cell killing (δ_1) is crucial for setting the scale of the response. For small δ_1 a Th1 response can be very large. There is also a threshold effect when $\sigma_1 < 1$; for small initial x_1 we relax back to the no-response state $x_1 = 0$, while for larger initial x_1 we move to the non-zero stable state x_1^*. In the limit $\delta_1 \to 0$ (i.e. no Fas/FasL expression on Th1 effectors), the threshold value of x_1 is $(1 - \sigma_1)/\pi_1$, and $x_1^* \to \infty$.

- A mixed state $M_1 = (x_1^{**}, x_2^{**})$. This exists when activation signals σ_1 and σ_2 are comparable; it is illustrated in Fig. 2.

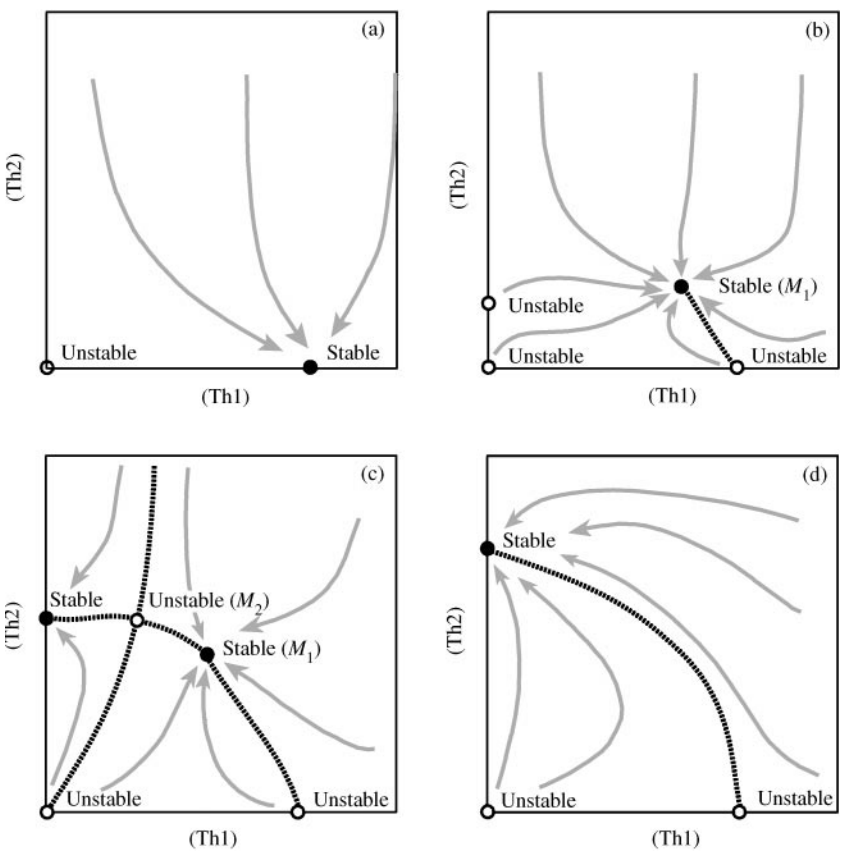

FIG. 2. The creation and disappearance of a stable, mixed Th1/2 population with increasing Th2 stimulus (σ_2). In these schematic phase diagrams, open circles represent unstable states and filled circles are stable states. The Th1 activation signal σ_1 is constant (> 1) throughout, ensuring that there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

Besides, we see from the above that we can switch from an ongoing Th1 to a Th2 response by changing the activation parameter σ_2. On the other hand, increasing σ_1 strengths the Th1 arm of the co-existent state but does not eliminate the Th2 component. We return to this asymmetry below.

3.1. THE EFFECT OF AICD FOR TH2 CELLS

It we suppose that Th2 cells are susceptible to AICD (that is, $\delta_2 \neq 0$), the exclusively Th1 response persists and its stability is enhanced. Clearly, the eigenvalue λ_1 in the x_1 direction is unaffected and that corresponding to the eigenvector with the component in the x_2 direction becomes.

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

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

3.2. THE NATURE OF THE Th1 Fas–FasL INTERACTION

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

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

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

3.3. THE INFLUENCE OF TGF-β AND IL-10

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

$$\frac{dx_1}{d\tau} = \sigma_1 \frac{x_1}{(1 + x_2)^2} + \pi_1 \frac{x_1^2}{(1 + x_2)} - \delta_1 \frac{x_1^3}{1 + x_2} - x_1, \tag{10}$$

$$\frac{dx_2}{d\tau} = \sigma_2 \frac{x_2}{1 + x_2} + \pi_2 \frac{(\rho(1 + x_2) + x_1)x_2^2}{1 + x_1 + x_2} - x_2. \tag{11}$$

If $\rho > 0$ then the Th2 population diverges for x_2 sufficiently large. However, if we neglect the influence of IL-4 as an autocrine growth factor for Th2 cells (set $\rho = 0$), this divergence is suppressed. This is because removing the term proportional to ρ means that at large x_2 the dominant contribution to eqn (11) is the death term. We then regain the no-proliferation Th2-dominated state $(0, x_2^*)$ where $x_2^* = \sigma_2 - 1$. In this case, we observe large, transient Th2 spikes which then relax to this non-proliferative Th2 state [see Fig. 3(a)]. The suppressive effect of TGF-β has no influence on the magnitude of the exclusively Th1 response, as in our model it is produced only by Th 2/3 cells.

Next, we explore the effect of removing the inhibition of T helper cell activation by IL-10. This corresponds to removal of a factor of $(1 + x_2)^{-1}$ from the activation terms (those

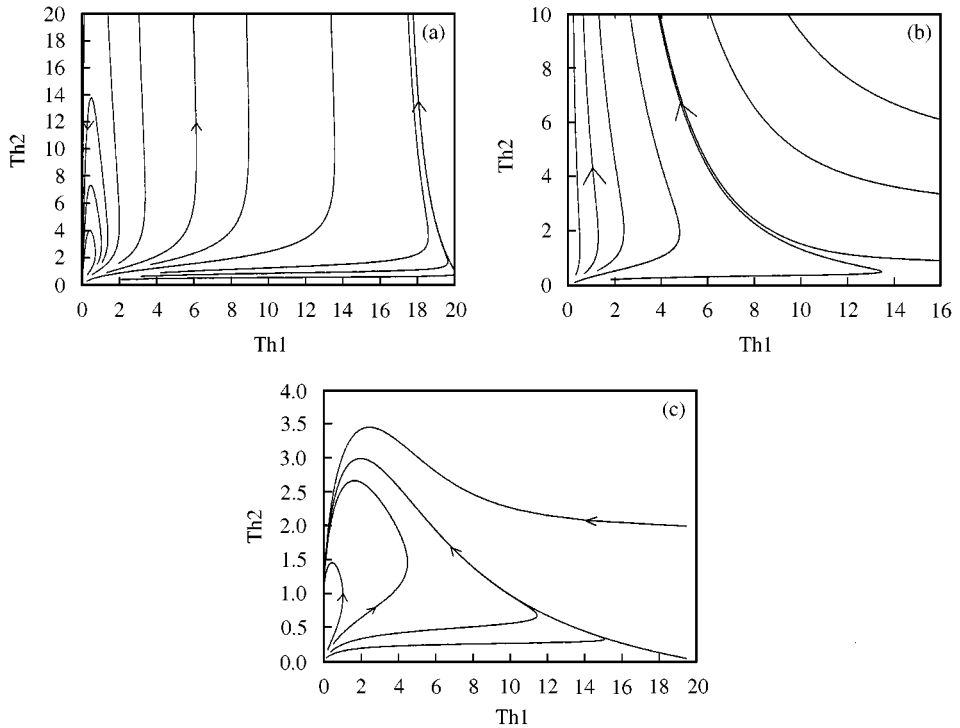

FIG. 3. (a) The effect of the absence of TGF-β suppression. The dynamical equations are eqns (10) and (11) and the parameters are $\sigma_1 = \sigma_2 = 2$, $\pi_1 = \pi_2 = 2$, $\delta_1 = 0.1$, $\delta_2 = 0$, $\rho = 0$. There is a saddle at ($x_1 \simeq 20$, $x_2 \sim 0$) and a stable point at ($x_1 = 0$, $x_2 = \sigma_2 - 1 = 1$). Notice the progression Th1 $\rightarrow$ large Th2 response $\rightarrow$ non-proliferative Th2 response. (b) Removing IL-10 suppression. The stable Th2 state ($x_1 = 0$, $x_2 = \sigma_2 - 1$) is removed and we have uncontrolled Th2 responses. (c) The same parameter set with TGF-β suppression and IL-10 inhibition included [eqns (6) and (7)].

proportional to σ_1 and σ_2). The dynamical equations are then

$$\frac{dx_1}{d\tau} = \sigma_1 \frac{x_1}{(1+x_2)} + \pi_1 \frac{x_1^2}{(1+x_2)^2} - \delta_1 \frac{x_1^3}{1+x_2} - x_1, \tag{12}$$

$$\frac{dx_2}{d\tau} = \sigma_2 x_2 + \pi_2 \left[\rho + \left(\frac{x_1}{1+x_2}\right)\right] \times \frac{x_2^2}{1+x_1+x_2} - x_2. \tag{13}$$

As with TGF-β, IL-10 has no influence on the size of Th1 responses as it is not produced by these cells. For all initial conditions with a non-zero Th2 population, we see explosion of the Th2 term and the Th1 population vanishes [Fig. 3 (b)]. Note the shift from Th1 to Th2 dominance if we start with small Th2 numbers.

We see then that it is only the combination of both suppression of proliferation by TGF-β and inhibition of activation by IL-10 which ensures that activated Th2 population sizes are always limited [Fig. 3(c)]. Experiments indicate the importance of TGF-β in the regulation of Th2 populations. For example, it has been shown that protection against Th2-induced autoimmunity is TGF-β dependent (Bridoux *et al.*, 1997). Further, the function of regulatory T cells, which suppress both Th1 and Th2 immune responses in the mucosa, is dependent on *both* IL-10 and TGF-β (Mason & Powrie, 1998) and the results from our model are in keeping with this observation.

3.4. ANTIGEN DOSE

How does varying the antigen dose influence the type of response? The activation efficiency of

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

Let us assume, for example, that a given antigen is slightly more likely to favour a Th1 than a Th2 response. We set $\sigma_1/\sigma_2 = c > 1$. Changing the antigen dose alters the stimulation strengths σ_1 and σ_2 but keeps their ratio c constant. For low doses of this antigen ($\sigma_2 < 1$), we have stable pure-Th1 states, and with growing activation parameters the Th1 dominance is taken over by an ever-increasing Th2 population. This is illustrated in Fig. 4, where $c = 2$.

This is consistent with the experimental observation that low-doses favour type 1 responses and intermediate to high doses favour type 2 responses (Menon & Bretscher, 1998; Doherty & Coffman, 1996). The model does not predict Th1 dominance at very high antigen concentrations.

Note, however, that if Th2 cells are susceptible to Fas-mediated apoptosis ($\delta_2 > 0$), the Th1-dominated steady state does not lose its stability and it is then simply the initial conditions which determine whether we end up in a pure type 1 or 2 state.

3.5. SUMMARY

We have a model in which

1. We assume that a non-primed individual mounts a response starting at a state with few antigen-specific T cells.
2. In the absence of Fas-mediated cell death (AICD) for Th2 cells, and for wide ranges of the remaining parameters with $\sigma_2 > 1$, we

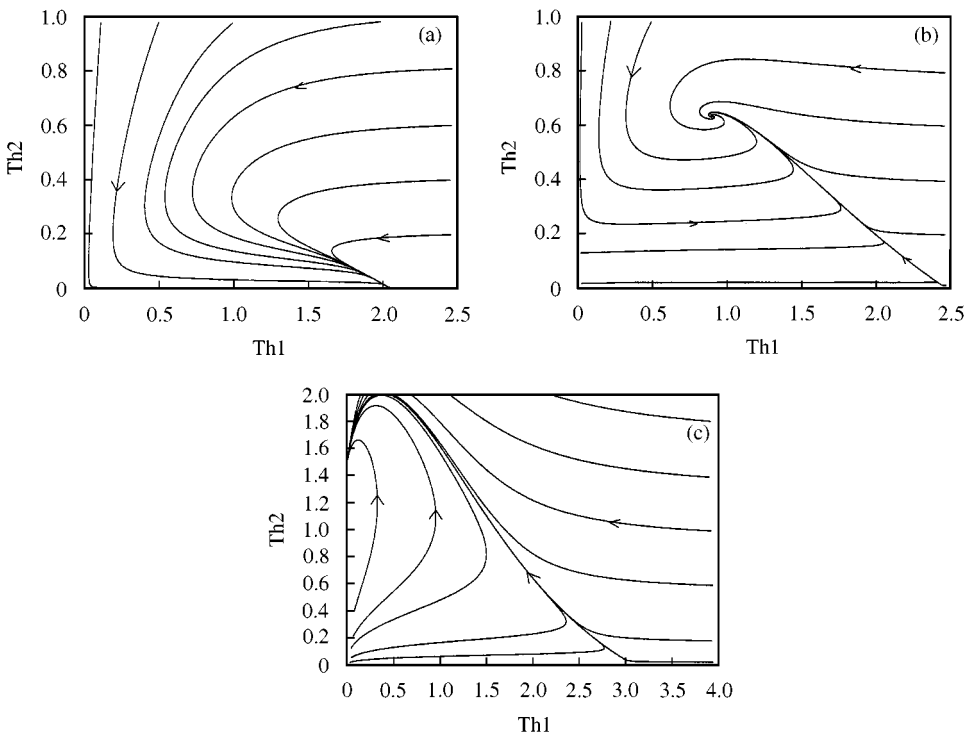

FIG. 4. Phase portraits for varying antigen levels, in the absence of Th2 AICD. (a) Low dose. The parameters are $\sigma_1 = 1.1$, $\sigma_2 = 0.55$, $\pi_1 = \pi_2 = 2$, $\delta_1 = 1$, $\rho = 0.1$, $\delta_2 = \theta_1 = \theta_2 = 0$. The only stable point is $(x_1^*, 0)$, where $x_1^* \simeq 2$; no stable Th2-dominated state or coexistence state exists, as $\sigma_2 < 1$. Clearly, low antigen dose favours a Th1 response. (b) Intermediate antigen dose. We double σ_1 and σ_2 (keeping their ratio constant); other parameters as before. The fixed point $(x_1^*, 0)$ loses its stability in a transcritical bifurcation: with increasing σ_2 a stable coexistence state moves up into the positive quadrant. (c) High antigen dose. When we again double the activation parameters ($\sigma_1 = 4.4$, $\sigma_2 = 2.2$), the mixed state is lost and the steady state $(0, x_2^*)$ becomes stable. Here, then, high antigen dose leads to Th2 dominance.

see only steady state at $(0, x_2^*)$ and dynamics corresponding to an initial Th1 response followed by a chronic (stable) Th-2-dominated state. If $\sigma_2 < 1$ there is Th1 dominance with no transient Th2 response;
3. The absence of Fas/FasL interactions leads to uncontrolled inflammatory (type 1) responses. Further, a suicidal AICD process is not sufficient to regulate Th1 cell numbers and a cell–cell interaction is required;
4. The susceptibility of Th2 cells to AICD (parameterised by δ_2) stabilises the existence of exclusive Th1 responses. For wide ranges of parameters we have stable Th1 and Th2 responses and an unstable coexistent state, with the areas of the basins of attraction governed by the relative sizes of the stimuli σ_1 and σ_2, as expected;
5. TGF-β is essential to avoid explosive expansion of Th2 numbers if any autocrine (IL-4-driven) growth among these cells takes place. However, if Th2 cells require *both* IL-2 and IL-4 for growth ($\rho = 0$), the absence of the suppressive effect of TGF-β leads to a large, transient but bounded Th2 response;
6. IL-10 is an essential component of the control mechanism for Th2 populations;
7. For large regions of parameter space, with Th2 cells less susceptible to AICD than Th1, we see transient Th1-dominance followed by a stable Th2 response;
8. Lower antigen doses tend to favour Th1 responses; higher doses favour Th2.

4. Further Refinements

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

4.1. IL-2 PRODUCTION BY Th0

The effect of IL-2 production by Th0 cells is modelled by the parameter χ_0 in eqns (4) and (5). It represents the number of IL-2 producing Th0 cells present. The pure-Th2 response $(0, x_2^*)$, where

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

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

The magnitude of the pure Th1 response is

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

Here the interplay between enhancement of proliferation and AICD is more complex. The overall effect is to diminish the pure Th1 response slightly with increasing χ_0.

4.2. T HELPER-INDEPENDENT ACTIVATION SIGNALS

As described in Section 2.2, components of the innate immune system and the local microenvironment may modify APC activation states and need a "bootstrap" mechanism for Th1 or Th2 self-promotion. We model this by including the terms proportional to θ_1 and θ_2 in eqns (4) and (5).

The most obvious influence of θ_1 and θ_2 is to remove the origin as a fixed point and to ensure that the axes are no longer nullclines. Over a wide range of parameters, when both Th1 and Th2 cells are susceptible to AICD there exist near-exclusive Th1 and Th2 responses and an unstable mixed-response state [Fig. 5(b)] as in the case where θ_1 and θ_2 are zero.

4.2.1. *Triggering a Th1 response*

Altering the rate of AICD for Th2 cells (δ_2) can give rise to profoundly different types of behaviour. Let us look at a situation where the only stable steady state of the system is Th2-dominated, which we have seen is a robust feature of the system help to trigger a Th1 or Th2 reaction.

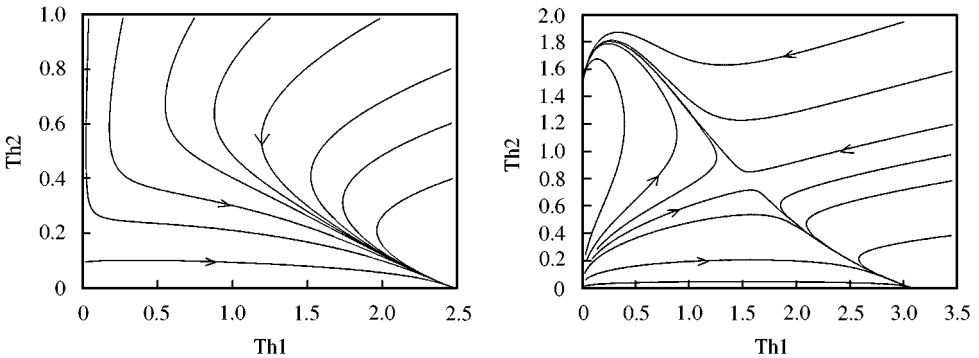

FIG. 5. The influence of antigen dose when Th2 cells are susceptible to Fas-induced cell death. We set $\delta_2 = 0.5$, with other parameters as in the previous figure. For low antigen doses, AICD does not alter the system behaviour and there is Th1 dominance as in Fig. 5 (a). For intermediate antigen concentrations, however, Th1 dominance is still the only steady state (left); at high doses, there are both pure-Th1 and Th2 responses, divided by the stable manifold of the unstable mixed Th1/2 state.

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

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

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

Despite this effect on the location of the steady state, θ_1 influences the dynamics by promoting transient Th1 dominance (Fig. 6). Even small, external pro-Th1 influences result in large detours towards a Th1-biased response which later settles into the Th2 state. This assumes of course that antigen clearance has not taken place in the meantime.

If Th2 cells are susceptible to AICD, the system exhibits a behaviour that is more intuitively

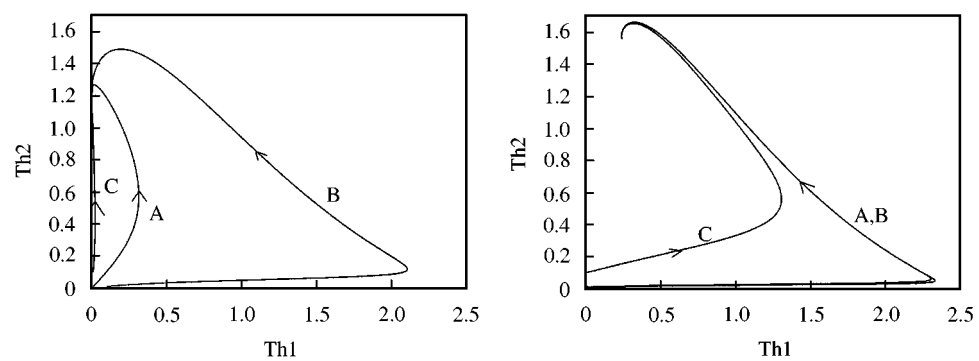

FIG. 6. The influence of a Th1 stimulus θ_1 on steady states and orbits. The parameters are $\sigma_1 = \sigma_2 = 2$, $\pi_1 = \pi_2 = 2$, $\delta_1 = 1$, $\delta_2 = 0$, $\rho = 0.1$, $\chi_0 = 0$, $\theta_2 = 0$; θ_1 is set to 0 and 0.2 in the left and right plots, respectively. We integrate orbits A, B and C for the initial conditions (0.01, 0.01), (0.1, 0.01), and 0.01, 0.1), respectively. Note that small θ_1 values do not substantially alter the position of the Th2-dominated state but *do* affect the transient. Th1 dominance.

obvious. Small pro-Th1 influences do not lead to noticeable effects, and in theory very high Th-1 stimuli lead to global stability of the pure Th1 response. We assume, however, that this is not biologically realistic due to saturation of both cytokine effects and the activating capacity of antigen-presenting cells. Thus, purely increasing θ_1 (e.g. through exogenous IL-12) is never sufficient to induce a permanent switch from a Th2 to a Th1 response (see Section 4.3).

4.2.2. *Triggering a Th2 response*

We start with a parameter set that describes a Th1-dominated system ($\sigma_1 > 1$, $\sigma_2 < 1$, $\pi_1 \sim \pi_2 \sim 2$, $\delta_1 = 1$, $\delta_2 = 0.5$, $\rho = 0.1$, $\theta_1 = \theta_2 = \chi_0 = 0$). Increasing the Th2 stimulus θ_2 leads to coexistence and/or bistability with a pure Th2 response and mixed Th1/Th2 populations and then finally to Th2 dominance, independent of the susceptibility of Th2 cells to AICD. This is illustrated in Fig. 7.

The instructive role of inmate immunity and its contribution to the decision-making process are still unclear. However, we see from the simple observations above that there is a fundamental asymmetry in the Th1–Th2 interactions that manifests itself under chronic stimulus. Short-term changes in the nature of the immune response can be induced by changing θ_1 or θ_2 in an intuitively obvious way, however.

4.3. TREATMENT WITH IL-12 TO INDUCE A TH2 TO TH1 SWITCH

Here we address the question of whether we can alter the Th1/Th2 outcome of an immune response by treatment with cytokines. We begin by supposing that, for example, constant treatment with IL-12 would simply change the Th1-independent activation parameter θ_1 (see Section 4.2).

Experiment has shown that treatment with IL-12 alone may not be sufficient to shift an established Th2 response to Th1 and that reduction of the antigen dose is also needed (Nabors *et al.*, 1995). This is in good agreement with out observations regarding θ_1 in the previous section and is illustrated in Fig. 8.

Note that a switch from Th2 to Th1 dominance can only be induced when Th2 cells are susceptible to AICD ($\delta_2 \neq 0$). The results of Nabors *et al.* (1995) in conjunction with those from our model would indicate that Th2 cells *do* undergo Fas-induced cell death to at least some small degree.

4.4. OSCILLATORY BEHAVIOUR

Beginning from a Th1-dominated state with $\sigma_1 \neq 0$ and $\sigma_2 = 0$, increasing the Th2 stimulus σ_2 drives the system into a mixed state. This evolves into a stable, globally attracting limit cycle corresponding to oscillatory Th1–Th2 responses. Further increases in the Th2 activation signal lead us into a stable Th2-dominated state (Fig. 9).

Conversely, if we begin with $\sigma_2 \neq 0$, $\sigma_1 = 0$, and increase σ_1, no new fixed points or qualitative changes in behaviour appear; Th2 responses always dominate eventually, even with a strongly Th1-biased initial condition. The increased Th1 signal strength leads to an increase in the switching time, however. If we were to include the

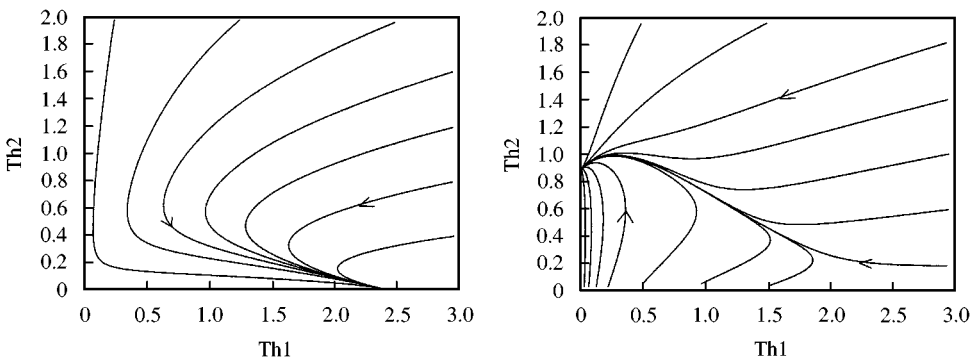

FIG. 7. Shifting from Th1 to Th2 dominance by increasing the pro-Th2 co-stimulatory signal θ_2 from 0 (left) to 1 (right). The remaining parameters are $\sigma_1 = 2$, $\sigma_2 = 0.8$, $\pi_1 = \pi_2 = 2$, $\delta_1 = 1$, $\delta_2 = 0.5$, $\rho = 0.1$, and $\theta_1 = \theta_2 = \chi_0 = 0$.

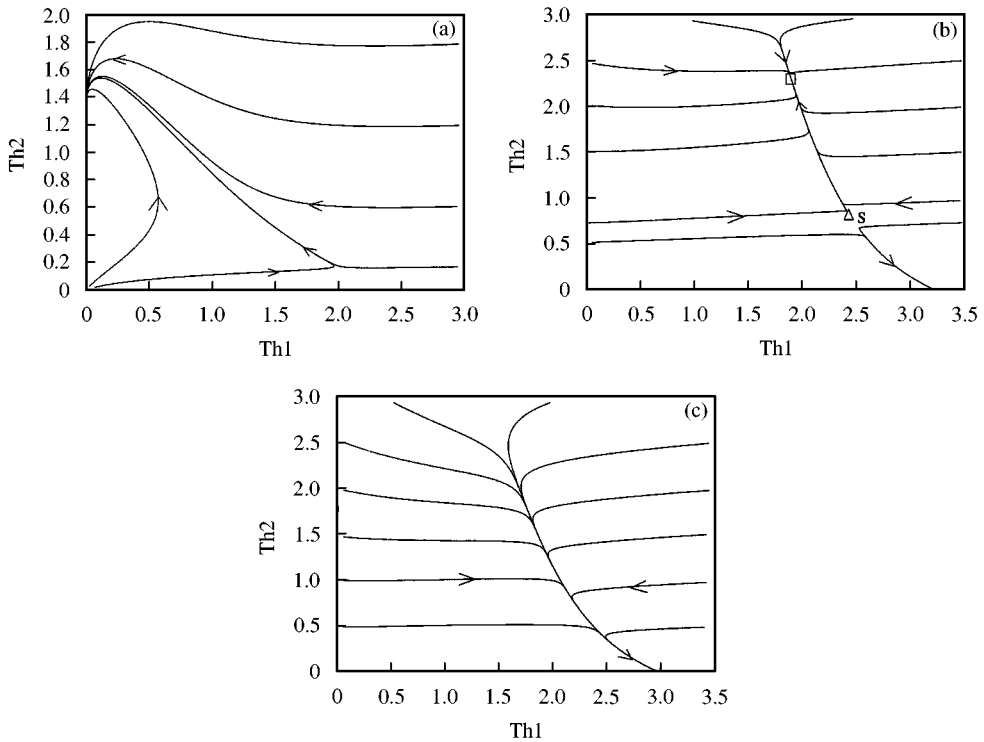

FIG. 8. Modification of a response with externally added cytokines. We start with parameters $\sigma_2 = \sigma_1 = 2$, $\pi_1 = \pi_2 = 2$, $\delta_1 = 1$, $\delta_2 = 0.2$, $\rho = 0.1$, $\chi_0 = 0.1$, $\theta_1 = \theta_2 = 0$. The only attractor is a pure Th2 response and no exogenous IL-12 is present (a). Injecting IL-12 (b) increases the value of the parameter $\theta_1 \to 5$. All other parameters are as before. The stable manifold of the saddle S divides the phase space and a pure Th1 state now exists. Note that starting at the pure Th2 state leads to coexistence of the two phenotypes and not to inversion of the Th1/Th2 ratio. However, if we additionally decrease the antigen dose ($\sigma_1 = \sigma_2 = 1.5$) all orbits end up in the pure Th1 state (c).

effects of antigen clearance, then quite possibly the infection would be cleared successfully by a type 1 response before the switch. However, the model indicates that when Th2 cells are not subject to significant AICD (δ_2 is small), eventually a Th2 response dominates, if the Th1 response fails to resolve the infection. In a sense, a type 2 response appears to be the "default".

A common feature of biological systems appears to be the progression from stability through to oscillatory behaviour and back to stability with increasing stimulus. This is in contrast to mechanical dynamical systems which tend to progress from stability to oscillatory behaviour through to chaos if their dimensionality is high enough. For example, oscillations in levels of the cytokine TNF-α have been observed in the eye following corneal allograft rejection over an intermediate range of antigen concentration (Chan *et al.*, 1999). In Fig. 9, we see that in the absence of Th2 AICD, low levels of Th2 stimulation lead to Th1 responses; intermediate levels give rise to oscillations in the Th1 and Th2 cell numbers and higher Th2 stimulation stabilizes a Th2 response.

In effect, the system is unable to switch cleanly from one stable state to the other as the stimulus is altered. Whether this could be a general feature of cytokine control systems, or a consequence of breakdown of regulatory mechanisms, is an open question.

4.5. Th1 → Th2 SWITCHES

One could say that the only parameter in our model which profoundly alters the qualitative behaviour is the susceptibility to Fas death of Th2 effectors, δ_2. If they are much less susceptible than Th1 cells ($\delta_2 \ll \delta_1$) and $\sigma_2 > 1$, our model predicts that Th1–Th2 switches are generic features of chronic infections and that dynamic Th2–Th1 switches are not possible. This

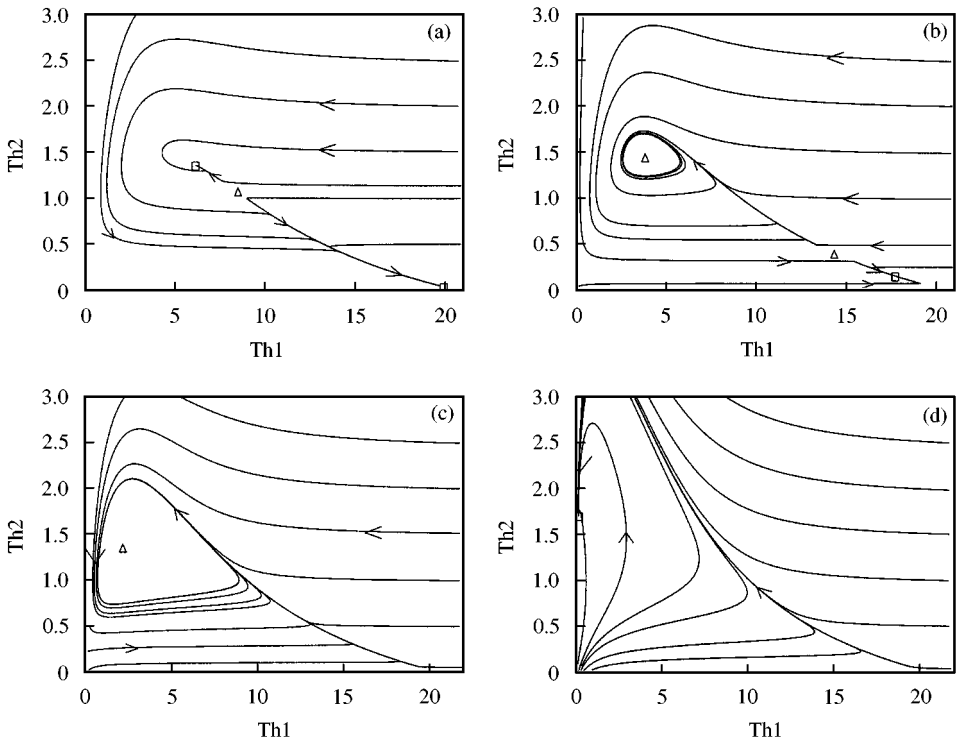

FIG. 9. Illustrating the switching from a Th1-dominated state to a Th2-dominated one through an intermediate, oscillatory phase as we change the Th2 stimulus σ_2. In (a) the parameters are $\sigma_1 = 2$, $\sigma_2 = 0.27$, $\pi_1 = \pi_2 = 2$, $\delta_1 = 0.1$, $\rho = \theta_1 = \theta_2 = \chi_0 = 0.1$, $\delta_2 = 0$. Stable equilibria are denoted with squares, unstable equilibria with triangles. Even though a stable coexistent state exists, the basin of attraction for the Th1 response covers the vicinity of the origin. Now we increase the Th2 activation signal to $\sigma_2 = 0.5$ and a Hopf bifurcation at $\sigma_2 = 0.45$ creates a stable limit cycle (b). Note that the stable Th1 state is about to be annihilated, but its basin of attraction is still dominant for a naive response. We increase the Th2 activation signal further [$\sigma_2 = 0.8$, (c)], and now the stable limit cycle is the only attractor for the system. This limit cycle decreases in size with increasing σ_2 and vanishes in a second Hopf bifurcation at $\sigma_2 \simeq 1.1$. A stable, near-exclusive Th2 dominated state remains [$\sigma_2 = \sigma_1 = 2$(d)]. Notice here the Th1 $\rightarrow$ Th2 switches in the absence of AICD for Th2 cells ($\delta_2 = 0$). This scenario is structurally stable to small increases in δ_2, which create a narrow basin of attraction for a stable, pure Th1 response near the Th1-axis.

asymmetry is rooted in the interplay between cross-regulation and differences in susceptibility to AICD and Fas/FasL expression; the interactions tend to favour early Th1 dominance, while lack of AICD for Th2 destabilises the pure Th1 state and drives the system eventually to mount a Th2 response. Work on Th1–Th2 switches *in vitro* has revealed that even polarized, committed Th1 populations from murine lymph nodes contain undifferentiated cells that are capable of becoming either Th1 or Th2 cells (Mocci & Coffman, 1997) and this is in keeping with our findings.

However, a recent study (Lopes *et al.*, 1999) has noted that Fas-deficient mice mount an excessive Th2-driven response to *Trypanosoma cruzi* infection and fail to clear to parasite, whereas successful elimination of the infection occurs through Th1 help and macrophage activation, in combination with upregulated Fas/FasL expression and high levels of AICD. This suggests that at least some AICD among Th2 cells is normal in wild-type individuals. The most likely scenario is that there is more complex, differential regulation of the Fas pathway than we have proposed here; however, we believe that the basic structure of the interactions, with Th1 cells preferentially expressing FasL over Th2, is most in keeping with the experimental observations.

5. Discussion

The true role of Fas and Fas ligand in immune regulation remains to be uncovered but our

model here demonstrates that with a biologically detailed, simple dynamical model we can reproduce broad features of the immune response. Understanding the interplay of several cooperative and competing biological mechanisms is a task obviously suited to mathematical modelling.

Our model raises both new issues and complements the now significant body of work on Th1/2 dynamics. These models differ in their selective emphasis on the various cytokine interactions and the underlying biological assumptions. Fishman & Perelson (1994), building on an earlier model of T cell and antigen-presenting cell interactions (Fishman & Perelson, 1993), include distinct Th1 and Th2 activation signals and cross-regulatory interactions in their model. In contrast to their study, we do not include antigen clearance and we consider the effect of feedback from the proliferating effector cells on the APC activation states, as well as a cytokine-regulated death mechanism. Additionally, the roles and extent of influence of IL-2 and IL-4 as growth factors are treated differently—we assume asymmetric roles for the two, and make the further assumption that secreted growth factors are available to the whole proliferating pool rather than just the cells producing them (i.e. paracrine rather than autocrine in nature). The structure of the cross-suppressive interactions that they include leads naturally to the instability of mixed populations—only polarized Th1 or Th2 responses are supported in their model. This work is developed further in (Fishman & Perelson, 1999) to account for multi-clonality in a response, with essentially the same robust conclusions derived from principles of competition between clones for activation and cross-suppression of proliferation. Their predictions for the dose dependence of the response are largely in agreement with those here and in the experimental literature. Muraille & Kaufman (1995) focus more (as we do here) on the asymmetries inherent in Th1/2 interactions and mechanisms of switching responses from Th1 to Th2 or vice versa, as well as addressing issues of control of T cell clone sizes, although primarily through control of proliferation and activation rather than apoptosis. Morel *et al.* (1992) use a model of Th1/2 dynamics to clarify the roles of IL-2 and IL-4 in proliferation and we draw on their conclusions here. Lev Bar-Or & Segel (1998) investigate the roles of different regulatory mechanisms in the T helper system in the context of autoimmune disease; a non-specific, cytokine-mediated suppression and a specific cell–cell interaction mechanism. This takes place through the presentation of T cell receptor peptides rather than through pro-apoptotic surface molecules, however, and so the *structure* of the suppressive interactions they put forward is broadly similar to those presented here, if not their immunological basis. They find that the cells' average sensitivities on Th1- and Th2-derived cytokines have an important role in the decision-making process. Carneiro *et al.* (1995) also address Th1/2 differentiation without direct treatment of antigen clearance and focus the early, decision making aspect of a T helper response. They use the concept of an invariant proliferative driving capacity or antigenic "niche" that is indifferent to the Th1/2 composition of the proliferating lymphocyte pool. They do not address the antigen presentation step in detail, but rather propose that the Th1/2 decision is made primarily by the dynamics of the T cell populations themselves rather than being directed by cogent choices of costimulatory signals offered by APCs. Our results, however, indicate an important role for both these processes in determining the final outcome of a response. Each of the models described above displays features that shed light on various experimental data; further, they differ significantly in their structure and areas of emphasis. Together, these are an indication of the complexity of the Th1/2 system.

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

1. There are differences between the population control mechanisms of the two major T helper subsets. Homeostatic regulation of Th1 effector populations is dominated by the cytokine-regulated, Fas-mediated AICD mechanism (active control). Th2 regulation appears to be through inhibition of activation and proliferation (passive control).
2. Cell–cell killing (fratricide) and not cell suicide appears to be the dominant mechanism for Fas-mediated AICD.

3. A critical parameter in our model is the susceptibility of Th2 effectors to AICD (δ_2). Asymmetries in AICD susceptibility lead to non-intuitive system behaviour. Comparable susceptibility leads to support of exclusive type 1 or 2 responses; substantially lower Th2 susceptibility leads to generic Th1 $\rightarrow$ Th2 switches in the absence of antigen clearance.
4. Contributory factors to autoimmune diseases may be defective Th1 AICD mechanisms, or differential Fas/FasL expression on the two subsets. The latter can lead to oscillatory Th1/Th2 responses or "failed decision making".
5. Low levels of antigenic stimulation tend to favour Th1 responses, higher doses favour Th2.
6. Signals from the inmate immune system can help to bias a response towards one helper subset, or switch the nature of an ongoing response. The long-term behaviour of the system, however, is again critically dependent on the relative susceptibilities of Th1 and Th2 cells to Fas-mediated cell death.

Much work remains to be done in understanding the decision-making process made at the antigen presentation stage. Our model addresses this process in a necessarily simplified way—strict control of the co-stimulatory or "context" signals θ_1 and θ_2 is probably instrumental for the effective choice of differentiation pathway, at least in the very early stages of a response. A more sophisticated model would treat antigen presentation and differentiation in more detail. However, the model shows how asymmetries in the dynamics of T cell proliferation and regulation, which are subject to cytokine control, can contribute to the final outcome just as significantly as the apparent choice of differentiation pathway made at antigen presentation. The multitude of cytokine interactions in Th1/2 system, many of which seem redundant, may help to make this process of choosing a response more robust. They may have evolved in parallel with viral strategies for subverting the immune system by interference with cytokine signalling or expression of co-stimulatory signals.

We thank Arne Akbar, Penelope Morel and Lee Segel for helpful comments. We also thank Yuri Kuznetsov and Bard Ermentrout for the use of their dynamical systems analysis packages CONTENT and XPPAUT. Financial support came from the EPSRC/BBSRC joint initiative in mathematical biology, grant reference 39/MMI09771, and the BOEHRINGER INGELHEIM FONDS.

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