Th1/Th2 Cross-Regulation

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We present and analyze a model for the cross–regulation of the Th1 and Th2 T helper cell subsets during an immune response by the regulatory cytokines interferon-γ (IFN–γ) and interleukin-10 (IL–10). IFN–γ, secreted by Th1 cells, can inhibit the proliferation of Th2 cells. Interleukin-10, secreted by Th2 cells, inhibits cytokine production by Th1 cells. Our model, based on these properties shows that responses are expected to be dominated by either Th1 cells or Th2 cells but not both. Which type dominates is shown to depend principally on the relative efficiencies of activation of the responding Th1 and Th2 cells. However, our model as well as numerous experiments show that perturbations of the system allow one to switch from a Th2 to a Th1 response, or vice versa. Our model can account for observed outcomes of parasitic infection and may also contribute to our understanding of immune responses to HIV infection as well as to tolerance to self components. Our model predicts that in certain parameter ranges vaccination with low doses of live parasites can provide protection against subsequent encounters with high doses that normally induce disease. Experiments by Bretscher *et al.*, *Science* **257**, 539 (1992) on *Leishmania major* infection are consistent with this prediction. A similar strategy may also be relevant for the design of an AIDS vaccine. Lastly, our results indicate that Th1/Th2 cross–regulation is capable of generating a "sneaking through" phenomenon, and hence it may play a role in tumor immunity.

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1. Introduction

In response to antigenic challenge, both humoral and cell-mediated immunity can be induced. Some antigens/protocols of immunization result in antibody formation, whereas others predominantly induce a type of cell–mediated response known as delayed type hypersensitivity (DTH) (Parish, 1972). Even the simplest invading pathogen presents a broad spectrum of antigenic determinants to the immune system. Some of these determinants induce DTH responses, while others result in antibody production (Scott *et al.*, 1989). However, when a pathogen is encountered an animal generally responds with either a humoral or cell–mediated response, not both (Parish, 1972; Katsura, 1977; Scott *et al.*, 1989; Müller *et al.*, 1989a).

A possible reason why cellular and humoral immune responses are mutually exclusive has emerged from studies of T helper cell clones. Two types of helper cell clones, called Th1 and Th2, have been defined on the basis of their pattern of cytokine secretion (Street & Mosmann, 1991; Romagnani, 1991). These two types of helper cells appear to have dramatically different functions. Th1 cells, because of the factors that they produce, help in the induction of DTH responses via macrophage activation and generation of cytotoxic T lymphocytes (CTL). Th2 cells cannot induce DTH but rather induce B cells to make and secrete antibody. Under some circumstances Th1 cells can also provide B cell help, but Th2 cells are much more efficient (Mosmann & Moore, 1991). Although both Th1 and Th2 cells secrete a large number of factors (see Table 1), only Th1 cells secrete interleukin–2 (IL–2), tumor necrosis factor–beta (TNF–β) and interferon–γ (IFN–γ), molecules involved in inducing macrophage activation in DTH responses and CTL proliferation (Cher & Mosmann, 1987; Stout & Bottomly, 1989). Only Th2 cells secrete interleukins 4, 5, 6, molecules important in controlling B cell proliferation and differentiation (Killar *et al.*, 1987; Coffman *et al.*, 1988; Esser & Radbruch, 1990), and interleukin 10 (IL–10), a molecule that appears to be important in preventing DTH responses (Fiorentino *et al.*, 1989; Mosmann *et el.*, 1991).

Of the various cytokines produced by Th1 and Th2 cells, we shall focus on two, IL–10 and interferon–γ, that exhibit cross–regulatory effects. IFN–γ, a product of Th1 cells, inhibits proliferation of Th2 clones (Fernandez–Botran *et al.*, 1988; Gajewski & Fitch, 1988). *Leishmania major* inoculation protocol—which normally leads to a DTH response—results in a humoral response when combined with an injection of anti–IFN–γ monoclonal

antibody (Coffman *et al.*, 1991). IL–10, a cytokine produced by Th2 clones, inhibits cytokine production by Th1 clones (Fiorentino *et al.*, 1989, 1991). Although IL–10 does not affect Th1 cell growth factor responsiveness, IL–10 induced reduction of IL–2 synthesis can result in decreased Th1 proliferation (Magilavy *et al.*, 1989). Enhanced production of IL–10 and reduced production of IFN–γ is observed in chronic (Th2 dominated) helminth infections. Production of IFN–γ can be increased by injecting anti–IL–10 mAb (Mosmann *et al.*, 1991), indicating that IL-10 cross-regulation may be involved in promoting a Th2 response *in vivo*.

The cross–inhibitory effects of IL–10 and interferon–γ, as well as the cross–stimulatory effects of IL–2 and IL–4 (Fernandez–Botran *et al.*, 1988), have led to the suggestion that Th1 and Th2 cell populations *in vivo* interact via cytokine production in such as a way as to have one population dominate (cf. Mosmann & Moore, 1991). If the dominant population were Th1 then one would expect a cellular response, whereas if the dominant population where Th2, a humoral response would be expected. Thus the relationship between cell–mediated and humoral responses may be explained on the basis of cytokine activities. If this is in fact the case, and if such regulation can be understood quantitatively, then the potential exists for controlling immune responses by modifying cytokine concentrations *in situ*. This is of great practical importance because disease or health in parasitic and retroviral diseases may depend on the type of the response the immune system mounts (cf. Sher *et al.*, 1992; Salk *et al.*, 1993). This paper is a theoretical attempt to understand the features that determine whether a response is cell mediated or humoral.

Below we formulate and analyze a model of Th1/Th2 cell cross–regulation mediated by IL–10 and interferon–γ in the context of an immune response. This model is an extension of a previous model, addressing T helper cell–antigen presenting cell interactions (Fishman & Perelson, 1993), referred to below as the *DTH model*. Before presenting the model we briefly discuss some of the important biological features of antigen presenting cells and cytokines that play a role in our model.

2. Antigen Presenting Cells

While dendritic cells (DC) are markedly more efficient at antigen presentation than either B cells (Metlay *et al.*, 1989) or macrophages (Langhoff & Steinman, 1989), all three types of antigen presenting cells (APC) can promote T helper cell activation in cell culture. However, *in vivo* they have distinctly different functions.

Dendritic cells play a major role in antigen presentation to resting T cells, which results in T cell activation (Steinman, 1991). Dendritic cells are bone marrow derived, express both MHC class I and II molecules at high density and occur in small numbers in most lymphoid and nonlymphoid tissues (Hart & McKenzie, 1990). Detection and uptake of antigen—primarily by endocytosis (Levine & Chain, 1992)—is followed by migration of the DC to the T–dependent areas of the lymphatic tissue (Steinman, 1991). Migration places the DC in the path of the recirculating T cell pool and hence increases their probability of encountering an antigen specific T cell. Lastly, motile, antigen bearing, dendritic cells acquire the ability to temporarily bind T cells in an antigen independent fashion (Inaba *et al.*, 1989). In this way a large number of T cells can be surveyed. When there is complementarity between an antigen presented by a dendritic cell and a T cell's receptor, the T cell is retained and stimulated to secrete lymphokines and to proliferate (Flechner, *et al.*, 1988). This process is called *sensitization* or more simply activation.

Once the sensitization of the resting T helper cells is accomplished, sensitized Th2 cells interact with B cells in the production of antibody (Vitetta *et al.*, 1989). Sensitized Th1 cells interact with antigen primed macrophages. This interaction results in activation of macrophages for more efficient phagocytosis and killing of the invading pathogens (Stout & Bottomly, 1989).

3. Cytokines Involved in Th1/Th2 Cross–Regulation

3.1 Interleukin–2 (IL–2) and Interleukin–4 (IL–4)

Interleukin–2 is a T cell growth factor produced by Th1 cells (Table 1). Interleukin–4 (IL–4) is a multifunctional cytokine produced by Th2 cells (Table 1) that acts as an autocrine growth factor for Th2 cells and affects antibody synthesis by B cells. Both Th1 and Th2 cells can use IL–2 and IL–4 to support their growth, however Th2 cells are more efficient, than Th1 cells, in utilization of IL–4 (Fernandez–Botran *et al.*, 1988).

3.2 Interferon–gamma (IFN–γ)

IFN–γ has a variety of stimulatory activities, including activation of macrophages (Pace *et al.*, 1983; Pace *et al.*, 1985), and induction of increased surface expression of MHC class I and class II molecules (King & Jones, 1983; Wong *et al.*, 1983). IFN–γ can also mediate inhibitory effects such as interference with viral replication (Vogel *et al.*, 1982; Spitalny & Havell, 1984) and with IL–4 induced B cell activation (Coffman & Carty, 1986; Reynolds *et al.*, 1987). More important in the regulation of Th1 and Th2 cells is the fact that IFN–γ partially inhibits proliferation of Th2 clones, but not of Th1 clones, in a dose–dependent manner (Fernandez–Botran *et al.*, 1988; Gajewski & Fitch, 1988). IFN–γ induced inhibition appears to be saturable (Fernandez–Botran *et al.*, 1988; Gajewski & Fitch, 1988), and thus may not be complete at any IFN–γ concentrations. Fernandez–Botran *et al.* (1988) found that recombinant IFN–γ could only inhibit 40–60% of the IL–2 and/or IL–4 mediated proliferation of Th2 cells. Further, IFN–γ did not shift dose–response curves in which proliferation was measured as a function of IL–2 or IL–4 concentration (Fernandez–Botran *et al.*, 1988; Gajewski & Fitch, 1988). Thus, the mechanism of action does not appear to involve competition for IL–2 or IL–4 receptors. Gajewski & Fitch (1988) showed that while IFN–γ affects the ability of Th2 cells to proliferate in presence of T cell growth factors (IL–2/IL–4), it does not affect the ability of Th2 cells to secrete normal amounts of cytokines.

3.3 Interleukin–10 (IL–10)

Interleukin–10 belongs to the family of regulatory cytokines. This molecule is produced by the Th2 subset of the $CD4^+$ cells, but not by cells in the Th1 subset (Fiorentino *et al.*, 1989). Although IL–10 has multiple biological activities (cf. Chen & Zlotnik, 1991;

Oswald *et al.*, 1992; Pecanha *et al.*, 1992; Ralph *et al.*, 1992), the function addressed here is the inhibition of IFN–γ (and in certain cases, IL–2) synthesis by Th1 cells, at both mRNA and protein levels (Fiorentino *et al.*, 1991). Because of these activities, IL–10 when first isolated was called cytokine synthesis inhibitory factor (Fiorentino *et al.*, 1989). The net effect of IL–10 on Th1 cells is to inhibit their proliferation, probably via reduced IL–2 synthesis. However, because the negative effect on proliferation is caused by inhibition of cytokine synthesis, Th1 cells inhibited by IL–10 retain the capacity to proliferate in the presence of externally added T cell growth factors (IL–2/IL–4) (Fiorentino *et al.*, 1989).

The ability of IL–10 to inhibit cytokine synthesis by Th1 cells depends on the presence of live antigen presenting cells (Fiorentino *et al.*, 1989, 1991). It has been demonstrated that macrophage mediated, but not B cell mediated, T cell activation is subject to IL–10 suppression (Fiorentino *et al.*, 1991; Ding & Shevach, 1992). The effects of IL–10 on Th1 cell–dendritic cell interactions are somewhat more complex. Previous publications (Fiorentino *et al.*, 1989; Seder *et al.*, 1992)—on which our model is based—have reported that Th1–DC interactions are not subject to IL–10 inhibition. However, a recent report (Macatonia *et al.*, 1993) indicates that while IL–10 does not inhibit DC dependent Th1 cell proliferation, it may inhibit DC dependent IFN–γ production.

In the model given below we envision that the *in vivo* effects of IL-10 predominately act on macrophage-Th1 cell interactions. One possible scenario that might apply to a localized infection occurring in a tissue is that migrating dendritic cells would pick up the antigen, process and present it to resting T cells in the draining lymph node, resulting in T cell sensitization. Sensitized T cells and macrophages will then accumulate at the site of infection. The macrophages will process and present the antigen to both sensitized T cells and activated T cells that have returned to the resting state. IL-10 would influence these interactions and inhibit the ability of Th1 cells activated by interacting with macrophages from secreting IFN-γ. We do not model these interactions in detail, and assume for for simplicity that IL-10 inhibits the ability of activated Th1 cells to secrete cytokines. In appendix C we demonstrate that addition of IL–10 mediated inhibition of the DC dependent IFN–γ production to our model does not changes its basic predictions.

4. Model

The experimental observations on IL–10 and IFN–γ emphasize cross–suppression, rather than cross–stimulation, between Th1 and Th2 clones. Here, as a first approximation, we neglect the cross–stimulation effects mediated by IL–2 and IL–4, and model a system for which T cell proliferation depends on the utilization of endogenously produced growth factors. As discussed in detail in Fishman & Perelson (1993), one can model T cell growth as depending on the bulk concentration of cytokine in the medium surrounding a cell population. Alternatively, one can assume that since activated Th1 cells produce IL–2 and activated Th2 cells produce IL–4, the IL–2 and IL–4 concentrations will be highest in the neighborhood surrounding the producing cell. As the distance from the secreting cell increases the cytokine concentration will decrease to a bulk concentration level. In Fishman & Perelson (1993) we showed that the qualitative behavior of the model did not change if one assumed that growth depended on the local concentration of IL–2 or the bulk concentration. Here we model the case in which T cell growth depends on the local concentration rather than the bulk concentration. Since this local concentration can be viewed to be constant for each secreting cell, it can be incorporated into the net growth rate of an activated cell leading to a rather simple formulation of the growth equations. We summarize the salient features of our model in Figure 1.

The model that we develop below is an extension of the Th1–APC model developed in Fishman & Perelson (1993). As in that paper, we consider as an antigen presenting cell (APC) a dendritic cell that has taken up, processed, and is presenting antigen fragment–MHC complexes on its surface. Antigen presenting cells, at concentration C, are derived from the interaction of dendritic cell precursors, at concentration C_p, with antigen at concentration Ag. We assume, as in Fishman & Perelson (1993), that each antigen presenting cell has n mutually independent T cell binding sites at which T cells can interact with peptide–MHC complexes. Given the independence of the T cell binding sites, we can change variables from APCs to T cell binding sites (Fishman & Perelson, 1993).

Let S_0 be the concentration of unoccupied sites on APCs, S_1 be the concentration of the sites on the APCs occupied by the Th1 cells, S_2 be the concentration of the sites on the APCs occupied by the Th2 cells, and let $S = S_0 + S_1 + S_2$ be the total concentration of sites. In these terms we have the following interaction scheme

Scheme 1

$$
\begin{array}{ccccccccc}
 & & & k_{b_1} & & k_{a_1} & & & \\
 & & T_1 & \rightleftharpoons & S_1 & \rightarrow & S_0 & + & T_1^* \\
 & + & & k_{d_1} & & & & & \\
S_0 & & & & & & & & \\
 & + & & k_{b_2} & & k_{a_2} & & & \\
 & & T_2 & \rightleftharpoons & S_2 & \rightarrow & S_0 & + & T_2^* \\
 & & & k_{d_2} & & & & &
\end{array}
$$

Here k_{b_i} $(i = 1,2)$ is the rate coefficient for the binding of a T_i cell to a T cell binding site on an APC. A free site is generated by the dissociation of the T_i cell from a site–T_i cell conjugate, leading either to the release of the T_i cell without activation, with rate coefficient k_{d_i}, or to the generation of an activated T_i cell (T_i^*), with rate coefficient k_{a_i}. Here k_{a_i} is taken to be a constant representing the rate with which T_i cells become activated, once the clonotype specific binding of the T cell to the antigen presenting site on the APC is accomplished.

We consider Th1 and Th2 cells in various states: at rest, at concentrations T_1 and T_2; activated, at concentrations T_1^* and T_2^*; and inhibited. Recall that IL–10 inhibits cytokine secretion by Th1 cells. Since only activated cells are expected to secrete cytokines, only activated cells can be inhibited. The concentration of activated Th1 cells that are non–secretors due to IL–10 inhibition is denoted T_1^{*ns}. Interferon–γ prevents proliferation of Th2 cells. The concentration of activated Th2 cells that are inhibited from proliferating due to IFN–γ is T_2^{*np}. Since both IL–10 and IFN–γ act in a dose–dependent manner, we shall keep track of the concentrations of these cytokines. We describe the kinetics of the interactions among APCs, Th1, and Th2 cells as follows:

$$\frac{dS_0}{dt} = nbC_pAg - k_{b_1}S_0T_1 + (k_{a_1} + k_{d_1})S_1 - k_{b_2}S_0T_2 + (k_{a_2} + k_{d_2})S_2 - d_CS_0 \ , \qquad (1a)$$

$$\frac{dS_1}{dt} = k_{b_1}S_0T_1 - (k_{a_1} + k_{d_1})S_1 - d_CS_1 \ , \qquad (1b)$$

$$\frac{dS_2}{dt} = k_{b_2}S_0T_2 - (k_{a_2} + k_{d_2})S_2 - d_CS_2 \ , \qquad (1c)$$

$$\frac{dT_1}{dt} = a_1 - d_T T_1 - k_{b_1} S_0 T_1 + k_{d_1} S_1 + (\mu k_p + k_r) T_1^* + k_{r_{ns}} T_1^{*ns} \ , \qquad (1d)$$

$$\frac{dT_2}{dt} = a_2 - d_T T_2 - k_{b_2} S_0 T_2 + k_{d_2} S_2 + (\mu k_p + k_r) T_2^* + k_{r_{np}} T_2^{*np} \ , \qquad (1e)$$

$$\frac{dT_1^*}{dt} = k_{a_1} S_1 - \left(k_p + k_r + k_{ns} \frac{IL_{10}}{K_{IL_{10}} + IL_{10}} \right) T_1^* \ , \qquad (1f)$$

$$\frac{dT_2^*}{dt} = k_{a_2} S_2 - \left(k_p + k_r + k_{np} \frac{IFN}{K_{IFN} + IFN} \right) T_2^* \ , \qquad (1g)$$

$$\frac{dT_1^{*ns}}{dt} = k_{ns} \frac{IL_{10}}{K_{IL_{10}} + IL_{10}} T_1^* - k_{r_{ns}} T_1^{*ns} \ , \qquad (1h)$$

$$\frac{dT_2^{*np}}{dt} = k_{np} \frac{IFN}{K_{IFN} + IFN} T_2^* - k_{r_{np}} T_2^{*np} \ , \qquad (1i)$$

$$\frac{dIFN}{dt} = p_{IFN} T_1^* - d_{IFN} IFN \ , \qquad (1j)$$

$$\frac{dIL_{10}}{dt} = p_{IL_{10}} (T_2^* + T_2^{*np}) - d_{IL_{10}} IL_{10} \ , \qquad (1k)$$

$$\frac{dAg}{dt} = \left[q - e_1 T_1^* - e_2 (T_2^* + T_2^{*np}) \right] Ag \ , \qquad (1l)$$

In eqn (1a), b is the rate coefficient for recruitment of APCs from precursors, C_p, in the presence of antigen. Because the uptake of antigen by dendritic cell precursors is primarily via endocytosis (Levine & Chain, 1992), we assume that the rate at which APCs are generated may be approximated by the product of the antigen concentration and precursor concentration. In situations where the antigen concentration may get very high one may wish to replace the antigen concentration by a nonlinear function of the antigen concentration that saturates. The next two terms in the equation relate to the binding of a T_i ($i = 1, 2$) cell to a T cell binding site on an APC (scheme 1). The last term in eqn (1a) is the rate of decay of an APC site, due to cell death and/or loss of peptide-MHC complexes. Equations (1b) and (1c) describe the rate of generation, and the rate of decay, of APC site–Th1 cell conjugates and APC site–Th2 cell conjugates, respectively.

In eqns (1d) and (1e), a_1 and a_2 are the rates of influx of resting, antigen specific Th1 and Th2 cells from precursors. Resting T cells are assumed to die at rate d_T or become activated by interaction with an APC. The next term corresponds to clonal expansion by cell division, where k_p represents a rate constant for proliferation and μ represents the

amplification associated with proliferation. Because activated T cells can undergo several divisions prior to return to a rest state (Smith *et al.*, 1983), μ may be > 2. The last two terms in eqn (1d) are the rates at which activated Th1 cells, T_1^*, and IL-10–inhibited non-secreting Th1 cells, T_1^{*ns}, return to the rest state without division. In these terms k_r and $k_{r_{ns}}$ are the rate constants for non–proliferative relaxation to the rest state of the activated (T_1^*) and of the non-secreting (T_1^{*ns}) T cells, respectively.

The inhibited T_1^{*ns} do not produce cytokines and do not proliferate in absence of externally added growth factors. One might expect that *in vivo* there would be some ambient level of cytokines that might allow T_1^{*ns} cells to divide. For IL–10 to be effective this ambient level must be sufficiently low that it does not produce substantial proliferation. In fact, since cytokines such as IL–1, IL–2 and IL–4 can support T cell growth in the absence of antigen stimulation (Gajewski & Fitch, 1988), high levels of lymphokine would lead to uncontrolled cell growth. Here we assume lymphokines levels are low so that such effects can be neglected. In appendix B we consider a case where an ambient level of cytokines supports proliferation of the T_1^{*ns} cells. Equation (1e) for T_2 is analogous to eqn (1d) for T_1.

Based on experiments by Fernandez–Botran *et al.*(1988), Gajewski & Fitch (1988), and Fiorentino *et al.* (1989), we model the effects of IL–10 and IFN–γ on Th1 and Th2 cells by saturation functions with half–saturation constants $K_{IL_{10}}$ and K_{IFN}. In modeling IL–10 and IFN–γ effects, cytokine dose–dependent terms are multiplied by rate constants that determine the maximum rate of inhibition. In eqns (1f) and (1h), k_{ns} is the maximum rate for the IL–10 mediated T_1^* to T_1^{*ns} transition. Similarly, in eqn (1g) and (1i), k_{np} is the maximum rate for the IFN–γ mediated T_2^* to T_2^{*np} transition. Notice that even at saturating concentrations of IFN–γ and IL–10 only a fraction of the activated cells will be inhibited. For IFN–γ this fraction is $f_{IFN} \equiv k_{np}/(k_p + k_r + k_{np})$. Fernandez-Botran *et al.* (1988) show that only 40-60% of cells are inhibited by IFN–γ, and parameters must be chosen accordingly. This is discussed further in section 7.

Equations (1h) and (1i) describe the rate of change of IL-10 inhibited Th1 cells, the non-secretors T_1^{*ns}, and the IFN-γ inhibited Th2 cells, the non-proliferators, T_2^{*np}. We assume that inhibited cells are created in a dose-dependent manner, as described above, and ultimately return to their uninhibited resting states at rates $k_{r_{ns}}$ and $k_{r_{np}}$, respectively. The rates of return to the rest state are sufficiently rapid compared with the lifespan of

T cells that we have ignored the possibility that inhibited cells die before returning to the rest state. This assumption is based on data by Fernandez–Botran *et al.* (1988) showing that IFN–γ interferes with growth factor utilization by Th2* but does not decrease their viability. Also, Fiorentino *et al.* (1989) and Ding & Shevach (1992) showed that IL-10 inhibited Th1* cells proliferate in presence of exogenous IL-2 indicating that they remain viable.

Equations (1j) and (1k) monitor the concentrations of IFN–γ (IFN) and IL–10 (IL_{10}). In eqn (1j), p_{IFN} is the per capita rate of IFN–γ production by T_1^* cells, and d_{IFN} is a rate constant for the loss of interferon. Similarly, in eqn (1k), $p_{IL_{10}}$ is the per capita rate of IL–10 production by T_2^* and T_2^{*np} cells, and $d_{IL_{10}}$ is a rate constant describing IL–10 loss. Here we have assumed that the IFN-γ inhibited, non-proliferative Th2 cells secrete IL-10 at the same rate per cell as uninhibited Th2 cells. This may be a simplification , but in the absence of quantitative data on secretion rates, this assumption allows us to focus on the primary effect of IFN-γ, which is to inhibit the proliferation of Th2 cells. Here we have also assumed that cytokine loss is by breakdown, by diffusion away from the region of interest, or by binding to cytokine receptors that occur on various cells throughout the body, and hence is independent of the local Th1 and Th2 cell population densities. A more refined model might include extra loss terms that take into account the uptake of cytokine by the target Th cells.

Equation ($1l$) deals with antigen dynamics. We assume that the antigen can grow, with q being the pathogens' rate of proliferation in the absence of an antigen specific Th1 or Th2 response. Thus, q incorporates any non-specific antigen elimination mechanisms. Systems in which $q < 0$ are of marginal interest since a specific immune response is unnecessary. Thus, we restrict our attention to systems in which $q > 0$.

The stimulation of Th1 and Th2 cells lead to the generation of cell mediated and humoral immune responses against the antigen. Following the details of these responses is outside the scope of this paper. However, since the effects of these responses are to reduce the antigen population, we introduce rate coefficients e_1 and e_2 that summarize the rate at which antigen is eliminated due to a Th1 and Th2 response, respectively. The coefficients e_1 and e_2 are expected to vary with the antigen since some antigens are readily susceptible to a cell mediated response (cf. Scott *et el.*, 1989) while others are more readily eliminated by a humoral response (cf. Urban *et al.*, 1992). In Fishman & Perelson (1993) we present

a more general analysis of the effects of helper cell activity on antigen elimination and show, that in the DTH model, the per antigen rate of elimination, eT^*, can be replaced by any monotonically increasing function of the helper cell concentration without changing the qualitative behavior of the model. We believe that this is also the case here but do not pursue this more general avenue of analysis. Notice that we have assume that T_2^{*np} are as effective at antigen elimination as their non-inhibited counterparts, T_2^*. Because the effects of helper cells are mediated through lymphokine secretion this assumption is consistent with the one we made above about rates of IL-10 secretion and is made for similar reasons. One could of course relax this assumption and introduce a third coefficient e_3 to characterize antigen elimination by T_2^{*np}. For reasons of simplicity we do not pursue this approach here.

5. Scaling

Analysis of eqns (1a)-(1l) can be significantly simplified by scaling the variables. System (1) involves two major time scales: a "fast" time scale on the order of hours or days that corresponds to the turnover rate for S–T cell interactions [$(1/(k_d + k_a)$ is of the order of 8–16 hours, Flechner et al. (1988)] and a "slow" time scale determined by the half-life of T lymphocytes, which is on the order of several weeks (Gray & Matzinger, 1991; von Boehmer, 1992). By scaling time in terms of the T cell half–life, we can separate these time scales.

To help simplify the analysis, we will choose $k_{a_1} = k_{a_2} = k_a$. On the long time-scale that will be of interest, any differences in activation time of Th2 cells relative to Th1 cells becomes insignificant. Also, as we will see the effects of T cell interaction with APC will be summarized by a modified equilibrium constant, $K = k_b/(k_a + k_d)$, so that differences in activation rates between Th1 and Th2 can be accounted for as differences in rate of binding or dissociation with the APC.

In defining dimensionless parameters it is convenient to work with ratio parameters that characterize Th1 and Th2 cells. We thus have arbitrarily chosen to write parameters such as η, which characterizes the efficiency of antigen elimination, as the Th2 parameter divided by the Th1 parameter, i.e. $\eta = e_2/e_1$. Clearly, if one wishes to study systems in which $e_1 = 0$ or $K_1 = 0$ a different nondimensionalization is needed.

The dimensionless form of the system of eqns (1) that we adopt is

$$\epsilon s_0' = \epsilon\sigma(g - s_0) - (s_0x_1 - s_1) - \Lambda\kappa^{-1}(s_0x_2 - s_2), \tag{2a}$$

$$\epsilon s_1' = (s_0x_1 - s_1) - \epsilon\sigma s_1, \tag{2b}$$

$$\epsilon s_2' = \Lambda\kappa^{-1}(s_0x_2 - s_2) - \epsilon\sigma s_2, \tag{2c}$$

$$x_1' = \alpha_1 + (x_1^* - x_1) - \beta\chi(s_0x_1 - s_1) - \frac{\chi}{\mu - 1}[s_1 - x_1^* - \Gamma x_1^{*ns}], \tag{2d}$$

$$x_2' = \alpha_2\kappa + (x_2^* - x_2) - \beta\chi\Lambda(s_0x_2 - s_2) - \frac{\chi}{\mu - 1}[\kappa s_2 - x_2^* - \rho x_2^{*np}], \tag{2e}$$

$$\epsilon x_1^{*\prime} = \gamma\big[s_1 - (1 + \xi\frac{l_{IL_{10}}}{\Omega\phi\kappa + l_{IL_{10}}})x_1^*\big], \tag{2f}$$

$$\epsilon x_2^{*\prime} = \gamma\big[\kappa s_2 - (1 + \zeta\frac{l_{IFN}}{\Omega + l_{IFN}})x_2^*\big], \tag{2g}$$

$$\epsilon x_1^{*ns\prime} = \gamma\big[\xi\frac{l_{IL_{10}}}{\Omega\phi\kappa + l_{IL_{10}}}x_1^* - \Gamma x_1^{*ns}\big], \tag{2h}$$

$$\epsilon x_2^{*np\prime} = \gamma\big[\zeta\frac{l_{IFN}}{\Omega + l_{IFN}}x_2^* - \rho x_2^{*np}\big], \tag{2i}$$

$$\epsilon l_{IFN}' = \delta_{IFN}(x_1^* - l_{IFN}), \tag{2j}$$

$$\epsilon l_{IL_{10}}' = \delta_{IL_{10}}\big[(x_2^* + x_2^{*np}) - l_{IL_{10}}\big], \tag{2k}$$

$$g' = \pi\big[\theta - x_1^* - \eta\kappa^{-1}(x_2^* + x_2^{*np})\big]g, \tag{2l}$$

where $' \equiv d/d\tau$; $\tau = d_Tt$; $K_i = k_{b_i}/(k_a + k_{d_i})$, $i = 1, 2$;

$$(s_0, s_1, s_2) = \frac{(\mu - 1)k_ak_pK_1}{d_T(k_p + k_r)}(S_0, S_1, S_2);\ x_i = K_iT_i\ ;\ g = \frac{(\mu - 1)k_ak_pK_1}{d_T(k_p + k_r)}\frac{nbC_p}{d_C}Ag\ ;$$

$$(x_1^*, x_1^{*ns}) = \frac{(\mu - 1)k_pK_1}{d_T}(T_1^*, T_1^{*ns})\ ;\ (x_2^*, x_2^{*np}) = \frac{(\mu - 1)k_pK_2}{d_T}(T_2^*, T_2^{*np})\ ;$$

$$l_{IFN} = \frac{(\mu - 1)k_pK_1}{d_T}\frac{d_{IFN}}{p_{IFN}}IFN\ ;\ l_{IL_{10}} = \frac{(\mu - 1)k_pK_2}{d_T}\frac{d_{IL_{10}}}{p_{IL_{10}}}IL10\ ;\alpha_1 = \frac{a_1K_1}{d_T}\ ;$$

$$\alpha_2 = \frac{a_2K_1}{d_T}\ ;\ \beta = \frac{k_a + k_{d_1}}{(\mu - 1)k_a}\ ;\ \gamma = \frac{k_p + k_r}{k_a + k_{d_1}}\ ;\ \delta_{IFN} = \frac{d_{IFN}}{k_a + k_{d_1}}\ ;\ \delta_{IL_{10}} = \frac{d_{IL_{10}}}{k_a + k_{d_1}}\ ;$$

$$\epsilon = \frac{d_T}{k_a + k_{d_1}}\ ;\ \xi = \frac{k_{ns}}{k_p + k_r};\ \zeta = \frac{k_{np}}{k_p + k_r}\ ;\ \sigma = \frac{d_C}{d_T}\ ;\ \kappa = \frac{K_2}{K_1}\ ;\Lambda = \frac{k_{b_2}}{k_{b_1}}\ ;\ \pi = \frac{e_1}{(\mu - 1)k_pK_1}$$

$$\nu = 1 + \frac{\zeta}{\rho} = \frac{k_{np} + k_{r_{np}}}{k_{r_{np}}} \; ; \; \theta = \frac{(\mu - 1)k_p K_1}{d_T} \frac{q}{e_1} \; ; \; \Gamma = \frac{k_{r_{ns}}}{k_p + k_r} \; ; \; \rho = \frac{k_{r_{np}}}{k_p + k_r} \; ; \; \eta = \frac{e_2}{e_1}$$

$$\chi = \frac{k_p + k_r}{k_p} \; ; \; \Omega = \frac{(\mu - 1)k_p K_1}{d_T} \frac{d_{IFN} K_{IFN}}{p_{IFN}} \; ; \; \phi = \frac{d_{IL_{10}}}{d_{IFN}} \frac{K_{IL_{10}}}{K_{IFN}} \frac{p_{IFN}}{p_{IL_{10}}} \; .$$

Here time is scaled in terms of T cell life–span, $1/d_T$. The choice of scales for the dependent variables is discussed in detail in Appendix A. As discussed above, we take the half–life of T cells ($1/d_T$) as 30–70 days, and the turnover rate for S–T cell interactions $[1/(k_d + k_a)]$ as 8–16 hours. Thus, $\epsilon = d_T/(k_d + k_a)$ ranges from about 0.0005 to 0.02, and can be used as a small parameter in formal perturbation analysis.

To study phenomena that occur on a time scale of days to weeks, rather than hours, it suffices to restrict our attention to the long time scale. Mathematically, this is done by setting $\epsilon = 0$ in eqn (2) and studying the resulting equations. From eqns (2b), (2c), and (2f)-(2k), with $\epsilon = 0$, we obtain

$$s_0 x_1 = s_1, \tag{3a}$$

$$s_0 x_2 = s_2, \tag{3b}$$

$$s_1 = \left(1 + \xi \frac{l_{IL_{10}}}{\Omega\phi\kappa + l_{IL_{10}}}\right) x_1^*, \tag{3c}$$

$$\kappa s_2 = \left(1 + \zeta \frac{l_{IFN}}{\Omega + l_{IFN}}\right) x_2^*, \tag{3d}$$

$$x_1^{*ns} = \frac{\xi}{\Gamma} \frac{l_{IL_{10}}}{\Omega\phi\kappa + l_{IL_{10}}} x_1^*, \tag{3e}$$

$$x_2^{*np} = (\nu - 1) \frac{l_{IFN}}{\Omega + l_{IFN}} x_2^*, \tag{3f}$$

$$l_{IFN} = x_1^*, \tag{3g}$$

$$l_{IL_{10}} = x_2^* + x_2^{*np}. \tag{3h}$$

In addition, in the $\epsilon = 0$ limit there will remain four differential equations, which we discuss after further simplification of the above algebraic equations.

Equations (3a) and (3b) yield

$$s = s_0 + s_1 + s_2 = (1 + x_1 + x_2)s_0 \ . \tag{4a}$$

Thus

$$s_0 = \frac{s}{1 + x_1 + x_2} \ , \quad s_1 = \frac{sx_1}{1 + x_1 + x_2} \ , \quad s_2 = \frac{sx_2}{1 + x_1 + x_2} \ . \tag{4b}$$

Adding eqns (2a)-(2c), we see that s obeys the differential equation

$$s' = s_0' + s_1' + s_2' = \sigma(g - s) \ . \tag{4c}$$

From eqn (3) we obtain

$$x_2^* = \frac{(\Omega + x_1^*)\kappa s_2}{\Omega + (1 + \zeta)x_1^*}, \tag{5a}$$

$$x_2^{*np} = \frac{(\nu - 1)\kappa s_2 x_1^*}{\Omega + (1 + \zeta)x_1^*}, \tag{5b}$$

$$l_{IL_{10}} = \frac{(\Omega + \nu x_1^*)\kappa s_2}{\Omega + (1 + \zeta)x_1^*}, \tag{5c}$$

$$x_1^{*ns} = \frac{(\xi/\Gamma)(\Omega + \nu x_1^*)s_2 x_1^*}{\Omega(\Omega\phi + s_2) + \big[(1 + \zeta)\Omega\phi + \nu s_2\big]x_1^*} \ . \tag{5d}$$

Substitution of eqn (5) into eqn (3) yields

$$q_0 x_1^{*^2} + q_1 x_1^* - q_2 = 0, \tag{6a}$$

where

$$q_0 = (1 + \zeta)\Omega\phi + (1 + \xi)\nu s_2, \tag{6b}$$

$$q_1 = \Omega\big[\Omega\phi + (1 + \xi)s_2\big] - \big[(1 + \zeta)\Omega\phi + \nu s_2\big]s_1, \tag{6c}$$

$$q_2 = \Omega(\Omega\phi + s_2)s_1. \tag{6d}$$

Thus,

$$x_1^* = \frac{\sqrt{q_1^2 + 4q_0 q_2} - q_1}{2q_0}. \tag{7}$$

Substitution of eqns (4)–(5) into eqn (2) yields the four differential equations that characterize the long-time behavior of the system

$$s' = \sigma(g - s) = f_1(s, x_1, x_2, g) \,, \tag{8a}$$

$$x_1' = \alpha_1 + x_1^* - x_1 = f_2(s, x_1, x_2, g) \,, \tag{8b}$$

$$x_2' = \alpha_2\kappa + x_2^* - x_2 = f_3(s, x_1, x_2, g) \,, \tag{8c}$$

$$g' = \pi\left[\theta - x_1^* - \eta\kappa^{-1}(x_2^* + x_2^{*np})\right]g = f_4(s, x_1, x_2, g) \,. \tag{8d}$$

Here x_1^* is defined by eqns (6)–(7) and x_2^*, x_2^{*np} are defined by eqn (5a,b) in terms of x_1^*.

By restricting out attention to a time scale of days to weeks, which should be appropriate for studying the immune system's response to a growing pathogen, we have been able to reduce a problem involving 12 differential equations to one involving only 4. We present the analysis of the reduced system of equations, eqn (8), below.

In our subsequent analysis of the dynamics of the response to a growing antigen we shall assume that the system is initially in the unique steady state assumed by the system in the absence of antigen. This steady state, which we call E_{00} below, is when perturbed by the introduction of antigen at dose $g(0)$.

6. Equilibrium Points and their Stability

System (8) has four equilibrium points. These equilibrium points are

$$E_{00} = (s^{00}, x_1^{00}, x_2^{00}, g^{00}) = (0, \alpha_1, \alpha_2\kappa, 0), \tag{9a}$$

$$E_{10} = (s^{10}, x_1^{10}, x_2^{10}, g^{10}) = (\theta + 1, \theta, 0, \theta + 1) \,, \tag{9b}$$

$$E_{01} = (s^{01}, x_1^{01}, x_2^{01}, g^{01}) = \frac{1}{\eta\kappa}\Big(\eta + \theta\kappa, 0, \theta\kappa^2, \eta + \theta\kappa\Big) \,, \tag{9c}$$

and

$$E_{11} = (s^{11}, x_1^{11}, x_2^{11}, g^{11}) = \big(R(z), z, Q(z), R(z)\big) \,, \tag{9d}$$

where

$$z = \frac{-p_1 - \sqrt{p_1^2 - 4p_0p_2}}{2p_0} ,$$

with

$$p_0 = (1+\zeta) - (1+\xi)\kappa ,$$

$$p_1 = [(1+\xi)(\theta - \Omega) + \eta\Omega\phi]\kappa - [(1+\zeta)(\theta + \eta\Omega\phi) - \Omega] ,$$

$$p_2 = \Omega\left[[(1+\xi)\theta + \eta\Omega\phi]\kappa - (\theta + \eta\Omega\phi)\right] ,$$

$$R(z) = \frac{[\Omega + (1+\zeta)z]\left[(\eta\nu - \kappa)z^2 + [\eta(\Omega+\nu) + (\theta - \Omega)\kappa]z + \Omega(\eta + \theta\kappa)\right]}{\kappa\eta(\Omega + z)(\Omega + \nu z)} ,$$

and

$$Q(z) = \frac{\kappa}{\eta}\frac{(\Omega + z)(\theta - z)}{(\Omega + \nu z)}.$$

Here E_{00} represents the state of the system in absence of antigen. It will be called a virgin or uninfected steady state. E_{10} represents a state in which antigen and Th1 cells are present but the Th2 cells are absent. In this state the antigen's proliferation is balanced by a Th1 dominated immune response. Such "coexistence" states, in which the immune system keeps a pathogen in check but does not eliminate it, are well documented (cf. Müller *et al.*, 1989b). E_{01} represents a second antigen coexistence state, here antigen is kept in check by Th2 cells. Finally, E_{11} represents a third coexistence state in which antigen growth is balanced by both Th1 and Th2 responses. Thus, in this state the cross–suppression between the responding Th1 and Th2 cells does not result in an effective elimination of either Th subset.

In solving for the steady states E_{10}, E_{01}, and E_{11} we have used the approximation $\alpha_i = 0$, $i = 1, 2$. Recall that α_i represents the rate of influx of cells into T_i cell subset. Thus, we have assumed that the number of T cells supplied via this influx is negligible when compared to the number of T cells created by T cell proliferation in the three steady states in which antigen is kept in check by a T cell response. A detailed analysis of this approximation was performed in the context of the DTH model by Fishman & Perelson (1993).

An implicit assumption made in the derivations of E_{01} and E_{11} is that $0 < \eta < \infty$, where $\eta = e_2/e_1$. Hence we have assumed that both cellular and humoral responses play a role in antigen suppression. However, they need not play an equal role. If η is not

of order one, i.e. $e_1 \gg e_2$ or $e_1 \ll e_2$, there will be marked differences in efficiency of immune elimination by Th1 (cell mediated) and Th2 (antibody mediated) responses, and consequently in the magnitude of the pathogen population, associated with the various coexistence steady states. For example: in infections by the intracellular protozoan parasite *Leishmania major*, protection against which appears to be effected by a cellular response, the Th1 dominated response is associated with resistance, while Th2 domination leads to non–healing and ultimately lethal disease (Müller *et al.*, 1989a,b; Scott *et al.*, 1989). Hence with *L. major* as the antigen we expect $\eta \ll 1$. Conversely, in infections by intestinal nematode parasites—protection against which is implemented by humoral–immunity, the Th2 subset is associated with resistance, while a Th1 dominated response leads to chronic disease (Else *et al.*, 1992; Urban *et al.*, 1992). Hence, for nematode infection $\eta \gg 1$. In such cases it may be of interest to take e_1 (e_2) equal to zero in eqn (1). Below we analyze the case $0 < \eta < \infty$, and address the special case $e_2 = 0$ in section 8.2.

Although four steady state solutions exist for eqn (8), only the stable steady states would be observed in an animal. To determine the stability of each of these steady states, we perform standard linear stability analysis by calculating the characteristic polynomials and their associated eigenvalues, λ (cf. Willems, 1970).

For E_{00} we obtain the characteristic polynomial $p_{00}(\lambda)$,

$$p_{00}(\lambda) = (\lambda + \sigma)(\lambda + 1)^2(\lambda - \pi\theta) \; . \tag{10}$$

The characteristic equation, $p_{00}(\lambda) = 0$, has three negative real roots, -1, -1, and $-\sigma$, and a single positive real root, $\pi\theta \equiv \partial f_4(E_{00})/\partial g$. Hence one root is positive and the uninfected state is unstable. Because the positive root depends on antigen it is easy to see that the uninfected state is only unstable to antigenic perturbations. In fact, if antigen were always absent when we could consider the eqn (8a)–(8c) with $g = 0$. For this system, the state $(s^{00}, x_1^{00}, x_2^{00})$ is stable. Thus in the absence of antigen, i.e. in a true virgin state, E_{00} is stable. However, once antigen is encountered, E_{00} is no longer stable, the antigen will grow, an immune response will be generated, and the system will approach one of the coexistence steady states.

The characteristic polynomial $p_{10}(\lambda)$, associated with E_{10}, the Th1 dominated state, is

$$p_{10}(\lambda) = \left(\lambda + U_{10}\right)\left[\lambda^3 + \left(\sigma + \frac{\theta}{\theta+1}\right)\lambda^2 + \left(\frac{\theta}{\theta+1} + \pi\theta\right)\sigma\lambda + \pi\theta\sigma\right] , \tag{11a}$$

where

$$U_{10} = 1 - \left[\frac{\Omega + \theta}{\Omega + (1+\zeta)\theta}\right]\kappa \ . \tag{11b}$$

For E_{10} to be stable, two conditions need to be met:

(i) $U_{10} > 0$ or

$$\kappa < \frac{\Omega + (1+\zeta)\theta}{\Omega + \theta} \equiv \kappa_U \ , \tag{11c}$$

(notice that since $\zeta > 0$, $\kappa_U > 1$)
and (ii)

$$\sigma > \frac{\pi - \theta/(\theta+1)}{\pi(\theta+1)+1} \equiv r(\pi,\ \theta) \ . \tag{11d}$$

Condition (ii) is obtained by application of the Routh–Hurwitz criterion (cf. Willems, 1970) to the cubic polynomial in eqn (11a).

The characteristic polynomial $p_{01}(\lambda)$, associated with E_{01}, the Th2 dominated state, is

$$p_{01}(\lambda) = \left(\lambda + U_{01}\right)\left[\lambda^3 + \left(\sigma + \frac{\theta\kappa}{\theta\kappa+\eta}\right)\lambda^2 + \left(\frac{\theta\kappa}{\theta\kappa+\eta} + \pi\theta\right)\sigma\lambda + \pi\theta\sigma\right] \ , \tag{12a}$$

where

$$U_{01} = 1 - \left[\frac{\eta\Omega\phi + \theta}{\eta\Omega\phi + (1+\xi)\theta}\right]\kappa^{-1} \ . \tag{12b}$$

Using the same methods of analysis, the stability conditions for E_{01} are

$$\kappa > \frac{\eta\Omega\phi + \theta}{\eta\Omega\phi + (1+\xi)\theta} \equiv \kappa_L \ , \tag{12c}$$

and

$$\sigma > \frac{\pi\eta - \theta\kappa^2/(\theta\kappa+\eta)}{\pi(\theta\kappa+\eta)+\kappa} \equiv q(\eta,\ \kappa,\ \pi,\ \theta) \ . \tag{12d}$$

Notice that since $\xi > 0$, $\kappa_L < 1$. Hence $\kappa_L < \kappa_U$.

For every positive value of η, π, κ and θ, the functions $r(\pi,\theta)$ and $q(\eta,\kappa,\pi,\theta) < 1$. Available evidence (Steinman, 1991) indicates that antigen–primed dendritic cells persist for 5–10 days. T cells live considerably longer. Hence, $\sigma \equiv d_C/d_T > 1$ for dendritic cells, and the stability conditions (11d) and (12d) are satisfied for all values of η, π, κ and θ.

It remains to examine the significance of the stability conditions (11c) and (12c). Equations (11c) and (12c) imply that E_{10} is unstable and E_{01} is stable if $\kappa \geq \kappa_U > \kappa_L$. Hence for large values of κ we should observe Th2 and not Th1 dominated responses. Recall that $\kappa \equiv K_2/K_1$, where $K_i = k_{b_i}/(k_a + k_{d_i})$, $i = 1, 2$. Thus κ is a measure of the relative efficiency of interaction of Th2 and Th1 cells with APC. Hence our analysis gives the not surprising conclusion that if Th2 cells interact more efficiently than Th1 cells with APC responses will tend to be Th2 dominated.

Equations (11c) and (12c) also imply that E_{10} is stable and E_{01} is unstable if $\kappa \leq \kappa_L < \kappa_U$. This situation has a similar interpretation to that given above. If κ is small, Th2 cells interact less efficiently than Th1 cells with APC and a Th1 dominated response is expected.

The most interesting situation obtains when $\kappa_L < \kappa < \kappa_U$. In this case both E_{10} and E_{01} are stable (Fig. 2), and hence either a Th1 or a Th2 dominated response can occur. This situation will be studied in more detail in the next section.

The parameter range, $\kappa_L < \kappa < \kappa_U$, is also interesting for another reason. The equilibrium point E_{11} is well defined ($s^{11} \geq 0, x_1^{11} \geq 0, x_2^{11} \geq 0, g^{11} \geq 0$) only when $\kappa_L < \kappa < \kappa_U$ [eqn (9d)]. Numerical bifurcation analysis using the computer program AUTO (Doedel, 1981) indicates that E_{11} is an *unstable* equilibrium point that bifurcates from E_{01} at κ_L and merges with E_{10} at κ_U (Fig. 3). Thus over its entire range of existence E_{11} is unstable and hence long-term responses with both Th1 and Th2 mediated help should not be observed in a biological system. This prediction is consistent with the empirical evidence (Katsura, 1977; Scott *et al.*, 1989), which indicates that the Th1–dependent cellular responses and the Th2–dependent humoral responses are mutually exclusive.

7. Dynamical Behavior of the Model

7.1 Estimation of parameters

Since $CD4^+$ T cells interact with peptide presented on an MHC class II molecule the efficiency of T cell—APC interaction depends on (a) the affinity of the T cell receptor

(TCR) for MHC class II–peptide complex; and (b) the density of these TCR ligands on the APC's surface (Matis *et al.*, 1983). The density of complexes depends on the MHC–peptide binding constant (Adorini & Nagy, 1990), and thus differs among MHC variants. An APC typically exhibits many antigenic determinants, derived by processing of an antigen, and thus is capable of interacting with several T cell clonotypes (TCR specificities). Clones of the Th1 and the Th2 subsets, responding to a given antigenic stimulus, typically appear to be directed to different molecular determinants (Scott *et al.*, 1988) or, when directed to the same molecular determinant, to exhibit different affinities (Danska *et al.*, 1990; Evavold *et al.*, 1992). Thus for different antigens the constants K_i (i=1,2) characterizing the interactions between T_i cells and dendritic cells, are expected to have different magnitudes. Furthermore, due to the extensive genetic variability of the MHC molecules, the interaction constants, associated with a given antigenic stimulus is expected to vary among individuals of an out bred population. The parameter $\kappa = K_2/K_1$, which summarizes the relative efficiency of interaction of the responding Th1 and Th2 cells with dendritic cells, is thus expected to vary extensively.

Similarly to the activation of T cells, the contributions of the responding T cells to antigen elimination involve T cell–APC interactions. In the case of Th1 cells, these interactions are mainly T cell–macrophage interactions, whereas in the case of Th2 cells, these interactions are mainly T cell–B cell interactions. Thus in addition to the relative efficiency of activation (κ), the relative contribution to antigen elimination (η), of the responding Th1 and Th2 cells, must be considered over a range of values. Finally, because η represents the relative efficiency of interaction with macrophages (for Th1 cells), or B cells (for Th2 cells), and the relative efficiency of the effector mechanisms induced by these interactions, while κ is determined by T cell–dendritic cell interactions, the values of κ and η are expected to be independent.

Other parameters, which do not depend on the particular antigen or MHC type of the host, are estimated as follows:

The "virgin" steady state population size of an average T_i ($i = 1, 2$) cell clone, a_i/d_T, is approximately 10^4 cells/liter (i.e. 10 cells in a mouse circulatory system of volume 1 cc). A typical value of $K_i = k_{b_i}/(k_{d_i} + k_a)$ is of the order of magnitude of 10^{-8} liters/cell (Bujdoso *et al.*, 1989). Thus, as a typical value we take $\alpha_i \equiv a_i K_i/d_T = 10^{-4}$. However, since the rate of creation of Th1 cells, a_1, may not equal the rate of creation of Th2 cells,

a_2, we will examine the effects of varying the magnitudes of α_1 and α_2 in regimes where $\alpha_1 \neq \alpha_2$.

Antigen–primed dendritic cells persist for 5–10 days (Steinman, 1991; Hopkins *et al.*, 1989), whereas the life–span of T cells is several weeks (Gray & Matzinger, 1991; von Boehmer, 1992). Thus $\sigma \equiv d_C/d_T \simeq 10^1$. In subsequent numerical simulations we take $\sigma = 10$.

Estimates of the dimensional parameter e_1 (section 5), which measures the rate at which activated Th1 cells lead to the elimination of antigen, are not available. One would expect this parameter to depend on the antigen and the efficiency of the cellular immune effector functions in suppressing that particular antigen. Thus, the dimensionless parameters π and θ can not be estimated with precision. However, $\pi\theta = q/d_T$ (section 5) and $q/d_T \simeq .1 - 10$ for antigens such as *Leishmania* or *Listeria* (Müller *et al.*, 1989a; Dannenberg, 1991). We have established (Fishman & Perelson, 1993) that variation of θ and π, within reasonable limits, does not changes the results significantly. In the numerical solutions discussed below, we use $\pi = \theta = 2$.

To estimate $\zeta \equiv k_{np}/(k_p + k_r)$ from experiments in which IFN–γ decreases Th2 cell–proliferation we use the fact that this inhibition is saturable [cf. eqn (1)]. Let f_{IFN} denote the fraction of growth inhibition due to IFN–γ at saturating concentrations. Then from eqn (1g)

$$f_{IFN} = \frac{k_{np}}{k_p + k_r + k_{np}} , \tag{13a}$$

and thus

$$\zeta \equiv \frac{k_{np}}{k_p + k_r} = \frac{f_{IFN}}{1 - f_{IFN}} . \tag{13b}$$

Similarly [eqn (1f)]

$$\xi \equiv \frac{k_{ns}}{k_p + k_r} = \frac{f_{IL_{10}}}{1 - f_{IL_{10}}} . \tag{13c}$$

In experiments using cloned Th1 and Th2 lines, the maximum inhibition of proliferation induced by IFN–γ is $\simeq 0.5$ (Fernandez–Botran *et al.*, 1988; Gajewski & Fitch, 1988), thus $\zeta = 1$. The maximum inhibition induced by IL–10 is $\simeq 0.9$ (Fiorentino *et al.*, 1989), thus $\xi = 9$. Lacking direct information about the effects of IL–10 and IFN–γ *in vivo*, we shall assume that these values of ζ and ξ are also appropriate for the *in vivo* situation. If this is the case, then $\xi \gg \zeta$, and Th2 cells would be more efficient at suppressing proliferation of

Th1 cells than the reverse. Consequently, except for small values of κ, a response involving both T helper cell subsets should be dominated by the Th2 subset. We shall return to this point later.

To estimate ν we note from its definition that

$$\nu \equiv 1 + \frac{k_{np}}{k_{r_{np}}} = 1 + \frac{k_{np}}{k_p + k_r} \Big/ \frac{k_{r_{np}}}{k_p + k_r} = 1 + \zeta/\rho$$

Assuming $k_{r_{np}} \simeq k_r \simeq k_p$, i.e. that the return to the rest state takes approximately one cell cycle time, then $\rho \equiv k_{r_{np}}/(k_p + k_r) = 0.5$. Hence, $\nu = 1 + 2\zeta = 3$. We will also compare cases in which $k_{r_{np}}$ is bigger or smaller than k_r.

To estimate Ω we note that the sensitivity of Th2 cells to IFN–γ cross–regulation implies Ω, the non–dimensional half–saturation constant [eqn (2g)], must be of order one. Below we take $\Omega = 1$.

To estimate ϕ, recall that

$$\phi \equiv K_{IL_{10}} \frac{d_{IL_{10}}}{p_{IL_{10}}} \Big/ K_{IFN} \frac{d_{IFN}}{p_{IFN}} , \tag{14}$$

where K_{IFN} and $K_{IL_{10}}$ are the respective half–saturation constants for IFN–γ's action on T_2^* and IL–10's action on T_1^*, $p_{IL_{10}}$ and p_{IFN} are the per capita production rates of IL–10 (by T_2^* and T_2^{*np} cells) and of IFN–γ (by T_1^* cells), while $d_{IL_{10}}$ and d_{IFN} are their respective removal rates. The importance of ϕ is due to a fact that among the dimensionless parameters of our model ϕ is the parameter most amendable to experimental manipulation—see section 8.1. In absence of evidence to the contrary, we shall assume that the respective half–saturation constants (K_{IFN} and $K_{IL_{10}}$) and the respective production to removal ratios ($d_{IL_{10}}/p_{IL_{10}}$ and d_{IFN}/p_{IFN}) are of the same order(s) of magnitude. Thus, we take $\phi = 1$. In section 8 we consider the effects of the magnitude of ϕ.

7.2 Antigen Threshold

In section 6 we derived four steady state outcomes of an antigenic challenge of the system of equations (8): an uninfected state (E_{00}); a coexistence state in which the antigen

persists but is kept in check by an immune response, that is implemented by the Th1 cells (E_{10}); a coexistence state dominated by the Th2 response (E_{01}); and a Th1–Th2 state (E_{11}) in which both T helper cell subsets have representation in the response to the persisting antigen. We also showed that the uninfected state, E_{00}, and the Th1–Th2 state, E_{11}, are unstable, while the two antigen coexistence states, E_{10} and E_{01}, are stable. The obvious conclusion, that all perturbations by antigen will lead to states in which antigen persists is not correct. In our previous work (Fishman & Perelson, 1993), we found that large doses of antigen approach a stable antigen coexistence state in an oscillatory manner with the antigen concentration initially decreasing. This initial concentration decrease, predicted by our differential equation model, can be over 20 orders of magnitude in amplitude. To maintain biological reality, we introduced an "effective zero" threshold with the property that if the antigen concentration falls below this level, it is set to zero. Thus, trajectories that approach a coexistence state through very low values of the antigen are forced to return to the uninfected state. In this manner the model predicted elimination of antigen or stable coexistence depending upon parameter values and antigen dose. In this model we also introduce an effective zero antigen concentration, which we choose to be equivalent to 0.01 organisms per animal. In nondimensional units this threshold corresponds to $g = 10^{-6}$ (see Fishman & Perelson, 1993 for details).

7.3 Outcome of Antigen Challenge

Beginning with a system in the virgin, E_{00}, steady state, we find that there are three possible outcomes of an antigenic challenge: (a) The invading pathogens are eliminated and the system returns to the uninfected steady state E_{00}. The elimination occurs because the antigen concentration is driven to the lower threshold. This is shown in Fig. 4a. (b) The pathogens are not eliminated and the system approaches a coexistence state dominated by Th1 cells—steady state E_{10} (Fig. 4b). (c) The pathogens are not eliminated and the system approaches a coexistence state dominated by Th2 cells—steady state E_{01} (Fig. 4c). In these figures we illustrate the antigen dynamics, $g(t)$, as well as the levels of activated Th1 cells, x_1^*, and Th2 cells, x_2^* and x_2^{*np}. Both x_2^* and x_2^{*np} are assumed to contribute to antigen elimination. Thus, we have plotted the total, activated, Th2 population. Further, because we are interested in their contribution to antigen elimination we have multiplied the Th2 population by the constant $\eta\kappa^{-1}$, which in the nondimensional equation (8d)

is the coefficient for the Th2 contribution to antigen elimination. In the nondimensional equation (8d) the Th1 population's contribution to antigen elimination is simply x_1^*. Which of these possible outcomes occurs depends on the size of the initial antigen challenge, $g(0)$, and the dimensionless parameters κ and η, which measure the relative efficiencies of Th1 and Th2 activation by the antigen presenting cells and the relative efficiencies of pathogen elimination by the responding Th1 and Th2 clones.

Figure 5 shows the outcomes predicted by the model as κ and the initial antigen dose, $g(0)$, are varied, while η is kept constant at $\eta = 1$, i.e. equal efficiencies of Th1 and Th2 mediated antigen elimination. As κ increases from zero there are four distinct regions of behavior. In region (I), defined for low values of κ, $0 \leq \kappa < 0.212$ for the parameters used here, low doses of antigen lead to Th1 dominated coexistence, whereas higher doses are eliminated. This is similar to the Th1 only responses described in Fishman & Perelson (1993). For this range of κ values, there is no significant activation of the Th2 cells and thus there is no cross–regulation of the Th1 response, i.e., the response is essentially an unperturbed Th1 response. This is documented more fully in Figs. 4a and 4b, where we show the dynamics of a response in region I. In Fig. 4b antigen initially grows, and stimulates both a Th1 and Th2 response. Because $\kappa < 1$, Th1 stimulation is more vigorous than Th2 stimulation and the Th1 population responds strongly and begins eliminating the antigen. The Th2 contribution to antigen elimination is about five orders of magnitude less than the Th1 cell's contribution, and hence this response can be thought of as Th1 only. As the antigen decreases, the Th1 stimulation decreases, and ultimately the antigen recovers and reaches a steady state in which its growth is balanced by the Th1 response. In Fig. 4a, we see a similar scenario except the initial antigen dose is higher. In this case the Th1 response is larger and the antigen is driven to elimination before the Th1 response falls substantially.

In region (II) of Fig. 5, defined for higher values of κ, here $0.212 < \kappa < 0.292$, the increase in κ causes the dose dependent activation of the Th2 cells to become significant and cross–suppression of the Th1/Th2 clones begins to moderate the total response. In this κ range, low antigen doses lead to coexistence, and larger doses lead to elimination , as in region I. However in region II, further increases of the antigen dose lead to coexistence. This change is due to Th2 suppression of the Th1 response and is analyzed in more detail below. This behavior is reminiscent of the "sneaking through" of tumors. The similarity may not be incidental; some tumors elicit both cellular and humoral responses (Greenberg,

1991) and hence Th1/Th2 cross–regulation may be involved. Finally, in region (II), very high doses are predicted to lead to antigen elimination. This prediction is probably not realistic because very high doses of the antigen may kill the animal, a phenomenon outside the scope of this model. Within region II, further increase of κ, here $0.283 < \kappa < 0.292$, causes the intermediate domain of antigen elimination to disappear and sneaking through is no longer possible.

In region (III), where κ is increased even further, here $0.292 < \kappa < 0.525$, the Th2 coexistence state E_{01} is added to the range of the possible outcomes. The predicted outcomes are now: low antigen doses lead to E_{10}, intermediate doses lead to E_{01}, and high doses lead to the Th2 dominated elimination of the antigen.

The largest values of κ correspond to region (IV), here $\kappa > 0.525$. In this region low antigen doses cause Th2 dominated coexistence and higher doses lead to antigen elimination. Thus, for sufficiently high values of κ there is essentially a Th2 response unregulated by Th1 cells.

In Fig. 6 we examine the behavior of our model in the "sneaking through" region of Fig. 5 in more detail. We plot the maximum value attained by x_1^*, i.e., the maximal Th1 contribution to suppression of the antigen during the response; the maximum value attained by $\eta\kappa^{-1}(x_2^* + x_2^{*n_p})$, i.e., the maximal Th2 contribution to the antigen's suppression; and the maximum values of the sum of these quantities which we call the total suppression, as a function of the antigen dose for $\kappa = 0.27$, which is in the sneaking through domain. We see that for low antigen doses the total response increases with the dose—eventually leading to responses that promote elimination of antigen rather then convergence to coexistence. As antigen dose is increased further, the activation of the Th2 cells becomes significant and the total response falls. Even at a low level of activation, where their contribution to pathogen suppression is negligible, Th2* cells induce a profound reduction of Th1 response. The resultant decrease in total response leads to the reintroduction of the convergence to coexistence (Fig. 5).

In Fig. 7a we illustrate how the various regions defined in Fig. 5 vary with the value of η. For clarity of presentation the E_{10}/E_{01} separators are omitted. It can be seen that regions I and II do not vary qualitatively with η. The most significant variations occur in regions III and IV at the boundary between the domain of the Th2 dominated coexistence

state E_{01} and the domain of the Th2 dominated antigen elimination. This change is due to the fact that for regions III and IV the immune response is Th2 dominated. Recall that $\eta \equiv e_2/e_1$, and thus the per capita contribution of the responding Th2 cells to antigen elimination decreases with η. Thus, as η decreases, Th2 cells are less effective at antigen elimination and a greater range of antigen doses lead to coexistence rather than elimination.

In Fig. 7b we illustrate how the coexistence/elimination separating curves vary with ν, where the magnitude of

$$\nu \equiv 1 + \frac{k_{np}}{k_{r_{np}}} = 1 + \frac{k_{np}}{k_p + k_r} \Big/ \frac{k_{r_{np}}}{k_p + k_r} = 1 + \zeta / \frac{k_{r_{np}}}{k_p + k_r}$$

depends on the ratio of the relaxation rate coefficient ($k_{r_{np}}$) of the inhibited, non–proliferating, T_2^{*np} cells, to the sum of the proliferative (k_p) and nonproliferative (k_r) relaxation rate coefficients of the uninhibited, T_2^* cells. Because no direct evidence on the magnitude of that ratio is available, we examine the effects of changing the magnitude of $k_{r_{np}}$ relative to k_r ($\approx k_p$). We plot coexistence/elimination separating curves for $\nu = 21$, $\nu = 3$, and $\nu = 1.2$. We see that decreasing ν shifts the curve toward increasing κ values. However, quantitative differences resulting from an order of magnitude change in the value of $k_{r_{np}}$ are relatively minor. Thus, the magnitude of $k_{r_{np}}$ does not seem to have a profound influence on overall dynamics of cross–regulation.

A simplification implicit in our model is that we only consider Th1 and Th2 type cells. Th1 and Th2 are phenotypes that characterize lymphokine secretion patterns of mature T cells. Naive T cells upon *in vitro* activation do not follow these secretion patterns (see Discussion). Because of uncertainty about the origins of Th1 and Th2 cells, it was of interest to examine the dependence of our results on the initial population sizes. In Fig. 7c we present three curves in the κ–$g(0)$ plane, where we vary the $\alpha_1 : \alpha_2$ ($a_1 : a_2$) ratio from 1:19 to 1:1 to 19:1, but keep the total influx ($\alpha_1 + \alpha_2 = 2 \cdot 10^{-4}$) constant. We see that increasing the influx of one set of T cells at the expense of an order of magnitude decrease in the influx to the other set shifts the curves relative to κ–axis without changing the basic characteristics of the model.

8. Potential Applications to Therapy

8.1 Treatment of autoimmune disorders

Fowell and coworkers (1991) and Powrie & Coffman (1993) review evidence indicating a role for Th1/Th2 cross–regulation in autoimmunity. In our model the relative efficiencies of Th1 and Th2 activation and the efficiencies of Th1 and Th2 cells in antigen elimination are mutually independent. Thus, given a component of self which induces both Th1 and Th2 responses with a κ value at which both E_{10} and E_{01} are stable [eqn (11c)–(12c)] and an η value that is not of order one, there will be different magnitudes of antigen elimination associated with the steady states E_{10} and E_{01}. Domination by a weakly suppressive T helper cell subset could therefore represent a non–pathological condition or tolerance of self. Conversely, domination by a strongly suppressive T helper cell subset may result in pathological autoimmunity. For example, in autoimmune diabetes—in which autoimmune destruction is implemented by $CD8^+$ cells (and thus is Th1–dependent)—pathology is associated with a Th1 dominated response, whereas antigen–specific Th2 cells confer protection (Fowell *et al.*, 1991). Similarly, in graft–versus–host (GVH) reactions pathology is associated with the Th2 dominated response (de Wit *et al.*, 1993), whereas antigen–specific Th1 cells confer protection (Sykes *et al.*, 1993). Thus, if methods for switching between Th1 and Th2 dominated states in humans can be found, they might be of use in the therapy of autoimmune diseases (Powrie & Coffman, 1993)..

One of the techniques for treating autoimmune disorders in experimental animals is the injection of "regulatory" $CD4^+$ cells (cf. Cohen, 1986). Below we examine the requirements for perturbing Th1 (Th2) dominated steady states by combining injection of Th2 (Th1) antigen–specific cells with an infusion of anti–IFN–γ or anti–IL-10 monoclonal antibody (mAb). In the context of the current model, infusion of anti–IL-10 or anti–IFN–γ mAb can be viewed as corresponding to an increase in the respective lymphokine's removal rate (d_{IFN} or $d_{IL_{10}}$) [eqn (1)], and thus a change in the magnitude of ϕ [eqn (14)]. Other treatments under consideration (Powrie & Coffman, 1993) include the use of cytokine antagonists or soluble cytokine fragments that would compete for cytokine binding, or agents that inhibit cytokine synthesis. These various treatments all can be viewed as effecting the value of ϕ [eqn (14)].

In Fig. 8a we present the minimal requirements for perturbations of E_{10} into E_{01} by injection of antigen–specific Th2 cells as a function of ϕ, for two different κ values. For a given value of κ, a dose of Th2 cells that is below the appropriate curve in Fig. 8a will not lead to a change in state, whereas a dose above that curve will switch the system from Th1 dominated response to a Th2 dominated response. Similarly, Fig. 8b depicts the

requirements for perturbations of E_{01} into E_{10} by injection of antigen–specific Th1 cells as a function of ϕ for two κ values. The figure shows that the dose of the cross–regulating T helper cells required for switching the steady states depends on the value of κ. The dose required decreases with κ for the E_{10} to E_{01} transition, and increases with κ for the E_{01} to E_{10} transition. Recall that $\kappa = K_2/K_1$, where $K_i = k_{b_i}/(k_a + k_{d_i})$, $i = 1, 2$. Thus κ is a measure of the relative efficiency of interaction of Th2 and Th1 cells with APC. Hence our analysis gives the not surprising conclusion that stability of an coexistence state depends on the efficiency of the interaction between the dominating T helper subset and antigen presenting cells. However, the required dose can be reduced by modifying ϕ. Decrease in ϕ facilitates the E_{10} to E_{01} transition, and increase in ϕ facilitates the E_{01} to E_{10} transition.

8.2 Treatment of parasitic diseases

Parasitic protozoa and helminths are a diverse group of organisms that are a major cause of infectious disease in humans. Studies, particularly in animal models, have shown that Th1–Th2 cross-regulation may play a crucial role in determining the outcome of infection. One disease on which much experimental attention has been focused is leishmaniasis. Leishmaniasis is a chronic protozoan disease caused by several different species of *Leishmania* parasites. Infection occurs when a sandfly transmits the *promastigote* stage of the parasite to a susceptible mammalian host. The parasites then invade macrophages, transform to *amastigotes* and divide, eventually rupturing the cell and invading other macrophages. This leads to a spectrum of clinical diseases, depending on the infecting species.

Leishmania major infections in mice have been used as an experimental model of human leishmaniasis. In this system $CD4^+$ T cells have the potential to mediate both disease susceptibility and resistance (Scott *et al.*, 1989). BALB/c mice appear to be innately susceptible to *L. major* infection. These mice mount an antibody response, which is ineffective in clearing the parasite. Other strains, such as CBA/J, which are resistant mount a stable cell-mediated response. Isolation and cloning of parasite specific $CD4^+$ T cells from susceptible and resistant strains showed that a predominance of Th1 cells correlates with protection, whereas the predominance of Th2 cells correlates with disease susceptibility (Scott *et al.*, 1989, Müller *et al.*, 1989a,b).

As discussed in section 6, in controlling infections by intracellular parasites in general, and leishmanial infections in particular, humoral (Th2 dependent) responses have much lower effectiveness than the cellular (Th1 dependent) responses, i.e., $e_2 \ll e_1$. To illustrate this point we study the extreme case, $\eta = e_2/e_1 = 0$. Since the rest of the nondimensional parameters do not depend on e_2, they remain as defined in sections 5–7. It is straightforward to verify that, with $\eta = 0$, system (8) has three steady state solutions. E_{00} and E_{10} remain unchanged, E_{01} no longer exists, and E_{11} is transformed into an equilibrium point $\tilde{E}_{11}$ given by

$$\tilde{E}_{11} = (\tilde{s}^{11}, \tilde{x}_1^{11}, \tilde{x}_2^{11}, \tilde{g}^{11}) = (U, \theta, V, U) \ ,$$

where

$$U = [\Omega + (1+\zeta)\theta]\left(\frac{\theta+1}{\kappa(\Omega+\theta)} + \Omega\phi\frac{[\Omega+(1+\zeta)\theta] - \kappa(\Omega+\theta)}{(\Omega+\nu\theta)\left[(1+\xi)\kappa(\Omega+\theta) - [\Omega+(1+\zeta)\theta]\right]}\right) \ ,$$

and

$$V = \Omega\phi\kappa\frac{(\Omega+\theta)}{(\Omega+\nu\theta)}\frac{[\Omega+(1+\zeta)\theta] - \kappa(\Omega+\theta)}{(1+\xi)\kappa(\Omega+\theta) - [\Omega+(1+\zeta)\theta]} \ .$$

Note, $\tilde{E}_{11}$ is well defined ($\tilde{s}^{11}, \tilde{x}_1^{11}, \tilde{x}_2^{11}, \tilde{g}^{11} \geq 0$) only when

$$\frac{\Omega+(1+\zeta)\theta}{(1+\xi)(\Omega+\theta)} < \kappa < \frac{\Omega+(1+\zeta)\theta}{\Omega+\theta} \ .$$

As in section 6, E_{10} is stable, whereas E_{00} and $\tilde{E}_{11}$ are unstable. If Th1 cells dominate the response, there are two possible outcomes of infection: either the invading pathogens are eliminated and the system returns to the uninfected steady state E_{00} (cf. Figure 4a) or the pathogens are not eliminated and the system approaches a coexistence state E_{10} (Figure 9a). On the other hand, if Th2 cells dominate the response, pathogens escape elimination and expand ultimately leading to the death of the infected animal (a phenomena outside the scope of this model) (Figure 9b). This occurs because Th2 cells do not contribute to the elimination of the invading pathogens. Figure 10a depicts the variation in these outcomes in κ–$g(0)$ plane for $\eta = 0$ (cf. Fig. 5 for the $\eta = 1$ case).

Monoclonal anti-cytokine antibodies are able to modulate the outcome of parasitic infection (cf. Coffman *et al.*, 1991; Mosmann *et al.*, 1991). This can be explained within the context of our model. In Fig. 10b we depict the variation in outcomes, elimination or coexistence versus escape of the pathogen from immune control, for three values of ϕ,

$\phi = 0.1, 1.0, 10.0$. An increase in ϕ causes a shift of the curve toward higher values of κ without changing its overall form. In particular, for a fixed value of κ the outcome of an inoculation with a given antigen dose which lays in the domain of escape for $\phi = 1$ can be shifted to the domain of coexistence or elimination by increasing ϕ (injecting anti–IL-10 mAb). Conversely, by decreasing ϕ (injecting anti–IFN–γ mAb) an escape from immune control can be induced. Since a single injection of antibody will not result in a permanent change in ϕ, the above interpretation is subject to criticism. However, it is clear that a permanent change is not required, one only needs to modify the parameters long enough to cause a trajectory to move from one domain of attraction to another.

In the case of *L. major*, Coffman *et al.* (1991) report that they were unable to switch a Th2 response to a protective Th1 response in BALB/c mice by injection of the anti-IL-10 antibody SXC-1. Coffman *et al.* argue that these results should be considered preliminary since the effectiveness of the antibody they used has not been clearly demonstrated in adult mice. According to our model, another explanation is that the value of κ maybe sufficiently high that changing ϕ still keeps the system in the Th2 domain. For example, at the point $\kappa = 0.5$, g(0)=250 in Fig. 10b, a change in ϕ from 1 to 10 will still keep the system in the Th2 domain. Coffman *et al.* also look at the effects of anti-IFN-γ. They show that a protective Th1 response can be converted to a disease progressing Th2 response in accord with our predictions. For example, in Fig. 10b the point $\kappa = 0.25$, $g(0) = 250$ is in the domain of pathogen elimination for $\phi = 1$ but moves to the domain of disease progression if ϕ is decreased to 0.1.

While anti-IL-10 therapy may not be effective in *L. major* infection, Sher *et al.* (1991) show that in schistosomiasis IL-10 appears to be at least in part responsible for down-regulating the Th1 cytokine response. In this system, addition of neutralizing anti-IL-10 mAb to antigen-stimulated spleen cell cultures taken from infected mice caused a dramatic augmentation in IFN-γ synthesis.

In addition to using monoclonal antibodies to change the course of a parasitic disease, our model suggests that vaccination with the appropriate dose of live parasite may lead to protective immunity. Let us once again consider the case of *L. major* infection, say with $\kappa = 0.5$. As shown in Figs. 9a and 10a, low doses of antigen (e.g. $g(0) \approx 1$) lead to a protective Th1 response with ultimate approach to the E_{10} coexistence state, whereas high doses (e.g. $g(0) \approx 10$) lead to a Th2 response and disease (Fig. 9b). However, because low

doses lead to the E_{10} steady state, one would expect that a second challenge with a dose of antigen that normally would lead to disease would be controlled by an animal that had an ongoing Th1 response. This is shown explicitly in Fig. 11, where a relatively high dose $g(0) = 100$, that normally leads to antigen escape (cf. Fig. 10a), is controlled.

Some of the behavior expected from our model has been seen in recent experiments by Bretscher *et al.* (1992) in which BALB/c mice were injected with different numbers of parasites. In these experiments, injection of 10^2 to 10^4 parasites seemed to lead to a successful DTH response as measured by footpad swelling. With 10^4 parasites the footpad size increased and reached a steady or gently undulating state, in which the mice "probably had a substantial number of parasites whose rapid and further proliferation was contained". This described steady state corresponds to the E_{10} state. Doses of parasite higher than 10^4 lead to progressive increase in foot size and generated the production of antibodies that were detectable two months after challenge, whereas lower dose of parasite gave much smaller amounts of antibody that were barely detectable. Further, vaccination with low doses of parasite provided protection, consistent with the model. BALB/c mice that are normally susceptible to disease became resistant by expose to low doses, i.e., initial exposure to 10^2 or 10^3 parasites rendered the mice resistant to exposure to 10^7 parasites 105 days later. Resistance took several month to establish. Recall that a unit of the nondimensional "time" τ, used in our model, corresponds to the T cell lifespan of roughly one month. Thus, in our model it also takes approximately one to three months for the establishment of the E_{10} state and resistance. Lastly, in this model, $g(0)$ is the nondimensional antigen dose, where $g = 1$ corresponds to 10^4 organisms. In Bretscher *et al.* the threshold for the switch from a Th1 dominated to a Th2 dominated response occurs at a dose of approximately 10^4 parasites. This occurs in our model at $\kappa \approx 0.5$.

8.3 Treatment of AIDS

Clerici and Shearer (cf. Clerici & Shearer, 1993; Salk *et al.*, 1993) have suggested that Th1 responses are associated with resistance to HIV infection and/or progression to AIDS, and that Th2 responses are associated with disease susceptibility. Their hypothesis is based on the observations that peripheral blood mononuclear cells from seronegative, but HIV exposed, individuals respond to HIV envelope antigens by producing IL-2, a Th1 response. Because HIV infection is likely to be caused by transfer of HIV-infected

cells, cell-mediated immunity could be more important than a humoral response upon infection. Thus, individuals mounting a successful Th1 response may be protected from disease. Also, as asymptomatic, HIV-seropositive individuals progress towards AIDS, they shift from a Th1 to a Th2 cytokine pattern. Thus after seroconversion IL-2 and IFN-γ production decrease, while IL-4 and IL-10 production increases. If Clerici and Shearer are correct about the protective role of Th1 cells, then methods of treatment that favor a Th1 response would be important to develop.

The principles that we discussed in section 8.2 for switching a Th2 response to a Th1 response in parasitic infections would also apply to HIV. Thus, our theory suggests that immunization with low doses of antigen or increasing ϕ, say by infusion of anti-IL-10 mAbs, could potentially lead to Th1 responses. As discussed by Salk *et al.* (1993), there are already experimental indications that low dose immunization might lead to a Th1 response. Their experiments show that administration of high doses of SIV to macques results in infection and antibody production with a minimal cell-mediated response, whereas lower doses elicit a strong cell-mediated response, but does not generate antibody production or infection. They also find that mice given high doses of inactivated HIV generate a transient DTH response followed by antibody production, whereas giving lower doses generates only a DTH response.

9. Discussion

The 1980's was a decade of exciting advances in immunology. Among the notable achievements were the elucidation of the role of accessory cells in antigen presentation to B and T cells (Vitetta *et al.*, 1989; Tew *et al.*, 1990; Steinman, 1991) and the discovery of the existence of the two different types of $CD4^+$ T helper cells, Th1 cells and Th2 cells with different patterns of lymphokine secretion (Street & Mosmann, 1991). Th1 cells secrete IL–2 and IFN–γ, cytokines important in mediating delayed type hypersensitivity and cytotoxic T cell responses. Whereas Th2 cells secrete IL–4, IL–5 and IL–6, cytokines important in generating antibody mediated responses. Due to these differences it has been suggested that the observation that immune responses tend to be either cell mediated or antibody mediated might be due to the type of helper cells stimulated in the response.

Here we present a model accounting for possible cross–regulatory phenomena of the Th1/Th2 helper cell subsets during an immune response. The model is built on the results of our previous model (Fishman & Perelson, 1993) of T cell–antigen presenting cell interactions. In order to focus on the Th1/Th2 cross–regulation, we did not introduce explicit details of T helper cell–effector cell interactions. In our previous model we had shown that T helper cell dynamics can be modeled independently of the effector dynamics. In the present model a self–renewing source of antigenic stimulus—that may be taken to represent either invading pathogens or tumor cells—is initially detected by dendritic cells (DC). These cells take up and process the antigen, converting it into the immunogenic form of peptide–MHC complexes. Interaction between an immunogen bearing DC and a $CD4^+$ T cell with the appropriate receptor specificity induces that T cell's activation. A common attribute of the above antigenic stimuli is simultaneous induction of both cellular and humoral immune responses (Scott *et al.*, 1989; Greenberg, 1991; Fowell *et al.*, 1991). However, even though both Th1 and Th2 cells may be stimulated by interaction with antigen presented on the surface of a dendritic cell, responses ultimately tend to be either cellular (Th1 mediated) or humoral (Th2 mediated).

Responding Th1 and Th2 cells are cross–suppressive. IFN–γ, a product of Th1 cells, inhibits proliferation of Th2 clones (Fernandez–Botran *et al.*, 1988; Gajewski & Fitch, 1988). Interleukin-10 (IL–10), a cytokine produced by Th2 clones, inhibits cytokine production by Th1 clones (Fiorentino *et al.*, 1989, 1991) and thus, in the absence of externally added cytokines, inhibits Th1 cell proliferation.

Antigenic stimuli typically present a broad spectrum of antigenic determinants to the immune system. $CD4^+$ cells of the Th1 and the Th2 subsets, responding to a given antigenic stimulus, appear to be either directed to different molecular determinants (Scott *et al.*, 1988); or, when directed to the same molecular determinant, to exhibit different affinities (Danska *et al.*, 1990; Evavold *et al.*, 1992). Thus, Th1 and Th2 cells responding to the same antigen will typically become activated to different degrees at a particular antigen concentration. Further, different antigens have different susceptibilities to cell mediated and humoral responses. Thus, Th1 and Th2 cells will differ in their per capita contribution to immune elimination. These two differences have profound consequences in our model. By scaling our system of equations (section 5) we obtained two critical dimensionless parameters: κ, which represents the efficiency of activation of the responding Th2 cells relative to efficiency of activation of the responding Th1 cells, and η, a parameter

that represents the per capita rate of the responding Th2 cells' contribution to immune elimination relative to the Th1 cells' contribution. In section 7.3 we have shown that the behavior of our model of the Th1/Th2 system strongly depends on values of κ and η. Values κ and η depend on the antigenic stimulus and MHC haplotype (section 7.1), and thus are expected to be highly variable among individuals of an out bred population. Our model is thus consistent with the variability seen in natural infections.

In our investigation of the scaled system of equations (section 6) we established the existence of four equilibrium points. One equilibrium point represents the state of the system in the absence of an antigenic stimulus. It might be called an uninfected steady state or cured steady state since the system will return to this point after a successful response that totally eliminates the antigen. We showed that this state is unstable due to its sensitivity to antigenic perturbations. Thus, introduction of antigen is expected to cause the system to move away from the uninfected steady state and approach one of the other equilibrium points of the system in which antigen continues to survive in the face of an ongoing immune response. However, this conclusion was shown to depend on the fact that our model involves continuous ordinary differential equations assumed to be valid down to arbitrarily small antigen concentrations. Introduction of a truncation procedure in which we set to zero very small antigen concentrations that would correspond to well less than one organism per individual (section 7.2), restores the capacity of the system to return to the uninfected state after an effective immune response (Fig. 4a). The instability of the uninfected state to antigenic perturbations also reflects our choice of antigen dynamics. Thus, the growth law that we use is appropriate for pathogens that grow even when introduced into animals at very small numbers. There may exist pathogens that only grow if more than a critical number of pathogens are present. This, for example, may occur in sexually reproducing organisms. For such pathogens a different type of growth law would be required and with such growth laws the uninfected state could be stable to antigenic perturbation (M. Kaufman, personal communication).

If an antigenic challenge does not result in elimination of antigen, the system may converge to a *coexistence* state in which the antigen's proliferation is balanced by an immune response. Whether the response will be dominated by Th1 or Th2 cells depends on the values of κ, η and the antigen dose (Figs. 5, 7a). Coexistence states, in which the immune system keeps a population of pathogens in check but does not eliminate it, are well documented (cf. Müller *et al.*, 1989b). Similarly in the case of tumors, dormant

states exist where potentially lethal tumor cells persist for prolonged periods with little or no growth. Although little is known about the mechanisms of dormancy, in many cases it has been suggested that the dormant state reflects control of tumor growth by immune mechanisms (cf., Stewart & Wheelock, 1992; Kuznetsov *et al.*, 1993).

The fourth equilibrium point of the model is an antigen coexistence state in which both Th1 and Th2 subsets are expressed and contribute to the response. For the parameters we have studied this state is unstable, consistent with observations indicating that over the long–term cellular and humoral immune responses appear to be mutually exclusive (Parish, 1972; Katsura, 1977; Scott *et al.*, 1989).

Our model, although quite complicated, has simplified many of the details of the Th1–Th2 system. For example, we have assumed that IL-2 and IL-4 are autocrine growth factors and thus we have neglected their potential cross-stimulatory role in Th1–Th2 regulation. This feature of the system is being investigated by Morel *et al.* (1993). In appendix B we show that if IL-2 and IL-4 have paracrine effects in addition to their autocrine effects the same qualitative behavior is to be expected of our model.

Another simplification implicit in our model is that we only consider Th1 and Th2 type cells. Th1 and Th2 are phenotypes that characterize lymphokine secretion patterns of mature T cells. Naive T cells upon *in vitro* activation do not follow these secretion patterns and it has been suggested that there exist precursor populations, common to both Th1 and Th2 cells, that secrete only IL-2 or IL-2 and IL-4 (cf., Swain *et al.*, 1991; Hsieh *et al.*, 1992; Seder *et al.*, 1992; Röcken *et al.*, 1992). However, none of the Th1/Th2 clones isolated to date (Scott *et al.*, 1989; Danska *et al.*, 1990; Evavold *et al.*, 1992) have been shown to share the same exact receptor specificity, even when directed to the same peptide, a result consistent with an independent origins for Th1 and Th2 cells. Our model has neglected populations other than Th1 and Th2, and has ignored the question of whether Th1 and Th2 cells are predetermined lineages or arise from a common precursor. In our model, we assume that before antigen challenge Th1 and Th2 cells exist at their E_{00} steady state levels, given by a_1/d_T and a_2/d_T, respectively. One view of our model is when that it applies to secondary responses, with the antigen specific Th1 and Th2 populations having differentiated and going into a memory state during the primary response. Alternatively, because our model and its analysis has been been geared to understanding long-term responses, one can assume that the differentiation of Th1 and Th2 cells during a primary

response is rapid on the time scale of interest, and neglecting these early dynamics will have little consequence. Recall that our dimensionless time unit corresponds to the lifetime of a T cell and hence is of order weeks to months. Because of the uncertainty about the origins of Th1 and Th2 cells, it was of interest to examine the dependence of our results on the initial population sizes. In Fig. 7c we demonstrated that the basic characteristics of our model do not change as the initial population is varied from 5% Th1, 95% Th2, to equal initial populations, to 95% Th1, 5% Th2.

Our model has also simplified the issue of antigen presenting cells. Besides dendritic cells, macrophages and B cells can present antigen to T cells. As discussed in Fishman & Perelson (1993), macrophages (and B cells) would be characterized by different parameters than dendritic cells, and hence a model with multiple APC populations could have richer behavior. For example at very low antigen doses, antigen specific B cells may be the only cells capable of binding enough antigen to stimulate T cells. Thus, antigen dose response curves may reflect different efficiencies of antigen presentation by different APC populations (Pfeiffer *et al*., 1991). Also, along similar lines, we have neglected the interactions between Th1 and Th2 cells and B cells and macrophages needed to generate effector responses and the interactions between Th1 cells and macrophages that are inhibited by IL-10. Thus, in essence, we have assumed that macrophages and B cells are present at levels required to generate the phenomena we model.

Among the dimensionless parameters that we derived in section 5, the parameter ϕ is of particular importance. This is due to the fact that ϕ, which represents the relative effects of the cross–regulating lymphokines, IL$-$10 and IFN$-\gamma$, in terms of the lymphokine–producing T cells, is amendable to external manipulation. The magnitude of ϕ is proportional to the product of the ratios of the *in vivo* removal rates of IL$-$10 and IFN$-\gamma$, production rates and dose-response half-saturation constants. All of these ratios can be influenced by external agents. For example, production rates may be influenced by drugs or cytokine therapy, removal rates can be increased by infusion of anti–IL$-$10 or anti–IFN$-\gamma$ monoclonal antibodies, and half-saturation constants may be influenced by competitive inhibitors of the cytokine receptors. All of these agents are not yet available but work is proceeding to develop them (Powrie & Coffman, 1993). In section 8 we examined the consequences of varying ϕ in context of perturbations of (a) Th1 or Th2 dominated coexistence states (b) uninfected state. For perturbations of Th1 or Th2 dominated coexistence states our results indicate that the magnitude of the perturbation essential for

obtaining the desired result (switching from one coexistence state another) can be significantly decreased by changing the magnitude of ϕ. For perturbations of uninfected state (antigenic challenges) our results indicate that an eventual outcome can be influenced by keeping the antigen dose fixed while changing the magnitude of ϕ.

In our model Th1/Th2 cross–regulation is functional over a relatively narrow range of κ values—regions II and III in Fig. 5. We expect that the bulk of immune reactions will be characterized by parameters that place them into either the cellular immune response region (I) or the humoral response region (IV) in Fig. 5—in which no substantial cross-regulation is occurring. Nevertheless, the list of immune responses in which the Th1/Th2 cross–regulation was found to play a role is being continuously expanded (cf. *Immunol. Rev.* volumes 123 and 127).

Th1/Th2 cross–regulation plays a role in a variety of parasitic and viral infections. Parasitic infection is frequently accompanied by a downregulation of the host's cell–mediated immunity. The Th2 subset dominates in many situations of chronic or exacerbated parasitic infection and is thought to suppress Th1 function as a consequence of the cross-regulatory activity of IL–10. This hypothesis is supported by experiments demonstrating that mAb–mediated neutralization of IL–10 reverses suppressed cellular responses in spleen cells from mice with helminth infections (Sher *et al.*,1991, 1992).

In parasitic diseases, such as *Leishmania major* infection, in which Th1/Th2 cross-regulation is an important determinant of disease outcome our model suggests that vaccination with live parasites may be an important potential therapeutic approach. As shown in Figures 5 and 9, for values of κ that correspond to region III, low doses of antigen lead to E_{10}, Th1 dominated coexistence state, whereas higher doses lead to E_{01}, the Th2 dominated coexistence state. For *L. major* infection as well as other parasitic and nematode infections, Th2 responses are not protective and lead to disease, whereas Th1 responses lead to cure. Since only low doses of this parasite lead to a Th1 response, high doses are usually disease inducing and in many cases fatal. Our model suggests that protection against doses that normally induce disease can be effected by vaccination. The rationale is simple. First giving a low dose, leads to the establishment of the E_{10} state. Once a Th1 dominated state is established, further encounters with the parasite lead to effective response against even very high doses of the parasite (Fig. 11). Thus vaccination is predicted to be a valuable protocol for diseases such as *Leishmania*. Bretscher *et al.* (1992)

have shown that in BALB/c mice vaccination with low doses of *L. major* does in fact lead to effective protection against normally disease producing doses.

While distant from parasites in their biology and phylogeny, some retroviruses also appear to induce an over–production of IL–10, an event closely associated with the onset of immunodeficiency. Thus, in an animal model involving infection of mice with LP–BM5 MuLV and in human HIV infection, Th2 (IL–10 and/or IL–4) cytokine synthesis is increased while Th1 (IFN–γ and/or IL–2) cytokine production is suppressed (Clerici & Shearer, 1993). These observations suggest that cytokine–mediated cross–regulation may play a role in the pathogenesis of acquired immune deficiency disease, contributing both to the progression of retroviral infection and the increase in susceptibility to opportunistic infections and malignancy. If this in fact the case, then the same type of low dose vaccination procedure suggested for *Leishmania*, should be applicable to AIDS (Salk *et al.*, 1993).

Finally, one of the results of our model is that for a set of κ and initial antigen dose values, Th2 induced suppression of the Th1 response is more significant than the Th2 contribution to antigen elimination—leading to a net decrease in the total immune response with increasing antigen dose (Fig. 6). Thus, in this interval of κ values (region–II, Fig. 5), low antigen doses lead to coexistence, and larger doses lead to the antigen's elimination. However, further increases of the antigen dose lead to coexistence. This behavior is reminiscent of the "sneaking through" of tumors. The similarity may not be incidental—some tumors elicit both cellular and humoral responses (Greenberg, 1991), and this may indicate that Th1/Th2 cross–regulation plays a role in tumor immunology.

Th1/Th2 cross–regulation appears to play a role in a wide variety of infectious diseases caused by organisms as diverse as helminths, protozoa and retroviruses (Sher *et al.*, 1992). This type of regulation may also play a role in autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE) (Baron *et al.*, 1992), arthritis (Schlaak *et al.*, 1992; Miltenburg *et al.*, 1992), autoimmune diabetes (Fowell *et al.*, 1991), allergies (Powrie & Coffman, 1993) and graft–versus–host (GVH) reactions (de Wit *et al.*, 1993; Sykes *et al.*, 1993). Given the wide spread importance of Th1/Th2 regulation in determining health or disease both theoretical and experimental approaches to increase our understanding of this system are needed. Here we have provided an initial analysis of this regulatory system

and shown how various potential therapies using low dose immunization or anti-cytokine mAbs may operate to switch between the dominant regulatory subtype of T cells.

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APPENDIX A. SCALING

As detailed in the text, the system described by the eqn (1) involves several distinct time scales. There are the "fast" time scales on the order of minutes to days that corresponds to the turnover rate for S–T cell interactions, the persistence of the activated state of T cells, and lymphokine dynamics. [$(1/(k_{d_i} + k_a)$ is of the order of 8–16 hours. $1/(k_p + k_r)$ is one to two days Flechner *et al.*, (1988), and $1/k_{r_{ns}}$, $1/k_{r_{np}}$ are of the same order of magnitude. Finally, $1/d_{IFN}$, $1/d_{IL_{10}}$ are on a (diffusional) time scale $\simeq$ minutes.]. There is also a "slow" time scale determined by the half–life of T lymphocytes, which is on the order of several weeks. On the scale of the T cells life–span $[1/d_T]$ S–T, S–T*, and lymphokine interactions, detailed in eqn (1) achieve a *quasi steady state.*

Thus

$$p_{IFN}T_1^* = d_{IFN}IFN \ , \tag{A.1a}$$

$$p_{IL_{10}}(T_2^* + T_2^{*np}) = d_{IL_{10}}IL_{10} \ , \tag{A.1b}$$

$$k_{b_i}S_0T_i = (k_a + k_{d_i})S_i \ , \ i = 1,2 \tag{A.1c,d}$$

$$k_aS_1 = \left(k_p + k_r + k_{ns}\frac{IL_{10}}{K_{IL_{10}} + IL_{10}}\right)T_1^* \ , \tag{A.1e}$$

$$k_aS_2 = \left(k_p + k_r + k_{np}\frac{IFN}{K_{IFN} + IFN}\right)T_2^* \ , \tag{A.1f}$$

$$k_{ns}\frac{IL_{10}}{K_{IL_{10}} + IL_{10}}T_1^* = k_{r_{ns}}T_1^{*ns} \ , \tag{A1.g}$$

$$k_{np}\frac{IFN}{K_{IFN} + IFN}T_2^* = k_{r_{np}}T_2^{*np} \ , \tag{A1.h}$$

Let us denote the dimensionless variables and the scales by

$$' = d/d\tau \ ; \ \tau = d_Tt \ ; \ K_i = k_{b_i}/(k_a + k_{d_i}) \ , \ i = 1,2 \ ; \ g = \Upsilon_{Ag}^{-1}Ag$$

$$(s_0, s_1, s_2) = \Upsilon_S^{-1}(S_0, S_1, S_2) \ ; \ x_i = \Upsilon_{T_i}^{-1}T_i \ , \ i = 1,2 \ ; \ x_i^* = \Upsilon_{T_i^*}^{-1}T_i^* \ , \ i = 1,2 \ ; \tag{A.2}$$

$$x_1^{*ns} = \Upsilon_{T_1^{*ns}}^{-1}T_1^{*ns} \ ; \ x_2^{*np} = \Upsilon_{T_2^{*np}}^{-1}T_2^{*np} \ ; \ l_{IFN} = \Upsilon_{IFN}^{-1}IFN \ ; \ l_{IL_{10}} = \Upsilon_{IL_{10}}^{-1}IL_{10} \ .$$

Substitution of eqn (A.2) into eqn (A.1) yields

$$\Upsilon_{T_1^*}\frac{p_{IFN}}{d_{IFN}}x_1^* = \Upsilon_{IFN}l_{IFN} \ , \tag{A.3a}$$

$$(\Upsilon_{T_2^*}x_i^* + \Upsilon_{T_2^{*np}}x_2^{*np})\frac{p_{IL_{10}}}{d_{IL_{10}}} = \Upsilon_{IL_{10}}l_{IL_{10}} \ . \tag{A.3b}$$

$$\Upsilon_{T_i}K_i s_o x_i = s_i \ , \ i = 1,2 \tag{A.3c,d}$$

$$\frac{k_a}{k_p + k_r}\Upsilon_S s_1 = (1 + \xi\frac{l_{IL_{10}}}{K_{IL_{10}}\Upsilon_{IL_{10}}^{-1} + l_{IL_{10}}})\Upsilon_{T_1^*}x_1^* \ , \tag{A.3e}$$

$$\frac{k_a}{k_p + k_r}\Upsilon_S s_2 = (1 + \zeta\frac{l_{IFN}}{K_{IFN}\Upsilon_{IFN}^{-1} + l_{IFN}})\Upsilon_{T_2^*}x_2^* \ , \tag{A.3f}$$

finally

$$\frac{\xi}{\Gamma}\Upsilon_{T_1^*}\frac{l_{IL_{10}}}{K_{IL_{10}}\Upsilon_{IL_{10}}^{-1} + l_{IL_{10}}}x_1^* = \Upsilon_{T_1^{*ns}}x_1^{*ns} \ , \tag{A.3g}$$

$$\frac{\zeta}{\rho}\Upsilon_{T_2^*}\frac{l_{IFN}}{K_{IFN}\Upsilon_{IFN}^{-1} + l_{IFN}}x_2^* = \Upsilon_{T_2^{*np}}x_2^{*np} \ . \tag{A.3h}$$

We shall select the scales Υ_S through Υ_{Ag} such that the dimensionless variables will be of the same order of magnitude (after the first transient).

To assure that l_{IFN} and x_1^* are of the same order of magnitude we select [eqn (A.3a)]

$$\Upsilon_{IFN} = \frac{p_{IFN}}{d_{IFN}}\Upsilon_{T_1^*} \ , \tag{A.4a}$$

similarly [eqn (A.3b)]

$$\Upsilon_{IL_{10}} = \frac{p_{IL_{10}}}{d_{IL_{10}}}\Upsilon_{T_2^*} \ . \tag{A.4b}$$

In selecting the scales for T_1^{*ns} and T_2^{*np} we have a choice of incorporating the ratios ξ/Γ and ζ/ρ [eqn (A.3g,h)] into the respective scales. However, it is straightforward to verify that such incorporation does not leads to any changes in the system [eqn (3)–(7)]. Thus, for the sake of simplicity, we select

$$\Upsilon_{T_1^{*ns}} = \Upsilon_{T_1^*} \ ; \ \Upsilon_{T_2^{*np}} = \Upsilon_{T_2^*} \ . \tag{A.5a,b}$$

To assure that s_0, s_1, and s_2 are of the same order of magnitude we select [eqn (A.3c,d)]

$$\Upsilon_{T_1} = K_1^{-1} \; ; \; \Upsilon_{T_2} = K_2^{-1} \; . \qquad (A.6a,b)$$

From eqn (A.3e) we have

$$\Upsilon_S = \frac{k_p + k_r}{k_a} \Upsilon_{T_1^*} \; . \qquad (A.7)$$

To select Υ_{Ag} we notice [eqn (1a,...,c)]

$$\frac{d}{dt} S = \frac{d}{dt}(S_0 + S_1 + S_2) = nbC_p Ag - d_C S \; , \qquad (A.8a)$$

thus

$$s' = \frac{d_C}{d_T}\left(\frac{nbC_p}{d_C} \Upsilon_{Ag} \Upsilon_S^{-1} g - s\right) \; , \qquad (A.8b)$$

hence we select

$$\Upsilon_{Ag} = (d_C / nbC_p) \Upsilon_S \; . \qquad (A.8c)$$

Finally, substitution of eqn (A1)–(A2) into eqn (1d,e) yields

$$x_1' = \alpha_1 + \frac{(\mu - 1) k_p K_1}{d_T} \Upsilon_{T_1^*} x_1^* - x_1 \; , \qquad (A.9a)$$

and

$$x_2' = \alpha_2 \kappa + \frac{(\mu - 1) k_p K_2}{d_T} \Upsilon_{T_2^*} x_2^* - x_2 \; , \qquad (A.9b)$$

since $\alpha_1, \; \alpha_2 \; \ll \; 1$ we select

$$\Upsilon_{T_1^*} = \frac{d_T}{(\mu - 1) k_p K_1} \; ; \; \Upsilon_{T_2^*} = \frac{d_T}{(\mu - 1) k_p K_2} \; . \qquad (A.9c,d)$$

Therefore [eqn (A.4)-(A.8)]

$$\Upsilon_{IFN} = \frac{d_T p_{IFN}}{(\mu - 1) d_{IFN} k_p K_1} \; ; \; \Upsilon_{IL_{10}} = \frac{d_T p_{IL_{10}}}{(\mu - 1) d_{IL_{10}} k_p K_2} \; ; \; \Upsilon_S = \frac{d_T (k_p + k_r)}{(\mu - 1) k_a k_p K_1} \; ;$$

$$\Upsilon_{Ag} = \frac{d_T (k_p + k_r)}{(\mu - 1) k_a k_p K_1} \frac{d_C}{nbC_p} \; ; \; \Upsilon_{T_1^{*ns}} = \frac{d_T}{(\mu - 1) k_p K_1} \; ; \; \Upsilon_{T_2^{*np}} = \frac{d_T}{(\mu - 1) k_p K_2} \; . \qquad (A.10)$$

Appendix B. Paracrine effects of IL–2 and IL–4

In formulating our model in section 4 we assumed that the ambient growth factor (IL–2 and IL–4) concentration is too low to permit proliferation of IL–10–inhibited Th1 cells, T_1^{*ns}. Here we examine the effects of that assumption by modeling an extreme case in which the ambient growth factor concentration supports maximum proliferation of the T_1^{*ns} cells. Thus, as discussed for T_1^* cells in section 4, the proliferation rate of T_1^{*ns} cells is assumed to be $k_p T_1^{*ns}$.

In adopting the probably unrealistic assumption, of maximal proliferation of the non-secreting T_1^{*ns} cells, we are motivated by the following considerations. Details of *in vivo* paracrine growth factor utilization are not well understood at present. In fact, intensive investigation and modeling of the effects of IL–2 and IL–4 in Th1/Th2 cross–regulation is currently in progress (Morel *et al.*, 1993) Here we wish to see if paracrine proliferation of T_1^{*ns} cells changes the basic characteristics of our model. Toward that end it is most efficient to consider an extreme case.

For reasons of mathematical simplicity, we assume $k_{r_{ns}} = k_{r_{np}} = k_r$ and $k_{a_1} = k_{a_2} = k_a$. The system of equations is when

$$\frac{dS_0}{dt} = nbC_pAg - k_{b_1}S_0T_1 + (k_a + k_{d_1})S_1 - k_{b_2}S_0T_2 + (k_a + k_{d_2})S_2 - d_CS_0, \qquad (B.1a)$$

$$\frac{dS_1}{dt} = k_{b_1}S_0T_1 - (k_a + k_{d_1})S_1 - d_CS_1, \qquad (B.1b)$$

$$\frac{dS_2}{dt} = k_{b_2}S_0T_2 - (k_a + k_{d_2})S_2 - d_CS_2, \qquad (B.1c)$$

$$\frac{dT_1}{dt} = a_1 - d_TT_1 - k_{b_1}S_0T_1 + k_{d_1}S_1 + (\mu k_p + k_r)(T_1^* + T_1^{*ns}), \qquad (B.1d)$$

$$\frac{dT_2}{dt} = a_2 - d_TT_2 - k_{b_2}S_0T_2 + k_{d_2}S_2 + (\mu k_p + k_r)T_2^* + k_rT_2^{*np}, \qquad (B.1e)$$

$$\frac{dT_1^*}{dt} = k_aS_1 - \left(k_p + k_r + k_{ns}\frac{IL_{10}}{K_{IL_{10}} + IL_{10}}\right)T_1^*\ , \qquad (B.1f)$$

$$\frac{dT_2^*}{dt} = k_aS_2 - \left(k_p + k_r + k_{np}\frac{IFN}{K_{IFN} + IFN}\right)T_2^*, \qquad (B.1g)$$

$$\frac{dT_1^{*ns}}{dt} = k_{ns}\frac{IL_{10}}{K_{IL_{10}} + IL_{10}}T_1^* - (k_p + k_r)T_1^{*ns}, \tag{B.1h}$$

$$\frac{dT_2^{*np}}{dt} = k_{np}\frac{IFN}{K_{IFN} + IFN}T_2^* - k_r T_2^{*np}, \tag{B.1i}$$

$$\frac{dIFN}{dt} = p_{IFN}T_1^* - d_{IFN}IFN, \tag{B.1j}$$

$$\frac{dIL_{10}}{dt} = p_{IL_{10}}(T_2^* + T_2^{*np}) - d_{IL_{10}}IL_{10}, \tag{B.1k}$$

$$\frac{dAg}{dt} = \left[q - e_1 T_1^* - e_2(T_2^* + T_2^{*np})\right]Ag, \tag{B.1l}$$

Here the variables and the parameters are as described in section 4. Scaling, as described in section 5, yields

$$\epsilon s_0' = \epsilon\sigma(g - s_0) - (s_0 x_1 - s_1) - \Lambda\kappa^{-1}(s_0 x_2 - s_2), \tag{B.2a}$$

$$\epsilon s_1' = (s_0 x_1 - s_1) - \epsilon\sigma s_1, \tag{B.2b}$$

$$\epsilon s_2' = \Lambda\kappa^{-1}(s_0 x_2 - s_2) - \epsilon\sigma s_2, \tag{B.2c}$$

$$x_1' = \alpha_1 + (x_1^* + x_1^{*ns} - x_1) - \beta\chi(s_0 x_1 - s_1) - \frac{\chi}{\mu - 1}[s_1 - x_1^* - x_1^{*ns}], \tag{B.2d}$$

$$x_2' = \alpha_2\kappa + (x_2^* - x_2) - \beta\Lambda\chi(s_0 x_2 - s_2) - \frac{\chi}{\mu - 1}[\kappa s_2 - x_2^* - \frac{\chi - 1}{\chi}x_2^{*np}], \tag{B.2e}$$

$$\epsilon x_1^{*\prime} = \gamma\left[s_1 - (1 + \xi\frac{l_{IL_{10}}}{\Omega\phi\kappa + l_{IL_{10}}})x_1^*\right], \tag{B.2f}$$

$$\epsilon x_2^{*\prime} = \gamma\left[\kappa s_2 - (1 + \zeta\frac{l_{IFN}}{\Omega + l_{IFN}})x_2^*\right], \tag{B.2g}$$

$$\epsilon x_1^{*ns\prime} = \gamma\left[\xi\frac{l_{IL_{10}}}{\Omega\phi\kappa + l_{IL_{10}}}x_1^* - x_1^{*ns}\right], \tag{B.2h}$$

$$\epsilon x_2^{*np\prime} = \gamma\left[\zeta\frac{l_{IFN}}{\Omega + l_{IFN}}x_2^* - \frac{\chi - 1}{\chi}x_2^{*np}\right], \tag{B.2i}$$

$$\epsilon l_{IFN}' = \delta_{IFN}(x_1^* - l_{IFN}), \tag{B.2j}$$

$$\epsilon l_{IL_{10}}' = \delta_{IL_{10}}\left[(x_2^* + x_2^{*np}) - l_{IL_{10}}\right], \tag{B.2k}$$

$$g' = \pi\left[\theta - x_1^* - \eta\kappa^{-1}(x_2^* + x_2^{*np})\right]g, \tag{B.2l}$$

Separation of time scales yields

$$s' = \sigma(g - s) = f_1(s, x_1, x_2, g) \,, \tag{B.3a}$$

$$x_1' = \alpha_1 + \frac{s x_1}{1 + x_1 + x2} - x_1 = f_2(s, x_1, x_2, g) \,, \tag{B.3b}$$

$$x_2' = \alpha_2 \kappa + x_2^* - x_2 = f_3(s, x_1, x_2, g) \,, \tag{B.3c}$$

$$g' = \pi\big[\theta - x_1^* - \eta\kappa^{-1}(x_2^* + x_2^{*np})\big] g = f_4(s, x_1, x_2, g) \,. \tag{B.3d}$$

Here

$$x_1^* = \frac{\sqrt{q_1^2 + 4 q_0 q_2} - q_1}{2 q_0}, \tag{B.4a}$$

where

$$q_0 = (1 + \zeta)\Omega\phi + (1 + \xi)\big(1 + \frac{\zeta\chi}{\chi - 1}\big) s_2, \tag{B.4b}$$

$$q_1 = \Omega\big[\Omega\phi + (1 + \xi) s_2\big] - \big[(1 + \zeta)\Omega\phi + \big(1 + \frac{\zeta\chi}{\chi - 1}\big) s_2\big] s_1, \tag{B.4c}$$

$$q_2 = \Omega(\Omega\phi + s_2) s_1. \tag{B.4d}$$

Finally, x_2^* and x_2^{*np} are given by

$$x_2^* = \frac{(\Omega + x_1^*)\kappa s_2}{[\Omega + (1 + \zeta) x_1^*]} \;;\; x_2^{*np} = \frac{\zeta\chi}{\chi - 1} \frac{\kappa s_2 x_1^*}{[\Omega + (1 + \zeta) x_1^*]} \,, \tag{B.5a, b}$$

Equilibrium Points and their Stability

System (B.3) has four equilibrium points.

$$\widehat{E}_{00} = (s^{00}, x_1^{00}, x_2^{00}, g^{00}) = (0, \alpha_1, \alpha_2\kappa, 0) \,, \tag{B6.a}$$

$$\widehat{E}_{10} = (s^{10}, x_1^{10}, x_2^{10}, g^{10}) = (\theta + 1, \theta, 0, \theta + 1) \,, \tag{B6.b}$$

$$\widehat{E}_{01} = (s^{01}, x_1^{01}, x_2^{01}, g^{01}) = \frac{1}{\eta\kappa}\Big(\eta + \theta\kappa, 0, \theta\kappa^2, \eta + \theta\kappa\Big) \,, \tag{B6.c}$$

and

$$\widehat{E}_{11} = (s^{11}, x_1^{11}, x_2^{11}, g^{11}) = \Big(1 + U + V, U, V, 1 + U + V\Big) \,, \tag{B6.c}$$

Here

$$U = \frac{\kappa - 1}{1 + \zeta - \kappa}\Omega\left[1 + \xi\frac{[\Omega + (1+\zeta)\theta] - \kappa(\Omega + \theta)}{\eta\Omega\phi(1 + \zeta - \kappa) + [\Omega + (1+\zeta)\theta] - \kappa(\Omega + \theta)}\right] ,$$

and

$$V = \frac{\kappa}{\eta}\frac{\chi - 1}{\chi\kappa - 1}\frac{[\Omega + (1+\zeta)\theta] - \kappa(\Omega + \theta)}{1 + \zeta - \kappa} .$$

Notice that since $\chi = 1 + k_r/k_p > 1$ and $x_1^{*11} = (\kappa - 1)\Omega/(1 + \zeta - \kappa)$, $\widehat{E}_{11}$ is well defined only when

$$1 < \kappa < \frac{\Omega + (1+\zeta)\theta}{(\Omega + \theta)} = \kappa_U . \qquad (B.7)$$

Procceding as in section 5 we obtain the characteristic polynomials associated with $\widehat{E}_{00}$, $\widehat{E}_{10}$ and $\widehat{E}_{01}$.

$$\widehat{p}_{00}(\lambda) = (\lambda + \sigma)(\lambda + 1)^2(\lambda - \pi\theta) = p_{00}(\lambda) , \qquad (B.8a)$$

i.e., $\widehat{E}_{00}$ is stable whenever E_{00} is stable.

$$\widehat{p}_{10}(\lambda) = \left(\lambda + U_{10}\right)\left[\lambda^3 + \left(\sigma + \frac{\theta}{\theta + 1}\right)\lambda^2 + \left(\frac{\theta}{\theta + 1} + \pi\theta\right)\sigma\lambda + \pi\theta\sigma\right] = p_{10}(\lambda) , \qquad (B.8b)$$

i.e., $\widehat{E}_{10}$ is stable whenever E_{10} is stable.

Finally,

$$\widehat{p}_{01}(\lambda) = (\lambda + 1 - \kappa^{-1})\left[\lambda^3 + \left(\sigma + \frac{\theta\kappa}{\theta\kappa + \eta}\right)\lambda^2 + \left(\frac{\theta\kappa}{\theta\kappa + \eta} + \pi\theta\right)\sigma\lambda + \pi\theta\sigma\right] = \qquad (B.8c)$$

$$= \frac{(\lambda + 1 - \kappa^{-1})}{(\lambda + U_{01})}p_{01}(\lambda) .$$

Thus, similarly to E_{00}, $\widehat{E}_{00}$ is unstable to antigenic perturbations. $\widehat{E}_{10}$ is (asymptotically) stable when $\kappa < \kappa_U$, and $\widehat{E}_{01}$ is stable whenever $\kappa > 1$. Thus, both $\widehat{E}_{10}$ and $\widehat{E}_{01}$ are stable when $1 < \kappa < \kappa_U$.

Next we examine stability of $\widehat{E}_{11}$. To demonstrate instability of an equilibrium point it is sufficient to show that the associated characteristic polynomial has negative coefficients (cf. Willems, 1970). The determinant of the Jacobian of system (B.3) evaluated at $\widehat{E}_{11}$ (free term of the associated characteristic polynomial—$\widehat{p}_{11}(\lambda)$), denoted by D_{11}, is given by

$$D_{11} = -\frac{\pi\Omega\sigma(\kappa-1)}{\eta(1+\zeta-\kappa)^2\sqrt{D}}[\Omega+(1+\zeta)\theta-\kappa(\Omega+\theta)]R\,, \qquad (B.8d)$$

where $R = (1+\xi)[\Omega+(1+\zeta)\theta-\kappa(\Omega+\theta)]+\eta\Omega\phi(1+\zeta-\kappa)$, and $D = q_1^2+4q_0q_2$ as defined by eqn (B.4)-(B.6). Since by (B.7), $1 < \kappa < [\Omega+(1+\zeta)\theta]/(\Omega+\theta) < 1+\zeta$, $D_{11} < 0$. Thus $\widehat{E}_{11}$ is unstable.

Thus, the model still has four equilibrium points; the uninfected state ($\widehat{E}_{00}$) and the co–coexistence ($\widehat{E}_{11}$) states are unstable, whereas the two coexistence ($\widehat{E}_{10}$ and $\widehat{E}_{01}$) states are stable, similarly to system 8. Moreover, similarly to E_{11}, $\widehat{E}_{11}$ is defined for κ values, for which both $\widehat{E}_{10}$ and $\widehat{E}_{01}$ are stable.

By allowing an unrealistic effectiveness to paracrine proliferation, we have, in effect, abrogated the suppressive effect of T_2^* cells on T_1^* cells. Thus, Th1/Th2 cross–suppression is reduced to a one–directional suppression of T_2^* cells by T_1^* cells—leading to an overall domination of the Th1 mediated responses (Figure 12). In analytical terms, the domain of stability of the Th2 dominated coexistence equilibrium is shifted toward higher κ values. From $\kappa_L < \kappa$ where

$$\kappa_L = \frac{\eta\Omega\phi+\theta}{\eta\Omega\phi+(1+\xi)\theta} < 1;\ \eta,\phi > 0$$

to $1 < \kappa$ (note, for η and ϕ of order 1, κ_L is of order 10^{-1}). This shift in domination, which occurs whenever exogenous growth factors are available to promote proliferation of IL–10 inhibited (non–secreting) activated Th1 cells, may be the underlying mechanism of the successful inhibition of the (Th2 dependent) graft–versus–host reaction by IL–2 injections (Sykes *et al.*, 1993).

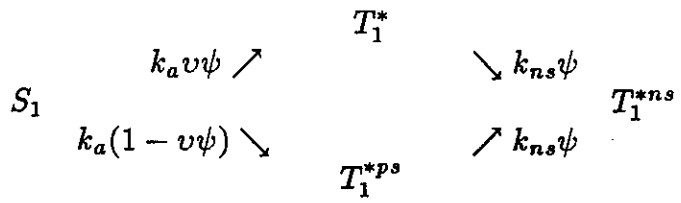
APPENDIX C: Inhibition of DC–dependent Th1 activation by IL–10

Let us denote by T_1^{*ps} the partially inhibited Th1* cells that proliferate, i.e., produce IL–2, but do not produce IFN–γ, and thus do not contribute to the elimination of antigen or Th2 cell inhibition.

We model IL–10 action on DC–dependent Th1 activation as follows. IL–10 acts on DC–Th1 cell complexes (Fiorentino *et al.*, 1989, 1991), represented by S_1 in our model. We shall assume that in the presence of IL–10 Th1 cells interacting with DC can differentiate into T_1^{*ps} cells, with probability $k_a v\psi$; $\psi = IL_{10}/(K_{DIL_{10}} + IL_{10})$, or into T_1^* cells, with probability $k_a(1 - v\psi)$. Here $K_{DIL_{10}}$ is the half–saturation constant for IL–10 effects on DC dependent Th1 cell activation. Note, $0 < v < 1$, [$v \approx 0.55 - 0.85$ (Macatonia *et al.*, 1993)] so the expressions above are well defined.

The possibility that T_1^{*ps} cells may be further inhibited by IL–10 into non–secretion of IL–2, similar to the inhibition of T_1^* can not be ruled out and we include it in the model below (scheme 2), in which we assume $K_{DIL_{10}} = K_{IL_{10}}$. We also follow appendix B and assume $k_{r_{ns}} = k_{r_{np}} = k_r$.

Scheme 2



The system of equations that describe this model is

$$\frac{dS_0}{dt} = nbC_pAg - k_{b_1}S_0T_1 + (k_a + k_{d_1})S_1 - k_{b_2}S_0T_2 + (k_a + k_{d_2})S_2 - d_CS_0 \ , \qquad (C.1a)$$

$$\frac{dS_1}{dt} = k_{b_1}S_0T_1 - (k_a + k_{d_1})S_1 - d_CS_1 \ , \qquad (C.1b)$$

$$\frac{dS_2}{dt} = k_{b_2} S_0 T_2 - (k_a + k_{d_2}) S_2 - d_C S_2 \ , \qquad (C.1c)$$

$$\frac{dT_1}{dt} = a_1 - d_T T_1 - k_{b_1} S_0 T_1 + k_{d_1} S_1 + (\mu k_p + k_r)(T_1^* + T_1^{*ps}) + k_r T_1^{*ns} \ , \qquad (C.1d)$$

$$\frac{dT_2}{dt} = a_2 - d_T T_2 - k_{b_2} S_0 T_2 + k_{d_2} S_2 + (\mu k_p + k_r) T_2^* + k_r T_2^{*np} \ , \qquad (C.1e)$$

$$\frac{dT_1^*}{dt} = k_a (1 - \upsilon \frac{IL_{10}}{K_{IL_{10}} + IL_{10}}) S_1 - \left(k_p + k_r + k_{ns} \frac{IL_{10}}{K_{IL_{10}} + IL_{10}} \right) T_1^* \ , \qquad (C.1f)$$

$$\frac{dT_2^*}{dt} = k_a S_2 - \left(k_p + k_r + k_{np} \frac{IFN}{K_{IFN} + IFN} \right) T_2^* \ , \qquad (C.1g)$$

$$\frac{dT_1^{*ps}}{dt} = k_a \upsilon \frac{IL_{10}}{K_{IL_{10}} + IL_{10}} S_1 - \left(k_p + k_r + k_{ns} \frac{IL_{10}}{K_{IL_{10}} + IL_{10}} \right) T_1^{*ps} \ , \qquad (C.1h)$$

$$\frac{dT_1^{*ns}}{dt} = k_{ns} \frac{IL_{10}}{K_{IL_{10}} + IL_{10}} (T_1^* + T_1^{*ps}) - k_r T_1^{*ns} \ , \qquad (C.1i)$$

$$\frac{dT_2^{*np}}{dt} = k_{np} \frac{IFN}{K_{IFN} + IFN} T_2^* - k_r T_2^{*np} \ , \qquad (C.1j)$$

$$\frac{dIFN}{dt} = p_{IFN} T_1^* - d_{IFN} IFN \ , \qquad (C.1l)$$

$$\frac{dIL_{10}}{dt} = p_{IL_{10}} (T_2^* + T_2^{*np}) - d_{IL_{10}} IL_{10} \ , \qquad (C.1l)$$

$$\frac{dAg}{dt} = \left[q - e_1 T_1^* - e_2 (T_2^* + T_2^{*np}) \right] Ag \ , \qquad (C.1m)$$

Scaling and separating the time scales as in section 4 (the scale for T_1^{*ps} is chosen equal to scale of T_1^*) yields

$$\epsilon s' = \sigma (g - s) \ , \qquad (C.2a)$$

$$x_1' = \alpha_1 + x_1^* + x_1^{*ps} - x_1 \ , \qquad (C.2b)$$

$$x_2' = \alpha_2 \kappa + x_2^* - x_2 \ , \qquad (C.2c)$$

$$g' = \pi [\theta - x_1^* - \eta \kappa^{-1} (x_2^* + x_2^{*np})] \ . \qquad (C.2d)$$

Where

$$x_1^{*ps} = \frac{\upsilon G s_1 s_2}{\Omega \phi F + (1 + \xi) V_3 s_2} \ ; \ x_2^* = \frac{E}{F} \kappa s_2 \ ; \ \frac{\zeta \chi}{\chi - 1} \frac{x_1^*}{F} \kappa s_2 \ , \qquad (C.3a, b, c)$$

here

$$E = \Omega + x_1^* \ ; \ F = \Omega + (1+\zeta)x_1^* \ ; \ G = \Omega + \frac{\zeta\chi}{\chi - 1}x_1^* \ .$$

Finally, x_1^* is the real positive solution of

$$r_0 x_1^{*^2} + r_1 x_1^* - r_2 = 0, \qquad (C.4a)$$

where

$$r_0 = (1+\zeta)\Omega\phi + (1+\xi)\big(1 + \frac{\zeta\chi}{\chi - 1}\big)s_2, \qquad (C.4b)$$

$$r_1 = \Omega\big[\Omega\phi + (1+\xi)s_2\big] - \big[(1+\zeta)\Omega\phi + (1-\upsilon)\big(1 + \frac{\zeta\chi}{\chi - 1}\big)s_2\big]s_1, \qquad (C.4c)$$

$$r_2 = \Omega[\Omega\phi + (1-\upsilon)s_2]s_1. \qquad (C.4d)$$

It is straightforward to verify that system (C2) has four steady state solutions. E_{00}, E_{10} and E_{01} remain unchanged and retain their stability properties. The fourth equilibrium point—$\hat{E}_{11}$, corresponding to E_{11} of system (8) is given by

$$\hat{E}_{11} = (\hat{s}, \hat{x}_1, \hat{x}_2, \hat{g}) = \left(W, \frac{R\hat{x}_1^*}{Q}, \frac{\kappa EH}{\eta G}, W\right) . \qquad (C5a)$$

where

$$R = \kappa E[\eta\Omega\phi + (1+\xi)H] \ ; \ Q = R - \upsilon FH \ ; \ W = \frac{F}{\kappa E}\left(1 + \frac{R\hat{x}_1^*}{Q}\right) + \frac{FH}{\eta G} \ . \qquad (C5b)$$

here $H = \theta - \hat{x}_1^*$, and $\hat{x}_1^*$ is given by

$$\hat{x}_1^* = \frac{-p_1 - \sqrt{p_1^2 - 4p_0p_2}}{2p_0} \ ,$$

with

$$p_0 = (1+\zeta) - (1+\xi)\kappa \ ,$$

$$p_1 = [(1+\xi)(\theta - \Omega) + \eta\Omega\phi]\kappa - [(1+\zeta)(\theta + \eta\Omega\phi) - \Omega] \ ,$$

$$p_2 = \Omega\big[[(1+\xi)\theta + \eta\Omega\phi]\kappa - (\theta + \eta\Omega\phi)\big] \ .$$

It is straightforward to verify that (i) $0 < \hat{x}_1^* < \theta$ if and only if $\kappa_L < \kappa < \kappa_U$, (ii) $\hat{s}, \hat{x}_1, \hat{x}_2, \hat{g} \geq 0$ if $0 < \hat{x}_1^* < \theta$. Thus, $\hat{E}_{11}$ exists only when both E_{10} and E_{01} are stable.

Finally, numerical bifurcation analysis using the computer program AUTO (Doedel, 1981) [cf. Fig 3] indicates that $\hat{E}_{11}$ is an *unstable* equilibrium point that bifurcates from E_{01} at κ_L and merges with E_{10} at κ_U.

Thus, we see that inclusion of IL–10 mediated inhibition of the DC dependent activation of Th1 cells does not change the qualitative properties of our model. Figure 13 compares the outcomes predicted by eqn (C2) as κ and the initial antigen dose, $g(0)$, are varied, with the outcomes predicted by eqn (8). We see that the quantitative differences are confined to the high antigen dose domain of region–II. As discussed in section 7, antigen doses depicted in that region may kill the animal—a phenomenon outside the scope of this model.

FIGURE CAPTIONS

Fig. 1. A schematic illustration of interactions between Th1 and Th2 clones. APC precursors (Cp) detect antigen (Ag) and convert it into an immunogenic stimulus, which consists of peptide–MHC class II complexes presented on their membrane. These complexes are when presented to helper T cells (T_1 and T_2) by the APC (C). The APC–T cell interaction induces T cell activation (sometimes called sensitization). Activated Th1 cells (T_1^*) secrete IL–2 and IFN–γ (IL–2 and IFN). Activated Th2 cells (T_2^*) secrete IL–4 and IL–10 (IL–4 and IL–10). IL–10 inhibits production of cytokines by activated Th1 cells. The resulting, inhibited, non-secreting cells (T_1^{*ns}) cannot produce cytokines and thus, can not proliferate in absence of externally added growth factors. IFN–γ inhibits cytokine utilization by T_2^* cells—inducing cells that produce cytokines but can not utilize them. Thus, these cells are non-proliferative (T_2^{*np}). Activated cells, T_1^* and T_2^*, proliferate by utilizing endogenously produced growth factors (IL–2 or IL–4), and ultimately relax to the resting state. Inhibited cells, T_2^{*np} and T_1^{*ns}, relax to the resting state without proliferation.

Fig. 2. Projection of the stability domains of E_{10} and E_{01} onto the η–κ plane. $\alpha_1 = 10^{-4}$, $\alpha_2 = 10^{-4}$, $\pi = 2$, $\theta = 2$, $\sigma = 10$, $\xi = 9$, $\zeta = 1$, $\Omega = 1$, $\phi = 1$, $\nu = 3$. For these parameter values, E_{11} only exists in the intermediate parameter domain defined by $\kappa_L < \kappa < \kappa_U$, and as we show in Fig. 3 is unstable.

Fig. 3. Projections of the bifurcation in κ for system (8) onto (a) κ–x_1 plane, and (b) κ–x_2 plane. BP denotes a bifurcation point. A log scale is used for x_2 in (b). $\alpha_1 = 10^{-4}$, $\alpha_2 = 10^{-4}$, $\pi = 2$, $\theta = 2$, $\sigma = 10$, $\xi = 9$, $\zeta = 1$, $\Omega = 1$, $\eta = 1$, $\phi = 1$, $\nu = 3$.

Fig. 4. Dynamics of the system of equations (8) under antigenic perturbation. Time is scaled in terms of the T cell life–span, so that one unit $\simeq$ 50 d. $\alpha_1 = 10^{-4}$, $\alpha_2 = 10^{-4}$, $\pi = 2$, $\theta = 2$, $\sigma = 10$, $\xi = 9$, $\zeta = 1$, $\eta = 1$, $\Omega = 1$, $\phi = 1$, κ and g(0) are varied. (a) Elimination of the invading pathogens (convergence to E_{00}); $\kappa = 0.15$, $g(0) = 110$. (b) Convergence to the Th1 dominated coexistence (E_{10}); $\kappa = 0.15$, $g(0) = 10$. (c) Convergence to the Th2 dominated coexistence (E_{01}); $\kappa = 0.75$, $g(0) = 10$.

Fig. 5. Domains of the outcomes E_{00}, E_{10} *and* E_{01} in κ–g(0) space. $\alpha_1 = 10^{-4}$, $\alpha_2 = 10^{-4}$, $\pi = 2$, $\theta = 2$, $\sigma = 10, \xi = 9$, $\zeta = 1$, $\Omega = 1$, $\phi = 1$, $\eta = 1$, $\nu = 3$. Domain

separating lines represent the minimal (within the limits of precision) g(0) values at which transitions occur. Whenever two curves diverge from a single point, or converge to a point, that point is marked by a •.

Fig. 6. Maximum values of: x_1^*, Th1 cells' contribution to antigen–suppression; $\eta\kappa^{-1}(x_2^* + x_2^{*np})$, Th2 cells' contribution to antigen–suppression; and $x_1^* + \eta\kappa^{-1}(x_2^* + x_2^{*np})$, total suppression. $\alpha_1 = 10^{-4}$, $\alpha_2 = 10^{-4}$, $\pi = 2$, $\theta = 2$, $\sigma = 10$, $\xi = 9$, $\zeta = 1$, $\nu = 3$, $\Omega = 1$, $\eta = 1$, $\kappa = 0.27$.

Fig. 7. (a) Variation of the domains of attraction of E_{00}, E_{10} *and* E_{01} in κ–g(0) space with η, $\alpha_1 = 10^{-4}$, $\alpha_2 = 10^{-4}$, $\pi = 2$, $\theta = 2$, $\sigma = 10$, $\xi = 9$, $\zeta = 1$, $\Omega = 1$, $\phi = 1$, $\nu = 3$. For clarity of presentation the separation of the coexistence region into domains of E_{10} and E_{01} is omitted. (b) Comparison of the curves separating coexistence from the return to the uninfected state for $\nu = 21$ ($k_{r_{np}} = .1k_r$), $\nu = 3$ ($k_{r_{np}} = k_r$), and $\nu = 1.2$ ($k_{r_{np}} = 10k_r$). $\alpha_1 = 10^{-4}$, $\alpha_2 = 10^{-4}$, $\pi = 2$, $\theta = 2$, $\sigma = 10$, $\xi = 9$, $\zeta = 1$, $\Omega = 1$, $\phi = 1$, $\eta = 3$. (c) Comparison of the curves separating coexistence from the return to the uninfected state for three values of α_1 to α_2 (a_1 to a_2) ratio. $\pi = 2$, $\theta = 2$, $\sigma = 10$, $\xi = 9$, $\zeta = 1$, $\Omega = 1$, $\phi = 1$, $\nu = 3$ $\eta = 1$. Here we vary the *relative* magnitudes of α_1 and α_2 but keep the total influx ($\alpha_1 + \alpha_2 = 2 \cdot 10^{-4}$) constant.

Fig. 8. Perturbation of the stable coexistence states E_{10} and E_{01}. (a) Perturbation of E_{10} into E_{01} with antigen–specific Th2 cells. ($\eta = 0.1$). (b) Perturbation of E_{01} into E_{10} with antigen–specific Th1 cells ($\eta = 10.$). The solid lines in (a) and (b) indicate the *minimal* effective dose of T cell required to effect the transition. $\alpha_1 = 10^{-4}$, $\alpha_2 = 10^{-4}$, $\pi = 2$, $\theta = 2$, $\sigma = 10$, $\xi = 9$, $\zeta = 1$, $\nu = 3$, $\Omega = 1$.

Fig. 9. (a) Dynamics of convergence to E_{10}. (b) Dynamics of pathogens escape from immune control. $\alpha_1 = 10^{-4}$, $\alpha_2 = 10^{-4}$, $\pi = 2$, $\theta = 2$, $\sigma = 10$, $\xi = 9$, $\zeta = 1$, $\nu = 3$, $\Omega = 1$, $\kappa = 0.516$, $\eta = 0$.

Fig. 10. (a) Domains of attraction of E_{00}, E_{10} and escape from immune control in κ–g(0) space when $\eta = 0$, $\phi = 1$. (b) Domains of attraction of E_{00}, E_{10} and escape from immune control in κ–g(0) space for three ϕ values with $\eta = 0$. Other parameters are $\alpha_1 = 10^{-4}$, $\alpha_2 = 10^{-4}$, $\pi = 2$, $\theta = 2$, $\sigma = 10$, $\xi = 9$, $\zeta = 1$, $\nu = 3$, $\Omega = 1$.

Fig. 11. Dynamics of "immunization" with a low dose ($g(0) = 1$) of viable pathogens. $\alpha_1 = 10^{-4}$, $\alpha_2 = 10^{-4}$, $\pi = 2$, $\theta = 2$, $\sigma = 10$, $\xi = 9$, $\zeta = 1$, $\nu = 3$, $\Omega = 1$, $\kappa = 0.516$, $\eta = 0$. At $\tau = 2$, a high dose of pathogen ($g(0) = 100$) is given. This dose if given initially leads to antigen escape (Fig. 10a) but now is controlled.

Fig. 12. Domains of attraction of E_{00}, E_{10} *and* E_{01} in κ–g(0) space under assumption that the ambient growth factor concentration supports maximum proliferation of the T_1^{*ns} cells. $\alpha_1 = 10^{-4}$, $\alpha_2 = 10^{-4}$, $\pi = 2$, $\theta = 2$, $\sigma = 10, \xi = 9$, $\zeta = 1$, $\Omega = 1$, $\phi = 1, \eta = 1$, $\nu = 3$.

Fig 13. Comparison of the outcomes E_{00}, E_{10} and E_{01} predicted by eqn (8) and eqn (C2) in κ–g(0) space. $\alpha_1 = 10^{-4}$, $\alpha_2 = 10^{-4}$, $\pi = 2$, $\theta = 2$, $\sigma = 10, \xi = 9$, $\zeta = 1$, $\Omega = 1$, $\phi = 1$, $\eta = 1$, $\nu = 3$. Domain separating lines represent the minimal (within the limits of precision) g(0) values at which transitions occurs.

Table 1

Cytokine Secretion Phenotypes of Mouse T Cells*

	Th1	Th2
IL–2	++	–
IFN–γ	++	–
TNF	++	+
IL–4	–	++
IL–5	–	++
IL–6	–	++
IL–10	–	++

* Adapted from Mosmann & Moore (1991).

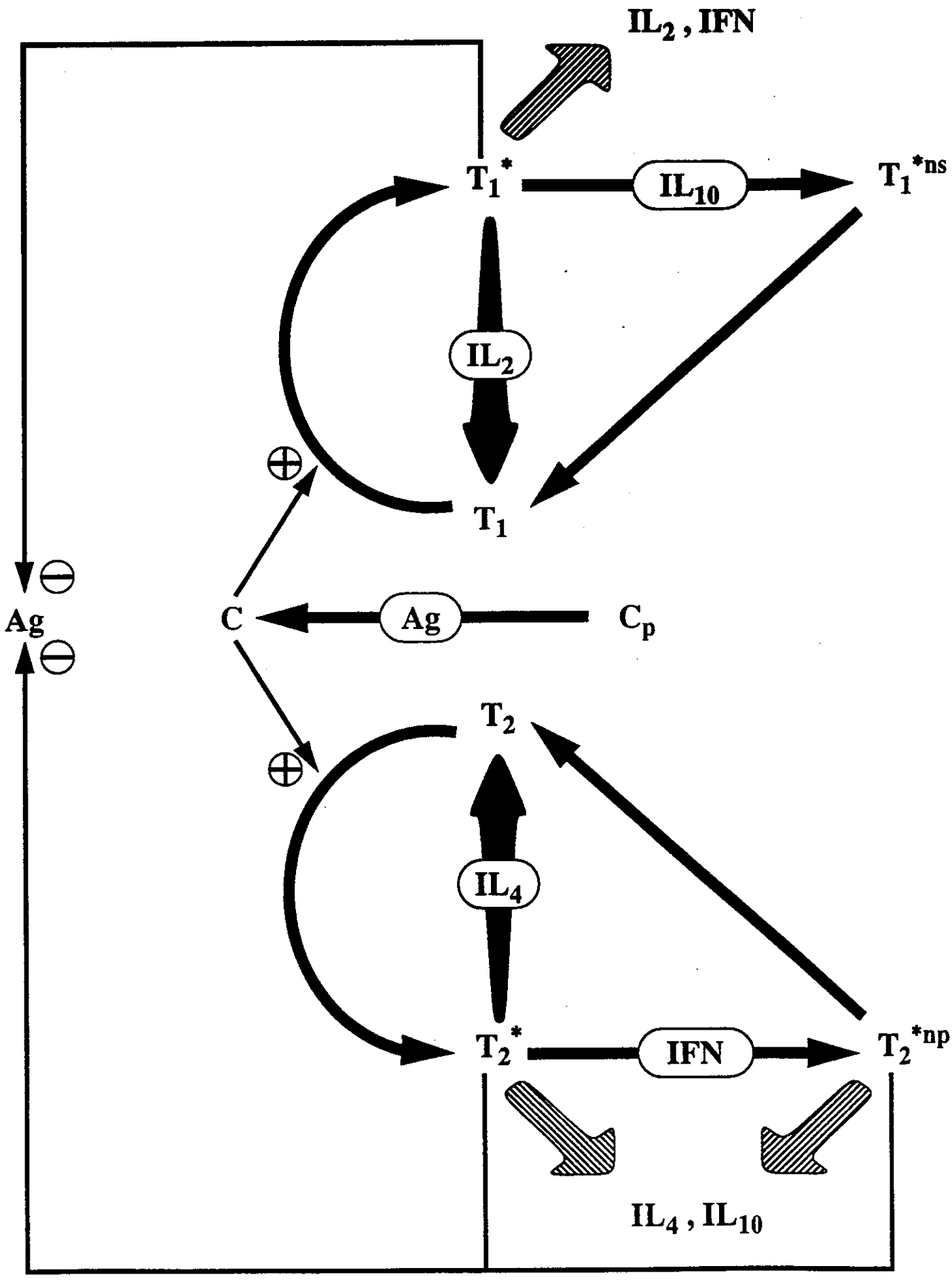


Figure 1

Figure 2

Stability in parameter space

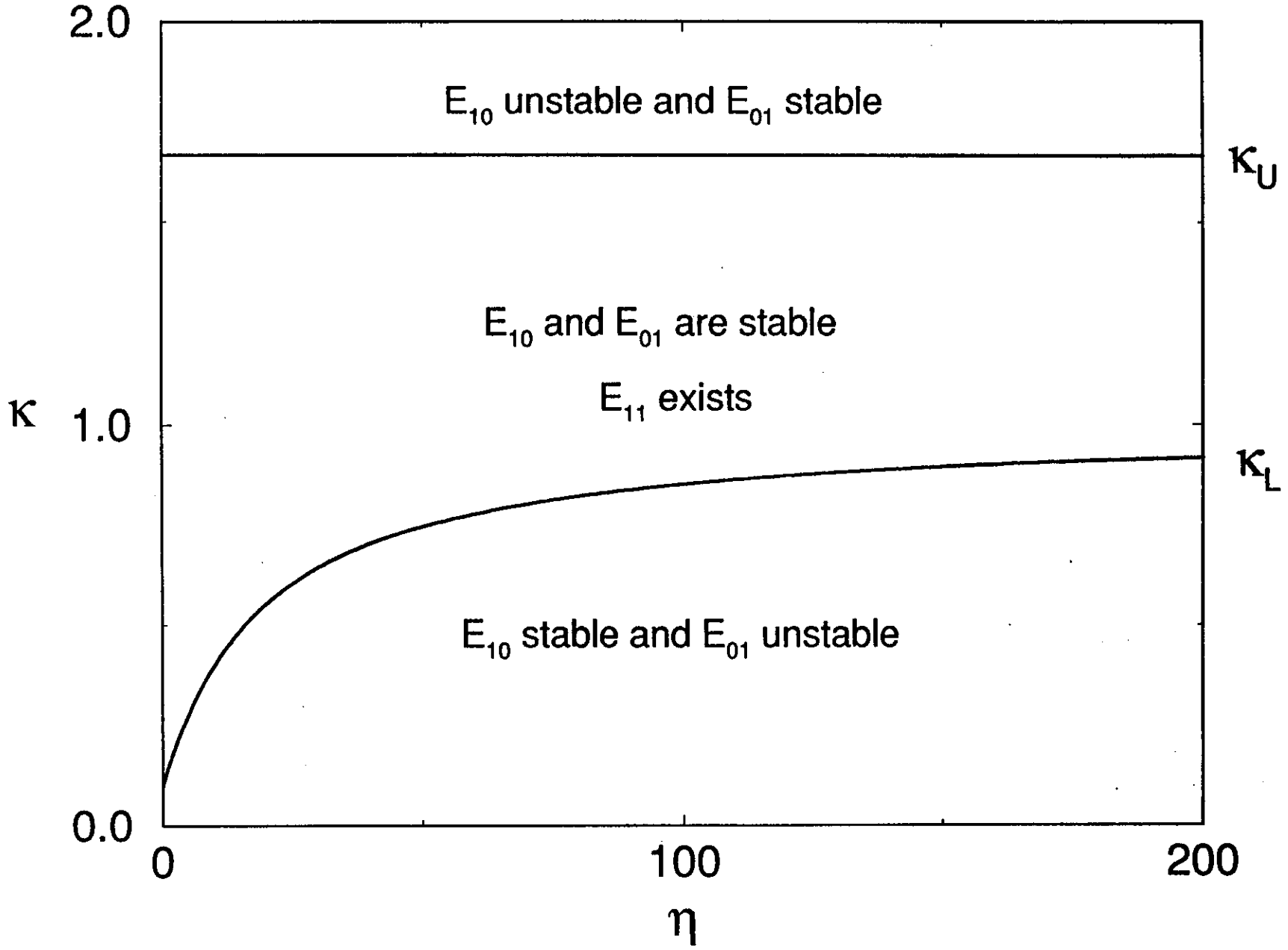


Figure 3a

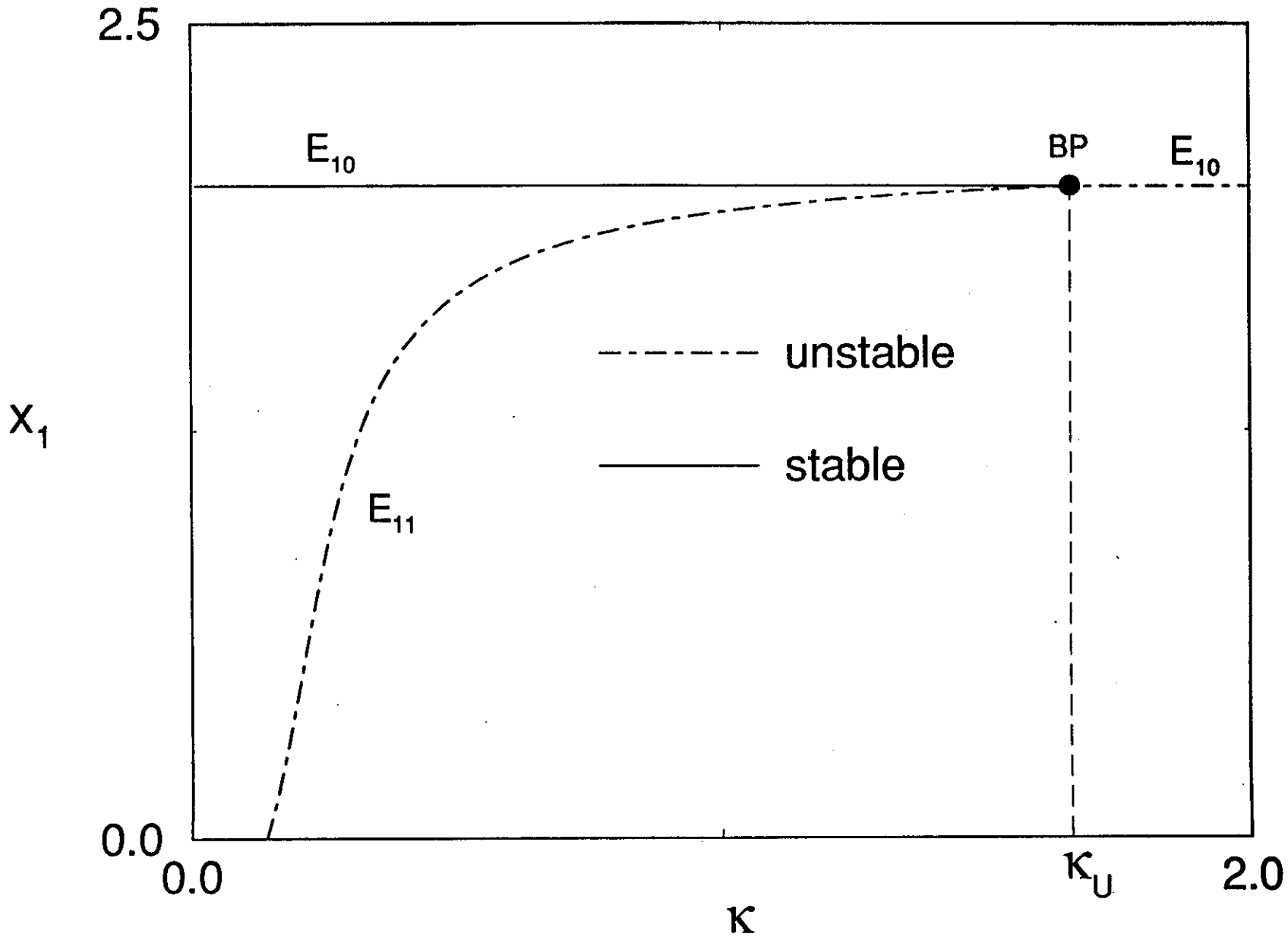


Figure 3b

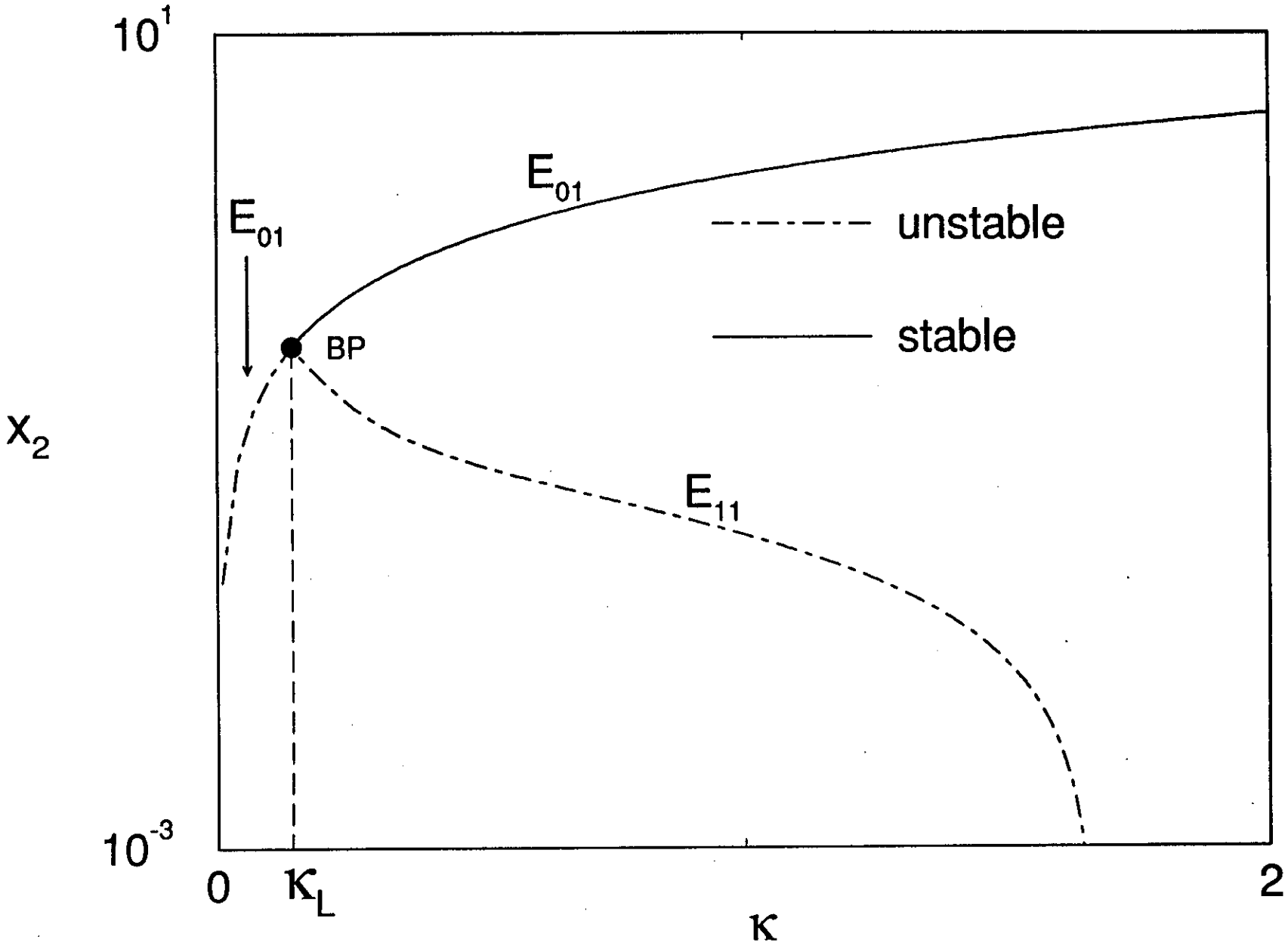


Figure 4a

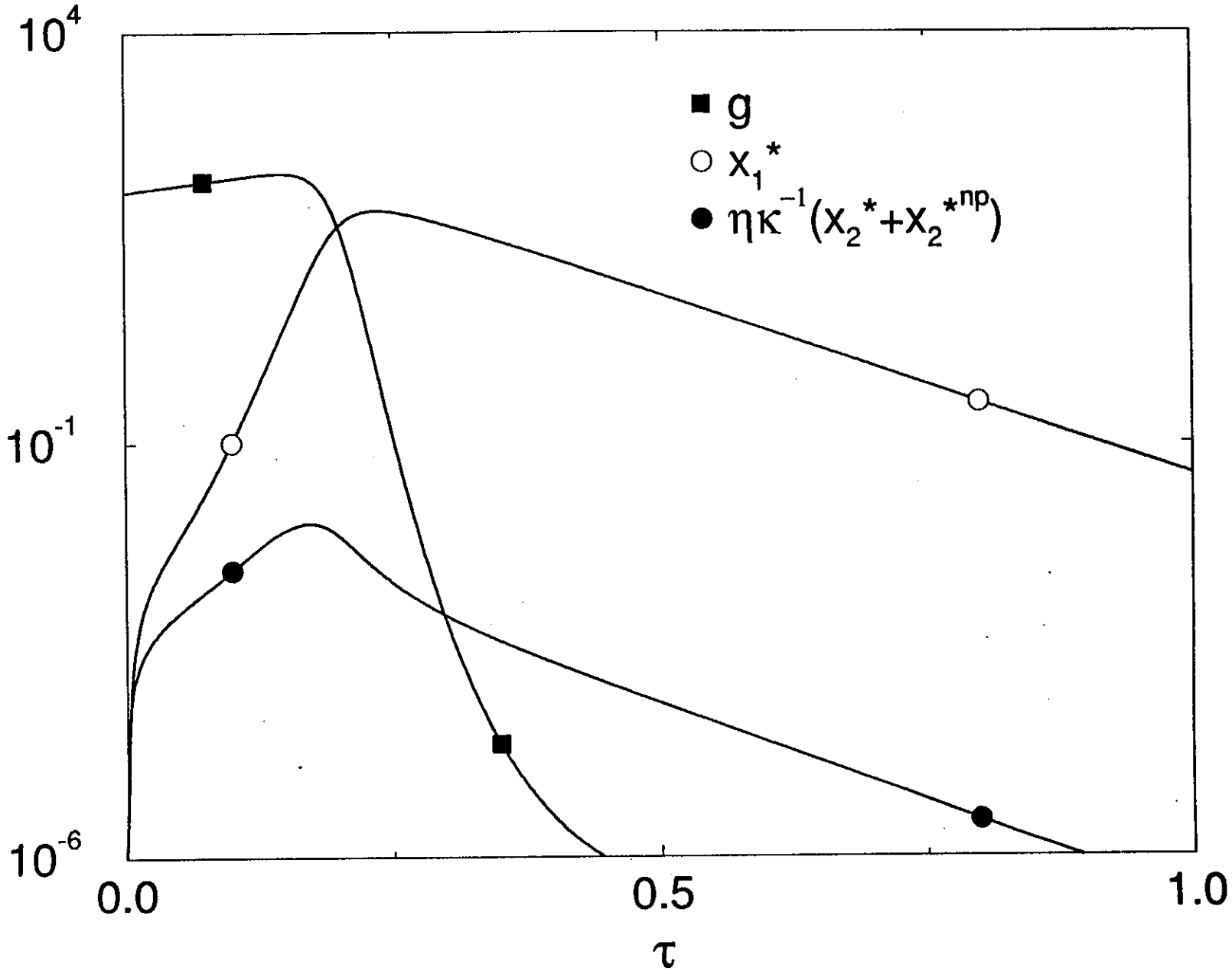


Figure 4b

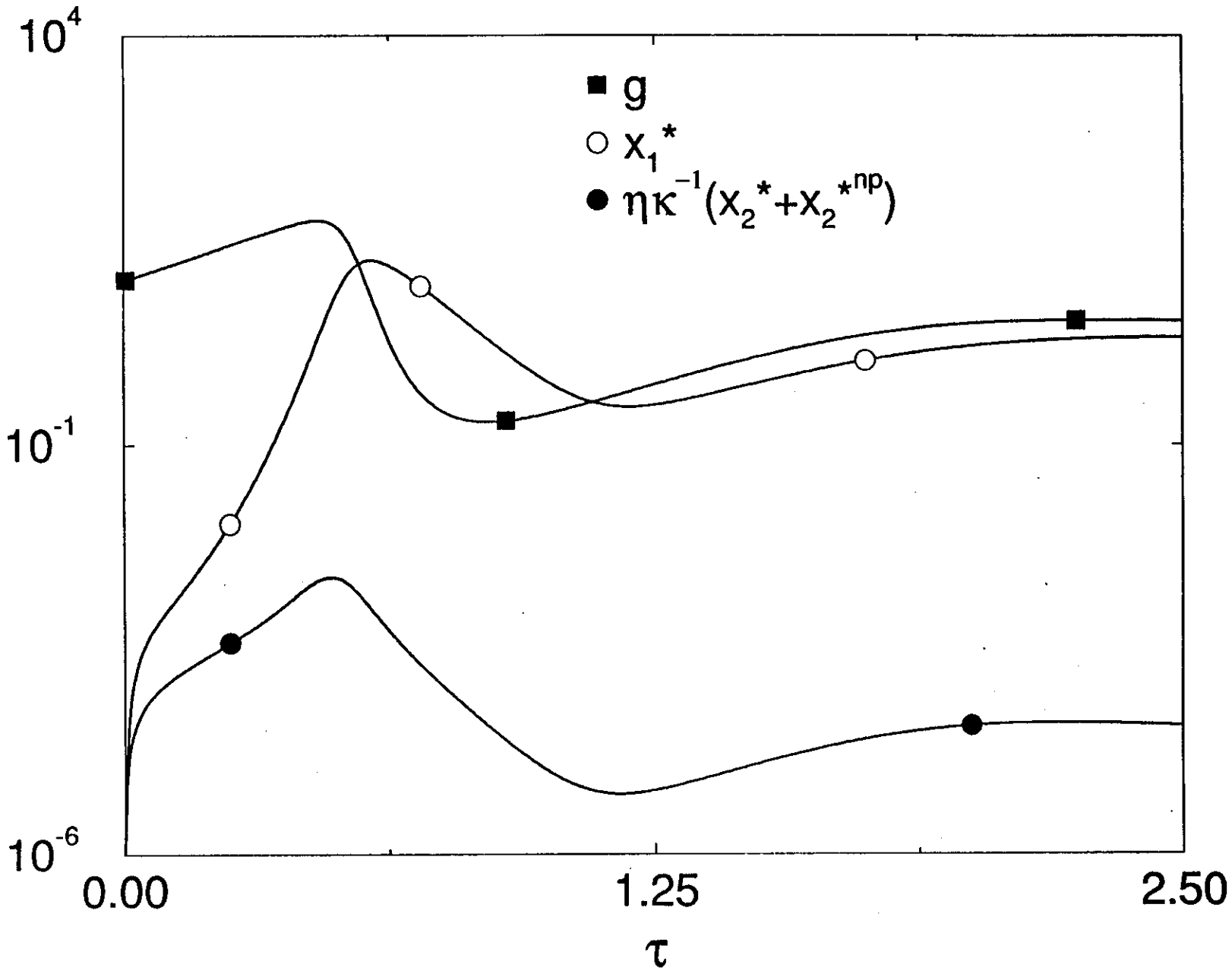


Figure 4c

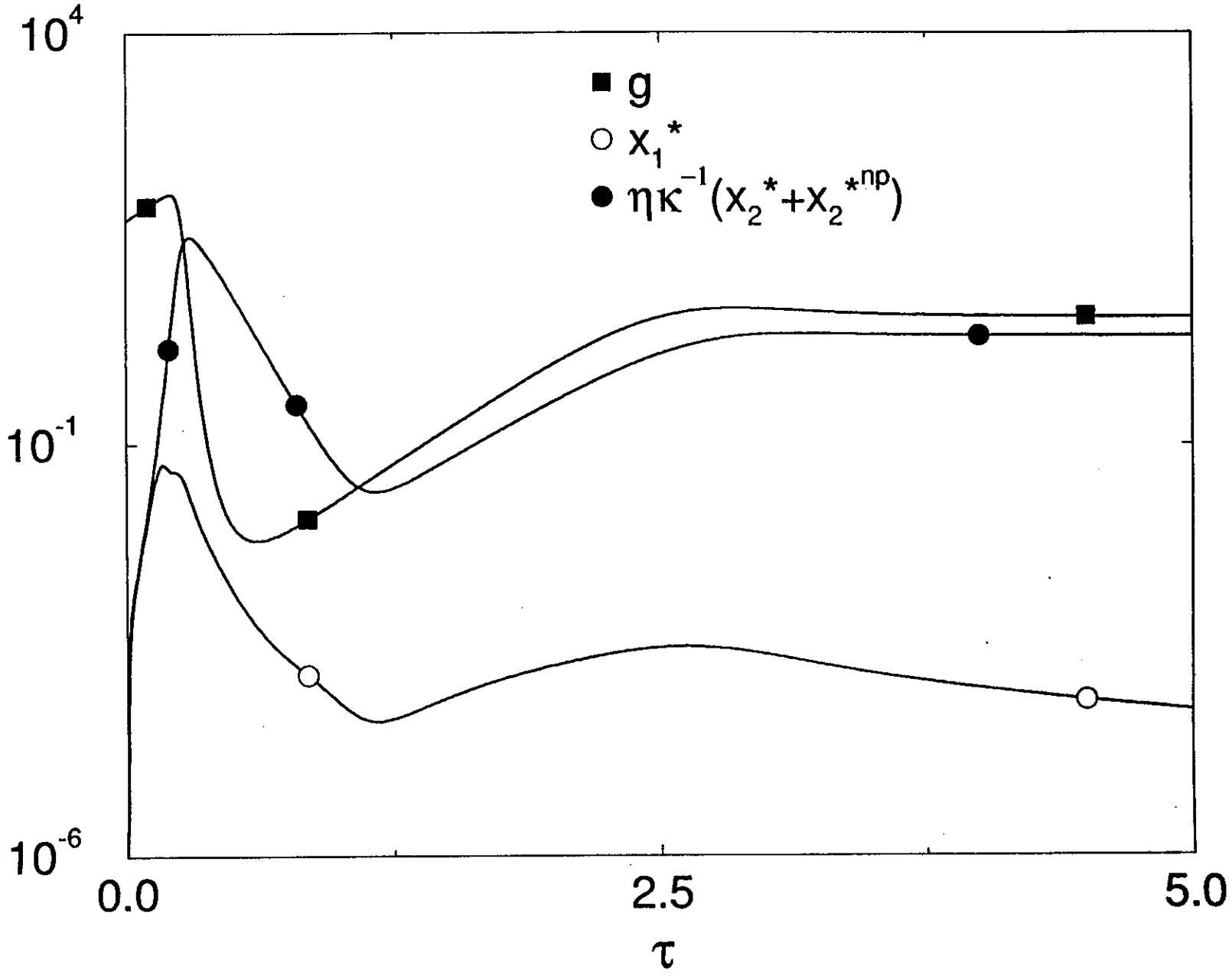


Figure 5

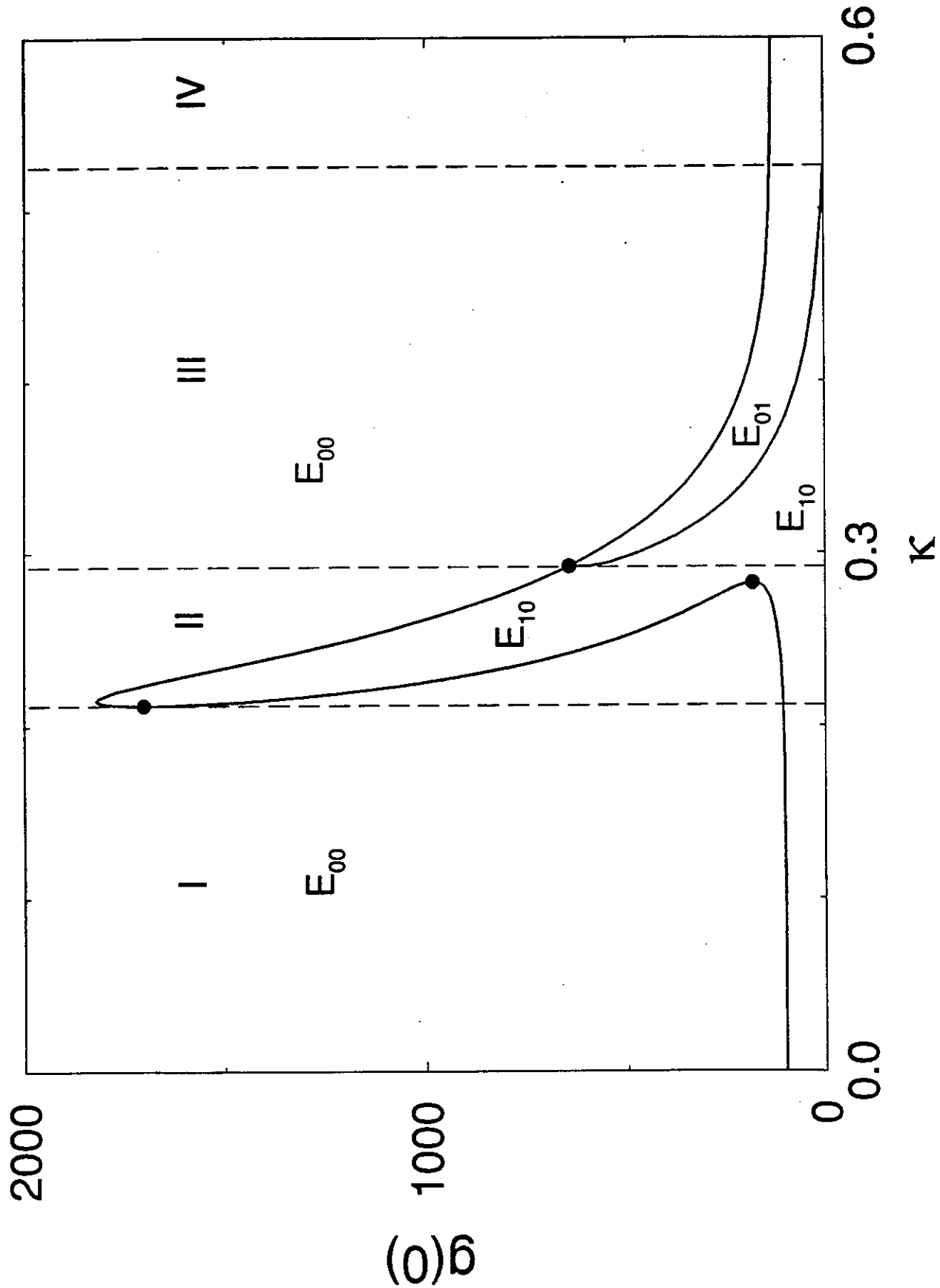


Figure 6

Contributions to suppression

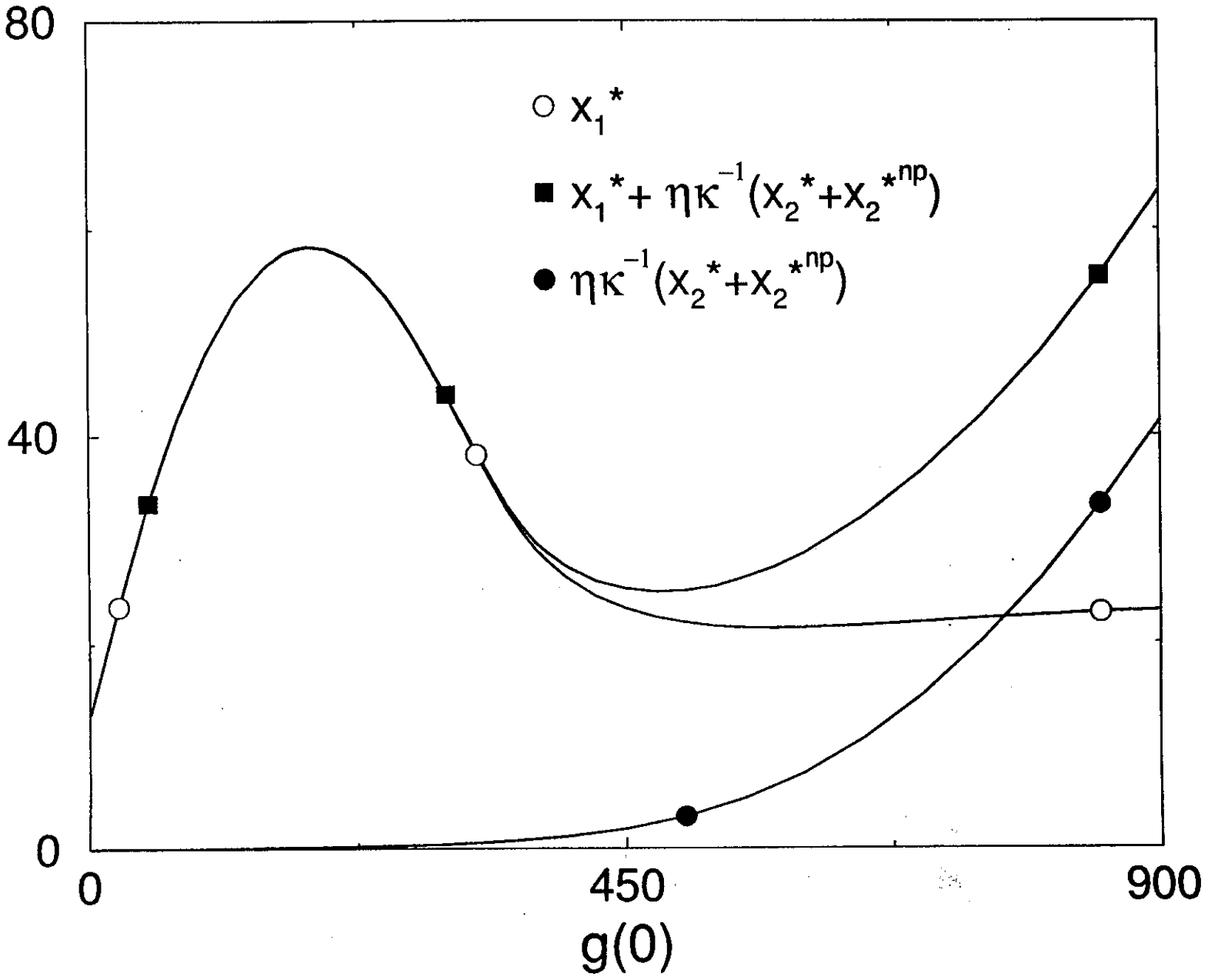


Figure 7a

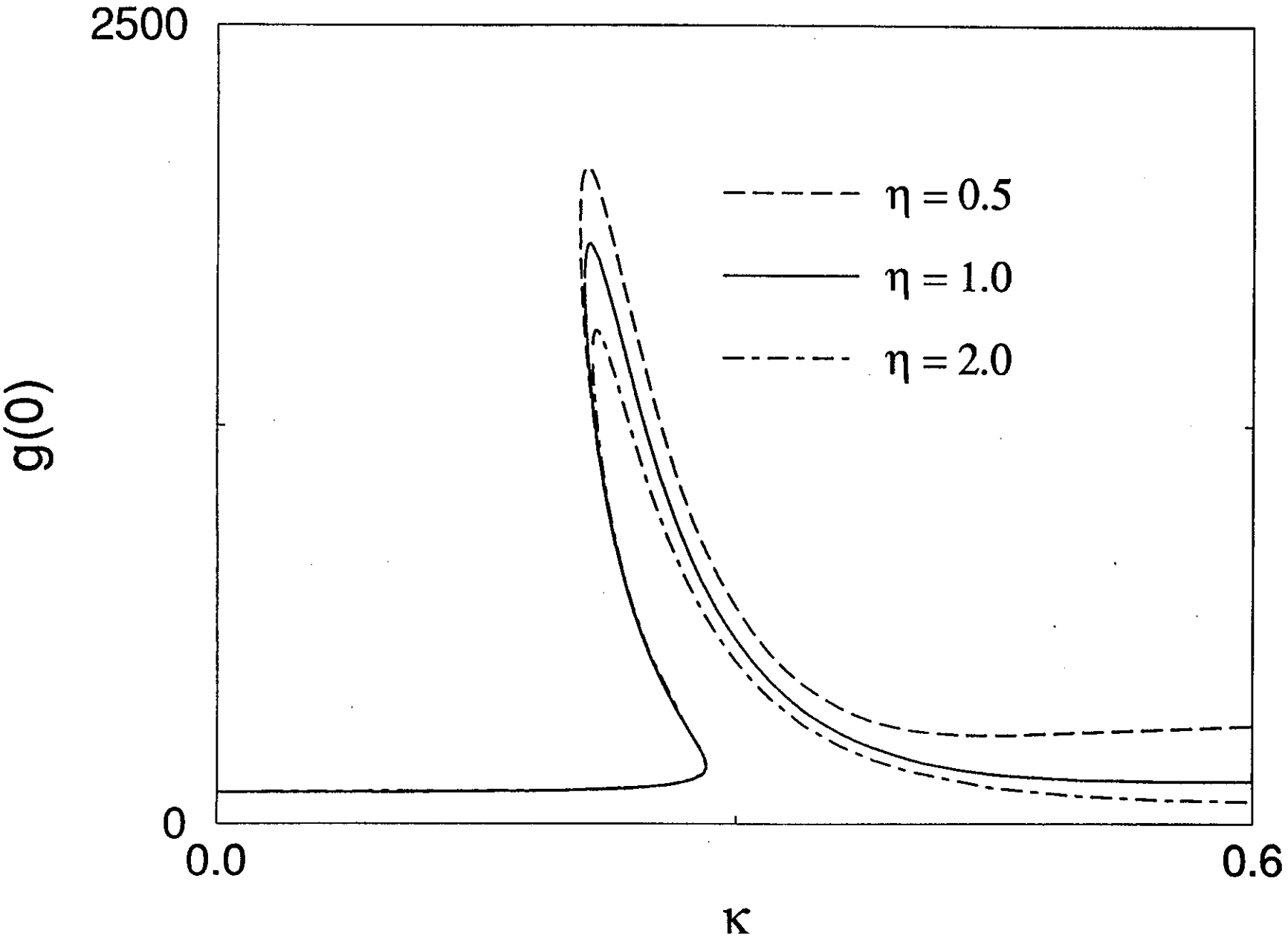


Figure 7b

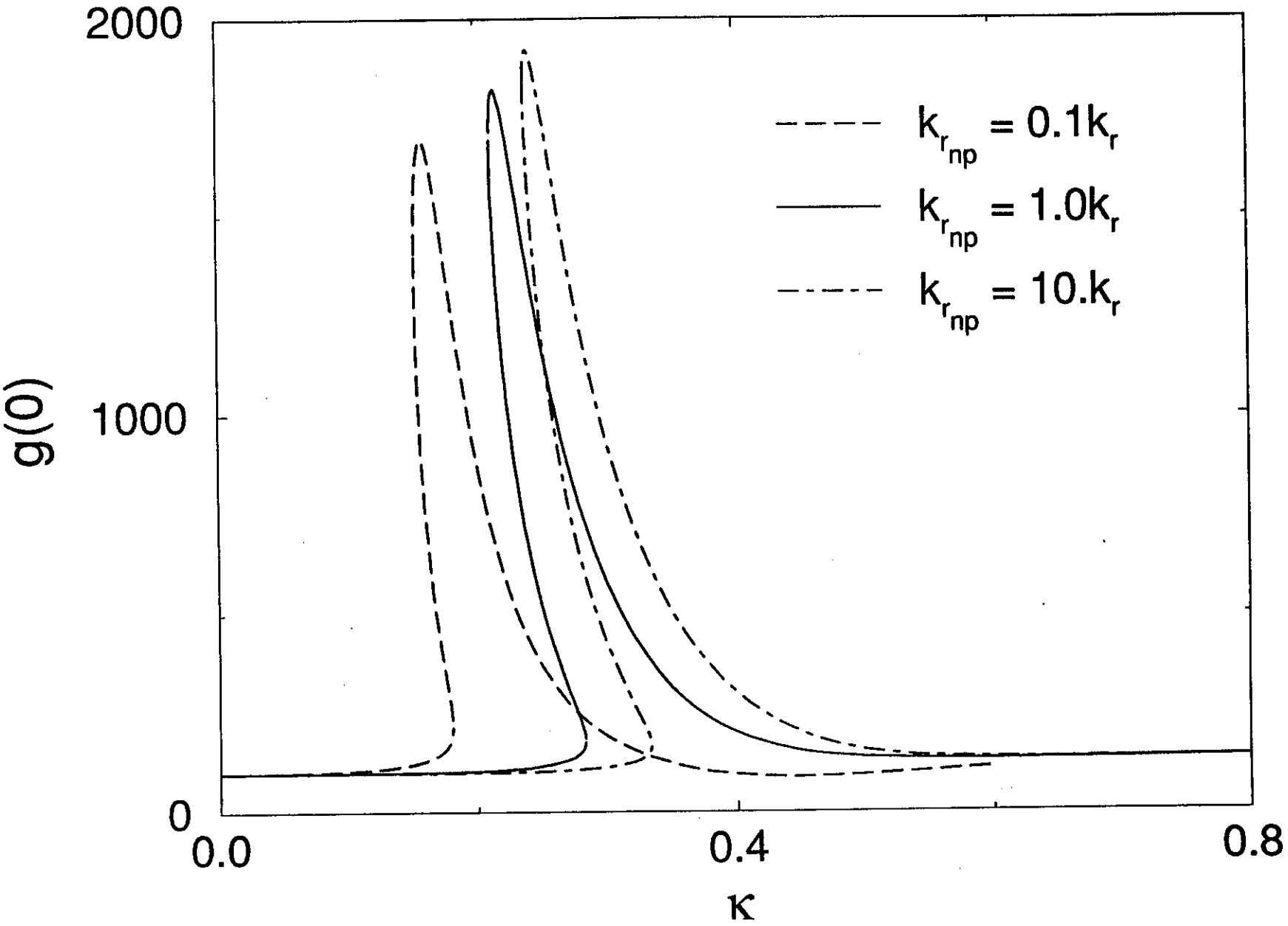


Figure 7c

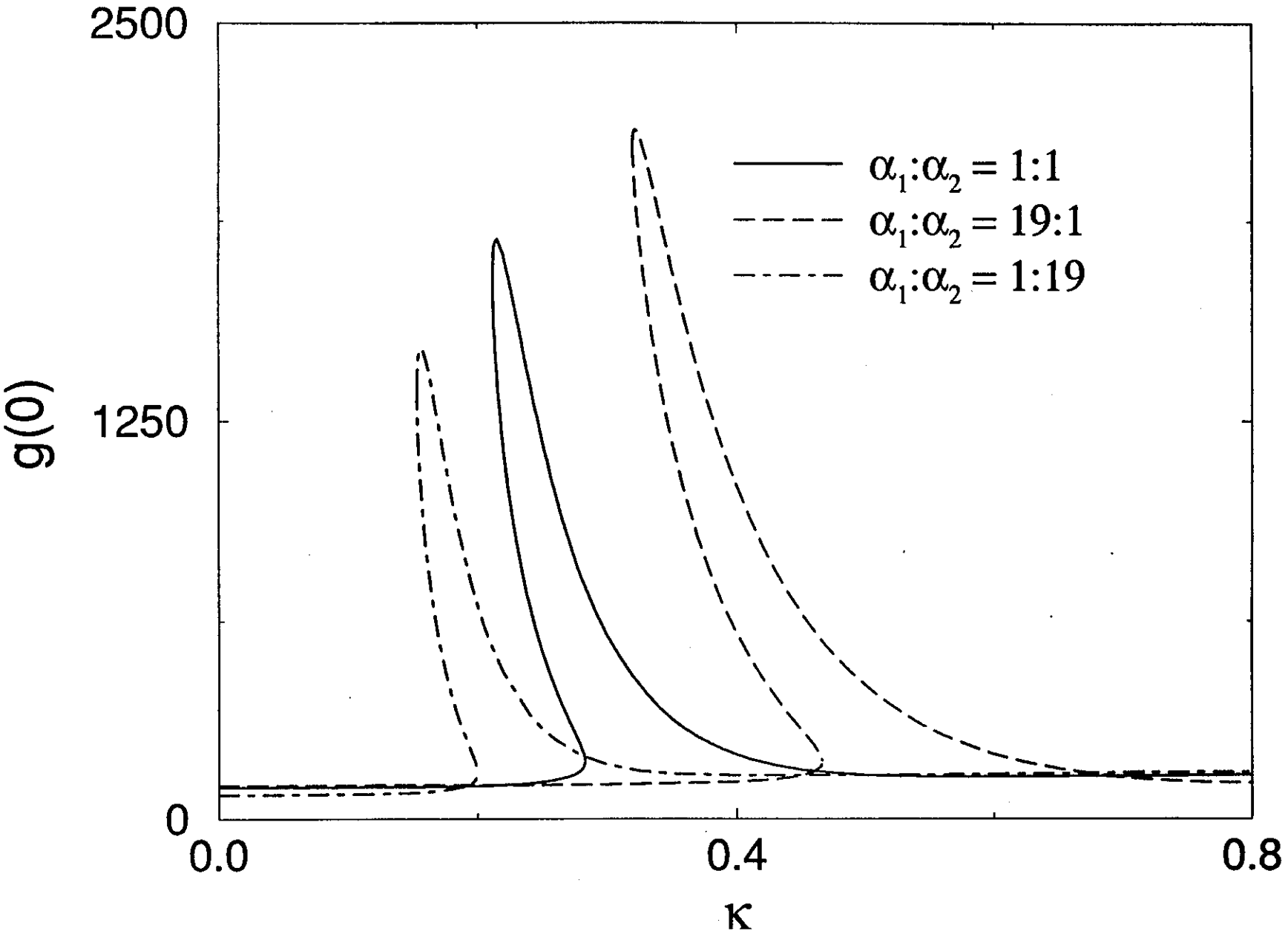


Figure 8a

Perturbations of E_{10}

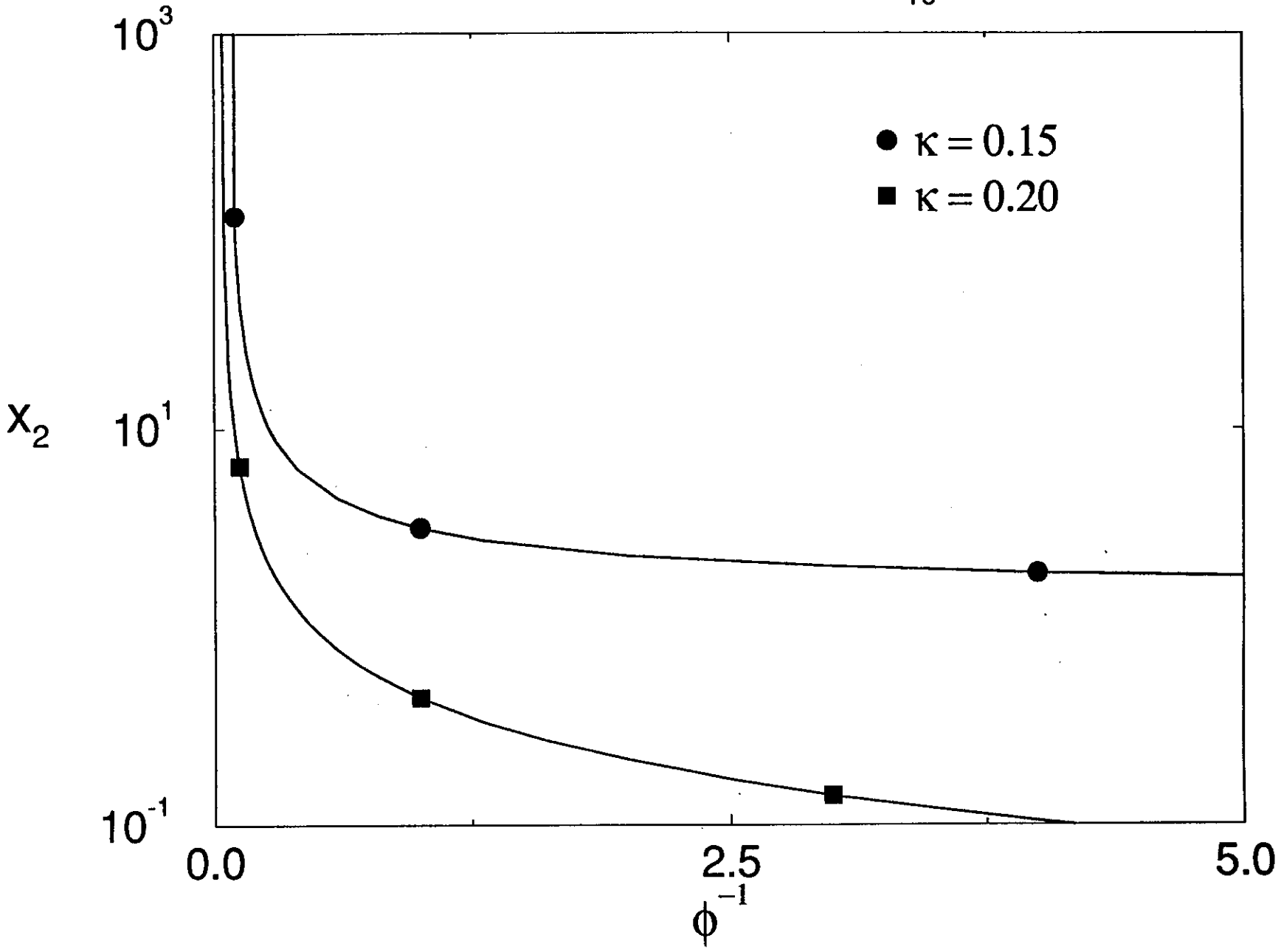


Figure 8b

Perturbations of E_{01} ($\eta = 10$)

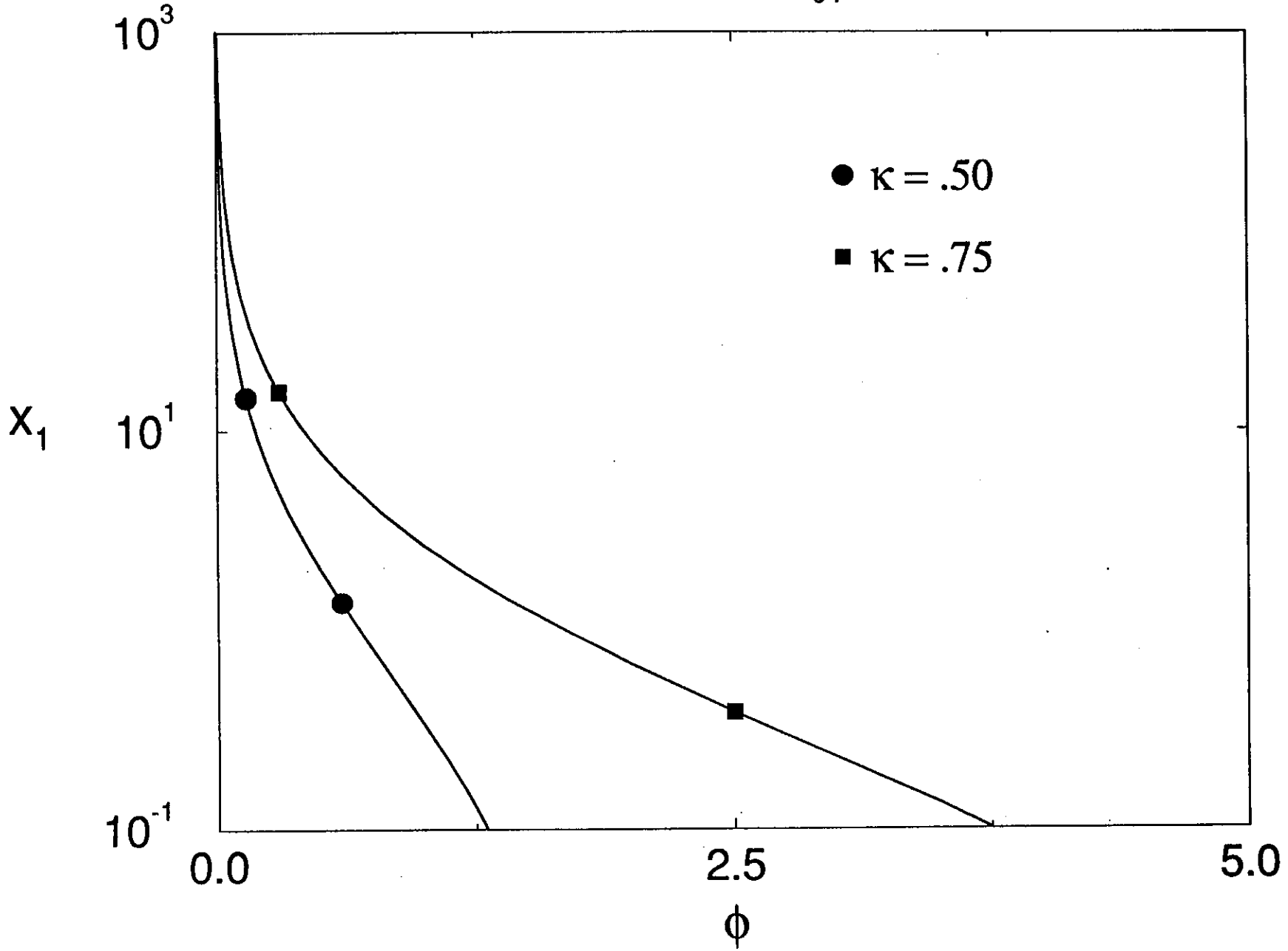


Figure 9a

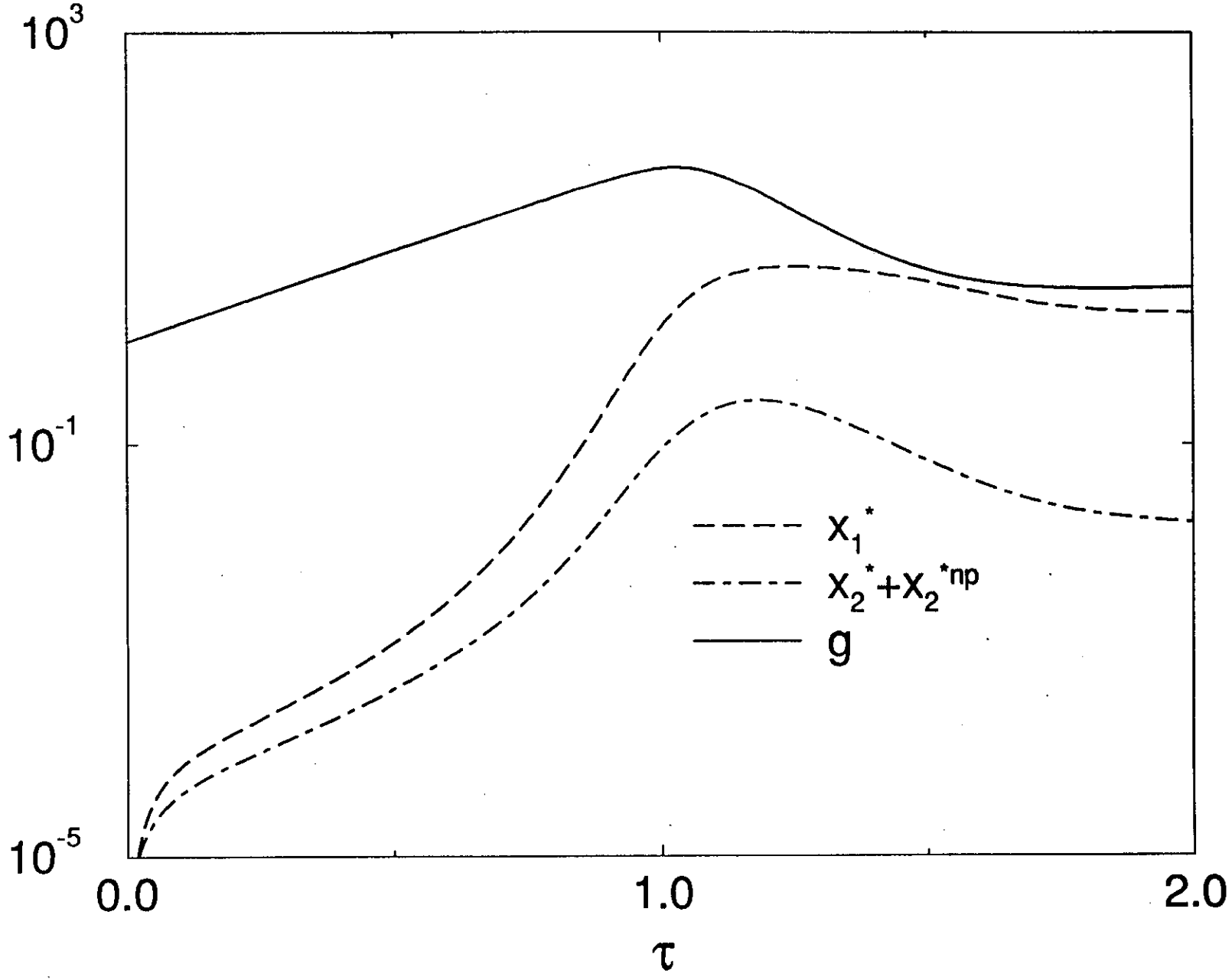


Figure 9b

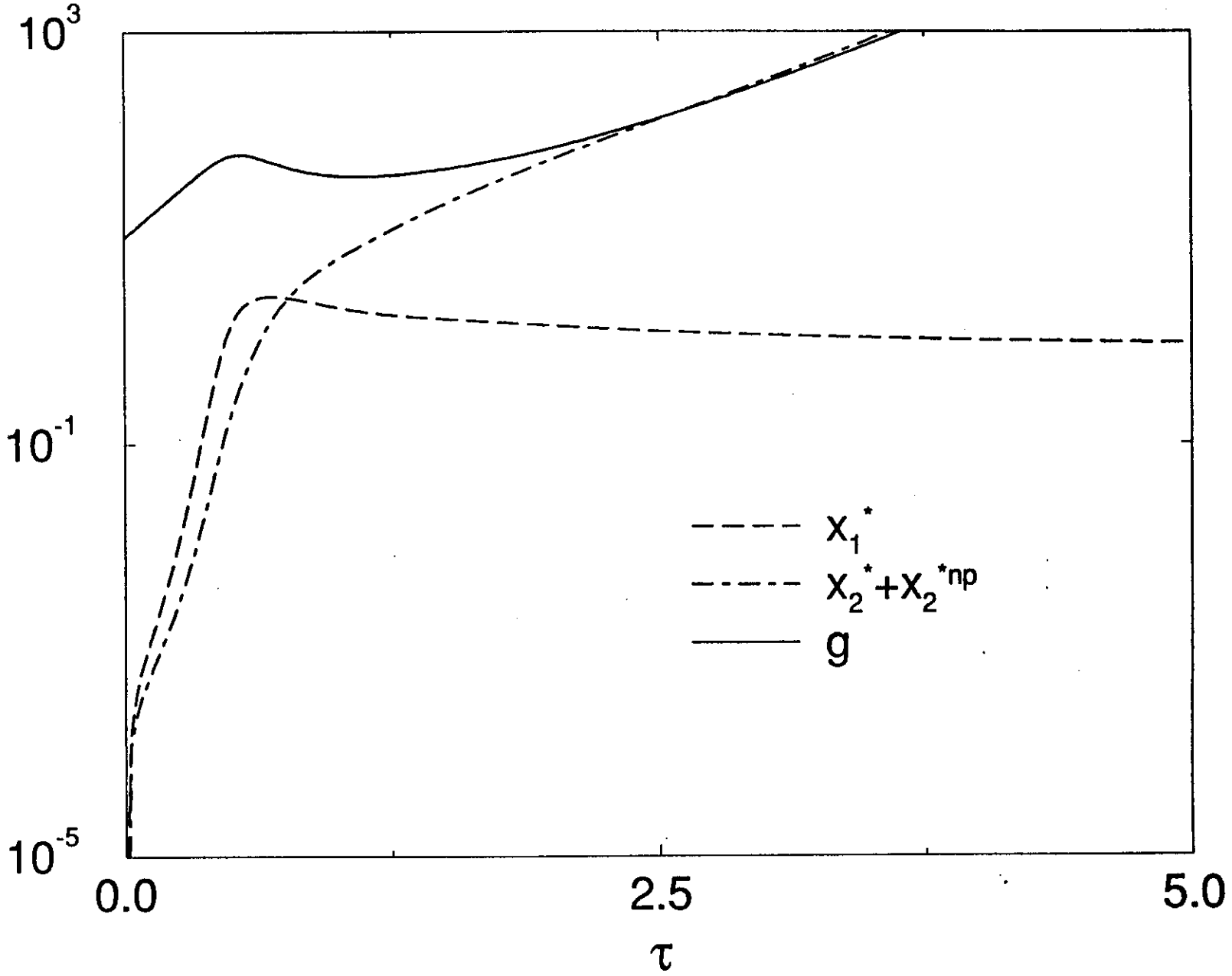


Figure 10a

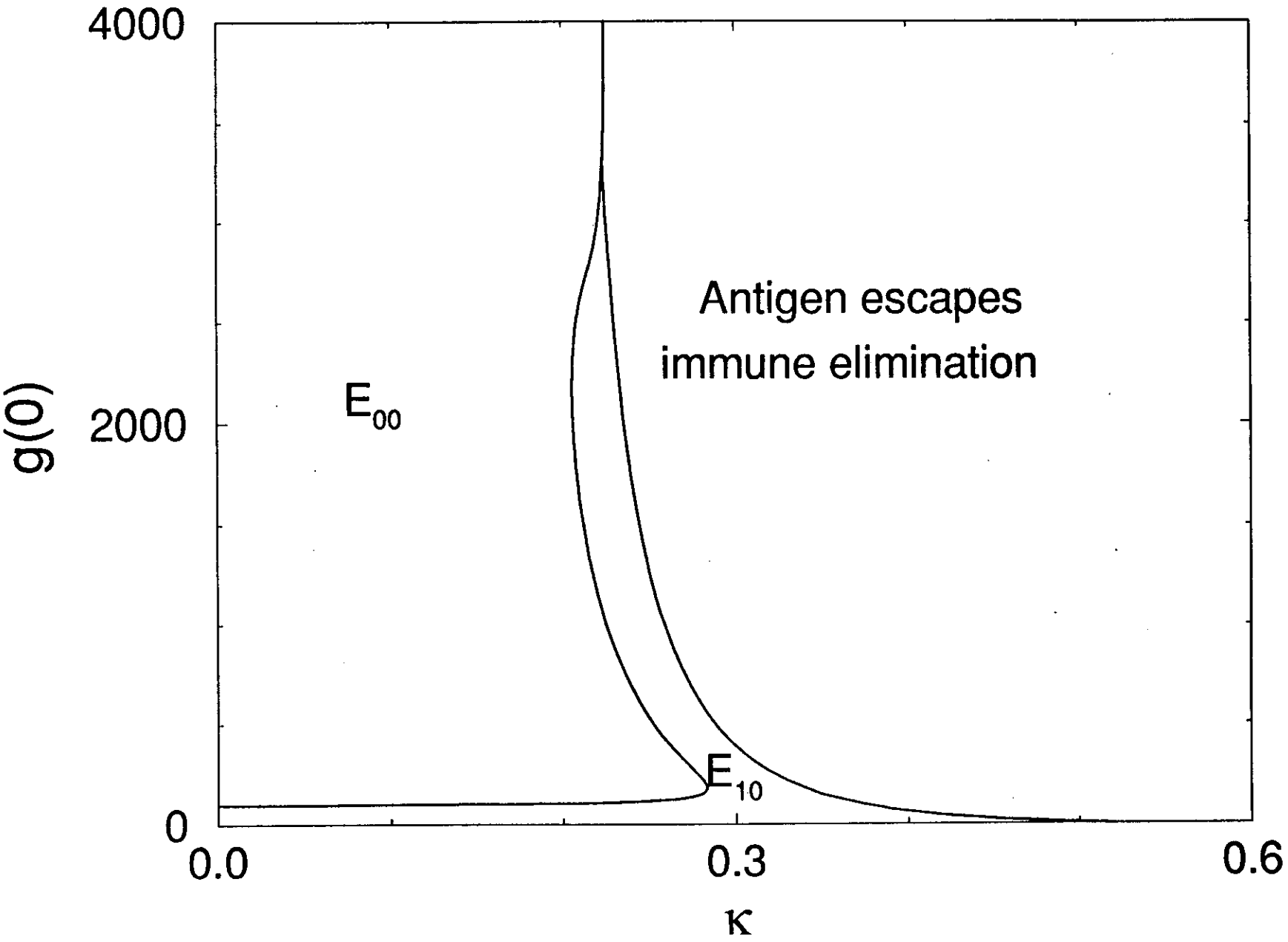

4000
2000
0
g(0)
0.0
0.3
0.6
κ
E00
E10
Antigen escapes
immune elimination

Figure 10b

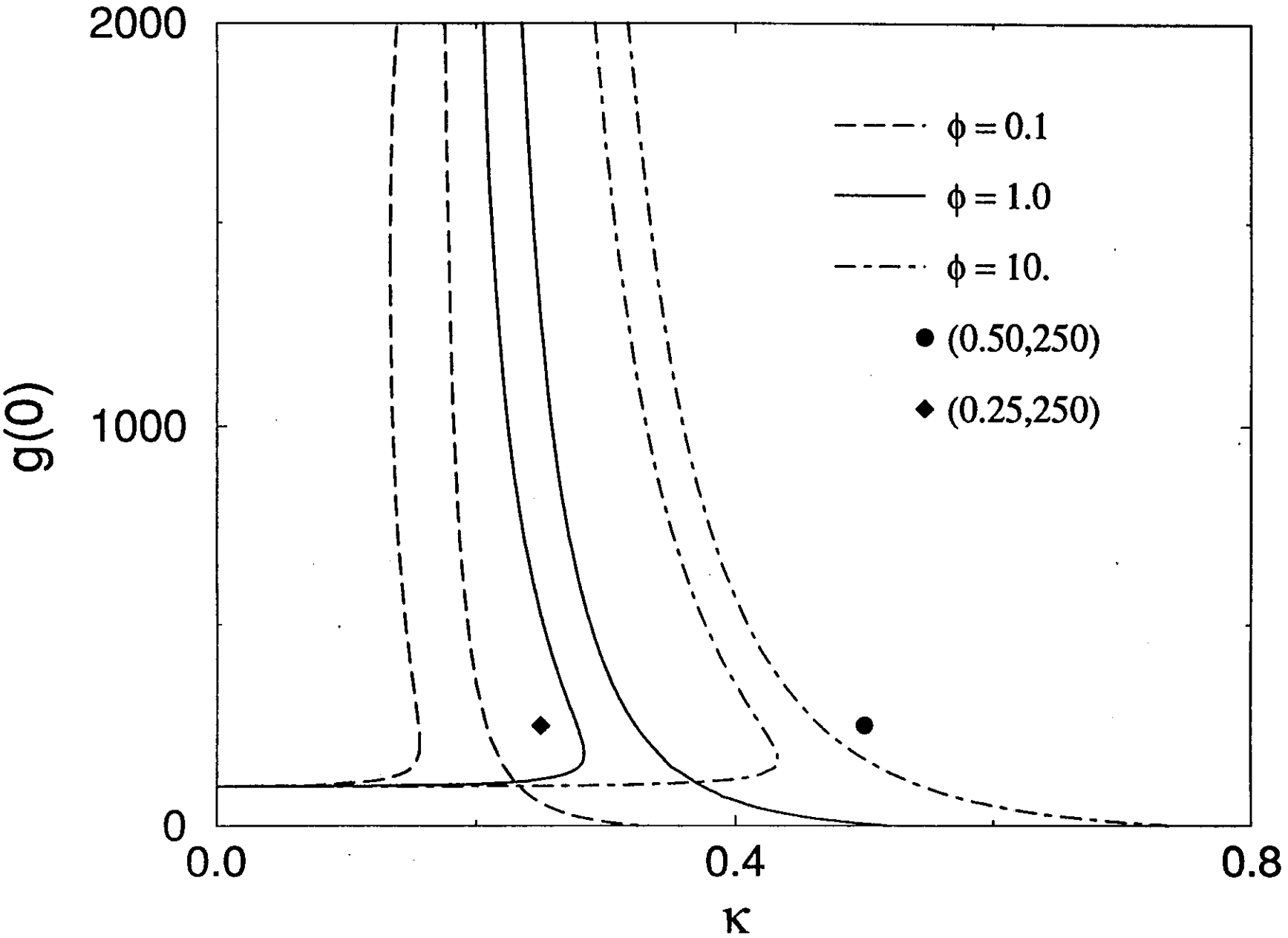


Figure 11

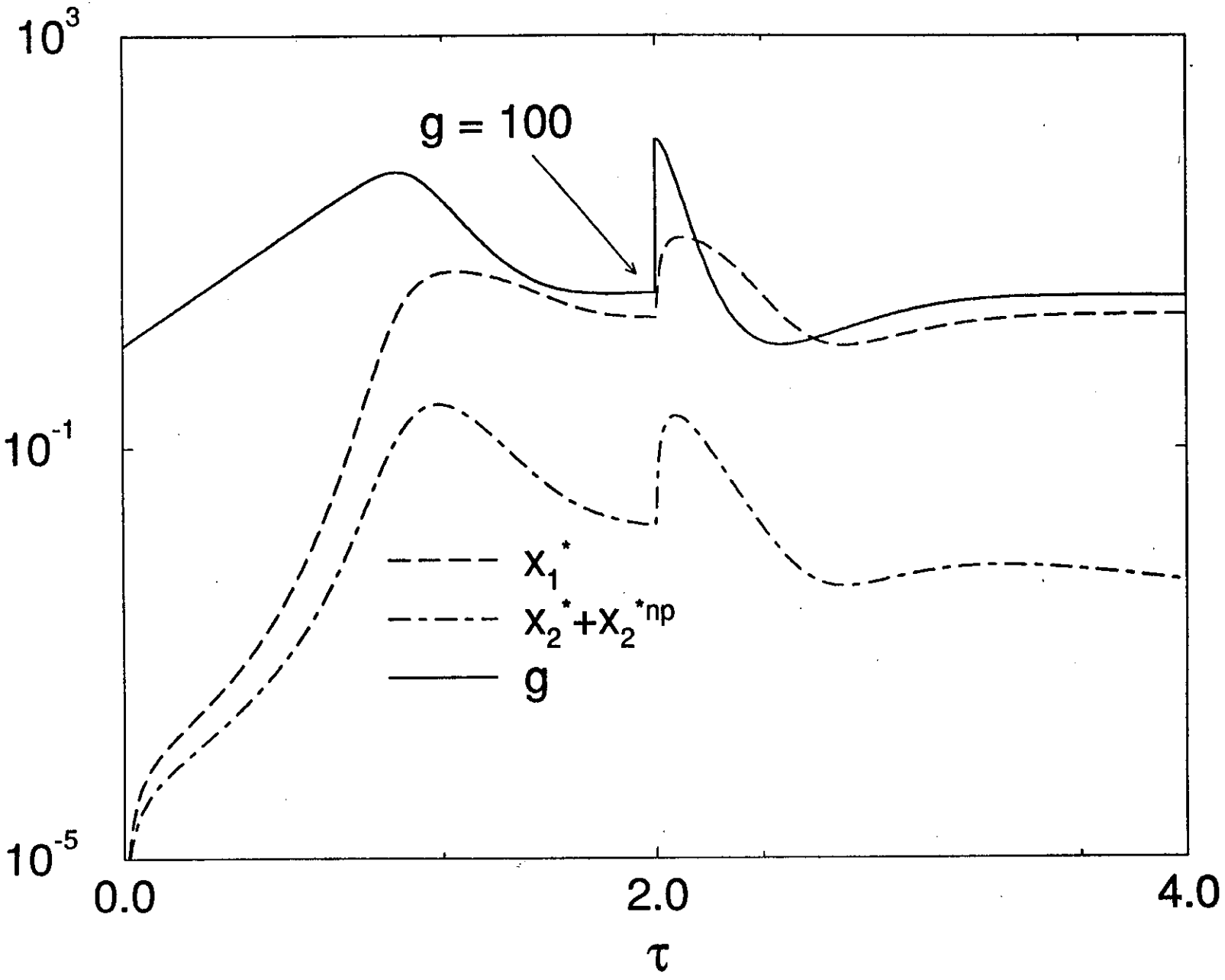


Figure 12
Paracrine effects

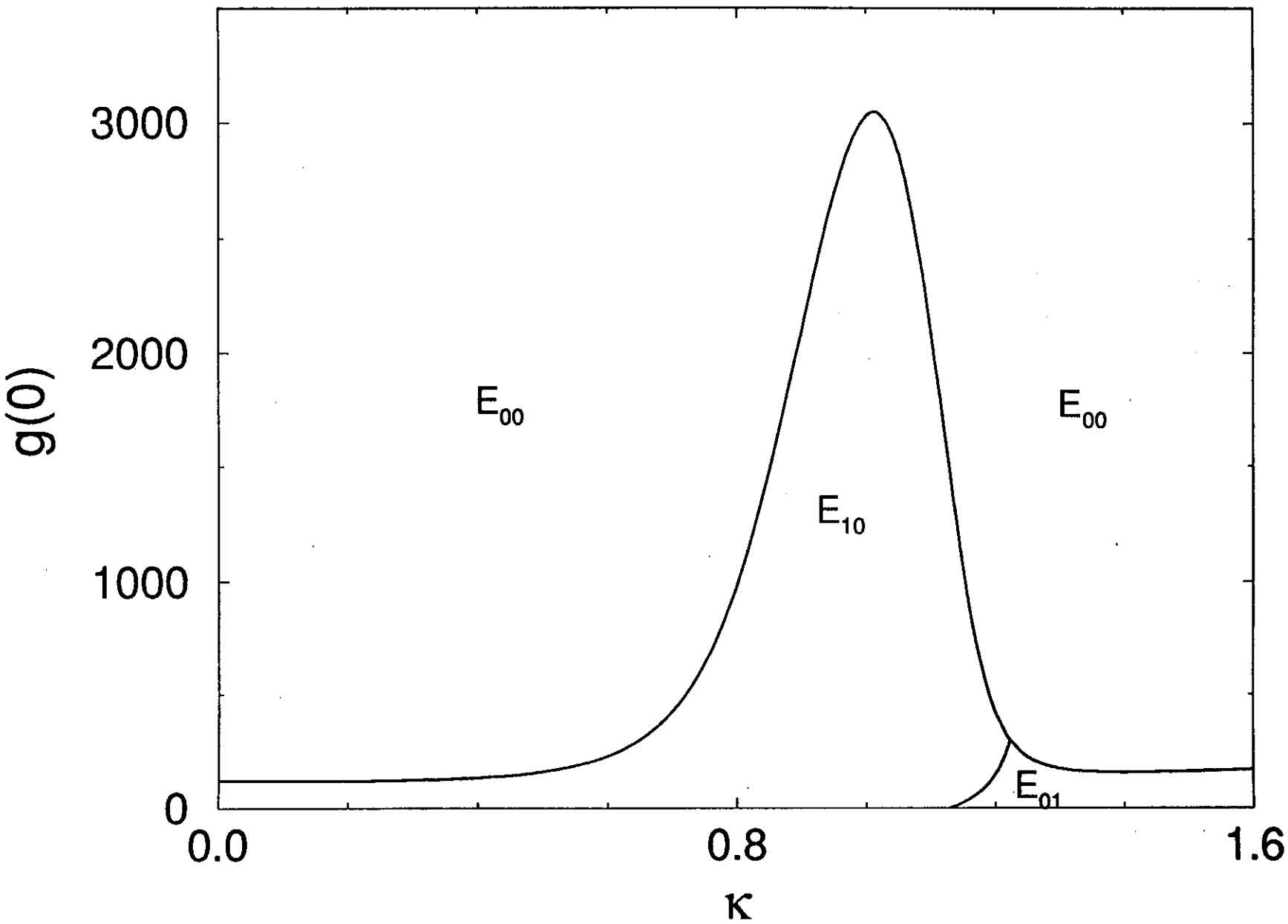


Figure 13

Inhibition of DC-Th1 interactions