Modelling T-cell-Mediated Suppression Dependent on Interactions in Multicellular Conjugates

KALET LEÓN* †‡, ROLANDO PERÉZ*, AGUSTIN LAGE* and JORGE CARNEIRO†

*Centro de Immunología Molecular, P.O. Box 16040, Habana 11600, Cuba and  †Instituto Gulbenkian de Ciencia, Apartado 14, 2781-901, Oeiras, Portugal

(Received on 7 February 2000, Accepted in revised form on 10 August 2000)

Tolerance to peripheral body antigens involves multiple mechanisms, namely T-cell-mediated suppression of potentially autoimmune cells. Recent in vivo and in vitro evidence indicates that regulatory T cells suppress the response of effector T cells by a mechanism that requires the simultaneous conjugation of regulatory and effector T cells with the same antigen-presenting cell (APC). Despite this strong requirement, it is not yet clear what happens while both cells are conjugated. Several hypotheses are discussed in the literature. Suppression may result from simple competition of regulatory and effector cells for activation resources on the APC; regulatory T cells may deliver an inhibitory signal to effector T cells in the same conjugate; or effector T cells may acquire the regulatory phenotype during their interaction with regulatory T cells. The present article tries to further our understanding of T-cell-mediated suppression, and to narrow-down the number of candidate mechanisms. We propose the first general formalism describing the formation of multicellular conjugates of T cells and APCs. Using this formalism we derive three particular models, representing alternative mechanisms of T-cell-mediated suppression. For each model, we make phase plane and bifurcation analysis, and identify their pros and cons in terms of the relationship with the large body of experimental observations on T-cell-mediated suppression. We argue that accounting for the quantitative details of adoptive transfers of tolerance requires models with bistable regimes in which either regulatory cells or effectors cells dominate the steady state. From this analysis, we conclude that the most plausible mechanism of T-cell-mediated suppression requires that regulatory T cells actively inhibit the growth of effector T cells, and that the maintenance of the population of regulatory T cells is dependent on the effector T cells. The regulatory T cell population may depend on a growth factor produced by effector T cells and/or on a continuous differentiation of effector cells to the regulatory phenotype.

© 2000 Academic Press

1. Introduction

Several mechanisms contribute to immunological tolerance to body tissues. Thymic deletion purges the emergent T cell repertoire from those lymphocytes that recognize, with high affinity or avidity, peptides expressed intrathymically (von Boehmer, 1991; Huang & Crispe, 1992). Mechanisms such as T cell ignorance (Ohashi et al., 1991), T cell anergy (Schwartz, 1997) or clonal exhaustion (Moskophidis et al., 1993; Rocha et al., 1995) may contribute to the peripheral unresponsiveness to tissue-specific antigens, which are not expressed in the thymus. However,
these mechanisms seem to be insufficient to explain tolerance to continuous stimulation by self-antigens, as they can easily be overcome during the course of immune responses and inflammation (Cahill et al., 1997). Dealing with this chronic stimulation by peripheral antigens seems to call into action yet another, maybe complementary, mechanism of tolerance, based in regulatory lymphocytes that actively suppress (auto) immune responses. The latter form of tolerance has been uncovered by adoptive transfers to naive recipients of T cells of donors that are either tolerant or responsive to some antigen. If in many instances the recipient becomes responsive to the antigen, in others they are reconstituted but display the “phenotype” of the tolerant donor (Sakaguchi et al., 1985, 1995; Fowell et al., 1991; Smith et al., 1992; Modigliani et al., 1995; Powrie et al., 1997). By analogy with the phenotype inheritance in genetics this form of tolerance, which can be transferred by T cells, has been named dominant in opposition to the previous mechanisms that have been named recessive (Le Douarin et al., 1996).

Although many different experiments of adoptive transfer of tolerance reveal the existence in normal healthy individuals of a CD4+ T cells subpopulation with suppressive properties, these regulatory T cells have not yet been isolated or cloned. This has prevented the full characterization of their phenotype and of their mechanism of action. Nevertheless, some clues on how the regulatory CD4+ T cells suppress the response of other cells have been derived from well-correlated in vitro and in vivo experiments (Takahashi et al., 1998; Thornton & Shevach, 1998). These studies suggest that T-cell-mediated suppression is not mediated by soluble factors and requires cell-to-cell contact. Moreover, these studies indicate that regulatory CD4+ T cells can only suppress the response by other cells if the ligands of both cells are expressed by the same antigen-presenting cell (APC) (Cobbold et al., 1996; Davies et al., 1996a; Frasca et al., 1997; Wise et al., 1998). Because of this fact, the mechanism of regulation was baptized “linked suppression”. Despite this strong requirement, it is not yet clear what the nature of this mechanism is. Suppression may be the result of simple competition for activation resources provided by APC (Lombardi et al., 1994; Waldmann & Cobbold, 1998). Regulatory and effector cells may exchange signals while they are simultaneously conjugated with the same APC. Thus, it has been suggested that regulatory cells may give an inhibitory signal to the responder T cells (Suri-Payer et al., 1998; Itoh et al., 1999), or alternatively the responder cells may themselves become regulatory cells during this interaction (Qin et al., 1993; Modigliani et al., 1996a). Alternatively, the regulatory cells may suppress the capacity of APCs to activate the effector cells (Taams et al., in press; Cederbom et al., 2000), avoiding the requirement for simultaneous conjugation. The attempts to discriminate experimentally these different hypotheses hitherto failed to provide a definitive picture. Nevertheless, the fact that in vitro suppression assays are typically performed using “fixed” APC renders the suppression via the APC rather implausible. The aim of the present article is to further our understanding on the mechanisms of linked suppression and its role in dominant tolerance, by exploring its putative requirement of a simultaneous interaction between three cell types. This sets up another goal, that is to develop a model of interactions in multicellular conjugates.

Mechanisms of intercellular cooperation involving the interaction of more than two cells simultaneously have been suggested before in Immunology (e.g. the classical view of cooperation between CD4 and CD8 T (Mitchison & O’Malley, 1987; Mitchison, 1990), but they are not common in biology. No mathematical model has been developed to study the quantitative implications of such interactions on cell population dynamics. Available mathematical models of T-cell-mediated suppression have addressed the possibility that this suppression is mediated by soluble factors, as in the class regulation models of Fishman & Perelson (1994) and others, or they have addressed the possibility of suppression mediated by a direct cell contact between a suppressor clone and an antigen-specific responder clone, as in classical idiotypic network models (Cohen & Atlan, 1989; Perelson, 1989) or in the more recent T cell vaccination models (Borghans & De Boer, 1995; Segel et al., 1995). Here we report the development and analysis of a mathematical model, in which the regulatory
interactions between T cells take place only in multicellular conjugates with their APC. We use this model to study the implications of this interaction on linked suppression and dominant tolerance.

2. The Basic Model

2.1. Biological System and Basic Postulates

The biological system that we are going to model has the following cellular components: the responder T lymphocytes that upon activation will trigger effector function (hereafter referred to as “effectors” and abbreviated as E), the regulatory T cells, (R), and the APCs (A). The major postulates in the model are:

(1) Regulatory T cells (R) and effector T cells (E) interact only during simultaneous conjugation with an APC.

Since we are interested in studying the quantitative implication of interactions amongst T cells in multicellular conjugates with APC, we impose that in the model. We note that T cell suppression may be mediated by other mechanisms. Antidiotypic regulatory cells may conjugate directly with the effector cells and suppress them; regulatory cells can synthesize suppressive cytokines acting on the effector cells in a paracrine fashion; and regulatory cells can act on the APCs inhibiting their capacity to stimulate effector cells. A comparative study of these alternative mechanisms represents a broader research plan, for which this is the first step.

(2) Antigen-presenting cells are a homogeneous population with fixed size.

The population of APCs in vivo is certainly not homogeneous (Cella et al., 1997). First, it is formed by cells of different lineage, which exhibit different capacities to stimulate T cells. Second, APCs from the same lineage may differ on the level of expression of the relevant antigenic peptide, and may be in different stages of activation and/or differentiation (Sprent, 1995; Kapsenberg et al., 1999). Even in vitro it may be difficult to achieve homogeneity of the APCs. Also, in vivo the total number of APCs may change and is perhaps a function of the T cells themselves (Takashima & Kitajima, 1998). This postulate allows us to obtain a simpler mathematical model, which hopefully captures those features of the real system depending on the average properties of the APC population. Those properties that depend on the APC heterogeneity are beyond the scope of the present work and may represent an area for future investigation.

(3) Each APC has a finite and fixed number of conjugation sites, which can be occupied by a single cell, irrespective of its phenotype being either regulatory or effector.

De Boer & Perelson (1994) introduced the concept of antigenic site to study T cell–APC interactions. The notion that the number of T cells that can be simultaneously conjugated with a single APC is limited is an intuitive and reasonable one: there must be some sort of steric hindrance. The strong simplification here (also present in De Boer & Perelson, 1994) is to assume that these conjugation sites are independent and equivalent, meaning that conjugation of a T cell in a particular conjugation site does not affect the conjugation of another T cell in an adjacent site. This postulate rules out any possible cooperativity effects (positive or negative) in the process of T cell–APC conjugation. Again, this assumption results in a simpler mathematical model that must be understood as a first approximation to the complexity of the real system. The number of conjugation sites per APC is defined in the model as an integer parameter s, which is varied within a realistic range of values.

(4) The process of formation and dissociation of a conjugate between a T cell and APC can be captured by the same formalism of first-order association reaction of ligand and receptor, leading to a conjugation constant K that is formally analogous to an affinity constant.

The process of T cell–APC conjugation is quite complex involving the concerted expression of many different adhesion molecules and some sort of synchronization of the signalling events in both cells (Dustin & Springer, 1991; Clark
We simplify all these processes assuming that the kinetics of formation of a T cell–APC conjugate is first order in both the concentration of APC conjugation sites (see postulate 3) and the concentration of T cells, and that the kinetics of dissociation of the conjugates is first order in the concentration of conjugates. This leads to an equilibrium constant, formally analogous to the \( k \) in receptor–ligand complex formation, that herein is termed the conjugation constant. This terminology is appropriate because it distinguishes the molecular affinity of the TCR for its ligand from the conjugation constant in the interaction between a T cell and its APC.

2.2. A System of Ordinary Differential Equations

The dynamics of populations of cells is described by a generic set of ordinary differential equations (ODE) in the following variables: the total number of regulatory cells \( R \), the total number of effector cells \( E \), the total number of APCs \( A \), and the number of multicellular conjugates involving one APC with \( i \) effector cells and \( j \) regulatory cells \( A_{ij} \).

For E and R cells we have, respectively,

\[
\frac{dE}{dt} = \sigma_E + \sum_{i=1}^{s} \sum_{j=0}^{s} z_E(i,j)A_{i,j} - \delta_E E_F, \tag{1}
\]

\[
\frac{dR}{dt} = \sigma_R + \sum_{j=1}^{s} \sum_{i=0}^{s} z_R(i,j)A_{i,j} - \delta_R R_F, \tag{2}
\]

where the involved quantities are defined in Table 1.

The equations for E [eqn (1)] and R [eqn (2)] have three terms. The first term represents the influx of new T cells, which is assumed to be constant. The second term accounts for the dynamical consequences of the interaction of R or E cells with the remaining cells in the system. This term is a linear combination of the conjugates formed by APCs with both classes of T cells, implementing postulate 1. Different settings of the coefficients \( z_E(i,j) \) and \( z_R(i,j) \), as detailed later are used to account for alternative interaction mechanisms. The third term represents the process of cell death that is assumed to be a simple exponential decay of the free T cells. Note that because only free T cells can die the conjugation with the APC acts as a survival factor.

The variables counting the multicellular conjugates \( A_{i,j} \) are only defined when \( (i,j) \in \{N, N\} \) and \( (i + j) \leq s \) giving rise to \( s(s + 1)/2 \) equations defined generically as

\[
\frac{dA_{i,j}}{dt} = (s - i - j + 1)c(A_{i-1,j}E_F + A_{i,j-1}R_F) - (d_Ei + d_Rj)A_{i,j} - (s - i - j)c(E_F + R_F)A_{i,j} + (d_E(i + 1)A_{i+1,j} + d_R(j + 1)A_{i,j+1}) \tag{3}
\]

and a conservation equation that accounts for postulate 2, and that allows us to drop the differential equation on the non-conjugated APC cells \( (A_{0,0}) \):

\[
A = \sum_{j=0}^{s} \sum_{i=0}^{s} A_{i,j}, \tag{4}
\]

The equations for \( A_{i,j} \) [defined generically according to eqn (3)] have four terms which represent: (I) the increase in the number of conjugates \( A_{i,j} \) formed by the association of free E or R cells with the conjugates of lower order \( A_{i-1,j} \) or \( A_{i,j-1} \), respectively; (II) the decrease in the number of conjugates \( A_{i,j} \) by dissociation of either E or R cells to these conjugates; (III) the decrease in \( A_{i,j} \) by association of either free E or R cells to these conjugates; (IV) the increase of the number of conjugates \( A_{i,j} \) by dissociation of either E or R cells from the conjugate of order \( A_{i+1,j} \) or \( A_{i,j+1} \), respectively.

Note that the formulation of eqn (3) is the most synthetic we have found, but it requires an appropriate definition of the quantities \( A_{i,j} \) (other than the variables that count the numbers of conjugate in each class (see Table 1), such that whenever the combination \( i, j \) is biologically unreasonable its value is set to zero. For example, when \( i + j \) is larger than \( s \) the corresponding conjugate does not exist and therefore the value of \( A_{i,j} \) is set to zero (Table 1). After substituting in eqn (3) the latter type of biological constraints the final system of equations for the conjugates \( A_{ij} \) is formed. Note that in this process some terms are lost in the equations for the conjugates in the extreme part of the system \( (i \text{ or } j) = 0 \) and \( (i + j) = s \).
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Biological meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E$</td>
<td>$\in \mathbb{R}^+$</td>
<td>Total number of effector T cells</td>
</tr>
<tr>
<td>$R$</td>
<td>$\in \mathbb{R}^+$</td>
<td>Total number of regulatory T cells</td>
</tr>
<tr>
<td>$A_{i,j}$</td>
<td>$\in \mathbb{R}^+$ if $(i + j) \leq s$ Number of APC cells conjugated with $i$ E cells and $j$ R cells</td>
<td></td>
</tr>
</tbody>
</table>

### Intermediate variables

| $E_{t}$ | $= \sum_{i=1}^{s} \sum_{j=0}^{s} iA_{i,j}$ | Total number of conjugated E cells |
| $R_{t}$ | $= \sum_{i=1}^{s} \sum_{j=0}^{s} jA_{i,j}$ | Total number of conjugated R cells |
| $E_{f}$ | $= E - E_{t}$ | Total number of free E cells |
| $R_{f}$ | $= R - R_{t}$ | Total number of free R cells |

### Parameters

| $S$ | $\in \mathbb{N}$ | Total number of conjugation sites per APC |
| $\sigma_{E}, \sigma_{R}$ | $\in \mathbb{R}^+$ | Influx of new E and R cells, respectively |
| $\delta_{E}, \delta_{R}$ | $\in \mathbb{R}^+$ | Rate constant of the death of free E and R cells, respectively |
| $c$ | $\in \mathbb{R}^+$ | Rate constant for the formation of a conjugate between T cells and APC sites |
| $d_{E}, d_{R}$ | $\in \mathbb{R}^+$ | Rate constants for the dissociation of E or A cells from an APC site |
| $\alpha_{E}(i,j)$ | $\in \mathbb{R}$ | Interaction coefficients determining the effect on the populations of E or R cells of conjugates of $i$ E cells, $j$ R cells and an APC. Different models can be represented as different sets of these coefficients (see Section 2.3 in text) |

#### 2.3. A NON-DIMENSIONAL SYSTEM OF ODES

The ODE system was rendered non-dimensional by dividing the number of cells in each class and the number of conjugates by the total number of APC $A$, and by substituting real time by the non-dimensional time $x$, divided by the death rate constant of effector cells $\delta_{E}$. This yields

$$\frac{dE}{dx} = \sigma_{e} + \sum_{i=1}^{s} \sum_{j=0}^{s} \alpha_{E}(i,j) a_{i,j} - e_{f}, \quad (5)$$

$$\frac{dR}{dx} = \sigma_{r} + \sum_{j=1}^{s} \sum_{i=0}^{s} \alpha_{r}(i,j) a_{i,j} - r_{f}, \quad (6)$$

The conservation equation (4) is transformed to the normalization condition for the distribution of frequencies $a_{ij}$ (fraction of APCs conjugated with $i$ E cells and $j$ R cells)

$$\theta_{a} \frac{da_{i,j}}{dx} = (s - i - j + 1)K_{e}(a_{i-1,j}e_{f} + a_{i,j-1}r_{f}) - (i + \delta j)a_{i,j} - (s - i - j)K_{e}(e_{f} + r_{f})a_{i,j} + (i + 1)a_{i+1,j} + \delta(j + 1)a_{i,j+1}. \quad (7)$$

$$1 = \sum_{j=0}^{s} \sum_{i=0}^{s} a_{i,j}. \quad (8)$$
The non-dimensional parameters and variables are defined as follows:

\[ e = \frac{E}{A}, \quad r = \frac{R}{A}, \quad x = \delta_E t, \quad a_{i,j} = \frac{A_{i,j}}{A}, \]

\[ e_f = e - \sum_{i=1}^{s} \sum_{j=0}^{s} i a_{i,j}, \quad r_f = r - \sum_{j=1}^{s} \sum_{i=0}^{s} j a_{i,j}, \]

\[ \sigma_e = \frac{\sigma_E}{\delta_E A}, \quad \sigma_r = \frac{\sigma_R}{\delta_R A}, \quad K_e = \frac{c}{\delta_E A}, \quad K_r = \frac{c}{\delta_R A}, \]

\[ \alpha_e(i,j) = \frac{\alpha_p(i,j)}{\delta_E}, \quad \alpha_r(i,j) = \frac{\alpha_r(i,j)}{\delta_R}, \quad \beta = \frac{K_e}{K_r}, \]

\[ \theta_e = \frac{\delta_E}{\delta_R}, \quad \theta_r = \frac{\delta_E}{\delta_R}. \]

2.4. A QUASI-STEADY-STATE APPROXIMATION

The processes of formation and dissociation of conjugates are relatively fast as compared to the overall dynamics of the T cell populations. While a T cell remains conjugated with its APC for a few hours, the T cell mitotic cycle lasts on average 12 hr and the T cell lifespan is at least several days. Under these conditions, it is reasonable to assume that the fraction of conjugates \( a_{i,j} \) is in quasi-steady state, reducing the system to two ODEs in the non-dimensional variables \( e \) and \( r \):

\[ \frac{de}{dx} = \sigma_e + \sum_{i=1}^{s} \sum_{j=0}^{s} \alpha_e(i,j) a_{i,j}(e,r) - e_f, \quad (9) \]

\[ \theta_r \frac{dr}{dx} = \sigma_r + \sum_{j=1}^{s} \sum_{i=0}^{s} \alpha_r(i,j) a_{i,j}(e,r) - r_f. \quad (10) \]

The distribution of the conjugates is now defined as

\[ 1 = \sum_{j=0}^{s} \sum_{i=0}^{s} a_{i,j}(e,r), \quad (11) \]

where \( a_{i,j}(e,r) \) is for each pair of values of \( e \) and \( r \), the frequency of APCs which are conjugated with \( i \) E cells and \( j \) R cells, when the processes of formation and dissociation of conjugates reach the equilibrium. In principle, this frequency can be calculated by solving a system of algebraic equations:

\[ 0 = (s-i-j+1)K_e(a_{i-1,j}(e,r)e_f + a_{i,j-1}(e,r)r_f) - (i + \delta j)a_{i,j}(e,r) - (s-i-j)K_e(e_f + r_f) \]

\[ \times a_{i,j}(e,r) + (i+1)a_{i+1,j}(e,r) \]

\[ + \delta(j+1)a_{i,j+1}(e,r). \quad (12) \]

This system of equations is quadratic in \( a_{i,j}(e,r) \) and there is no general analytic solution available for it. The following section proposes an analytic solution for our particular case, based on its particular simplifying properties.

2.5. AN ANALYTIC SOLUTION FOR \( a_{i,j}(e,r) \)

Taking into account the homogeneity of the APCs and the independence of their conjugation sites, the task of obtaining \( a_{i,j}(e,r) \) can be divided into two separate problems. First, to obtain the total number of effector and regulatory cells conjugated with the APC sites, respectively, \( E \) and \( R \), and second to distribute them among the individual APC sites.

The first problem has been addressed before by De Boer and co-workers (De Boer & Perelson, 1994, 1995, 1997), who derived several approximations for the general problem of calculating the number of conjugates formed by \( n \) classes of T cells with \( m \) classes of APCs. From our own experience none of these approximations is good enough in the problem at stake. Fortunately, in the particular cases of just a few different T cell populations (less than three) it can be solved analytically, as demonstrated in Appendix A. Briefly, the solution is obtained by first computing the total number of free antigenic sites (F) at equilibrium. The latter quantity can be expressed in general as an algebraic equation of order equal to the number of T cells species in the system plus 1. In the particular case of two T cell classes E and R the following third-order equation for non-dimensional variables is derived, where \( f \) is now the number of free sites per APC \((F = F/A)\):

\[ 0 = \{K_e K_r\} f^3 + \{K_e + K_r - K_e K_r (s - r - e)\} f^2 \]

\[ + \{1 - K_e (s - e) - K_r (s - r)\} f - s. \quad (13) \]
The three roots for eqn (13), although complicated, can be obtained analytically. Only one is biologically meaningful and is used to obtain the number of conjugated E and R cells per APC in the system according to the following relations:

\[ e_c = \frac{fK_e}{1 + fK_e} \quad e_c, \quad r_c = \frac{fK_r}{1 + fK_r} \quad r_c. \]  

(14)

The second problem is more complex and hitherto has not been addressed in biological modelling. However, it has some well-known analogues in mathematics and physics. For instance, the work on a lattice gas model (Roman et al., 1996) has given us clues to address it. The desired quasi-steady-state distribution \( (a_{i,j}(e, r)) \) represents the probability of finding an APC conjugated with \( i \) E cells and \( j \) R cells, given the total number of APC A, the number of conjugated E cells and the number of conjugated R cells, respectively, \( E_c \) and \( R_c \). This quantity can be obtained in two steps, considering the equivalence of conjugated E and R cells. First, we obtain the probability of having an APC with \( i + j \) T cells of any kind. And then we multiply it by the probability that within a random sample of \( i + j \) T cells, drawn from a population of \( E_c \) and \( R_c \) cells, \( i \) are E cells and \( j \) are R cells. Both steps of the problem are of the same nature corresponding to the classical combinatorial problem of sampling without replacement. The solution to this problem can be found elsewhere (Hoel, 1963) and is expressed in terms of the hyper-geometric distribution. Using the latter analysis the following expression for \( a_{i,j}(e, r) \) is obtained:

\[ a_{i,j}(e, r) = Hyp(i + j, (e_c + r_c)A, sA, s) \times Hyp(i, e_cA, (e_c + r_c)A, i + j), \]  

(15)

where \( Hyp \) is the hypergeometric distribution defined as

\[ Hyp(N, N_0, N, L) = \binom{N_0}{N} \binom{M - N_0}{L - N} \binom{M}{L}. \]  

(16)

Equations (15) and (16) allow us to calculate the \( a_{i,j}(e, r) \) needed in eqns (9)–(11). This distribution is illustrated in Fig. 1. We have confirmed the validity of the latter approach by comparing the expression obtained here and the numeric solution of the set of eqns (12) (data not shown).

2.6. QUALITATIVELY DIFFERENT MECHANISMS OF SUPPRESSION CAN BE MODELLLED BY SETTING THE VALUES OF THE PAIRS \( a_e(i, j) \) AND \( a_r(i, j) \)

The basic model developed here for the population dynamics of regulatory and effector T cell populations can be used to represent different putative mechanisms by which regulatory T cells suppress the activation and proliferation of effector T cells. This can be achieved by setting the pair of parameters \( a_e(i, j) \) and \( a_r(i, j) \) in such a
FIG. 2. Cartoon illustrating the different hypothetical mechanisms by which regulatory cells may suppress effector cells while both participate in multicellular conjugates with the APC.

One of the main results of this paper is the actual formalism to deal with multicellular conjugates of T cells with APC. In the following sections, we report the results of applying this formalism to model the three candidate mechanisms of interaction between effector and regulatory cells. For each mechanism we formulate a particular model, analyse its phase plane and dependence on parameters. The properties of each model are interpreted and discussed in the context of experimental observations.

3.1. MODEL 1: E AND R CELLS COMPETE FOR CONJUGATION SITES

The first hypothesis concerning the mechanism of T-cell-mediated immune-regulation is competition: R cells are simply cells that are themselves unable to trigger effector function but may interfere with E cells by competing with them for the use of some limited growth factor. This has been made explicit and investigated experimentally by Lechler (Lombardi et al., 1994) and Waldmann (Cobbold et al., 1996), who proposed that anergic T cells could play the role of regulatory cells. Because anergic cells do not produce IL-2, but express high levels of IL-2 receptor they could act as a sink for IL-2, which is required for the growth of normal T cells. In our model, competition between E and R cells for a growth factor stems naturally from the fact that both classes of cells require conjugation with the APC in order to survive or proliferate. The fixed number of conjugation sites acts as a limiting resource (De Boer & Perelson, 1994; Borghans et al., 1999).

The equations describing this system are obtained by defining $x_e(i,j) = \pi_e i$ and $x_r(i,j) = \pi_r j$, where $\pi_e$ and $\pi_r$ are the ratios between proliferation rate and death rate constants, respectively, for E and R cells. This yields

$$\frac{dx}{dx} = \pi_e e - e_f,$$ (17)

$$\frac{dr}{dx} = \pi_r r - r_f.$$ (18)

The quantities $e_r$ and $r_r$ are calculated as described in the previous section. Note that
and conjugates \( a_{i,j} \) do not appear in these equations because there is no direct interaction amongst the different T cells; the E and R cells can only interfere by occupying the conjugation sites on the APC, giving rise to a competitive interaction which is symmetric.

3.1.1. Modelling results

Competition models have been extensively studied in theoretical ecology and more recently in theoretical immunology. It is easier to analyse the model when there is no influx of new cells, i.e. when \( \sigma_e \) and \( \sigma_r \) are set to zero, and then to study the effect of a small influx. This approach will be followed here and in the following sections.

In the absence of influx, the nullclines for \( e \) and \( r \) in the system are, respectively,

\[
e = 0
\]

and

\[
\frac{1}{\pi_e + 1} e + \frac{1}{(K_e/K_r)\pi_e + 1} r = s - \frac{1}{K_e\pi_e} \quad (19)
\]

and

\[
r = 0
\]

and

\[
\frac{1}{(K_e/K_r)\pi_r + 1} e + \frac{1}{\pi_r + 1} r = s - \frac{1}{K_r\pi_r} . \quad (20)
\]

Heretofore, we will refer to the nullclines in which the concentrations of \( r \) or \( e \) are zero or positive, respectively, as the trivial and the non-trivial nullclines. The non-trivial nullclines are two straight lines [eqns (19) and (20)], which do not intersect in positive ranges unless they are identical. The condition for identity is

\[
K_r\pi_r = K_e\pi_e. \quad (21)
\]

The system has three possible steady states at the intersection between the nullclines in the space \((r, e)\): one trivial steady state \((0, 0)\) and two non-trivial steady states defined as

\[
\begin{pmatrix}
(s(\pi_r + 1) - \frac{1}{K_r} - \frac{1}{K_r\pi_r}, 0)
\end{pmatrix}
\]

and

\[
\begin{pmatrix}
0, s(\pi_e + 1) - \frac{1}{K_e} - \frac{1}{K_e\pi_e}
\end{pmatrix}. \quad (22)
\]

The latter are only meaningful \([(e, r) \in (R^+_e, R^+_r)]\), respectively, when the following conditions are fulfilled:

\[
sK_e\pi_e > 1 \quad \text{and} \quad sK_r\pi_r > 1. \quad (23)
\]

Conditions (21) and (23) define three regions in the parameter space \( K_e\pi_e \text{ vs. } K_r\pi_r \) corresponding to three biologically distinct situations (Fig. 3). In region O neither \( R \) nor \( E \) populations are sustainable, in region I the growth capacity of the effector population is higher than the one of the regulatory population such that the latter is excluded, and in region II the growth capacity of the regulatory population is higher than the one of the effector population and the latter is excluded. Representative phase planes corresponding to regions I and II are illustrated also in Fig. 3.

The basic feature of the model is that, irrespective of the parameter settings, it has always a globally stable equilibrium. If the subpopulations of T cells are sustainable, then only one of them will persist competing out the other one. Which population remains either regulatory or effector cells, depends on the parameter regime.

The algebraic analysis of the system when the source term is positive is quite complicated, but from numerical and graphical analysis it is easy to understand that the qualitative properties of the system remain the same (dashed lines in the phase planes in Fig. 3). Now there are only two nullclines in the system, one for \( e \) and one for \( r \). The nullcline of \( r \) (or \( e \)) is a curve that tends asymptotically to \((\sigma_r, \sigma_e)\) when \( e(r) \) tends to infinity and tends asymptotically to the nullcline defined by eqn (19) [eqn (20)] when \( e(r) \) tends to zero. Under these conditions the trivial and the unstable steady states observed with a null source term, disappear and there is only one equilibrium point, which is stable, in the intersection between the nullclines. The source term changes the numbers or regulatory and effector cells at the equilibrium and also changes slightly the conditions on the parameters separating regions I and II.
3.1.2. Biological interpretation

The interpretation of the results of the present model and also in the ones that follow requires an additional assumption on the meaning of the equilibria dominated by either regulatory or effector lymphocytes. As in our previous proposal (Carneiro et al., 1995), we will interpret the equilibrium dominated by regulatory cells as tolerance or unresponsiveness and the state dominated by effectors as an effective immune response or autoimmunity. The rationale behind this assumption is that the T cells themselves do not mediate the effective immune response, but they trigger it.

The major outstanding property of this model is that for any interesting parameter regime there is only a globally stable equilibrium characterized by one of the T cell populations excluding by competition the other one. In general, models with this property can only explain different qualitative responses to antigen such as tolerance and unresponsiveness and the state dominated by effectors as an effective immune response or autoimmunity. The rationale behind this assumption is that the T cells themselves do not mediate the effective immune response, but they trigger it.

The major outstanding property of this model is that for any interesting parameter regime there is only a globally stable equilibrium characterized by one of the T cell populations excluding by competition the other one. In general, models with this property can only explain different qualitative responses to antigen such as tolerance and unresponsiveness and the state dominated by effectors as an effective immune response or autoimmunity. The rationale behind this assumption is that the T cells themselves do not mediate the effective immune response, but they trigger it.

The major outstanding property of this model is that for any interesting parameter regime there is only a globally stable equilibrium characterized by one of the T cell populations excluding by competition the other one. In general, models with this property can only explain different qualitative responses to antigen such as tolerance and unresponsiveness and the state dominated by effectors as an effective immune response or autoimmunity. The rationale behind this assumption is that the T cells themselves do not mediate the effective immune response, but they trigger it.
tissues and immunity to invading pathogens. This example could be matched with the ideas of the danger theory of Matzinger and co-workers (Matzinger, 1994).

Although these two hypotheses are immunologically plausible, they are challenged by experimental observations on dominant tolerance. The basic experimental procedure in dominant tolerance is the adoptive transfer into naive recipients of mixtures of purified T cells from tolerant and immune donors, sources, respectively, of regulatory and effector cell populations. The recipients will become either tolerant or immune after reconstitution of their T cell compartment, depending on the initial composition of the inoculum. The only experimental variables in these experiments are the numbers of effector and regulatory cells, which map to the variables in our model. All the other parameters being kept constant, different initial proportions of effector and regulatory cells lead to qualitatively different steady states, making it hard to accept that the system in vivo would be globally stable. These considerations render the first hypothesis less straightforward,* and readily reject the second hypothesis.

Overall, any model predicting a globally stable system can be interpreted in terms of dominant tolerance in a non-straightforward way that requires additional assumptions on how parameters are controlled. Moreover, these additional assumptions are controversial or even not supported by experimental observations. These considerations open the way to the next model in which a bistability parameter regime is found, and that leads to a more straightforward interpretation of the results of adoptive transfers.

### 3.2. Model 2: R Cells Inhibit the Proliferation of E Cells

The second candidate model assumes that R cells inhibit the proliferation of E cells during simultaneous conjugation with APC. This inhibitory interaction, in contrast to the basic competition for conjugation sites, is asymmetric. This hypothesis is perhaps the most popular in the field of dominant tolerance. It stems from the observation that in vitro proliferation of normal E cells is inhibited by anergic/regulatory T cells (Lombardi et al., 1995; Read et al., 1998; Takahashi et al., 1998; Thornton & Shevach, 1998) and that the extent of expansion of donor cells in recipient animals in the presence or absence of regulatory cells is different (Powrie et al., 1997; Annacker et al., 2000). The equations describing this particular mechanism are obtained from the general model by setting $a_e(i,j) = \omega(i,j)\pi_e i$ and $a_r(i,j) = \pi_r j$, where $\omega(i,j)$ is an appropriate function that determines the proliferation rate of E cells as a function of the number of R cells present on the same conjugate. To simplify, it is assumed here that the presence of a single R cell in a conjugate is enough to abrogate the proliferation of all the E cells on the same conjugate. This approximation maximizes the advantage of the R cells over the E cells. Mathematically, we have $\omega(i,j) = 0$ and $\omega(i,0) = 1$ with $j > 1$. The differential equations are:

$$\frac{de}{dx} = \sigma_e + \pi_e \sum_{i=1}^{s} i a_{i,0}(e, r) - e_f, \quad (24)$$

$$\theta_r \frac{dr}{dx} = \sigma_r + \pi_r r - r_f \quad (25)$$

with (see Appendix B):

$$\sum_{i=1}^{s} i a_{i,0}(e, r) = \text{Hyp}(0, r_e A, s A, s) \frac{s}{s - r_e} e_c. \quad (26)$$
FIG. 4. Phase planes and parameter regions of Model 2 in which regulatory and effector T cell populations compete for conjugation with APC and regulatory cells inhibit the proliferation of effector cells during co-conjugation. (a) Typical phase planes corresponding to parameter regions I, II and III in b. The meaning of the lines and the circles is the same as Fig. 1. Particular parameters: (I) \( n_e = 14, n_r = 0.4 \); (II) \( n_e = 8, n_r = 5 \); (III) \( n_e = 14, n_r = 1 \). The remaining parameters are common: \( s = 5, K_e = K_r = 1, \sigma_e = \sigma_r = 0 \) or 1. (b) The parameter regions in the model are defined in the plane \( k_e n_e \) vs. \( k_r n_r \) with \( s = 3 \) and \( \sigma_e = \sigma_r = 0 \): regions 0, I, II result in globally stable regimes similar to equivalent regions in the model of Fig. 1; the new parameter region III corresponds to a bistable regime (illustrated in A-III).

Note that eqn (25) describing the dynamics of R cells, remains unchanged as compared to eqn (18), because the only way the E cells can interfere with their dynamics is by competition for conjugation sites. Also, note that in the particular case when \( s = 1 \) this model is reduced to the simple competition model [eqn (20)] (Fig. 4). The deviation of this nullcline from linearity results from the higher sensitivity of the inhibition of the growth of E cells to an increase in the number of R cells (Fig. 4). The curvature of the nullcline for \( e \) allows it to intersect the nullcline for \( r \) under some parameter regimes, giving rise to the emergence of bistability.

The second model shows four qualitatively different phase planes corresponding to four parameter regions [Fig. 4(b)]. Three of the phase planes, corresponding to regions 0, I and II, are comparable to the situations observed in the simple competition model: either both cell populations collapse or there is only a globally stable equilibrium composed of either R or E cells (Fig. 4). However, there is a new parameter region III where the non-trivial nullclines for \( e \) and \( r \) cross each other such that the system becomes bistable. The corresponding phase plane [Fig. 4(a)] shows four equilibrium points: the trivial equilibrium, the coexistence equilibrium (at the intersection of the non-trivial nullclines), and two equilibria composed exclusively of either R or E cells (at the intersections between non-trivial nullcline of \( r \) and trivial nullcline of \( e \) and vice versa). The first two equilibria are unstable and the last two are stable. Although the coexistence equilibrium cannot be calculated analytically the equilibria composed of either R or E cells are defined as in eqn (22).

The conditions on the parameters defining region III are the ones that allow persistence of both E and R populations [eqn (23)] and the condition for the intersection of the non-trivial nullclines in the positive values of \( e \) and \( r \).
are given as follows:

\[ K_e \pi_e < K_e \pi_e < \frac{1}{s H_{\text{yp}}(0, (s - 1/K_e \pi_e)A, sA, s)}. \]

(27)

Note that because of the particular shape of the non-trivial nullclines the two conditions in eqn (27) correspond to their intersection at \( r = 0 \) (left-hand inequality) and their intersection at \( e = 0 \) (right-hand inequality). These conditions define, respectively, the interfaces between the parametric regions II and III and the parametric regions III and I in Fig. 4(b).

The whole parameter dependence of this model, as well as in the previous one, could be represented in the plane \( K_e \pi_e \text{ vs. } K_e \pi_e \) [Fig. 4(b)] for a given value of the number conjugation sites per APC (s). Increases in the value of s increase the size of the observed region III (data not shown). When s is reduced to 1 region III in the parameter space collapses, as expected from the fact that the model is reduced to a simple competition one.

The major feature of this model as compared to the model of simple competition is that it has a parameter region where bistability exists. Some additional features of interest are related to the effect of the total number of antigen-presenting cells (A). Depending on the initial conditions and the remaining parameters a change in A can have two qualitatively different effects: a bifurcation in which the system switches between phase planes of types I and II; or a change in the relative size of the domains of attraction of the two stable equilibria within the phase plane of type III.

In order to understand the effects of the number of APCs (A) it may be useful to recall that in the non-dimensional system (Section 2.3) the variables \( e \) and \( r \) are relative to A, and that the parameters \( K_e \) and \( K_r \) are defined as the product of the conjugation constants of either E or R cells by A. Under these conditions, changes in A alone correspond to change in the coordinates in the space \( (K_e \pi_e \text{ vs. } K_r \pi_r) \) along a straight line that passes necessarily through the origin.

With this consideration in mind, it is easy to understand why changes in the number of APCs can lead to a bifurcation between phase planes of types I and III. Any straight line connecting the origin and any point within parameter region III will necessarily pass by region I [Fig. 4(b)]. Note that changes in the number of APCs cannot lead to a bifurcation between regions III and II or between regions II and I. Straight lines connecting the origin and any point in parameter region II do not cross either region I or III. Note also that in the simple competition model, as can be easily inferred from Fig. 3, it is not possible there to switch between regions I and II by changing the number of APC cells.

The second interesting effect of A is on the basins of attraction of the stable equilibria within the bistability regime III (therefore an effect in which no bifurcation is involved). This may be relevant because the final equilibrium reached depends critically on the initial conditions. Thus, relatively high values of A as compared to \( E + R \) — low values of \( e + r \) — will tend to result in the equilibrium dominated by E cells, while relatively low values of A — high values of \( e + r \) — tend to lead to the equilibrium dominated by R cells (Fig. 5). This result is systematically observed independently of the initial proportions of R and E cells, as long as both populations of cells are initially not null.

As in the previous case, the introduction of a small source term of E and R cells leads to the disappearance of the trivial nullclines. The new resulting nullclines are illustrated as dashed lines in the phase planes of Fig. 4(a) and (b). Graphical and numerical analysis (not shown) indicates that all the properties pointed out here are generic except for the existence of the trivial state (0, 0), which is not present when there is a source term.

The results discussed in this section are systematically observed in models in which the effector cells can only interfere with the growth of regulator by competition for conjugation sites on the APC, but regulatory cells have an extra inhibitory term which reduces the rate of proliferation of the effector cells (data not shown). Mathematically, this is obtained by setting \( \omega(i, j) < 1.0 \) with \( j > 0 \) and \( \omega(i, 0) = 1.0 \). Moreover, when the value \( \omega(i, j) \) is negative then the model accommodates a biologically distinct mechanism according to which regulatory cells actively kill the effector cells.
3.2.2. Biological interpretation

Again we consider here that the state dominated by regulatory cells is tolerance and the state dominated by effector corresponds to (auto)immunity.

The major property of this model is the existence of parameter region III where bistability is possible. Operating in this region the model may overcome the difficulties discussed in the previous section for models with a globally stable equilibrium. Nevertheless, studies in vitro suggest that regulatory cells when stimulated with APCs in the absence of effector cells do not proliferate (Lombardi et al., 1995; Read et al., 1998; Suri-Payer et al., 1998; Takahashi et al., 1998). This observation raises a problem for the present model. Hence, if the proliferation rate of the regulatory population is set to zero then we will be in a region of the parameter space in which effector cells will always dominate [region I in Fig. 4(b)], i.e. we will be in the presence of global stable equilibrium. Therefore, the present model, as the one before, may be inconsistent with the phenomenon of dominant tolerance when the expansion of the regulatory population alone is experimentally undetectable.

Another property that is relevant for the biology of dominant tolerance is the predicted influence of the number of APCs on the basins of attraction of the steady states of the system. According to this model it is always possible to switch from a state dominated by regulatory cells to a system dominated by effector cells (and vice versa) by increasing the number of antigen-presenting cells. The latter tendency has been shown by in vitro titration of APCs by both Sakaguchi (Takahashi et al., 1998) and Lechler (Lombardi et al., 1994). Also, it is well known that adoptive transfers of tolerance may fail if the total number of cells in the inoculum is low (Le Douarin et al., 1996; Modigliani et al., 1996b). Typically, this is interpreted as the result of the absence of regulatory cells in the inoculum. The present model suggests an alternative interpretation according to which what is being done in those experiments is to decrease the values of e and r, such that the dominance of effector cells is favoured. This interpretation also provides a straightforward account for the well-documented observation that partial depletion of a normal T cell compartment may lead to autoimmunity (Sakaguchi et al., 1989, 1994). Also, it is interesting to recall here that auto-reactivity and a reconsideration of horror autotoxicus was only possible after the discovery of adjuvants by Freund. Different animal models of autoimmunity were discovered by injecting self-antigens in CFA, the most notable example being experimental allergic encephalomyelitis (EAE). One of the effects of adjuvants is simply promoting the influx of antigen-presenting cells to the place where the antigen is and their local activation. The present model, but also the one discussed in the next section, gives a natural interpretation to this well-known fact.
3.3. MODEL 3: THE GROWTH OF R CELLS IS DEPENDENT ON E CELLS

The third particular case of the general model encloses in a single mathematical formalism several different mechanisms of interest. It is obtained by setting \( z_{e,i}(i,j) = \omega(i,j) \pi_{r}i - \eta \mu ij \) and \( z_{r,i}(i,j) = \pi_{r}j + \mu ij \) in eqns (9)–(11), where \( \omega(i,j) \) is defined as in the previous section. This yields

\[
\frac{de}{dx} = \sigma_{e} + \pi_{e} \sum_{i=1}^{s} j a_{i}o(e,r) - \eta \mu \sum_{i=1}^{s} \sum_{j=1}^{s} ij a_{i}j(e,r) - e_{f},
\]

(28)

\[
\frac{dr}{dx} = \sigma_{r} + \pi_{r} r_{e} + \mu \sum_{i=1}^{s} \sum_{j=1}^{s} ij a_{i}j(e,r) - r_{f},
\]

(29)

where we have (see demonstration in Appendix B)

\[
\sum_{i=1}^{s} \sum_{j=1}^{s} ij a_{i}j(e,r) = \frac{s-1}{s} A e_{r} \approx \frac{s-1}{s} e_{r},
\]

(30)

The latter is a good approximation for values of \( A \) big enough. In the following analysis of this model, values of \( A \) greater than 100 are systematically used (even when studying the dependence on \( A \)).

From the comparison of eqns (28) and (29) and eqns (24)–(26), it is noticeable that this model adds a new interaction term to the previous one (being reduced to it when \( \mu = 0 \)). In order to explore the properties that are unique to this new term of interaction we will set \( \pi_{r} \) to zero, which will naturally account for the observation that regulatory cells do not proliferate when cultivated alone with APC.

The new interaction can be interpreted in different ways depending on the values of \( \mu \) and \( \eta \). Two particular cases with biological interest are the following. In the case \( \eta = 0 \) the new interaction term is understood as an effector-dependent proliferation of the regulatory cells. In the case \( \eta = 1/\theta_{r} \) [note that the relationship between the time-scales in eqns (28) and (29) is given by \( \theta_{r} \)] the equations represent a regulatory-dependent differentiation of effector to regulatory cells, as it has been proposed by Waldmann et al., (Qin et al., 1993) and others (Modigliani et al., 1995). The same parameter setting would also account for the possibility that regulatory cells actually kill the effector cells but are stimulated to proliferate during this interaction. Any other combination of the values of \( \mu \) and \( \eta \) can of course be interpreted as a mixture of these elementary mechanisms.

3.3.1. Modelling results

As in the previous sections, we are going to first analyse the model in the simplest situation where the influx of new T cells is zero and only then generalize the major qualitative properties to the situation when this influx is positive but small.

In this last particular model, in the absence of influx, the non-trivial nullcline for \( e \) is similar to the one in the previous section; however, the non-trivial nullcline for \( r \) is different, reflecting the effector-dependent growth of R cells (Fig. 6). The latter is a parabolic-curve that never crosses the trivial nullcline of \( e \), i.e. both arms of the curve cross the trivial nullcline of \( r \) (Fig. 6). The shape of this nullcline reflects the most distinctive property of the model, which is the impossibility of sustaining an R cell population in the absence of E cells (a direct consequence of setting \( \pi_{r} \) to zero).

The model shows four types of phase planes in \((e,r) \in (R_{0}^{0},R_{0}^{+})\), depending on how the parameters determine the positions of the nullclines and their intersections (Fig. 6b). As we will pinpoint, they show some properties which are analogous to the ones of the previous models. For this reason we will call them again as parameter regions O, I, II and III. Nevertheless, there are some different properties that will also be indicated.

In the parameter regime O, the phase plane is identical to the ones in all previous models. It is characterized by the presence of only the trivial nullclines in the quadrant \((R_{0}^{0},R_{0}^{+})\), such that the only steady state is the trivial one \((0,0)\).

The phase plane in parameter region I is characterized by the non-trivial nullcline of \( e \) passing in the quadrant \((R_{0}^{0},R_{0}^{+})\) where it does not intersect the non-trivial nullcline of \( r \) (whether or not this one is in the same quadrant). Under these conditions the model has one unstable trivial steady state \((0,0)\) and one globally stable steady state defined by the intersection of the non-trivial nullcline of \( e \) and the trivial nullcline of \( r \) (Fig. 6). The qualitative properties in this parameter
region are therefore very similar to the ones of the previous models in analogous region, namely by the fact that independently of the initial conditions the population of effector cells will always exclude by competition the population of regulatory cells R.

In parameter region II, the non-trivial nullclines of $r$ and $e$ are both in the quadrant $(e, r) \in (R_r^0, R_r^0)$ where they cross only once. Under these conditions there is an additional steady state in the intersection between the two non-trivial nullclines. This is a focus, that is globally stable, because the steady state that was stable in parameter region I, now is unstable. Similar to parameter region II in the previous models, a globally stable steady state is predicted where regulatory cell population persists. However, while in the previous models this steady state was characterized by the exclusion or extinction of the effector cell populations, in this final model, the steady state in which regulatory cells can dominate requires also the persistence of effector cells.

In the phase plane corresponding to parameter region III the non-trivial nullclines of $r$ and $e$ intersect twice, such that a fourth steady state appears. This fourth steady state is a saddle point. In this parameter regime, the steady state dominated by $e$ cells is again stable as in region I, and the focus, where $r$ cells persist, which was always stable in region II, can now be stable or unstable depending on the value of $\eta$. When $\eta = 0$ the focus is always stable and when $\eta > 0$ it can be either stable or unstable. This parameter region is similar to region III in the previous model because it displays four steady states and can show bistability. It has, however, two major differences that were already mentioned. First, the focus can actually be unstable such that bistability is not observed (see Fig. 7), and second, when the focus is stable both regulatory and effector cell populations persist.

Although this final model is extremely nonlinear it is interesting that parameter regimes O, I, II and III can be simply represented in the plane $K_e \pi_e$ vs. $K_r \mu$, for different values of $s$ [Fig. 4(b)] and for different ratios $\eta' = \eta K_e / K_r$ [Fig. 6(c)]. The boundaries between region II and regions I and III can be derived analytically as in the case of the previous models, being defined by the following conditions on the parameters:

$$s (K_e \pi_e)^2 - s (s - 1) (K_e \pi_e) (K_r \mu) + (s - 1) (K_r \mu) = 0.$$  

(31)

These conditions correspond, respectively to, on the one hand, the intersection of the lower arm of the non-trivial nullcline of $r$, to the upper arm of the non-trivial nullcline of $e$ at the axis $r = 0$, and on the other hand, the intersection of the upper arm of the non-trivial nullcline of $r$, to the upper
In Model 3 the influx of new cells to the effector cell population stabilizes the steady state dominated by regulatory cells, within parameter region III. In the absence of an influx of effector cells ($\sigma_e = 0.0$) the dynamics of the system (---) can range from damped oscillations with low amplitude (a), to damped oscillations with unrealistic amplitude (b) or even divergent oscillations are obtained, when the equilibrium is unstable (c). A small influx ($\sigma_e = 0.1$) into the effector cell populations stabilizes the dynamics for the system in all these cases (---). Initial conditions: (a) $r(0) = 1, \ e(0) = 0.1$; (b) $r(0) = 1, \ e(0) = 0.1$; (c) $r(0) = 1, \ e(0) = 0.7$. Parameters: (a) $\pi_e = 14, \ \eta = 1, \ \mu = 2, \ s = 5, \ K_e = K_r = 1, \ \sigma_e = 0$; (b) $\pi_e = 14, \ \eta = 1, \ \mu = 2, \ s = 5, \ K_e = K_r = 5, \ \sigma_e = 0$; (c) $\pi_e = 14, \ \eta = 5, \ \mu = 6, \ s = 5, \ K_e = K_r = 1, \ \sigma_e = 0$.

While changing $\eta'$ does not affect the existence of the four regions in the plane $K_e, \pi_e$ vs. $K_r, \mu$, when $s$ is unitary both regions II and III collapse. This is obviously expected from the assumption that growth of the regulatory cell population is dependent on interactions between effector cells and regulatory cell in multicellular conjugates.

Despite the structural differences in the phase planes of this model and the previous one, they show some similarities: both models can show the same three types of phase planes/parameter regimes (two globally stable regimes dominated by either effector cells or regulatory cell, and bistability with dominance of either regulatory or effector cells); both models show a bifurcation which allows to switch the system between parametric regions III and I by changing parameter A alone; and finally within bistable regime III it is always possible to favour the dominance of E cells by increasing A (reciprocally to favour the dominance of R cells by decreasing A).

As in the previous cases, the addition of a small source term for effector and regulatory cells, does not change the qualitative properties of the model. The only exception is that the introduction of a source term of effector cells but not of regulatory cells is sufficient to stabilize the equilibrium point dominated by regulatory cells (dashed lines Fig. 7). This stabilization is related to an introduction of a curvature in the nullclines crossing in the equilibrium dominated by regulatory cells. As discussed next, this may be a feature which is highly relevant immunologically.

3.3.2. Biological interpretation

The model studied in this section displays bistability and essentially the same qualitative behaviour as the one discussed in the previous section: it accounts for titration of tolerance by titration of regulatory cells, for the influence of antigen-presenting cells, etc. In addition, this final model naturally accommodates the observation of the non-trivial nullcline of $e$ at the axis $r = 0$. As to the boundary between regions I and III we were unable to derive it analytically. The curve in Fig. 6(b) was derived by computing numerically the parameters defining the intersection between the two non-trivial nullclines in the positive quadrant.
that regulatory cells do not proliferate \textit{in vitro} when cultivated alone in the presence of APCs (Lombardi \textit{et al.}, 1994; Read \textit{et al.}, 1998; Takahashi \textit{et al.}, 1998; Thornton & Shevach, 1998), even within the bistability regime. While this observation was, as we mentioned, problematic for the previous model, it is a prerequisite of this final model, which assumes that the expansion of the population of regulatory cells is dependent on the presence of effector cells.

The other property of this last model that is relevant for a comparison with experimental data is the coexistence of both effector and regulatory cells in a state dominated by regulatory cells. This is only predicted by this last model and was not present in any of the previous ones. Experimental reports indicate that populations of both regulatory and effector cells coexist in tolerant animals as can be revealed by selective induction of autoimmunity or tolerance in recipient animals receiving different subpopulations of cells. Many of these experiments are performed in euthymic animals where a residual population of effector cells could be maintained by a continuous influx of thymic-derived cells. All candidate mechanisms that we specified and analysed here are qualitatively compatible with this observation. However, in some cases, the same observation has been made in athymic animals (Modigliani \textit{et al.}, 1996b), and this will strongly favour the mechanisms in which the growth of the regulatory cells population is dependent on continuous interactions with effector cells.

Also interesting from the point of view of the relationship with experimental observations is the finding that the influx of cells can determine the robustness and the stability of the steady state dominated by regulatory cells. Interestingly, it is well known that thymectomized animals, as compared to euthymic animals, are more susceptible to procedures that induce autoimmunity (irradiation, stimulation, etc.). The traditional interpretation is that the maintenance of peripheral tolerance is dependent on a continuous influx of new regulatory cells from the thymus. Interestingly, our results suggest a less intuitive interpretation. Thymectomy would deprive the periphery of new coming effector cells switching the system from a parameter regime in which the state dominated by regulatory cell is stable to a regime in which this state is now unstable or less resistant to perturbations. That is to say, it is the influx of new effector cells from the thymus which reinforces the stability of the tolerant state and not the influx of regulatory cells.

4. Discussion

The goal of the present article was to further our understanding on the mechanisms of linked suppression and its putative role in dominant tolerance, by exploring its requirement of a simultaneous interaction between three cell types. To achieve this goal we developed a mathematical tool to follow the interactions in multicellular conjugates. To our knowledge, this report is the first mathematical analysis of this type of cell interaction.

The dynamical models that take into account the interaction between more than three cell types must \textit{a priori} be quite complex. As we have seen, the number of variables and differential equations, which count the number of high-order conjugates being formed and dissociated, is controlled by the number of conjugation sites on the APC, $s$. Actually, the number of equations increases with the square of $s$. The quasi-steady-state approach we followed overcomes this difficulty proposing an algebraic solution for the quasi-steady-state distribution of the high-order conjugates between two classes of T cells and their APC. This allowed us to reduce the number of equations to the actual number of cell types. The strategy involves two steps: the calculation of the number of T cells in the conjugates and their distribution by the APCs. This approach will hold as long as the conjugation sites are independent. Moreover, it can be generalized for more than two T cell subpopulations. However, there is an analytic solution only for systems with up to three subpopulations, since the roots of a polynomial with order equal to the number of subpopulations plus I are involved in calculating the number of cells in each class that are conjugated. For orders greater than 3 some other approximation can be used (De Boer & Perelson, 1995); however, this should be systematically compared with numerical solutions.

The basic model we proposed may represent a very general tool to deal with simultaneous
interactions between more than two cell types since different detailed mechanisms can be specified by an appropriate setting of the interaction coefficients determining the consequences of each high-order conjugate (Table 1). The application of this formal tool in situations other than the current application in dominant tolerance can be quite straightforward. For example, it was proposed recently that the interaction between CD4+ T cells and CTLs does not involve the simultaneous interactions between the two cells on the same antigen-presenting cell, as classically proposed (Mitchison & O'Malley, 1987; Mitchison, 1990), but that the APC may act as a temporal bridge (Bennett et al., 1998; Ridge et al., 1998; Schoenberger et al., 1998). The formalism presented here can be very easily adapted to analyse in detail the quantitative implications of the two different mechanisms (Leon & Carneiro, unpublished data).

An important question at this point is of course: how do these mathematical models help in understanding dominant tolerance mediated by linked-suppression? The experimental systems that reveal and investigate the mechanism of dominant tolerance are very diverse. Many experiments are performed in normal animals while others make use of experimentally manipulated animals. The latter always raise the possibility that we are dealing with some consequence of the manipulation rather than a natural mechanism. In adoptive transfers of tolerance different authors use different T cell subpopulations as sources of effector and regulatory T cells. For example, as source of effector and regulatory cells, respectively. Powrie (Powrie et al., 1997) typically uses CD45RBlow and low CD4+ T cells while Sakaguchi (Sakaguchi et al., 1995) uses CD25− and CD25+ CD4+ T cells. Although, in general, both CD25+ and CD45Rblow cells are considered to be “antigen experienced”, the relationship between the two populations is not straightforward (Read et al., 1998). Under these conditions, it is very difficult to provide a unifying hypothesis. Instead, we illustrated how the general model, made particular for specific cases, can be useful to interpret experimental observations and to narrow down the alternative candidate explanations. We evoked different experimental observations and tried to indicate the conditions under which they can be consistent with the alternative mechanisms of linked suppression. Overall, the modelling results reported here and the whole set of observations that we discussed would strongly favour two candidate mechanisms. These are the ones that are translated by the final model: regulatory T cells inhibit the proliferation of effector cells while they are nevertheless dependent on a growth factor that the latter produce; or regulatory cells inhibit the expansion of the population of effector cells because they convert them to the regulatory phenotype. Either mechanism or both may be operative in vivo, maybe even dependent on the life history of the effector cells. Modigliani et al. (1996b) actually demonstrated that regulatory cells from thymic epithelium chimeras can both suppress the responses of mature effector cells and promote the differentiation of recent thymic emigrants into the regulatory phenotype.

The experimental reports on dominant tolerance in vivo are most of the time qualitative rather than quantitative. For this reason, this paper explored the generic qualitative properties of the different models. In vitro suppression assays are more quantitative than in vivo adoptive transfers, and therefore they represent a better area to try to test the quantitative predictions of the models. Elsewhere, we analyse the experimental data of linked suppression in vivo. We explore the simple fact that there must be a limit in the frequency of encounters leading to multicellular conjugates, and show that only a few mechanisms could lead to the levels of in vitro suppression reported (Leon et al., unpublished data).

The full understanding of dominant tolerance may eventually require a consideration of the clonal diversity of the T cell repertoire. Lafaille et al. (1994), and Olivares-Villagomez et al. (1998) reported that the onset of autoimmunity mediated by an auto-reactive transgenic population of T cells is prevented by regulatory T cells with an endogenous TCR. A consideration of this type of phenomenon suggests an immediate extension of our present model to consider the dynamics and potential heterogeneity of the population of APCs and clonal composition of regulatory and effector T cell populations. This extension is not only experimentally motivated but also has an important theoretical justification. Hence,
the most serious theoretical problem faced by any candidate model of dominant tolerance is to explain how an organism is able to mount effective immune responses to invading pathogens and remains, nevertheless, self-tolerant (Carneiro, 1997). Although some qualitative considerations about the role of repertoire diversity in tolerance can be found in the literature, the issue has never been addressed properly, and therefore represents an area for development of the modelling strategy reported here.

We thank Jose Faro and Joao Sousa for many useful discussions, during the realization of this work and John Stewart for critically reading this manuscript.

This work is supported by Program Praxis XXI of the Ministério para Ciência e Tecnologia, Portugal (grant Praxis/P/BIA/10094/1998). JC is supported by Fundação para a Ciência e Tecnologia—Program Praxis XXI (fellowship BPD/11789/97). KL is partially financed by the Fundação Calouste Gulbenkian.

REFERENCES


Combining eqns (A.2) and (A.1), we obtain two terms of constants we of E and R cells and their respective conjugation base to obtain the following third-order algebraic equation:

\[ 0 = \{K_E K_R\} F^3 + \{K_E + K_R - K_E K_R\} \times (sA - R - E) F^2 + \{1 - K_E (sA - E) - K_R (sA - R)\} F - sA. \quad (A.6) \]

This equation has three roots, but only one is biologically meaningful. Although this root of eqn (A.6) can be expressed algebraically it is very complex and will not be presented here. \( E_c \) and \( R_c \) are then obtained by replacing \( F \) in eqns (A.5) by the root of eqn (A.6), and \( E_F \) and \( R_F \) are obtained by replacing \( E_c \) and \( R_c \) in eqns (A.3).

We demonstrate that there is only one biologically reasonable root of eqn (A.6) by reduction to the absurd. Assume that there are two roots of \( F \) which are biologically reasonable denoted by \( F_1 \) and \( F_2 \). Assume that \( F_1 > F_2 \). If the latter is true then eqn (A.3) implies

\[ (E_c + R_c) > (E_c 1 + R_c 1). \quad (A.7) \]

On the other hand, by using eqn (A.5) the following relationship is derived between the value of \( E_c + R_c \) and the corresponding value of \( F \):

\[ E_c + R_c = \frac{FK_E}{1 + FK_E} E + \frac{FK_R}{1 + FK_R} R. \quad (A.8) \]

This relationship expresses a monotonously increasing dependence between \( F \) and its corresponding value of \( E_c + R_c \), since both belong to \( R_0^+ \). The bigger the \( F \) the bigger the corresponding \( E_c + R_c \). So if inequality (A.7) and relation (A.8) are true then necessarily \( F_2 > F_1 \). The latter is the negation of the original premise \( F_1 > F_2 \) demonstrating the impossibility of having two different biologically reasonable solutions for this equilibrium.

The procedure described here to obtain the equilibrium numbers of APC-conjugated and free cells in a mixture of several T cell populations is easily extended to more than two populations, eventually \( N \) of them. In this case, the equation for \( F \) is obtained as an algebraic equation of order equal to \( N - 1 \).
APPENDIX B

Analysing the properties of the distribution of T cells in APC sites

In Section 2.1, it was shown that if the antigenic sites in a single APC are assumed to be independent (postulate 4), then the frequency of APC containing \( i \) E cells and \( j \) R cells, is given by eqn (15). The latter equation is composed of two hypergeometric distributions defined according to eqn (16). Substituting eqn (16) into eqn (15) and simplifying it yields:

\[
a_{i,j,k}(e, r, h) = \frac{(eC_A)^i (rC_A)^j (s - eC - rC)^k}{(sA)^{i+j+k}}
\]  
\[
\text{free sites per APC and the number of free sites in APC containing} i \text{E and} j \text{R cells. This gives us a straightforward procedure to extend expression (B.1) to the case of more than two types of T cells. For instance, for three types of cells E, R, H the following is obtained:}
\]

\[
a_{i,j,k}(e, r, h) = \frac{(eC_A)^i (rC_A)^j (hC_A)^k (s - eC - rC - hC)^{i+j+k}}{(sA)^{i+j+k}}
\]  

Let us study now some simplifications of interest in expression (B.1). Particularly, we are interested in the following relation:

\[
\sum_{i=0}^{s-j} i a_{i,j}(e, r) = \text{Hyp}[j, rC_A, sA, s] \frac{s-j}{s-rC} e_C
\]  

Note that the particular case of this expression, when \( j = 0 \) is used in Section 3.2. To demonstrate that eqn (B.3) holds it is enough to multiply and divide eqn (B.1) by

\[
\left( \frac{(s - rC) A}{s - j} \right)
\]

obtaining:

\[
a_{i,j}(e, r) = \left( \frac{rC_A}{j} \right) \left( \frac{s - rC}{s - j} \right)
\]

\[
\times \left( \frac{(eC_A)^i (s - eC - rC)^{i+j+k}}{(sA)^{i+j+k}} \right)
\]

which according to eqn (15) is equivalent to

\[
a_{i,j}(e, r) = \text{Hyp}[j, rC_A, sA, s]
\]

\[
\times \text{Hyp}[i, eC_A, (s - rC)A, s - j].
\]  

Substituting then eqn (B.4) on the left-hand side of eqn (B.3) we get

\[
\sum_{i=0}^{s-j} i a_{i,j}(e, r) = \text{Hyp}[j, rC_A, sA, s]
\]

\[
\sum_{i=0}^{s-j} i \text{Hyp}[i, eC_A, (s - rC)A, s - j].
\]  

Inserting into eqn (B.5) the expression for the value of the median in hypergeometric distribution reported elsewhere:

\[
\langle N \rangle = \sum_{N=0}^{K} N \text{ Hyp}[N, No, M, K] = NoK/M.
\]  

We get the expression proposed in eqn (B.3), which for the particular case \( j = 0 \) stands

\[
\sum_{i=0}^{s} i a_{i,0}(e, r) = \frac{\text{Hyp}[0, rP_A, sA, s] s}{s - rC} e_C
\]  

\[
\sum_{i=0}^{s} i a_{i,0}(e, r) = \frac{\text{Hyp}[0, rP_A, sA, s] s}{s - rC} e_C
\]
Note that the latter expression has a very simple interpretation: the numerator is the number of free sites in APC with \( j = 0 \) R cells, while the denominator is the total free sites that can be occupied by the E cells “once the conjugated R cells are already distributed”.

The other particular relation of interest for us in this work is

\[
\frac{s}{j=0} \sum_{i=0}^{s-j} t_i a_{i,j}(e, r) = \frac{(s-1)A}{(sA-1)} e_c r_c. \tag{B.8}
\]

To demonstrate this relation let us first substitute eqn (B.3) on the left-hand side of eqn (B.7) getting

\[
H = s \sum_{j=0}^{s-1} j a_{i,j}(e, r)
= \sum_{j=0}^{s} \text{Hyp}[j, rC A, sA, s] \frac{s-j}{s-r_c} e_c
= \sum_{j=0}^{s} j \text{Hyp}[j, rC A, sA, s] \frac{s}{s-r_c} e_c
- \sum_{j=0}^{s} j^2 \text{Hyp}[j, rC A, sA, s] \frac{1}{s-r_c} e_c. \tag{B.9}
\]

Using expression (B.6) here we get

\[
H = s \frac{r_c e_c}{s-r_c} - \frac{e_c}{s-r_c} \sum_{j=0}^{s} j^2 \text{Hyp}[j, rC A, sA, s]. \tag{B.10}
\]

Finally, obtaining \( \langle N^2 \rangle \) from the expression of the standard deviation of the hypergeometric distribution:

\[
A^2 N = \langle N^2 \rangle - \langle N \rangle^2 = \frac{N_0 K (M - K)}{M (M - 1)} \left( 1 - \frac{N_0}{M} \right). \tag{B.11}
\]

Substituting it into eqn (B.10), we recover eqn (B.8), demonstrating the validity of the latter expression.