



A Model of the Immune Network with B-T Cell Co-operation. I—Prototypical Structures and Dynamics

JORGE CARNEIRO,^{†§} ANTÓNIO COUTINHO,[†] JOSE FARO[‡] AND JOHN STEWART[†]

[†] *Unité d'Immunobiologie, CNRS URA 1961, Institut Pasteur, Paris, France and* [‡] *Departamento de Física Aplicada, Universidad Salamanca, Spain*

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Hitherto, “second generation” network models of the immune system have all been restricted to B-lymphocytes and the Ig molecules they produce. These models have not so far been able to provide a convincing mechanism for the distinction between a “Central Immune System” (CIS) composed of a connected network of lymphocyte clones which couple with “self” antigens in a tolerant mode, and a “Peripheral Immune System” (PIS) composed of clones with little or no supra-clonal organization and which produce classical immune responses when interacting with “non-self” antigens. Here, we present a new network model which explicitly incorporates B-T cell co-operation. In this model, B-cell activation is dependent on T-cell help, and activated T-cells are down-regulated by engagement of their TCRs by soluble Ig. We discuss the underlying biology on which we base the system of ordinary differential equations which defines the present network model. We then illustrate some basic features of the model by examining several prototypical situations with a small number of clones. Depending on the idiotypic connectivity structure, the model exhibits two distinct modes of coupling with antigens: an “immune response” mode in which T- and B-cell clones grow exponentially; and a “tolerant” mode in which T-cell clones are controlled by inclusion of all TCRs in the repertoire of an idiotypic B-cell network. Finally, we discuss the simplifying assumptions of the present model and argue that its range of validity is indeed the region of the state-space of the system where the discrimination between the CIS and the PIS takes place.

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1. Introduction

It has been suggested (Huetz *et al.*, 1988a; Coutinho, 1989) that the immune system (IS) is composed of two compartments, a Central system (CIS) and a Peripheral system (PIS), which differ essentially in their supra-clonal organization. The CIS is conceptualized as a connected network of activated lymphocyte clones which are dynamically constrained to frequencies or concentrations that preclude their engagement in typical immune responses. Antigens which are available throughout ontogeny and which interact with the components of the CIS are

[§] Author to whom correspondence should be addressed at: Unité d'Immunobiologie, Institut Pasteur, 25, rue du Dr. Roux, 75724 Paris Cedex 15, France.
E-mail: carneiro@pasteur.fr

integrated in its repertoire, and participate in its stable dynamics. The PIS is the set of lymphocytes which are decoupled from the CIS and show shallow or no supra-clonal organization. The individual clones in the PIS are free to grow exponentially and differentiate when stimulated, and are thus able to follow classical immune responses when interacting with available antigens.

The conceptual distinction between a CIS and a PIS is based on a large body of experimental evidence (Coutinho, 1989), and has already proven its heuristic value. However, the fact is that, at present, this distinction is essentially descriptive; to date no convincing *mechanism* has been proposed which could give rise to the CIS/PIS distinction as an emergent self-organized property of the IS. The “second

generation" network models of the immune system (Varela & Coutinho, 1991) designed to date have all been restricted to B-lymphocytes and the Ig molecules they produce (Varela *et al.*, 1988; De Boer & Perelson, 1991; Faro & Velasco, 1993b). The results of these studies raise the real possibility that such models may not be capable of illustrating the interplay between the CIS and the PIS. The inherent problems are dual: (i) the developing network tends to expand by the continuous recruitment of new clones until it becomes virtually complete, so that there is no appropriate partitioning of the potential repertoire into network and disconnected fractions (De Boer & Perelson, 1991; Stewart & Varela, 1991; Detours *et al.*, 1994); and/or (ii) the network is not reliably stable when coupled to antigens that are continuously available (optimally stimulated clones tend to produce immune responses and to disconnect from the network) (Calenbuhr *et al.*, 1995; Detours *et al.*, 1994). It is true that the available models have not been explored to their fullest potential, and we cannot rigorously exclude the possibility that they may yet manage to show how the CIS/PIS distinction can be achieved. However, we have developed the conviction that by restricting the models to a single lymphocyte compartment we may be over-simplifying and missing essential features afforded by co-operation with other components of the IS. This conviction is based not only on the behaviour of current "second generation" models, but also on compelling experimental evidence.

The active participation of $\alpha\beta$ T-lymphocytes in the selection of the immune B-cell repertoire is very well established, as they are strictly required for efficient immune responses (Hill & Chapel, 1993), and/or autoimmune diseases (either "spontaneous" or their experimental models) (Traugott *et al.*, 1983; Botazzo *et al.*, 1985; Holmdahl *et al.*, 1985; Londei *et al.*, 1985; Zamvil *et al.*, 1985; Bendelac *et al.*, 1987; Lindstrom *et al.*, 1988; Singer & Theofilopoulos, 1990; Marguerie *et al.*, 1992). Even when autoimmune pathology is mediated by antibodies, the active role of T-lymphocytes in the process is made evident by the high frequency of class-switch and somatic mutation (Marion *et al.*, 1992; Randen *et al.*, 1992; Zouali, 1992). Similarly, the co-participation of B- and T-lymphocyte compartments in the establishment of the pre-immune repertoire, and, thus, the establishment of the CIS, is suggested by the "autonomous" activity of the IS in the absence of external challenges. Non-immunized specific pathogen-free, germ-free or antigen-free mice have near-normal levels not only of natural antibodies and activated B-cells (Hooijkaas *et al.*, 1984) but also of activated T-cells (Pereira

et al., 1985, 1986). Such "background" activity in the B-cell compartment is not independent of that in the T-cell compartment, as is shown by the recursive selection of the actual repertoires of activated B- and T-cells (Marcos *et al.*, 1988; Pereira *et al.*, 1989), and the T-cell dependence of natural B-cell activation (Huetz *et al.*, 1988b).

In the present article we therefore question the assumption, underlying previous "second generation" network models, that T-cell help is never a limiting factor for B-lymphocyte proliferation or Ig-production. In an extended version of the model proposed by Varela and co-workers (Varela *et al.*, 1988), we make the activation of B-lymphocytes explicitly dependent on co-operation with activated T-lymphocytes. We explore the major qualitative changes in the behaviour of the model introduced by this amendment, paying special attention to the modes of coupling between the system and antigens. Many previous immune network models started by studying network properties in the complete absence of antigenic perturbation (De Boer & Perelson, 1991; Stewart & Varela, 1991); by contrast, the present model includes antigens as one of its variables from the outset. Understanding the distinction between the CIS and the PIS starts by understanding how the network develops and co-exists stably with the somatic antigens.

In this article we formulate a minimal model of the dynamics of a normal "pre-immune" lymphoid system. We discuss the underlying biology (Section 2.1) on which we base a system of ordinary differential equations (Section 2.2). We then present some basic features of the model by illustrating them in simple prototypical situations with a small number of clones, paying special attention to the innovative features of the model as compared with previous "second generation" immune networks. In particular, we show that depending on the idiotypic connectivity structure, the present model is capable of both CIS and PIS modes of coupling with antigens. Finally, we discuss the simplifying assumptions of the present model and argue that the domain in which these assumptions are valid is indeed the region of the state-space of the system where the discrimination between the CIS and the PIS takes place.

In a companion article (Carneiro *et al.*, 1996) we give substance to these considerations by showing that an appropriate distinction between a CIS and a PIS can indeed be achieved in numerical simulations of a model with continuous generation and metadynamical recruitment of new clonotypes. The results

of these simulations lead to a reconsideration of some classical concepts such as the “immunological self” and the selective pressure for generation of diversity.

2. The Model

2.1. BIOLOGICAL BACKGROUND AND QUALITATIVE POSTULATES

The components of the model presented here are: peripheral B-lymphocytes and the Ig molecules they produce; peripheral T-helper lymphocytes; and available antigens in the body. B- and T-lymphocytes are continuously produced from precursors in bone marrow and thymus respectively. The fate of each peripheral B- or T-lymphocyte depends on its clonal receptor ligation and its interactions with other cells in the IS: it will either be activated (to proliferate and perform effector functions), or else it will die within a few days.

B-lymphocytes are rescued from death if they are activated following productive ligation of their membrane Ig-receptors (mIg). This process involves two steps: firstly, crosslinking of mIg leads to adequate expression of membrane proteins (adhesion molecules and co-receptors) that promote and enable co-operation with activated T-cells (the *induction step*); secondly, full activation of the B-cell follows actual co-operation with activated T-cells (the *activation step*). Once activated, B-lymphocytes both proliferate (resulting in clonal expansion) and secrete soluble Ig-molecules. Induced B-cells that are not fully activated will revert to a resting state.

T-cell activation depends on the specific TCR-dependent interaction of resting T-cells with antigen presenting cells (APC). Once activated, T-cells proliferate, and they can also trigger and modulate the activation of B-cells, both by specific cell-to-cell co-operation and by cytokine production.

Multivalent Ig-molecules, secreted in soluble form by activated B-cells, attain a homogeneous concentration in the body fluids, and contribute to the induction of B-cells and/or the regulation of activation state of both B- and T-lymphocytes by binding their receptors. Similarly, antigens available in the body can contribute in their native conformation to the induction of B-cells; once processed and presented by the major histocompatibility complex protein as (MHC) + peptide assembled complex on the membrane of unspecific APCs, they can also drive T-cell activation.

In addition to these general assumptions, the model we present here is based on a further set of six qualitative postulates that are stated below and

illustrated in Figs 1 and 2; the biological justification for these postulates is presented in the Appendix. We would like to emphasize that this set of simplifying assumptions is only meant to be valid when considering variable region-specific interclonal interactions during a “pre-immune” steady state in a normal IS. In conditions in which the immune system is rapidly and strongly perturbed (for example by antigenic challenges that lead to clonal expansion and dominance) some of these simplifications almost certainly fail to hold. Moreover, mechanisms for activating Ig-production by B-cells in the absence of T-lymphocytes exist and may operate in the autonomous activities in germ-free animals. To date there is no conclusive evidence that naturally activated antibody repertoires are different in T-cell competent and deficient mice (Freitas *et al.*, 1989; Malanchère *et al.*, 1995).

- (i) There are no specific interactions between resting lymphocytes. Productive interactions occur only between “co-operation prone” activated T-cells and induced (or activated) B-cells.
- (ii) B-lymphocyte induction and T-cell activation require the cross-linking or polymerization of their clonal receptors.
- (iii) Primary activation of resting T-lymphocytes is triggered by co-operation with unspecific APCs, involving the specific recognition of a set of dominant and frequent antigenic peptides in the context of MHC molecules. The contribution of B-lymphocytes is irrelevant.
- (iv) Primary induction of resting B-lymphocytes follows cross-linking of mIg by multivalent ligands: these are either common antigens, or soluble idiotypic Ig molecules.
- (v) In order to engage and sustain co-operation with an activated T-cell, an induced B-cell must somehow engage its TCR either by presenting MHC + peptide complexes that are specifically recognized by that T-cell, or by direct mIg-TCR interactions.
- (vi) Soluble Ig-molecules are inhibitory for T-cells.

It is worth pointing out explicitly that in the present model, this inhibition by anti-TCR soluble Ig is the *only* possible down-regulatory influence on activated T-cells. This is of course a major simplification.

2.2. THE DIFFERENTIAL EQUATIONS AND QUANTITATIVE POSTULATES

The translation of the qualitative model outlined above into an explicit set of quantitative differential equations which can serve as the basis for computer simulations intrinsically involves a second set of

simplifying assumptions. We will comment on these additional postulates in the presentation of the equations themselves. As in any modelling work, we aim at relating just two levels of description: an underlying “micro” level of cell physiology and molecular interactions, and an emergent “macro” level of the global behaviour of the system (tolerance, immune responses and so on). The differential equations and functional forms used below to describe the underlying “micro” level are purely *ad hoc* and phenomenological; they do not even attempt to reflect the “sub-micro” mechanisms which in turn give rise to the properties of the underlying components as emergent phenomena in their own right.

2.2.1. The variables

The basic variables in our model immune system are the size of the T-lymphocyte clones (T); the size of the B-lymphocyte clones (B) and the concentration of Ig-molecules they produce (F); and the effective concentration of the available antigens (A). At any given time the state of the system is defined by its composition:

$$\begin{aligned} T_l & \quad l = 1, \dots, N_T \\ B_i, F_i & \quad i = 1, \dots, N_B \\ A_k & \quad k = 1, \dots, N_A \end{aligned}$$

In the present model, the units of the clonal sizes (T) and (B) are “cell numbers”. It is assumed that cell-cell interactions and lymphocyte activation occur locally in the lymphoid organs; and furthermore, that the induction of B-cells and the activation of T-cells is the limiting factor for their productive encounter. The effective concentrations of Ig-molecules (F) and available antigens (A), which diffuse freely, are those in peripheral blood and lymph. This model thus takes into account, to some extent, the spatial organisation of the immune system. A more fully realistic modelling of spatial organisation, including the possibility of stochastic effects, may or may not affect the dynamics and stability of the interactions in the model. This is clearly a potential area for future work in the field not only theoretical but also experimental; in this first approach we have adopted the approach of maximal simplification in order to establish a point of reference.

In the presentation below we will use the following notation: (i) the main variables are identified by indexed roman capital letters; (ii) intermediate variables are identified by small case greek letters; (iii) parameters are identified by small case roman letters (with indicative suffices); (iv) finally interaction

coefficients between the main variables X_i and Y_j are denoted as M_{ij}^{XY} where XY identifies the nature of the interacting variables and i and j identify the particular clonotypes.

2.2.2. The basic equations

The size of a given T-lymphocyte clone (T_l) decreases exponentially with a constant Death rate (k_{DT}), and increases as a function of its average proliferation rate and thymic output. The effective Proliferation rate is proportional (k_{PT}) to the number of activated cells (function α_T), which is determined by the combined effect of both stimulatory (π_l) and inhibitory (η_l) signals (see below). The term $\xi(l, t)$ corresponds to the thymic output rate for cells of the clone l ; $\xi(l, t)$ is a function which describes the result of processes of gene rearrangement and thymic selection, which may vary with time (t).

$$\frac{dT_l}{dt} = -k_{DT} \cdot T_l + k_{PT} \cdot \alpha_T(\pi_l, \eta_l, T_l) + \xi(l, t) \quad (1)$$

The size of a given B-lymphocyte clone (B_i) decreases exponentially with a constant Death rate (k_{DB}), and increases as a function of its average proliferation rate and *de novo* bone-marrow production. The effective proliferation rate is proportional (k_{PB}) to the number of activated B-cells in the clone (function α_B), which is determined by the amplitude of induction signals (σ_i) and the number of specific activated T-lymphocytes available for co-operation (τ_i) (see below). The contribution of the bone-marrow, resulting from random gene rearrangement and possible local selection, is represented by an appropriate function $\zeta(i, t)$.

$$\frac{dB_i}{dt} = -k_{DB} \cdot B_i + k_{PB} \cdot \alpha_B(\sigma_i, \tau_i, B_i) + \zeta(i, t). \quad (2)$$

The concentration of soluble Ig-molecules (F_i) produced by clone i decreases at a rate proportional to their removal either as free molecules (k_{DF}), or as the complexes they form with available ligands ($k_{DC} \cdot \sigma_i$), and increases proportionally (k_{SF}) to the number of Ig-producing (activated) B-cells [the same function α_B as in eqn (2)].

$$\frac{dF_i}{dt} = -(k_{DF} + k_{DC} \cdot \sigma_i) \cdot F_i + k_{SF} \cdot \alpha_B(\sigma_i, \tau_i, B_i). \quad (3)$$

Although the proliferating and Ig-secreting sub-populations of a B-lymphocyte clone are not always coincident, and this distinction was included in previous mathematical models (Varela *et al.*, 1988), the present model assumes that they are identical (α_B)

in order to reduce the dimension of the parameter space.

2.2.3. *Antigens*

The present model essentially aims at understanding the structural and dynamical features of clonally specific B- and T-lymphocytes, that bring about a stable network mode of coupling with the somatic antigens in the body, and nevertheless make available a significant repertoire of disconnected clones (that, at least potentially, could be driven by other antigens). Since the body has a rather constant composition of endogenous somatic antigens, we will assume that these have a fixed concentration in time (A_k), and constitute the pool of available antigens. Additional antigens can be introduced at various times in the simulated ontogeny of the system.

2.2.4. *Stimulatory and inhibitory signals: T-cells*

The activation state of T-lymphocytes is determined by the combined effect of both stimulatory and inhibitory signals.

As we have argued [postulate (iii) above], in a steady-state IS the principal stimulatory signals are the dominant and frequent MHC + peptide complexes presented by unspecific APC. The process by which the list of somatic antigens “maps” to a set of MHC-presented peptides is very complex and depends (at least) on the following factors: the relative representation of the antigens in the body and their efficiency in being captured by APCs; the differential peptide composition of each antigenic molecule and the nature and efficiency of degradation; the relative efficiency with which the different peptides are processed, assembled with MHC molecules and transported to the cell surface; and finally the relative half-life of each MHC + peptide pair on the membrane. Consequently, the terms π_i in eqn (1) are, in principle, functions ϕ_i of at least the antigen concentrations A_k and the interaction coefficients M^{T^A} :

$$\pi_i = \phi_i(A_k; M_{ik}^{T^A}). \tag{4}$$

The terms π_i are thus akin to intermediate variables, and for this reason they are identified notationally by a Greek letter. However, in line with our previous assumptions concerning the pool of available antigens, the loading of the somatic antigens on the MHC molecules on the membrane of unspecific APCs must be constant in time (as long as we assume that the dynamics of lymphocytes does not significantly interfere with that function). In these conditions, the stimulatory signals perceived by a given T-cell clone

1 may also be considered constant. We therefore propose to bypass the establishment of precise equations for all these processes by simply assuming, for each and every T-cell clone 1, that the terms π_i in eqns (1) and (4) are arbitrary constants with values between zero and one which correspond to the proportion of T-cells in clone 1 that can be *potentially* activated. The proportion of cells which are *actually* activated will be modulated by the inhibitory signals η_i .

Following postulate (vi), the inhibitory signals η_i are calculated as a linear sum of the anti-TCR

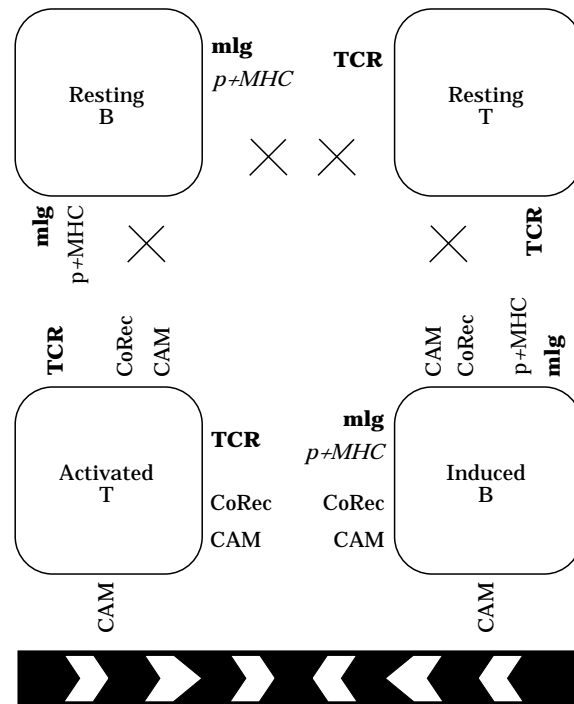


FIG. 1. Interclonal interactions occur only between co-operation prone activated T-lymphocytes and induced (or activated B) lymphocytes. Constitutively expressed clonal receptors (mIg and TCR) are depicted in bold type; (MHC + p)peptide is depicted in italics to emphasize that although MHC molecules are expressed constitutively, membrane representation of a particular peptide is contingent. “CoRec” stands for pan-clonal co-receptors (such as CD40 in B-lymphocytes or B7 family in T-lymphocytes) whose expression is activation or induction dependent; “CAM” stands for cell adhesion molecules (such as CD44 in B-lymphocytes) which are upregulated upon receptor engagement. Constitutive co-receptors or adhesion molecules (such as CD28 in B-lymphocytes or CD40L on T-lymphocytes) are not depicted. The respective sets of CoRec and CAM upregulated in induced B and activated T-lymphocytes are complementary, and thus enable and stabilize B-T cell co-operation. The pattern of CAM expression also allows appropriate interactions with other cells and the extracellular matrix such that co-operation prone lymphocytes follow “convergent” pathways of migration. The down-regulation of CoRec and CAM in resting lymphocytes prevents them from consistently co-operating with other lymphocytes; the lack of such molecules is indicated by the sign “X”.

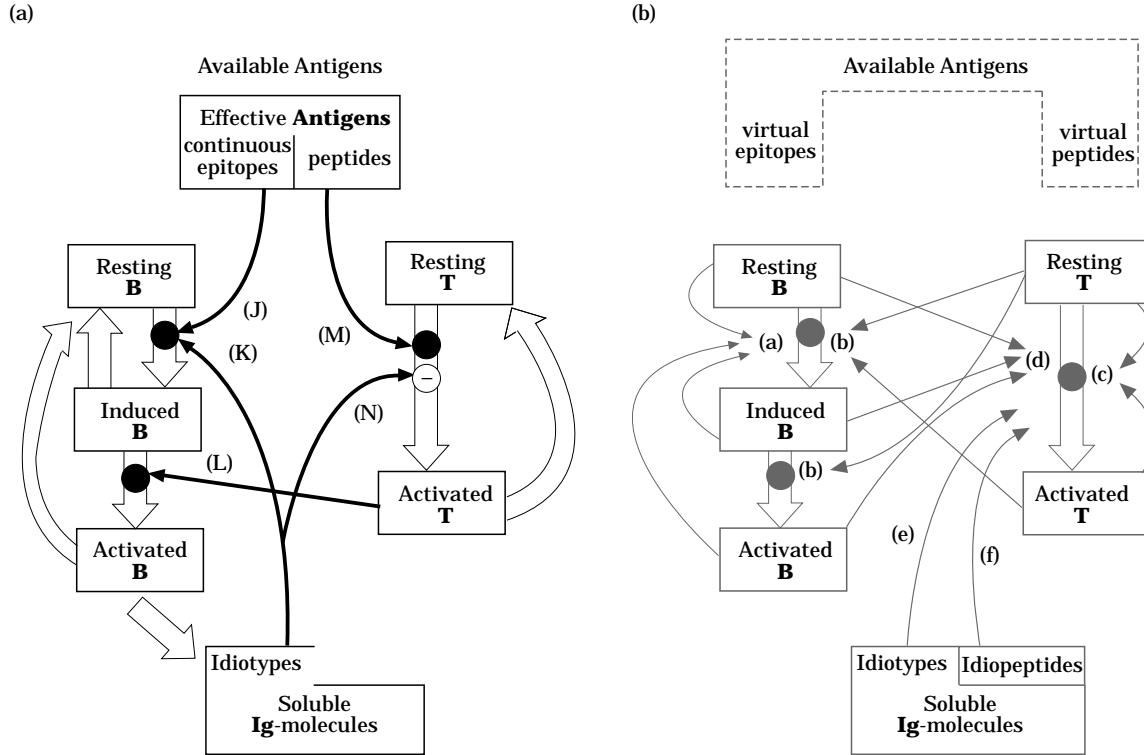


FIG. 2. (a): the components and interactions included in the immune network model. B-lymphocytes: induction of resting B-lymphocytes depends on the engagement of the mIg by native epitopes on antigens (J), and/or by soluble Ig molecules produced by other B-lymphocytes (K); full activation requires co-operation with activated T-lymphocytes (L) mediated either by specific MHC + peptide complexes or by anti-TCR mIg molecules; activated B-lymphocytes can (i) produce soluble Ig molecules, (ii) divide, and/or (iii) revert to the resting state. T-lymphocytes: resting T-lymphocytes are activated following co-operation with APCs which present dominant antigenic peptides (M); this activation event can be inhibited by soluble anti-TCR Ig molecules produced by B-lymphocytes (N); activated T-cells can (i) activate induced B-lymphocytes, (ii) divide, and/or (iii) revert to the resting state. (b): putative components and interactions which have been explicitly omitted from the model on account of their low probability *in vivo*. Induction of resting lymphocytes by specific interactions (a) with other B-lymphocytes or (b) with T-lymphocytes. Specific activation of T-lymphocytes by interactions (c) with T-lymphocytes, or (d) with B-lymphocytes. Activation of resting T-lymphocytes by interactions (e) with anti-TCR Igs polymerized on the membrane of APCs or (f) with MHC + idiopeptides presented by APCs.

Ig-molecules weighted by their respective affinities:

$$\eta_l = \sum_{j=1}^{N_B} M_j^{TF} \cdot F_j. \quad (5)$$

We assume that these inhibitory signals only become effective when they are above a given threshold concentration. We employ a one-sided log-normal function for this inhibitory response curve [eqn (6b)], by analogy with the suppressive side of the cross-linking curve previously postulated for B-cells [see eqn (7) below].

Thus, the number of activated T-cells in our model can be calculated as:

$$\alpha_T(\pi_l, \eta_l, T_l) = \pi_l \cdot T_l; \quad \text{if } \log(\eta_l) < a_{T1} \quad (6a)$$

$$\alpha_T(\pi_l, \eta_l, T_l) =$$

$$\exp \left[- \left(\frac{\log(\eta_l) - a_{T1}}{a_{T2}} \right)^2 \right] \cdot \pi_l \cdot T_l; \quad \text{if } \log(\eta_l) \geq a_{T1} \quad (6b)$$

where π_l is the l -specific constant obtained according to simplification in eqn (4), and a_{T1} and a_{T2} are absolute constants.

This activation function is depicted in Fig. 3(a).

2.2.5. B-cell activation

The activation of B-lymphocytes is a two-step event requiring first the induction by cross-linking of the mIg, and then co-operation with specific T-cell clones.

Step 1. The degree of cross-linking as a function of the concentration of soluble (multivalent) ligand has been previously well studied in biophysical

models, and follows a general bell-shaped curve. According to Faro & Velasco (1993a, 1994), the actual value of the kinetic constant of dissociation has a strong impact in the precise shape and positioning of the activation curve. However, in a maximal simplification, we consider here (as in previous models) that the bell-shaped curve is independent of the affinity and dissociation constants. Thus, the number of *induced* cells in a given B-cell clone i is calculated as:

$$\beta_i = \exp \left[- \left(\frac{\log(\sigma_i) - b_1}{b_2} \right)^2 \right] \cdot B_i \quad (7)$$

where σ_i is the sum of the concentrations of all the multivalent ligands for the Ig of the clone i [soluble Ig-molecules produced by other lymphocytes (F_j) or

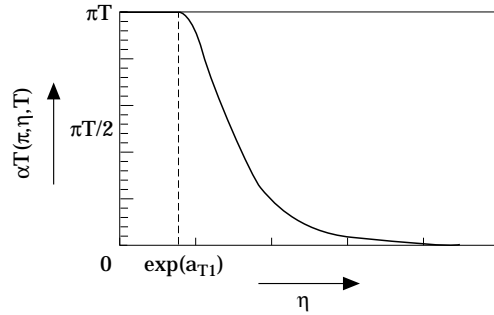
antigen (A_k)], weighted by their respective pairwise affinities:

$$\sigma_i = \sum_{j=1}^{N_B} M_{ij}^{BF} \cdot F_j + \sum_{k=1}^{N_A} M_{ik}^{BA} \cdot A_k. \quad (9)$$

Step 2. The fraction of fully activated cells in B-cell clone i is then calculated as a function of β_i , the number of induced cells in the clone, and τ_i , the number of activated T-cells that are “available” to co-operate specifically with them. In general, the bigger the size of the B-cell clone the more T-cells will be required to activate the same proportion of cells, since a single T-cell can only co-operate with a limited number of B-cells at a time.

$$\alpha_B(\sigma_i, \tau_i, B_i) = \frac{\tau_i \cdot \beta_i}{\tau_i + \beta_i}. \quad (10)$$

(a) Activation Function for T-Lymphocytes: $\alpha_T(\pi, \eta, T)$



(b) Activation Function for B-Lymphocytes: $\alpha_B(\sigma, \tau, B)$

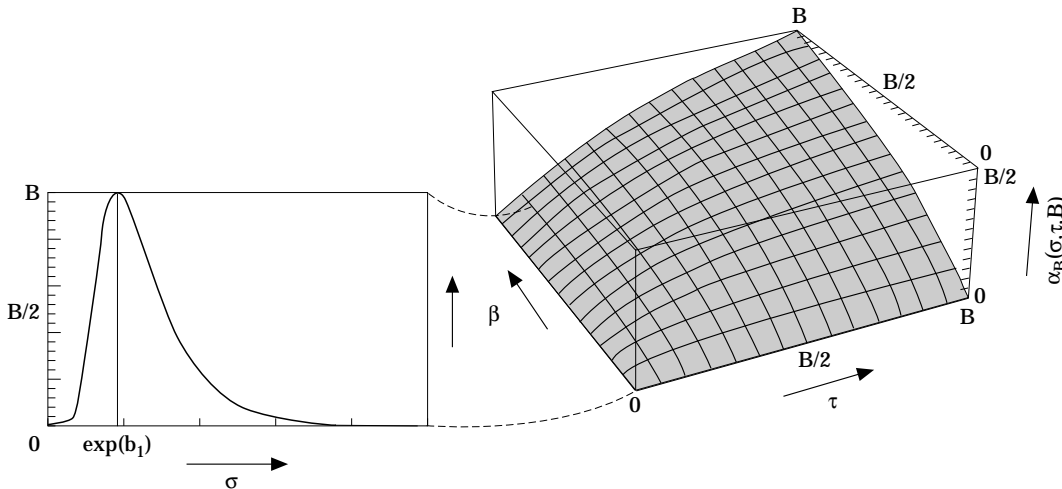


FIG. 3. Top: the inhibition function of T-lymphocyte clones as a function of η , the summed inhibitory signals from anti-TCR Ig.—Bottom: the activation of B-lymphocytes as a function of the field σ and specific T-cell help τ . (See text for full definitions of variables.)

This formula is comparable to that derived by De Boer & Perelson (1995) for T-cell proliferation functions.

It may be noted at this point that when $\tau_i \gg \beta_i$ we have $\alpha_B \approx \beta_i$ which renders the eqns (2) and (3) formally identical to the ones proposed by Varela *et al.* (1988); in other words, when specific T-lymphocyte help is freely available and not limiting, the dynamics of B-lymphocytes [eqn (2)] and Ig-molecules [eqn (3)] reduces to that of a typical “second generation” network model, in a coherent general scheme. Innovative features can be expected in the present network model when $\tau_i \leq \beta_i$, since in this case competition between B-cells for T-lymphocyte co-operation will become significant.

The induction and activation functions for B-lymphocytes are depicted in Fig. 3(b).

Finally, the number of “available” activated T-cells (τ_i) depends necessarily on the other clones that can receive specific “help” from them: all the candidate B-lymphocytes will compete amongst each other for the co-operation sites, and their relative fitness must be proportional to the interaction strength of the particular pair B_i-T_i .

Thus, the quantity τ_i in eqn (10) can be calculated as:

$$\tau_i = \sum_{l=1}^{N_T} \frac{\$_{il}^{BT}}{m} \cdot \frac{\$_{il}^{BT} \cdot \beta_i}{\sum_{j=1}^{N_B} \$_{jl}^{BT} \cdot \beta_j} \cdot v \cdot \alpha_T(\pi_l, \eta_l, T_l) \quad (11)$$

where the term v takes into account the number of “co-operation sites” per activated T-cell; i.e. it determines how many B-cells can co-operate simultaneously with a single activated T-cell, and m is a constant which normalizes the affinity coefficients $\$_{il}^{BT}$ (we assume these terms to be fixed parameters). $\$_{il}^{BT}$ is the interaction strength of the particular pair B_i-T_i ; notationally, the cross-bar on the \$ indicates that this term has two distinct forms depending on whether the co-operation is achieved and maintained by direct mIg-TCR recognition, or by MHC + Ag-peptide presentation.

In the first case the interaction strength will be essentially the affinity of the two molecules:

$$\$_{il}^{BT} = M_{il}^{BT}. \quad (12a)$$

In the second case the situation is more complex, since the affinity of the mIg for the native antigen, the efficiency of processing and presentation of the peptides, and the concentration of the antigen

itself (both in absolute terms and relative to its antigenic or peptidic competitors) all influence the stability of the co-operation B_i-T_i . In a maximum simplification we will assume that the strength of the interaction is proportional to all these quantities, and thus we have:

$$\$_{il}^{BT} = s \cdot \sum_{k=1}^{N_A} M_{ik}^{TA} \cdot M_{ik}^{BA} \cdot A_k \quad (12b)$$

where s is a non-dimensional constant that brings the two modes of B-T co-operation into the same scale.

2.2.6. Parameter settings

As a consequence of trying to stay as close as possible to the biology, the model proposed here is fairly complicated, and has a considerable number of parameters. A systematic exploration of the parameter space is not the issue in this first approach; we aim at identifying certain qualitative emergent features of the model (in particular, the CIS vs. PIS issue), and with that in mind we have chosen a reference set of parameter values that exhibit the relevant behaviour. The present model is an extension of the model of Varela *et al.* (1988), and this guided us in the choice of many of the parameters. Thus, the reference parameters for the dynamics of B-lymphocytes and Ig-molecules are in typical ranges previously proposed for that model [$k_{PB} = 0.3$, $k_{DB} = 0.1$, $k_{DF} = 0.04$, $k_{DC} = 0.008$, $k_{SF} = 4.0$, $b_1 = \log(80.0)$ and $b_2 = \log(2.1)$ (Detours *et al.*, 1994)].

For T-lymphocyte clones, we chose values that would make their intrinsic dynamics reasonable when compared to those of B-lymphocytes [proliferation and decay rates comparable to those of B-cells; the reference values were $k_{PT} = 0.2$, $k_{DT} = 0.15$, $a_{T1} = \log(80.0)$, $a_{T2} = \log(2.1)$]. This amounts to assume that the Ig-mediated inhibition of T-lymphocytes follows a curve as a function of ligand that is in the same range of the “suppressive” region of cross-linking curve in B-lymphocytes; this assumption is reasonable, since a concentration of Ig which is sufficient to saturate the membrane Igs in a B-cell on a “one-to-one” basis should also be sufficient to “block” the TCR molecules in a T-lymphocyte. Also, in the initial investigations to be reported here, the terms π_l are all simply set at the maximal value 1.0.

Finally, the most difficult parameters to estimate are those that regulate B-T co-operation, namely v and s , since we have no good experimental estimation of their actual values. We will only suggest for the

moment a reference range. v is the parameter that determines how many B-cells can co-operate simultaneously with a single activated T-cell; clearly, at least for obvious geometric reasons, this value must be finite and small (we have assumed that it is in the interval $[1, 10]$). In fact, this parameter amounts in practice to a scaling factor between the size of B- and T-lymphocyte clones, so it can be expected not to influence the general qualitative behaviour of the system. In our initial investigations, it is set at 1.0. The parameter m , which normalizes the affinity coefficients S_{it}^{BT} , is set at 1.0 since the ‘‘affinities’’ employed here are typically of this order, in coherence with the parameters governing the dynamics (Detours *et al.*, 1994; Calenbur *et al.*, 1995).

The parameter s , which corresponds to the relative efficiency of the two modes of T-cell help, is assumed to be in the interval $[s \leq \exp(-b_1)]$. There is some experimental evidence which suggests that anti-TCR B-cells may have some competitive advantage over the MHC-presenting ones (Tite *et al.*, 1986; Martinez-A. *et al.*, 1988). That is in fact an *a priori* expectation since the ‘‘valency’’ of the B-lymphocyte (mediated by either MHC or mIg) for the T-cells must be the major determinant of the value of s . It is known that the total number of mIg binding sites on the surface of a B-cell [about 10^5 molecules per cell, ref. (Resch, 1976)] will usually be some orders of magnitude higher than the number of individual peptides actually presented by available MHC molecules [potentially between 1 and 10^5 peptide copies per cell, but usually in the order of a few hundreds (Harding & Unanue, 1990)]. Moreover, as already discussed, mIg molecules are constitutively expressed by the B-lymphocytes, and tuned according to their intrinsic cell physiology, while the surface representation of a particular MHC-peptide complex is essentially contingent and dependent on other candidate peptides in the process of processing and presentation (Manca *et al.*, 1991; Harding & Geuze, 1993; Kroon & McDonnel, 1993; Lehman *et al.*, 1993; West *et al.*, 1994). Qualitatively, the value $s = \exp(-b_1)$ implies that a B-lymphocyte that is optimally induced by cross-linking antigen and presents its peptides to specific T-cells, will have a B-T interaction strength which is comparable to that of an anti-TCR B-lymphocyte with unitary affinity; smaller values imply that a B-lymphocyte needs higher concentrations of antigen to present peptides with the same efficiency.

3. Results: Preliminary Studies in Small Prototypical Systems with Fixed Connectivity Structures

3.1. DYNAMICAL CONSEQUENCES OF THE TWO MODES OF B-T CO-OPERATION

In this section we describe some basic dynamical properties of a pair of co-operating B and T-lymphocyte clones that derive rather directly from the inbuilt postulates of the model. The two clones of such a pair can couple in two prototypical modes: on the one hand through (MHC + peptide)—TCR interactions, on the other through mIg—TCR interactions (Fig. 4).

In the case where the members of a B-cell clone target the members of a given T-cell clone by presenting ‘‘specific’’ peptides on their membrane MHCs, there is no particular relationship between the clonal receptors of the two types of lymphocytes. The Ig-molecules produced by the B-cell following activation will not interfere directly with the TCR of the T-cells, and therefore will not interfere with the state of activation of that clone [Fig. 4(a)]. The consequence of this mode of B-T co-operation is immediate: both lymphocyte clones will grow exponentially as long as the other stimuli they require for activation are maintained.

In order to analyse this mathematically, we consider a prototypic system (prototype I) consisting of one B cell clone (B_1), the Ig it produces (F_1) and the T-cell that it co-operates with (T_1). The interaction coefficient between F_1 and T_1 is null. We keep both $\sigma_1 = \exp(b_1)$ and $\pi_1 = 1.0$, i.e. constant and optimal stimuli. To render the system as simple as possible, the interaction coefficient S_{it}^{BT} [defined according to eqn (12b)] and v are unitary, and the source terms $\xi(1)$ and $\zeta(1)$ are null. Equations (1–12) in this simplified system reduce to:

$$\frac{dT_1}{dt} = (k_{FT} - k_{DT}) \cdot T_1 \quad (13)$$

$$\frac{dB_1}{dt} = -k_{DB} \cdot B_1 + k_{PB} \cdot \frac{T_1 \cdot B_1}{T_1 + B_1} \quad (14)$$

$$\frac{dF_1}{dt} = -(k_{DF} + k'_{DC}) \cdot F_1 + k_{SF} \cdot \frac{T_1 \cdot B_1}{T_1 + B_1} \quad (15)$$

where: $k'_{DC} = k_{DC} \cdot \exp(b_1)$.

It is easy to see mathematically that this system either collapses or is unstable. In Fig. 4(a) we illustrate the behaviour of this prototypical system using the standard set of parameters. In the bottom graph, several ‘‘explosive’’ trajectories in state-space are depicted.

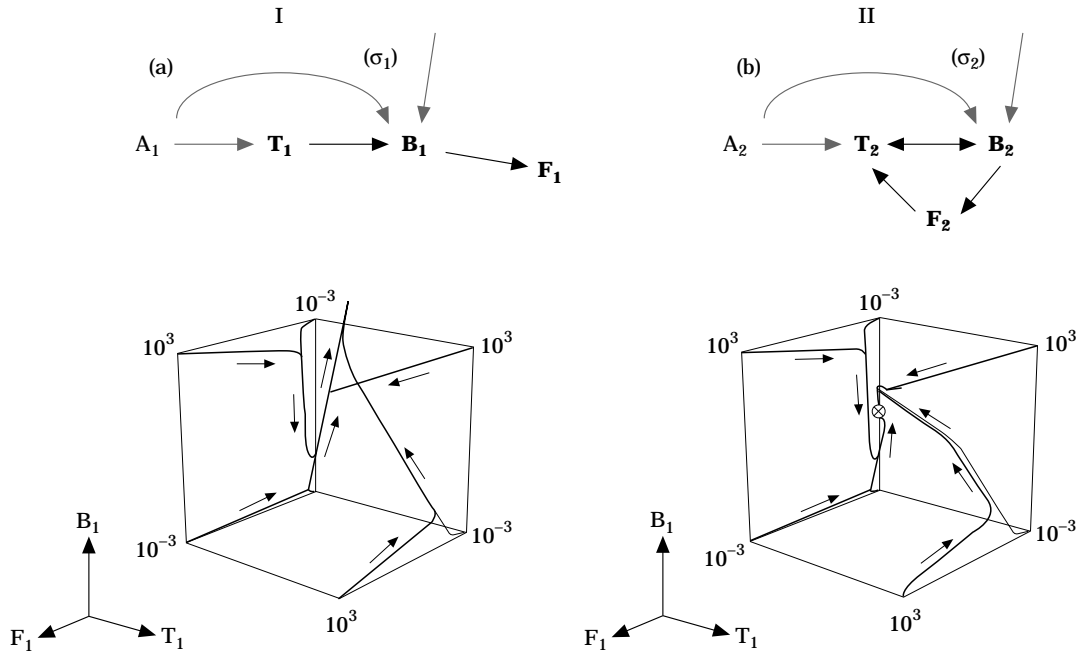


FIG. 4. Top panel: two prototypical modes of B-T co-operation. I—A B-cell clone (B_1) and a T-cell clone (T_1) which are both specific for antigen A_1 are stimulated, and their co-operation is mediated by MHC + peptide presentation by the B-cell clone. The immunoglobulins (F_1) produced by B_1 do not recognize T_1 directly, so the latter are not controlled and grow exponentially. II—The B-cell clone (B_2) and the T-cell clone (T_2) are both specifically stimulated by a common antigen A_2 as in I, but their co-operation is now also mediated by direct mutual recognition. The immunoglobulins (F_2) produced by B_2 recognize directly T_2 , and inhibits its growth, defining a negative feed-back loop that renders the system stable. Bottom panel: several trajectories in phase-space from numerical simulations of the two models, leading (I) to explosion and (II) to a fixed-point attractor. These representations were obtained using the package GRIND by R. J. De Boer; the parameter values are those defined in the text.

We will now discuss the second major prototype of interaction, where a B-cell clone co-operates with a T-cell clone through direct mIg-TCR interaction [Fig. 4(b)]. The Ig molecules produced by the activated B-lymphocytes specifically recognize the TCR of their T-cell counterparts. Since free anti-TCR Ig molecules constitute inhibitory signals for the T-cell, the B-T pair will be dynamically stabilized by a negative feedback loop. Thus, even when the other stimuli they require are optimally stimulatory, the B-T pair will evolve towards a situation in which the anti-TCR Ig molecules (both free and membrane-bound) produced by the B-cells maintain a certain level of T-cell activity, which in turn is just sufficient to sustain the B-cells. This equilibrium is stable, because if the B-cell activity were to increase, the additional anti-TCR Igs would inhibit the T-cells, and the reduced T-help would decrease the B-cell activity back to the equilibrium point.

Mathematically, we may consider as a minimal system with heuristic value the prototype II composed of a single B cell clone (B_2), the Ig it produces (F_2) and the T-cell that it co-operates with (T_2), but now the interaction coefficient between F_2

and T_2 is not null ($M_{22}^{FT} > 0$). We keep both σ_2 and π_2 constant and optimal. To render the system as simple as possible, the interaction coefficient M_{22}^{BT} [in this case idiotypic affinity, according to eqn (12a)] and v are unitary and the source terms $\zeta(2)$ and $\zeta(2)$ are null. Equations (1–12) in this simplified system reduce to:

$$\frac{dT_2}{dt} = (k_{PT} \cdot \alpha'_T(F_2) - k_{DT}) \cdot T_2 \quad (16)$$

$$\frac{dB_2}{dt} = -k_{DB} \cdot B_2 + k_{PB} \cdot \frac{\alpha'_T(F_2) \cdot T_2 \cdot B_2}{\alpha'_T(F_2) \cdot T_2 + B_2} \quad (17)$$

$$\frac{dF_2}{dt} = -(k_{DF} + k'_{DC}) \cdot F_2 + k_{SF} \cdot \frac{\alpha'_T(F_2) \cdot T_2 \cdot B_2}{\alpha'_T(F_2) \cdot T_2 + B_2} \quad (18)$$

where:

$$k'_{DC} = k_{DC} \cdot \exp(b_1); \quad \alpha'_T(F_2) = \frac{\alpha_T(\pi_2, \eta_2, T_2)}{\pi_2 \cdot T_2}.$$

This system has a single non-trivial fixed point when the following conditions are satisfied:

$$k_{DB} < k_{PB} \quad \text{and} \quad k_{DT} < k_{PT} \quad \text{and} \quad \alpha'_T(F_2) = k_{DT}/k_{PT}.$$

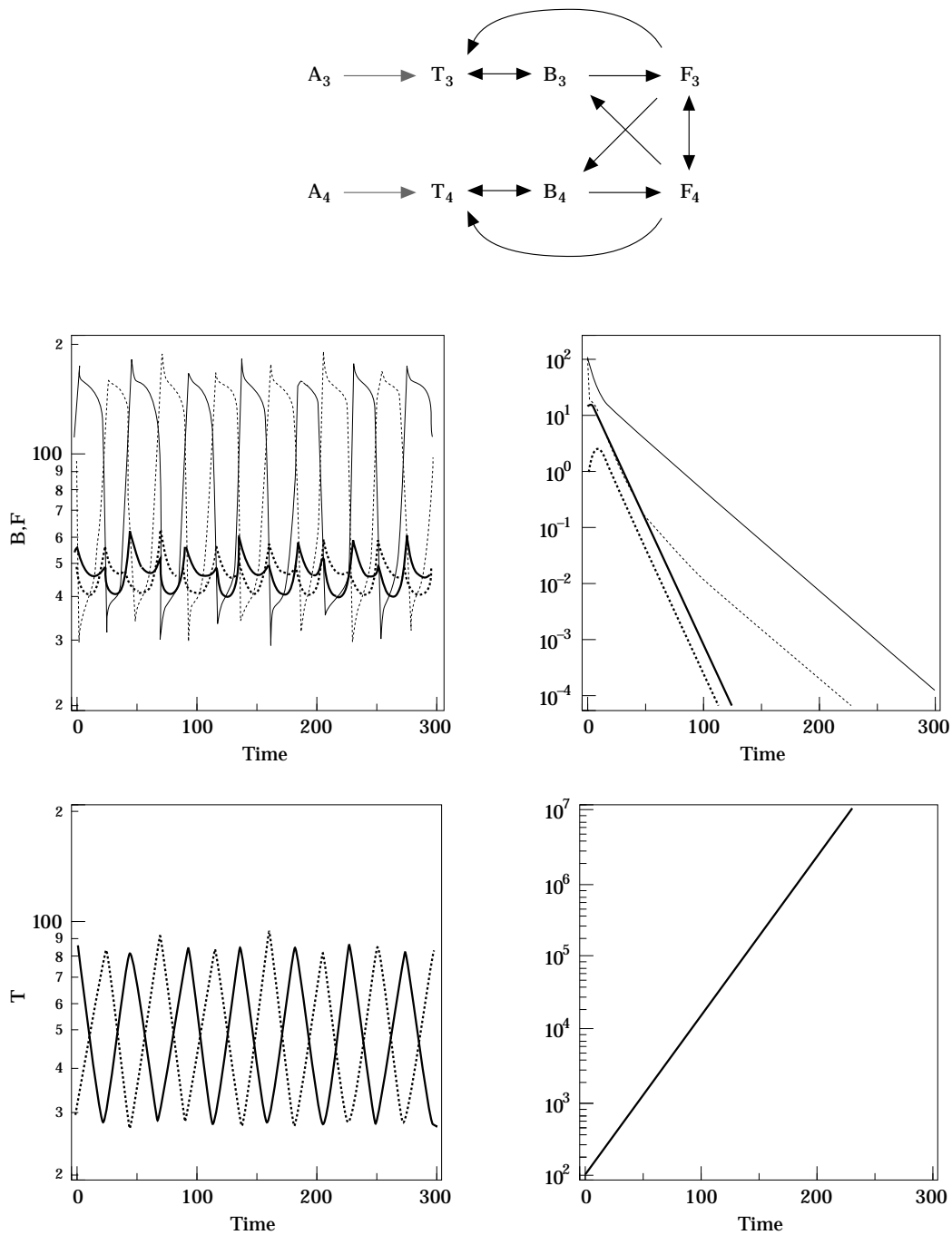


FIG. 5. Prototype III. T-cell clones T_3 and T_4 are stimulated by specific antigens A_3 and A_4 . B-cell clones B_3 and B_4 co-operate by direct mutual recognition with clones T_3 and T_4 respectively. The induction of the two B-cell clones is ensured by mutual recognition through their immunoglobulins (F_3 and F_4). The structure is symmetrical. The fate of this system is strongly dependent on the initial conditions: the system can either fall into a locally stable oscillatory regime in which all the variables are non-null (left); alternatively, a failure in establishing a dynamical equilibrium between the two B clones results in collapse of the variables B_3 , F_3 , B_4 and F_4 with concomitant exponential growth of the T_3 and T_4 components (right). The two situations are illustrated by time-plots of numerical simulations of the mathematical models described in the main text. First row: time-plots of B_3 (dashed thick), F_3 (dashed thin), B_4 (plain thick) and F_4 (plain thin); Second row: time-plots of T_3 (plain) and T_4 (dashed). Simulations were performed using GRIND; the parameter values are those defined in the text.

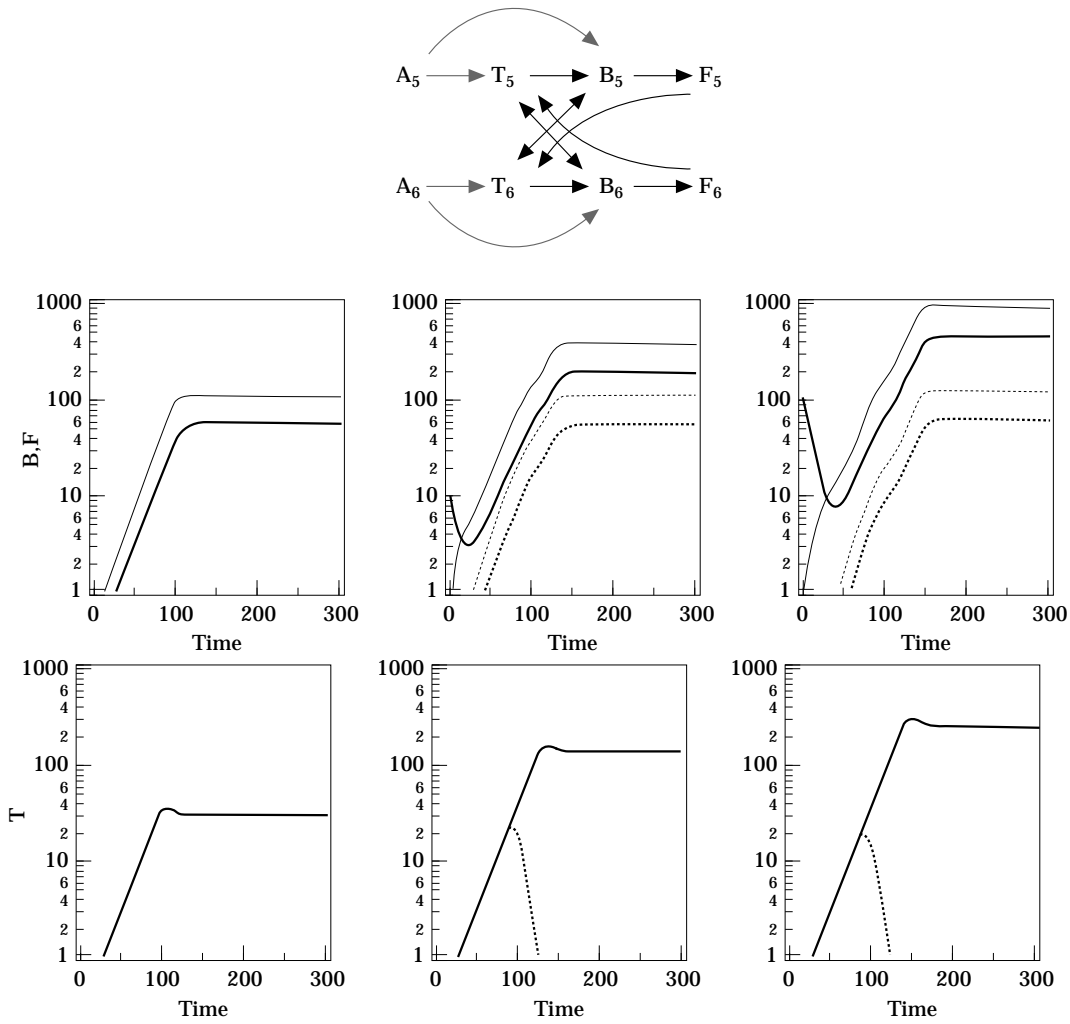


FIG. 6. Prototype IV. Two pairs of B- and T-cell clones $[(B_5, T_5)$ and $(B_6, T_6)]$ are specifically stimulated by antigens A_5 and A_6 respectively. B_5 also recognizes clone T_6 directly; symmetrically, B_6 directly recognizes clone T_5 . Clearly, F_6 will inhibit T_5 and F_5 will inhibit T_6 . The structure is symmetrical (diagram on top). The system regularly attains a fix point; however, the equilibrium composition depends on the initial conditions. This behaviour is illustrated by time-plots of numerical simulations in which the symmetry in the initial composition is progressively broken by increasing the initial value of B_5 (left: $B_5 = 0.1$; center: $B_5 = 10$; and right: $B_5 = 100$), while keeping the initial values of other variables constant at 0.1). First row: time-plots of B_5 (plain thick line), F_5 (plain thin), B_6 (dashed thick) and F_6 (dashed thin); Second row: time-plots of T_5 (dashed) and T_6 (plain). Simulations were performed using GRIND; the parameter values are those defined in the text.

The equilibrium values at the fixed point are thus:

$$F_2 = \alpha'_T{}^{-1} \left(\frac{k_{DT}}{k_{PT}} \right) \quad (19)$$

$$B_2 = \frac{k_{PB} \cdot (k_{DF} + k'_{DC})}{k_{SF} \cdot k_{DB}} \cdot F_2 \quad (20)$$

$$T_2 = \frac{k_{PT} \cdot k_{PB} \cdot (k_{DF} + k'_{DC})}{k_{DT} \cdot k_{SF} \cdot (k_{PB} - k_{DB})} \cdot F_2 \quad (21)$$

According to the Routh–Hurwitz criterion (Murray,

1989), the prototype II equations are locally stable at the non-trivial fixed point as long as the inequality $k_{PB} > k_{DB} + k_{DT}$ is satisfied.

In Fig. 4(b) we illustrate the behaviour of this prototypical system using the standard set of parameters. In the bottom graph, several stable trajectories are drawn in the 3D state-space.

The two basic examples just presented suggest that the present model of B-T lymphocyte networks can show some clear-cut correlations between the structure of the interactions and the dynamical behaviour. Prototypes I and II constitute the basic building blocks of the model which by anticipation

correspond to the putative ‘PIS’ and ‘CIS’ respectively. However, extrapolating from these situations to more complex systems is certainly not trivial. Two additional prototypical systems will illustrate this point clearly.

Consider a simple model (prototype III) with two B-lymphocyte clones (B_3 and B_4) which induce each other through the Ig-molecules they produce (F_3 and F_4), and get to be activated by co-operating with two optimally stimulated T-cell clones (T_3 and T_4) that they recognize through idiotypic interactions (Fig. 5-top). In order for this system to demonstrate self-sustaining behaviour, the initial concentrations of F_3 and/or F_4 must be in the inductive range; in addition, the system can only maintain all its components in a concentration above the virgin one if it is dynamically stable. Illustrative examples of stable and unstable dynamics obtained with this model are depicted in the left and right panels of Fig. 5.

Finally, let us consider a fourth situation (prototype IV) represented again by two B-lymphocyte clones (B_5 and B_6) which are optimally induced by different antigens and which do not interact directly with each other (Fig. 6). Nevertheless, they interact indirectly because each B-cell clone obtains help from the same two antigen-specific T-lymphocytes (T_5 and T_6). Thus, B_5 presents antigenic peptides to clone T_5 but also recognizes clone T_6 by direct mIg-TCR interaction; conversely B_6 presents antigenic peptides to clone T_6 and recognizes specifically clone T_5 . Clearly, F_6 will inhibit T_5 and F_5 will inhibit T_6 . For simplicity let us assume that the structure of the model is perfectly symmetrical. The behaviour of this symmetric model is particularly interesting: it regularly approaches a fixed point. However, the system has a “metastable” character: there are an indefinite number of fixed points, and the precise composition of the asymptotic equilibrium depends on the initial conditions.

4. Discussion

The model of the normal non-immunized IS presented here is based on a restricted set of mechanisms of lymphocyte co-operation; this set is far from exhausting all the possibilities that have been proposed, or actually demonstrated in particular experimental settings (Mazel *et al.*, 1990). We have made a strict selection of those mechanisms that have experimental support, and conform to observations of clonal sizes, repertoire diversity and ongoing activity in the peripheral lymphoid compartment of

normal unmanipulated individuals. In this section we will discuss how this simplified model, which has no pretention to be complete, may nevertheless account for that level of organization where the distinction between CIS and PIS actually comes about.

As the simple prototypic systems II–IV illustrate, the articulation of this restricted set of interactions can potentially lead to a stable self-regulating immune network. Such dynamic stability effectively ensures that clonal dynamics will evolve below the thresholds of frequency and dominance that may reasonably be supposed to be necessary for an entire panoply of additional mechanisms of intercellular interaction or co-operation processes to come into play. In other words, under certain conditions (notably pertaining to the connectivity structure of the network), the organization of the model can by itself ensure that it will remain within its own range of validity.

In other conditions (illustrated by prototype I), exponential expansion of clones can proceed in our model with no upper limits. Clearly in the real IS, clonal expansion during an immune response does not continue indefinitely: it is regulated, and can give rise to immunological memory, by processes made

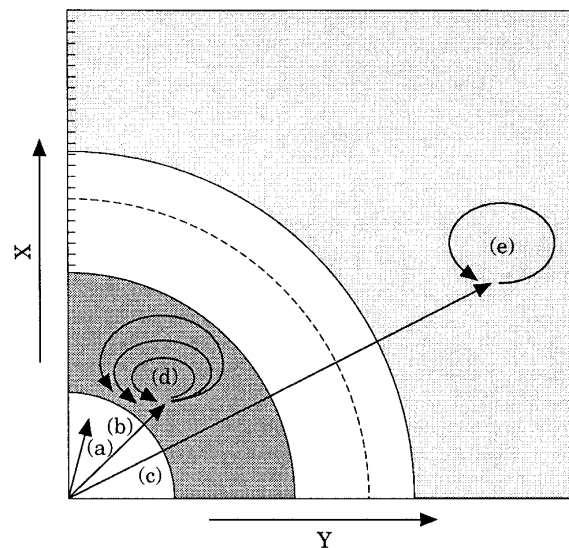


FIG. 7. A “phase-space” diagram illustrating the domain of operation of the immune network model as a function of two co-operating components X and Y (for example a B- and T-lymphocyte clone). The domain of validity of the model is illustrated by the dashed line that imposes a limit on the magnitude of the components (for example clonal size or frequency), such that the simplifying assumptions detailed in Fig. 2 (right) are reasonable. We postulate that the operation of the network during normal physiology progresses below such limits (inside dark grey ring). The internal dynamics of the immune system will define under which conditions the trajectories of the components, (a), will either: (b) reach the domain of the network and enter into its stable dynamics (d); or alternatively (c) escape and give rise to a typical immune response dynamics (e). Note that although the immune response dynamics (outside the light grey ring) is not included in the simplified model, its initiation is explicitly taken into account.

possible and viable only when clonal frequencies or dominance are high enough. These processes do *not* form part of our model. However, we would like to emphasize, that the *initiation* of the “unlimited growth” that makes them possible, *is* a part of our extremely simplified model. In the course of their expansion from a single bone-marrow precursor, the clones responsible for immune responses traverse the domain of operation of the network; only if they escape the potential “attractor” represented by the “stable” mode will they depart from the domain of validity of the present model. In this way, our model includes the critical domain in which the differentiation between two distinct modes of coupling to antigen occurs (Fig. 7).

At this point a question naturally arises as to whether the fully-fledged model system, endowed with free metadynamical recruitment of new clones, develops in such a way that these two modes both appear spontaneously; and if so, whether these two modes can co-exist and give rise to a meaningful distinction in the repertoire corresponding to that between a CIS and a PIS. These questions are addressed in the companion article (Carneiro *et al.*, 1995).

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APPENDIX

The Biological Justifications for the Six Major Simplifying Postulates of the Model

- (i) There are no specific interactions between resting lymphocytes. Productive interactions occur only

between “co-operation prone” activated T-cells and induced (or activated) B-cells.

Following induction by adequate engagement of mIg by ligand, B-lymphocytes modulate the expression of a battery of adhesion molecules and co-receptors. Similarly, activation of T-lymphocytes also changes their patterns of membrane protein expression. The newly expressed molecules in both cells endow them with the potential to engage in cell-to-cell co-operation; a process that requires interactions with adequate affinity, “valency” and duration, involving a concerted engagement of clonal receptors, non-clonal co-receptors and adhesion molecules, and a harmonious co-evolution of the physiology of both cells (Dustin & Springer, 1991; Clark & Ledbetter, 1994). In addition, activated lymphocytes also produce chemotactic factors (Kornfeld *et al.*, 1985; Clinchy & Möller, 1994) which target other sensitive cells to them. Finally, the particular set of adhesion molecules expressed in induced or activated lymphocytes modifies the nature of interactions with their environment (other cells and the extracellular matrix) such that they home to the lymphoid organs, where they follow selective pathways and kinetics of migration, and participate in special processes of morphogenesis in lymphoid organs (Dustin & Springer, 1991; Foy *et al.*, 1994).

Resting lymphocytes do not participate actively in any of these processes. We conclude that in the normal steady-state IS, interclonal interactions effectively only take place between “co-operation prone” lymphocytes, that is, activated T-cells and induced or activated B-cells. Due to their “homing” capacities, increased mobility and “convergent” migration pathways, the probability of a meeting event is increased; and due to their increased intercellular complementarity and avidity, the probability of productive engagements in any such encounter is also increased. Hence, the overall rate of productive encounters between activated T-lymphocytes and induced B-lymphocytes is postulated to be increased by several orders of magnitude over that between resting cells. Conversely, it is assumed that specific interactions involving resting lymphocytes are relatively improbable (both because of a reduced frequency of encounters, and because of a reduced

probability and efficiency of engagement), and are therefore negligible from a network point of view.† Simplifying it is assumed that the probability of productive encounters between “co-operation prone” cells is 1.0, and between resting cells is 0.

(ii) B-lymphocyte induction and T-cell activation require the cross-linking or polymerization of their clonal receptors.

The model assumes that activation of lymphocytes is strictly dependent on “cross-linking” or “polymerisation” of their clonal receptors (B-cell mIg or T-cell TCR). B- and T-cells, however, are known to display qualitatively different activation pathways and ligand requirements. Actually, in view of the different degrees of valency for antigenic-determinants of mIg and TCR molecules, B- and T-cells can *a priori* be expected to be sensitive to qualitatively different stimuli. While any ligand with valence higher than two can potentially lead to the polymerisation of bi-valent mIg on B-lymphocyte membrane (Mongini *et al.*, 1989; Faro & Velasco, 1993a), the extensive aggregation of monovalent TCR effectively requires a ligand which is already “polymeric”. Hence, B-lymphocytes can be induced *in vitro* by anti-mIg Ab either in soluble (free) or plate-coated forms. By contrast, T-cell activation cannot be achieved *in vitro* with anti-TCR Ab in solution; it can only be triggered when the ligand is “polymerised” on coated plates or on the membrane of an APC [for example, MHC + peptide complexes, mIg on specific anti-TCR B-cells (Tite *et al.*, 1986), or anti-TCR IgG polymerised by binding Fc γ receptor (Ceuppens *et al.*, 1985; Clement *et al.*, 1985; Stohl *et al.*, 1987) or covalent attachment to cell surfaces (Kranz *et al.*, 1984)]. Concomitantly, it is to be noted that soluble anti-TCR Abs are excellent *inhibitors* of T-lymphocyte responses (Reinherz *et al.*, 1980; Hoffman *et al.*, 1985; Shaw *et al.*, 1985; Pantaleo *et al.*, 1987), a point to which we shall return.

(iii) Primary activation of resting T-lymphocytes is triggered by co-operation with unspecific APCs, involving the specific recognition of a set of dominant and frequent antigenic peptides in the context of MHC molecules. The contribution of B-lymphocytes is irrelevant.

We assume that T-cell activation *in vivo* is triggered by TCR aggregation induced by MHC + peptide complexes (with the concomitant engagement of non-clonal receptors and adhesion molecules) during cell-to-cell co-operation with antigen presenting cells (APC) (Grusby *et al.*, 1993). As mentioned in postulate (i) and in spite of the fact that specific

† Two corollaries of this postulate are that T-cell activation is virtually B-cell independent and that B-cell induction is virtually T-cell independent. Note that some immediate consequences of this assumption are similar to those of “two signal models” of lymphocyte activation [see Cohn (1994) for a review]; notice however that at variance with “two signal models”, we pay special attention to the frequency of specific encounters *in vivo*.

activated B-lymphocytes seem to be very proficient APCs (Lanzavechia, 1985), they are in such low frequencies, both in absolute terms and as compared to other “unspecific” APCs (dendritic cells, macrophages, etc.), that their contribution to the activation of resting T-cells is likely to be insignificant (Lassila *et al.*, 1988; Ronchese & Hausmann, 1993). Similarly, induced or activated B-cells are just frequent enough to co-operate with activated T-cells, but are unable to activate resting T-cells. Again, the frequency of individual specificities in serum Ig is so low that the potential stimulatory effect of anti-TCR Ig when “polymerized” on the surface of an APC (following binding by the Fc-receptor) is also insignificant. Finally, for the reasons given above, the model assumes that “idiopeptides” from clonal receptors (Mazel *et al.*, 1990) are also negligible. In short, the model postulates that T-lymphocytes are activated primarily when they recognize a set of frequent and dominant antigenic peptides presented by unspecific APCs.

(iv) Primary induction of resting B-lymphocytes follows cross-linking of mIg by multivalent ligands: these are either common antigens, or soluble idiotypic Ig molecules.

Soluble multivalent Ig-molecules can contribute to the induction of a resting B-lymphocyte because, first, their normal concentrations are not negligible and, second, they can polymerize the bivalent mIg receptors. However, according to postulate (i), the contribution of clonal membrane receptors of B- and T-lymphocytes to the primary activation of resting B-lymphocytes is not significant. It is worth noting also that if induction occurs through the cross-linking of receptors, as postulated, this automatically accommodates the finding that high concentrations of ligand do not lead to clonal maintenance (Nemazee & Buerki, 1989), since high concentrations of ligand results in monovalent binding.

(v) In order to engage and sustain co-operation with an activated T-cell, an induced B-cell must somehow engage its TCR either by presenting MHC + peptide complexes that are specifically recognized by that T-cell, or by direct mIg-TCR interactions.

According to postulate (i), only activated T-cells have a significant probability of meeting and engaging complementary induced or activated B-cells. Productive cell-to-cell co-operation between B- and T-cells can only take place, moreover, if the B-cell is

able to aggregate the TCR complexes of the co-operating T-lymphocyte at the intercellular interface.

An induced B-lymphocyte can ligate TCR molecules of an activated T-lymphocyte using MHC + peptide complexes (as classical restriction elements), as long as the specific peptides are sufficiently represented on its membrane. Since induced B-cells do not themselves activate the T-lymphocytes [postulate (iii)], they must present the same antigenic peptide(s) that drove the activation of the T-cell by unspecific APC (or potentially other cross-reactive peptides); in practical terms this implies recognizing the corresponding antigenic protein, processing it and presenting its peptides. Clearly, the surface representation of a particular MHC + peptide complex on a B-cell is essentially contingent, and dependent on the metabolism and total proteins being processed.

A B-lymphocyte can also aggregate the receptor molecules of a T-lymphocyte if its mIg-molecules are specifically complementary to the TCR. As in the previous case, the B-cell in order to co-operate must have been induced *and* must recognize a previously activated T-lymphocyte. Hence, its mIg must cross-react both with a frequent multivalent ligand (either antigenic protein or soluble Ig-molecules produced by other lymphocytes [postulate (iv)] *and* with a TCR molecule that recognizes a dominant and frequent MHC + peptide complex [postulate (iii)].

(vi) Soluble Ig-molecules are inhibitory for T-cells.

Since TCR-molecules are monovalent they cannot be polymerized by soluble Ig. Moreover, soluble anti-TCR Ig not only fails to activate resting T-lymphocytes, but it actually inhibits (*in vivo* or *in vitro*) both the proliferative response and effector functions of T-lymphocytes (Reinherz *et al.*, 1980; Hoffman *et al.*, 1985; Shaw *et al.*, 1985; Pantaleo *et al.*, 1987). T-lymphocyte inhibition by soluble anti-TCR Igs is due to a variety of mechanisms, namely: blocking the interaction of the TCR with the MHC + peptide complex on the APC; uncoupling the signals transduced by the TCR complex and other signals required for full T-cell activation (Mueller *et al.*, 1989); or by down modulating the expression of TCR molecules on the membrane (Pantaleo *et al.*, 1987; Moretta *et al.*, 1989). As already discussed, the fraction of Ig-molecules polymerized on the surface of an APC (by Fc-receptor ligation) is negligible due to the diversity of serum Ig-molecules.