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Chapter 1.3

Immunological Discrimination between Self and Non-Self by Precursor Depletion and Memory Accumulation

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We study processes by which T-lymphocytes "learn" to discriminate "self" from "non-self". We show that intrinsic features of the T cell activation and proliferation process are sufficient to tolerize (self) reactive T-lymphocyte clones. Self vs non-self discrimination therefore develops without any down-regulatory (e.g. suppressive) interactions. T-lymphocyte clones will expand by proliferation only if the IL2 concentration is high enough to induce a proliferation rate larger than the rate of cell decay. This concentration is the proliferation threshold. Because effector T cells are short-lived the proliferation threshold must be quite high. Such high numbers of cells producing IL2 are achieved only when sufficient (memory) precursors are activated. Self and non-self antigens differ with respect the number of (memory) precursor cells they accumulate, as a result of two processes, i.e. precursor depletion and memory accumulation, and can thus be discriminated. Precursor depletion: the dynamics of long-lived precursors can cause tolerization. In neonatal circumstances precursor influx is still low, newborn cells reacting with self antigens are immediately activated, generating (few), i.e. fewer than the proliferation threshold, effectors that decay rapidly. Thus total lymphocyte numbers remain low, yielding self tolerance. Conversely, large doses of similar antigens introduced in mature systems push "their" lymphocyte clone over the proliferation threshold because a large (accumulated) precursor population is rapidly activated. Small doses are however low zone tolerized. Memory accumulation: peripheral T-lymphocyte populations in fact consist of a mixture of virgin precursors and memory cells. If the formation process of (long-lived) memory cells is taken into account and virgin precursors are made short-lived, the proliferation threshold again accounts for self non-self discrimination. Memory cells accumulate when antigenic restimulation is low; it is low when the antigen concentration and/or the antigen affinity is low. Therefore self antigens, which are present in relatively high concentrations, fail to accumulate high affinity memory cells, and are hence tolerated. Memory cells crossreacting to self antigens with low affinity, however accumulate neonatally, pushing those clones over the proliferation threshold whenever "their" high affinity antigen enters the immune system. Thus the model generates differences in the antigenicity (i.e. memory precursor frequency) of self and non-self. We conclude that the neonatal T cell repertoire is strongly influenced by the self environment: clones with high affinity to self remain small and unresponsive whereas clones with low self-affinity are enriched. Such enlarged memory clones can be precursor depleted again by low doses of antigen, thus yielding low zone tolerance.

Introduction

The most fascinating aspect of immune systems is their ability to discriminate between "foreign" antigens to which they respond, and "self" antigens which they

tolerate. An immune system consists of a large set of different receptor molecules; these receptors are embedded in the cell membrane of lymphocytes (or released by lymphocytes, as, e.g., antibody molecules); lymphocytes that share identical receptors form a clone. The diversity of receptor molecules is generated by (1) the combination of a multiplicity of germline gene segments (Early *et al.*, 1980; Chien *et al.*, 1984), and (2) somatic mutation (Griffiths *et al.*, 1984; Berek *et al.*, 1985). Both mechanisms can give rise to receptors that have sufficient affinity to self antigens to activate a lymphocyte. When somatic mutation is involved such receptors inevitably arise; interestingly somatic mutation appears to be absent in T-lymphocytes (which are of regulatory importance) (Barth *et al.*, 1985), whereas it plays an important role in the generation of the (secondary) B-lymphocyte repertoire (Berek *et al.*, 1985). In order to prevent immunological self destruction (auto immunity), lymphocytes with such anti-self receptors have to be eliminated and/or impaired by (partly unknown) tolerization processes.

It is known experimentally that immunological tolerance to antigens arises when these are present during the neonatal development of the immune system (i.e. "neonatal tolerance") (Owen, 1945; Burnet & Fenner, 1949; Billingham *et al.*, 1953). Mature immune systems are reported to tolerate antigens that are introduced either in very small doses ("low zone tolerance") or in very large doses ("high zone tolerance") (Mitchison, 1965; Weigle, 1971). Generally, in research on immunological tolerance "down-regulatory" processes are considered to be responsible for the functional deletion of autoreactive lymphocyte clones. There is considerable experimental evidence that suppression plays a role in the inactivation of self reactive clones (Gorczynski & MacRae 1979*a*,*b*; Hilgert, 1979; Fazekas de St Groth *et al.*, 1984; Stockinger, 1984; Tilkin *et al.*, 1985; Stockinger *et al.*, 1986). The matter however remains open to discussion because clonal inactivation can also be caused in the absence of suppression (Nossal & Pike, 1981; Good *et al.*, 1983; McCarthy & Bach, 1983; Feng *et al.*, 1983; Carnaud *et al.*, 1984; Gammon *et al.*, 1986).

Here we present models which develop tolerance and immunity as a result of differential kinetics of growth factor (IL2) production, and not by suppression. This means that we concentrate on helper T cells (that produce IL2). T cell tolerance differs essentially from B cell tolerance because T cells recognize antigens in the context of (highly variable) major histocompatibility complex (MHC) molecules. T cells are restricted to antigens presented together with the self MHC (Zinkernagel & Doherty, 1975). Thus education of T cells involves two learning processes: (1) T cells must learn to be restricted to self MHC and (2) T cells must learn to tolerate conventional self antigens (i.e. non-MHC antigens). The existence of two, possibly separate, processes obscures the interpretation of the literature on T cell tolerance. For clarity we define the terms we use as follows: (1) self MHC, i.e. the inherited MHC; (2) self conventional antigens, e.g. heart muscle antigens; and, most importantly, (3) self antigen, i.e. self MHC plus self conventional antigen.

The process generating a T cell repertoire that recognizes self MHC molecules from the primary (possibly random) repertoire is a positive selection process. Only those cells with sufficient affinity to the self MHC pass through. The thymus is the most likely environment for the occurrence of this process (Zinkernagel *et al.*, 1978;

Smith, 1984*b*). Conversely, the process which eliminates T cells that recognize (conventional) self antigens is a negative selection process. Because many of the conventional self antigens are most probably absent from the thymus (e.g. heart muscle antigens) we suppose this (tolerance) process to take place in the periphery. Note however that circulating conventional antigens can be expressed in the thymus (Kyewski *et al.*, 1986). We here investigate the peripheral process, i.e. we assume that the virgin precursor cells in our models are already self MHC restricted. It will appear that, despite the absence of down regulatory interactions, these T cells tolerate antigens presented in sufficiently low or high concentrations and antigens presented during the neonatal development of the immune system, but that they react vigorously to foreign antigens introduced in mature immune systems.

Previously we analyzed low zone tolerance to infinitely expanding antigens (i.e. tumours) (De Boer & Hogeweg, 1985, 1986*a*,*b*). In those models, tolerance evolves because low antigen doses slowly activate the (long-lived) precursors. In the proliferative systems (De Boer & Hogweg, 1986*a*,*b*) net helper T cell proliferation requires a sufficiently high IL2 concentration; otherwise the proliferation rate is lower than the rate of helper cell decay. These immune systems respond only when precursor activation suffices for generating an IL2 producer population that is sufficiently high for initiating proliferation, i.e. one that is larger than the proliferation threshold. Once proliferation has started it continues autonomously as an autocrine process (Meuer *et al.*, 1984). Low antigen concentrations activate the precursors slowly, generating only a few effectors that decay rather than proliferate; thus lymphocyte numbers decline (by precursor depletion), disabling the system so that it can never pass the proliferation threshold again. It has been demonstrated (De Boer & Hogeweg, 1986*a*,*b*) that the development of (low zone) tolerance depends crucially on: (1) the transition from long-lived precursors to short-lived effectors, and (2) on the degree of antigenicity.

We investigate here whether simple immune systems with a proliferation threshold can account for self non-self discrimination. Whether or not immune systems will pass the proliferation threshold, i.e. will develop an immune response, is determined by the quantity of virgin and memory precursor cells present. Long-lived precursors decrease by precursor depletion, memory precursors increase by memory accumulation or decrease by "memory precursor" depletion. Thus neonatal, low zone, and high zone tolerance can be generated in absence of any down-regulatory process.

The Models

We here investigate two proliferative models, i.e. systems capable of infinite (repeated) proliferation, see Fig. 1 and Table 1. The first (simple) model incorporates long-lived precursor cells and short-lived effectors, but no memory cells; the second (memory) model specifies an equal (short) life-span for virgin precursors and effectors but incorporates long-lived memory cells. The two different models show that: (1) if memory cells and virgin precursors are lumped into one (mixed) population of long-lived cells the requirements for the development of tolerance reside in just the few interactions incorporated in the simple model, and (2) if

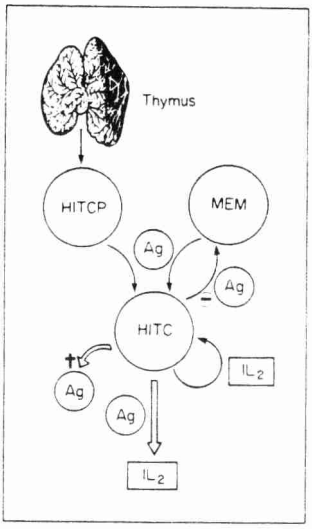

FIG. 1. The interactions incorporated in the models. MEM: memory cell, HITC: helper independent cytotoxic T cell, HITCP: HITC precursor, IL2: T cell growth factor, AG: antigen. In the simple model memory cells are discarded.

memory cells are explicitly incorporated, the model again accounts for self non-self discrimination, but now by memory accumulation. Both models generate low zone tolerance by (memory) precursor depletion, i.e. the process pinpointed most closely in the simple model. Down regulatory interactions, e.g. suppression or other explicit silencing mechanisms, have been deliberately omitted from the models. Only by omitting explicit down regulatory interactions are we able to investigate whether tolerance does indeed depend on down regulation.

The model immune systems are derived from models described before (De Boer *et al.*, 1985; De Boer & Hogeweg, 1986*a*,*b*). For reasons of simplicity, they specify cytotoxic T-lymphocytes that produce their own growth factor (IL2). Such cells, i.e. Helper Independent Cytotoxic T Cells (HITC), have actually been described (Widmer & Bach, 1981; Roopenian *et al.*, 1983). In the models precursors (HITCP) are activated when stimulated with antigen (AG) and subsequently become capable of (a) IL2 production and (b) proliferation in response to IL2.

FORMAL DESCRIPTION

Immunocompetent precursors (HITCP) appear in the periphery (arriving from the thymus) at a constant rate I; precursors have a constant decay rate DP, see Table 1. The unprimed, steady-state precursor populations thus equal I/DP. Upon antigenic activation (AP), precursors become cytotoxic effectors capable of IL2 production (HITC); effectors have a constant decay rate DE. Antigenic stimulation terms (SM and SH) are defined as saturation functions: the maximum rate at which precursors or memory cells are stimulated is AP or AM per day. Memory cells (if

TABLE 1

Helper-independent-Cytotoxic-T-cells (HITC), HITC precursors (HITCP), memory cells (MEM), and the antigenic cell population (AG) are modelled as ordinary differential equations. Growth factor (IL2) kinetics proceed relatively fast; IL2 is incorporated as a quasi steady-state variable. Memory activation (SM), precursor and effector activation (SH), the killing term (SK), and proliferation (KI) follow conventional Michaelis-Menten kinetics. The models are further explained in the text and in Fig. 1. In the simple model the memory equation is discarded. In order to increase the readability of the tables, mnemonics are used for the variables of the model

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SH = AA*AG/(KH+AA*AG)
SM = AA*AG/(KM+AA*AG)
SK = AA*AG/(KK+AA*AG)
IL2 = HITC*SH
d(MEM)/dt = M*HITC*(1-SH) - AM*MEM*SM - DM*MEM
d(HITCP)/dt = I - AP*HITCP*SH - DP*HITCP
d(HITC)/dt = AM*MEM*SM + AP*HITCP*SH + P*HITC*IL2/(KI+IL2)
             - DE*HITC - M*HITC*(1-SH)
d(AG)/dt = R*AG - B*AG*AG - K*HITC*SK
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they are incorporated, i.e. if $M > 0$) are only generated when antigenic restimulation (SH) is sufficiently low. Antigen (AG) grows logistically, its maximum size being R/B (consider e.g. an organ). Antigen is eliminated (cells are lysed) by effectors (K*HITC); killing follows Michaelis-Menten kinetics (Merrill, 1982). IL2 is produced by effectors when they are restimulated by antigen (Meur *et al.*, 1984; Miller *et al.*, 1986). IL2 is scaled to one unit produced per cell per day. Effectors proliferate in response to IL2. When IL2 concentrations are high ($IL2 \gg KI$) proliferation proceeds at rate P per day.

PARAMETERS

We assume that activation of resting (memory) cells takes longer than that of "active" virgin precursor cells (i.e. $AM = 5 < AP = 25$, see Table 2); memory cells also require a higher antigen concentration for maximum activation ($KM = 10^5 > KH = 10^3$). It has been proved experimentally that reactivation of resting T cells indeed requires more steps than that of activated cells (Manger *et al.*, 1985). Proliferation (cell division) takes quite a long time, i.e. about 16 hours (Look *et al.*, 1981) ($P = 1{\cdot}5$, in the models the maximum proliferation possible is a 10-fold increase in about 5 days). Unstimulated effectors quickly return to the memory stage ($M = 5$). Cytotoxic effectors lyse a maximum of 50 antigenic cells per day ($K = 50$). Brunner *et al.* (1981) report high cytotoxicity values for cytotoxic T-lymphocyte clones. In addition, several cytotoxic effector cell types should profit from the helper cells (here the HITC). Maximum cytotoxicity is achieved at relatively high antigen concentrations ($KK = 10^5$). Antigen, e.g. an organ, expands more slowly than the T-lymphocytes ($R = 0{\cdot}1$), compare De Boer *et al.* (1985).

TABLE 2

The parameter settings are derived from models presented before [*De Boer* et al., 1985; *De Boer & Hogeweg, 1986b*], *which, in turn, were based on various sources in the literature. The parameters presented here were chosen in a reasonable order of magnitude; the models do not represent any specific immune system. In the simple model precursors are long-lived* ($DP = 0{\cdot}01$), *and memory cells are ignored* ($M = 0$). *In the memory model precursors have a short life span* ($DP = 1$) *and long-lived memory cells* ($DM = 10^{-3}$) *are incorporated* ($M = 5$). *Effectors are always short-lived* ($DE = 1$). *Maximal proliferation proceeds at an equivalent rate in both models, i.e. a 10-fold increase in about 5 days* ($P = 1{\cdot}5$). *Antigenicity is defined as the reactivity of precursors, which, in turn, can be defined as* (1) *the number (or the percentage) of reactive precursors arriving daily from the thymus, and* (2) *the number of circulating reactive precursors. The former definition* (1) *is independent of precursor life-span, but the latter one gives different antigenicity values in both models for an equal precursor influx. The thymic output of a mouse is about* $5*10^6$ *cells per day* [*Rocha* et al., 1983]; *an influx of 10 cells* ($I = 10$, *used throughout*) *thus corresponds to a reactivity of* 1:500 000 *fresh precursors. Experimental data on helper T precursor reactivity are scarce; the peripheral frequency of the helper precursors specific to the herpes simplex virus (a conventional antigen) in unprimed mice was, for instance, reported to be below* 1:100,000 [*Prymowicz* et al., 1985]. *Although these numbers seem within the range of our models, their interpretation is difficult since peripheral population sizes are influenced by:* (1) *unknown life-span of precursors,* (2) *precursor depletion by cross reactivity in the simple model, and* (3) *accumulation by cross reactivity in the memory model*

AA	10^{-12} to 1·0	affinity	
AP	25·0	precursor activation rate	per day
AM	5·0	memory reactivation rate	per day
B	10^{-9}	contact inhibition rate	per cell per day
DE	1·0	effector decay	per cell per day
DM	10^{-3}	memory decay	per cell per day
DP	0·01 or 1	precursor decay	per cell per day
I	0·1 to 100	precursor influx	cells per day
K	50·0	killing capacity	cells per cell per day
KI	50·0	growth factor saturation	units
KH	10^3	activation saturation	cells
KK	10^5	killing saturation	cells
KM	10^5	reactivation saturation	cells
M	0 or 5	memory cell generation	per day
P	1·5	proliferation rate	cells per cell per day
R	0·1	maximum tissue growth rate	per day

In the models the life-time of the T-lymphocyte subpopulations is varied. It is generally accepted that effectors are short-lived (Jerne, 1984); we assume a life is one day in both models (DE = 1). The life-time of virgin precursors is uncertain (Rocha *et al.*, 1983; Jerne, 1984). We make them short-lived (DP = 1) and incorporate long-lived memory cells (DM = 10^{-3}), or we (simply) make them long lived (DP = 0·01) and ignore memory cells. Because effectors are short-lived (DE = 1) and because proliferation depends on the IL2 concentration (i.e. KI > 0), a proliferation threshold emerges in both models. At high antigen concentrations, in the absence of precursors, about 100 effectors are needed to produce sufficient IL2 for net proliferation. This number corresponds to KI = 50.

For each different antigen, influx (I) represents the number of reactive cells arriving daily from the thymus, i.e. the clone renewal rate. Influx (I) thus represents the qualitative antigenicity of the antigen (De Boer *et al.*, 1985; De Boer & Hogeweg, 1986*c*), i.e. I determines the number of circulating unprimed precursors ready to respond upon the introduction of the antigen.

ANALYSIS

The models are investigated by means of dynamic and static analysis, i.e. by numerical integration and by O-isocline (Segel, 1984) analysis respectively. We have previously discussed the advantages of using numerical integration in combination with numerical phase state analysis (De Boer & Hogeweg, 1986*a*). We use GRIND (De Boer, 1983) to investigate the models. GRIND enables the user to analyze the static properties of models by the numerical computation of O-isoclines, and to analyze the dynamic behaviour of models by numerical integration. The integrator implemented in GRIND is ROW4A (Gottwald & Wanner, 1981).

Results

THE SIMPLE MODEL

We first investigate a model that ignores memory cells, and, instead, specifies precursor cells to be long-lived (i.e. M = 0, DP = 0·01). In fact such a population can be considered as a mixture of short-lived precursors and long-lived memory cells. The generation of these memory cells is however ignored in this (simple) model, but will be incorporated in the next (memory) model. We will show here that the simple model accounts for neonatal and low zone tolerance by means of precursor depletion.

Model behaviour: rejection

Consider the immune response of a mature immune system to a large amount of antigen. In mature, unprimed systems precursor influx (I) and efflux (DP) equilibrate, yielding (steady-state) precursor populations of I/DP cells. These cells circulate in the periphery ready to respond upon introduction of "their" antigen. In Fig. 2(a) we depict the mature unprimed situation between day 0 and 10; at day 10 AG is

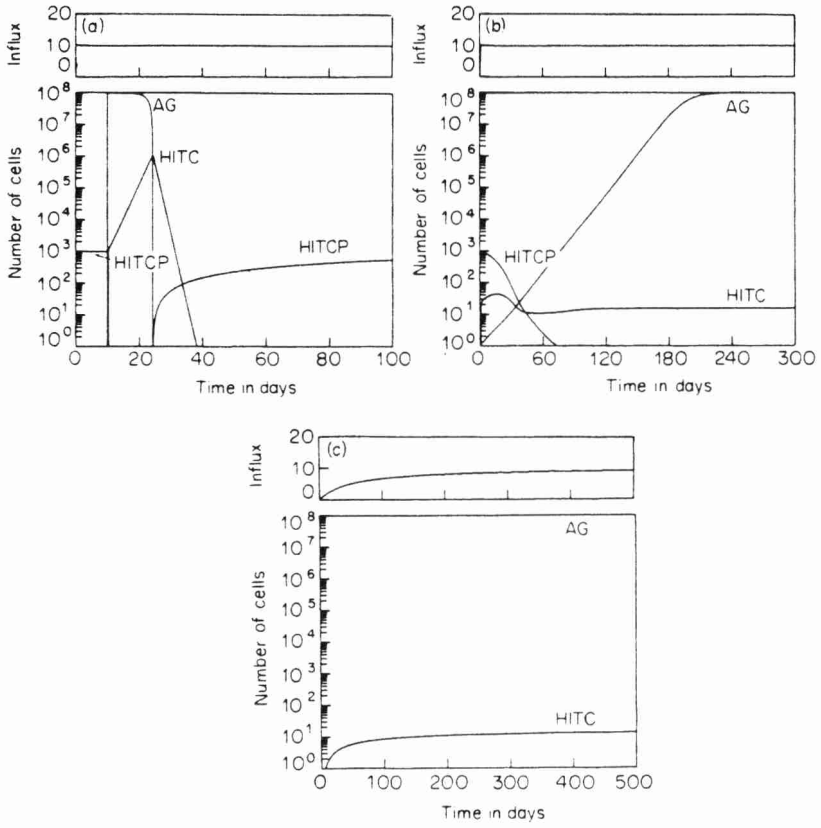

FIG. 2. The behaviour of the simple model as revealed by numerical integration. AG rejection (a): a large dose of antigen ($AG = 10^8$) is rejected when it is introduced (at day 10) into a mature immune system, i.e. a system with a steady-state precursor population (day 0–10). Low zone tolerance (b): a small dose of antigen ($AG = 1$) slowly depletes the steady-state precursor population (day 0–70), after which antigen expands in an unresponsive system. Neonatal tolerance (c): in the upper panel the slow increase in precursor influx is shown; immigrating precursors are immediately activated ($AG = 10^8$), generating an unresponsive (i.e. small) effector population. Parameters as in Table 2 ($I = 10$, $DP = 0{\cdot}01$, $M = 0$).

introduced in a dose of 10^8 cells. Such a situation would arise with an organ transplantation. Introduction of antigen induces rapid activation of the ($I/EP = 1000$) precursors: in a few hours almost every precursor becomes one effector. This immediately depletes the precursor population; the effectors proliferate until antigen is rejected (day 23). Following antigen elimination, effectors decay and precursors slowly recover.

Model behaviour: low zone tolerance

If, instead, a small dose of antigen ($AG = 1$) is introduced into a mature system, the system develops tolerance and antigen is allowed to expand to values that

normally (see above) evoke immune reactions. Between day 0 and 70, when antigen concentrations are intermediate, precursors are activated at a slow rate and hence decrease slowly. Effectors are therefore only induced slowly; moreover, because effector numbers are low, IL2 concentrations remain low. As a consequence effector proliferation proceeds at a very slow rate, i.e. at a rate slower than the rate of effector decay (DE). Thus the number of effectors increases initially by the activation of precursors, but never by proliferation. Because effectors have a shorter life than precursors, transition to the effector stage corresponds to a decrease in the total number of lymphocytes (if proliferation is absent). At the time the antigen concentration becomes high, most precursors are activated (and depleted), but effector numbers are still too low for net proliferation. The antigen can expand without any further increase in the immune reaction. Thus low doses of antigen induce a slow transition of long-lived cells to short-lived cells, resulting in low IL2 concentrations, absence of proliferation, and a reduction in total lymphocyte numbers. This phenomenon, which has been referred to as "precursor depletion", (De Boer & Hogeweg, 1986*a*,*b*), accounts for low zone tolerance. It takes place in the complete absence of down regulation (e.g. suppression).

Model behaviour: neonatal tolerance

We define the neonatal development of the model immune systems as a gradual increase in the influx of immunocompetent lymphocyte precursors (HITCP). The characteristics of neonatal precursors are thus identical to those of their mature counterparts. At day zero, the antigen (e.g. an organ) is fully developed (i.e. at its maximum size), their are no lymphocytes in the system (influx equals zero, upper panel Fig. 2(c)). Then influx is gradually increased and precursors slowly arrive in the system. These few precursors are immediately stimulated by the antigen (which is present in high concentrations) and turn into short-lived effectors; precursors thus never accumulate. Effectors accumulate slowly, and again remain below the critical number required for net proliferation (the proliferation threshold). In conclusion, effectors accumulate slowly by activation of few precursors, proliferation remains absent, and precursors remain depleted by antigenic activation. At the final stage effector numbers are small, i.e. below the proliferation threshold. This means that the antigen is tolerated. Thus precursor depletion accounts for neonatal tolerance in the absence of down regulation (e.g. suppression).

Static analysis

The dichotomy in the behaviour of the model (immunity vs. tolerance) is analyzed graphically in Fig. 3. The relationship between the three variables of the simple model (i.e. HITCP, HITC and AG) is depicted, for $I = 10$, in the 3-D state spaces of Fig. 3 by means of the O-isocline planes of the three variables (see e.g. Segel (1984) for an explanation of the isocline method). In each 3-D panel one of the isocline planes is shaded. When antigen is at its maximum size ($R/B = 10^8$) the AG isocline (Fig. 3(c)) runs vertical; sufficiently high effector numbers (i.e. in the upper region of the cubes) cause AG to decrease. The precursor$' = 0$ isocline plane (Fig. 3(a)) depends on AG only, at low antigen concentrations (on the left) the HITCP$' = 0$

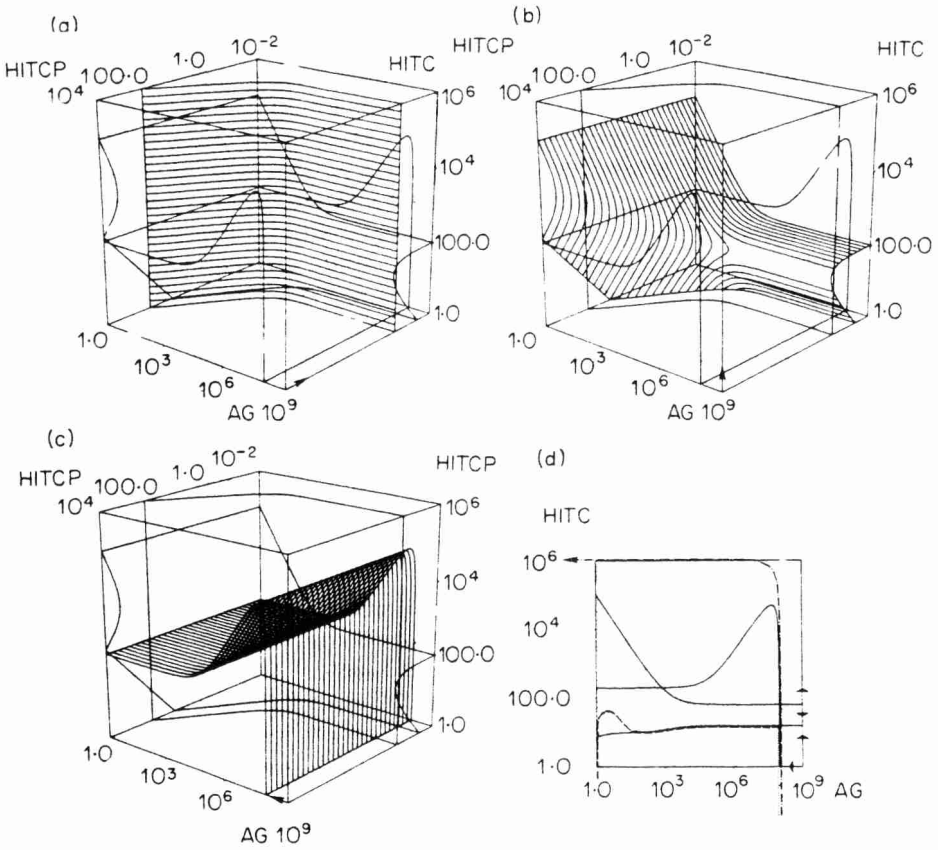

FIG. 3. The O-isocline planes of the three variables of the simple model. The three cubes are identical; only the shading differs: in (a), (b) and (c) we have shaded HITCP, HITC, and AG respectively. Arrows indicate the local direction of trajectories. The implications of the forms and positions of the planes are discussed in the text. In (d) the isoclines are projected into a 2-D state space by a quasi steady-state assumption for HITCP. The intermediate $HITC' < 0$ region is shaded. The behaviour of the model (as depicted in Fig. 2), is indicated in the HITC-AG state space: dashed line, low zone tolerance; dotted line, neonatal tolerance; dash-dotted line, antigen rejection. The tolerance trajectories end up in the (stable) intersect between the lower part of the $HITC' = 0$ isocline and the vertical part of the $AG' = 0$ isocline. Parameters as in Table 2 ($I = 10$, $DP = 0{\cdot}01$, $M = 0$).

isocline bends asymptotically towards the unprimed steady-state value ($I/DP = 1000$); this is only partly visible. At high antigen concentrations the plane reaches the minimum HITCP value achieved by maximum activation ($I/(DP+AP)$). At sufficiently low precursor numbers the effector plane (Fig. 3(b)) is folded: low effector numbers increase (due to precursor activation), intermediate numbers decrease (due to decay), whereas high numbers increase again (due to proliferation). Trajectories which have passed the intermediate $HITC' < 0$ region, i.e. those that have passed the upper part of the folded $HITC' = 0$ isocline, enter the region of endless proliferation. The upper part of the folded HITC isocline, i.e. the critical

number of effectors required for endless (net) proliferation, is referred to here as the proliferation theshold. Trajectories situated at low precursor and effector numbers turn downwards in the intermediate $HITC' < 0$ region until the stable, lower part of the HITC isocline is reached. Such trajectories never pass the proliferation threshold again and correspond to tolerance.

We can reduce this quite complicated 3-D state space to a 2-D state space by making a quasi steady-state assumption for the precursors. Figure 3(d) clearly shows the separation of the effector isocline into a high (instable) proliferation threshold and a low stable (tolerance) part. Both enclose a (shaded) region of effector decrease. The two separate regions of effector increase correspond to the dichotomy in the model behaviour: high effector numbers proliferate infinitely; small effector populations remain confined to a low stable equilibrium, unable to bridge the intermediate $HITC' < 0$ gap.

The rejection trajectory (i.e. the time plot of Fig. 2(a)) is represented by the dash-dotted line. The trajectory starts at $AG = 10^8$ and $HITC = 0$; it (rapidly) moves upwards, passing the proliferation threshold; HITC numbers increase due to repeated proliferation until AG is rejected. Apparently the quasi steady-state assumption is not fulfilled during the activation stage: the rapid activation of the steady-state precursor pool enables the system to bridge the gap and to pass the proliferation threshold. The neonatal tolerance trajectory (dotted line) also starts at $AG = 10^8$ and $HITC = 0$; however because it moves upwards slowly, the quasi steady-state assumption is fulfilled (due to slow precursor influx). The system is thus unable to bridge the $HITC' < 0$ gap, and remains at the stable intersect of the (lower) $HITC' = 0$ and the $AG' = 0$ isocline. The low zone tolerance trajectory (dashed line) starts at the left (at low AG concentrations); turns upwards (initially in the shaded $HITC' < 0$ region) but is unable the bridge the gap, turns downwards, and encounters the lower (stable) $HITC' = 0$ isocline, then moves rightwards and stops at the $AG' = 0$ isocline.

In conclusion, rapid activation of the precursors generates an effector population that is capable of net proliferation. Slow activation allows effectors to decay before sufficient additional effectors are generated. Note that the size of the effector population generated after precursor activation depends on the (initial) size of the precursor populations, which, in turn, corresponds to the degree of antigenicity.

Antigenicity

The effect of antigenicity is studied statically (Fig. 4(a)) and numerically (Fig. 4(b)). In Fig. 4(a) we analyze the effect that antigenicity (i.e. precursor influx: I) has on the separation of the $HITC' = 0$ isocline (Fig. 3). It appears that for weak antigens (low I values) the separated HITC isocline diverges, i.e. the $HITC' < 0$ region expands. The immune system becomes unable to bridge the $HITC' < 0$ gap, and weak antigens are tolerated whatever their initial concentration (proliferation cannot start). Note that the proliferation threshold, i.e. the horizontal upper part of the HITC isocline, is independent of antigenicity (if antigens are sufficiently weak).

Sufficiently strong antigens (high I values) eliminate the intermediate $HITC' = 0$ region: the influx of precursor cells always suffices for generating an effector population larger than the proliferation threshold (the proliferation threshold

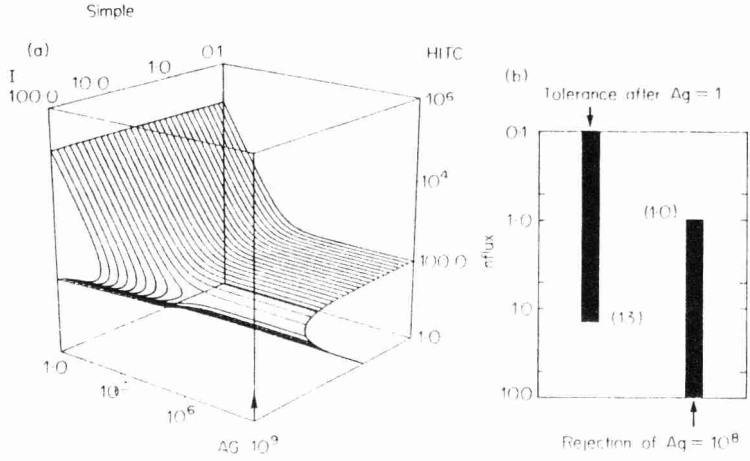

FIG. 4. The effect of antigenicity on tolerance in the simple model. In (a) the 2-D HITC′ = 0 isocline is depicted for several antigenicity (I) values. It appears that the lower part of the HITC isocline climbs upwards as I increases; when I becomes sufficiently large it coincides with the proliferation threshold (the horizontal upper part). For such antigens tolerance becomes impossible. (b) depicts the outcome of the dynamic analysis of antigenicity. The left bar indicates the antigenicity range in which small antigen doses (AG = 1) fail to induce proliferation; the right bar indicates the range in which large doses ($AG = 10^8$) do cause proliferation. Weak antigens are thus always tolerated, strong antigens always rejected. In the interesting overlapping range ($1 < = I < = 13$), antigens are rejected in high doses but tolerized in low doses (or in neonatal circumstances). Parameters as in Table 2 (DP = 0·01, M = 0).

becomes invisible in the isocline plot because the intermediate HITC′ < 0 region is eliminated). Such antigens therefore always induce a proliferative immune response; they cannot be tolerated.

The numerical analysis of the role of antigenicity is depicted in Fig. 4(b). The left bar in the figure represents the antigens that can be low zone tolerized, i.e. the antigens that fail to induce proliferation when they are introduced as a single cell. The right bar represents all antigens that do induce proliferation after being introduced in a large dose ($AG = 10^8$, e.g. a full-grown organ). Antigens that induce proliferation are always rejected; the lymphocytes can expand infinitely whereas the maximum antigen size is limited. In the interesting antigenicity range (i.e. $1 < = I < = 13$) the antigens can be tolerated (neonatally or in low doses) or rejected (sufficiently large doses in mature systems). If the self antigens belong to this group, they require explicit tolerization in order to prevent autoimmunity. Precursor depletion provides such a tolerance process.

THE MEMORY MODEL

Because precursor depletion hinges upon the longevity of the precursor cells and because (at least part of the) precursors are reported to be short-lived (Rocha *et al.*, 1983; Jerne, 1984), we next investigate a model that divides the precursors into short-lived virgin (unprimed) cells and long-lived (primed) memory cells (i.e. M = 5, DP = 1, $DM = 10^{-3}$). We will show that this "memory" model accounts for (1)

neonatal, low zone and high zone tolerance; and (2) generates a difference in the antigenicity of self and non-self antigens. The major restriction of the (previous) simple model, i.e. that self antigens must be relatively weakly antigenic ($I < = 13$), is thus removed.

Model behaviour: high zone tolerance

If a large dose of the same antigen as used in the simple model ($I = 10$, see Fig. 2(a)) is introduced into a mature system with short-lived precursors (Fig. 5(a)), the antigen is tolerated instead of rejected. Because precursors are short-lived, steady-state virgin precursor populations are small (i.e. $I/DP = 10$ cells, day 0–10); too small for pushing the system over the proliferation threshold, even if antigen is introduced in a large dose ($AG = 10^8$). Precursors are depleted by activation, effector populations reach a small stable equilibrium, and memory cells are never generated because the antigen concentration is too high.

Model behaviour: memory accumulation

A small dose of this antigen ($I = 10$, Fig. 5(b)) slowly activates (and depletes) the precursor population (day 0–60). Because the (re)stimulatory conditions are

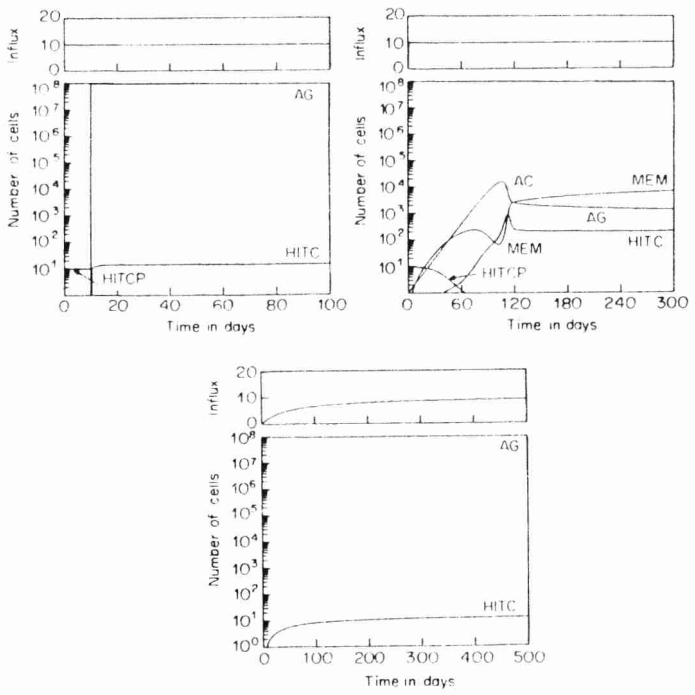

FIG. 5. The behaviour of the memory model. (a) depicts the tolerization of a large dose ($AG = 10^8$) of the antigen $I = 10$, which was rejected in the simple model (Fig. 2(a)). A low dose ($AG = 1$) however induces proliferation because memory cells accumulate (day 0–100; antigen hence regresses and the system settles into a stable equilibrium (b). The large antigen dose ($AG = 10^8$, a complete organ) is also tolerized in neonatal circumstances (c). Parameters as in Table 2 ($I = 10$, $DP = 1$, $M = 5$).

poor (antigen is small), the generated effectors return to the memory stage. Memory cells are protected from the high precursor and effector decay rate ($DM = 10^{-3}$), and hence accumulate. Thus the clone size increases; memory cells acquire a population of 230 cells around day 70. Proliferation remains absent in this period because effectors quickly become memory cells again, and thus remain below the proliferation threshold. Once antigen becomes sufficiently large (around day 100) for restimulating effectors and reactivating memory cells however, the 230 memory cells push the effectors over the proliferation threshold and proliferation commences. The clone size now increases steeply (by proliferation) and the antigen, as a consequence, regresses. The system settles into a stable equilibrium with a low (harmless?) antigen concentration ($AG = 10^3$), few effectors ($HITC = 200$) and many memory cells ($MEM = 10^4$). The system is immune to subsequent introduction of antigen.

In conclusion this antigen ($I = 10$) is tolerized in a high dose but induces proliferation and immunity when it is introduced in a smaller dose; this phenomenon corresponds to high zone tolerance. Immunity develops by virtue of memory accumulation; memory cells accumulate when stimulatory conditions are poor.

Model behaviour: neonatal tolerance

During neonatal life precursor influx is low but stimulatory conditions are good, i.e. antigen (the organ) is fully developed ($AG = 10^8$, Fig. 5(c)). Thus effector restimulation is high and memory cells fail to accumulate, hence the organ is tolerated. We conclude that self antigens fail to accumulate memory cells because stimulatory conditions are good. This failure extends to mature life as long as the self antigen remains present in the same (relatively) high concentration.

Static analysis

The model still switches between tolerance and proliferation (Fig. 5(a) vs 5(b)), but in reversed circumstances. We again investigate this switch statically: Fig. 6 depicts the isocline planes of the memory model. This model consists of four variables; we project these into the 3-D state space (for $I = 10$) by making quasi steady-state assumptions for memory cells (Fig. 6(a), (b)) or for precursor cells (Fig. 6(c), (d)). In the 2-D state space (Fig. 6(e)) both quasi steady-state assumptions are made (for $I = 10$). The antigen is identical to that of the simple model and is hence not shaded. The precursor plane (Fig. 6(a) resembles that of Fig. 3(a): it is almost identical at high antigen concentrations but its position at low antigen concentrations is shifted backwards 100-fold (precursor longevity was decreased 100-fold). As a consequence the left side of the precursor plane is situated behind the effector plane (i.e. in the $HITC' < 0$ region); this is not the case in Fig. 3(a). Moreover, the precursor plane bridges a far smaller region in the state space, i.e. precursor kinetics are far more restricted. Both features explain why precursor kinetics fail to push the system over the proliferation threshold (Fig. 5(a)). The effector plane of Fig. 6(b) is very similar to that of Fig. 3(b); large effector populations at low antigen values, however, decrease here because the cells become memory cells; i.e. the upper slope of the plane is steeper here (almost vertical). The form of the memory plane (Fig. 6(c)

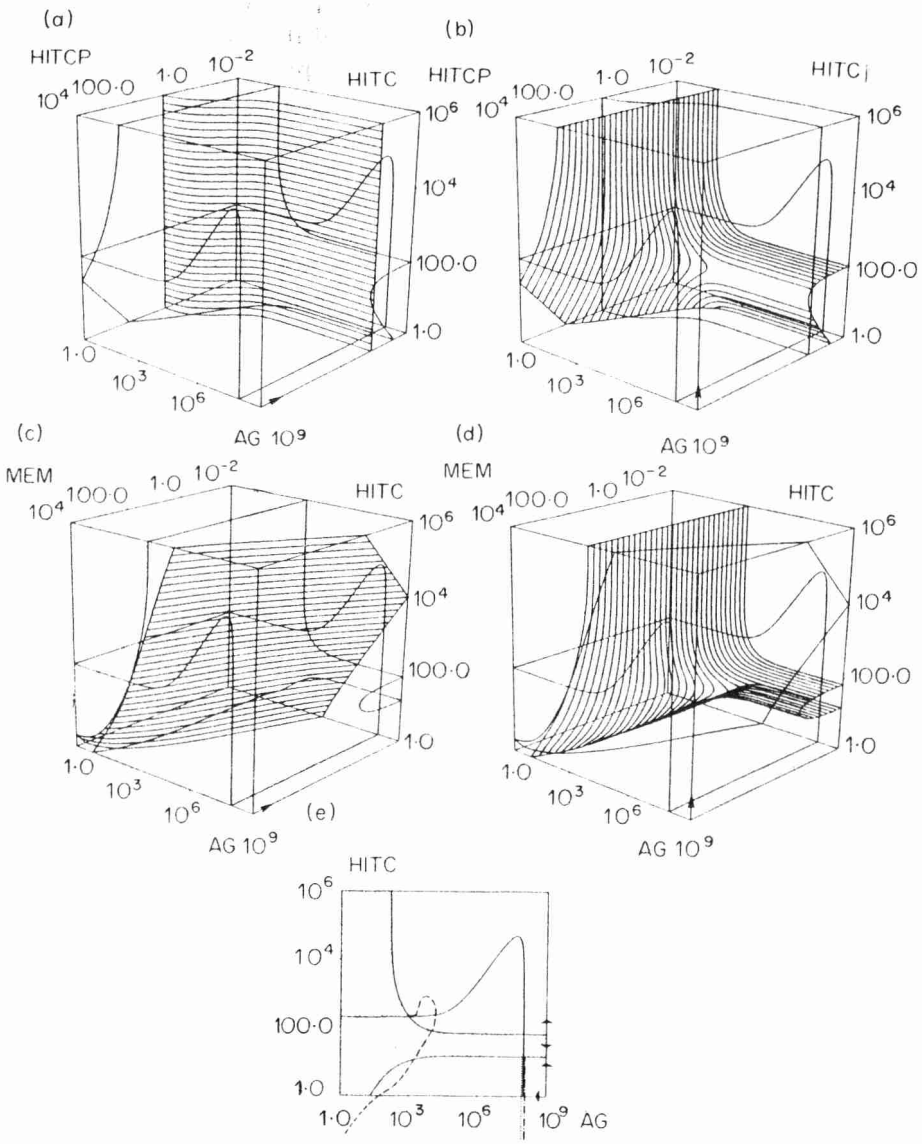

FIG. 6. The 0-isocline planes of the (4) variables of the memory model. Quasi steady-state assumptions are made for memory cells in (a) and (b); for precursors in (c) and (d); and for memory and precursors in (e). (b) and (d) depict the effector planes, (a) the precursor, and (c) the memory′ = 0 isocline plane (for I = 10). (e) shows the 2-D state space: shading indicates the effector decrease zone. Arrows indicate the local direction of trajectories. The time plots of Fig. 5 are indicated in the 2-D state space: dashed line: low dose memory accumulation; dotted line: neonatal tolerance; dash-dotted line: high zone tolerance. Parameters as in Table 2 (I = 10, DP = 1, M = 5).

illustrates that large steady-state memory populations require antigen to be small; large effector populations increase that steady-state memory population. The form of the effector plane (Fig. 6(d)) is similar to that of the previous effector planes, the fold however is more pronounced. Note that the proliferation threshold disappears (the fold closes) at sufficiently high memory numbers: accumulated memory populations push the system over the proliferation threshold.

The reduction into two dimensions (Fig. 6(e)) generates a very similar isocline plot for the antigen I = 10 (compare Fig. 3(d)). The figure shows a high proliferation threshold and a low tolerance isocline enclosing a (shaded) region of effector decline. The trajectory of Fig. 5(a) (high zone tolerance) is indicated by the dash-dotted line. In contrast to Fig. 3(d), this same antigen in the same dose fails to bridge the intermediate $HITC' < 0$ zone. In the absence of memory cells quasi steady-state assumptions are apparently more reliable here, because precursor kinetics are more restricted (see Fig. 6(a)).

The low zone memory accumulation trajectory (Fig. 5(b)) is represented by the dashed line. By accumulating memory cells the model passes the proliferation threshold, (apparently) ignoring the quasi steady-state assumptions; by proliferation the trajectory crosses the $AG' = 0$ isocline leading to antigen regression, and as a consequence, but again ignoring the quasi steady-state assumptions, leading to effector decline. Effector increase in the $HITC' < 0$ region is due to the rapid activation of accumulated memory cells (day 75–100); effector decrease in the upper $HITC' > 0$ region is due to rapid effector return to memory cells (day 120). Effectors and antigen decline until the stable intersect between the proliferation threshold and the $AG' = 0$ isocline is reached (this same intersect is instable in the simple model).

The neonatal trajectory (dotted) line ends up in the tolerance equilibrium, and is identical to that of the simple model. Here, however, the large dose (dash-dotted) is also tolerated.

Antigenicity

Figure 7 is the memory equivalent of Fig. 4. The static analysis (Fig. 7(a)) yields comparable results, i.e. an isocline plane similar to that of Fig. 4. The model behaviour (numerical analysis, Fig. 7(b)) however differs considerably: the left (tolerance) and the right (rejection) bar no longer have an overlapping antigenicity range. In the intermediate zone between the bars large doses are tolerized whereas smaller doses induce proliferation (e.g. I = 10, Fig. 5(a) and 5(b)). Thus if they are to be rejected, large antigen doses need to be more antigenic ($I >= 14$) than small doses ($I > 9$). Small doses need to be sufficiently weak ($I <= 9$) for to induce tolerance (i.e. absence of proliferation); such tolerized antigens expand to their intrinsic size.

Memory accumulation

In the memory model slow activation of virgin precursors no longer reduces the clone size (i.e. the precursor depletion phenomenon of the simple model) because precursors and effectors now have an equal life span. Conversely, slow precursor

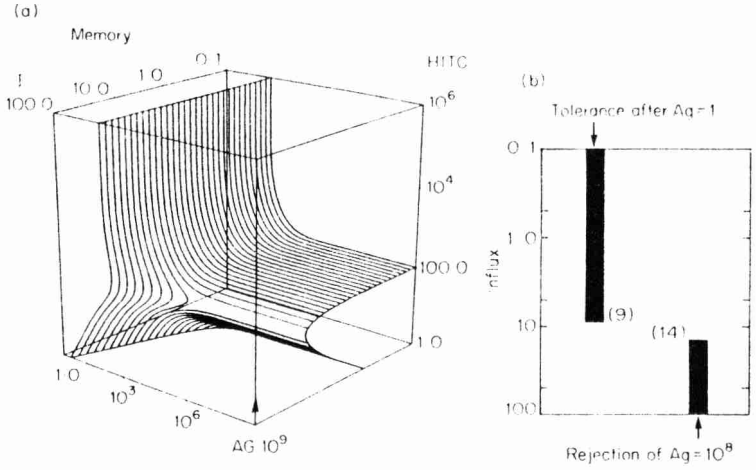

FIG. 7. The effect of antigenicity on tolerance in the memory model. Figure 7 is the memory equivalent of Fig. 4. Although the static analysis (a) yields similar results, the dynamic analysis (b) is totally different. Weak antigens are again always tolerized, and strong antigens always rejected, but the bars no longer overlap: in the intermediate zone ($9 < I < 14$) antigens induce proliferation in small doses but are (high zone) tolerized in large doses. Parameters as in Table 2 (DP = 1, M = 5).

activation (in low dose circumstances) now coincides with rapid memory accumulation, i.e. with an increase in clone size. And indeed, the situation is reversed: large antigen doses are tolerized (in both neonatal and mature conditions) and smaller doses evoke immunity. Returning to the topic of self non-self discrimination we have seen that self antigens fail to accumulate memory cells (Fig. 5(c)). Self antigens can thus be differentiated from non-self if non-self antigens accumulate memory cells; moreover if they do so, non-self antigens facilitate the development of "their" immune response because the clone size increases. Non-self specific memory cells accumulate when stimulatory conditions are poor; we suggest that this is the case in low affinity cross reactions, i.e. due to multispecificity (of the first kind (Jerne, 1984)).

Cross reactivity

In order to account for cross reactivity we have to incorporate the affinity of the interaction between the T-lymphocyte receptor and the antigen. As we did before (De Boer *et al.*, 1986*c*), we suppose that low affinity reduces the intensity of the interaction, i.e. for low affinity clones a far larger antigen concentration is required for maximum stimulation (corresponding to the multiplication of Ag by its affinity). In Fig. 8(a) we depict the steady-state memory population that is reached when large doses ($AG = 10^8$) of different antigens varying in affinity are presented to three clones of T-lymphocytes ($I = 0.1$, $I = 1$, and $I = 10$ respectively). Intermediate affinity antigens evoke the largest memory populations: low affinity antigens fail to activate precursors whereas high affinity antigens block memory generation. The size of the steady-state memory population depends almost linearly on the daily precursor

influx (I); the maximum reached when I = 0·1 is about 30 cells, this is insufficient to push the clone over the proliferation threshold even if the cells are activated by a high affinity antigen (Fig. 8(b), the right bar). We conclude that memory populations easily accumulate by cross reactivity, and that, for a sufficiently high precursor influx (I > = 0·4, Fig. 8(b)), memory accumulation enables the clones to cross the proliferation threshold whenever "their" high affinity antigen enters the immune system. Clones with an insufficient daily influx easily accumulate sufficient memory cells whenever they cross react (with low affinity) during a proliferative immune response, because they then profit from the local IL2 production (not shown). This corresponds to elongation of the right bar in Fig. 8(b).

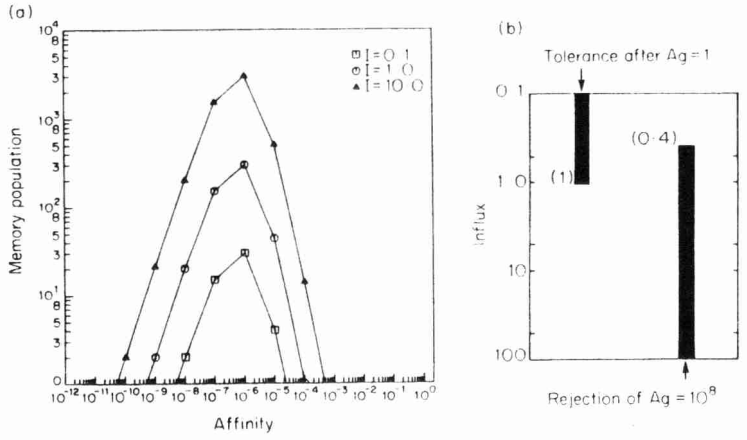

FIG. 8. (a) the steady-state memory population arising by cross reactivity, as revealed by simulation. It appears that intermediate affinity antigens evoke the largest steady-state memory populations. A 10-fold increase in antigenicity (I) approximately corresponds to a 10-fold increase in the steady-state memory population (about 100 memory cells are required for pushing any clone over the proliferation threshold). (b) the effect of accumulation on tolerance. The figure is similar to Fig. 7b but memory cells are first allowed to accumulate by a low affinity interaction ($AA = 10^{-6}$). Large antigen doses become rejectable by memory accumulation and low doses are less often tolerized (compare Fig. 7(b)). However, the bars now overlap (for 0·4 < = I < = 1), which corresponds to low zone tolerance (compare Fig. 4(b)). Parameters as in Table 2 (DP = 1, M = 5).

Clones cross reacting with low affinity to self antigens (i.e. by multispecificity) should already start to accumulate memory cells neonatally; note however that the clones with high self affinity fail to do this (Fig. 5(c)). Thus the neonatal T cell repertoire is strongly influenced by the self environment: it is depleted of self reactive clones but enriched in self cross-reactive clones. Experimental determination of the antigenicity of foreign antigens should therefore yield relatively high (memory) precursor frequencies. The major shortcoming of the simple model, namely that self antigens had to be weakly antigenic, is thus evaded: self antigens are indeed relatively weak because non-self antigens appear as stronger antigens due to memory accumulation.

Low zone tolerance

Accumulated memory populations are "precursor" depleted when they are slowly activated by a high affinity antigen. Introduction of a high affinity antigen in a low dose (AG = 1) activates the memory cells slowly, yielding only a few effectors which decay rather than proliferate. Thus the clone size decreases, because long-lived memory cells are replaced by short-lived effectors, i.e. by (simple) precursor depletion. This is shown by the left bar in Fig. 8(b): in the antigenicity range where the bars overlap small doses (AG = 1) induce tolerance whereas large doses ($AG = 10^8$) induce proliferation. This accounts for low zone tolerance. We conclude that in the memory model neonatal tolerance can occur for all antigens for which $I < 14$ (Fig. 7(b)); high zone tolerance for $9 < I < 14$ (Fig. 7(b)), and low zone tolerance for $0{\cdot}4 < = I < = 1$ (for this particular affinity ($AA = 10^{-6}$), Fig. 8(b)). Neonatal tolerance thus develops most easily. Note that in the simple model neonatal and low zone tolerance coincide (Fig. 4(b)).

The humped curves of Fig. 8(a) demonstrate that memory cells no longer accumulate in low dose circumstances. The introduction of a low dose of a high affinity antigen increases the stimulatory conditions markedly; the maximum accumulated memory population should therefore decline.

Self non-self discrimination

The actual differentiation of self and non-self is demonstrated in a memory model with two lymphocyte clones: one (HITCa, see Table 3) with high affinity to self and low affinity to non-self (i.e. AA = 1 and $AV = 10^{-6}$), and another (HITCv) that cross reacts with low affinity to self but which has high affinity to the foreign antigen (i.e. $VA = 10^{-6}$ and VV = 1). The foreign antigen (VR) can, for instance, be a virus infecting the immune system during mature life (at day 1000, Fig. 9). In the model we suppose that IL2 remains restricted to the local compartment of production (e.g. a lymph node), i.e. the clones do not profit from each other's IL2. During the neonatal development, i.e. in presence of self (AG) and in the absence of virus (VR), virus specific memory cells (MEMv) accumulate (Fig. 9(c)). The AG (self) specific clone (Fig. 9(a)) behaves similarly to that of Fig. 5(c); we again consider a clone with I = 10. Although the daily influx of virus specific cells is 10 times lower (IV = 1) than the influx of the self-clone the VR-clone reaches a population of about 250 cells (the self-clone 15, see Fig. 9(a)). Upon the introduction of a large dose of the virus ($VR = 10^7$, day 1000, Fig. 9(d)) this MEMv population is rapidly activated, pushing the virus specific clone over the proliferation threshold. Note that proliferation would never have started had the clone size not increased by memory accumulation (see Fig. 7, or compare the self antigen, AG). After about 18 days of proliferation the virus is rejected by about 10^5 effectors (HITCv). The effectors subsequently become memory cells, leaving a huge, slowly declining memory population. The presence of about 350 virus specific, but self cross-reacting effectors (HITCv), poses no threat to the self antigen: the self affinity of these cells (VA) is insufficient for self destruction (killing). Thus this two-clone immune system discriminates self from non-self: the virus is rejected by virtue of self cross-reactivity, whereas clones with

TABLE 3

We consider two memory clones: the populations with the suffix "a" are specific for the self antigen AG; those with the suffix "v" for the virus VR. The clones cross react symmetrically. AA is the affinity fo the a-clone to AG, AV its affinity to VR; VA is the affinity of the v-clone to AG, VV its affinity to VR (AA and VV = 1, AV and VA = 10^{-6}). The maximum antigenic stimulation values are still 1·0 (as in the single clone model): stimulation is defined as the Michaelis–Menten saturation of the sum of the different antigen concentrations multiplied by their respective affinities. We assume that IL2 remains restricted to the local production site, i.e. the clones do not profit from each other's IL2. Target cell lysis is similarly reduced by low affinity interactions. Note however that the total killing increases when the number of clones increases. Parameters are as in Table 2 (EP = 1, M = 5)

```
SMa = (AA*AG + AV*VR)/(KM + AA*AG + AV*VR)
SMv = (VA*AG + VV*VR)/(KM + VA*AG + VV*VR)
SHa = (AA*AG + AV*VR)/(KH + AA*AG + AV*VR)
SHv = (VA*AG + VV*VR)/(KH + VA*AG + VV*VR)
IL2a = HITCa*SHa
IL2v = HITCv*SHv
d(MEMa)/dt = M*HITCa*(1 - SHa) - AM*MEMa*SMa - DM*MEMa
d(MEMv)/dt = M*HITCv*(1 - SHv) - AM*MEMv*SMv - DM*MEMv
d(HITCPa)/dt = IA - AP*HITCPa*SHa - DP*HITCPa
d(HITCPv)/dt = IV - AP*HITCPv*SHv - DP*HITCPv
d(HITCa)/dt = AM*MEMa*SMa + AP*HITCPa*SHa + P*HITCa*IL2a/(KI + IL2a)
              - DE*HITCa - M*HITCa*(1 - SHa)
d(HITCv)/dt = AM*MEMv*SMv + AP*HITCPv*SHv + P*HITCv*IL2v/(KI + IL2v)
              - DE*HITCv - M*HITCv*(1 - SHv)
d(AG)/dt = R*AG - B*AG*AG - K*HITCa*AA*AG/(KK + AA*AG + AV*VR)
           - K*HITCv*VA*AG/(KK + VA*AG + VV*VR)
d(VR)/dt = R*VR - B*VR*VR - K*HITCv*VV*VR/(KK + VV*VR + VA*AG)
           - K*HITCa*AV*VR/(KK + AV*VR + AA*AG)
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high self affinity never evoke immunity. Intermediate doses of the virus ($10^3 < VR < 10^7$) also induce proliferation but settle into the same equilibrium as does the antigen in Fig. 5(b). Lower doses are however low zone tolerized. Low doses of immunogenic viruses (e.g. IV = 10) however always induce proliferation (see Fig. 8(b), the left bar).

We conclude that the simple and the memory model generate similar phenomena, i.e. neonatal and low zone tolerance, by precursor depletion and memory accumulation. The memory model in addition (1) accounts for high zone tolerance, and, (2) generates relatively weak antigenicity for self antigens. The latter was a condition in the simple model.

Discussion

The interactions incorporated in the models follow conventional Michaelis–Menten saturation kinetics. The relation between the proliferative response and the

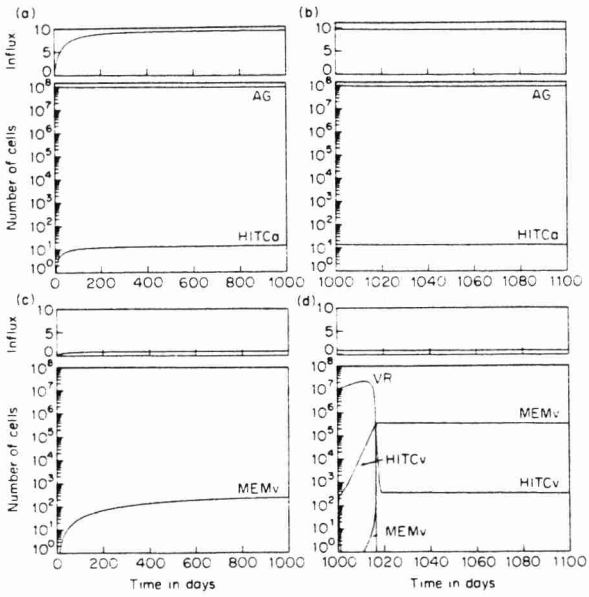

FIG. 9. Self non-self discrimination: rejection of a weakly antigenic virus (IV = 1) in combination with the tolerization of a more antigenic self antigen (IA = 10). During the neonatal development virus (VR) specific memory cells (MEMv) accumulate, whereas self reactive memory cells (MEMa) remain absent. At day 1000 the virus is introduced: the accumulated population of 250 memory cells pushes the virus clone over the proliferation threshold, leading to proliferation and virus rejection. The self antigen and the self reactive clone remain untouched. Parameters as in Table 2 (IA = 10, IV = 1, AA = 1, VV = 1, AV = 10^{-6}, VA = 10^{-6}, DP = 1, M = 5).

IL2 dose was however reported to be logistic (Cantrell & Smith, 1984; Hooton *et al.*, 1985); quantitative analysis however demonstrates that the slope parameter of that logistic function approximates 1·3 (Hooton *et al.*, 1985). A slope of 1·0 yields the conventional Michaelis–Menten function (used in our models), and the functions with slope 1·0, or 1·3 differ hardly. If the logistic IL2 dose response is incorporated in our models (e.g. with slope 2), tolerance development is slightly facilitated: in the simple model the interesting tolerance region covers $0{\cdot}8 <= I <= 18$, in the memory model high zone tolerance develops for $11 < I < 19$ and low zone tolerance for $0{\cdot}3 <= I <= 2$.

The combination of numerical integration and isocline analysis has proved to be a powerful method: the static analysis provided the proliferation threshold, and the dynamic analysis the conditions under which steady-state assumptions were ignored and the proliferation threshold was passed. Moreover the differences in numerical behaviour (Fig. 7(b) vs 4(b)) in combination with the similarity in the static analysis (Fig. 7(a) vs 4(a), or 3 vs 5) leads us to conclude methodologically, that for valid conclusions to be drawn static analysis has to be accompanied by (numerical) behaviour analysis (simulation).

Proliferation always requires an effector population sufficiently large for maintaining an IL2 concentration that is large enough to induce a cell division rate that exceeds the rate of cell decay. This is the proliferation threshold. If the proliferating cells are short-lived, the proliferation threshold is situated at relatively high effector numbers. Such a large effector population can only be achieved by activation of the precursors (cells are not yet proliferating); immune systems are therefore only responsive when full activation of the unprimed precursor populations plus (possibly) the memory cells generates an effector population larger than the proliferation threshold. Otherwise, e.g. for weak antigens (Figs 4 and 7), the antigen is tolerated whatever the dose in which it is introduced.

In responsive systems with long-lived precursors, (1) slow activation of unprimed precursors or (2) rapid activation of slowly accumulating precursors can result in a reduced total lymphocyte clone because the replacement effectors have a shorter life-span than the precursors (proliferation remains absent when effectors accumulate slowly). Once such a precursor population is smaller than the minimum required for generating an effector population larger than the proliferation threshold, proliferation remains absent whatever the subsequent antigen concentration. This corresponds to the development of tolerance in circumstances where other conditions evoke immunity.

If precursors are short-lived the reverse may occur: unresponsive systems become responsive when memory cells accumulate. Memory cells accumulate during poor stimulatory conditions, i.e. in low dose or low affinity circumstances, when activation proceeds relatively slowly. Antigens that fail to accumulate memory cells are tolerated (if they are sufficiently weak). Memory cells accumulate by low affinity cross reactivity to the self environment, thus yielding a T cell repertoire consisting of relatively large clones with non-self reactivity. Non-self antigens therefore appear to be fairly antigenic. We have shown that self antigens fail to induce memory cells, causing neonatal (self) tolerance. Low antigen doses may evoke proliferation in the same circumstances in which high doses yield tolerance; this combination corresponds to high zone tolerance. By contrast, accumulated memory populations can be "precursor" depleted by low doses of high affinity antigens, thus yielding to low zone tolerance.

The models thus generate two mechanisms for (self) tolerance, and although the mechanisms differ, the model behaviour is comparable at the macroscopic level. Both models account for tolerance in similar (and experimentally described) circumstances. Both mechanisms are intrinsic features of T cell activation and proliferation process and hence require no suppressive interactions. In fact the processes yield a form of clonal anergy [Nossal, 1983] in which the activity of each individual cell is preserved. The fact that proliferative T-lymphocyte clones can be silenced without receiving any down regulatory signal is strongly counterintuitive, but emerges as a robust property of models with a proliferation threshold. The major implication of a proliferation threshold is that small clones (i.e. clones activated by weak antigens) are silent; precursor depletion silences larger clones, memory accumulation wakes

up silent ones. It remains to be established experimentally whether biotic immune systems develop self tolerance by means of a proliferation threshold, precursor depletion and/or memory accumulation.

EXPERIMENTAL DATA

In accordance with the model behaviour is the fact that the prolonged maintenance of tolerance requires the continuous presence of the antigen (Humphrey, 1964; Boyse *et al.*, 1970, 1973), and that tolerance corresponds to a reduction in the number of antigen binding cells, i.e. in reduced clone size (Siskind, 1984; Fazekas de St Groth *et al.*, 1984). The fact that in the models tolerance develops due to a paucity in IL2 fits in with the general observation that antigenic stimulation (signal 1) in the absence of helper T cell factors (signal 2) generates unresponsiveness (Bretscher & Cohn, 1970; Metcalf, 1976, 1977; Teale *et al.*, 1979; Claman, 1979; Cleveland & Claman, 1980). We show here that the absence of signal 2 (IL2) can be caused by insufficient precursor or memory accumulation.

The major problem concerning the present results is however the sensitivity of these tolerance processes to external IL2 supply, e.g. IL2 produced by other (possibly distant) immune reactions to unrelated antigens. If the models tolerance easily terminates when such an external source of IL2 is incorporated, e.g. if the two clones of the Table 3 memory model (Fig. 9) were to exchange IL2 the self reactive clone would cross the proliferation threshold, yielding autoimmunity. Some experimental data however reveal similar phenomena: (1) the induction of neonatal tolerance can be abrogated by IL2 administration (Malkovskỳ *et al.*, 1984; Malkovskỳ & Medawar, 1984), (2) administration of IL2 enhances the development of (spontaneous insulin-dependent) autoimmunity in BB rats (Kolb *et al.*, 1986), and (3) T cell tolerance to *Mycobacterium leprae* in leprosy patients is known to terminate upon the administration of IL2 (Haregewoin *et al.*, 1984). However, in other experimental situations tolerant lymphocyte clones cannot be stimulated again (Streilein & Gruchalla, 1981; Gruchalla & Streilein, 1982; Feng *et al.*, 1983; Carnaud *et al.*, 1984).

A possible explanation for the fact that IL2 derived from distant immune reactions fails to push self reactive clones over the proliferation threshold is the high turnover of IL2 in the blood (Smith, 1984*a*; Lotze *et al.*, 1985*a*,*b*). We must therefore assume, as we did in Fig. 9, that IL2 effects are mediated locally, i.e. in the particular lymph node that has trapped the reactive lymphocytes. This however seems a quite reasonable assumption.

Systemic administration of IL2 to e.g. cancer and AIDS patients should however lead to autoimmunity. An explanation for the absence of such clinical data (Lotze *et al.*, 1985*a*,*b*) would be: (1) again the high turnover of the IL2 in the blood and (2) the absence of virgin precursors due to involution of the thymus. After puberty the thymus regresses, yielding a reduction in the influx of virgin precursors (Weksler & Siskind, 1984). Once the influx becomes zero our clonal anergy turns into a clonal abortion (Burnet & Fenner, 1949) (thus eliminating the controversy on anergy and abortion), because self reactive clones, lacking memory accumulation, depend

entirely on thymic efflux. The absence of precursors would account for the immunity of the tolerance process to systemic administration of IL2.

ANTIGEN PRESENTATION: IL1

Experiments have shown that antigen presentation and IL1 production by antigen presenting cells play a crucial role in tolerance development: the bypassing of antigen presentation markedly facilitates tolerization (Unanue, 1984). For the sake of simplicity we have omitted antigen presentation from our models. Although it is generally assumed that bypassing antigen presentation induces suppressor T cells which account for the observed tolerance (Germain *et al.*, 1980; Unanue, 1984), such results can be reinterpreted in terms of a proliferation threshold. IL1 is a co-factor in T-lymphocyte proliferation: it induces IL2 receptor expression and it increases IL2 production (Oppenheim *et al.*, 1986). The IL1 concentration therefore determines the height of the proliferation threshold. Low IL1 concentrations correspond to high proliferation thresholds and hence to increased tolerance development. We conclude, again, that the explanation involving suppression can be avoided. (Note that antigen presentation is low in neonatal circumstances (Argyris, 1984; Unanue, 1984.)

MHC RESTRICTION

In this paper we consider the negative selection process which eliminates MHC-restricted peripheral T-lymphocytes that recognize conventional self antigens. We have concluded that down regulation is not a prerequisite for (conventional) self tolerance.

Here we would like to speculate on the positive selection process which selects T-lymphocytes with sufficient affinity to the self MHC. We restrict ourselves to the discussion of helper T cells (i.e. to class II MHC restriction) because helper T cell unresponsiveness should also imply tolerance for the helper dependent cell types (cytotoxic T-lymphocytes, B-lymphocytes), see e.g. De Boer & Hogeweg (1986*a*,*b*). Suppose that this process occurs within the thymus (Zinkernagel *et al.*, 1978; Smith, 1984*b*); after their arrival from the bone marrow, most T-lymphocytes die in the thymus; a few survive and are expanded by (intrathymic) proliferation (Scollay *et al.*, 1984). Suppose that the only T-lymphocytes that proliferate are those that are sufficiently stimulated by the intrathymic MHC (in analogy with peripheral stimulatory requirements for proliferation), and that non-stimulated cells, lacking proliferation, decay (in analogy with the peripheral proliferation threshold). This would account for a peripheral T-lymphocyte repertoire ranging from intermediate to high affinity to self MHC.

The high affinity subset of these cells is expected to respond to the MHC (class II) expressed on several pheripheral cell types. If such high affinity cells are sufficiently rare, i.e. if their influx (I) is sufficiently low, these high affinity T-lymphocytes can be "eliminated" by precursor depletion and/or failure of memory accumulation. (The situation is not different from that in conventional self antigens). The frequency of T cell receptors with such high affinity to one of the very many

different MHC molecules (i.e. the self MHC) can indeed expected to be low. Once the virgin T-lymphocytes with high affinity to self MHC are eliminated, the peripheral T cell repertoire will consist only of cells with intermediate affinity to self MHC. (See Grossman (1982, 1984) and Dröge (1981*a*,*b*) for different explanations for the same end result.)

It is tempting to speculate that this intermediate affinity to self MHC suffices for memory accumulation, thus increasing the size of all non-self reactive clones. Moreover, note that cells with high affinity to foreign MHC are not eliminated by the processes, i.e. high alloreactivity is compatible with the current results. High alloreactivity suggests that alloantigens cross react with self MHC or with self MHC plus foreign antigen (i.e. self antigen), resulting in memory accumulation. The occurrence of the latter cross reaction is supported by data (Wilde *et al.*, 1984; Ashwell *et al.*, 1986).

Suppose that the cells with intermediate self MHC affinity do not respond to the peripheral MHC (note that they did respond to intrathymic MHC), i.e. suppose that peripheral conditions are less stimulatory than intrathymic conditions (e.g. due to the lack of thymic hormones). Sufficient affinity can then be achieved when conventional antigens are co-expressed with MHC antigens. Antigenic fragments associated with MHC should indeed transform the intermediate affinity interaction into a high or low affinity interaction. The former situation leads to an immune response, the latter to absence of interaction. In conclusion, peripheral T cells with high affinity to self MHC plus conventional self antigens are silenced by precursor depletion and/or kept silent by the failure of memory accumulation. The other clones expand, constituting the well-known peripheral T cell repertoire that consists of cells that respond to self MHC plus foreign antigen.

ANTIGENICITY

In our previous analysis of the low zone tolerance phenomenon we demonstrated that precursor depletion accounts solely for tolerance to weak antigens (De Boer & Hogeweg, 1986*a*,*b*). If conventional self antigens are only weakly antigenic, i.e. if they belong to the group of antigens that are always tolerated (i.e. $I < 1$ or $I < 9$ in the simple and memory model respectively), the proliferation threshold is in itself a sufficient explanation for self tolerance. Although it is conceivable that receptors that recognize conventional self are eliminated by genetic selection, which would indeed lead to very few self reactive precursors (i.e. weakly antigenic self antigens), this possibility is uninteresting because it would mean tolerization of self antigens whatever the circumstances in which they are presented to the immune system. In the simple model the interesting self tolerance region, i.e. the region in which both rejection and tolerance occur, ranges from $1 <= I <= 13$ (Fig. 4(b)). If the self antigens belong to this group, explicit tolerization is indeed required for avoiding self destruction (autoimmunity); this can occur during neonatal life and precursor depletion can account for it.

The memory model suggests an even more interesting possibility: all antigens, self or non-self, may be too weakly antigenic to initiate proliferation by (virgin)

precursor activation alone. Then cross reactivity and memory accumulation must be responsible for pushing immune reactions to any (possibly foreign) antigen over the proliferation threshold. In contrast to all other antigens, self antigens are tolerated because they fail to accumulate memory cells. Moreover, this conceptualization of immune systems explains thymic involution: once the periphery is fully seeded with memory cells, immune dynamics operate by memory accumulation, due to cross reactivity (multispecificity), totally independently of virgin precursor influx.

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REFERENCES

ARGYRIS, B. F. (1984). *Immunol. Today* **5**, 34.
ASHWELL, J. D., CHEN, C. & SCHWARTZ, R. H. (1986). *J. Immunol.* **136**, 389.
BARTH, R. K., SIM, B. S., LAN, N. C., HUNKAPILLER,T., SOBIECK, N., WINOTO, A., GERSHENFELD, H., OKADA, C., HANSBURG, D., WEISSMAN, I. L. & HOOD, L. (1985). *Nature* **316**, 517.
BEREK, C, GRIFFITHS, G. M. & MILSTEIN, C. (1985). *Nature* **316**, 412.
BILLINGHAM, R. E., BRENT, L. & MEDAWAR, P. D. (1953). *Nature* **172**, 603.
BOYSE, E. A., LANCE, E. M., CARSWELL, E. A., COOPER, S. & OLD, L. J. (1970). *Nature* **227**, 901.
BOYSE, E. A., CARSWELL, E. A., SCHEID, M. P. & OLD, L. J. (1973). *Nature* **244**, 441.
BRETSCHER, P. & COHN, M. (1970). *Science* **169**, 1042.
BRUNNER, K. T., MCDONALD, H. R. & CEROTTINI, J. C. (1981). *J. Exp. Med.* **154**, 362.
BURNET, F. & FENNER, F. (1949). *The Production of Antibodies*, 2nd edn. London: Macmillan.
CANTRELL, D. A. & SMITH, K. A. (1984). *Science* **224**, 1312.
CARNAUD, C., ISHIZAKA, S. T. & STUTMAN, O. (1984). *J. Immunol.* **133**, 45.
CHIEN, Y., NICHOLAS, R. J. G., KAVALER, J., LEE, N. E. & DAVIS, M. M. (1984). *Nature* **309**, 322.
CLAMAN, H. N. (1979). *Cell. Immunol.* **48**, 201.
CLEVELAND, R. P. & CLAMAN, H. N. (1980). *J. Immunol.* **124**, 474.
DE BOER, R. J. (1983). *GRIND: Great Integrator Differential Equations.* Bioinformatics Group, University of Utrecht, The Netherlands.
DE BOER, R. J., HOGEWEG, P., DULLENS, H. F. J., DE WEGER, R. A. & DEN OTTER, W. (1985). *J. Immunol.* **134**, 2748.
DE BOER, R. J. & HOGEWEG, P. (1985). *J. theor. Biol.* **113**, 719.
DE BOER, R. J. & HOGEWEG, P. (1986*a*). In: *Lecture Notes in Biomathematics, Vol. 65.* (Hoffman, G. W. & Habra, T. eds). p. 120. Berlin: Springer-Verlag.
DE BOER, R. J. & HOGEWEG, P. (1986*b*). *J. theor. Biol.* **120**, 331.
DE BOER, R. J., MICHELSON, S. & HOGEWEG, P. (1986*c*). *J. Immunol.* **136**, 4319.
DRÖGE, W. (1981*a*). *Cell. Immunol.* **57**, 251.
DRÖGE, W. (1981*b*). *Cell. Immunol.* **64**, 381.
EARLY, P., HUANG, H., DAVIS, M., CALAME, K. & HOOD, L. (1980). *Cell* **19**, 981.
FAZEKAS DE ST GROTH, B., BASTEN A. & LOBLAY, R. (1984). *Eur. J. Immunol.* **14**, 228.
FENG, H. M., GLASEBROOK, A. L., ENGERS, H. D. & LOUIS, J. L. (1983). *J. Immunol.* **131**, 2165.
GAMMON, G., DUNN, K., SHASTRI, N., OKI, A., WILBUR, S. & SERCARZ, E. E. (1986). *Nature* **319**, 413.
GERMAIN, R. N., PIERRES, M. & BENACERRAF, B. (1980). In: *Macrophage Regulation of Immunity.* (Unanue, E. R. & Rosenthal, A. P. eds). p. 15. New York: Academic Press.
GOOD, M. F., PYKE, K. W. & NOSSAL, G. J. V. (1983). *Proc. natnl. Acad. Sci. U.S.A.* **80**, 3045.
GORCZYNSKI, R. M. & MACRAE, S. (1979*a*). *J. Immunol.* **122**, 737.
GORCZYNSKI, R. M. & MACRAE, S. (1979*b*). *J. Immunol.* **122**, 747.
GOTTWALD, B. A. & WANNER, G. (1981). *Computing* **26**, 355.
GRIFFITHS, G. M., BEREK, C., KAARTINEN, M. & MILSTEIN, C. (1984). *Nature* **312**, 271.
GROSSMAN, Z. (1982). *Eur. J. Immunol.* **12**, 747.
GROSSMAN, Z. (1984). *Immunol. Rev.* **79**, 119.
GRUCHALLA, R. S. & STREILEIN, J. W. (1982). *Immunogenetics* **15**, 111.
HAREWOIN, A., MUSTAFA, A. S., HELLE, I, WATERS, M. F. R., LEIKER, D. L. & GODAL, T. (1984). *Immunol. Rev.* **80**, 77.

HUMPHREY, J. H. (1964). *Immunology* **7**, 449.
HILGERT, I. (1979). *Immunol. Rev.* **46**, 27.
HOOTON, J. W. L., GIBBS, C. & PAETKAU, V. (1985). *J. Immunol.* **135**, 2464.
ISHIZAKA, S. T. & STUTMAN, O. (1983). *Eur. J. Immunol.* **13**, 936.
JERNE, N. K. (1984). *Immunol. Rev.* **79**, 5.
LOOK, A. T., SCHRIBER, T. J., NAWROCKI, J. F. & MURPHY, W. H. (1981). *Immunol.* **43**, 677.
LOTZE, M. T., FRANA, L. W., SHARROW, S. O., ROBB, R. J. & ROSENBERG, S. A. (1985*a*). *J. Immunol.* **134**, 157.
LOTZE, M. T., MATORY, Y. T., ETTINGHAUSEN, S. E., RAYNER, A. A., SHARROW, S. O., SEIPP, C. A. A., CUSTER, M. C. & ROSENBERG, S. A. (1985*b*). *J. Immunol.* **135**, 2865.
KOLB, H., ZIELASEK, J., TREICHEL, U., FREYTAG, G., WRANN, M. & KIESEL, U. (1986). *Eur. J. Immunol.* **16**, 209.
KYEWSKI, B. A., FATHMAN, C. G. & ROUSE, R. V. (1986). *J. exp. Med.* **163**, 231.
MALKOVSKY, M. & MEDAWAR, P. B. (1984). *Immunol. Today* **5**, 340.
MALKOVSKY, M., MEDAWAR, P., HUNT, R., PALMER, L. & DORE, C. (1984). *Proc. R. Soc. Lond. Ser. B* **220**, 439.
MANGER, B., WEISS, A., WEYAND, C., GORONZY, J. & STOBO, J. D. (1985). *J. Immunol.* **135**, 3669.
MCCARTHY, S. A. & BACH, F. H. (1983). *J. Immunol.* **131**, 1676.
MERRILL, S. J. (1982). *Math. Biosci.* **62**, 219.
METCALF, E. S. & KLINMAN, N. R. (1976). *J. exp. Med.* **143**, 1327.
METCALF, E. S. & KLINMAN, N. R. (1977). *J. Immunol.* **118**, 2111.
MEUER, S. C., HUSSEY, R. E., CANTRELL, D. A., HODGDON, J. C., SCHLOSSMAN, S. F., SMITH, K. A. & REINHERZ, E. L. (1984). *Proc. natn. Acad. Sci. U.S.A.* **81**, 1509.
MILLER, R. A., ROZANS, M. K., YTHIER, A. A. & STROM, T. B. (1986). *J. Immunol.* **136**, 977.
MITCHISON, N. A. (1965). *Proc. R. Soc. Lond. Ser. B* **161**, 275.
NOSSAL, G. J. V. (1983). *Ann. Rev. Immunol.* **1**, 33.
NOSSAL, G. J. V. & PIKE, B. L. (1981). *Proc. Natn. Acad. Sci. U.S.A.* **78**, 3844.
OPPENHEIM, J. J., KOVACS, E. J., MATSUSHIMA, K. & DURUM, S. K. (1986). *Immunol. Today* **7**, 45.
OWEN, R. D. (1945). *Science* **102**, 400.
PRYMOWICZ, D., MOORE, R. N. & ROUSE, B. T. (1985). *J. Immunol.* **134**, 2683.
ROCHA, B., FREITAS, A. A. & COUTINHO, A. A. (1983). *J. Immunol.* **131**, 2158.
ROOPENIAN, D. C., WIDMER, M. B., OROSZ, C. G. & BACH, F. H. (1983). *J. Immunol.* **130**, 542.
SCOLLAY, R., BARTLETT, P. & SHORTMAN, K. (1984). *Immunol. Rev.* **82**, 79.
SEGEL, L. A. (1984). *Modelling Dynamic Phenomena in Molecular & Cellular Biology*. Cambridge: Cambridge University Press.
SISKIND, G. W. (1984). In: *Fundamental Immunology* (Paul, W. E., ed.). p. 537. New York: Raven Press.
SMITH, K. A. (1984*a*). *Ann. Rev. Immunol.* **2**, 319.
SMITH, K. A. (1984*b*). *Immunol. Today* **5**, 83.
STOCKINGER, B. (1984). *Proc. natn. Acad. Sci. U.S.A.* **81**, 220.
STOCKINGER, B., DARJES, H. & KRAMMER, P. H. (1986). *Eur. J. Immunol.* **16**, 301.
STREILEIN, J. W. & GRUCHALLA, R. S. (1981). *Immunogenetics* **12**, 161.
TEALE, J. M. & LAYTON, J. E. & NOSSAL, G. J. V. (1979). *J. exp. Med.* **150**, 205.
TILKIN, A. F., BEGUE, B., GOMARD, E. & LEVY, J. P. (1985). *J. Immunol.* **134**, 2779.
UNANUE, E. R. (1984). *Ann. Rev. Immunol.* **2**, 395.
WEIGLE, W. O. (1971). *Clin. Exp. Immunol.* **9**, 437.
WEKSLER, M. E. & SISKIND, G. W. (1984). *Monogr. Devl. Biol.* **17**, 110.
WIDMER, M. B. & BACH, F. H. (1981). *Nature* **294**, 750.
WILDE, D. B., PRYSTOWSKY, M. B., BELLER, D. I., GOLDWASSER, E., IHLE, J. N., VOGEL, S. N. & FITCH, F. W. (1984). *J. Immunol.* **133**, 1992.
ZINKERNAGEL, R. M. & DOHERTY, P. C. (1975). *J. exp. Med.* **141**, 1427.
ZINKERNAGEL, R. M., CALLAHAN, G. N., ALTHAGE, A. COOPER, S., KLEIN, P. A. & KLEIN, J. (1978). *J. exp. Med.* **147**, 882.