J. theor. Biol. (1985) **113**, 719–736 *Chapter 1.1*

Tumor Escape from Immune Elimination: Simplified Precursor Bound Cytotoxicity Models

R. J. De Boer

Bioinformatics Group, University of Utrecht, Padualaan 8, 3584 *CH Utrecht, The Netherlands and, Department of Pathology, University of Utrecht, Pasteurstraat* 2, 3511 *HX Utrecht, The Netherlands*

AND

P. Hogeweg

Bioinformatics Group, University of Utrecht, Padualaan 8, 3584 *CH Utrecht, The Netherlands*

(*Received* 6 *December* 1983, *and in revised form* 2 *November* 1984)

In this paper we present a series of models on cytotoxic T-cell activation derived, by successive simplifications, from the model for Tumor Escape from Immune Elimination of Grossman & Berke (1980). In their model Grossman & Berke (1980) investigate the "sneaking through" phenomenon, by which they mean that small tumors grow progressively, medium-sized tumors are rejected and large ones break through again. We define precursor bound cytotoxicity models as systems incapable of infinite proliferation. We show that sneaking through can occur in a broad class of very simple precursor bound cytotoxicity models due to the depletion of the precursor cells. The simplest process by which precursors can be depleted is long-lasting antigenic stimulation. We conclude that in precursor bound cytotoxicity models sneaking through does not need the rather intricate combination of counteracting feedback loops, memory and blocking described by Grossman & Berke (1980).

1. Introduction

Grossman & Berke (1980) describe a simple mathematical model for the interactions between a tumor and a part of the cell-mediated immune response, i.e. the activation of cytotoxic T-lymphocytes. The immune response to a tumor involves several different cell populations, e.g. macrophages, lymphocytes (B-, T-, NK-, K-cells), polymorphonuclear cells and mast cells. Simple kinetic models (mini models) of the anti-tumor response tend to take only one aspect of this reaction into consideration and are therefore always incomplete from an immunological point of view.

In the Grossman & Berke model a tumor is attacked by cytotoxic T-lymphocytes (CTL) that are generated upon T-cell contact with the tumor. It is indeed generally accepted that the cell-mediated immune response plays an important role in tumor immunology. The effector cells that are generally considered to play a role in cellular immunity to tumors are: cytotoxic T-lymphocytes (Miller & Heppner 1979; Schirrmacher *et al.*, 1979), cytotoxic macrophages (Haskill, Proctor & Yamamura 1975; Peri *et al.*, 1981) and natural killer (NK) cells (Herbermann, 1983).

Current concepts about T-cell activation involve a separation of T-lymphocyte populations (into helper and cytotoxic cells), antigen presentation by macrophages to lymphocytes and lymphocyte activation by factors derived from macrophages. The Grossman & Berke model thus only represents one aspect and not necessarily the most important aspect of the anti-tumor response (i.e. CTL induction). Furthermore, the process of CTL induction is known to be more complex than is specified in their model. Nevertheless, the Grossman & Berke model has proved to be a good paradigm system (as defined by Hogeweg & Hesper, 1978, 1981) for the study of non-monotonic growth patterns of tumors such as the sneaking through phenomenon.

This "sneaking through" phenomenon, "where small amounts of tumor cells grow progressively, medium-sized amounts are rejected, and large ones break through again" (Grossman & Berke, 1980) is one of the most interesting features of the model. Since spontaneously arising tumors always start from a single cell (tumors are monoclonal—Currie, 1976) it might be argued that the sneaking through phenomenon is only important in experimental situations. However, as Grossman & Berke argue, tumors during their life time exhibit several related non-monotonic or multiphasic growth patterns, e.g. rapid growth after a period of latency. The Grossman & Berke model was designed in order to study whether such phenomena could be due simply to the kinetics of the lymphocyte activation system itself and therefore would not need more complex explanations like an alteration in the tumor's antigenic determinants (Boyse & Old, 1969). In this paper we investigate whether models simpler than the Grossman & Berke model can also account for such non-monotonic growth patterns.

The model, as formulated by Grossman & Berke (1980), see Fig. 1, incorporates two features which in combination enable "a small tumor to bridge the gap between the low and high zone, without eliciting a strong response" (p. 269). The two factors are:

1. A positive feedback loop in the activation pathway of cytotoxic cells which in the case of medium-sized tumors rapidly generates a large number of precursor cells; moreover, the tumor size has to exceed some critical

value, 'the second activation threshold', before the rate of second activation necessary for the generation of cytotoxic T cells outweighs the rate at which once-activated cells return to the resting stage.

2. A negative feedback caused by blocking factors (i.e. shed antigen molecules) which are slowly released by living tumor cells and which are released after lysis of tumor cells. The latter process is quantitatively more important.

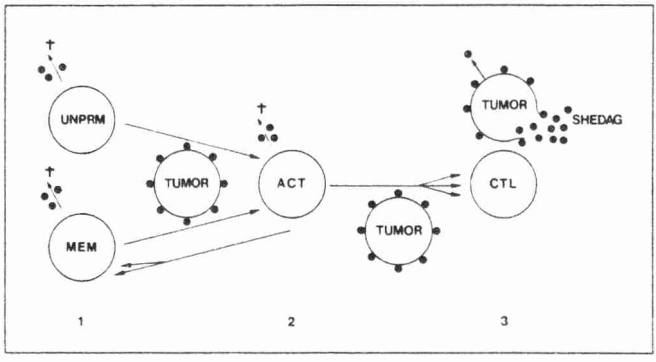

FIG. 1. Schematic illustration of the Grossman & Berke model: 1 = precursor stage, 2 = activation stage, 3 = effector stage, UNPRM = unprimed cells, MEM = memory cells, ACT = once-activated cells, CTL = cytotoxic T-lymphocyte, TUMOR = tumor cells (coated with antigen), SHEDAG = shed antigen.

The present paper shows that the presence of these two counteracting feedback mechanisms is not a prerequisite for sneaking through behaviour. Several models which lack these feedback loops but incorporate the assumption that cytotoxic cells emerge (after one proliferation step) from a rate-bound precursor population also show sneaking through behaviour. In these models an initially small tumor eventually grows in an uncontrolled manner after the tumor has depleted the precursor population. In models in which precursors can be generated by endless proliferation (as in the Grossman & Berke model) however, sneaking through needs a more complex explanation. Such a proliferation cycle will have to be shut off by some blocking factor.

2. Methods

In this paper we use a "multi-model-fixed parameter" approach to investigate the circumstances in which sneaking through behaviour occurs. The

various models were investigated by means of two program systems: GRIND (De Boer, 1983) enables the user to analyse the static properties of a model by numerical computation of 0-isoclines, as well as to analyse the dynamic behaviour by numerical integration; DEASIM (Stafleu, 1979) allows numerical integration of models incorporating time delays. The integrator implemented in GRIND is ROW4A (Gottwald & Wanner, 1981), the one implemented in DEASIM is the algorithm of Bulirsch & Stoer (1966).

3. Precursor Bound Cytotoxicity Models

(A) INTRODUCTION

We define precursor bound cytotoxicity models as activation schemes based on the assumption that the population of cytotoxic effector cells does not sustain itself by cell division but depends on the influx of new cells from one or more precursor populations. Proliferation in precursor bound cytotoxicity systems is a "once-only" occurrence, i.e. after cells have proliferated they do not proliferate again. By contrast, "proliferative systems" (to which the Grossman & Berke model with its particular parameter setting belongs) can generate an infinite number of effector cells from a limited precursor population by repeated proliferation. The maximum cytotoxic response of precursor bound cytotoxicity models is limited by the size of the precursor population: tumors large enough to cope with the maximum response therefore always break through. Progressive growth of small tumors and the rejection of a set of larger ones thus suffice to demonstrate sneaking through in such models. Note that the saturation term in the killing rate of the current models enhances the breakthrough of large tumors. In order to pinpoint more closely the factors responsible for the progressive growth of small tumors in precursor bound cytotoxicity models we develop a series of simplifications of the Grossman & Berke model.

(B) THE GROSSMAN & BERKE MODEL

The Grossman & Berke (1980) model is based on the following three main assumptions (see Fig. 1):

1. Two-step activation is necessary to transform a precursor cell into an effector (cytotoxic) cell.
2. Memory cells (functionally identical with precursor cells) are formed, upon proliferation, from activated cells that are not stimulated again.
3. Shed antigen molecules released by the tumor, especially following tumor cell lysis, block the activation of the non-effector cells.

Formal representation† of the Grossman & Berke model:

$$d(\mathrm{UNPRM})/dt = s - e' * \mathrm{UNPRM} - a * \mathrm{UNPRM} * (\mathrm{TUMOR} + \mathrm{SHED}) \quad (1)$$

$$d(\mathrm{MEM})/dt = e * \mathrm{ACT}(t - t') - a * \mathrm{MEM} * (\mathrm{TUMOR} + \mathrm{SHED}) - e' * \mathrm{MEM} \quad (2)$$

$$\mathrm{PREC} = \mathrm{UNPRM} + \mathrm{MEM} \quad (3)$$

$$d(\mathrm{ACT})/dt = a * \mathrm{PREC} * \mathrm{TUMOR} - a' * \mathrm{ACT} * (\mathrm{TUMOR} + \mathrm{SHED}) - d * \mathrm{ACT} \quad (4)$$

$$d(\mathrm{CTL})/dt = a'' * \mathrm{ACT}(t - t'') * \mathrm{TUM}(t - t'') - c * \mathrm{CTL} \quad (5)$$

$$d(\mathrm{TUMOR})/dt = b * \mathrm{TUMOR} - \beta * \mathrm{TUMOR} * \mathrm{CTL}/(\beta/\alpha + \mathrm{TUMOR}) \quad (6)$$

$$d(\mathrm{SHED})/dt = b' * \mathrm{TUMOR} - b'' * \mathrm{SHED} + k * \beta * \mathrm{TUMOR} * \mathrm{CTL}/(\beta/\alpha + \mathrm{TUMOR}) \quad (7)$$

Note that for the sake of our argument the equations have been rewritten so that:

—cells emerging from the positive feedback loop (MEM) are formulated explicitly,

—shed antigen (SHED) is treated as a separate entity (and not as part of the total amount of antigen),

—the killing term shows the conventional Michaelis Menten constant (β/α) (cf. Merrill, 1982).

Unprimed cells (UNPRM) have a constant influx (s) and efflux (e'). They become activated on encountering tumor cells (TUMOR), or become blocked by free antigen molecules (SHED) (1). Like uprimed cells, activated cells (ACT) can be stimulated (after which they become cytotoxic) or blocked (4). If, however, ACT cells fail to encounter antigen (TUMOR or SHED) they return to the memory (MEM) stage. Effector cells (CTL) are formed from stimulated activated cells after a proliferation ($a'' > a'$) stage of t'' hours (5). Memory cells (MEM), on the other hand, are formed from unstimulated activated cells after some proliferation ($e > d$) for t' hours (2). The total precursor population (PREC) is composed of unprimed and

† In order to increase readability, mnemonics are used for the variables of the model; parameter names are identical to those in Grossman & Berke (1980).

memory cells (3). Tumor cells (TUMOR) grow exponentially, and are killed by cytotoxic effector cells (6). This killing term has a Michaelis–Menten limitation ($KM = \beta/\alpha$). Shed antigen molecules (SHED) are released by living tumor cells, and upon tumor cell lysis (7). They are removed or decay, at a rate b''.

The parameters of the Grossman & Berke model were chosen on the basis of experimentally known orders of magnitude (Grossman & Berke, 1980; see appendix). Note that the fact that $a' \ll a$ and that $d \gg e'$ enlarges the positive feedback loop and, consequently, increases the sneaking through possibilities of the model. The sneaking through behaviour of the model is demonstrated for two different parameter settings (their Fig. 3–6 and Fig. 6–7 respectively). In the second setting the influx of unprimed cells is increased 100-fold, and the tumor grows 1·5-times faster (the various parameter settings are specified in the appendix).

The sneaking through phenomenon can be described as follows: (1) a small tumor grows slowly initially and has elicited only a weak cytotoxic response by the time it reaches its second activation threshold; subsequently its growth accelerates in an increasingly uncontrolled manner; (2) a medium-sized tumor is quickly eliminated by a large population of cytotoxic cells (it may increase during the first few days); and (3) a large tumor grows undisturbed from the beginning.

With regard to the counteracting feedback loops in the Grossman & Berke model, we observe that almost no memory cells (i.e. the positive feedback loop) are formed by large tumors because once-activated cells (ACT) are immediately restimulated. An intermediate tumor is likely to be rejected because the precursor population, and therefore the maximum cytotoxic response, increases due to the memory feedback loop. The negative feedback (i.e. the blocking factor) is most important in the case of small tumors because a large amount of shed antigen molecules prevents the memory cells formed from becoming cytotoxic when the tumor reaches its second activation threshold. The blocking factors have accumulated during the first growth phase and their amount increases steeply when the first cytotoxic cells emerge (i.e. at the second activation threshold).

(C) MODEL WITHOUT THE MEMORY FEEDBACK

So far little is understood about the process of memory T-cell induction and their subsequent restimulation. See Jerne (1984) for an interesting discussion on the factors controlling the longevity of T-cells. Smith (1984), for instance, suggested that memory T cells are the same as effector cells but have lost their interleukin 2 (IL2, growth factor) receptor. Cells require

antigenic restimulation for the (re)expression of the IL2 receptors. By contrast, Lefrancois *et al.* (1984) demonstrate that memory T-cells can be reactivated simply through the addition of IL2. Memory cells in the Grossman & Berke model however are identical to precursor cells, i.e. they require antigenic restimulation (twice) for maturation into effector cells. Moreover, MEM cells derive from antigen-primed cells (ACT), and not from effector cells. In order to investigate the role of the positive feedback loop in the generation of sneaking through, we omit the memory cells; the problems mentioned above are thus evaded. If memory is omitted from the model, i.e. the second activation threshold is deleted, the model still performs sneaking through behaviour (see Fig. 2).

Formal representation, no memory:

$$\mathrm{d(PREC)}/\mathrm{d}t = s - e' * \mathrm{PREC} - a * \mathrm{PREC} * (\mathrm{TUMOR} + \mathrm{SHED}) \tag{8}$$

$$\mathrm{d(ACT)}/\mathrm{d}t = a * \mathrm{PREC} * \mathrm{TUMOR} - a' * \mathrm{ACT} * (\mathrm{TUMOR} + \mathrm{SHED}) - d * \mathrm{ACT} \tag{4}$$

$$\mathrm{d(CTL)}/\mathrm{d}t = a'' * \mathrm{ACT}(t - t'') * \mathrm{TUM}(t - t'') - c * \mathrm{CTL} \tag{5}$$

$$\mathrm{d(TUMOR)}/\mathrm{d}t = b * \mathrm{TUMOR} - \beta * \mathrm{TUMOR} * \mathrm{CTL}/(\beta/\alpha + \mathrm{TUMOR}) \tag{6}$$

$$\mathrm{d(SHED)}/\mathrm{d}t = b' * \mathrm{TUMOR} - b'' * \mathrm{SHED} + k * \beta * \mathrm{TUMOR} * \mathrm{CTL}/(\beta/\alpha\ \mathrm{TUMOR}) \tag{7}$$

The parameters we use are the same as those in the first Grossman & Berke parameter setting (see the appendix), with two exceptions: the influx of precursor cells was increased (10-fold) because the precursor population is incapable of further expansion, and the efflux of once-activated cells (d) was made equal to that of precursor cells (e'). Note that d only represents decay here, and not the rate of transformation into memory cells.

The small tumor (Fig. 2(a)) still sneaks through because 1) the accumulated shed antigen molecules block the precursor cells and 2) the precursors are depleted by activation during the long period of latency (latency in this model is caused by slow tumor growth outweighing a weak attack). The generated once-activated cells decay as long as the tumor is sufficiently small (i.e. when $a' * \mathrm{ACT} * \mathrm{TUM} \ll \mathrm{d} * \mathrm{ACT}$). The generation of a threshold in the response is therefore an intrinsic feature of a two-step activation. The threshold in this model, however, occurs at a much smaller tumor size than the threshold in the models with memory feedback. A medium-sized tumor (Fig. 2(b)) is rejected because it generates many CTLs, whereas a larger tumor (Fig. 2(c)) is able to survive this attack and continue its growth.

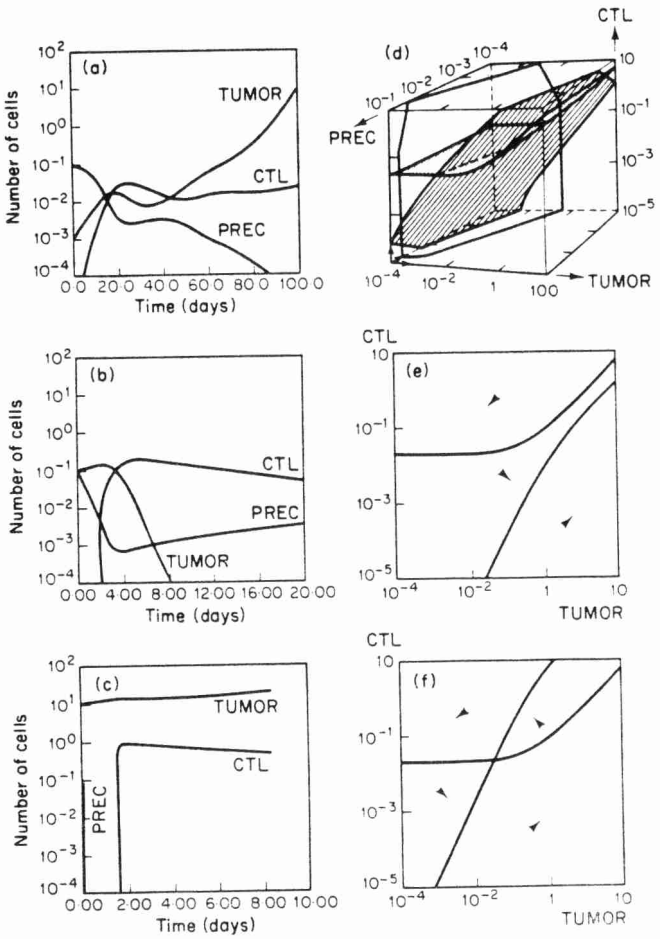

FIG. 2. Sneaking through in the model without the memory cycle. Parameters as in Grossman & Berke setting 1 (see the appendix) but: $s = 0{\cdot}002$, $d = 0{\cdot}02$. Time plots of the number of precursor cells (PREC), cytotoxic cells (CTL) and tumor cells (TUMOR): a) tumor escape for TUMOR = 0·001 in 100 days; b) tumor rejection for TUMOR = 0·1 in 20 days; c) tumor break through for TUMOR = 10 in 8 days; d) phase portrait showing the PREC′ = 0 (white), CTL′ = 0 (dotted), and the TUMOR′ = 0 (striped) isocline plane, for ACT and SHED at their steady state value; and e) and f) the two-dimensional representation of the isoclines at the back and the front of this cube respectively. Indicated are the TUMOR′ = 0 and the CTL′ = 0 isocline, and the direction of trajectories. Fig. 2(e) shows the position of the isoclines after precursor depletion.

A phase portrait of this model is shown in Fig. 2(d). The isocline planes of PREC′ = 0, CTL′ = 0 and TUMOR′ = 0 are depicted; ACT (equation (4)) and SHED (equation (7)) are assumed to be at their steady state values. In the absence of antigen the size of the precursor population equals s/e', depicted at the front of the cube: all trajectories start there at CTL = 0. Henceforth we indicate the initial direction of the trajectories in the phase space by three arrows in the left-hand corner of the side of the cube at which TUMOR = 10^{-4}, CTL = 10^{-5} and PREC = initial value. Because both TUMOR and CTL increase below their respective isocline planes (i.e. when CTL is small), tumor rejection can occur only if the CTL′ = 0 plane is above the TUMOR′ = 0 plane: only then can the trajectory penetrate into the region where TUMOR decreases. The picture shows the effect of a decrease in the precursor population (by depletion and/or blocking): tumor rejection becomes impossible when the trajectory moves from the front of the cube, where the TUMOR′ = 0 and the CTL′ = 0 plane intersect (Fig. 2(f)), to the back, where the CTL′ = 0 plane falls below the TUMOR′ = 0 plane (Fig. 2(e)). Tumor rejection is facilitated by a larger influx of precursor cells (cf. the 10-fold increase in the current parameter setting) because the volume of the region where CTL increases and TUMOR decreases is larger at larger PREC values.

(D) MODEL WITHOUT BLOCKING FACTORS

The role that shed antigen plays in blocking the immune response is open to discussion. Shed antigen may induce suppression by activating suppressor T cells (Nepom, Hellström & Hellström, 1982), or it may block the activity of effector cells or the activation of precursor cells. Grossman & Berke (1980) incorporated the latter possibility in their model. Shed antigen seems however to serve as a "general purpose explanation" for the failure of immune responses in tumor immunology. We further simplify by omitting the blocking factor, again evading the problems mentioned above.

Formal representation, no blocking:

$$\mathrm{d(UNPRM)}/\mathrm{d}t = s - e' * \mathrm{UNPRM} - a * \mathrm{UNPRM} * \mathrm{TUMOR} \tag{9}$$

$$\mathrm{d(MEM)}/\mathrm{d}t = e * \mathrm{ACT}(t - t') - a * \mathrm{MEM} * \mathrm{TUMOR} - e' * \mathrm{MEM} \tag{10}$$

$$\mathrm{PREC} = \mathrm{UNPRM} + \mathrm{MEM} \tag{3}$$

$$\mathrm{d(ACT)}/\mathrm{d}t = a * \mathrm{PREC} * \mathrm{TUMOR} - a' * \mathrm{ACT} * \mathrm{TUMOR} - d * \mathrm{ACT} \tag{11}$$

$$\mathrm{d(CTL)}/\mathrm{d}t = a'' * \mathrm{ACT}(t - t'') * \mathrm{TUM}(t - t'') - c * \mathrm{CTL} \tag{5}$$

$$\mathrm{d(TUMOR)}/\mathrm{d}t = b * \mathrm{TUMOR} - \beta * \mathrm{TUMOR} * \mathrm{CTL}/(\beta/\alpha + \mathrm{TUMOR}) \tag{6}$$

The deletion from the model of the inhibitory effects that are due to shed antigen increases the model's response. As a compensation we decreased the influx of unprimed cells (10-fold) and increased the growth rate of the tumor (2-fold), as compared with the Grossman & Berke parameter setting.

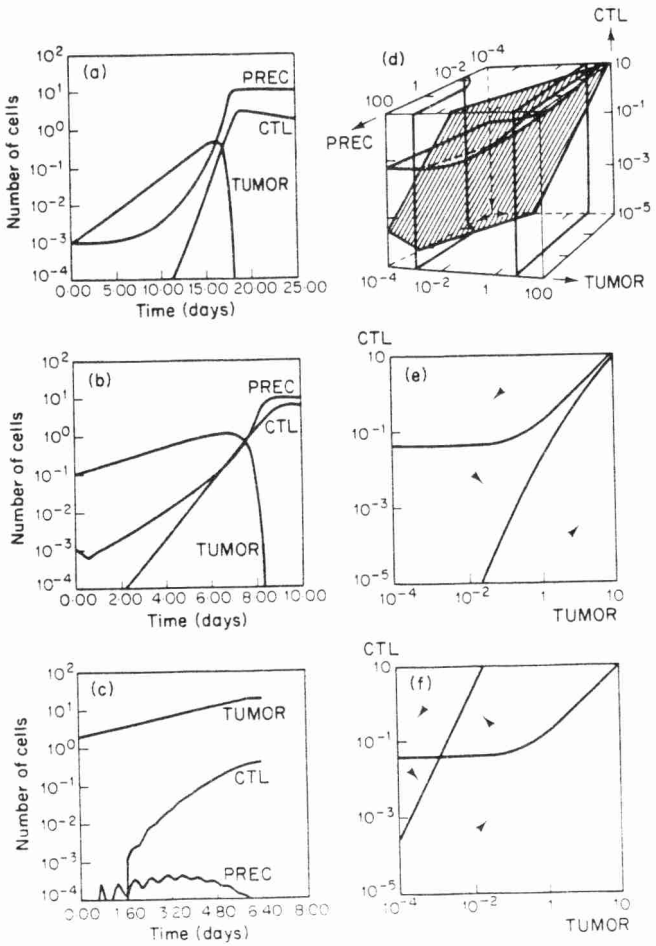

FIG. 3. Time plots and phase portrait of the model deprived of blocking factors. Parameters as in Grossman & Berke setting 1 but: $s = 0{\cdot}00002$, $b = 0{\cdot}4$. a) Tumor rejection for TUMOR = 0·001 in 25 days; b) tumor rejection for TUMOR = 0·1 in 10 days; c) tumor breakthrough for TUMOR = 2 in 8 days; d) phase portrait showing the PREC′ = 0 (white), CTL′ = 0 (dotted), and the TUMOR′ = 0 (striped) isocline plane, for ACT assumed to be at its steady state value; and e) and f) the two-dimensional representation of the isoclines at the back and the front of this cube respectively. Indicated are the TUMOR′ = 0 and the CTL′ = 0 isocline, and the direction of trajectories.

For this (adjusted) parameter setting, small tumors are always rejected due to the unlimited generation of memory cells during the first growth phase. The huge pool of memory cells generates a large wave of cytotoxic cells when the tumor reaches its second activation threshold (Fig. 3 day 15). So only tumors over a certain size will be able to break through in this particular system, i.e. one which generates such a large amount of memory cells. However, if we examine the time scale on which rejection occurs we find that medium-sized tumors regress far earlier than smaller tumors (compare Fig. 3(a) and 3(b)): a small tumor first has to reach its second activation threshold before it will elicit a cytotoxic response.

The phase portrait of this model (Fig. 3(d)) and that of the previous one are very similar. However, because the precursor population increases for a large range of tumor sizes (due to the formation of memory cells), the initial value of PREC is depicted at the back here. Trajectories thus move towards the front. Correspondingly, in the initial situation the $CTL' = 0$ plane lies below the $TUMOR' = 0$ plane (Fig. 3(e)). The trajectories of small and intermediate tumors move forwards, and the tumor is rejected.

According to this parameter specification an unlimited number of memory cells can be formed at a high rate. This rules out sneaking through since these cells are not blocked by shed antigen. However, the generation of memory cells does not in itself imply the elimination of sneaking through; it only does so if it results in the accumulation of precursor cells. This model does perform sneaking through behaviour when the rate of memory cell induction is sufficiently small (e.g. when $e = 1$ and all other parameters are identical to those of the model described below).

(E) MODEL WITHOUT MEMORY AND WITHOUT BLOCKING FACTORS

If both the positive and the negative feedback are removed, we are left with a simple model which is still capable of displaying sneaking through behaviour.

Formal representation, no memory, no blocking:

$$\mathrm{d(PREC)}/\mathrm{d}t = s - e' * \mathrm{PREC} - a * \mathrm{PREC} * \mathrm{TUMOR} \tag{12}$$

$$\mathrm{d(ACT)}/\mathrm{d}t = a * \mathrm{PREC} * \mathrm{TUMOR} - a' * \mathrm{ACT} * \mathrm{TUMOR} - d * \mathrm{ACT} \tag{11}$$

$$\mathrm{d(CTL)}/\mathrm{d}t = a'' * \mathrm{ACT}(t - t'') * \mathrm{TUM}(t - t'') - c * \mathrm{CTL} \tag{5}$$

$$\mathrm{d(TUMOR)}/\mathrm{d}t = b * \mathrm{TUMOR} - \beta * \mathrm{TUMOR} * \mathrm{CTL}/(\beta/\alpha + \mathrm{TUMOR}) \tag{6}$$

This model pinpoints the most essential element for sneaking through

behaviour in this simplification series: the reason why initially small tumors (Fig. 4(a)) eventually break through is that, during the period of latency, the precursor population is depleted by activation. The generated activated cells decay. If the precursor population is depleted by the time the rate of

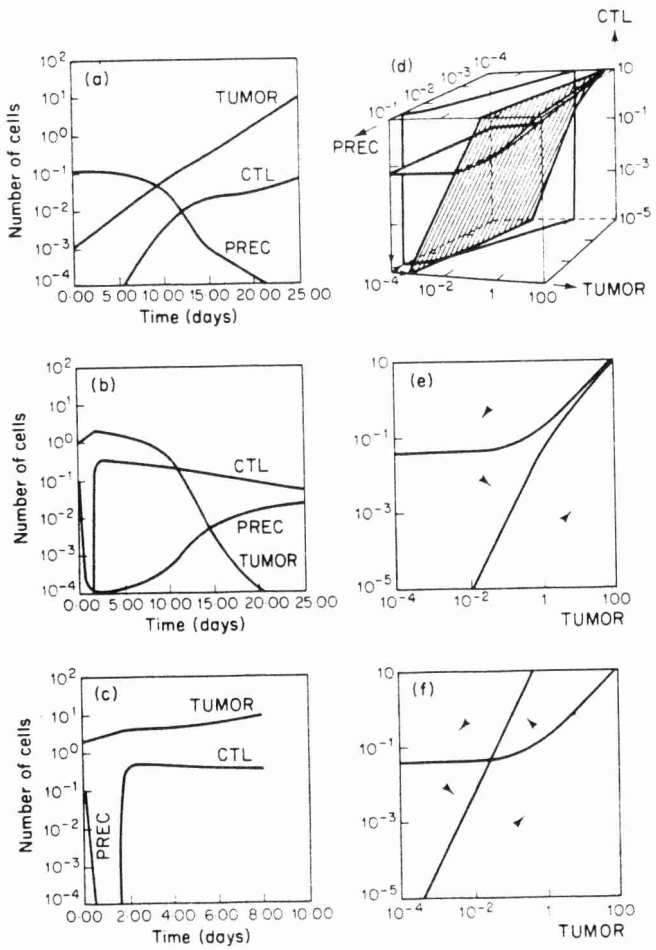

FIG. 4. Sneaking through in the model without memory and blocking factors. Parameters as in Grossman & Berke setting 1 but: $s = 0{\cdot}002$, $d = 2$, $b = 0{\cdot}4$. a) Tumor escape for TUMOR = 0·001 in 25 days; b) tumor rejection for TUMOR = 1 in 5 days; c) tumor breakthrough for TUMOR = 2 in 8 days; d) phase portrait showing the PREC′ = 0 (white), CTL′ = 0 (dotted), and the TUMOR′ = 0 (striped) isocline plane, for ACT assumed to be at its steady state value; and e) and f) the two-dimensional representation of the isoclines at the back and the front of this cube respectively. Indicated are the TUMOR′ = 0 and the CTL′ = 0 isocline, and the direction of trajectories.

second activation outweighs this decay, the tumor grows without eliciting a cytotoxic response. Medium-sized tumors (Fig. 4(b)), again, have to cope directly with the much larger steady state precursor population and are therefore rejected.

Figure 4(d) is essentially analogous to Fig. 2(d): trajectories begin on the CTL = 0 line of the front plane of the cube (Fig. 4(f)), and depletion of precursors causes the CTL′ = 0 isocline plane to disappear below the TUMOR′ = 0 plane (Fig. 4(e)), thus allowing sneaking through of the tumor. Large tumors start in a region with a fast increase of CTL; trajectories thus move upwards, pass through the TUMOR′ = 0 isocline plane and the tumor is rejected.

We used the first Grossman & Berke parameter setting, but increased the influx of precursor cells (10-fold) as in section 3(C) in order to compensate for the lack of precursor expansion via the memory pathway, and we increased the growth rate of the tumor (2-fold) as in section 3(D) in order to compensate for the lack of blocking factors (see the appendix). In addition, the decay of once activated cells was made 100-fold larger than the decay of precursor cells in order to enlarge the threshold effect responsible for sneaking through in this model. Note that the efflux of ACT cells was even larger in the original Grossman & Berke setting.

(F) MODEL WITHOUT TWO-STEP ACTIVATION

In order to analyse whether sneaking through depends on a threshold caused by a two-step activation, we deleted the once-activated cells. This leads to the model described below.

Formal representation, single step activation:

$$\mathrm{d(PREC)}/\mathrm{d}t = s - e' * \mathrm{PREC} - a * \mathrm{PREC} * \mathrm{TUMOR} \tag{12}$$

$$\mathrm{d(CTL)}/\mathrm{d}t = prol * a * \mathrm{PREC}(t - t'') * \mathrm{TUM}(t - t'') - c * \mathrm{CTL} \tag{13}$$

$$\mathrm{d(TUMOR)}/\mathrm{d}t = b * \mathrm{TUMOR} - \beta * \mathrm{TUMOR} * \mathrm{CTL}/(\beta/\alpha + \mathrm{TUMOR}) \tag{6}$$

This model performs sneaking through behaviour when the degree of proliferation, affecting the magnitude of the maximum response, and decay of cytotoxic cells are large. The few cytotoxic cells generated per unit of time by a small tumor have little effect and decay quickly: the tumor (initially of size 0·001) sneaks through in 190 days. A medium-sized tumor generates a large wave of CTLs and is quickly eliminated (see Fig. 5(b)).

The parameters are the same as in the previous model ($b = 0{\cdot}4$, $s = 0{\cdot}002$) except for the decay of cytotoxic cells (c) which was increased 10-fold, and

for the proliferation, which was increased 2-fold (*prol* = 20) (see the appendix). This parameter setting was chosen in order to obtain a phase space (Fig. 5(d)) qualitatively analogous to that of the previous model (i.e. a CTL′ = 0 plane moving from above (Fig. 5(f)) to below (Fig. 5(e)) the

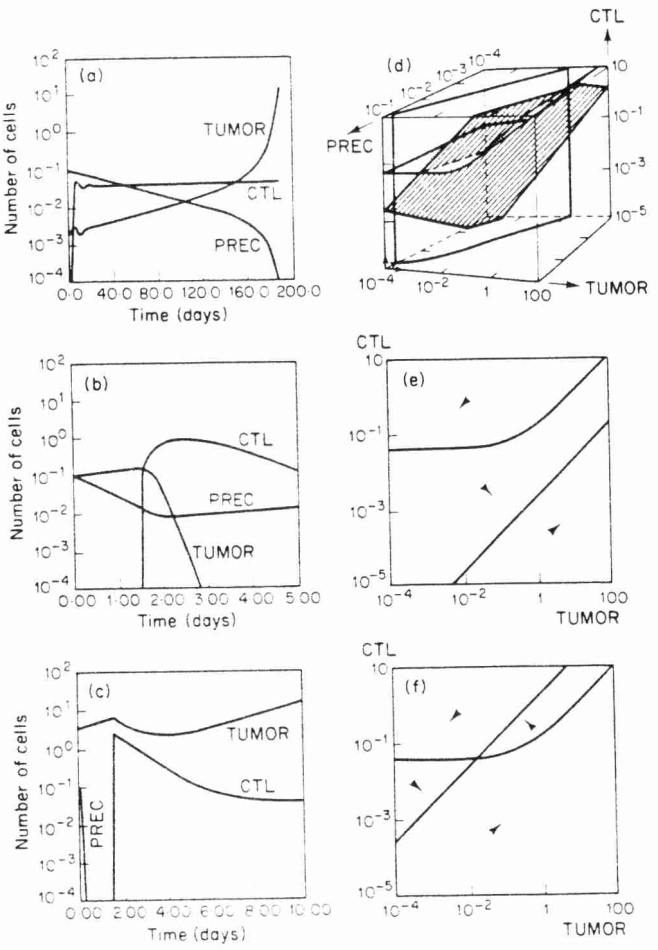

FIG. 5. Sneaking through in the model without memory, wihout blocking factors and without a two-step activation. Parameters as in Grossman & Berke setting 1 but: $s = 0{\cdot}002$, $b = 0{\cdot}4$, *prol* = 20, $c = 1$. a) Tumor escape for TUMOR = 0·001 in 200 days; b) tumor rejection for TUMOR = 0·1 in 5 days; c) tumor breakthrough for TUMOR = 4 in 10 days; d) phase portrait showing the PREC′ = 0 (white), CTL′ = 0 (dotted), and the TUMOR′ = 0 (striped) isocline plane; and e) and f) the two-dimensional representation of the isoclines at the back and the front of this cube respectively. Indicated are the TUMOR′ = 0 and the CTL′ = 0 isocline, and the direction of trajectories.

TUMOR′ = 0 isocline plane along the PREC dimension). This high rate of CTL decay is however quite reasonable because cytotoxic effector T-cells are reported to be short-lived (Hobart & McConnel, 1975 p. 95; Jerne, 1984).

(G) MODELS WITHOUT TIME DELAYS

The deletion of time delays does not affect the sneaking through capacities of any of the models.

4. Discussion

The purpose of the stepwise simplifications of the Grossman & Berke model was to pinpoint the minimal requirements for the occurrence of the sneaking through phenomenon. After we deleted those processes that are apparently superfluous to sneaking through we show that in precursor bound cytotoxicity models precursor depletion by long-lasting activation suffices for the generation of the phenomenon. Thus we did not intend to improve the model from an immunological point of view: much larger models are needed for that (e.g. De Boer *et al.*, in press). However, the insight obtained with the help of the "mini models" studied here markedly facilitates the analysis of sneaking through in such more complex models (manuscript in prep.).

We concentrated on the existence of one phenomenon (sneaking through) in a variety of models based on a uniform set of parameters. The set of models consists of a series of simplifications in which all changes of parameter values have been kept within the range of the original model. It is extremely difficult to determine experimentally the precise pattern of direct interactions between variables (e.g. cell types) in systems like the immune system. On the other hand, the order of magnitude of the total contribution of (lumped) subsystems (e.g. the lymphocyte system) can usually be assessed more easily. For example, cell counts on the different cell types provide an easy estimate of the relative importance of each variable. In such a situation experimentally known phenomena can best be studied by a multi-model-fixed-parameter approach, as introduced here. The interaction structure of the (minimal) models that generate the experimentally observed phenomena provides insight into these phenomena, and the model's interaction structure should serve as a guideline for further experimental work on the direct interactions in the biotic system.

In all the precursor bound cytotoxicity models developed here sneaking through occurs after depletion of the population of effector cell precursors. Long-lasting activation of the precursor population is the most simple

process by which this depletion can occur. When memory cells cause an expansion of the precursor population (i.e. in the proliferative models) sneaking through will occur only if these extra precursor cells are blocked by shed antigen.

Current views on T-lymphocyte activation cast doubt on whether these activation systems in fact belong to the class of precursor bound cytotoxicity models. T-lymphocytes are known to be composed of two functionally different populations, namely helper T-cells and cytotoxic T cells (Cantor & Boyse, 1975*a*, *b*). The signal initiating proliferation after antigenic stimulation is interleukin 2 (IL2, growth factor), which is produced by activated helper T cells (see Wagner *et al.*, 1980 for a review). Cells generated by IL2 induced proliferation do not revert to a precursor stage (as they do in the Grossman & Berke model) but divide repeatedly upon restimulation by IL2 and antigen (Smith, 1984). The latter is required for the re-expression of the IL2 receptors (Smith, 1984). The most important difference between such a (proliferative) activation scheme and precursor bound cytotoxicity systems is that an unlimited number of effector cells can be formed from a limited number of effector cells (Gillis *et al.*, 1979). Thus, depletion of precursors would seem to be of no importance because an allergic reaction, once started up, is able to free itself from the influence of precursor cells because of endless proliferation. However we have found (manuscript in prep.) that a form of precursor depletion is responsible for sneaking through in models that do specify T cell populations with endless proliferation (De Boer *et al.*, in press).

Recent reports suggest that a lymphoid differentiation factor is required for maturation of proliferating cells of the CTL line (Raulet & Bevan, 1982; Wagner *et al.*, 1982). This was convincingly demonstrated before for B-cells, see Melchers & Andersson (1984) for a review. Antigenic restimulation serves as such a "maturation signal" in the Grossman & Berke model. Systems in which the proliferating cells mature into non-dividing effector cells are prone to precursor depletion due to "over-maturation" (Grossman, 1982). If such a system is challenged with high doses of antigen, then the rate of maturation of activated cells far exceeds the proliferation rate of these cells. Consequently, precursors become depleted and relatively few effector cells are generated (Grossman, 1982). Precursor depletion that causes sneaking through is different since it occurs when the system is challenged with low doses of antigen. Furthermore, our precursor bound systems lack the antagonistic distinction between a proliferation cycle and a maturation step (see Grossman, 1982). (Note that this discussion concerns CTL differentiation from its proliferation stage to its effector stage only: differentiation from the precursor to the proliferation stage (Finke *et al.*,

1983; Hancock, Kilburn & Levy, 1981; Männel, Falk & Dröge, 1983) is implicit in the first activation step.)

Precursor depletion may, additionally, be a valuable concept for the analysis of the macrophage or the natural-killer response to tumors. Macrophage populations for instance, are not self-sustaining, but depend on their bone marrow-derived monocyte precursors (Blusse van Oud Alblas, Matti & Van Furth, 1983). Monocytes may undergo a division shortly after their arrival in the tissue but do not proliferate at the effector stage (Van Furth *et al.*, 1980). It is generally accepted that macrophages play a role in the anti-tumor immune response (Hibbs, 1974; Den Otter, Evans & Alexander, 1972; Den Otter *et al.*, 1977). We have recently investigated models in which interactions between proliferative T-lymphocyte systems and a precursor bound macrophage system generate anti-tumor immune responses (De Boer *et al.*, in press; De Boer & Hogeweg, in prep.).

This work in tumor immunology has been done in close collaboration with the Department of Experimental Pathology. We thank Prof. W. Den Otter, Dr H. F. J. Dullens and Dr R. A. De Weger for discussing many problems and for critically reading the manuscript. Dr Roel De Weger played an important role in shaping our current outlook on the immune system. We highly appreciate the helpful criticisms of Drs Grossman & Berke. We thank an anonymous reviewer for his/her suggestions. We thank Dr D. Lauffenburger for discussing preliminary results. We are grateful to Miss S. M. McNab for linguistic advice and to Mr D. Smit for drawing the three-dimensional phase plots.

REFERENCES

BLUSSE VAN OUD ALBLAS, A., MATTI, H. & VAN FURTH, R. (1983). *Cell Tissue Kinet.* **16,** 211.
BOYSE, E. A. & OLD, L. J. (1969). *Ann. Rev. Genet.* **3,** 269.
BULIRSCH, R. & STOER, J. (1966). *Numerische Mathematik* **8,** 1.
CANTOR, H. & BOYSE, E. A. (1975*a*). *J. exp. Med.* **141,** 1376.
CANTOR, H. & BOYSE, E. A. (1975*b*). *J. exp. Med.* **141,** 227.
CURRIE, G. (1976). *Biochim. Biophys. Acta* **458,** 135.
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. (in press). *J. Immunol.*
DEN OTTER, W., EVANS, E. & ALEXANDER, P. A. (1972). *Transplantation* **14,** 220.
DEN OTTER, W., DULLENS, H. F. J., VAN LOVEREN, H. & PELS, E. (1977). In *The Macrophage & Cancer* (James, McBrick & Stuart, eds.), p. 119. Edinburgh: Econoprint.
FINKE, J. H., SCOTT, J., GILLIS, S. & HILFIKER, M. L. (1983). *J. Immunol.* **130,** 763.
GILLIS, S., UNION, A. A., BAKER, P. E. & SMITH, K. A. (1979). *J. exp. Med.* **149,** 1460.
GOTTWALD, B. A. & WANNER, G. (1981). *Computing* **26,** 355.
GROSSMAN, Z. & BERKE, G. (1980). *J. theor. Biol.* **83,** 267.
GROSSMAN, Z. (1982). *Eur. J. Immunol.* **12,** 747.
HANCOCK, E. J., KILBURN, D. G. & LEVY, J. G. (1981). *J. Immunol.* **127,** 1394.
HASKILL, J. S., PROCTOR, J. W. & YAMAMURA, Y. (1975). *J. Natn. Cancer Inst.* **54,** 387.
HERBERMAN, R. B. (1983). *Adv. Host Defense Mech.* **2,** 241. Raven Press: New York.
HIBBS, J. B. (1974). *J. Natn. Cancer Inst.* **53,** 1487.

HOBART, M. J. & MCCONNEL, I. (1975). *The Immune System.* Oxford: Blackwell.
HOGEWEG, P. & HESPER, B. (1978). *Comput. Biol. Med.* **8,** 319.
HOGEWEG, P. & HESPER, B. (1981). *J. theor. Biol.* **93,** 411.
JERNE, N. K. (1984). *Immunol. Rev.* **79,** 5.
LEFRANCOIS, L., KLEIN, J. R., PAETKAU, V. & BEVAN, M. J. (1984). *J. Immunol.* **132,** 1845.
MÄNNEL, D. N., FALK, W. & DRÖGE, W. (1983). *J. Immunol.* **130,** 2508.
MERRILL, S. J. (1982). *Math. Biosci.* **62,** 219.
MELCHERS, F. & ANDERSSON, J. (1984). *Cell* **37,** 715.
MILLER, F. R. & HEPPNER, G. H. (1979). *Cancer Immunol. Immunother.* **7,** 77.
NEPOM, G. T., HELLSTRÖM, I. & HELLSTRÖM, K. E. (1982). *Experientia* **39,** 235.
PERI, G., POLENTARUTTI, N., SESSA, C., MANGIONI, C. & MANTOVANI, A. (1981). *Int. J. Cancer* **28,** 143.
RAULET, D. H. & BEVAN, M. J. (1982). *Nature* **296,** 754.
ROBSON MCDONALD, H. (1982). *Immunol. Today* **3,** 183.
SCHIRRMACHER, V., BOSSLET, K., SHANTZ, G., CLAUER, K. & HUBSCH, D. (1979). *Int. J. Cancer* **23,** 245.
SMITH, K. A. (1984). *Ann. Rev. Immunol.* **2,** 319.
STAFLEU, G. (1979). *DEASIM, Differential Equations Easily Simulated.* Bioinformatics Group, University of Utrecht, The Netherlands.
VAN FURTH, R., DIESSENHOFF-DEN DULK, M. M. C., RAEBURN, J. A., VAN ZWET, T. L., CROFTON, R. W. & BLUSSE VAN OUD ALBLAS, A. (1980). In, *Mononuclear Phagocytes—Functional Aspects* (van Furth, R. ed.), p. 279. The Hague: Martinus Nijhoff Publications.
WAGNER, H., HARDT, C., HEEG, K., PFIZENMAIER, K., SOLBACH, W., BARTLETT, R., STOCKINGER, H. & RÖLLINGHOFF, M. (1980). *Immunol. Rev.* **51,** 215.
WAGNER, H., HARDT, C., ROUSE, B. T., RÖLLINGHOFF, M., SCHEURIG, P. & PFIZENMAIER, K. (1982). *J. exp. Med.* **155,** 1876.

APPENDIX

Parameter setting of the models

	Grossman and Berke		Simplified Models			
	Setting 1	Setting 2	No MEM	No SHED	No SHED No MEM	No SHED No MEM Single
s	0·0002	0·02	0·002	0·00002	0·002	0·002
e'	0·02	0·02	0·02	0·02	0·02	0·02
a	10	10	10	10	10	10
a'	1	1	1	1	1	—
a''	10	10	10	10	10	$prol = 20$
d	5	5	0·02	5	2	—
e	15	15	—	15	—	—
c	0·1	0·1	0·1	0·1	0·1	1·0
b	0·2	0·3	0·2	0·4	0·4	0·4
b'	0·5	0·5	0·5	—	—	—
b''	0·1	0·1	0·1	—	—	—
α	10	10	10	10	10	10
β	3·33	2	3·33	3·33	3·33	3·33
k	0·05	???	0·05	—	—	—
t'	0·5	0·5	0·5	0·5	0·5	0·5
t''	1·5	1·5	1·5	1·5	1·5	1·5