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Modelling the macrophage invasion of tumours: Effects on growth and composition

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Even in the early stages of their development, tumours are not simply a homogeneous grouping of mutant cells; rather, they develop in tandem with normal tissue cells, and also recruit other cell types including lymphatic cells and the endothelial cells required for the development of a blood supply. It has been repeatedly seen that macrophages form a significant proportion of the tumour mass, and that they can have a variety of effects upon the tumour, leading to a delicate balance between growth promotion and inhibition. This paper develops a model for the early, avascular growth of a tumour, concentrating on the inhibitory effect of macrophages due to their cytolytic activity. It is shown that such an immune response is not sufficient to prevent growth, due to it being a second-order process with respect to the density of the tumour cells present. However, the presence of macrophages does have important effects on the tumour composition, and the authors perform a detailed bifurcation analysis of their model to clarify this. An extended model is also considered which incorporates addition of exogenous chemical regulators. In this case, the model admits the possibility of tumour regression, and the therapeutic implications of this are discussed.

Keywords: cancer; cytolysis; immunotherapy; macrophage; tumour composition.

1. Introduction

It has long been known that tumours contain a variety of cell types. In addition to the mutant cells that drive tumour growth, there is often a high component of normal-tissue cells that proliferate along with mutant cells at the tumour site. Moreover, there is also additional recruitment from other sites of a variety of cell types, including lymphocytes, macrophages, and endothelial cells—which are required for a growing tumour to develop its own blood supply. Such tumour infiltrate is found to contain a large proportion of macrophages, as much as 80% in breast carcinomas (Götlinger *et al.*, 1985), which are the result of the maturation in tissue of a type of white blood cell known as a monocyte. In this context they are referred to as tumour-associated macrophages (TAMs), which can affect tumour vascularization, growth rate, cytostasis, connective-tissue formation and dissolution, and killing of mutant cells. Tumour cells and TAMs both release factors which can affect each other's activity, and so the details of this regulation can have important consequences for the survival of tumours. In particular, the possibility that macrophages can destroy tumour cells makes

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them attractive in the context of immunotherapy. For detailed reviews of these interactions and their mechanisms see papers by Esgro *et al.* (1990), Hamilton & Adams (1987), Mantovani (1989, 1990), and Mantovani *et al.* (1992). The importance of macrophages to general homeostasis is illustrated by the fact that animals can survive without an intact lymphocyte system, but not without their macrophage system (Esgro *et al.*, 1990).

We will concentrate here on the inhibitory effects of macrophages during the early stages of growth, when the tumour is small enough for its nutritional and respiratory needs to be satisfied by diffusion from nearby blood vessels—such as after an initial mutation giving a proliferative advantage (for models concentrating on the details of the mutation see Sherratt & Nowak, 1992; Wheldon, 1975), and also in situations where one or more metastatic cells infiltrate tissues and proliferate (for a discussion of the role of macrophages in such cases see Esgro *et al.*, 1990). Thus the interactions we consider are cell proliferation, aggregation of macrophages at the tumour site, and their killing of mutant cells. A number of mathematical models of tumour immunology have been proposed, typically with a generic representation of the immune response (Adam, 1993; Albert *et al.*, 1980; Hiernaux & Lefever, 1988; Sherratt & Nowak, 1992). More specific modelling by Kuznetsov *et al.* (1993) focused on interactions between different immune-cell types.

Macrophages in normal tissue do not proliferate significantly, but at least in some cases TAMs differ in this respect due to the production by mutant cells of a substance known as macrophage colony stimulating factor (M-CSF), and enhanced expression of receptors for M-CSF on the surface of TAMs (Bottazzi *et al.*, 1990). This evidence is restricted to a few mouse sarcoma cell lines, and in humans M-CSF seems to promote macrophage survival, but it is not a good proliferative stimulus (Mantovani *et al.*, 1992).

The basic mechanism by which macrophages are recruited to a tumour site is chemotaxis. Monocytes circulate in the bloodstream, attaching themselves to capillary walls where the concentration of chemotactic chemical is sufficiently high, and enter the tissue, where they mature into macrophages. Along with the macrophages which are already resident, they move up chemical gradients towards the tumour. There is a large body of experimental work on the production by mutant cells of macrophage chemoattractants. Results include the identification of monocyte chemotactic protein (MCP-1) (Bottazzi *et al.*, 1983), followed more recently by the related proteins MCP-2 and MCP-3 (van Damme *et al.*, 1992), and other chemoattractants including M-CSF (Wang *et al.*, 1987, 1988). An important correlation has repeatedly been found between the extent of chemotactic activity, as determined by *in vitro* assays or imposed by genetic alteration, and the proportion of TAM within *in vivo* tumours (Bottazzi *et al.*, 1983, 1985, 1992; Walter *et al.*, 1991).

Macrophages are able to lyse tumour cells in preference to normal cells. This is dependent on biochemical activation (Mantovani, 1990). A two-stage activation process has been postulated, in which newly recruited macrophages are first 'primed' by interferon-γ, and subsequently activated to full competence by a variety of bacterial products such as lipopolysaccharide (Hamilton & Adams, 1987). Other cytokines such as tumour necrosis factor and M-CSF have been shown to induce or augment macrophage activation (Mace *et al.*, 1988; Mantovani, 1990; Sampson-Johannes & Carlino, 1988), and recent evidence suggests that cytokine-independent pathways may also be involved (Keller, 1993). Some of these macrophage-activating factors are produced by certain tumour cell lines (Mantovani, 1990; Mantovani *et al.*, 1992). Esgro *et al.* (1990) have noted that once macrophages have

been activated and then lysed a tumour cell, they revert to an inactivated state ready for reactivation.

Once activated, the first step in tumour-cell killing is the formation of a macrophage–tumour cell complex. This direct cell–cell contact is a key part of the lysis (Hamilton & Adams 1987; Jonjic *et al.*, 1992), and it may also upregulate macrophage activation (Mantovani, 1993). Many different mechanisms have been proposed for the actual lysis process within the complex, all involving the secretion of cytolytic factors. In particular, a nitrogen-monoxide-mediated mechanism has been determined in detail (Hibbs, 1991), although this may not be important in humans (Schneemann *et al.*, 1993). A detailed review of cytolytic mechanisms has been given by Hamilton & Adams (1987).

Crucially, this tumour-cell destruction can occur independently of antibodies, although there is evidence for an additional antibody-mediated mechanism (Hamilton & Adams, 1987). This makes macrophage-mediated tumour cytotoxicity particularly interesting from a clinical viewpoint, since it is selective for mutant cells, yet not dependent on specific antibodies. This leads to the hope that the mechanism could be enhanced to be therapeutically effective against a wide range of heterogeneous chemotherapy resistant tumours, both primary and secondary.

2. Modelling the macrophage–tumour interaction

We propose an ordinary differential equation (ODE) model, focusing on the temporal dynamics of the tumour–macrophage interaction. In this spatially independent framework, the effect of chemotaxis is incorporated as an additional influx of macrophages due to extravasation from the blood supply.

Macrophages destroy tumour cells by binding to form a complex and then lysing the mutant cell. Quiescent macrophages must be biochemically activated before complex formation and lysis are possible, as discussed above. We make the simplifying assumption that there is a single generic chemical regulator, concentration $f(t)$, responsible for macrophage activation, chemotaxis, and proliferative control. This is a reasonable simplification, since the various factors involved in these processes all derive primarily from mutant cells. We assume that the rate of complex formation is linear with respect to the concentration of this chemical, and also with respect to the macrophage and tumour-cell densities. In addition, we assume that the complex returns viable macrophages after lysis of the mutant cell (Esgro, *et al.* 1990). Schematically this can be represented in the form

$$f + l + m \xrightarrow{k_1} c + f, \qquad c \xrightarrow{k_2} l + \text{debris}.$$

Here $l(t)$, $m(t)$, and $c(t)$ are the densities of the macrophages, the mutant cells, and the complex, respectively; k_1 and k_2 are positive constants. We must stress that there is no definitive experimental data on the details of this tumour cell lysis, so that the above pathway is of necessity generic. We have considered some alternative formulations, such as the separation of activation and complex formation or degradation of the chemical, and the preliminary results suggest that these changes do not alter the main qualitative features of the model predictions. The dynamics for the macrophage–mutant cell complex are completed by a linear death term $\delta_c c$. Our model framework involves one other variable in addition to $l(t)$, $m(t)$, $c(t)$, and $f(t)$; this is the density of normal-tissue cells, denoted by $n(t)$.

The remaining assumptions that we make with regard to macrophages are: (i) macrophages proliferate only in the presence of the chemical regulator (Bottazzi *et al.*, 1990), and such proliferation increases linearly with the concentration; (ii) such growth is limited by the crowding effect of all cell types; (iii) there is a background influx from capillaries, which increases linearly with the regulator concentration to reflect chemotaxis; and (iv) macrophages die with some constant rate per cell. In the absence of chemoattractants, the normal background level of tissue macrophages is maintained by a constant influx, at a rate denoted by I.

We use a term for the proliferation of macrophages with the form

$$\text{chemical concentration} \times \text{macrophage density} \times \text{crowding term}.$$

The most common representation of crowding effects on cell division is the logistic term, which has been used in a wide range of models (Fisher, 1937; Sherratt & Murray, 1990). In this case it is inappropriate, since the structure of our system would mean that the carrying capacity k was not an upper bound for the overall cell density, because of the chemotactic influx of macrophages, leading to a negative growth term which would be chemically promoted; this is quite unrealistic. Instead we use the term

$$r(x) = (N + N_e)/(N + x),$$

where N_e is the total equilibrium density of all cell types in normal tissue and N is a measure of the crowding response. In the absence of detailed biological data, only the qualitative form of this function is known. Note that our model allows the total cell density to increase above N_e. Biologically this can arise in a number of different ways, including: (i) a high density of cells that are typically smaller than normal; (ii) a thickening of the epithelium in carcinomas (cancers of the epithelium), which represent the vast majority of clinically observed tumours; (iii) closer cell packing in sarcomas (cancers of connective tissue) and adenocarcinomas (cancers of glandular epithelium)—here the normal tissue is relatively acellular. Most tumours arise initially from a mutation that affects the control of cell division. This can be due to the expression of an oncogene or due to the loss of a tumour-suppressor gene (Fearon & Vogelstein, 1990; Volpe, 1990; Weinberg, 1989); in either case the result is a proliferative advantage for the mutant cell over its peers. Previous mathematical models have focused on the different types of mutation and their consequences (Sherratt & Nowak, 1992; Wheldon, 1975). Here our focus is on the role of macrophages during the early stages of tumour growth, and thus we use a very simple model in which the dynamics of mutant and normal cells are alike except for the removal of mutant cells by macrophages, and we scale the mutant-cell growth rate by $\xi > 1$ to model their proliferative advantage. We use the same crowding term as discussed above, and a growth rate in normal tissue of δ, balanced by an equal rate of cell death.

As discussed above, the sole source of our generic chemical regulator is mutant cells, and we assume a constant secretion rate β per unit of mutant-cell density, and a linear natural decay with rate δ_f. Together, these various assumptions lead to the following system of five coupled differential equations:

$$\frac{dl}{dt} = \frac{\alpha f l(N + N_e)}{N + l + m + n} + I(1 + \sigma f) - k_1 f l m + k_2 c - \delta_l l, \tag{1a}$$

$$\frac{dm}{dt} = \frac{\xi\delta m(N+N_e)}{N+l+m+n} - \delta m - k_1 flm, \tag{1b}$$

$$\frac{dn}{dt} = \frac{\delta n(N+N_e)}{N+l+m+n} - \delta n, \tag{1c}$$

$$\frac{df}{dt} = \beta m - \delta_f f, \tag{1d}$$

$$\frac{dc}{dt} = k_1 flm - k_2 c - \delta_c c. \tag{1e}$$

Although already greatly simplified, this model is still complicated to study analytically, and it can profitably be reduced to three equations by making pseudo-steady-state approximations for the chemical concentration and the complex density. These approximations are based on the assumption that their dynamics are relatively fast compared to those of macrophages, and mutant and normal cells. This is biologically reasonable since the dynamics of the cell populations will be related to cell-cycle times, which have a time scale of days, which can be compared to the time scales of minutes for chemical production and hours for cell lysis (Hamilton & Adams, 1987). The rationale behind these approximations is that, since the dynamics are fast, $f(t)$ and $c(t)$ can be expected to settle quickly to steady values as a function of the other variables. Applying this principle gives

$$f = \frac{\beta}{\delta_f} m, \qquad c = \frac{k_1}{k_2+\delta_c} flm = \frac{k_1\beta}{(k_2+\delta_c)\delta_f} lm^2.$$

We rescale time by the normal-cell death rate, and rescale the cell densities by the equilibrium density N_e, giving the nondimensionalization

$$l^* = l/N_e, \quad m^* = m/N_e, \quad n^* = n/N_e, \quad t^* = \delta t, \quad A = \frac{\alpha\beta N_e}{\delta_f \delta}, \quad N^* = N/N_e,$$

$$I^* = I/(N_e\delta), \quad S = \frac{\sigma\beta N_e}{\delta_f}, \quad K_l = \frac{\delta_c k_1 \beta N_e^2}{(k_2+\delta_c)\delta_f\delta}, \quad \delta_l^* = \delta_l/\delta, \quad K_m = \frac{k_1\beta N_e^2}{\delta_f\delta},$$

and, after omitting the asterisks for notational simplicity, this gives the nondimensional model

$$\frac{dl}{dt} = \frac{Alm(N+1)}{N+l+m+n} + I(1+Sm) - K_l lm^2 - \delta_l l, \tag{2a}$$

$$\frac{dm}{dt} = \frac{\xi m(N+1)}{N+l+m+n} - m - K_m lm^2, \tag{2b}$$

$$\frac{dn}{dt} = \frac{n(N+1)}{N+l+m+n} - n. \tag{2c}$$

There is insufficient experimental data available to determine the various model parameters for any particular tumour type. Therefore, we list here generic order-of-magnitude

estimates for the parameters, with a brief justifications, bearing in mind that we have now scaled time by the normal-cell death rate.

$\delta_l \sim 10^{-1}$, macrophages survive in tissue for weeks or months, which can be compared to a turnover time of days for normal cells, so that macrophage death rate is expected to be at least an order of magnitude smaller than that for normal cells.

$I \sim 10^{-2}$, the condition $I < \delta_l$ must hold for the normal-tissue steady state to be non-negative. In addition, the proportion of macrophages in normal tissue is relatively small.

$A \sim 10^{-2}$, macrophages are a mature form of blood monocyte, which reside in tissues and do not normally proliferate. With certain stimuli, including some tumour-derived chemicals, they may proliferate, but at a low level (Bottazzi *et al.*, 1990), particularly in humans (Mantovani *et al.*, 1992).

$N \sim 10^1$, N is a measure of the initial growth rate, and of the subsequent response to crowding, and it should be of the same order of magnitude as the normal-cell death rate.

$S \sim 10^0$–10^2, S varies with the response of macrophages to chemoattractants, and with mutant-cell chemical-production rates. These characteristics change for different cell lines, and it is anticipated that the value of S will have a crucial bearing on tumour composition, and so it will be considered below to be the main bifurcation parameter.

$K_m \sim 10^1$–10^2, K_m is a measure of the rate of activation and complex formation, which has a time scale of hours (Hamilton & Adams, 1987), and hence a rate one or two orders of magnitude larger than the death rate of normal cells (a typical cell-cycle time is of the order of 100 hours).

$K_l \sim 10^1$–10^2, as for K_m, noting that the pseudo-steady-state hypothesis applied to the full model imposes the requirement that $K_l < K_m$.

$\xi \sim 10^1$, ξ represents the growth advantage of mutant cells, and thus must be greater than one; however, mutant-cell growth should still be of the same order of magnitude as for normal cells.

3. Steady states

Inevitably certain aspects of our model are somewhat arbitrary, such as the details of mutant-cell growth. Nevertheless, in this section we present a fairly detailed analysis of the steady-state structure of the model, with particular reference to the parameters governing macrophage activity. This analysis is an essential step in our study, providing a framework for numerical investigations of stability (see Section 4). We will show that there are four possible types of steady state for system (2a): (i) a state with only macrophages; (ii) normal tissue, with both normal cells and macrophages; (iii) a tumour state with only mutant cells and macrophages, which is henceforth referred to as a mutant-only state; and (iv) a tumour state in which all species coexist. We will begin by considering the nontumour steady states which are easy to deal with, and move on to the steady states containing mutant cells, which are more complicated, and for which we treat the cases $n = 0$ and $n \neq 0$ separately. In these latter cases the chemotactic response parameter S is a key bifurcation parameter.

3.1 *Steady states with* $m = 0$

Straightforward examination of (2a) shows that there are two steady states with $m = 0$; namely, $(l, m, n) = (I/\delta_l, 0, 0)$ and $(I/\delta_l, 0, 1 - I/\delta_l)$. The second of these corresponds to normal tissue, and, as expected intuitively, the ratio of the macrophage influx to the death rate determines the normal level of macrophages. The first state corresponds to a population of macrophages in isolation, also maintained by the influx rate I.

3.2 *Steady states with* $m \neq 0$, $n = 0$

Substitution of $n = 0$ into (2a) gives a pair of coupled nonlinear algebraic equations in l and m. Analysis shows that there must always be between one and three positive solutions, but explicit expressions for these steady states cannot be derived. Extensive numerical studies suggest that for parameter values in the ranges suggested for the model, only one such solution exists. It is interesting mathematically to note that for other ranges of parameters (in particular for unrealistically high values of A, the parameter governing macrophage proliferation) three steady states can exist. Numerical investigation using *Auto* (Doedel *et al.*, 1991) shows that this leads to fold bifurcations from one to three steady states and from three back to one steady state, which exhibit hysteresis. The existence of only one solution for small A is evidenced by a parameter continuation which shows that as A decreases the two-fold bifurcations coalesce, until three states are no longer possible.

It is also possible to show analytically that where there is only one mutant-only steady state its proportion of macrophages increases as S increases, in agreement with experimental evidence that chemotactic activity correlates with the macrophage level in a tumour (Bottazzi *et al.*, 1983, 1985, 1992; Walter *et al.*, 1991). Figures 2 and 5 are examples of numerically calculated bifurcation structures, showing the single mutant-only steady state, and the variation in macrophage proportion with S.

3.3 *Steady states with* $m \neq 0$, $n \neq 0$

In this section we will determine three bifurcation points where the number and type of coexistence steady states changes. The location of these bifurcations is important because we will see in Section 4 that they are also the points at which the stability of the steady states changes. We deliberately choose S as our bifurcation parameter, since biologically it is the dependence on parameters governing the immune response which is of most interest, particularly the effect of varying the chemotactic response parameter S in the influx term.The first bifurcation point at $S = \mathcal{S}_{crit}$ concerns a change from zero to two coexistence steady states, and the remaining two bifurcation points ($\mathcal{S}_1$ and $\mathcal{S}_2$) correspond to the steady states changing from being positive to having a negative component.

The equation for normal cells gives $n = 1 - l - m$, and substitution into (2a) followed by straightforward algebra gives a quadratic equation for m independent of l. There are no solutions for $S < \mathcal{S}_{crit}$, and there are two solutions for $S > \mathcal{S}_{crit}$, which we denote by $l = l_\pm$ and $m = m_\pm$. Here

$$\mathcal{S}_{crit} \equiv \frac{K_l(\xi - 1)}{K_m I} - \frac{A^2(\xi - 1)}{4\delta_l K_m I} - \frac{A}{2\delta_l} - \frac{I K_m}{4\delta_l(\xi - 1)}. \tag{3}$$

When $S = S_{crit}$, $m_+ = m_-$ is positive, but as S is increased further m_- changes from positive to negative. As well as requiring non-negative solutions for m, the solutions for l and n must also be non-negative. Given a solution for m, l is determined by $l = (\xi - 1)/K_m m$, and then n is determined as $1 - l - m$. Therefore l and m have the same sign, and non-negative n requires $l + m \leqslant 1$. We will show that this leads to a pair of transitions, again with conditions on S. These transitions will occur when $l + m = 1$, and substituting for l as above gives

$$\frac{\xi - 1}{K_m m} + m = 1. \tag{4}$$

Multiplying by the denominator gives a quadratic equation in m which has two positive roots,

$$m_1 \equiv \frac{1}{2}\left(1 - \sqrt{1 - \frac{4(\xi - 1)}{K_m}}\right) < m_2 \equiv \frac{1}{2}\left(1 + \sqrt{1 - \frac{4(\xi - 1)}{K_m}}\right),$$

provided $1 > 4(\xi - 1)/K_m$. If this last condition is not satisfied, then $l + m$ must always be greater than unity, and so no non-negative solutions with nonzero n can exist except the normal-tissue steady state. This makes sense biologically, since if ξ, the mutant-cell growth-advantage parameter, is too high, coexistence of normal and mutant cells should not occur (Sherratt & Nowak, 1992). In the same way, if the rate of complex formation is too low, the tumour growth is not sufficiently suppressed to allow coexistence.

Since m_1 and m_2 are the only values of m for which $l+m = 1$, it is clear that $n = 1-l-m$ cannot change sign except at these values. As $m \to 0$ and as $m \to \infty$, $l + m \to \infty$, so that steady states corresponding to $m_\pm \notin (m_1, m_2)$ have $n < 0$. Thus the condition for non-negative steady states is $m_1 < m_\pm < m_2$.

It is convenient to think in terms of variations with the parameter S, since this affects the steady-state levels but not m_1 and m_2. The transitions to and from non-negative steady states are at $m_\pm = m_1$ and $m_\pm = m_2$, and simple substitution shows that this occurs at $S = S_1$ and $S = S_2$, defined by

$$S_i \equiv \frac{K_l(\xi - 1)}{K_m I} - \frac{\delta_l}{I} + \underbrace{\left(\frac{K_m \delta_l}{(\xi - 1)I} - \frac{A}{I} - \frac{K_m}{\xi - 1}\right)}_{\text{positive for realistic parameters}} m_i \qquad (i = 1, 2). \tag{5}$$

From the discussion previously of the orders of magnitude of the various parameters, the braced term is expected to be positive, since δ_l/I is considerably larger than the other terms. This means that $S_1 < S_2$ (recall $m_1 < m_2$), and simple manipulation shows that $S_{crit} < S_1$. Thus the transition that occurs as S increases through S_1 is from a positive to a negative value of n if $l_\pm + m_\pm|_{S=S_1} < 1$, and the reverse is true otherwise. Figure 1 illustrates both cases. Note that at $S = S_1, m_+ = m_-, l_+ = l_-$, and the sum of their common values is given by

$$\Sigma = \frac{2\delta_l(\xi - 1)}{A(\xi - 1) + I K_m} + \frac{A(\xi - 1) + I K_m}{2\delta_l K_m}.$$

Table 1 summarizes the dependence of the coexistence steady-state structure on Σ and

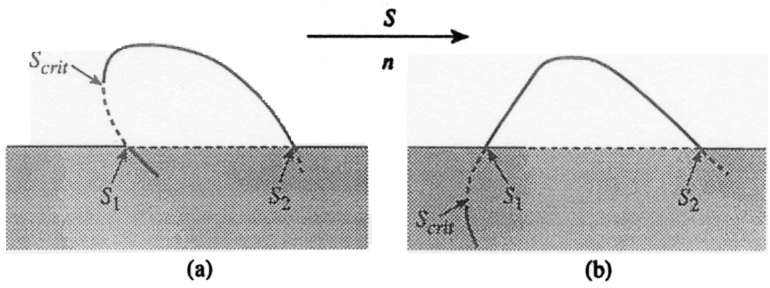

FIG. 1. A schematic representation of the two possible bifurcation structures: (—) stability, and (- - -) instability. The shaded area indicates $n < 0$, and hence negative steady states. In both (a) and (b) the following holds: for $S < \mathcal{S}_{crit}$ and $S > \mathcal{S}_2$, only the mutant-only steady state is stable, with coexistence states having negative components; for $S \in (\mathcal{S}_1, \mathcal{S}_2)$ one coexistence state is non-negative, and is the only stable steady state. This covers the whole range of S for structure (b); but for structure (a), $S \in (\mathcal{S}_{crit}, \mathcal{S}_1)$ gives two non-negative coexistence steady states, one of which is stable, with the mutant-only steady state also remaining stable.

TABLE 1

The coexistence steady-state structure. These are steady states in which l, m, and n are all nonzero. The number of non-negative steady states is determined by the value of S, and whether Σ—which is determined by the other parameters—is greater or less than 1

	$\Sigma < 1$	$\Sigma > 1$
$S < \mathcal{S}_{crit}$	No steady-state solutions	
$\mathcal{S}_{crit} < S < \mathcal{S}_1$	Two non-negative states	Two negative states
$\mathcal{S}_1 < S < \mathcal{S}_2$	One non-negative, one negative state	
$\mathcal{S}_2 < S$	All negative states	

on the size of S in relation to $\mathcal{S}_{crit}$, $\mathcal{S}_1$, and $\mathcal{S}_2$. In subsequent discussions of numerical simulations, we will present results suggesting that, as well as delimiting regions of non-negative solutions, $\mathcal{S}_1$ and $\mathcal{S}_2$ correspond to points at which the stability of steady states changes.

It is interesting to note that the term $K_l(\xi - 1)/K_m I$ occurs in the expression for each of the transitions in this section. An order-of-magnitude analysis for the parameter ranges already discussed suggests that the points $\mathcal{S}_{crit}$ and $\mathcal{S}_1$ are both close to this value. Numerical work also supports this, and it demonstrates that the range, if any, where two non-negative coexistence steady states exist is very small.

The coexistence steady state corresponding to (l_+, m_+) has m_+ decreasing and l_+ increasing as S increases, so that the proportion of macrophages in a tumour will rise with the chemotactic activity. This is not true of the state corresponding to m_-, but, as discussed above, the range where this solution is non-negative is small, and in Section 4 we will show that it is unstable anyway.

Coexistence steady states are to be expected in a realistic model for tumour growth, since real tumours certainly contain a variety of cell types (Mantovani, 1990). Other models also predict this type of solution (Sherratt & Nowak, 1992; Wheldon, 1975), although no

previous model has looked in detail at the macrophage proportions. As we have discussed, if the growth advantage of mutant cells is too high, coexistence is not possible, and non-negative steady states of this type cannot exist. We have also given a series of transition points for changes in the number and type of coexistence steady states as the chemotactic response parameter S varies.

4. Stability

In this section we will examine the stability of the steady states discussed above, primarily by using the bifurcation package *Auto* (Doedel *et al.*, 1991). First, we consider the linearization of (2a) when $m = 0$, which corresponds to the macrophage-only and normal-tissue steady states. Straightforward analysis shows that both the macrophage-only and normal-tissue steady states are unstable. The cause of the instability is the proliferative advantage of mutant cells, which is not counteracted by the immune response, since this is second order in m. This second-order term is an important consequence of the activation step for macrophage cytotoxicity, so that when linearizing about a steady state with $m = 0$, the $K_m l m^2$ immune response term does not make a contribution.

It is clear then that, for the immune response to be effective in eliminating tumour growth, it must have a component which is first order in m. One method of achieving this which may be medically relevant is to amend the original equations to include a constant source of chemical regulator. We investigate this scenario in detail in a later section. Stability calculations for the mutant-only and coexistence steady states are algebraically rather complicated, and we have been unable to derive a complete analytical deduction of stability. However, numerical methods offer more complete results. We have used the bifurcation-analysis package *Auto* (Doedel *et al.*, 1991) to find the stability of the coexistence and mutant-only steady states as S varies. There are two basic cases to examine, when there are: (i) two non-negative coexistence steady states possible, and (ii) only one. These structures are illustrated schematically in Fig. 1(a, b)

In Fig 1(a) there are two non-negative coexistence steady states for $S \in (\mathcal{S}_{crit}, \mathcal{S}_1)$, one of which is stable, so that with the mutant-only steady state there are multiple stable steady states. In both cases the mutant-only state becomes unstable for $S \in (\mathcal{S}_1, \mathcal{S}_2)$. The structure in Fig. 1(b) is simpler, with unique non-negative stable states for all S. There is actually a third possibility, which is not illustrated in Fig. 1, in which non-negative coexistence states never exist, due to the mutant-cell growth advantage ξ being too high, or because the rate of complex formation is too low. In this case the mutant-only state is always stable.

In fact for realistic parameter ranges, the region with multiple stable states (as in Fig. 1(b) is often extremely small in comparison with the larger range of S over which the steady states vary. Biologically this means one would not expect such behaviour to be seen, even under carefully controlled conditions. Equations (3) and (5) give analytical expressions for $\mathcal{S}_{crit}$ and $\mathcal{S}_1$, respectively, with an order-of-magnitude analysis indicating this narrow range. Figure 2 shows the two structures in a real setting, using *Auto*; the multiple stable-steady-state region in Fig. 2(a) is clearly very small. Examples of numerical solutions of the model equations in the above cases are illustrated in Figs. 3 and 4.

Bifurcation diagrams corresponding to Fig. 2(a), with l and m plotted instead of n, show that the proportion of macrophages increases with S, while that of mutant cells falls, agreeing with the observations in the analysis of Section 3, and agreeing qualitatively with ex-

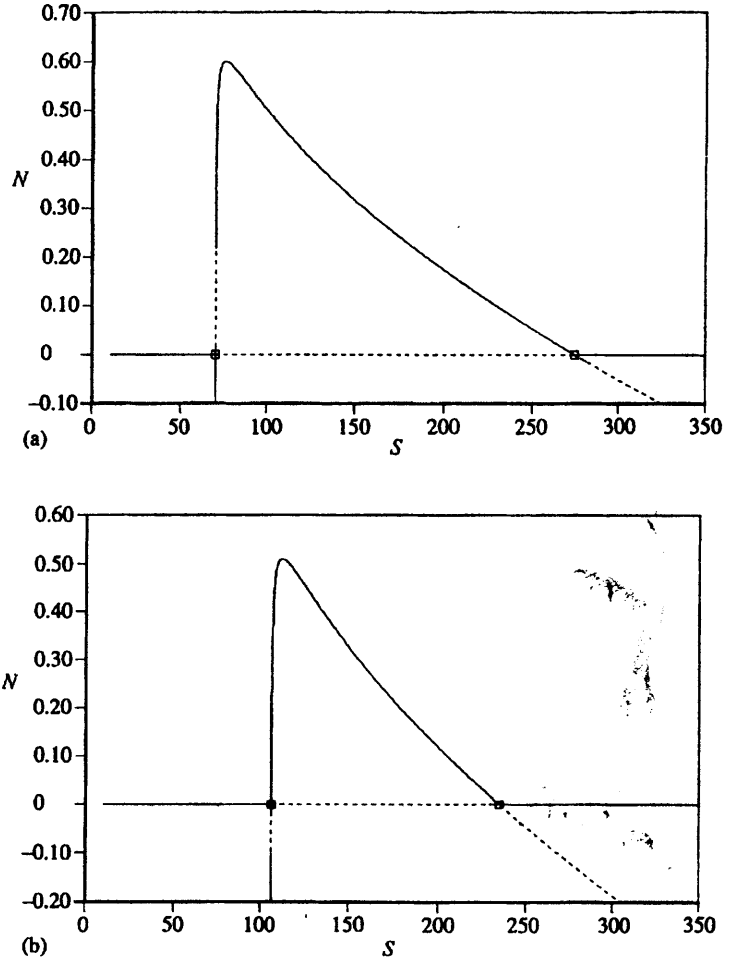

FIG. 2. Examples of numerically simulated bifurcation structures, determined using *Auto*, which should be compared with the schematic representation in Fig. 1: (—) stability, and (- - -) instability. (a) An example of the structure shown schematically in Fig. 1(a), with $\xi = 2.0$. (b) An example of the structure illustrated schematically in Fig. 1(b), with $\xi = 2.5$. The other parameters for both (a) and (b) were $A = 0.025$, $N = 1$, $I = 0.01$, $K_l = 17.857$, $\delta_l = 0.1$, and $K_m = 25.0$ which give $S_{crit} = 70.6723$, $S_1 = 70.7163$, $S_2 = 274.641$, and $\Sigma = 0.7823$.

perimental results (Bottazzi *et al.*, 1983, 1985, 1992; Walter *et al.*, 1991). We include just one example, in Fig. 5, with the same parameters as in Fig. 2(a).

It is easy to show that the analytical results on the stability of macrophage-only and normal-tissue steady states for the reduced three-variable model extend to the full model, and numerical work supports the extension of stability results for the other steady states. Simulations of the ODEs show slight differences between the reduced and full models over short time scales, but they converge to the same steady states (except in the case of

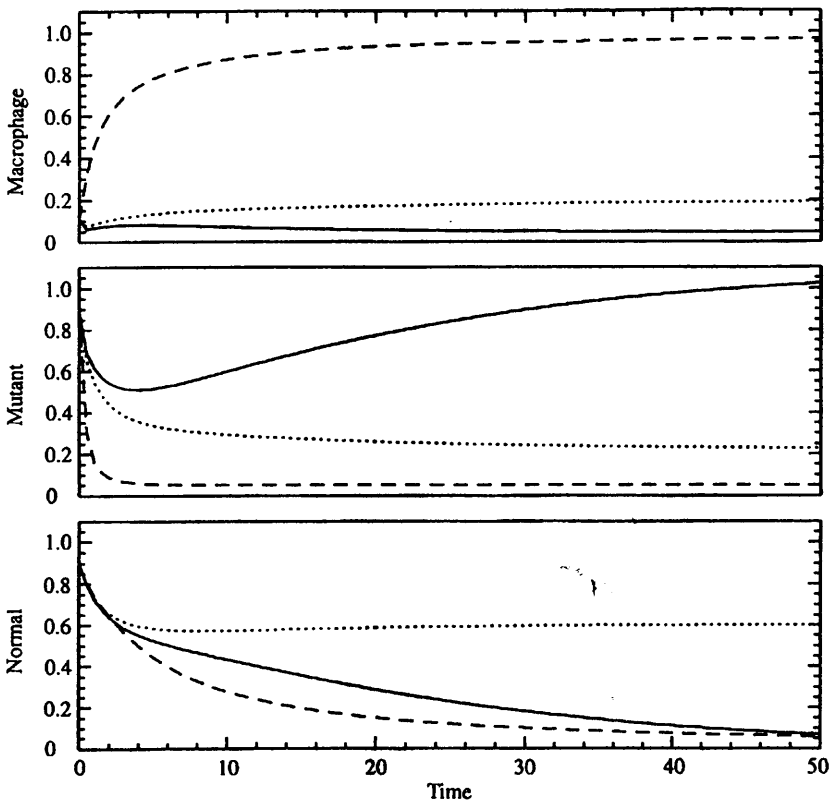

FIG. 3. Model solutions for $S < \mathcal{S}_{crit}$, $S \in (\mathcal{S}_1, \mathcal{S}_2)$, and $S > \mathcal{S}_2$ with the initial conditions $(I, m, n) = (0.1, 0.9, 0.9)$: (—) $S = 62.5$, $(\cdots)S = 75.0$, and (- - -) $S = 287.5$. Parameters were $A = 0.025$, $N = 1$, $I = 0.01$, $K_I = 17.857$, $\delta_I = 0.1$, $\xi = 2.0$, $K_m = 25.0$, giving $\mathcal{S}_{crit} = 70.6723$, $\mathcal{S}_1 = 70.7163$, $\mathcal{S}_2 = 274.641$, and $\Sigma = 0.7823$. These parameters give the bifurcation structure illustrated schematically in Fig.1(a), but the solutions shown lie outside the region of multiple stable steady states, and they are representative of both generic bifurcation structures. As expected, for $S < \mathcal{S}_{crit}$ and $S > \mathcal{S}_2$ the solution converges to a mutant-only steady state, and for $S \in (\mathcal{S}_1, \mathcal{S}_2)$ it converges to a coexistence steady state.

multiple stable steady states, where basins of attraction become important), as expected from the pseudo-steady-state hypothesis used to derive the three-variable model.

5. Enhancing the immune response

As mentioned previously, the immune response cannot stabilize the normal-tissue steady state to the introduction of a small number of mutant cells with a larger intrinsic growth rate, since it is second order in m. In this section we will examine the possibility of adding a first-order immune-response term by including an extra influx of chemical regulator, at a rate ϕ, say, in the original equations for the full five-variable model. This does not occur naturally, but it is a possible therapeutic approach, which has been investigated in some recent experimental studies (see Section 7). Use of the pseudo-steady-state hypothesis, as

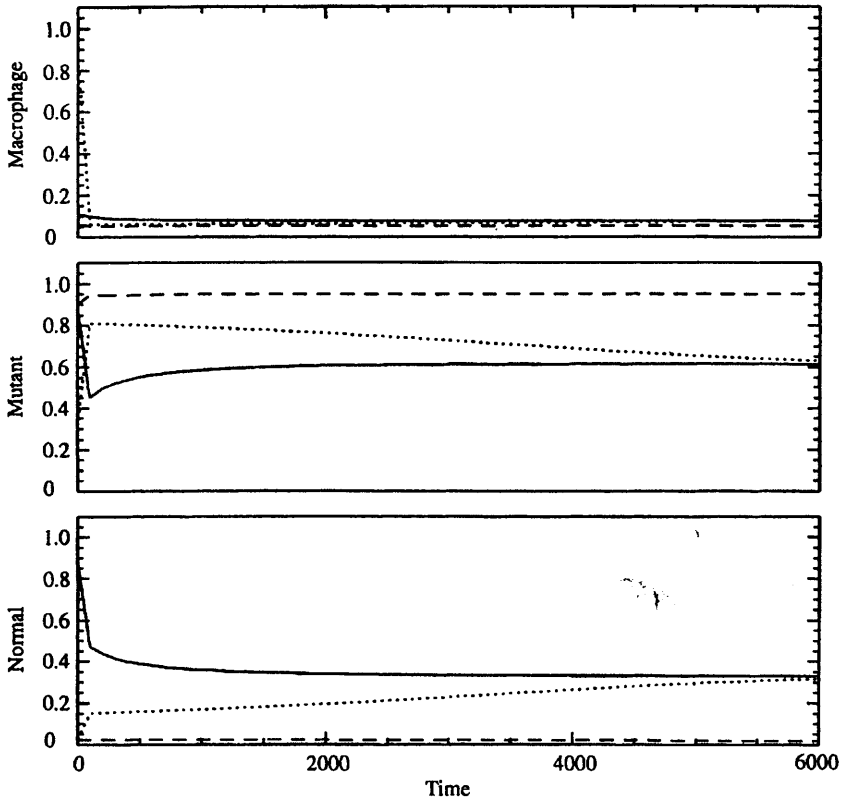

FIG. 4. Model solutions for $S = 70.7 \in (\mathcal{S}_{crit}, \mathcal{S}_1)$, for which there are two stable steady states, so that different initial conditions for (l, m, n) lead to different steady states: (—) (0.1, 0.9, 0.9), (· · ·)(0.9, 0.3, 0.01), and (- - -) (0.05, 0.9, 0.01). The parameters are the same as those in the legend to Fig. 3, giving $\mathcal{S}_{crit} = 70.6723$, $\mathcal{S}_1 = 70.7163$, $\mathcal{S}_2 = 274.641$, $\Sigma = 0.7823$, and the bifurcation structure illustrated schematically in Fig. 1(a).

in the original development of the three-variable model, leads to the system of equations

$$\frac{dl}{dt} = \frac{Al(m+F)(N+1)}{N+l+m+n} + I[1+S(m+F)] - K_l lm(m+F) - \delta_l l, \tag{6a}$$

$$\frac{dm}{dt} = \frac{\xi m(N+1)}{N+l+m+n} - m - K_m lm(m+F), \tag{6b}$$

$$\frac{dn}{dt} = \frac{n(N+1)}{N+l+m+n} - n, \tag{6c}$$

where $F = \phi/N_e\beta$. The dimensionless parameter F represents the scaled influx of chemical regulator. Clearly this system reduces trivially to the original system on setting $F = 0$, and for nonzero F there is a component of the immune response which is first order in m.

A similar analysis to that used in Section 4 shows that there are only two structures for the nature and stability of normal and macrophage-only steady states modified by the addition of extra chemical. The first is such that both remain unstable when F is small,

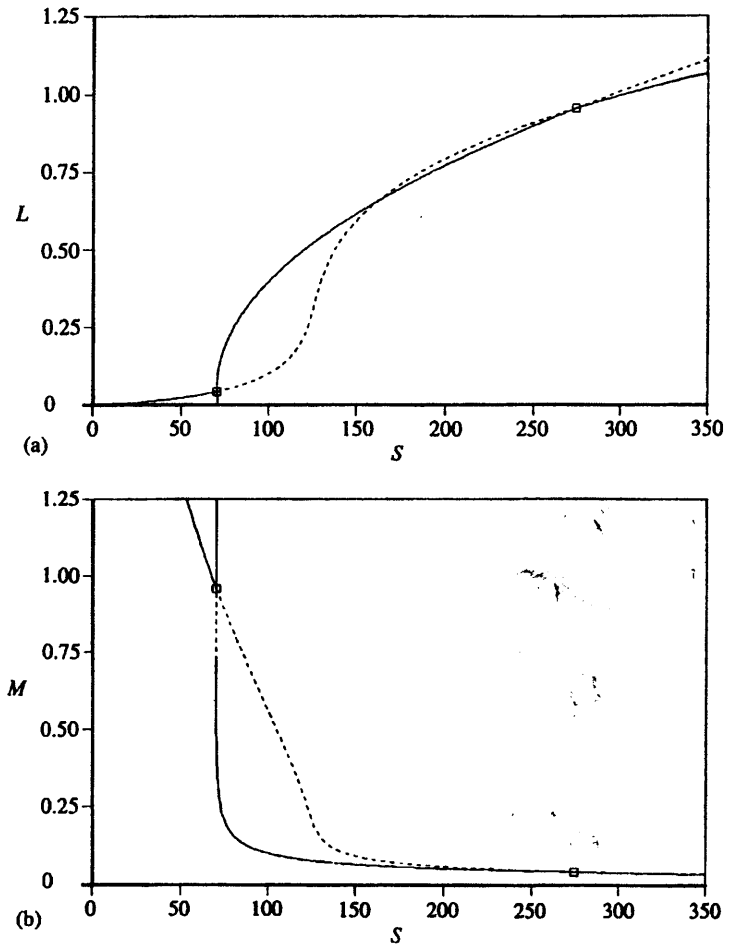

FIG. 5. Examples of numerically simulated bifurcation structures, determined using *Auto*, showing the variation with S of: (a) macrophage, and (b) mutant-cell levels. (—) Stability, and (- - -) instability. The parameters are the same as in Fig. 2(a). As the chemotactic response parameter S increases, the proportion of macrophages in the tumour steady states increases, in agreement with experimental evidence.

but as F increases through a transition point F_1 the modified normal state becomes stable until a second transition occurs at $F = F_3$, when simultaneously n becomes negative and the steady state becomes unstable, while the macrophage-only state becomes stable. In the second structure the modified normal state is unstable for all positive F, and n also becomes negative at $F = F_3$. Subsequently, at $F = F_2$, the macrophage-only steady state becomes stable. Table 2 summarizes both these structures; we have deliberately omitted the derivations (which are straightforward) for brevity.

At the same time as these relatively simple transitions take place, the steady states containing mutant cells undergo more complicated changes, so that one or more of them may

TABLE 2

The steady-state structure when extra chemical is added. Note that if $F_1 > F_3$ the modified normal steady state cannot *be stabilized by chemical addition. F_1 is the unique positive solution for F of $K_m ISF^2 + [KmI + A(\xi - 1)]F - \delta_I(\xi - 1) = 0$, and $F_3 = (\delta_I - I)/(IS + A)$. Analysis shows that F_2 is also uniquely determined, but with a very complicated algebraic form*

Modified normal-tissue steady state		Modified macrophage-only steady state	
$F < F_3$	Non-negative, stable for $F > F_1$	$F < \max\{F_2, F_3\}$	Unstable
$F > F_3$	Negative, unstable	$F > \max\{F_2, F_3\}$	Stable

be stable at the same time as one of the non-mutant steady states. Numerical results indicate that a wide range of behaviours are possible, which we have not attempted to classify.

Figures 6 and 7 illustrate model simulations of the effect of exogenous chemical addition. Figure 6, in particular, shows that the time at which addition is started is crucial in the case in which both a modified non-tumour and a modified tumour are stable. This is because the solution trajectory moves between the basins of attraction of the two steady states. Our model is of course inappropriate for tumours that have developed their own vasculature. Therefore the model is clinically most applicable to cases in which other therapies have been used initially to reduce the tumour size, and it indicates that the proportion of mutant cells remaining could be crucial in determining whether subsequent treatment of the type discussed in this section would be successful. This is consistent with the results of clinical trials using interferon-γ to treat ovarian carcinomas: patients with minimal residual disease (Colombo *et al.*, 1992) exhibited markedly enhanced anti-tumour activity compared to patients with a substantial tumour burden (Allavena *et al.*, 1990).

6. Summary

In this paper we have developed a model for the role of macrophages in avascular tumour growth, concentrating on the cytolysis of mutant cells. Analysis of the model showed the existence of four types of non-negative steady states: macrophage only, normal tissue, mutant-only, and coexistence.

Non-tumour steady states are always unstable because the macrophage immune response is second order in the density of mutant cells, and so it is unable to counteract their increased growth rate. At least one of the cancerous steady states is always stable, with the type varying with parameter values. We have determined these transitions analytically in terms of the parameter S, which is a measure of the rate of production by mutant cells of regulatory chemical, and the response of macrophages to the chemical. As the production rate or response increases, the proportion of macrophages in the tumour increases; this is qualitatively in line with experimental evidence that the proportion of TAMs in tumours increases with the chemotactic activity of the mutant cell line (Bottazzi *et al.*, 1983, 1985, 1992; Walter *et al.*, 1991).

We have also investigated the effect of the addition of extra chemical regulator, at some constant rate, on the stability of non-tumour steady states. Such chemical addition modifies

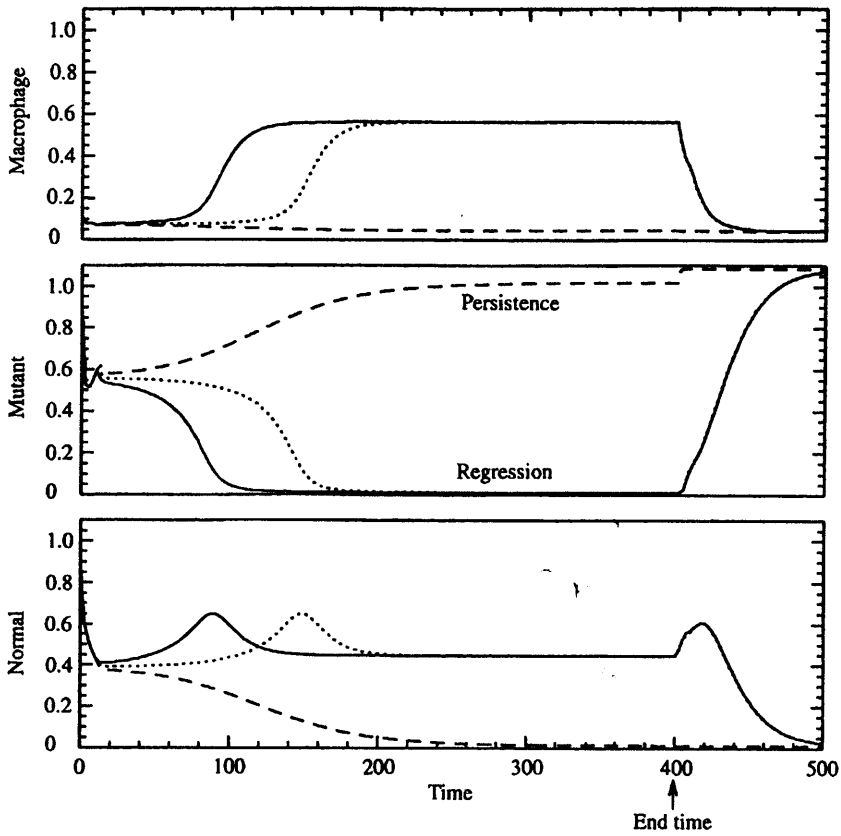

FIG. 6. Model simulations of the effect of exogenous addition of chemical regulator for treatment starting at three different dimensionless times: (—) 10, (···) 11, and (- - -) 12. The initial conditions were $(l, m, n) = (0.1, 0.9, 0.9)$; and the parameter values were $F = 0.072$, $A = 0.025$, $N = 1$, $I = 0.01$, $S = 62.5$, $K_l = 17.857$, $\delta_l = 0.1$, $\xi = 2.0$, and $K_m = 25.0$, which gave two stable steady states. Before the chemical was added the dynamics followed their normal course towards a mutant-only steady state. If the chemical was added sufficiently early, the solutions converged to the stabilized normal-tissue state. However, if it was added too late then the solutions already lay in the basin of attraction of a modified-tumour steady state, and hence they converged to that state. This indicates that such a therapy would be most effective after other methods have been used to reduce the tumour size. When the addition was stopped, at time $t = 400$, the solutions returned to the mutant-only steady state. This simply reflects the fact that the model is invalid at very low cell densities, due to its continuous nature.

the composition of steady states, and we have shown that modified non-tumour steady states can be stabilized with sufficient levels of chemical.

7. Discussion

The ability of the immune system to selectively kill some tumour cells has been known for many years, and it provides an attractive explanation for the rare spontaneous disappearance of human tumours (Kaiser, 1989). Recently this *immune surveillance hypothesis* has been widely disputed (Prehn, 1994), and our model is consistent with this scepticism since

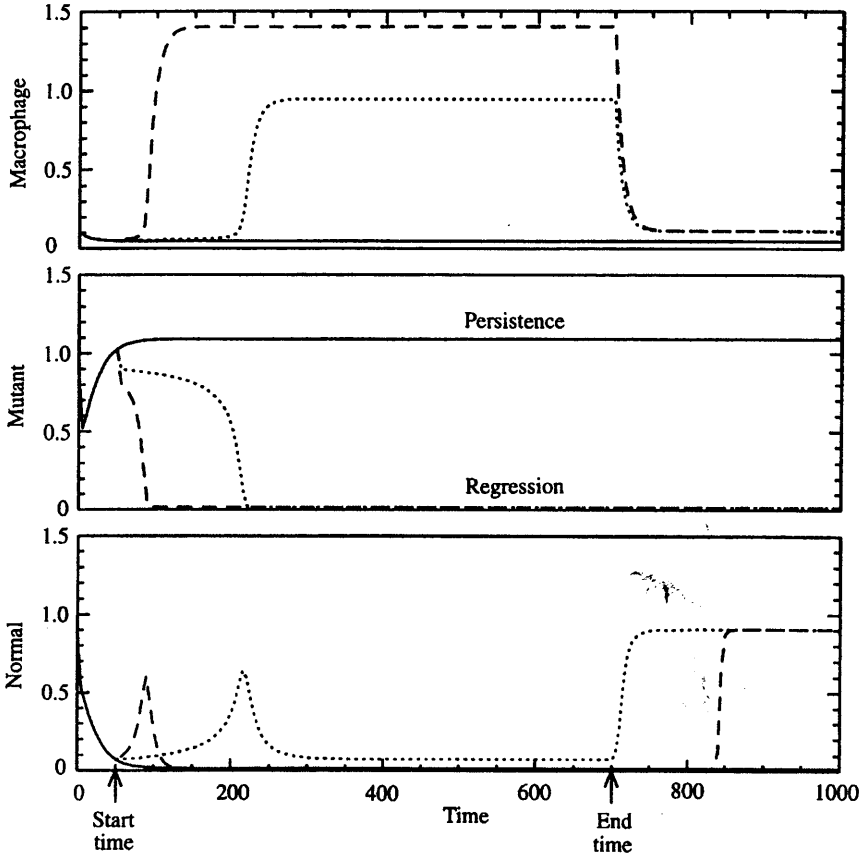

FIG. 7. Model simulations of the effect of exogenous addition of chemical regulator for three different dimensionless rates of chemical addition, F: (—) 0, (···) 0.13, (- - -) 0.2. The treatment start time was 50, and the end time was 700. The initial conditions and parameters were the same as in Fig. 6, except for the value of F. For $F = 0.13$ the modified normal-tissue steady state was stable, but for $F = 0.2$ the solutions converged to a modified-macrophage-only steady state. Note that when the treatment was removed both solutions converged to the normal-tissue steady state, indicating successful eradication of a tumour. After continuing the simulation for sufficient time the tumour will reappear, as explained in the legend to Fig. 6.

it predicts that macrophages are never able to eliminate a tumour without intervention. However, the possibility of manipulating and improving host responses is the basis of a number of rapidly developing cancer immunotherapies (Rosenberg, 1990). A variety of cell types have been used in immunotherapy, notably lymphokine-activated killer cells (Rosenberg *et al.*, 1987) and tumour-infiltrating lymphocytes (Rosenberg *et al.*, 1990).These cell types are used in *adoptive immunotherapy* methods, meaning that immune-system cells are removed from the body, cultured and activated *in vitro*, and then reinjected. These treatments have had some success, although the side-effects can be severe. Partly for this reason, macrophages are increasingly being considered as a vehicle for immunotherapy. In addition, tumour-infiltrating lymphocytes are specific to a particular antigen displayed

on a patient's cancer cells, whereas macrophages can select mutant cells for lysis without specific antigen recognition, and they are therefore seen as a possible weapon against the heterogeneity in the antigenicity and the chemotherapeutic susceptibility seen in metastatic cancer (Esgro, 1990).

Macrophage-based immunotherapies are of two main types. Some current adoptive treatments remove circulating blood monocytes, mature and activate them in culture with interferon-γ, and reintroduce what are now macrophages into the patient (Andreesen, 1993; Andreesen *et al.*, 1990). Other methods use immature monocytes (Stevenson *et al.*, 1988). Our model would need to explicitly include activated macrophages to be applicable here. The second main type is the local or systemic administration of macrophage-activating chemicals, such as MTP-PE (Alving, 1983: Fidler & Kleinerman, 1993), a synthetic analogue of a component of the bacterial cell wall, and lymphokines, such as interleukin-2 (Allavena *et al.*, 1987) and interferon-γ (Allavena *et al.*, 1990; Colombo *et al.*, 1992). Our simulations in Section 5 correspond to the latter approach. One application of our modelling approach would be to predict the optimal therapeutic regime in a particular case.

One of our principal motivations for modelling the macrophage–tumour interaction is to elucidate the important parameters determining the effect of macrophage infiltration in nascent tumours, and the possibility of manipulating the system to control it. Analysis of our model has shown that the natural macrophage response is incapable of preventing mutant cells with a proliferative advantage from forming a tumour; however, the steady-state structure agrees with the experimental evidence that there is a correlation between the proportion of macrophages in a tumour and the extent of chemotactic activity. Empirical studies typically use the same type of macrophages throughout a particular series of experiments, so that variations in the extent of chemotactic activity, which are assessed using Boyden-chamber assays, are due to differences in production by mutant cells of chemotactic factors. However, the model predicts a variation in tumour composition with the chemical production rate, and also with the macrophage sensitivity to these chemicals, which considering macrophage heterogeneity (Mantovani, 1989) is expected to vary appreciably. These predictions are amenable to experimental verification *in vitro*, using a controlled environment in which only the parameters of interest can be varied; multicell spheroids are a possible vehicle for such experimental work (Hofstader *et al.*, 1995; Durand, 1990).

There is a further question on the composition of tumours, regarding the proportion of normal cells. *In vivo*, the cellular composition of tumours is highly variable. Our model predicts the possibility of two different types of tumour, according to the presence or absence of normal cells. It also predicts that a key factor controlling the tumour type is the chemotactic activity.The bifurcations between these two types occur at well-defined values of the chemotactic parameter, which could be tested experimentally by selectively regulating the chemotactic response, and this is feasible for at least some cell types (Aznavoorian, *et al.*, 1990). In a separate paper (Owen & Sherratt, 1997), we will address the effect of macrophages on the structure of a spatially heterogeneous tumour.

We have concentrated on the inhibitory effects of macrophages on the growth of avascular tumours, but a number of processes that occur later in tumour development are stimulated by macrophage-derived factors, creating an overall balance between inhibition and promotion (Mantovani *et al.*, 1992). Data on the relationship between macrophage infiltration and tumour prognosis is conflicting (Mantovani *et al.*, 1992). Two examples of pro-

cesses upregulated by macrophages are tumour stroma development (Vignaud *et al.*, 1994) and angiogenesis; the latter effect has been extensively studied in breast cancer (Lewis *et al.*, 1995). Both are key steps in malignant progression (Chaplain, 1995). Vignaud *et al.* (1994) investigated the relationship between tumour, macrophages, and stromal tissue, finding paracrine promotion of stromal development by macrophages. With regard to angiogenesis, it is interesting to note that, in contrast to the above concepts of macrophage immunotherapy, Vukanovic & Isaacs (1995) have used treatment with linomide to reduce the numbers of macrophages, and hence the secretion of angiogenic factors, in an attempt to inhibit neovascularization.

Acknowledgements

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