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CONCOMITANT IMMUNIZATION BY THE FULLY ANTIGENIC COUNTERPARTS PREVENTS MODULATED TUMOR CELLS FROM ESCAPING CELLULAR IMMUNE ELIMINATION

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In a mathematical model of the cellular antitumor immune response, we studied the possible role of antigenic modulation as a tumor escape mechanism. Modulated tumor cells arise from normal (fully antigenic) tumor cells when the latter interact with antibodies. Modulated tumor cells demodulate when antibody concentrations are sufficiently low. Through modulation, tumor cells become less sensitive to cytotoxic macrophages (cell lysis) and contribute less to the stimulation of the immune system. These experimental data are incorporated in a model which we have analyzed previously. The model incorporates interactions between macrophages and T lymphocytes, which lead to cellular antitumor immune reactions (i.e., to cytotoxic macrophages). Parameters were derived from the immune resistance of DBA/2 mice to the SL2 tumor.

Although all parameters were chosen deliberately to favor the modulation process (i.e., modulation proceeds fast, demodulation slowly, and the killing rate is reduced 50-fold), modulation is found to be a poor tumor escape mechanism. Heterogeneous populations of modulated and normal tumor cells are easily rejected. Homogeneous populations of modulated cells do escape, however. We conclude that the impact of modulation as an escape mechanism remains small because modulated tumor cells do not appear until the immune system has been stimulated (immunized) by the fully antigenic tumor cells. Thus, the elimination of modulated tumor cells generally occurs merely as a side effect of the immune response which is directed primarily against the fully antigenic tumor cells.

Parameter sensitivity analysis shows that this conclusion holds true only for cellular immunity. Conversely, the parameter analysis suggests that antigenic modulation plays a deleterious role in cytotoxic antibody responses (e.g., monoclonal antibody therapy).

We have already investigated several mathematical models of antitumor immune reactions generated by interactions between macrophages and T lymphocytes (1–3). To determine the minimal requirements for the failure of antitumor immune responses, we omitted from these models explicit tumor escape mechanisms such as suppression, antigenic modulation, and antigenic heterogeneity. The failure of the immune response (tumor escape) or its success (tumor rejection) was found to be nonmonotonically related to the number of reactive helper T cells (i.e., helper T cell reactivity). Tumor antigenicity is therefore defined as helper T cell reactivity, i.e., as the number of helper T lymphocyte precursors (HTLP)[2] ready to become activated on introduction of the tumor (1). Irrespective of their initial size, weakly antigenic tumors (those with low helper reactivity) are never rejected, whereas tumors that are slightly more antigenic can be rejected even if introduced in large doses (1). Tumors may also escape in these models because the immune system becomes tolerant. Although all forms of immune suppression are excluded from the previous models, tolerance evolves when effector helper T cells are made short-lived. Tumors are (low zone) tolerated when they are introduced in a small dose (sneaking through) (2, 3).

Having determined the requirements for progressive tumor growth in the absence of explicit escape mechanisms, we are now interested in the effect of incorporating such mechanisms. An analysis of the effect of introducing new mechanisms provides insight into the role these mechanisms play in the model behavior (here the immune response) (4–6). Here we investigate the possible role of antigenic modulation as a tumor escape mechanism.

Antigenic modulation has been described experimentally as a decrease in the density of cell surface antigens in response to the exposure of the cells to antibodies (AB) with affinity to these antigens (7). The phenomenon was originally described for the TL antigen on a T cell leukemia (7), but there is increasing evidence that it affects many surface antigens (8). On modulation, (tumor) cells become resistant to cytotoxic AB (AB plus complement). As in the case of natural immune responses, however, no consensus has been reached concerning the role of cytotoxic AB in the antitumor immune response. The cellular immune response (NK cells, macrophages, and T lymphocytes), on the other hand, is generally thought to play an important role in tumor elimination (9, 10). AB

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[2] Abbreviations used in this paper: AB, antibody; AD, antigen density; ADCC, antibody-dependent cellular cytotoxicity; ANGRY, cytotoxic macrophage(s); APC, antigen-presenting cell(s); BCGF, B cell growth factor; BL, B lymphocyte(s); BLP, BL precursor(s); CTL, cytotoxic T lymphocyte(s); DEBRIS, tumor cell debris; DEM, demodulation rate; HTL, helper T lymphocyte(s); IB, B cell reactivity; HTLP, HTL precursor(s); IH, tumor antigenicity; MPH, normal macrophage(s); MOD, modulation rate; TAA, tumor-associated antigen.

may nevertheless maintain (part of) of the cellular reaction when tumor cells become coated with AB (AB-dependent cellular cytotoxicity (ADCC)) (9). However, AB also may be responsible for blocking the cellular response (11).

Even if cytotoxic AB play only a minor role in the antitumor immune response, modulation still constitutes an escape mechanism if it protects tumor cells against cellular immunity (e.g., ADCC). Cellular cytotoxicity is indeed reported to decline when tumor cells modulate. Modulated tumor cells are much more protected against cytotoxic AB, however, than against cellular cytotoxicity (12–15). Nevertheless, antigenic modulation is frequently quoted as a tumor escape mechanism (7, 9, 13, 16). Here we study the role of modulation by comparing the immune reactions to tumor cells capable of modulation with those to tumor cells incapable of modulation, for an otherwise identical parameter setting (Multi-Model Fixed-Parameter approach (4)). In addition, we compare immune reactions to mixed populations of modulated and demodulated tumor cells (such as to arise by "natural" modulation) with reactions to homogeneous populations of either normal (no modulation) or modulated (no demodulation) cells.

Note that these "experiments" are quite easy to perform in this model but would be impossible in wet immune systems. Experimental investigation of the antigenic modulation phenomenon has provided data on the modulation and demodulation rate, and on the reduction in cytotoxicity brought about by modulation. However, such data do not show that modulation is responsible for the observed tumor escape. Conversely, by investigation of mathematical immune systems incorporating antigenic modulation with its experimentally described parameters, we obtain insight into the influence of modulation on the immune reaction and tumor escape. We conclude that the concomitant presence of modulated tumor cells and normal (fully antigenic) tumor cells markedly reduces the impact of antigenic modulation.

MATERIALS AND METHODS

The model (Table I) and its parameter setting are almost the same as our previous models (1–3). However, we have excluded cytotoxic T lymphocytes (CTL) because they were previously shown to play only a negligible role in the model's immune response to lowly antigenic tumors (1). Here we only consider weakly antigenic tumors. If the CTL are (re)incorporated, results are unaffected (not shown).

To incorporate modulation, we now include B cells and AB. B lymphocyte precursors (BLP) are activated whenever they interact with tumor-associated antigen (TAA) on the tumor cell surface. Effector B lymphocytes (BL) proliferate in response to helper T cell-derived growth factor (FACTOR); they produce AB on restimulation by antigen (TUMOR). B cell maturation into plasma cells is excluded for reasons of simplicity. AB stimulate normal tumor cells (TUMOR) to modulate and prevent modulated tumor cells (TUMMOD) from demodulating. After modulation, tumor cells express a lower density (AD) of antigens: they are less sensitive to cytotoxic macrophages and contribute to a lesser degree to the activation of lymphocytes.

Parameter setting. We assume that modulation proceeds quickly, and that demodulation proceeds slowly and occurs in the absence of AB only; we choose modulated cells to be 50 times less sensitive to cytotoxic macrophages and to contribute 50 times less to the stimulation of the immune system. We opt for such an extreme specification of the modulation phenomenon, i.e., one that favors tumor escape, to facilitate extrapolation of our results to biotic immune systems.

Wolf et al. (14) show for DBA/2J mastocytoma cells that modulation reduces cellular cytotoxicity (for CTL) by a factor 2 or 3. Biddison and Palmer (13) present data for a mastocytoma that develops reduced susceptibility to cell-mediated cytotoxicity. The fact that this process is reversible suggests that it is modulation; however, it operates in the absence of AB. After this "modulation", cytotoxicity reduces, on the average, by a factor 10 (cytotoxicity drops maximally from 28% to 0%) (13). Dalianis et al. (17) present data of two sublines of the YAC (Moloney) lymphoma: the variants with reduced antigen expression are two to 13 times less sensitive to CTL. Lesley et al. (12) show that (tumor) cells with an antigen density too low for efficient lysis by AB and complement remain sensitive to cell-mediated lysis. In addition, macrophage cytostatic activity remains unaffected after modulation, whereas lysis by complement reduces (18). In our model, we opt for a 50-fold reduction in susceptibility to cell-mediated cytotoxicity.

Although modulation measured by susceptibility to lysis by complement readily becomes complete (100%), large amounts of antigens persist on the cell surface in such circumstances (8). Thus, antigen density, e.g., measured by immunofluorescence or radioimmunoassay, reduces by only 50 to 70% after modulation (8, 19). The immunizing capacity of modulated cells may thus remain quite high. Indeed, variants with reduced antigen expression do not differ from the original YAC lymphoma in this respect (17). The data of Wolf et al. (14) show that modulation brings about a fivefold reduction in the immunizing capacity. In the model, we opt for a 50-fold reduction (AD = 50).

According to the experimental data, the modulation time for thymus leukemia (TL) antigen and many other surface antigens averages from 1 (20) to 3 hr (21). Note that surface immunoglobulins modulate more rapidly (i.e., within minutes) (8), but that modulation of TAA takes at least 1 hr (21–26). We assume that modulation occurs within 1 hr (MOD = 25 modulations per cell per day). The data reveal a demodulation time (roughly) ranging from 3 or 4 hr (8) to 18 hr (19). We assume that demodulation takes about 1 day (DEM = 1 demodulation per modulated cell per day). Moreover, we assume that for demodulation a cell must be free of AB pressure. Therefore, as the concentration of AB decreases, the demodulation rate approaches its maximum. We choose the saturation coefficients of the modulation and demodulation process (KM1 and KM2), i.e., the AB concentration where modulation and demodulation proceed at one-half of their maximal rate, to be 5000 and 50,000, respectively. It seems reasonable to assume that modulated cells require higher AB concentrations for the saturation of their demodulation process than do normal cells for their modulation process (i.e., we assume KM2 > KM1). The results, however, are relatively insensitive for the values of these saturation coefficients (as well as for most other parameters) (see Fig. 5). The parameters are presented in Table II.

The model is analyzed by simulation (here numerical integration). Integration was performed by ROW4A (27), implemented in GRIND (28).

RESULTS

In Figure 1 we compare the immune responses to a tumor of an antigenicity corresponding to IH = 0.3 (HTLP = 15) in the absence of modulation (Fig. 1*a*) and in the presence of modulation (Fig. 1*b*). The tumor is introduced as a single cell; it is rejected when the cells do not modulate, and it escapes when they do. The largest tumor dose that can be rejected in the absence of modulation is 10^4 cells (see Fig. 4). The behavior of the model without modulation is very similar (approximately externally equivalent (5, 6)) to the previously presented models: in those models, the largest rejectable dose of this particular tumor consisted of 2×10^4 cells (1). The tumor growth curve (Fig. 1*a*) consists of two qualitatively different phases: expansion (days 1 to 14), and rejection due to a severe attack by macrophages (days 15 to 17). We consider a tumor to be rejected whenever it consists of less than one cell. (In fact, TUMOR was artificially set to 0 at day 18 in Fig. 1*a*; otherwise, it would have regrown like the tumor in Fig. 2*a*).

If B cells are introduced (Fig. 1*b*), the tumor becomes capable of modulation. At day 5, the first modulated tumor cells appear; at day 20, they exceed 10^8 cells, which is sufficient to kill the host (i.e., the mouse). We continued the simulation for another 10 days, although in a biotic system such a period would not exist. Note

TABLE I

Formal representation of the model[a]

ATUMMOD	= TUMMOD/AD
ATUMOR	= TUMOR + ATUMMOD
FACTOR	= HTL × ATUMOR/(KMT + ATUMOR)
AB	= BL × ATUMOR/(KMT + ATUMOR)
MODRATE	= MOD × TUMOR × AB/(KM1 + AB)
DEMRATE	= DEM × TUMMOD × (1 − AB/(KM2 + AB))
INFLAM	= H × FACTOR/(KMF + FACTOR)
APC	= (MPH + ANGRY) × DEBRIS/(KMD + DEBRIS)
d(BLP)/dt	= IB + IB × INFLAM − A × BLP × ATUMOR − EL × BLP
d(HTLP)/dt	= IH + IH × INFLAM − A × HTLP × APC − EL × HTLP
d(MPH)/dt	= IM + IM × INFLAM − A × MPH × FACTOR − EM × MPH
d(BL)/dt	= A × BLP × ATUMOR + P × BL × FACTOR/(KMF + FACTOR) − DL × BL
d(HTL)/dt	= A × HTLP × APC + P × HTL × FACTOR/(KMF + FACTOR) − DL × HTL
d(ANGRY)/dt	= A × MPH × FACTOR − DM × ANGRY
d(TUMOR)/dt	= R × TUMOR/(1 + TUMOR/KR) + DEMRATE − MODRATE − KILL × ANGRY × TUMOR/(KMK + ATUMOR)
d(TUMMOD)/dt	= R × TUMMOD/(1 + TUMMOD/KR) − DEMRATE + MODRATE − KILL × ANGRY × ATUMMOD/(KMK + ATUMOR)
d(DEBRIS)/dt	= D × ATUMOR + KILL × ANGRY × ATUMOR/(KMK + ATUMOR) − ED × DEBRIS

[a] We discuss the equations in order of appearance; for a more detailed discussion of this model, we refer to De Boer et al. (1). ATUMMOD represents the degree of quantitative antigenicity of the modulated tumor cells (TUMMOD). ATUMOR thus represents the degree of quantitative antigenicity of the total tumor population. FACTOR (helper T cell-derived lymphokines) and AB are quasi-steady-state variables (i.e., they are assumed to have a rapid turnover rate). The production of both FACTOR and AB depends on restimulation by antigen (TUMOR); these factors are produced at a maximal rate when ATUMOR ≫ KMT. Modulation (MODRATE) is regulated by the AB concentration; at high AB concentrations, modulation proceeds at a rate MOD per cell. Demodulation (DEMRATE) proceeds at its maximal rate (DEM) when the AB concentration equals 0. The inflammation reaction, i.e., an enhanced influx of precursor cells, depends on FACTOR production by the HTL. The rate at which macrophages present antigen is regulated by the accumulation of antigenic tumor cell debris (DEBRIS). BLP are activated by antigenic tumor cells (ATUMOR); HTLP by antigen-presenting cells (APC); and macrophages (MPH) by lymphokines (FACTOR). All precursors have a constant influx of "virgin" cells (IB, IH, and IM, respectively). The influx is increased with INFLAM during an inflammation reaction. Precursors have a constant efflux (EL and EM); the steady-state precursor populations therefore equal IB/EL, IH/EL, and IM/EM, respectively. Effector BL and HTL are generated on activation of their respective precursors; they proliferate in response to a growth factor (BCGF and IL 2, lumped into FACTOR); the lymphocyte effectors have a constant decay (DL). BL produce AB; HTL produce FACTOR. Cytotoxic macrophages (ANGRY) are generated by activation of precursors only (they do not proliferate); they also have a constant decay rate (DM). Both tumor subpopulations grow exponentially; large tumors grow as if they were only KR cells large (i.e., tumor growth levels off). TUMOR decreases by modulation, whereas TUMMOD increases; TUMOR increases by demodulation, corresponding to TUMMOD decrease. Antigenic tumor cell debris (DEBRIS) accumulates on normal death of antigenic tumor cells (D) and after lysis (KILL) of antigenic tumor cells by macrophages. DEBRIS has a constant decay rate (ED). For reasons of simplicity, all interactions are incorporated as first-order processes.

TABLE II

Parameter setting[a]

A	10^{-3}	activation rate	per cell per day
AD	50.0	antigen density	
D	10^{-3}	debris generation rate	units per cell per day
DEM	1.0	demodulation rate	cells per cell per day
DL	0.02 or 0.4	lymphocyte effector decay	per day
DM	1.0	cytotoxic macrophage decay	per day
ED	2.0	debris decay	per day
EL	0.02	lymphocyte precursor efflux	per day
EM	0.05	normal macrophage efflux	per day
H	9.0	inflammation constant	
IB	0.0 to 100	BLP influx	cells per day
IH	0.01 to 100	HTLP influx	cells per day
IM	125,000	macrophage influx	cells per day
KILL	10.0	killing capacity	cells per cell per day
KMD	10^7	presentation saturation	units
KMF	50.0	factor saturation	units
KMK	10^5	killing saturation	cells
KMT	10^3	restimulation saturation	cells
KM1	5,000	modulation saturation	cells
KM2	50,000	demodulation saturation	cells
KR	10^9	growth rate saturation	cells
MOD	25.0	modulation rate	cells per cell per day
P	1.0	proliferation rate	cells per cell per day
R	1.0	tumor growth rate	cells per cell per day

[a] The parameter setting of the model is based on experimental data for the immune response of DBA/2 mice to the SL2 tumor (38). These parameters have been discussed in greater detail previously (1). The parameters of the modulation process were derived from the literature; to avoid underestimating the role of modulation, we have set these parameters so that they favor tumor escape. The degree of antigenicity of the tumor is defined as the number of HTLP present on introduction of the tumor. Helper precursor influx (IH) is thus varied in order to represent different tumors. We vary the number of BL precursors (IB) in order to vary the (initial) intensity of modulation.

that in this proliferative model (2, 3), T lymphocyte densities can become lethal, too (at day 20, helper T lymphocytes (HTL) > 10^7).

For the first 10 days, the growth curves of the total tumor population (TUMOR + TUMMOD) are very similar in Figure 1*a* and *b*. During this period, however, TUMOR remains smaller in Figure 1*b* because the cells transform into modulated cells. Because TUMOR remains smaller, and because the replacement TUMMOD are less antigenic, helper T cell activation proceeds more slowly in Figure 1*b*. As a consequence, the first helper T cells appear at around day 11 in Figure 1*a* and around day 12

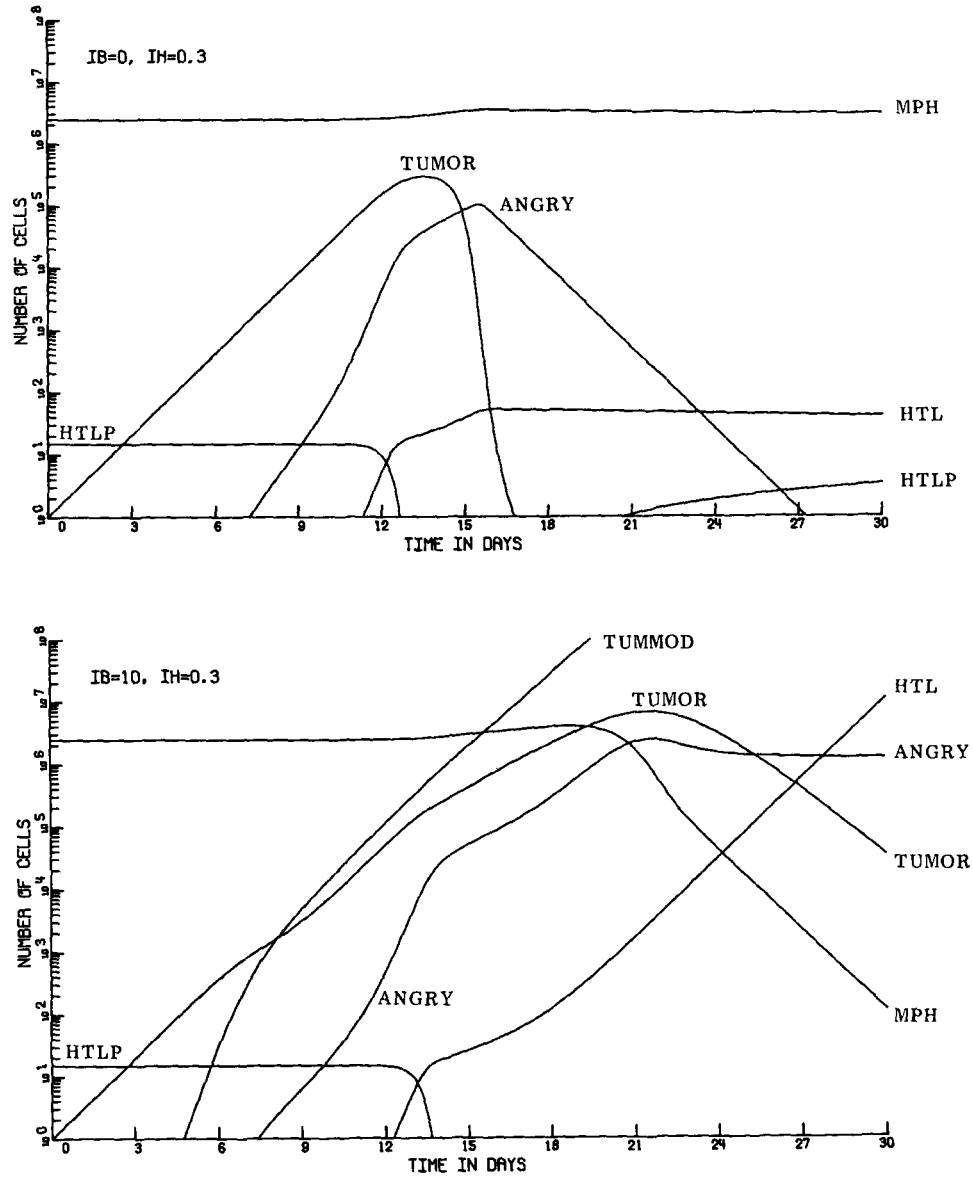

Figure 1. Tumor escape by antigenic modulation. Time plots of the immune response to a tumor of an antigenicity corresponding to IH = 0.3 (HTLP = 15). The tumor is introduced as a single cell. We compare tumor growth without modulation (*a*, no B cells, IB = 0) with the growth of cells capable of modulation (*b*, IB = 10, BLP = 500). The peritoneal cavity of DBA/2 mice, on which the parameter setting of the model is based (1), contains fewer than 5×10^6 B cells. BLP = 500 thus corresponds to a reactivity of at least one out of 10^4, which is quite high for TAA. Parameters are as in Table II, but IH = 0.3 and IB = 0 (*a*) or IB = 10 (*b*).

in Figure 1*b*. The initial ANGRY[2] population, therefore, also remains smaller in Figure 1*b*. Later, however (after day 16), ANGRY numbers in Figure 1*b* are larger than those in Figure 1*a*. By that time, however, the tumor has grown so large that it withstands this attack, expanding continuously. At day 20, when the host would normally die, TUMMOD is increasing but TUMOR starts decreasing. At that time, the fully antigenic tumor cells make up only 4% of the total tumor population.

In Figure 2, we consider a tumor which is slightly more antigenic (i.e., IH = 0.4, HTLP = 20). This tumor, again introduced as a single cell, is rejected whether modulation is incorporated or not. Thus, the effect of incorporating modulation is small as compared to the impact of tumor antigenicity (i.e., helper reactivity). Due to the increase in the antigenicity of the tumor, the tumor growth curve here consists of four phases: expansion (days 0 to 12), regression (days 13 to 17), regrowth (days 18 to 24), and a slow decline phase (days 25 to 135). At day 135, when the tumor is rejected, HTL = 3015 and ANGRY = 10354. The largest rejectable tumor dose now consists of 7×10^4 cells (see Fig. 4).

Increasing the antigenicity of the tumor capable of modulation hardly alters the development of the immune response during the tumor expansion phase (Fig. 1*b* vs Fig. 2*b*). At day 13, however, the TUMOR growth rate declines in Figure 2*b* because ANGRY is three times larger. ANGRY and HTL expand faster in Figure 2*b* because the HTL population is larger. TUMMOD regresses around day 19; TUMMOD is rejected at day 30. Comparing Figure 2*a* and *b*, we observe that although the tumor is capable of modulation, it is nevertheless rejected because the immune response increases considerably. The maximal number of cytotoxic macrophages in Figure 2*b* is 2.3×10^6, whereas the maximal number in Figure 2*a* is only 8.4×10^4. Hence, the immune system adapts (becomes stronger) whenever large TUMMOD populations arise. Adaptation, however, must occur quickly because otherwise the tumor already will have grown too large (Fig. 1*b*).

B cell reactivity. The impact of modulation is expected to vary with the intensity of the humoral response. Tumors activating larger fractions of all B cell precursors (B cell reactivity, IB) quickly evolve high antibody concentrations and should therefore escape more easily by modulation. Figure 3 shows the effect of three 10-fold in-

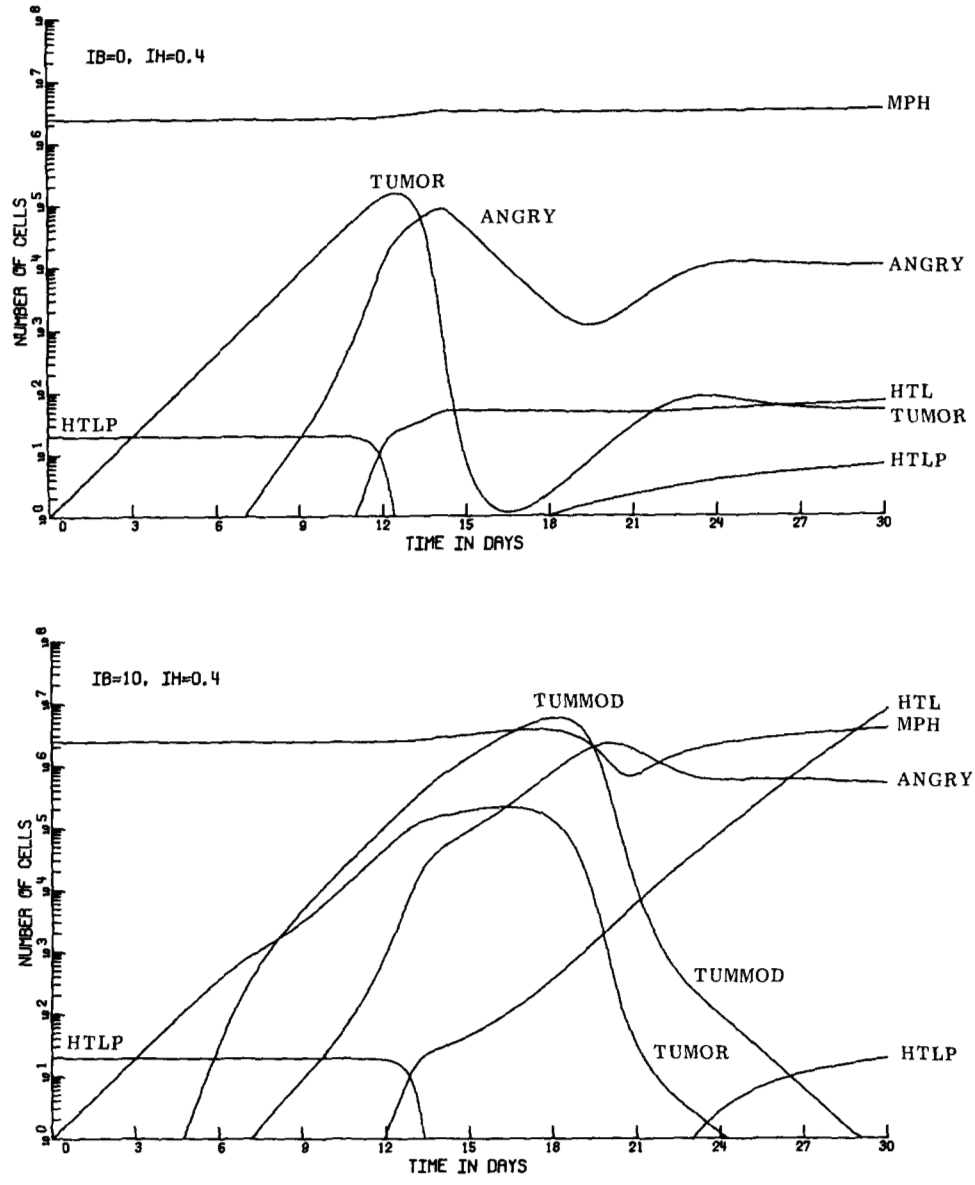

Figure 2. Rejection of a tumor capable of modulation. The antigenicity of this tumor corresponds to IH = 0.4 (HTLP = 20). (See legend to Fig. 1. Parameters are as in Table II, but IH = 0.4 and IB = 0 (*a*) or IB = 10 (*b*)).

creases in B cell reactivity. The curve on the left (IB = 0.1) is identical to a curve corresponding to absence of modulation (IB = 0; see Fig. 4). Tumors that activate 5000 B cell precursors, the curve on the right (IB = 100, i.e., a 1000-fold increase), require only a twofold increase in helper reactivity to regain their rejectability (i.e., from IH = 0.3 to IH = 0.7 for the first rejectable tumor). We conclude (again) that the impact of an increase in the intensity of modulation (by increasing B cell reactivity) remains negligible compared with an increase in helper T cell reactivity (i.e., tumor antigenicity).

Immunization by fully antigenic counterparts. In Figure 4, we compare the rejectability of homogeneous populations of either normal or modulated tumor cells (the curve on the left and on the right, respectively). Homogeneous populations of modulated cells, i.e., modulated cells incapable of demodulation, require an almost sixfold increase in helper T cell reactivity (i.e., from IH = 0.3 to IH = 1.7) to regain rejectability. This contrasts strongly with the rejectability of modulated tumor cells (protected by the same 50-fold reduction in killer susceptibility) that grow in the presence of fully antigenic tumor cells (triangles and circles).

The fact that modulated cells in the mixed populations are more easily rejected than homogeneous populations of modulated cells is not due to demodulation and subsequent eradication of the modulated subpopulation. The heterogeneity curve (circles) corresponds to mixed, but independent (i.e., MOD = 0 and DEM = 0), growth of modulated and fully antigenic tumor cells. Such a heterogeneous tumor is just as easily rejected as a "naturally" modulating tumor (triangles). In conclusion, it turns out that the mere presence of fully antigenic tumor cells facilitates the rejection of modulated cells (diamonds vs circles). The impact of antigenic modulation as an escape mechanism thus remains small because the development of modulated cells is always accompanied—even preceded—by the growth of normal tumor cells. The latter immunize the immune system against the former.

Immunization. We were able to test this conjecture by explicitly immunizing the model immune system with the fully antigenic subpopulation. Following our previous procedures (1), we immunized with two doses of 10^7 irradiated (nondividing) normal tumor cells, administered

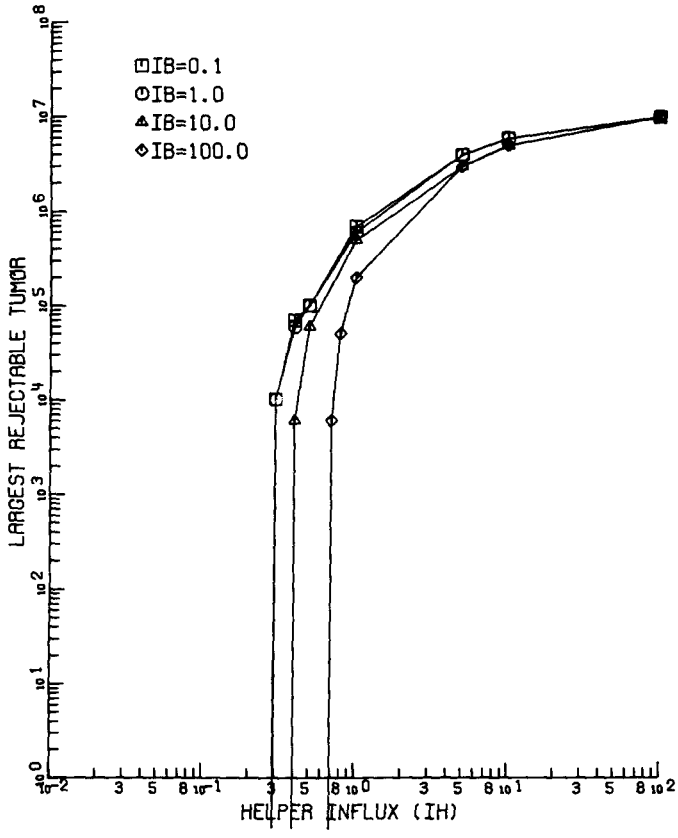

Figure 3. The size of the largest rejectable tumor as a function of tumor antigenicity (i.e., helper reactivity) for four different values of IB. The curve marked with squares (IB = 0.1) is identical to a curve corresponding to absence of modulation (IB = 0). Parameters are as in Table II; IH varies from 0.01 to 100 along the x-axis; IB varies 10-fold in each experiment.

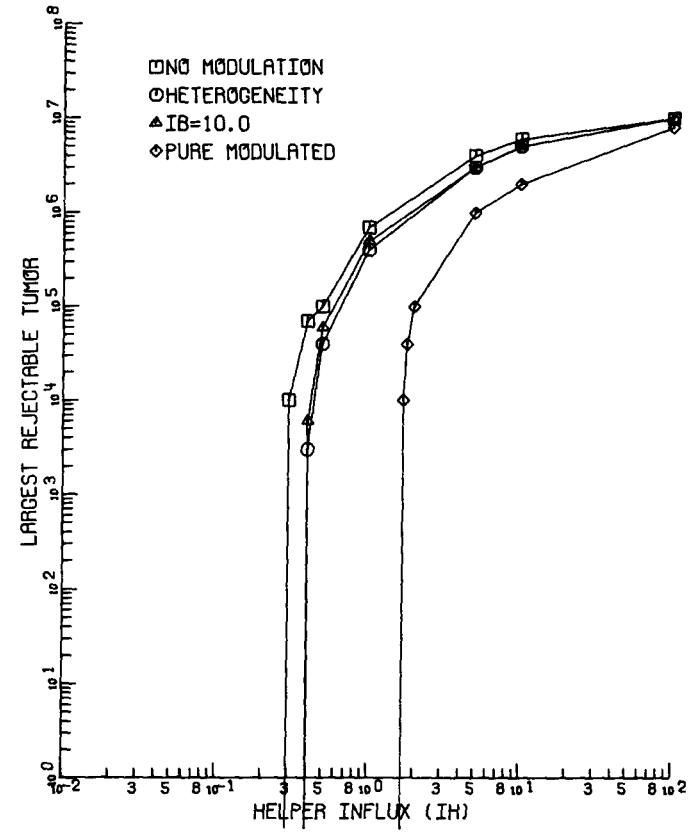

Figure 4. The size of the largest rejectable tumor as a function of tumor antigenicity (i.e., helper reactivity). □, Absence of modulation; we introduce normal tumor cells. ○, Absence of modulation and demodulation; we introduce a tumor which consists of one-half normal and one-half modulated cells; the populations thus grow independently (because the two subpopulations are "genetically stable", the curve is labeled "heterogeneous"). △, Natural modulation (MOD = 25 and DEM = 1); we introduce normal tumor cells (this curve is identical to that shown in Fig. 3). ◇, Absence of demodulation; we introduce modulated cells only (the tumor thus grows as a pure population of modulated cells).

at day 0 and day 10, respectively. At day 20, we introduced a homogeneous population of modulated tumor cells capable of cell division. For this experiment, we took the tumor corresponding to IH = 0.3, the first rejectable tumor in the absence of modulation (Fig. 4). It appears that after immunization (i.e., at day 20 when HTL = 984), the system is able to reject a dose of (at most) 7×10^6 modulated tumor cells of this tumor (formerly none). Thus, immunization with fully antigenic tumor cells increases the rejection of the modulated counterparts markedly.

Sneaking through. We have shown previously that tumors may escape immune elimination after the model immune system has been tolerized (2, 3). When HTL are made short-lived, a proliferation threshold comes into existence, below which HTL numbers are insufficient for (net) proliferation. Above the proliferation threshold, HTL are capable of infinite proliferation. During the regression phase of a normal immune response (e.g., Fig. 2*a*), HTL numbers decline (this is more pronounced when the HTL are short-lived); if they fall below the proliferation threshold, the system becomes tolerant and the tumor regrows progressively. Larger tumors are attacked more vigorously and may therefore be rejected in the regression phase. This phenomenon (low zone tolerance combined with rejection of larger tumors) is known as sneaking through (29). Sneaking through also occurs in the current model when modulation is absent, e.g., when DL = 0.4, IH = 0.6, for TUMOR = 1 and 3×10^4, respectively.

The incorporation of modulation, however, counteracts tolerization, i.e., this tumor breaks through accompanied by an ever-increasing immune response. Tumors slightly more antigenic (IH = 0.8), and capable of modulation, are rejected but never sneak through. This is explained, first because the transient period of tumor regression is absent from the growth curves of the tumors capable of modulation (Fig. 2*a* vs *b*). Both tumor populations are rejected in their regression phase in Figure 2*b*. Secondly, and probably the most important reason, the rejection of modulating tumors requires a strong immune response (i.e., high HTL numbers); it is therefore unlikely that the HTL numbers will subsequently drop below the proliferation threshold (situated at low HTL numbers). Thus, surprisingly, the incorporation of one escape mechanism (i.e., modulation) eliminates the possibility of escaping via the other (tolerance).

Parameter sensitivity. The fact that in this model, modulation plays only a negligible role, even for exaggerated parameter values, suggests that our conclusion can be extrapolated to a large class of wet immune systems (e.g., to immune systems other than DBA/2 mice and/or to other effector cells). We explore this possibility in Figure 5, in which we analyze the impact of modulation on tumor rejectability as a function of 1000-fold variations of the modulation parameters. The impact of modulation is measured by the increase in tumor antigenicity (IH) required for regaining rejectability, i.e., we plot the

antigenicity (the IH) of the weakest antigenic tumor that can be rejected as a function of the modulation parameter values. For the IB (B cell reactivity) parameter, for instance (Fig. 5*a*), the first rejectable tumors correspond to the intersects of the IB curves with the x-axis in Figure 3. It turns out (Fig. 5) that five (of six) parameters are insensitive: 1000-fold variations in the magnitude of modulation hardly influence tumor rejectability. The only parameter that markedly affects tumor rejectability is AD; AD values larger than a 100-fold protection against cytotoxicity and a 100-fold reduction in T lymphocyte activation generate a potent modulation phenomenon which solely allows rejection of extremely antigenic tumors. Thus, our conclusion holds true for AD values smaller than (or equal to) 100 only. AD values of cellular reactions are reported to vary around a 10-fold protection (in the model we choose for 50) (12–14, 17, 18), which easily fulfills this condition (AD ≤ 100). We therefore expect antigenic modulation to play a minor role in a diverse set of immune systems.

DISCUSSION

We have concluded that the role of antigenic modulation: 1) is small compared to that of tumor antigenicity (Fig. 1 vs Fig. 2); 2) is counterbalanced by an increase of the immune response (Fig. 2*a* vs Fig. 2*b*); and 3) remains small because the fully antigenic precursors provide immunity against the modulated subpopulation (Fig. 4, diamonds vs circles). To be able to extend these results to wet (biotic) immune systems, we exaggerated the effect of modulation in our model, i.e., we set all of the parameters so that they favor tumor escape. In addition, we assumed that modulated cells must be free of AB pressure in order to demodulate. Experimentally, it is very difficult to establish the demodulation rate in the presence of AB, because in these circumstances, demodulated cells will modulate again; the demodulation rate therefore will seem to be low. Moreover, we have assumed that after modulation, not only the tumor cell surface reduces in antigen density, but also that the debris of modulated cells, processed and presented by macrophages, is less antigenic. This is not necessarily the case, but at least our specification is extreme.

Stochastic models. Michelson (30) presents a stochastic model of the modulation phenomenon. In that model, the immune response is nonadaptable: it is incorporated as a fixed function which first increases (activation) and subsequently declines (due to, e.g., suppression). Tumors that survive this "immunity" period thus grow unhampered afterwards. Michelson (30) shows that antigenic modulation markedly increases the probability that tumor cells will survive this period. Tumors survive by "bouncing" between the modulated and the demodulated state (compartment). In the current model, however, modulation plays a negligible role because the immune response is adaptable (e.g., capable of immunization invoked by the fully antigenic precursors). Bouncing for prolonged periods affords no protection because the immune response, instead of declining, increases in the presence of (a small population of) tumor cells.

Monoclonal AB therapy. Our conclusions are valid for the cellular immune response, which is indeed generally supposed to be the predominant mode of tumor eradication. However, antigenic modulation may play an important role in humoral reactions, e.g., in the clinical situations in which monoclonal AB are administered to cancer patients. Monoclonal AB therapy is known to be severely hampered by antigenic modulation (21, 31). Monoclonal AB eliminate tumor cells by opsonization and/or by fixation of complement and subsequent lysis (32). Fixation of complement, however, requires high (local) antibody concentrations at the (tumor) cell surface (12). The antigen density on modulated tumor cells may thus be insufficient for complement fixation by (monoclonal) AB, and may hence provide resistance to cytotoxic AB, and in turn hamper monoclonal AB therapy. In the model, this would mean that for cytotoxic AB, the AD parameter is far larger than 50.

Interestingly, our parameter analysis (Fig. 5) proved the AD parameter to be the only sensitive parameter. We show (Fig. 5*b*) that if AD is gradually increased, the immune resistance of the model switches: above a certain threshold (AD > 100), only extremely antigenic tumors are rejectable. Cellular cytotoxicity AD values (AD ≤ 10)

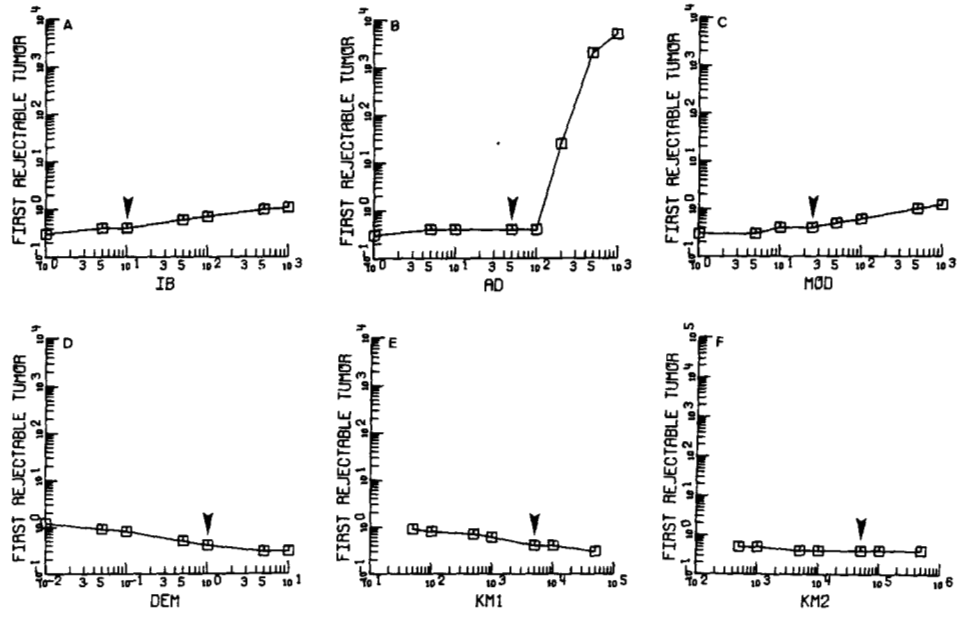

Figure 5. Parameter analysis. We study the role of modulation as a function of the modulation parameters. The role of modulation is measured by the increase in IH required for regaining tumor rejectability after incorporation of modulation. In the absence of modulation, the first rejectable tumor corresponds to IH = 0.3. Arrows indicate the parameter values used throughout this report (when one parameter is varied, others are kept at this value). We plot the antigenicity (IH) of the first (weakest antigenic) tumor that can be rejected as a function of 1000-fold variations in the modulation parameters: *a*, IB varies from one to 1000 cells per day; *b*, AD varies from a one- to 1000-fold reduction in cytotoxicity and T lymphocyte activation; *c*, MOD varies from one to 1000 modulations per cell per day; *d*, DEM, varies from one demodulation per cell per 100 days to 10 demodulations per cell per day; *e*, KM1, modulation saturation varies from 50 to 50,000 cells; and *f*, KM2, demodulation saturation varies from 500 to 500,000 cells.

lie well in the range of the easily rejectable tumors (12–14, 17, 18). AD values for humoral cytotoxicity (AB plus complement), however, are reported to be far larger (12–15) (protection against cytotoxic AB can become nearly complete (7)). Thus, although our results seem to be at variance with the general view that modulation is of utmost importance in monoclonal AB therapy (15, 21, 31), the model generates a similar contradiction on variation of one of its parameters. The nature and the range of variation of this parameter are fully consistent with the experimental data on the differences between cellular and humoral cytotoxicity. Note, however, that cytotoxic AB were excluded from this model.

Quantitative antigenicity. We have defined the degree of antigenicity of a tumor as the percentage of all helper precursors that can possibly recognize that tumor as alien. For the sake of the present discussion, we refer to this as the qualitative antigenicity of the tumor: the number of antigenic determinants (for HTLP). Through modulation, a tumor alters its quantitative antigenicity: the number of antigenic determinants remains identical, but their accessibility (e.g., their density) reduces. After modulation, an equal number of HTLP can react to the tumor, but this requires larger tumor (TUMMOD) numbers. Thus, T lymphocyte activation proceeds at a slower rate, and tumor cells are less likely to be recognized by cytotoxic macrophages. We do think that antigenic modulation, as it has been described experimentally (7), should correspond to a decrease in quantitative antigenicity. Our previous results (1), which stress the importance of helper T cell reactivity (IH), here translate into: qualitative antigenicity is far more important than quantitative antigenicity. Our present hypothesis that the role of modulation is small is thus in full agreement with these previous findings (modulation affecting quantitative antigenicity only).

The distinction between qualitative and quantitative antigenicity, however, becomes blurred if, for example, low affinity lymphocytes require a high antigen density per cell for their stimulation. If this is the case, these low affinity lymphocytes may fail to recognize tumor cells with a decreased antigen density (e.g., due to modulation). In the model, this would mean that the modulated tumor cells interact with a population of helper T cells comprising only part of the original subpopulation specific for the fully antigenic tumor cells, and not, as they do now, with the whole population at a low rate. This is kinetically almost identical, but conceptually resembles the antigenic heterogeneity phenomenon.

Antigenic heterogeneity. We consider "antigenic heterogeneity" to corresond to the (genetically stable) expression of qualitatively different antigens on different tumor subpopulations (33, 34). After mutation, tumor cells may, for example, cease to express one of their (several) tumor-associated antigenic determinants. This would mean that the T lymphocyte subpopulations activated by this antigen become unable to recognize the tumor as alien. The response of these T lymphocytes therefore wanes. There is convincing experimental evidence for qualitative alterations in the antigenic constitution of tumors during immune responses (35, 36).

We have investigated the antigenic heterogeneity phenomenon previously (unpublished results). In that model, we considered two tumor populations: one permanently expressing two antigens (to which two different T lymphocyte populations react); the other expressing only one of these two antigens (also permanently). The latter population is thought to arise from the former by mutation (i.e., to arise in limited numbers). The preliminary results resemble the conclusions drawn here for modulation: the immune response directed primarily against the population expressing two antigens also eliminate cells of the other population. This is especially true for the nonspecific part of the immune response (here the macrophages), which (by definition) cannot discriminate between the two tumor populations. In addition, however, the T lymphocytes that are induced by the persistent antigen expressed on the first tumor population are already activated and proliferating at the time the second population (bearing the same antigen) arises. Again, the new tumor subpopulation develops in an "immunized" system. We conclude that for genetically stable, weakly antigenic subpopulations of (antigenetically) heterogeneous tumors as well, the immune response to the most antigenic variant may, as a side effect, account for the rejection of the others.

Pecking order. The fact that such antigen-loss variants of primary tumors do escape in wet immune systems (35–37) may be explained by recent results which suggest that TAA have a "pecking order" (37). These data demonstrate that tumors expressing two distinct antigens induce an immune reaction to the "immunodominant" antigen only. Only the variants that have lost this antigen are able to induce an immune reaction to the persistent "immunorecessive" antigen (37). If this is generally the case, our hypothesis (i.e., specific immunization induced by the (qualitative) fully antigenic subpopulation) only holds true when the persistent antigen is the immunodominant one.

In conclusion, antigenic modulation and antigenic heterogeneity have in commmon the fact that new variants develop in systems previously immunized against them. This holds true for both the specific (here helper T cells) and the nonspecific (here macrophages) immune response. Therefore, these escape mechanisms must play only a minor role. A pecking order among the TAA may prevent specific immunization to antigen-loss variants of antigenically heterogeneous tumors, and is hence more likely to be responsible for tumor escape. However, even if the existence of a pecking order prevents specific immunization, nonspecific immunity will continue unhampered.

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