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*J Immunol* 2007; 179:5659-5668; doi: 10.4049/jimmunol.179.9.5659

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How Regulatory CD25⁺CD4⁺ T Cells Impinge on Tumor Immunobiology: The Differential Response of Tumors to Therapies

Kalet Leon,¹* Karina Garcia,* Jorge Carneiro,† and Agustin Lage*

Aiming to get a better insight on the impact of regulatory CD25⁺CD4⁺ T cells in tumor-immunobiology, a simple mathematical model was previously formulated and studied. This model predicts the existence of two alternative modes of uncontrolled tumor growth, which differ on their coupling with the immune system, providing a plausible explanation to the observation that the development of some tumors expand regulatory T cells whereas others do not. We report now the study of how these two tumor classes respond to different therapies, namely vaccination, immune suppression, surgery, and their different combinations. We show 1) how the timing and the dose applied in each particular treatment determine whether the tumor will be rejected, with or without concomitant autoimmunity, or whether it will continue progressing with slower or faster pace; 2) that both regulatory T cell-dependent and independent tumors are equally sensitive to vaccination, although the former are more sensitive to T cell depletion treatments and are unresponsive to partial surgery alone; 3) that surgery, suppression, and vaccination treatments, can synergistically improve their individual effects, when properly combined. Particularly, we predict rational combinations helping to overcome the limitation of these individual treatments on the late stage of tumor development. The Journal of Immunology, 2007, 179: 5659–5668.

Regulatory T (T_R)² cells, which are contained within the CD4⁺CD25⁺ pool and actively suppress the activity of other cells, are important factors for the immunobiology of some tumors (1–3). Depletion of these cells with mAbs (4, 5) leads to rejection of some transplantable syngenic tumors; T_R cells accumulate inside tumors or their adjacent lymph nodes, correlating with disease progression (6–12); and moreover, vaccination with tumor associated Ags can induce the expansion of CD4⁺CD25⁺ T_R cells accelerating tumor progression (13–16). Based on these observations T_R cells promise a better understanding and management of tumors though there are still many open questions. Particularly, it is quite alarming that the enrolment of T_R cells in tumor development could entail negative effects rather than clinical benefits to patients, in response to classical treatments, such as vaccines.

In a previous work (17), we studied the tumor-immune system dynamics in the presence of effector T (T_E) cells that can potentially reject or induce the rejection of the tumor, and T_R cells, by formulating a simple mathematical model for the interaction between a solid tumor and the lymphocytes on its adjacent lymph node. This model predicts that tumors could grow uncontrolled in two alternative modes differing on whether or not they involve T_R cells expansion, denoted GR+ or GR−, respectively. Tumors characterized by high specific growth rates, low immunogenicity and relative resistance to T_E cell-dependent destructive functions were predicted to induce the expansion of tumor-specific T_E cells that competitively exclude T_R cells. Tumors showing a slow specific growth rate, that are immunogenic, and/or that are highly sensitive to T cell effector function were predicted to sustain T_R cells that control T_E cell expansion. These results provide a natural interpretation for the observation that tumors are heterogeneous in respect to the selective expansion and role of T_R cells in transgenic mice models (18). This tumor heterogeneity regarding regulatory T cell function is also observed in humans if one considers the ensemble of reports. Although in some cases the development of the tumor seems to be associated with the concomitant expansion of Foxp3⁺ T_R cells (7, 12, 19) in other cases, the bad prognosis, is dissociated from regulatory cells activity (20–23). Although it is not unequivocal, at first sight the latter tumors could be interpreted as tumors progressing irrespective of T_R cells.

Furthermore, the latter theoretical results raised the intriguing possibility that the two classes of tumors may be susceptible to different immunotherapeutic strategies. In this work we directly address this possibility, exploring in our mathematical model the differential response of T_R cell-dependent (GR−) tumors and T_R cell-independent (GR+) tumors to different therapies. We simulated the responses of these tumors to vaccination, immune suppression, surgery, or different combinations of these therapies. Our results predict plausible strategies for the differential treatment of these tumors, providing clues for the rational design of more effective therapies.

Materials and Methods

Mathematical model

The mathematical model of tumor-immune system dynamics was developed in detail elsewhere (17). The following subsection briefly describes the main postulates and assumptions of this model, relegating for Appendix A the presentation of the model equations and parameter values.

Model structure in two spatial compartments

The model contemplates two spatial compartments (Fig. 1), representing, respectively, the solid tumor (T) and its adjacent lymph node (LN). Both
compartments have a variable volume that is proportional to the number of $T_E$ cells, $T_R$ cells, APCs, and, in the case of the tumor compartment, the tumor cells they contain at any given time. These two compartments are coupled via cell migration. APCs presenting tumor determinants are activated in the tumor and migrate to the LN. A fraction of the $T_E$ and $T_R$ cells activated at the lymph node migrate to the tumor compartment, whereas some tumor-infiltrating $T$ cells migrate back to the lymph node. Cellular migration between these compartments is assumed to be fast, reaching a quasi-steady-state equilibrium rapidly. (Note that this approximation does not preclude $T_E$ and $T_R$ cells to differentially accumulate inside the tumor node, it only implies fast migration and equilibrium. The model could be easily set to get at equilibrium, for instance, the $T_R$ cells being preferentially accumulated in the tumor site, just as experimentally reported for some tumors.)

Dynamics at the lymph node compartment

The LN compartment contains $T_E$ cells, $T_R$ cells, and APCs measured, respectively, as $E$, $R$, and $A$. Both $T$ cell subpopulations recognize the tumor-related self-Ags that are presented by the APCs. Conjugation and deconjugation of $T_E$ and $T_R$ cells with the APC antigenic sites are assumed to be fast processes that are in quasi-steady-state equilibrium. Interaction of both $T_E$ and $T_R$ cells to perform functions and to progress through the cell cycle is assumed to require interactions with cognate APCs, and further depends on interactions these $T$ cell types make with each other (Fig. 1). Particularly, $T_E$ and $T_R$ cells are assumed to interact indirectly by competition for access to cognate APCs and more directly by molecular processes that require their colocalization in the physical domains in the vicinity of these cognate APCs (inside an APC focus; see Ref. 24). We assume here for simplicity that each of these physical domains is composed of a cluster of spatially close APCs, collectively containing a constant number of independent and equivalent sites $s$ for $T$ cell conjugation and interactions.

The total number of APC clusters in the LN compartment is assumed to increase with tumors size, saturating asymptotically on a maximal value given by parameter $A_{\text{max}}$, i.e., the larger the tumor size the larger the number of APC clusters presenting tumor-related Ags, but when the tumor becomes too large the Ag presentation levels reach a plateau. Inside each APC cluster the following interactions are considered (Fig. 1): $T_E$ cells get activated and proliferate following productive interactions with the APC, but such proliferation is completely inhibited/suppressed if at least one $T_R$ cell gets activated inside the same cluster. In contrast $T_R$ cells get activated and proliferate depending on interactions with both APCs and $T_E$ cells colocalized in the same cluster. Upon activation, a fraction of both $T_E$ and $T_R$ cells migrate to the tumor compartment, and some of them can recycle back into the LN compartment.

Dynamics in the tumor compartment

The tumor compartment (Fig. 1) contains tumors cells, and infiltrating $T_E$ and $T_R$ cells, measured, respectively, as $T_U$, $E$, and $R$. The number of $T_E$ and $T_R$ cells in the tumor increases due to migration of activated lymphocytes from the LN and decreases due to cell death or migration back to the LN. The number of tumor cells $T_U$ is assumed to increase with charactristic proliferation rate, to decrease as cells die by nonimmune processes, and to be killed by immune effector functions, depending on the proportion of infiltrating $T_E$ and $T_R$ cells. $T_R$ cells are assumed to inhibit/suppress locally the $T_E$ cell-dependent killing of tumor cells.

Results

Two modes of uncontrolled tumor growth in the absence of therapy

As described before (17), simulations of the progression of an initially very small tumor in the absence of any treatment leads to three scenarios. The tumor can grow uncontrolled (Fig. 2), it can reach a stable equilibrium size; or it can be eliminated by the immune response it triggers (data not shown). The simulation results depend on the parameter values controlling tumor properties. Namely, the tumor-specific growth rate, the sensitivity of tumor
cells to the immune effector functions, and the tumor immunogenicity, defined as the intrinsic capacity of the tumor to induce the presentation of its Ags by the APCs in the adjacent LN.

For the purposes of studying cancer therapy we are interested in the simulations where tumors grow uncontrolled, which is predicted to happen in two modes depending on the engagement of regulatory T cells during the immune response (Fig. 2). In the GR− mode, T E cells predominate early on in the response to the tumor and eventually outcompete T R cells from the LN, although the tumor outgrowth this spontaneous immune reaction. In the GR+ mode, the tumor induces a balanced expansion of both T E and T R cells, and the latter continuously prevent tumor cell destruction by the former in the tumor compartment (Fig. 2b). The model predicts that tumors that grow fast, are poorly immunogenic, and are resistant to T cell destructive functions will grow in mode GR−; whereas tumors that grow slowly, are strongly immunogenic, and/or that are sensitive to destruction by T cells will grow in mode GR+.

Despite the latter differences, GR− and GR+ tumors share the same general phases of interaction with the immune T cells (see Fig. 1, a and b), and these phases, as we will see later, are relevant to interpret the response to therapies on simulations. In the initial phase (I) of tumor growth the dynamics of the T cells is not affected, because the tumor is too small. This is followed by a second phase (II) of strong interaction between the T cells and the growing tumor. In this phase GR− tumors break tolerance, expanding preferentially T E cells, while GR+ tumors expand also the regulatory population. Finally there is a third phase (III) where T cell dynamics reach a plateau while the tumor continues to grow. This plateau of the immune response follows the saturation of the tumor overgrowth this spontaneous immune reaction. In the GR+ mode, the tumor induces a balanced expansion of both T E and T R cells, and the latter continuously prevent tumor cell destruction by the former in the tumor compartment (Fig. 2b).

Response to therapy

Model responses to different therapies, simulated as particular dynamical perturbations, are studied here for tumors that grow, in the absence of treatment, either as GR+ or GR− tumors. Our aim is to establish whether therapies are differentially effective to treat these classes of tumors. We classify the results of the simulation of response to therapy by looking at the long term dynamics of different cell populations in the system (i.e., neglecting transient effects of perturbations). We found five generic classes of responses, despite the particular type of treatment studied. They are described below and extensively exemplified for the particular treatments studied on this work, in our home-maintained web site. (At site http://www.cim.sld.edu/articulos/Regulatory_T_Cells_Tumor_biology(kalet2007)/index.htm you may download several figures, which complement and extend the information provided in this original manuscript, as well as a Mathematica 4.2 notebook allowing you to reproduce our simulation of tumor growth kinetics in the presence or absence of treatments, for parameter values of your choice.)

1. Tumor rejection (symbol R) when tumor mass reduces to zero following treatment. In this class, we further check which T cells dominate the final state of the system. If dominated by T E (R < E) or by T R (R > E) cells we refer to them respectively as autimmune positive (symbol A+) and negatives (symbol A−) states.

2. Persistent tumor growth (symbol G) when tumor keeps growing despite treatment. In this class we further check whether this secondary growth expands preferentially T E cells (GR− like mode, symbol GR−) or expands along the T R cells (GR+ like mode, symbol GR+).

3. Accelerated tumor progression (gray shadow regions in figures), when tumor continues to grow but reaches the net size of 10⁶ cells faster than expected without treatment.

In the following sections we study how different treatments of interest elicit the possible responses as function of relevant control parameters in the simulations. We illustrate these dependencies with the tumors whose population dynamics, with no treatment, are shown in Fig. 2.

Response to vaccination

Cancer vaccines are being currently used as immunotherapeutic intervention in cancer. These vaccines consist in immunizing the patients with tumor Ags (or tumor cells) together with adjuvants. This has two distinct effects: on the one hand, it increases transiently the availability of tumor Ags on APCs, and in contrast it induces a massive maturation of professional APCs from precursor cells that will migrate to and accumulate transiently in the lymph node. Accordingly, we simulate cancer vaccines by an instantaneous and transient increase in the number of APCs presenting tumor related Ags in the lymph node compartment of the model (LN). The mathematical translation of this therapy is described in Appendix A, corresponding to the last term of Equation A4.

Our simulations of cancer vaccines are controlled by three parameters. The vaccine dose Dv sets the value for the net increase in the number of APCs induced by the vaccine. Parameter Tv sets the time of vaccine application because tumor growth initiation. Parameter dTv, determines the duration of the transient APC increase induced by the vaccine. Examples of typical population dynamics in the model after vaccinations are provided in our web site (See http://www.cim.sld.edu/articulos/Regulatory_T_Cells_Tumor_biology(kalet2007)/index.htm).

Vaccination can induce rejection of both GR− and GR+ tumors (region R in Fig. 3, a and b) when applied in a sufficiently high dose. This tumor rejection may (subregion A+ on Fig. 3, a and b) or may not be accompanied by the induction of a long lasting immune reaction to the self-related tumor Ags, i.e., it may or may not cause the induction of long lasting autoimmune response. However the minimal vaccine dose required to induce tumor rejection increases rapidly with tumor progression. In practice, vaccination will only be effective when applied early enough, inside the phase I and II of tumor development (see phase definitions in Results). If applied too late (phase III) or at a suboptimal dose it results in a sustained tumor growth, which for GR− tumors is always in a GR− fashion (region GR− on Fig. 3a), whereas overgrown GR+ tumors, after vaccination may switch to a GR+ like (region GR+ on Fig. 3b) or a GR− like (region GR− on Fig. 3b) mode of growth. Interestingly, for both types of tumors there is a subregion (shadow in gray) where suboptimal vaccination may lead to accelerated tumor growth.

Thus, the model predicts a strikingly similar response to vaccines for both regulatory T cell independent (GR−) and dependent (GR+) tumors. In both cases, vaccines could induce effective tumor elimination, when applied early enough in tumor development (phase I and II) and in a sufficiently high dose. Moreover, for both tumors, successful vaccination could result in persistent autoimmunity and suboptimal vaccination could lead to accelerated tumor progression rather than destruction.

Qualitatively, the capacity of vaccine treatments to eliminate GR− tumors is not surprising. In this case, the increase of Ag presentation derived from the treatment directly expands the T E cells that mediate tumor destruction. More difficult is to understand the success of vaccines on T R dependent tumors. There, vaccines could also expand T R cells, which turn down the immune response. The success of vaccines on these class of tumors derives from the
FIGURE 3. Parameter dependencies of tumor responses to treatments of vaccination (a and b) and T cell depletion (c and d). Different regions in these graphs, delimited parameter values (of those parameters indicated on the axes, which control treatment application) leading to qualitatively different dynamic outcomes after treatment in our numerical simulations. These regions are labeled according to the dynamic outcome classification provided in Results. Briefly, symbol R labels regions of parameter values leading to tumor rejection, while the subregions, inside this one, labeled as A+ demark parameter regions where there is induction of long lasting autoimmunity as a byproduct of tumor elimination. Symbols GR− and GR+ label region of parameters where treatment results on tumor sustained growth, that preferentially expand effector T cells (GR−) or regulatory T cells (GR+). Subregions, inside the latter’s, shadow in gray corresponds to the induction of an overall accelerated tumor progression by treatment application. a and b, the response of GR− (a) and GR+ (b) tumors to vaccination as a function of the dose (Dv) and the timing of application (Tv). Panels c and d, the response of GR− (c) and GR+ (d) tumors to T cell depletion treatment as a function of treatment dose (Dd) and timing of application (Td).

OTHER INTERESTING RESULT OF OUR SIMULATIONS IS THAT THE VACCINE Dose required to induce tumor rejection, rapidly increases with tumor development. This result can be understood, from the fact that tumor development gradually increases the number of APCs, T_E cells, and T_R cells at the LN, in the model. Thus, perturbations to the system based on increasing the number of APCs (like our vaccine) will be relevant if they are large enough relative to the preexistent number of APCs. Therefore with tumor progression the minimal vaccine required for efficient therapy increases and eventually reaches unfeasible values.

Response to T cell depletion/suppression treatments

In recent years, Ag-nonspecific T cell depletion or suppression with anti-CD4 or anti-CD25 Abs have been used successfully to treat tumors in animal models (5, 28, 29) or humans (30). These treatments are simulated here as an instantaneous and transient increase in T cell death rate. See last terms of Equations A1 and A2 of appendix A for the mathematical implementation. Note that we have assumed no preferential bias toward T_E or T_R cell depletion on this treatment.

In the simulations, three parameters control depletion treatment. The dose of immunosuppressor drug is controlled through parameter Dd that sets the increase in T cell death rate. The time of initiation of suppression is determined by the value of parameter Td which is relative to the initiation of the tumor. The parameter, DT0, which sets the duration of the transient effect of depletion. (parameter DT0 was explored for values ranging from 0.5 to 5, obtaining qualitative results similar to those reported here for DT0 = 4.) Examples of typical population dynamics in the model after T cell depletion are provided in our web site.

GR− tumors are rejected, in the simulations, following a strong T cell depletion during a small time window, that spans over phase I of tumor development (Fig. 3c). Such tumor rejection may or may not be concomitant with the induction of long lasting autoimmunity. Suboptimal treatments with either low dose of immunosuppressants or administered outside the sensitivity time window (on phase II and III) have no major implications in tumor growth, and may even accelerate tumor progression (Fig. 3c).

For GR+ tumors, there is also an initial time window, which spans over phase I and II of tumor development, where T cell suppression treatments can induce tumor rejection (Fig. 3d). This rejection may or may not lead to long lasting autoimmunity. Inside this sensitivity window, the response shows a dose dependency, similar to the one observed for GR− tumors, i.e., there is a
 minimal dose of treatment above which suppression is always effective. Suboptimal depletion dose most typically causes the tumor to continue to grow in a GR+ like mode, whereas treatment after the sensitivity time windows (phase III), always result in a sustained tumor progression, either in a GR+ like mode or in GR− like mode. Interestingly, as observed in Fig. 3d, for the parameter values used here (dT ∆ ≥ 5), this treatment appears unable to induce accelerated tumor progression of GR+ tumors. Although, if one deliberately extend the depletion period (dT ∆ > > 5), when applying a high dose depletion, such negative effect can be obtained mainly inside phase II of GR+ tumor development (data not shown).

Thus, in summary, the model predicts that transient depletion treatment can induce rejection of both T R cell dependent (GR+) and independent (GR−) tumors. This treatment will be effective when applied during an initial time window of tumor development and when applied with the appropriate dose ranges. However, GR+ tumors are better targets for this treatment than GR− tumors. On the one hand, GR+ tumors have a wider sensitivity time window than GR− tumors, and in contrast, the adverse consequence of accelerated tumor progression (region shadow in gray on Fig. 3, c and d) appears more easily in GR− tumors.

The predicted capacity of T cell depletion treatment to induce rejection of both GR+ and GR− tumors is counter intuitive, given that it eliminates equally the cells needed to destroy the tumor and the cells that can suppress the immune reaction. This result stems from the stringency of the regulation exerted by T R cells over T E cells, which in our model (as we explained before) is a function of the ratio of T R cells per APCs (value of R/A). Therefore, depletion treatment that, among others, reduces the number of R cells dynamically favors T E cell dominance, because it reduce the ratio R/A in the system, consequently relaxing tolerance.

Relevantly, like vaccines, depletion treatment is predicted to be ineffective on late phases of tumors development, although there are different explanations, for such effect on GR+ and GR− tumors. Treatment of GR− tumors becomes inefficient on phase II of tumor development; when the T E population is significantly expanded in the system. In this and later phases, after tolerance has been broken by tumor growth, depletion treatment has no qualitative impact, because there is no relevant T R activity left to be suppressed. However, for GR+ tumors, depletion treatment becomes inefficient only at phase III of tumor development, when the immune response has reached the plateau determined by the saturation of the tumor-dependent influx of APCs to the lymph node (control by parameter A M). In this condition, depletion could induce a T R cell expansion but with a limited size, determined by the maximal existent levels of Ag presentation. Thus, if the tumor is large enough, such limited response would be nevertheless ineffective to control its growth.

Response to incomplete surgery

Surgery is common practice in medical treatment of solid tumors. We simulate surgery as an instantaneous but incomplete reduction of tumor cell numbers, taking into account the practical possibility to remove all tumor cells either due to inefficient surgical procedures or due to the incapacity to identify all tumor cells among normal tissues. (Note that surgery could also eliminate T E and T R cells infiltrating the tumor, but we neglect this following the previous assumptions that migration of T cells between the lymph node and the tumor compartment is in rapid equilibrium and that the lymph node contains more T cells than the tumor compartment at any given time.) Incomplete surgery is controlled by two parameters in the simulations. The efficiency is controlled by parameter D S that sets the number of tumor cells that are left over by surgery. The timing of surgery is measured relative to tumor onset by parameter T S. Examples of typical population dynamics, in the model, after incomplete surgery, are provided in our web site.

T R cell-dependent (GR+) and T R cell-independent (GR−) tumors respond differently to incomplete surgery in the simulations. GR− tumors are sensitive to surgeries that are done on phase II and III of tumor development, provided that they reduce the tumor burden to a magnitude that is handled by the spontaneously expanded immune response (Fig. 4). Furthermore, successful surgery can induce a long lasting immune reaction (subregion A+) interpreted as autoimmunity, which will work also as an efficient anti-tumor immune memory (i.e., it prevents the growth of a new tumor of the same kind, data not shown). GR− tumors are insensitive to incomplete surgery during the phase I of their development when immune tolerance is still not broken (Fig. 4). Incomplete surgery at this phase can be seen as a resetting the tumor to its initial state, which can delay but will not prevent tumor progression.

In contrast with T R-independent tumors, GR+ tumors are insensitive to incomplete surgery at any phase of their development, irrespective of how many tumors cells are left. The rational to the resistance to surgery is that these tumors lead to a progressive increase of the T R cells and therefore decreasing tumor size will delay progression (see Fig. S4 for kinetics) but cannot imbalance the response toward T E cells due to the concomitant and fast decrease in Ag-presentation derived from surgical reduction of tumor size.

In summary, incomplete surgery alone can deal effectively with GR− tumors that have progressed over their first phase, but will not work with GR− tumors in their first phase of development or GR+ tumors at any phase of their development. Relevantly, this treatment shows no negative effects for the patients other than autoimmunity, because there are no conditions leading to accelerated tumor progression upon treatment.

Combination therapies

The monotherapies studied in previous sections could induce tumor elimination when targeting either GR+ or GR− tumors within a sensible phase of their development, and with a dose

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**FIGURE 4.** Parameter dependencies of the response of GR− tumors to surgery. Different regions, in the graphs, delimited parameter values leading to qualitatively different dynamic outcomes of numerical simulations. They are labeled as explained on Fig. 3. Parameter D S, stands for the amount of tumor cells remaining in the system after treatment (i.e., it sets the maximal efficiency of the surgery); and the parameter T S refers to the time relative to tumor progression when surgery is applied.
inside the appropriate effective range. If any of these prerequisites is not met then the treatment fails or, even worse, may lead to some negative effects for the patient. Thus vaccination and T cell depletion appear to fail on late phases of tumor development, while incomplete surgery of GR+ tumors is ineffective. An appealing possibility is that the combination of these therapies could broaden their individual effectiveness. Therefore, we have studied every possible pairwise combination therapy. We describe below the best combinations to target GR+ or GR− tumors.

The best therapy to target GR− tumors is the combination of vaccination plus surgery. Surgery was found to significantly increase efficacy of vaccination, by reducing the minimal dose of vaccine required to induce GR− tumor rejection (Fig. 5a). This combination clearly overcomes the difficulties of vaccines on late phases of tumor development, by reducing the tumor to a size that now becomes quite sensitive to vaccination. Moreover, the combination is effective with “suboptimal” surgeries, i.e., those surgeries that alone cannot induce tumor rejection because they do not remove enough cells ($D_S > 10^5$). Remarkably, the combination is more effective when surgery is applied simultaneously or shortly after vaccination (optimal schedule used to produce Fig. 5a). Such optimal combination scheme can be qualitatively understood as follows. GR− tumors spontaneously expand $T_P$ cells with a concomitant loss of $T_R$ cells, thus one must vaccinate as soon as possible either simultaneously or slightly before surgery. In this way, vaccination will help to expand the preexistent immune response, and thus better eliminate the tumor whose size is concomitantly reduced by surgery.

The best therapy we found to treat GR+ tumors is the combination of T cell depletion and surgery. Surgery increases the efficacy of T cell depletion by broadening the dose ranges of depletion that can induce GR+ tumor rejection (Fig. 5b). The effect observed is particularly relevant on the phase III of tumor development, which was unresponsive to depletion or surgery alone, but can be successfully treated by their combination. The best combination scheme is when depletion is applied some time before surgery (schedule used to produce Fig. 4b). Such optimal strategy for the combination can be qualitatively understood from the fact that depletion treatment alone can switch the tumor from the GR+ growth to the GR− growth mode when applied on phase III of tumor development (see Fig. 2d), which then similarly to GR− tumors (see Results), is very sensitive to incomplete surgery. Thus, this combination strategy relies in weakening regulatory activity with the T cell depletion treatment to transform a GR+ tumor into a GR− like tumor, to then apply surgery to eliminate it. A nice correlation for the time after suppression to optimally apply surgery comes from following the repopulation of T cells in the system. Once the system has been repopulated with T cells, the GR− like mode will have been established and the tumor will have become susceptible to surgery.

In summary, our results demonstrate that combinations significantly increase the effects of the individual vaccination, surgery and T cell depletion therapies. Particularly, the two combination treatments, described above, are effective at every phase of respectively GR+ or GR− tumor development. Such property is not observed on any of the individual therapies when applied alone. Moreover, these combination therapies are effective on wider and better dose ranges than the respective individual therapies when effective alone.

Discussion

The enrollment of regulatory T cells during tumor development is simultaneously alarming and promising of better understanding and new management strategies of cancer. Theoretical studies (17) suggested that slowly growing immunogenic tumors that are sensitive to the immune response would engage regulatory T cell and induce tolerance to themselves, while poorly immunogenic, fast growing tumors would progress in a regulatory T cell independent manner. These theoretical results provide a reasonable interpretation of the empirical fact that some tumors expand regulatory T cells (GR+) while other do not (GR−). Furthermore, it raises the possibility that tumors might be heterogeneous in their response to therapy, and that specific interventions might be designed toward each specific tumor class.

The theoretical results reported here indicate that GR− and GR+ tumor shows both similarities and differences on their qualitative response to monotherapies such as cancer vaccines, T cell depletion and surgical intervention. According to our model, these monotherapies can successfully destroy tumors if applied on the appropriate class of tumors, GR+ or GR−, with the appropriate dose ranges and within the appropriate phase of tumor-immune system interaction. This result foresees a great complexity for applying these therapies on current clinical setting, were tumors are

![FIGURE 5. Parameter dependencies of tumor response to combined treatments of surgery plus vaccination (a) or plus T cell depletion (b). Different parameter regions in the graphs correspond to different dynamic outcomes after treatment, being labeled as on Fig. 3. a. The response of GR− tumors to combined surgery and vaccination treatments, as a function of the dose ($D_V$) and timing of vaccine application ($T_V$), for the indicated efficiency ($D_S$) of the surgery, applied always at the same fixed time interval to the vaccine ($T_S = T_V + 1$). b, GR+ tumor responses to combined treatment of surgery and T cell depletion. This response is showed as a function of the dose ($D_d$) and timing of suppression application ($T_d$), for the indicated efficiency ($D_S$) of the surgery applied previous to the vaccine ($T_S = T_d + 3$).]
not classified neither in terms of their $T_R$ dependence or independence, nor in terms of the phase of their natural interaction with the immune system. (Note that currently we mainly classify tumors in the clinic by having or not some particular target of interest and according to their stage of progression, that essentially considered the geographical spreading of the tumor and not necessarily the stage of its interaction with the immune system.) Thus on these conditions the application of these therapies with a fixed time schedule and dose will at most benefit some particular sets of susceptible patients, while will be ineffective or will even worsen the clinical situation of the others. Our theoretical study suggests then two possible ways to improve the efficacy of these cancer therapies. The first one is to classify better the clinical tumors according to the type and the stage of their interaction with the immune system. Our description of the therapeutically relevant phases I, II and III of this interaction, in the model, could provide some clues on the relevant aspect required for this classification. The other possible way to proceed is to look for new therapies that are able to work on a more heterogeneous setting, for instance that work for either $T_R$-dependent and independent tumors or that could work at every phase of tumor-immune system interaction. Interestingly, in this sense, our results suggest that the appropriate combinations of the very same therapies would be effective on more heterogeneous settings. Particularly, we propose two combinations that are able to work at any phase of tumor development, respectively for $GR+$ and $GR-$ tumors.

Response of $GR-$ and $GR+$ tumors to vaccination

In contrast with our initial expectation, responses of $GR-$ and $GR+$ tumors to vaccination treatment are strikingly similar, despite their differential capacity to expand regulatory T cells. Both types of tumors show a complex and diverse response to vaccination, which can result in tumor rejection with or without the induction of concomitant autoimmunity, or might even result in an accelerated tumor progression. All these possibilities have been observed in experimental tumor models (see (14–16) for examples of accelerated progression and (31–33) for the complex relation with autoimmunity). Notwithstanding, our analysis further predicts how these alternative outcomes might originate as a function of the timing of vaccination with respect to tumor development and on the dose of the vaccine (which directly correlates here with the increase in Ag presentation). Particularly, our model proposes that: 1) Vaccination induces tumor rejection at high dose, although the minimal effective dose increases with tumor growth until it reaches unfeasible values; 2) Induction of an accelerated tumor progression results from suboptimal vaccination in early tumor states; thus, increasing the efficiency of vaccination in these situations might turn this negative effect to the desired tumor rejection; and 3) Autoimmunity induction may correlate very well with the development of effective anti-tumoral responses, although there are some ranges of intermediate vaccine doses were autoimmunity could be avoided.

Response of $GR-$ and $GR+$ tumors to T cell depletion treatments

The responses of $GR-$ and $GR+$ tumors to T cell depletion therapies also show some similarities according to our model. Both types of tumors show a complex and diverse response to this treatment, which results either in tumor rejection with or without concomitant autoimmunity, or in partially delayed or even accelerated tumor progression. Remarkable is the capacity of this treatment to induce tumor rejection, particularly given the fact that it has no bias toward a preferential regulatory T cell depletion, i.e., the treatment depleted both the cells that mediate the regulation and the cells that mediate the immune reaction. The latter result is of practical importance, because so far there is no cell marker which could be safely used for the ideal treatment of specific $T_R$ cell depletion. Thus, our theoretical results support the use of nonspecific treatments that deplete $T_R$ and $T_R$ cells simultaneously, which despite being less efficient and harder to predict, can achieve similar goals than treatments depleting specifically $T_R$ cells. These treatments may be achieved with several existent therapeutic agents. For instance, mAbs against CD4, CD3, and CD25 markers, or even some chemotherapeutic treatments that deplete dividing lymphocytes. Indeed, such predicted types of effect have been observed experimentally (28, 34–37), but they are often interpreted as an indication that the delivered treatment preferentially eliminated the $T_R$ cells and not as the result of a perturbation to the dynamical balance of $T_R$ and $T_R$ cell populations as favored by our model. It is nevertheless important to state that having a treatment that preferentially eliminate $T_R$ cells is always desirable, and in our simulations (data not shown) the stronger the bias toward depletion of $T_R$ cells the wider becomes the dose range leading to effective treatment.

Another important theoretical result of our simulations is that T cell depletion induces rejection in both $GR-$ and $GR+$ tumors only if performed within an initial time window during tumor development. If the treatment is applied either too early before tumor initiation or too late when the tumor is too large then it fail to induce tumor rejection. This complex dependence arrives from coupling the dynamics of normal tumor immune system interaction with the imbalance in the strength of the interaction among T cells induced by depletion. Because this imbalance is transient it cannot be done too early otherwise it will fade away before the tumor start having a non negligible impact on T cell dynamics, and amplifies it. Overall this result provides a simple and plausible interpretation for the findings (4, 5) that rejection of some transplantable tumors can be induced by treatment with anti-CD25 depleting Abs but only if applied shortly before or early enough after tumor implantation. Surprisingly, in our model, such dependence is not particular to $GR+$ tumors, where $T_R$ cells have a dominant role in the overall system dynamics, but is also observed in $GR-$ tumors, in which $T_R$ cells only participate in the very initial phase of response. Thus, according to our model, observing such phenomenology in an experimental tumor, although important, does not indicate per se that regulatory T cells play a relevant role on its overall tumor dynamics, i.e., it does not help to identify regulatory T cell-dependent tumors.

Despite similarities, there are also significant differences in the responses to T cell depletion treatments observed for $GR-$ and $GR+$ tumors. Particularly relevant is that, in our simulations, $GR+$ tumors appear better targets for this treatment than $GR-$ tumors. On the one hand, $GR+$ tumors have a wider sensitivity time window than the one observed in $GR-$ tumors; and in contrast, the adverse event of accelerated tumor progression is more easily obtained in $GR-$ tumors. This result can be qualitatively understood from the dominant role that regulatory T cells play in the dynamics of this mode of tumor growth. The suppression treatment aims to eliminate immune regulation by regulatory T cells. Therefore, if this phenomenon is more relevant in the dynamics of $GR+$ tumors, the treatment must be more effective for them.

Response of $GR-$ and $GR+$ tumors to surgery

$GR-$ and $GR+$ tumors strongly differ in their response to incomplete surgery. Although the former can be eliminated depending on the efficiency and timing of the surgical procedure (i.e., how much
it reduces tumor burden), the latter are expected to be always unresponsive. These results provide a novel interpretation to the failure of therapeutic surgery in tumors. Reappearance of the tumor following surgical intervention is commonly interpreted as a failure to remove all tumor cells. In line with this, medical doctors often choose not to operate when they foresee that removal of all or most malignant tissue is unlikely. Instead, our results suggest that different responses of tumors to surgery could rely solely on the differences in the pre-established tumor specific immune activity. Tumors that spontaneously induce a $T_R$-independent immune reaction will be sensitive to incomplete surgery; but tumors that are tolerated by the immune system will require a complete tumor extirpation to prevent a recurrent growth. Moreover, our results suggest that it may be useful to perform surgery even when it is known a priori that a significant number of malignant cells will be left over, as these may be susceptible to the immune response.

**Response of $GR^-$ and $GR^+$ tumors to combination therapies**

We explored potentially useful combination therapies searching to identify the dose and time schedules that maximize their beneficial effects. The theoretical results presented here suggest that combinations increase the effectiveness of therapy beyond that of vaccination, surgery, and $T$ cell depletion monotherapies. Particularly, we propose two combination strategies specifically effective when targeted to $GR^+$ and $GR^-$ tumor. These specific combinations are effective at every phase of each of two tumor classes, and thus may allow their treatment on the heterogeneous clinical settings. Moreover, they are effective on wider and better dose ranges than those of the monotherapies on which they are based, further sustaining their practical value.

We propose that $T_R^-$ independent tumors can be best managed by vaccination plus surgery. Moreover, the optimal combination requires vaccinating concomitantly or slightly before surgery. This strategy, intends to further expands, with the vaccine, the preexistent spontaneous immune reaction on this class of tumor, facilitating the immune mediated removal of the tumor mass remaining after surgery. Interestingly, this type of combination has been previously explored experimentally (38) being referred to as vaccination in adjuvant settings and was proven successful in some tumor models. Our results thus support the effectiveness of this combination, for $GR^-$ tumors. But more relevantly, they provide a rational for the scheme of these therapies combination that differs from the most classical scheme used on the practical settings, were vaccine is commonly applied after surgery and not before or simultaneously to it. Therefore our finding, suggest a rationale to optimize some of the adjuvant setting strategies used on animal models and the clinic.

We propose that therapy of choice for $T_R^-$ dependent tumors is the combination of surgery and $T$ cell depletion. As far as we know, this type of combination has never been proposed before or explored in experimental tumor models. Although one might try to reinterpret in this framework the effects of some chemotherapies when applied to adjuvant settings (39, 40), particularly if one stresses the fact that some chemotherapies could induce lymphocyte depletion. In any case, our simulations predict that such combination might be useful, although only in tumors that expand regulatory $T$ cell activity ($GR^+$ tumors). Thus, our result proposes a new type of combined therapy, whose optimal combination occurs when surgery is applied after $T$ cell depletion, when $T$ cells have just recovered from the depletion treatment. We believe this prediction is amenable for experimental testing and may be of practical value.

Finally, we would like to stress that our model is a radical simplification of tumor immunobiology, focusing on some of its aspects and neglecting many others. Some of these major simplifications and their consequences are worth emphasizing here. In a major simplification of reality, our model assumed that parameters controlling tumor dynamics (like growth rate, immunogenicity, sensitivity to immune effectors functions and $T_R$ or $T_K$ migration/accumulation inside the tumor site) remain constant all along tumor development. Based on this simplification we could highlight the interplay between $T_R$ and $T_K$ cells crossregulation and tumor dynamics. We simulated other model variants, for instance tumors growing with variable growth rate according to a Gompertz law. We found that in most cases, tumors could still be classified as purely $GR^+$ or $GR^-$, although we found also some switches in dynamic tumor classification, for instance obtaining tumors that initially evolve as $GR^+$ tumor and then switch "spontaneously" to $GR^-$ growth. In any case, hybrid tumors that behave transiently like $GR^+$ or $GR^-$ react to the simulated treatments in similar qualitative terms as the pure $GR^+$ and $GR^-$ tumors studied here. Therefore, although we acknowledge the simplicity of this first approach we believe it is a stepping stone to understand more realistic and eventually more complex cases.

Other major simplification of our model is that it excluded the multitude of components of the immune system that likely play a role in tumor development besides $T$ cells and APCs. Examples of putative relevant mechanism ignored in our model are the production of soluble factor by tumor cell that could impair $T$ cell activity on its microenvironments, the recruitment by the tumor of other regulatory cell populations, like myeloid dendritic cells or some NK cells. It is then very important to note that the model developed here focused on the minimal set of interactions influencing $T_R$ activity and maintenance, particularly those interactions related to their main function on controlling the pathologic autoimmune response to peripheral self-Ags. In principle, no tumor mostly may escape the influence of this elementary regulatory mechanism, at least in its initial and decisive phases of growth, because all tumors are mainly composed of self-Ag. This minimal model has shown an interesting, and quite complex dynamic behavior, particularly regarding tumor response to different treatments. It explains some known experimental facts and makes several counterintuitive, yet testable predictions. Adding further layers of complexity, by including now neglected regulatory mechanism, should improve model performance, better explaining particular tumors dynamics. Nevertheless, having understood the behavior of the minimal model developed here will be useful and instrumental to better analyze and interpret simulation result of more complex models. Our expectation is that adding new regulatory mechanism, will lead to further subdivision in different subclasses of tumors inside the broader $GR^+$ and $GR^-$ tumor classes defined here. These subclasses will differ on the response to some therapies, but most likely they will inherit some properties of the broader $GR^+$ or $GR^-$ tumor class they belong to.

Despite the latter simplifications it is fair to stress that our model is a step forward in respect to previous mathematical models (41–48) by including the regulatory $T$ cells, whose existence and relevance is well accepted by the immunological community. Our simulations then reveal the impact that regulatory $T$ cell population has for tumor immunobiology and therapy. Furthermore, we believe they contain sufficient realism to warrant some qualitative testable predictions, which may be used to improve tumor immunobiology modeling and may serve as a stepping stone for the rational-design of novel combination therapies.
Appendix A: Model equations and Parameters values.

In our numerical simulations, a nondimensional form of our previous model (17) was used to reduce significantly the number of parameters and to prevent the occurrence of numerical errors in the computation. This nondimensional model is obtained by introducing the following change of variables:

\[ t = x / d; E = e \cdot A_0; R = r \cdot A_0; T_u = t_u \cdot \delta \cdot A_0; A = a \cdot A_0 \]

This change of variables normalizes the time in the model to the death rate for lymphocytes (\( d \)) and normalizes the cell numbers for tumor cells (\( T_u \)), effector (\( E \)) and regulatory T cells (\( R \)) to the basal number of APCs (\( A_0 \)) in the absence of the tumor. It results in the following system of equations plus algebraic relationships

\[
\frac{de}{dx} = \phi_e + \pi \left( 1 - \frac{r_e}{sa} \right)^{r_e - 1} \cdot e_e - (e - e_e) - D_e \cdot H(x - T_e) \cdot H(T_u + dT_u - x) \cdot e
\]

\[
\frac{dr}{dx} = \phi_r + \frac{r_e - 1}{s} \cdot \mu \cdot e_e \cdot a \cdot (r - r_e)
\]

\[
\frac{d}{dx} (t_u) = g \cdot t_u - k_i \cdot e_f \cdot \left( \frac{e_f}{t_u + e_f + r_f} \right) \cdot \left( 1 - \frac{r_f}{t_u + e_f + r_f} \right) \gamma \cdot t_u
\]

\[
a = 1 + \frac{\lambda \cdot a_M \cdot t_u}{a_M + \lambda \cdot t_u} + D_{T_u} \cdot H(x - T_v) \cdot H(T_u + dT_u - x)
\]

\[
e_f = \pi \cdot \left( 1 - \frac{r_f}{sa} \right)^{r_e - 1} \cdot e_e \cdot r_f = \mu \cdot e_e \cdot a
\]

\[
t_e = \frac{k \cdot (s \cdot a + e + r)}{2 \cdot k} + \left( \frac{2 \cdot k}{2 \cdot k} \right) - \sqrt{(k \cdot (s \cdot a + e + r) + (a \cdot a + e + r))^2 - 4 \cdot k^2 \cdot s \cdot a \cdot (e + r)}
\]

Where \( H(x) \) is the heaviside function, defined as 1 for positive arguments and 0 for negative ones, and the nondimensional variables and parameters represent:

- \( e, r, a \) Rescaled number of \( T_u \) cells, \( T_R \) cell and APCs clusters at the lymph node.
- \( e_e, r_e \) Rescaled number of E and R conjugated to APCs at the lymph node.
- \( e_f, r_f \) Rescaled number of E and R infiltrating the tumor site.
- \( \phi_e, \phi_r \) Source term of new E and R cells incoming from the thymus.
- \( \pi, \mu \) Proliferation rates of E and R cells.
- \( \lambda \) Tumor immunogenicity, defined by its capacity to promote its Ag presentation at LN.
- \( a_M \) Rescaled maximal number of APC clusters attainable at the lymph node.
- \( k \) Conjugation constants of E and R cells with the APC site.
- \( s \) Number of conjugation sites for T cells on an APC cluster.
- \( \alpha \) Ratio between the per-cell, volume occupy by APCs and lymphocytes.

\( g \) Rescaled net growth rate of tumor in the absence of T cells.

\( k_i \) Rescaled rate at which immune effector T cells can destroy a tumor cell.

\( \gamma \) Coefficient controlling the efficiency of regulatory T cell inhibition of effectors T cell-dependent tumor cell destruction.

\( D_{T_u}, D_{T_v} \) Doses for vaccination and T cell depletion treatments, respectively.

\( T_v, T_u \) Timing for treatment application, either for vaccination or T cell depletion.

\( dT_u, dT_v \) Duration of direct treatment effects, either for vaccination or T cell depletion.

In the figures along this paper, nondimensional parameters were set to the following values, based on the analysis in our previous work (17) \( \pi = 21, \mu = 2.9, k = 7.0, \phi_e = 0.01, \phi_r = 0.01, s = 6, a_M = 10^5, \gamma = 8, \alpha = 1, k_i = 400, \delta = 1, A_0 = 10^5 \). These parameter values are compatible with a life span and doubling time for effector lymphocytes respectively of 7 days and 8 h. Therefore they result in a nondimensional time unit corresponding to a week of real time and a nondimensional cell-number unit corresponding to \( 10^5 \) real cells.

To simulate a typical GR− or GR+ tumor, we used different values for the tumor immunogenicity parameter (\( \lambda \)) and the tumor specific growth rate parameter (g). Particularly, in this paper’s figures, we use \( \lambda = 0.0001, g = 4.7 \) for GR− tumors, and \( \lambda = 0.01, g = 2.1 \) for GR+ tumors. However, we have explored many other parameter conditions selected inside the GR− and GR+ regions provided on Fig. 2 of reference (17) obtaining results for the effect of treatments, qualitatively similar to those explicitly reported here.

Acknowledgments

The work was supported by the Foundation for Science and Technology (Portugal): fellowships to KL (respectively BPD/11575/2002) and grants to JC (POCTI/MGI/46477/2002 with FEDER funds). We thank Dr. Rui Gardner for kindly revising this manuscript.

Disclosures

The authors have no financial conflict of interest.

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