REPORT

Mathematical modeling of the implications of dominant tolerance for tumor biology and the response to combination therapy

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ABSTRACT

The existence of regulatory T lymphocytes (Tregs) that can control effector lymphocytes within the context of autoimmune, infectious and tumoral diseases is definitely accepted in current immunological research. Tregs confirm the theory of dominant tolerance, which holds that the choice of antigen rejection or tolerance in the immune system is the result of a dynamic equilibrium between populations of effector and regulatory T lymphocytes. The present paper summarizes the result of a recent theoretical study using mathematical modeling to analyze the dynamic interplay between T lymphocyte populations in the absence or presence of tumors and in response to different therapeutic treatments. The resulting model, developed at the Center of Molecular Immunology, which received an award from the Cuban Academy of Sciences in 2002, includes tumor cells and can simulate the effect of antitumoral mono- or combination therapies, by taking into account the way in which certain dynamic properties of tumors can, under specific circumstances, lead to the spontaneous expansion of Tregs populations. One of the advantages of the model is the prediction of several new strategies for the differential treatment of tumors, depending upon their ability of inducing the expansion of regulatory T cells. This is the first model available for the study of the impact of Tregs on the growth of malignant tumors, with results supported by international publications. Additionally, the model predicts the practical effects of several combination therapies, including vaccines, the excision of the tumor and depletion of lymphocyte populations.

Keywords: Regulatory T cells, dynamics of tumor growth, tumor-immune system interaction, combination therapies, mathematical modeling

*I*ntroduction

The existence of regulatory T lymphocytes (Tregs) that can control effector lymphocytes within the context of autoimmune, infectious and tumoral diseases has become an accepted fact in current immunological research. Tregs confirm the theory of dominant tolerance, which holds that the choice of antigen rejection or tolerance in the immune system is the result of a dynamic equilibrium between populations of effector and regulatory T lymphocytes. However, and in spite of the existence of large experimental data, no biomathematical model is available for the prediction of the dynamic interaction between these cell populations in the context of an anti-tumor response.

The present biomathematical study deals with the role of Tregs in immunological tolerance, specifically in tumor biology, as well as with tumor response to different therapies. Several theoretical models are analyzed and contrasted with experimental data. These extend and complement the theoretical results on dominant tolerance in the regulation of the immune response (in the absence of tumors and their treatment) that have been developed at the Center of Molecular Immunology (CIM) since 1999 and have received the Cuban Academy of Sciences award in 2002 [1].

*R*esults and discussion

Theoretical study of the regulation of the immune response by dominant tolerance

Our study extends and complements other theoretical results that have been obtained at CIM since 1999: the development of a simplified formulation of the original cross-regulation model, with a much simpler mathematical implementation that still retains its basic properties; and the extension of its scope, evidencing its validity by the ability of the model to adjust to recent experimental data on the underlying mechanisms of the interaction between effector and regulatory T lymphocyte populations [2].

The reformulation of the model offers a number of advantages for theoretical research. The first model, although accurate, is complex and unwieldy both for numeric simulations and during analytical or semi-analytical characterizations of inter-parameter dependencies. Being a simplified version, the new model is more accessible to its target audience, whose education and training are often focused on biological rather than mathematical sciences. Also, while the interplay between effector and regulatory T lymphocytes in the original model was assumed to take place through their simultaneous interaction with the same antigen-presenting cell (APC), the actual molecular mechanism responsible for this was unknown and is still controversial. The main hypotheses on the mechanistic details of the effector-regulatory balance show a range that include its dependence on soluble factors synthesized locally at the cellular microenvironment, its requirement of direct cell-cell contact between the regulatory and effector lymphocytes, or taking place during a multi-stage mechanism by conditioning the APCs in their interaction with T lymphocytes. In order to discriminate among these hypotheses, computational simulations were performed based on agents for the different possibilities, which showed that the new reformulation can simulate all these variants regardless of the molecular details of the regulatory-effector cell interaction as long as it takes place in the

1. León K, Pérez R, Lage A. Modelo matemático de la regulación del sistema inmune mediante la tolerancia dominante. BA 2003;20:253-7.

2. Carneiro J, León K, Caramalho I, Van den Dool C, Gardner R, Oliveira V *et al*. When three is not a crowd: A cross regulation model of the dynamics and repertoire selection of regulatory CD4 T cells. Immunol Rev 2007;216:48-68.

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immediate vicinity of the APC, where T-lymphocyte activation occurs.

This new reformulation makes it possible to study the involvement of dominant tolerance in the development and shaping of the peripheral T-cell repertoire as a result of the interaction of lymphocytes with a diverse group of self antigens. One of the predictions of the cross-regulation model is that the peripheral T-lymphocyte repertoire is dynamically (spontaneously) structured into 3 sub-populations differentiated by their level of recognition: the first one would recognize self-antigens poorly presented by the APC, and would maintain the balance between regulatory T cells and self-perpetuating effector cells generated at the thymus by the continuous generation of new clones; the second, enriched in effector T lymphocytes, would recognize low levels of antigens at the APC and would be independent from the generation of new clones at the thymus for the maintenance of the regulatory-effector equilibrium (in spite of its diversity, this subpopulation would be composed of clones targeting antigens that are scarce and would therefore not produce an autoimmune response); and a third subpopulation, with clones targeting antigens that are presented at high levels by the APC, which would be enriched in Tregs for the continuous control of the expansion of potentially pathogenic effector T-lymphocytes recognizing highly represented antigens. This predicted structure is quantitatively compatible with the experimentally measured proportions of regulatory and effector T-cells and could facilitate the development of an immune response against new non-self antigens since, on probabilistic grounds alone, the first two subpopulations could recognize foreign antigens and additionally, the low levels of regulatory T cells in these two subpopulations would facilitate the expansion of effector T lymphocytes which would quickly outnumber regulatory T lymphocytes with the same specificity.

Implications of dominant tolerance for the development of malignant tumors

A new mathematical model was developed, which incorporated the mechanisms of cross-regulation previously studied and modeled, aimed at the simulation of the dynamic interaction between tumor cells and effector and regulatory T lymphocytes [3]. To the best of our knowledge, this is the first mathematical model for this interaction described in specialized literature that is geared for the design of anti-tumor therapies. This model allowed: 1) the prediction of two modes of tumor growth (in the absence of therapy) differentiated by the dynamics of their interaction with the immune system (Figures 1a and b); 2) the prediction and identification of those dynamic properties of tumors that determine whether their growth is Tregs-dependent (denominated as GR+ mode) or independent (GR- mode) (Figure 1c); and 3) the design of an experiment for distinguishing the GR+ from the GR- mode in models of transplantable tumors in laboratory animals.

In the first case, a tumor growing in a GR+ mode would result in a balanced expansion of regulatory and effector T-lymphocytes, which depends on suppressor activity. A tumor growing in the GR- mode would, on the other hand, break the balance between these two lymphocyte populations and lead to the expansion of effector cells, which would however be unable to restrain tumor growth. The existence of these two modes fits the evidence obtained from tumors in animal models and clinical cases, where growth and prognosis of tumors depend on Tregs (T CD4+CD25+) activity and expansion or on the lack of these.

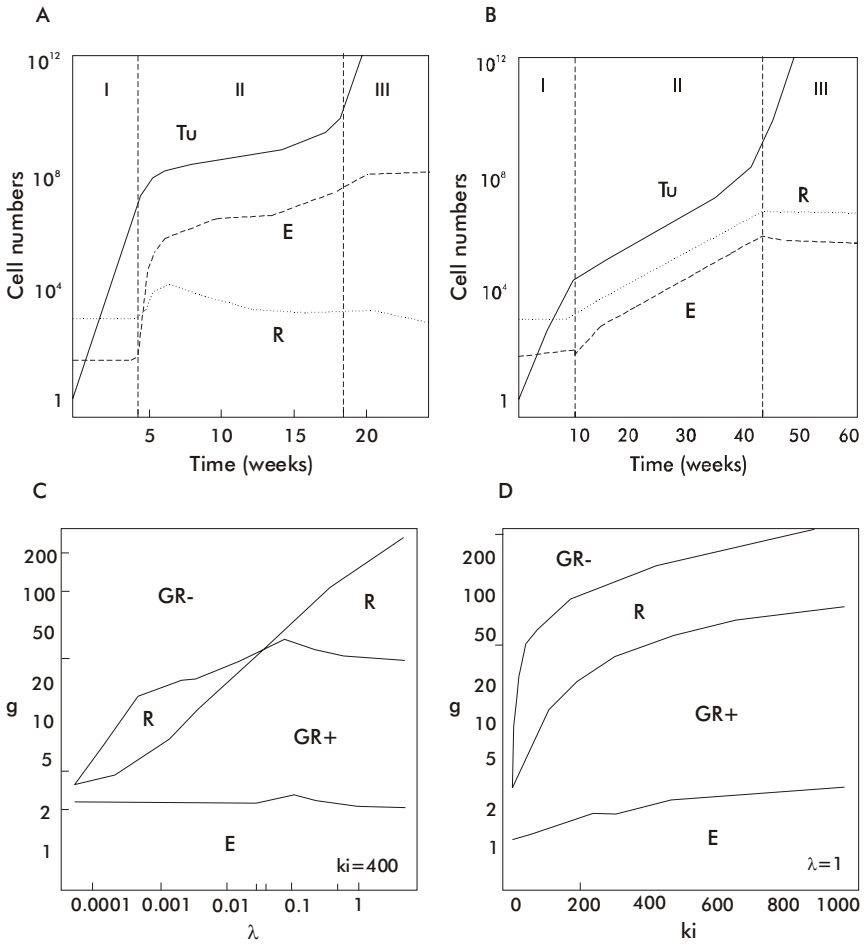

Figure 1. Typical growth kinetics of the number of tumor cells (Tu) during their interaction with effector (E) and regulatory (R) T-lymphocytes, in the GR- (a) or GR+ mode (b) according to our model. The vertical dotted lines define three functional stages shared by both tumor types during their interaction with the immune system, which are relevant for the interpretation of their response to different therapies. Graphs c) and d) show the regions in space defined by dynamic tumor parameters where the model predicts a GR+ or GR- growth mode. The region labeled R corresponds to spontaneous tumor regression, and the region labeled E corresponds to a stable balance of the tumor with the immune system. The parameters labeled g, λ and ki control the specific growth rate of tumor cells, tumor immunogenicity and sensitivity to effector immune functions, respectively.

Regarding the prediction and identification of dynamic properties in tumors, the model predicts that tumors with a low specific growth rate, relatively high immunogenicity (defined by the spontaneous induction of the presentation of their antigens in APC) and high sensitivity to effector immune functions (defined by their sensitivity to lysis mediated by effector T lymphocytes) will grow in the GR+ mode, while tumors with a high specific growth rate but low immunogenicity and sensitivity to effector immune functions would grow in the GR- mode. This important result implies that the growth mode and the ca-

3. León K, García K, Carneiro J, Lage A. How regulatory CD25+CD4+T cells impinge on tumor immunobiology? On the existence of two alternative dynamical classes of tumors. J Theor Biol 2007; 247:122-37.

pacity for recognition by peripheral Tregs of a specific tumor might not depend on its antigenic composition, but on intrinsic, dynamic properties of the tumor itself, which would explain recent observations in genetically engineered mice for the spontaneous development of breast tumors. These animals developed a large variety of tumors with a similar antigenic composition (determined by the genetic construction used for their modification) but large differences regarding their induction of Tregs expansion and their specific escape mechanisms.

The model predicts the properties that should be evaluated in actual tumors thus helping to understand why some cancers expand the Tregs populations while others leave them untouched, which has considerable clinical relevance in itself. In particular, the prediction that rapid growth tumors proliferate in the GR- mode has the corollary that most of the models of transplantable tumors used in animal experimentation would be GR- negative, and would therefore be inadequate to study the role of Tregs in tumor biology and the response of spontaneous tumors to immunotherapy, given that the latter usually have a much slower development *in vivo*.

The proposal for an experimental design to distinguish GR+ from GR- mode tumors in transplantable tumor models in laboratory animals is based on the study of the dependence of tumor growth on the number of initially implanted cells. According to the biomathematical model, GR+ mode tumors would have a bell-like dependency curve, with detectable growth when the implant contains either a few or a large number of cells, and tumor rejection as the outcome with intermediate-size inocula. Such a curve can be easily distinguished from that expected for GR- mode tumors, where detectable tumor growth would be attainable only with large numbers of implanted cells. It should be stressed that the type of dependence predicted for GR+ tumors were observed during the 1970s in certain tumoral models, which resulted in the adoption of the term 'sneaking through' to designate this phenomenon. The results of our model allow a reinterpretation of these observations within the context of the theory of dominant tolerance.

Modeling the tumor-immune system interaction and the effect of single or combination therapies

This stage of our work placed a special stress on understanding the differences in the therapeutic response between GR+ and GR- tumors [4]. Three types of therapies were studied, including every possible combination in pairs. The first therapy was vaccination, simulated in the model as an abrupt and transitory increase in the number of APCs presenting tumoral antigens. The second was the depletion of regulatory and effector T lymphocytes, simulated as a temporary increase in their death rate. The last therapy was surgery, simulated as an abrupt, but partial excision of tumor cells. The model predicted similarities and differences in the response to therapy of GR+ and GR- tumors, with combination therapies yielding a better result over the monotherapy in every case. The main results were:

1) The model predicted a surprisingly similar response to vaccination of GR+ and GR- tumors (Figures 2a and b). In both cases high doses of the vaccine are efficient, but the minimal dose required to induce rejection increases as the tumor grows. The obvious implication is that vaccination in itself is effective only when delivered at the initial stages of tumor

4. León K, García K, Carneiro J, Lage A. How regulatory CD25+CD4+T cells impinge on tumor immunobiology? On the differential response of tumors to immunotherapies. J Immunol 2007;179: 5659-68.

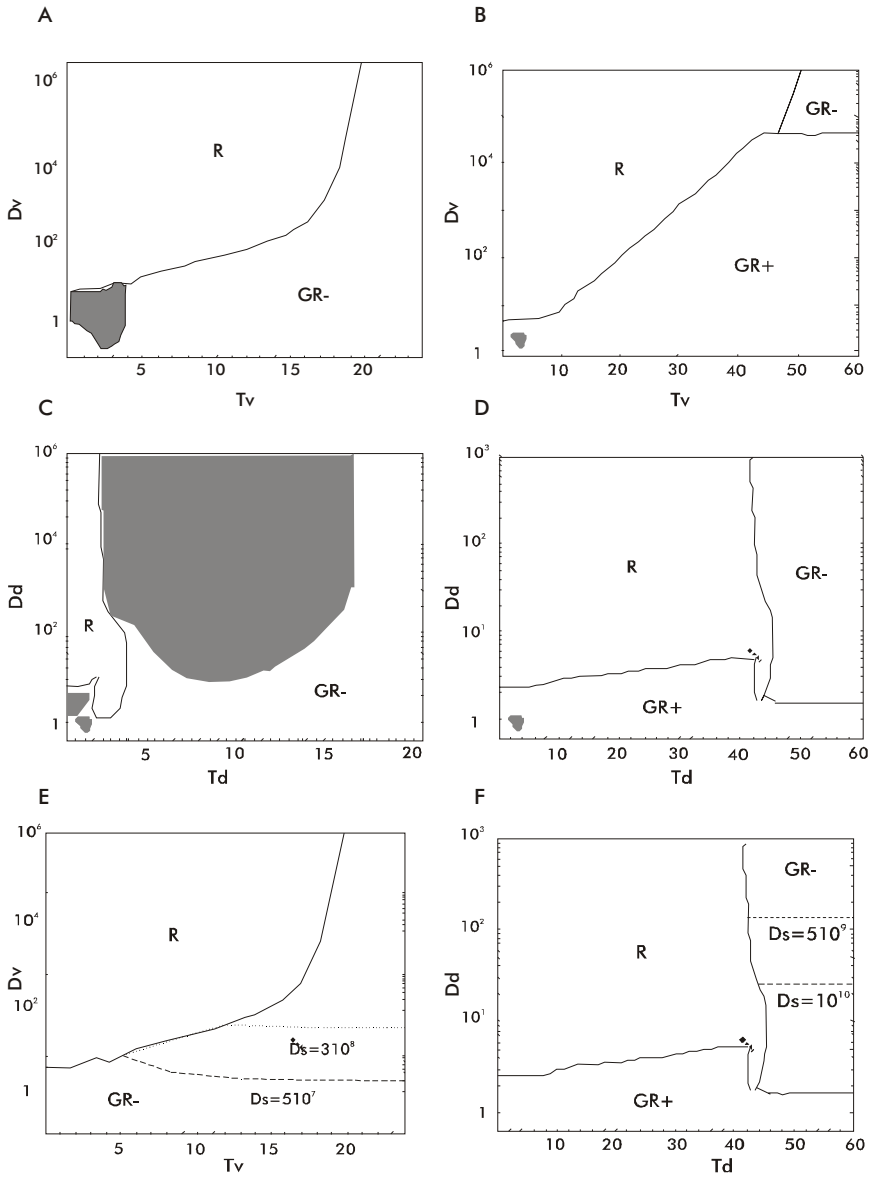

Figure 2. Response against single or combination therapies. 2a and b) Response against individual vaccination treatments; 2c and d) Depletion of T cells for tumors growing in GR- (left column, kinetics from Figure 1a) or GR+ (right column, kinetics from Figure 1b) mode in the absence of a treatment as a function of the dose (*Dv* for the vaccine and *Dd* for T cell depletion) and the time of treatment delivery in relation to tumor development. The treatments in which parameters (indicated at the axes) fall within the R regions lead to tumor rejection, whereas those falling in the GR+ or GR- regions lead to the continuous growth of the corresponding tumor. The regions shaded in gray correspond to treatment parameters that result in accelerated tumor growth. 2e) Response of a GR- tumor to a combination of vaccination and surgery. The continuous line represents the minimal required vaccine dose for the induction of tumor rejection at time *tv* in the absence of surgery, whereas the other two discontinuous lines correspond to the same dependency, but in the presence of surgery leading to a reduction of tumor size down to the number of cells shown in the chart (Ds), just before vaccination. 2f) Response of a GR+ tumor to a combination of T cell depletion and surgery. The continuous lines delimit the treatment parameter region for T cell depletion that leads to tumor rejection in the absence of surgery. The dotted lines show how this region expands when using a surgery that leaves behind the indicated number of tumor cells (Ds), after the depletion of T lymphocytes.

growth. Another important prediction was that using suboptimal doses of a vaccine at the early stages of the disease would be counterproductive, leading instead to faster tumor proliferation. This result proposes that conventional vaccines are typically more efficient when used against tumors in early stages or before their implantation, while regularly failing during more advanced stages. The prediction also agrees with recent reports on increased tumor growth as the result of the administration of therapeutic vaccines, suggesting the use of suboptimal doses as a likely cause of failure.

2) The response to the depletion of T lymphocytes is tumor rejection in both cases, but only if the depletion is delivered in high doses at the initial stages of tumor development (Figures 2c and d). The treatments with suboptimal dosages or that delivered outside the initial window of opportunity may speed up tumor growth. In spite of these similarities, GR+ malignancies are predicted to be more sensitive to these therapies than their GR- counterparts. In the GR+ case, the window of opportunity for treatment is wider and the treatment conditions leading to more rapid tumor proliferation are more specific. Such a prediction explains previous observations in animal models where the induction of tumor rejection by the depletion of T-lymphocytes with anti-CD25 antibodies was effective only during the initial stage of development of the implant. Our analysis suggests that such a behavior would be observed both for GR+ and GR- cases, in contrast to other interpretations published in the literature.

3) The predicted response to partial excision is different in each case. According to the model, GR+ tumors would be insensitive to partial surgery, which reduces their size only temporarily. On the other hand, GR- tumors are predicted to be highly sensitive to this treatment, since there is a considerable reduction in tumor volume at a time in which they have already triggered the expansion of an effector immune response. Our results suggest that this is the only therapy that would never result, under any circumstance, in an increase in tumor growth rate as an adverse event. Additionally, the predictions propose a reinterpretation of the phenomenon of tumor recurrence after surgery by taking into account the role of the immune system, unlike the traditional model that explains recurrence as the result of the incomplete elimination of all cancer cells. Another suggestion of this result is the possible convenience of surgical treatment, even under conditions where it is impossible to remove all or most of the cancer cells.

4) The simulations evidenced the complexity of the response to therapy in all three cases. Therapeutic efficacy depended, to a large extent, on dosage, the type of tumor (GR+ or GR-) and its stage of development. Frequently, a therapy that is effective at a specific dose against a particular tumor in a specific stage of development was predicted to be ineffective or counterproductive when used at a different development stage or against a different tumor at the same development stage. This scenario illustrates the difficulties in assessing similar treatments in a clinical setting where, in contrast to animal models, tumors tend to be highly heterogeneous from patient to patient, they cannot be classified as GR+ or GR-, and are not synchronized in respect to the different development phases of their interaction with the immune system. Overcoming this will require a better classification of malignancies and their stages during clinical trials and possibly custom-tailoring the treatment against specific tumor populations. In the first case, our model proposes a definition of three stages of tumor growth (Figure 1a and b) according to the interaction of the tumor with the immune system, which can assist in the evaluation and interpretation of responses to the treatments shown above. As for the second possibility, the model proposes therapeutic combinations having higher efficacy than monotherapies that would be effective against both tumor types (GR+ o GR-), in both early and late developmental stages.

5) The best predicted therapeutic combination for GR- tumors was vaccination together with surgery (Figure 2e). This combination can be optimized, according to the model, if the vaccine is delivered concomitantly or slightly before surgery. Also, the combination is potentially effective at all tumor stages and is more efficient than either therapy alone. Such a combination has already been explored in cancer research, where it is known as adjuvant scenario vaccination and has been effective in some cancer models. The novelty of our result, however, is the fact that the conditions currently employed for this therapy –surgery before therapeutic vaccination- are suboptimal; a prediction that can be easily tested in the future.

6) The best predicted therapeutic combination for GR+ tumors was T cell depletion combined later with surgery, once the cell populations have rebounded (Figure 2f). This combination is predicted as being effective at early and late stages of tumor development and is, therefore, better than either therapy alone. To the best of our knowledge such a combination has never been evaluated in clinical or preclinical studies, and is therefore a good candidate for future experimental studies at CIM.

Conclusions

The results of this work are a contribution to the theoretical understanding of the role of Tregs in the regulation of the immune response and, specifically, their influence in the development of malignant tumors. Our study proposes concrete strategies for differential tumor therapy that can be explored and implemented at the stages of preclinical and clinical experimentation. In addition, they represent a step forward in the application of computational modeling and simulation techniques to the disciplines of immunology and immunotherapy, thus evidencing their validity and potential.