Modeling the role of IL2 in the interplay between CD4+ helper and regulatory T cells: studying the impact of IL2 modulation therapies

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Abstract

Several reports in the literature have drawn a complex picture of the effect of treatments aiming to modulate IL2 activity in vivo. They seem to promote indistinctly immunity or tolerance, probably depending on the specific context, dose and timing of their application. Such complexity might derives from the dual role of IL2 on T-cell dynamics. To theoretically address the latter possibility, we develop a mathematical model for helper, regulatory and memory T-cells dynamics, which account for most well-known facts relative to their relationship with IL2. We simulate the effect of three types of therapies: IL2 injections, IL2 depletion using anti-IL2 antibodies and IL2/anti-IL2 immune complexes injection. We focus in the qualitative and quantitative conditions of dose and timing for these treatments which allow them to potentate either immunity or tolerance. Our results provide reasonable explanations for the existent pre-clinical and clinical data and further provide interesting practical guidelines to optimize the future application of these types of treatments. Particularly, our results predict that: (i) Immune complexes IL2/anti-IL2 mAbs, using mAbs which block the interaction of IL2 and CD25 (the alpha chain of IL2 receptor), is the best option to potentate immunity alone or in combination with vaccines. These complexes are optimal when a 1:2 molar ratio of mAb:IL2 is used and the mAbs have the largest possible affinity; (ii) Immune complexes IL2/anti-IL2 mAbs, using mAbs which block the interaction of IL2 and CD122 (the beta chain of IL2 receptor), are the best option to reinforce preexistent natural tolerance, for instance to prevent allograft rejection. These complexes are optimal when a 1:2 molar ratio of mAb:IL2 is used and the mAbs have intermediate affinities; (iii) mAbs anti-IL2 can be successfully used alone to treat an ongoing autoimmune disorder, promoting the re-induction of tolerance. The best strategy in this therapy is to start treatment with an initially high dose of the mAbs (one capable to induce some immune suppression) and then scales down slowly the dose of mAb in subsequent applications.

Keywords: dynamics of t cells, interleukin-2 therapies, mathematical modeling, regulatory t cells

Introduction

Several reports in the literature have drawn a complex picture of the effect of treatments aiming to modulate IL2 activity in vivo. They seem to promote indistinctly immunity or tolerance, probably depending on the specific context, dose and timing of their application. On the one hand, treatments that increase IL2 activity, just by injecting it (i.e. IL2-based treatments), have been shown to potentate the immune response to vaccines (1–4) and are a current medical practice to enhance the natural anti-tumor immunity in patients with melanoma. However, some reports in the literature have shown that HIV (5–8) and melanoma (9) patients treated with IL2, experience an increase in the frequency of the CD4+ CD25+ FoxP3+ regulatory T cells, which typically mediate natural immune tolerance. Moreover, pre-clinical studies have further documented a tolerogenic effect of IL2. IL2-based treatments show the prevention or amelioration of autoimmune responses in some animal models (10–12). Treatments which reduce natural IL2 activity, by sequestering it with anti-IL2 monoclonal antibodies, have been shown to induce autoimmune responses (13). Treatments intending to block IL2 activity, with non-depleting anti-IL2-receptor antibodies, are showed to have anti-tumoral effect in pre-clinical experiments (14). However, in clinical practice, the anti-IL2-receptor antibodies are used to ameliorate the autoimmune reaction in patients with neoplasia, autoimmune diseases and organ allograft rejection (15). Finally, further complexity to the latter picture has
been recently added with the pre-clinical assessment of treatments based on immune complexes formed by IL2 and monoclonal antibodies anti-IL2. They showed a much more potent in vivo effect than IL2, but they appear again to potentiate either immunity (16, 17) or tolerance (18), depending on the specific antibody used to form the immune complexes. In particular, the specific epitope in the IL2 recognized by the antibody has been postulated as critical for this phenomenon (19, 20).

Mechanistically, IL2 interact with many different cells types, which express the three known chains of the IL2 receptor. Particularly, relevant and complex is its relationship with the dynamics of the CD4 T lymphocytes. IL2 was originally described as a potent CD4+ T-cells growth factor (21), which should in consequence enhance overall T-cell immunity. However, several experiments have shown lately a critical role for this cytokine on the survival and proliferation of the CD4+ CD25+FoxP3+ T cells (regulatory T cells) (22, 23), which mediate the maintenance of natural and induced tolerance. The CD4+CD25–FoxP3– T cells (helper T cells) have been identified as the principal source of IL2 in vivo (24), suggesting that the regulatory T cells have to sequester the IL2 produced by these cells in order to proliferate and survive (25). Moreover, in vitro and in vivo experiments have shown that regulatory T cells inhibit the production of IL2 by the helper T cells (26), limiting in this way their own source of this essential cytokine. Thus, overall, it seems that IL2 has a dual role on its circuit of interactions with CD4+ T cells. It could promote the proliferation of the helper T cells, which may drive effective immunity and foster IL2 production. But, it could also promote the expansion of regulatory T cells, which may turn off the immune reaction, as well as the IL2 production on its own. The dynamic balance between these opposite forces might explain the complexity observed in the effect of treatments that modulate IL2 activity, either sequestering it or further increasing it.

To theoretically address the latter possibility, we study here the effect of different IL2 modulation treatments in a mathematical model for helper and regulatory T-cells dynamics, which account for most well-known facts relative to their relationship with IL2. The mathematical model used is based on the one previously developed in our group (27). Particularly, we focus here in the qualitative and quantitative conditions of dose and timing for treatments based on the injection of IL2, anti-IL2 antibodies or immune complexes of IL2/anti-IL2 mAbs, which allow them to potentiate either immunity or tolerance. Our results provide reasonable explanations to existent pre-clinical and clinical data and further provide interesting practical guidelines to optimize the future application of these types of treatments.

Methods

Introduction to the mathematical model

The mathematical model used in this paper is based on the one developed in (27) to describe the interaction between IL2 and helper (E) and regulatory (R) CD4+ T cells inside a lymph node. The model includes several physical compartments, which minimally capture the bio-distribution of T cells, IL2 and antibodies in the immune system (see Fig. 1). It includes several compartments, which represent different interactions with CD4+ T cells. It could promote the proliferation of the helper T cells, which may drive effective immunity and foster IL2 production. But, it could also promote the expansion of regulatory T cells, which may turn off the immune reaction, as well as the IL2 production on its own. The dynamic balance between these opposite forces might explain the complexity observed in the effect of treatments that modulate IL2 activity, either sequestering it or further increasing it.

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to their cognate APCs. The activation of E cells can be inhibited by the presence of co-localized R cells on the APCs.

ii. The activated E and R cells could become cycling cells following a dose-dependent response to cytokine-derived signals. The activated R cells get this signal from the interaction with available IL2 while the E cells could additionally use other homeostatic cytokines, which are referred in the model as ILx and are available inside the lymph node in a constant but limited amount. Note that, although other cytokines are able to stimulate Treg cells in vitro, several reports in the literature have indicated IL2 as the key cytokine for the proliferation and survival of Treg cells in vivo. The group of Freitas (24) have shown that the absence of CD4+ T cells capable of producing IL2 leads to the absence of Treg cells and to the development of autoimmune. Moreover, mice knockouts of IL2 or IL2 receptor components have been shown to lack the accumulation of Tregs in vivo, exhibiting once more an autoimmune phenotype (28, 29).

iii. In these latter scenarios of autoimmune mice, other cytokines besides IL2 are capable to maintain and expand the autoreactive effector CD4+ T cells (perhaps IL7, IL15 or IL21). In the absence of enough cytokine-derived signal, a fraction of the activated E or R cells revert to the resting state and the remaining fraction just die.

iv. The cycling E and R cells are fully committed to divide, producing two new resting cells. Thus, they are presumed to do so with a constant rate.

v. The model includes also the dynamics of a generic population of non-CD4 T cells, which binds weakly to the existent APCs, but proliferates in response to IL2 signal, with similar sensibility than the activated helper CD4+ T cells. This cells (referred as M cells) represent the memory CD8+CD44+ T cells, which have been recently shown (20) to proliferate in vitro and in vivo in response to IL2 without any requirements of activation by cognate APCs (see Fig. 3 and equations derived in Appendix B).

It is important to note that our model has focused on the control that IL2 exert on T-cell cycle progression, impacting both in T-cell proliferation and survival. But we have neglected some others reported roles of IL2 in T-cell differentiation. For instance, IL2 have been reported to increase the suppressive capacity of the regulatory T cells (12); to condition the differentiation of CD8 T cells into a memory phenotype (30, 31); to induce together with TGFβ the generation of the so called induced Tregs from naive CD4+ T cells (32). We believe these phenomena, although important in some experimental context, are not essential to understand the main phenomenology study in this paper.

The dynamics of IL2 molecules inside the lymph node takes into account the role of T cells in the production and degradation of this cytokine. The following processes are considered in the model, just as reported in (27) (see Fig. 2 and equation derived in Appendix B):

v. IL2 is produced by E cells upon activation. It is produced as a burst whenever a resting E cell becomes an activated E cell. Such production of IL2 is inhibited, together with the E cell activation, by the presence of co-localized R cells on the APCs.

vi. IL2 is degraded in the lymph nodes, after being internalized by the T cells in the form of complexes with the IL2 receptor at their cell surface.

Interaction between IL2 and T cells

Interactions of IL2 and T cells in the model are based on the expression by these cells, in the resting, activated or cycling state, of different levels of the IL2 receptor. These receptors mediate the binding of IL2, which provide a stimulatory signal in a dose-dependent fashion to the T cell. In the original model developed in (27), a single form of the IL2 receptor was considered, but in this work, the model is extended to include the three known chains of the IL2 receptor, alpha, beta and gamma (33). These three chains are combined dynamically at the cell surface, upon IL2 binding, to conform the two known signaling forms of the IL2 receptors. The following processes and known facts are considered in the model regarding this interaction (see Fig. 4 and equations derived in Appendix C):

i. IL2/IL2 receptor complexes formation is modeled as a multistep process: free, soluble, IL2 binds initially to the available free alpha or free beta chains of the receptor and only then can form dimmer or trimmers with the remaining IL2 receptor chains at the cell membrane. The gamma chain is assumed to be always in excess compared with the
amount of beta chain bound to IL2, either alone or together with alpha chain. Therefore, gamma chain joins immediately to these membrane complexes, forming the well-known intermediate (beta-gamma-IL2) or high affinity (alpha-beta-gamma-IL2) IL2-IL2 receptor complexes. These processes were independently modeled and calibrated to experimental data in our group (J. E. Hernández and K. León, in preparation).

ii IL2/IL2R receptor configurations, which include the beta and gamma chains (high-affinity alpha-beta-gamma and intermediate affinity beta-gamma receptor), trigger a signal into the T cells (19). Therefore, in the model, the mean number of such signaling receptors per activated E cell, R cell and M cell are counted. Then, the probability of getting enough signal as to become a cycling cell, for any particular activated E, R or M cell, is computed with a sigmoid dose response curve, of the mean signaling level. The use of a sigmoid dose response curve is based on direct experimental observations on in vitro culture of CD4+ T cells (34) stimulated with recombinant IL2.

iii Beta and gamma chain of the IL2 receptor are similarly expressed by E and R cells in all functional states, but the expression of the alpha chain is modulated with T-cell activation (35). R cells constitutively express the alpha chain in the resting state but further increase its expression level with activation. E cells do not express the alpha chain in the resting state but gain a significant expression level with activation.

iv The M cells are assumed to express a negligible amount of the alpha chain of IL2 receptor but have levels of the beta and gamma chain which are higher than those of helper and regulatory T cells (36).

**Simulation of different therapies**

Three types of treatments are simulated in the model to compare with available experimental information: injections of IL2; injections of anti-IL2 monoclonal antibodies and injections of immune complex composed of a mixture of IL2 and anti-IL2 antibodies with a specified constant proportion of them. One of three classes of antibodies is used, when this type of molecule is included in a given treatment. (i) The anti-IL2 mAbs, which bind and thus block the site in the IL2 surface implicated on the interaction with the alpha chain of the IL2 receptor (referred as the phase alpha mAbs); (ii) The anti-IL2 mAbs, which bind and thus block the site in the IL2 surface implicated on the interaction with the beta chain of the IL2 receptor (referred as phase beta mAbs); and (iii) the anti-IL2, which block the binding of IL2 to all chains of the IL2 receptor (referred as a fully blocking mAbs).

Treatments are simulated to represent a continuous infusion of the involved molecules in the blood for a defined period of time. This is implemented by setting on, transiently, the external source terms in the blood compartment of the molecules involved in a specific treatment (i.e. IL2 and/or anti-IL2 antibody). Two parameters always control treatment application: the 'dose,' which set the total amount per day of IL2 and/or anti-IL2 antibody infused in the blood; and the 'treatment duration,' which set the time period for which continuous infusion is maintained. In all cases, we explore how the dose and treatment duration determine the outcome of the system simulation. We study whether or not different treatments can condition a significant preferential expansion (dominance) of helper T cells or regulatory T cells or M cells in the lymph nodes.

**Parameter and variable values in model simulations**

Most parameters in the model were previously calibrated in (27). Few new parameters are added in this work, being related to few models modification explained above. The actual values of parameter used in our simulations are provided in Appendix D and Tables 1–3. The majority of the model parameters are fixed to values directly taken or derived from available independent experimental data; just a few parameters remain unknown, and their influence in result was explored inside a range of biologically reasonable values.

Given the realistic values and units of the most model parameters used in the simulations, we report in this paper the values of treatments doses in milligrams and the values of treatment duration in weeks. For this propose, we assumed a molar mass of 15 Kda and 150 Kda, respectively, for IL2 and anti-IL2 mAbs. However, the reader should note
that our model is only roughly calibrated, thus one should believe on the order of magnitude and general qualitative trends of the predicted effects. But, the exact values of dose and treatment duration reported here to cause a given effect in the simulations should not be taken as a solid prediction.

**Results**

**Basic model and simulations setup**

The model in this work is setup to study the basic homeostasis of the immune system of a mouse in the absence of particular infections or artificial immunization. For this aim, the APCs in the lymph node compartments of the model are interpreted as those APCs in the immune system, which present self-antigens to T cells in the absence of infections. One could think, for instance, of those APCs observed in mice raised in special germ-free conditions, which sustain the activation of up 10–15% of the existent T cells. In consequence, the CD4+ T cells in the model, both helper and regulatory cells, are separated in two populations, which differ on their capacity to interact with the available self-antigens: (i) the populations of E and R cells, which weakly recognize the existent self-antigens and thus barely interact with the available APCs and (ii) the populations of autoreactive E and R cells, which significantly recognizes the existent self-antigens and thus interact with the available APCs.

The typical lymph node compartment is set to represent an inguinal lymph node of a mouse with a volume of 1 μL containing about 10^7 APCs. This lymph node sustains about 10^7 CD4 T cells in normal, non-autoimmune and non-infected conditions. Therefore, 10 identical lymph nodes compartments are included in the simulations, to guaranteed a total of about 10^8 CD4+ T cell in the system, just as estimated for a normal, non-autoimmune and non-infected mouse. The thymic output for new CD4+ T cells is set to be about 10^6 new cells per day, as estimated in normal mice.

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Table 1. Variables and parameters appearing in the equations that model the dynamics in the blood compartment

<table>
<thead>
<tr>
<th>Variables</th>
<th>Definitions</th>
<th>Values used in simulations</th>
<th>References or range of values</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL2_s</td>
<td>Total number of IL2 molecules in the blood</td>
<td>—</td>
<td>0.1 ug/day – 1 mg/day^a</td>
</tr>
<tr>
<td>Ab_a</td>
<td>Total number of anti-IL2 mAb in the blood</td>
<td>10^3 molec/h</td>
<td></td>
</tr>
<tr>
<td>IL2_ab</td>
<td>Total number of IL2-mAb complexes in the blood</td>
<td>Ln(2)/10 min</td>
<td>(37)^b</td>
</tr>
<tr>
<td>Il</td>
<td>Total number of free anti-IL2 mAb (non-conjugated to IL2-IL2R complex at the cell membrane) in the lymph node</td>
<td>Ln(2)/2 h</td>
<td>Ln(2)/12 min – Ln(2)/2 h^d</td>
</tr>
<tr>
<td>VS</td>
<td>Total number of IL2 molecules in the blood</td>
<td>2.5 × 10^-3 L, 10^-6 L</td>
<td>—^a</td>
</tr>
<tr>
<td>Ve</td>
<td>Fraction of the lymph node volume, in which molecules and mAbs can diffuse</td>
<td>0.1</td>
<td>0.1–1^d</td>
</tr>
<tr>
<td>K_{on}, K_{off}</td>
<td>Association and dissociation constants of IL2-mAb complexes</td>
<td>Phase alpha mAb: 1.5 × 10^5 M^-1 s^-1, 1.4 × 10^-4 s^-1; phase beta mAb: 2.3 × 10^4 M^-1 s^-1, 6.6 × 10^-5 s^-1</td>
<td>Appendix C^e</td>
</tr>
<tr>
<td>Gamma</td>
<td>External influx of IL2, typically used to simulate IL2 addition treatment</td>
<td>—</td>
<td>0.01 ug/day – 1 mg/day^a</td>
</tr>
<tr>
<td>K_{pi}</td>
<td>Rate of IL2 production by helper CD4+ T cells upon activation</td>
<td>10^4 M</td>
<td></td>
</tr>
<tr>
<td>K_{di}</td>
<td>Elimination rate of IL2 in the blood</td>
<td>Ln(2)/3 days</td>
<td>(39)</td>
</tr>
<tr>
<td>N_{LN}</td>
<td>Total number of equivalent lymph nodes considered in the system</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>D_{ij}, D_ab</td>
<td>Diffusion rate for the exchange of IL2 and mAbs, between the blood and peripheral lymph nodes</td>
<td>Ln(2)/2 h</td>
<td></td>
</tr>
<tr>
<td>V_{LN}</td>
<td>Volume of the blood and lymph node compartments, respectively</td>
<td>10^4 M</td>
<td></td>
</tr>
</tbody>
</table>

^aExplored inside a range of values to simulate treatment.  
^bEstimated indirectly from data in literature.  
^cEstimated by the author.  
^dExplored inside a range of possible values, for which the qualitative predictions of the model are preserved.  
^eEstimated from data of our experimentalist at CIM.
Table 2. Variables and parameters appearing in the equations that model the T-cells dynamics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Definitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_N$, $E_A$, $E_C$</td>
<td>Total number (conjugated plus non-conjugated) of resting, activated and cycling E cells</td>
</tr>
<tr>
<td>$R_N$, $R_A$, $R_C$</td>
<td>Total number (conjugated plus non-conjugated) of resting, activated and cycling R cells</td>
</tr>
<tr>
<td>$M_A$, $M_C$</td>
<td>Total number (conjugated plus non-conjugated) of activated and cycling M cells</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intermediate variables</th>
<th>Definitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_r^E$, $E_r^A$, $E_r^C$</td>
<td>Number of resting, activated and cycling E cells conjugated to APCs</td>
</tr>
<tr>
<td>$E^E$</td>
<td>Total number of conjugated E cells: $E^E = E_r^E + E_a^E + E_c^E$</td>
</tr>
<tr>
<td>$E_r^E$, $E_r^A$, $E_r^C$</td>
<td>Number of resting, activated and cycling E cells non-conjugated to APCs: $E_r = E_r^E + E_r^A + E_r^C$, $\forall \ i \in (N, A, C)$</td>
</tr>
<tr>
<td>$R_r^A$, $R_r^A$, $R_r^A$</td>
<td>Number of resting, activated and cycling R cells conjugated to APCs</td>
</tr>
<tr>
<td>$R_r^A$, $R_r^A$, $R_r^A$</td>
<td>Number of resting, activated and cycling R cells non-conjugated to APCs: $R_r = R_r^A + R_r^B + R_r^C$, $\forall \ i \in (N, A, C)$</td>
</tr>
<tr>
<td>$M_r^E$, $M_r^A$, $M_r^C$</td>
<td>Number of activated and cycling M cells conjugated to APCs</td>
</tr>
<tr>
<td>$M_r^E$, $M_r^A$, $M_r^C$</td>
<td>Total number of conjugated M cells: $M_r^E = M_r^A + M_r^B$</td>
</tr>
<tr>
<td>$M_r^E$, $M_r^A$, $M_r^C$</td>
<td>Number of activated and cycling M cells non-conjugated to APCs: $M_r = M_r^E + M_r^B + M_r^C$, $\forall \ i \in (A, C)$</td>
</tr>
<tr>
<td>$F$</td>
<td>Total number of APC conjugation sites that remain free in the system</td>
</tr>
<tr>
<td>$SigE$, $SigR$, $SigM$</td>
<td>Number of bound cytokines signaling receptors at the surface of an activated E, R and M cells</td>
</tr>
</tbody>
</table>

Symbolic labels

- $i$: Symbolic label that denotes the possible functional states of the T cells: $i = N$ resting state, $i = A$ activated state and $i = C$ cycling state

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<th>Values used in simulations</th>
<th>References or range of values</th>
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<tbody>
<tr>
<td>$\Gamma$, $\Gamma_i$</td>
<td>Input rate of new resting self-reactive E and R cells from the thymus</td>
<td>$2.5 \times 10^4$ cells/day</td>
<td>—$^a$</td>
</tr>
<tr>
<td>$K_E^E$, $K_r^E$</td>
<td>Activation rate for resting E and R cells conjugated to APCs</td>
<td>Ln(2)/2 h, Ln(2)/6 h</td>
<td>Ln(2)/2 h – Ln(2)/9 h$^b$</td>
</tr>
<tr>
<td>$K_E^E$, $K_r^E$, $K_M^E$</td>
<td>Division rate for cycling E, R and M cells</td>
<td>Ln(2)/4 h</td>
<td>(40)</td>
</tr>
<tr>
<td>$K_E^S$, $K_r^S$</td>
<td>IL2 signaling-waiting rate for activated E and R cells</td>
<td>Ln(2)/2 h</td>
<td>Ln(2)/1 h – Ln(2)/2 h$^b$</td>
</tr>
<tr>
<td>$K_M^S$</td>
<td>IL2 signaling-waiting rate for activated M cells</td>
<td>Ln(2)/4 h</td>
<td>Ln(2)/1 h – Ln(2)/6 h$^b$</td>
</tr>
<tr>
<td>$K_E^A$, $K_r^A$, $K_M^A$</td>
<td>Death rate for free resting E and R cells and free activated M cells</td>
<td>Ln(2)/1 week</td>
<td>Ln(2)/1 week – Ln(2)/10 weeks$^c$</td>
</tr>
<tr>
<td>$A$</td>
<td>Number of total APCs</td>
<td>$2 \times 10^5$</td>
<td>Appendix C$^d$</td>
</tr>
<tr>
<td>$s$</td>
<td>Total number of conjugation sites per APC</td>
<td>5</td>
<td>2–8$^c$</td>
</tr>
<tr>
<td>$K_E^E$, $K_r^E$</td>
<td>Equilibrium conjugation constants ($K_{on}/K_{off}$) for E and R cells to the APC conjugation sites</td>
<td>$K_{on} = 10^{-13} L \times s^{-1} \times cell^{-1}$</td>
<td>(41, 42)$^b$</td>
</tr>
<tr>
<td>$K_M^E$</td>
<td>Equilibrium conjugation constants ($K_{on}/K_{off}$) for M cells to the APC conjugation sites</td>
<td>$K_{on} = 10^{-13} L \times s^{-1} \times cell^{-1}$</td>
<td>(43)$^b$</td>
</tr>
<tr>
<td>$g_E$, $g_R$</td>
<td>Fraction of activated E and R cells reverting to the resting state in the absence of cytokine-related signal</td>
<td>0.95</td>
<td>0–1$^c$</td>
</tr>
<tr>
<td>$h$</td>
<td>Hill coefficient at the sigmoid response curve</td>
<td>4</td>
<td>(34)$^b$</td>
</tr>
<tr>
<td>$S_E$, $S_R$, $S_M$</td>
<td>Sensitivities thresholds for E, R and M cells to cytokines signal</td>
<td>500</td>
<td>(34)$^b$</td>
</tr>
</tbody>
</table>

$^a$Estimated by the author.

$^b$Estimated indirectly from data in literature.

$^c$Explored inside a range of possible values, for which the qualitative predictions of the model are preserved.

$^d$Estimated from data of our experimentalist at CIM.

From the new CD4+ T cells, a small fraction (5%) is assumed to be significantly autoreactive. Half of this new autoreactive T cells are assumed to be of the regulatory phenotype (50). Two main problems are then studied in the model simulations: (i) The basic dynamics states of the system in the absence of treatments and (ii) The effect of perturbations...
which represent specific IL2 modulation treatments on the stability of these dynamics states.

**Tolerance and immunity as the basic model steady states (in the absence of treatment)**

The model has two stable steady states that can be interpreted as natural tolerance and autoimmune in the system. The steady state, which is interpreted as an autoimmune state (Fig. 5A), is one where autoreactive helper cells are significantly expanded while the autoreactive regulatory T cells are outcompeted from their cognate APCs. This steady state is also characterized by the existence of high levels of free IL2 and some subsequent expansion of the memory CD8+ T-cells population (M cells) in the lymph nodes. The steady state, which is interpreted as natural tolerance in the model, is one where the autoreactive E and R cells co-exist in a dynamic equilibrium (Fig. 5B). In this steady state, the expansion of the autoreactive helper cells is actively controlled by their interaction with the autoreactive regulatory T cells, the amount of free IL2 remains very low and the size of M cell population remains close to its basal homeostatic level. Interestingly, in both steady states, the number of weak autoreactive CD4+ T cells remains equal to the value set by the compensation of their thymic output and their basic life span (data not shown). These CD4+ T cells barely participate on the study dynamics, despite their supremacy in numbers inside the lymph node. They almost never get activated on the APCs, remaining in the resting state without significant expression of the IL2 receptor.

A key dynamical property of the model is the existence of a parameter regime where the steady states of tolerance and autoimmunity described above can coexist (Fig. 5C). This is a regime of bistable behavior, where the model could evolve dynamically into either to the autoimmune or the tolerant steady state but depending on the initial conditions used to seed the simulation without any change of parameter values (for instance changing the initial proportion of autoreactive E and R cells in the lymph nodes). The existence of such bistable regimen of parameters was extensively

### Table 3. Variables and parameters related with the dynamics of IL2/IL2R and IL2/mAb/IL2R complexes formation

<table>
<thead>
<tr>
<th>Variables</th>
<th>Definitions</th>
<th>Values used in simulations</th>
<th>References or range of values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C^j_{El}$, $C^j_{Rl}$, $C^j_{Ml}$</td>
<td>Number of IL2 molecules bound to j chain of IL2R, at the surface of the indicated T-cell type</td>
<td>$K_{off}^{a}=0.6 \text{ s}^{-1}$, $K_{on}^{a}=10^7 \text{ M}^{-1} \text{ s}^{-1}$, $K_{off}^{f}=3 \times 10^{-3} \text{ s}^{-1}$, $K_{on}^{f}=3.4 \times 10^{5} \text{ M}^{-1} \text{ s}^{-1}$</td>
<td>Appendix C&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>$CA^j_{El}$, $CA^j_{Rl}$</td>
<td>Number of IL2/mAb complexes bound to the j chain of IL2R, at the surface of the indicated T-cell type</td>
<td>ILa/Ea=10^3/Ea, ILm/Ma=10^3/Ma</td>
<td>10^4–10^5 b</td>
</tr>
<tr>
<td>$K_{off}^{i}$, $K_{on}^{i}$</td>
<td>Dissociation and association constant of IL2 to the j chain of the IL2R</td>
<td>0, 1</td>
<td>—</td>
</tr>
<tr>
<td>$N_j$</td>
<td>Switch parameter setting if the mAb blocks (=1) or not (=0) the interaction of IL2 with the j chain of the IL2R</td>
<td>$R_{off}^{Ab}=10$, $R_{off}^{Ba}=10^4$, $R_{on}^{Ab}=10^3$, $R_{on}^{Ba}=10^2$</td>
<td>(35, 44, 45)</td>
</tr>
<tr>
<td>$R_{off}^{j}$, $R_{on}^{j}$</td>
<td>Total number of alpha and beta chains of IL2R per E cells in the state I</td>
<td>$R_{on}^{j}=10^4$, $R_{off}^{j}=10^3$, $R_{on}^{j}=10^2$, $R_{off}^{j}=10^1$</td>
<td>(35, 44, 46, 47)</td>
</tr>
<tr>
<td>$R_{off}^{M}$, $R_{on}^{M}$</td>
<td>Total number of alpha and beta chains of IL2R per M cells in the state I</td>
<td>$R_{on}^{M}=10$, $R_{off}^{M}=10^1$</td>
<td>(45)</td>
</tr>
<tr>
<td>$K_{off}^{a}$, $K_{on}^{a}$</td>
<td>Association and dissociation rates for the interaction of free alpha chain to preformed IL2/alpha chain complexes, at the T-cell membrane</td>
<td>$K_{off}^{a}=2.2 \cdot 10^{-3} \text{s}^{-1}$, $K_{on}^{a}=3 \cdot 10^{-3} \text{s}^{-1}$</td>
<td>Appendix C&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>$K_{off}^{ba}$, $K_{on}^{ba}$</td>
<td>Association and dissociation rates for the interaction of free alpha chain to preformed IL2/beta chain complexes, at the T-cell membrane</td>
<td>$K_{on}^{ba}=0.6 \cdot 10^{-2} \text{s}^{-1}$, $K_{off}^{ba}=0.6 \text{s}^{-1}$</td>
<td>Appendix C&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>$K_m$</td>
<td>Internalization (degradation) rate of signaling IL2/IL2R complex by T cells</td>
<td>$K_m=0.04 \text{min}^{-1}$</td>
<td>(48, 49)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Estimated from data of our experimentalist at CIM.

<sup>b</sup>Explored inside a range of possible values, for which the qualitative predictions of the model are preserved.
Fig. 5. Steady states obtained from numerical simulations of the model and their dependence on parameter values. (A and B) Kinetic evolution of the total number of helper (E_T), regulatory (R_T) and memory (M_T) T cells and free IL-2 concentration (IL-2), where the system evolves into: (A) the immune/autoimmune steady state (IS) and (B) the tolerant steady state (TS). (C) The graph shows five regions delimited by the values of parameters Kpi and ILα, inside which the system can only evolves into the indicated types of steady states. The most relevant parameter region is the bi-stable region (upper right region) where both, tolerant and immune/autoimmune states, coexist in the system. The symbol B corresponds to the region of basal steady state, where the population of T cells cannot grow and evolves to their basal levels. Simulations in A and B were obtained for values of Kpi and ILα inside the bi-stable region (Kpi = 10^3 molec/h, ILα = 10^6) and different initial T-cells conditions ((A) R_T(0) = 10^6, (B) R_T(0) = 1). Other parameter values were taken from Tables 1–3. The remaining initial conditions are fixed at values: E_T(0) = 10^6, E_A(0) = 1, E_C(0) = 1, R_T(0) = 1, R_C(0) = 1, M_A(0) = 1, M_C(0) = 1, IL2(0) = 0.01 pM.

discussed in the original work of Garcia-Martinez and Leon (27). In this study, the value of the parameter ILα, which is the only non-calibrated parameter of the model, is taken to set the model inside this bistable parameter regime. Thus, in equilibrium, in the absence of treatments, the system will be either in the tolerant or the immune/autoimmune steady state referred above. This parameter choice is required, as discussed in (27), to explain properly with the model the dominant tolerance phenomenology. Particularly, the results of adoptive transfer experiments in mice, where transferring different proportions of CD4+CD25− (helper) and CD4+CD25+ (regulatory) T cells into immune deficient mice (those lacking T cells, Rag−/− or nu−/−), they either reconstitute a normal (tolerant to self-antigens) immune system or develop an autoimmune disease mediated by the uncontrolled expansion of the transferred autoreactive CD4+ T cells.

Response to treatments that modulate IL2 concentration
In following sections, the effects of different treatments, which aim to modulate IL2 activity either by injecting it or sequestering it, are studied. Treatments simulate a continuous infusion in the blood for a defined period of time of the involved molecules (IL2 and/or anti-IL2 antibody). Two parameters control their application: the ‘dose,’ which setup the total amount per day of IL2 and/or anti-IL2 antibody infused and the ‘treatment duration,’ which set the time period of sustained infusion. Treatments are always applied in a system that is previously set to a dynamic equilibrium. This is a system either in the tolerant or the autoimmune steady states discussed in previous sections. We explore by varying the dose and treatment duration, whether a given treatment is able to induce a significant change of the preexistent steady state. In particular, whether it could promote immunity inducing a transition from the tolerant state to the autoimmune state or whether it could promote tolerance, inducing a transition from the autoimmune state to the tolerant state.

Simulation of IL2-based treatments (IL2 injections). Simulations of IL2 injections show that, when this treatment is applied in a system with a preexistent autoimmune reaction (system initialized in the autoimmune steady state), it is unable to revert the system to the tolerant state, irrespectively of the dose and treatment duration chosen. Furthermore, it can promote transiently the expansion of the autoreactive E cells and the M cells (Fig. 6A) reinforcing the ongoing autoimmune response. However, when this treatment is applied to a previously tolerant system (system initialized in the tolerant steady state), it just transiently reinforces the preexistent tolerance, by further expanding the regulatory populations (Fig. 6B). The duration of the transient reinforcement of the preexistent tolerant or autoimmune state, which is induced by this treatment, relates directly to the treatment duration. As soon as the treatment is stopped, the reinforcement effect is lost.

Overall, the above results show that, in the model, IL2 injections will always reinforce the preexistent steady state, this is expanding transiently either the R or the E cells, respectively, for a preexistent tolerant or autoimmune situation (continue increasing the IL2 dose in a preexistent tolerant state, induce immunity by expanding the M cells; however, this effect is obtained for high unrealistic values of dose, i.e. applying 3 mg/day for 1 week). Consequently, this treatment is predicted as unable to induce a significant change of steady state either from tolerance to immunity or vice versa. This prediction is somehow counterintuitive, since in the model IL2 is the key molecule for immune regulation but still its injection appears with a little dynamical effect. However, a closer look to the model behavior qualitatively explains the obtained results. Briefly, in a preexistent autoimmune steady state, there is an excess of IL2 (see Fig. 5A) in the lymph node, thus is not lack of IL2 what limits regulatory T-cell expansion, is their competition with autoreactive E cells for the cognate APCs. In consequence, injecting IL2 would never reestablish tolerance. In a preexistent tolerant steady state, there is a small amount of IL2 in the lymph node (see Fig. 5B), which is almost exclusively used by the regulatory T cells, limiting their expansion. The helper T cells do not expand as result of the direct suppression of their activation exerted by the R cells. In this situation the injection of IL2, naturally leads to the enhanced expansion of R cells reinforcing the suppression over the E cells.
Modeling the role of IL2 in the interplay between CD4+ helper and regulatory T cells

Simulation of IL2 depletion treatments (injections of anti-IL2 mAbs). Simulations of the injection of monoclonal antibodies anti-IL2 shows that, when this treatment is applied in a previously tolerant system, it could induce a breakdown of tolerance (Fig. 7A), with the consequent transition of the system to the autoimmune steady state. This effect is obtained with all classes of anti-IL2 mAbs studied, irrespective of their capacity to block the IL2 interaction with one or other chain of the IL2 receptor. In all cases, the latter effect requires a minimal effective dose of the anti-IL2 mAb and treatment duration (Fig. 7C). There are some variations on the efficiency of this effect, regarding the type of mAbs used. It appears that phase alpha mAbs are significantly more efficient than fully blocking or phase beta mAbs (Fig. 7C) and the higher the affinity the mAbs the better in every case (Fig. 7E). Other relevant dependency on the efficiency of this effect was observed with the size of thymic output in the simulations (Note that changing the size of thymic output in the simulations modifies the amount of new regulatory T cells which arrive to the peripheral lymph nodes.) (Fig. 7E). The higher the size of thymic output the higher the minimal dose of mAbs required to break tolerance.

The effect of treatment with anti-IL2 mAbs in a system with a preexistent autoimmune reaction is also quite significant. In this case, the treatment is capable of resetting the system into the tolerant steady state (i.e. inducing tolerance) (Fig. 7B). This effect occur under quite restrictive treatment conditions: there is a minimal treatment duration required and the dose of the anti-IL2 mAbs used has to be set inside some particular intermediate range of values (Fig. 7D). The tolerogenic effect of the anti-IL2 mAbs is obtained when applied phase beta or fully blocking mAbs but not phase alpha mAbs (Fig. 7D) and the higher the affinity of the mAbs the higher their efficacy (Fig. 7F). The size of thymic output has also a strong impact on this treatment effect. The higher the thymic output the wider the ranges of mAbs doses capable to induce tolerance (Fig. 7F). Thymectomy could almost prevent the existence of this tolerogenic effect in the model.

Overall, the simulations of IL2 depletion treatments using anti-IL2 antibodies predict that this type of therapy is able to break a preexistent tolerant state, inducing an autoimmune response or to render tolerant a preexistent autoimmune system. This dual role of the treatment, apparently counts intuitive and of potential practical implications, results from the highly non-linear relation of IL2 with CD4+ T-cell dynamics. Qualitatively, the dominant effect of the mAbs on these simulations is similar in all cases. They appear to sequester the IL2 in the lymph node compartments taking it to the blood compartment, where it is cleared by renal elimination. The net effect of the mAbs is then to transiently accelerate the effective IL2 degradation rate, limiting the availability of IL2 capable to provide signal to the T cells in the lymph node. When the treatment is applied into an initially tolerant steady state, the initial effective concentration of IL2 is low (see Fig. SB) and it is further reduced to insignificant levels, where this cytokine is incapable to signal neither to E, R nor M cells. Therefore, if the treatment is sustained long enough, the number of R cells fall down to a minimum determined by the availability of the homeostatic cytokine of ILα, which they could use as alternative to IL2 signal. Therefore, once the injected mAbs are cleared, the autoreactive E cells could have some initial advantage in respect to the R cells, particularly when the thymic output is small enough and the amount of ILα is large enough, leading the T-cell expansion, which drive the system into the autoimmune steady state. However, when the treatment is applied to an initially autoimmune system, the effective concentration of IL2 is quite large (see Fig. 5A) and it is reduced by the presence of the antibody. This reduction in the IL2 concentration is only efficient in reducing the effective cytokine signal when the mAbs used block the signaling capacity of IL2 (phase beta or fully blocking mAbs), due to the large amount of this cytokine present in the system. For a very high antibody dose, the effective IL2 concentration falls to negligible values, which as before are unable to signal neither to E, R or M cells. Thus, the size of the autoreactive E cell population is reduced to the value set by the availability of ILα and the number of R
cell remains low in a value determined by the size of thymic output. When the injected antibody is cleared the system could return back to the autoimmune equilibrium. However, for some intermediate doses of the antibody, the effective IL2 concentration is reduced to values where it is unable to signal on the E and M cells, but it is still significant for the R cells, which are more sensible due to their higher expression of the alpha chain of the IL2 receptor. Thus, for those intermediate doses of mAbs, E cells' number tends to reduce to the values sustained by the available ILα, but the R cells can grow forcing the system to switch into the tolerant steady state.

The model prediction of a higher efficacy of treatments with phase alpha mAbs, to break a preexistent tolerant state, relates to the impact of this type of mAbs on the dynamics of the M cells. Phase alpha mAbs bind the available IL2 forming immune complex that can still signal through the intermediate affinity IL2 receptor (beta + gamma chain). This form of the receptor is prevalent in the M cells, thus phase alpha mAbs partially redirect IL2 signaling into the M cells expanding this population. This effect on the M cells condition some interference with the dynamics of CD4+T cells that dictate the advantages of this type of mAbs. The expansion of M cells contributes to consume the available IL2 and to reduce the capacity of CD4+ T cells to interact with the APCs.

Simulation of IL2/mAb immunocomplexes injections. We also simulate the effects of the injection of immune complexes of IL2 plus anti-IL2 mAbs, which has been recently highlighted as a novel therapeutic strategy (18, 20, 51). Intuitively, one should expect immune complexes to share properties with its basic components: IL2 and anti-IL2 mAbs. Although some of the properties might be potentate by the appropriate combination of these molecules in the immune complexes. This section will thus focus on the search of ‘synergistic’ effects of the immune complexes, as well as, the characterization of how the proportion of mAb/IL2 and the affinity of mAbs used in the complexes influence their properties.

Our model simulations show that, as the therapy with anti-IL2 mAbs, the therapy with immune complexes could modify any preexistent steady state. It could subvert a preexistent tolerant state, taking the system into the autoimmune steady state (Fig. 8A) and it could set back into the tolerant steady state a system initially set in the autoimmune steady state (data not shown). These effects can be obtained with immune complexes, which contain all type of anti-IL2 mAbs. But only in a few conditions they perform better than the therapy based on the anti-IL2 mAb alone. The capacity of immune complex to reset into tolerance a system initiated in the autoimmune steady state always requires larger amounts of mAbs than the one required by a therapy based on the mAb alone (data not shown). This is true irrespective of the type of mAb or the specific proportion mAb/IL2 used in the complex. The capacity of immune complexes to break tolerance taking the system into the autoimmune state is lower than the one of the therapy based on mAbs alone whenever phase beta or fully blocking mAbs are used to form the complexes (data not shown). However, immune complexes formed with phase alpha mAbs, can indeed be more efficient than the mAb alone to promote immunity (Fig. 8B). Such synergistic effect of the immune complexes is observed when the molar proportion mAb:IL2 is around a 1:2 ratio. Complexes with larger or lower amount of IL2

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Fig. 7. Simulations of transient IL2 depletion treatments, using anti-IL2 mAbs that block the interactions of this molecule with the alpha or beta chain of the IL2R. (A and B) Kinetics outcomes where this therapy is able to: (A) break a preexistent tolerant state, inducing an autoimmune response and (B) render tolerant a preexistent autoimmune system. The particular values of dose (DAc) and treatment duration (tac) used in these simulations, corresponding to phase alpha and phase beta mAbs, respectively, are: (A) DAc = 0.2 mg/day, tac = 1 week; (B) DAc = 0.3 mg/day, tac = 15 week. (C and D) Dose dependency predicted for both types of anti-IL2 mAbs: (C) Breakdown of tolerance requires a minimal effective dose and treatment duration. In this scenario, phase alpha mAbs (continuous line) are more efficient than phase beta mAbs (dashed line), (D) Reestablishment of tolerance requires minimal treatment duration and the dose has to be set inside an intermedite range of values. This effect is obtained when applied phase beta mAbs (continuous line), not phase alpha mAbs. (E and F) Dose dependency predicted considering a higher affinity of the antibody ($10^{-11}$ M) (continuous line) or a higher level of thymic output (10-fold) (dashed line): (E) using phase alpha mAbs for breaking tolerance and (F) using phase beta mAbs for reestablishing tolerance. The thin line in both graphics corresponds to the original curve showed in (C) and (D), respectively.
tend to be much less efficient. The affinity of the anti-IL2 mAb in the complexes also influences the latter effect by a simpler rule: higher affinity mAbs are just better (Fig. 8C).

The therapy with immune complexes also inherits some of the properties of the IL2-based therapy. Particularly, the capacity to reinforce a preexistent steady state. Our simulation shows that some immune complexes can be significantly more efficient than IL2 in reinforcing a preexistent tolerant steady state. Figure 8 (D and E) show how the injection for 3 days of immune complexes with a fixed amount of IL2 and variable quantities of mAbs chains the number of E, R and M cells in the lymph node compartment of the models. Interestingly, immune complexes formed with phase beta mAbs (Fig. 8D) induce a quite significant expansion of regulatory T cells (reinforcing the tolerant state). This increment of the regulatory T cells is larger than the one achieved with the injection of IL2 alone and is maximal for a proportion of mAb:IL2 in the complex close to a 1:2 ratio. Relevantly, immune complex formed with phase alpha mAbs have a quite different effect in these simulations (Fig. 8E). They could expand the R cells, but they expand much more the M cells in the lymph node. The capacity of this immune complex to expand M cells became larger as the proportion of mAbs in the complex is increased. Other relevant dependency on the latter effects is the one observed with the affinity of the mAbs for IL2 in the complexes. The capacity of phase alpha-based immune complexes to expand M cells is larger for antibodies of higher affinities (data not shown), but the capacity of phase beta-based immune complex to expand Tregs is maximal for mAbs with some intermediate affinity values (Fig. 8F).

To summarize, the results above show that immune complexes sometimes synergistically potentate the effects of IL2 and mAbs. Interestingly, phase alpha and phase beta
mAbs-based complexes have significantly different properties in this sense. Complexes based on phase alpha mAbs promote immunity primarily by expanding the M cells and leading ultimately to a quite efficient breakdown of a preexisting tolerant steady state. Complexes based on phase beta mAbs can efficiently reinforce tolerance, expanding significantly the R cells preexistent in the tolerant steady state. Although this latter conclusion is only valid for relatively low doses treatment because, as mentioned earlier, significantly higher doses of the same immune complexes (i.e. formed by phase beta mAbs +IL2) expand the M cells leading to a breakdown of tolerance. This apparently counterintuitive, low- versus high-dose dependence of the treatment with immune complexes, is similarly predicted for treatments with IL2 alone (but in much higher doses), explain the results reported by Tang et al. (11). There, using the NOD mice, a similar low- versus high-dose dependence of the treatment with immune complexes formed by IL2+JES6-1A12 mAb was observed to respectively prevent or exacerbate diabetes development.). Both complexes are better on their task, in molar proportions mAb:IL2 close to the 1:2 ratio. Phase alpha mAbs for immune complexes are better with a high affinity, but phase beta mAbs could be better for some intermediate affinity values.

Qualitatively, the effects of immune complexes can be explained based on two main dynamical properties in the model: (i) In the immune complex, the IL2 is protected from degradation. While bind to the mAbs, the IL2 have a live span of 3 days (like the mAbs), which is significantly larger than the live span of 10 min reported for free IL2. (ii) Immune complexes block different sites in the surface of IL2 conditioning its preferential interaction with different cell populations, according to their differential expression of the IL2 receptor chains. Phase alpha mAbs, form immune complexes that bind and signal through the beta + gamma pair of IL2 receptors. Thus, since beta chain is over-expressed by the M cells, this complex preferentially redirect the IL2 signal to these cells. Following this analysis, one could easily explain why this type of immune complex has a maximal efficiency when the affinity of the phase alpha mAbs used is high. With high-affinity mAbs, the IL2 is more protected from degradation, and the signaling is maximally redirected to the M cells. Phase beta mAbs form immune complexes unable to signal in any class of IL2 receptor. Thus, to mediate any biological activity, this type of complex have to partially dissociate, working as a controlled source of free IL2. If the affinity of the phase beta mAbs in the complex is too high then the IL2 is never released and the immune complex have no effect at all. If the affinity of the phase beta mAbs is too low, then injecting the complex is like injecting IL2 alone. However, if the affinity of the phase beta mAbs in the complex is larger than the affinity of the dimeric IL2 receptor (beta+ gamma chain) but lower than the affinity of the trimeric IL2 receptor (alpha + beta + gamma chain), the IL2 in the complex is easily release to provide signal through the high affinity trimeric IL2 receptor but not through the intermediate affinity dimeric IL2 receptor. In this way, the phase beta-based immune complexes provided a preferential signaling to the regulatory cells, which over-express the alpha chain of the IL2 receptor.

Discussion

The effect of therapies based on Interleukin-2 (IL2) modulations in clinical and pre-clinical experiments is still not well understood. The observed complexity most likely derive from the dual role that this cytokine has in the dynamics of T cells. To explain the contradictory results observed in these therapies, we develop a mathematical model based on the most relevant interaction involved in the dynamics of IL2 and helper, regulatory and memory T cells. This model is based on previously developed in our group (27). We simulate the effect of three types of therapies: IL2 injection, IL2 depletion using anti-IL2 antibodies and IL2/anti-IL2 complex injection. For each treatment case study, the concordance of our result with available clinical and pre-clinical observations is discussed, and the implications for a better treatment design or application are derived.

About the effect of IL2 injections

The model predicts that IL2 injections will always reinforce the preexistent steady state in the system. These model predictions are indeed compatible with existent experimental observations and further provide a guideline for its future practical application.

In the one hand, the reinforcement of ongoing immune reactions by IL2 injections, predicted by the model, explains classical observations on in vivo animal models, where IL2 have been shown to potentate immune reactions to viral infection (52) and to well-adjuvated vaccines (1–3). In these systems, the immune response induced to the involved foreign antigens, which are most probably loosely or just not controlled by regulatory T-cell activity, is further promoted by the injected IL2. Furthermore, the observed enhancement of immunity, in these experimental systems, might not relay just on the model predicted expansion of helper CD4+ T cells. It might also involve important direct effects of IL2 on memory CD8+ T cell and/or NK cells, which are known to be relevant in many of these particular systems. In any case, the model here will further predict that optimal application of IL2 for the purpose of enhancing immunity, will be obtained when providing IL2 after the immune reaction have already started and never before because some reminiscent of immune regulation might still exist and could be potentate by the added IL2.

On the other hand, the capacity of IL2 addition to reinforce natural tolerance mediated by regulatory T cells, predicted by the model, explains as well several experimental observations. Particularly, it explains clinical data stating that regulatory T-cells populations are significantly expanded, both in cancer (9, 53) and HIV (54) patients, treated with IL2. Such effect might be related to the poor efficacy observed in these clinical applications of IL2. Particularly, in the case of cancer, <20% of the treated patients show some anti-tumor effect, perhaps, according to the model here because just an small fraction of the patients happen to have a naturally preexistent immune response against tumor antigens, which could be further enhanced by the injected IL2. In the case of HIV patients, IL2-based therapy have led to the recovery of CD4+ T-cells counts, but the patients do not seem to recover their capacity to fight general infections, perhaps, according
to the model here because this treatment is just reinforcing tolerance mediated by regulatory T-cell activity.

Furthermore, this second model prediction also explains many results in pre-clinical animal models. It explains, for instance, that IL2 injections can prevent allograft rejection (10), attenuate the induction of experimental autoimmune encephalomyelitis (EAE) (10), or fully prevent the development of diabetes in the NOD mice (11). Interestingly, in the EAE and allograft reaction models, the latter effects are observed for scheme of IL2 applications where this cytokine is injected in the system before implanting the allogeneic tissue or before inducing the EAE. This is before there will be an ongoing immune/autoimmune reaction in the system, thus when preexistent natural tolerance mediated by regulatory T cells could be reinforced by the applied treatment. However, in the NOD mice model, recent data (12) have shown that IL2 treatment at the onset of diabetes could revert disease development. Interestingly, in this ‘therapeutically relevant scenario’ treatment efficacy is much lower than in the preventive settings. Only 40–60% of the NOD mice appear to be cured, while 100% of the NOD mice are cured when treating in the preventive settings. Whether or not at the onset of NOD mice diabetes, the balance between regulatory T cells and effectors T cells have been fully disrupted in favor of immunity, just as considered in our model simulations of an autoimmune disease therapeutic scenario, is a matter of discussion. Actually Grinberg-Bleyer et al. have shown that at the onset of NOD diabetes a significant amount of regulatory T cells can still be found in the pancreas and its draining lymph node. Unfortunately in the NOD mice, the acute nature of diabetes development (with a full irreversible destruction beta islet) invalidate any moving of treatment application toward a more advanced stage of the disease, to better compare with our model predictions.

About the effect of anti-IL2 mAbs injections

In the model, simulations of IL2 depletion treatments, using anti-IL2 mAbs, predict a dual role of this type of treatment. In this sense, is obtained that this therapy is able to break a preexistent tolerant state, inducing an autoimmune response or to render tolerant a preexistent autoimmune system. More interestingly, the latter model predictions are indeed compatible with existent experimental observations. On the one hand, the predicted capacity of treatments blocking IL2 activity to promote autoimmune/immunity explains observations where monoclonal antibodies against IL2 have been shown to promote effective immune responses to tumors (16) and to induce autoimmune disease in mice (13). In both cases, the model explains the observed effects as being associated to the treatment capacity to weaken regulatory cell activity, just as argued by their original authors. Moreover, the model suggests that the thymic activity lowers the pathologic effect of this therapy, by an apparent tolerating effect of thymic output. As far as we know, this last prediction has not been tested experimentally. Although, the role of thymus reinforcing peripheral regulation mediated by regulatory T cells has been argued (55, 56). In those works, adult thymectomy markedly increases the susceptibility of mice to immunosuppression-dependent autoimmunity.

On the other hand, the model predicted capacity of IL2 blocking therapies to reestablish tolerance in the context of ongoing immune/autoimmune reactions is less well documented in the literature. This prediction could explain some experimental data, which show a positive effect of anti-IL2 antibodies in the treatment of atherosclerosis (a disease of elderly people involving inflammation in the arteries). However, one must be aware that in the latter observation a direct participation of regulatory T cells, as proposed by the model here, has not been proved.

In any case, this model prediction is very interesting from the practical perspectives for the treatment of autoimmune diseases. However, the fact that the predicted treatment effect just occurs in the simulation for a particular intermediate range of drugs doses, applied during a relatively long period, makes difficult to implement this strategy. To overcome this difficulty, the model additionally suggests that an effective strategy to apply this therapy could be to use a large initial dose of the mAb, reducing it periodically with a low rate. Particularly, the model predicts that the initial mAb dose used must be large enough to induce significant immunosuppression, and the dose must be reduced by half every month.

About the effect of IL2/mAb immunocomplexes injections

Results obtained from simulations of IL2/mAb immunocomplexes injections predict different properties of the complexes depending on the type of mAb based. In this sense, is obtained that complexes based on phase alpha mAbs are efficient in breaking preexistent tolerant state and promote immunity, expanding the memory cells. On the other hand, complexes based on phase beta mAbs reinforce tolerance expanding the regulatory T cells.

The model results explain available experimental data on the use of immune complex. Our observations that immune complexes formed with phase alpha or phase beta mAbs expand different cell populations when injected in vivo into a normal (tolerant) mouse are fully compatible with the results reported in (18, 20, 51). In these experiments, the S4B6 mAb (a phase alpha mAbs) is shown to form immune complexes that strongly expands CD8+CD44+ T cells and to a lesser extent the R cells (20). This phase alpha immune complex has been used to promote immunity in combination with vaccines (17), showing a significantly higher efficiency than IL2 alone. Moreover, the group of Jonathan Sprent have shown that JES6-1 (a phase beta mAbs) induce a larger expansion of Tregs (CD4+CD25+Foxp3+ T cells) than the injection of IL2 alone in the same experimental setting (20). This other type of complex has been shown to reinforce preexistent tolerance, preventing graft rejection or autoimmune disease induction (18) in some pre-clinical model.

The simulations, however, propose some interesting guidelines to improve the therapeutic effect of immune complexes. They predict that in the case of complexes using phase alpha mAbs, the best strategy is to use mAbs with the higher affinity available. But in the case of immune complexes formed with phase Beta mAbs, the use of intermediate affinity mAbs is recommended. Other important prediction of our model simulations is that treatment with immune complexes based on phase beta mAbs are useful to
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reinforce a preexistent tolerant state preventing the induction of autoimmunity, but it would be quite inefficient to therapeutically treat an already established autoimmune disorder. For the latter task, the best strategy would be to use anti-IL2 mAbs alone following the strategies described in section 2.3.2.

Severe toxicity, i.e the appearance of the cytokine storm and the vascular leak syndrome, is perhaps the major limitation knows today for the practical application of IL2 modulation treatments in the clinics. Our model cannot be used to simulate directly the toxic effects of different IL2 modulation treatments. It could only be used to predict strategies that optimize the expected therapeutic efficacy regarding the balance between regulatory and effector CD4+ T cells. Interestingly, treatments using immune complex formed by IL2 plus phase alpha mAbs anti-IL2 have been recently shown as less toxic than IL2 injections in animal models. A recent report by the group of Boyman (57) have shown that vascular leak syndrome, which leads to severe pulmonary edema, is caused by the direct interaction of IL2 with its high-affinity receptor expressed in lung epithelial cells. They demonstrated that treatment with immune complexes of IL2 plus S4B6 mAbs (anti-IL2 mAb which interferes the union of IL2 to the alpha chain of IL2 receptor) prevents vascular leak syndrome while induces a potent antitumor response. This experimental observation supports the practical feasibility of some of our model predictions. Particularly, the use of immune complex formed by IL2 plus phase alpha mAbs anti-IL2 as a better strategy than IL2 injections to promote immunity. Whether the potential use of other types of IL2-mAbs immune complex or anti-IL2 mAbs alone, will face limitations on its clinical application derived from their toxicity, could be matter of futures studies.

Concluding remarks

Mathematical modeling of the IL2 and T-cells dynamics, considering the dual role of IL2 in their interaction with regulatory and helper CD4+ T cells, results sufficient to explain the complexity observed in the effect of IL2 modulating treatments. In this sense, we show that the model explain a large amount of available clinical and pre-clinical data. Moreover, it predicts optimal strategies for the future application of these treatments:

A) Immune complexes IL2/anti-IL2 mAbs, using mAbs that block the interaction of IL2 and CD25 (the alpha chain of IL2 receptor), are the best strategy to potentiate immunity alone or in combination with vaccines. These complexes are optimal when a 1:2 molar ratio of mAb:IL2 is used and the mAb has the largest possible affinity.

B) Immune complexes IL2/anti-IL2 mAbs, using mAbs which block the interaction of IL2 and CD122 (the beta chain of IL2 receptor), are the best option to reinforce preexistent natural tolerance, for instance to prevent allograft rejection. These complexes are optimal when a 1:2 molar ratio of mAb:IL2 is used and the mAb has intermediate affinities.

C) Phase beta or fully blocking mAbs anti-IL2 can be successfully used alone to treat an ongoing autoimmune disorder, promoting the reinduction of tolerance. The best strategy in this therapy is to start treatment with an initially high dose of the mAbs (one capable to induce some immune suppression) and then scales down slowly the dose of mAb in subsequent applications.

Appendix A.

Equations for the dynamics in the blood compartment

The dynamics of the number of molecules contained in the blood compartment (IL2, anti-IL2 mAbs and immune complexes of IL2 and anti-IL2 mAbs) is modeled using the following equations:

\[ \frac{d\text{IL2}_{S}}{dt} = K_{on}^{\text{IL2}} \cdot \text{IL2}_{S} - K_{off}^{\text{IL2}} \cdot \frac{\text{fve} \cdot \text{IL2}_{N}}{V_{S}} \cdot \text{IL2}_{S} \cdot \text{Ab_{S}} + N_{IL2} \cdot \left( D_{IL2} \cdot \frac{\text{IL2}_{S}}{\text{fve} \cdot \text{IL2}_{N}} - D_{IL2} \cdot \frac{\text{IL2}_{S}}{V_{S}} \right) - K_{di} \cdot \text{IL2}_{S} + \Gamma_{IL2} \]  \hspace{1cm} (A1)

\[ \frac{d\text{Ab}_{S}}{dt} = K_{on}^{\text{Ab}} \cdot \text{IL2}_{S} - K_{off}^{\text{Ab}} \cdot \frac{\text{fve} \cdot \text{Ab}_{N}}{V_{S}} \cdot \text{IL2}_{S} \cdot \text{Ab_{S}} + N_{IL2} \cdot \left( D_{Ab} \cdot \frac{\text{Ab}_{S}}{\text{fve} \cdot \text{IL2}_{N}} - D_{Ab} \cdot \text{Ab}_{S} \right) - K_{di} \cdot \text{Ab}_{S} + \Gamma_{Ab} \]  \hspace{1cm} (A2)

\[ \frac{d\text{IL2}_{Ab}^{\text{IL2}}}{dt} = - K_{on}^{\text{IL2}} \cdot \text{IL2}_{S}^{\text{IL2}} + K_{off}^{\text{IL2}} \cdot \frac{\text{fve} \cdot \text{IL2}_{N}}{V_{S}} \cdot \text{IL2}_{S} \cdot \text{Ab_{S}} + N_{IL2} \cdot \left( D_{IL2Ab} \cdot \frac{\text{IL2}_{S}^{\text{IL2}}}{\text{fve} \cdot \text{IL2}_{N}} - D_{IL2Ab} \cdot \text{IL2}_{S}^{\text{IL2}} \right) - K_{di} \cdot \text{IL2}_{S}^{\text{IL2}} \]  \hspace{1cm} (A3)

The dynamics of IL2 (IL2_{S}), and anti-IL2 mAbs (Ab_{S}) number is modeled using equations (A1) and (A2), respectively, while the dynamics of the number of immune complexes (IL2_{Ab}^{\text{IL2}}) is modeled by equation (A3). The variables and parameters involved in (A1–A3) are defined in Table 1. Parameters values were taken directly from, or indirectly estimated using, experimental data reported in literature (as explained below in Appendix D).

Equations (A1–A3) consider the increase in the number of IL2 and mAbs in the blood due to the dissociation process of immune complexes with a constant rate (K_{off}^{\text{IL2}}), which corresponds to a decrease in the amount of these complexes (first term in equations (A1–A3)). The process of formation of immune complexes, through the association of IL2 and mAb with a constant rate (K_{on}^{\text{IL2}}), is taken into account in the second term in equations (A1–A3). The exchange of molecules between blood and peripheral lymph nodes is modeled as a simple diffusion process that balance the molecule concentrations in both compartments (third term in equations). The number of molecules circulating in the blood decays exponentially with a constant rate (K_{di}, K_{off}) due to renal elimination in kidney (fourth term in equations). Finally, an external source for IL2 and mAbs is considered, which causes an increase in the number of these molecules in the blood (last term in equations (A1) and (A2)).
Appendix B.

Equations for the dynamics of T cells in the lymph node compartment

The dynamics of the number of T cells in the lymph node compartment is modeled as appears in the following equations:

The dynamics of the number of T cells in the lymph node compartment

\[
\frac{dE_N}{dt} = \Gamma_s - K_A^E \cdot E_N^B \cdot \left(1 - \frac{R^B}{s \cdot A}\right)^{\left(s-1\right)} + \alpha_E \cdot K_S^E \cdot \left(1 - \frac{(\text{SigE})^n}{(S_E)^n + (\text{SigE})^n}\right) \cdot E_A + 2 K_S^E \cdot E_C - K_S^E \cdot E_N^B,
\]

\[
\frac{dE_A}{dt} = K_A^E \cdot E_N^B \cdot \left(1 - \frac{R^B}{s \cdot A}\right)^{\left(s-1\right)} - K_S^E \cdot E_A,
\]

\[
\frac{dE_C}{dt} = K_S^E \cdot \left(\frac{(\text{SigE})^n}{(S_E)^n + (\text{SigE})^n}\right) \cdot E_A - K_S^E \cdot E_C,
\]

\[
\frac{dR_N}{dt} = \Gamma_r - K_A^R \cdot R^B + \alpha_R \cdot K_S^R \cdot \left(1 - \frac{(\text{SigR})^n}{(S_R)^n + (\text{SigR})^n}\right) \cdot R_A + 2 K_S^R \cdot R_C - K_A^R \cdot R^B + K_R^B \cdot R^B - K_S^R \cdot R^B + K_S^R \cdot R^B,
\]

\[
\frac{dR_A}{dt} = K_A^R \cdot R^B - K_S^R \cdot R^B + R^B - K_S^R \cdot R^B + K_S^R \cdot R^B,
\]

\[
\frac{dM_A}{dt} = - K_S^M \cdot \left(\frac{(\text{SigM})^n}{(S_M)^n + (\text{SigM})^n}\right) \cdot M_A + 2 \cdot K_S^M \cdot M_C - K_S^M \cdot M_A,
\]

\[
\frac{dM_C}{dt} = K_S^M \cdot \left(\frac{(\text{SigM})^n}{(S_M)^n + (\text{SigM})^n}\right) \cdot M_A - K_S^M \cdot M_C,
\]

\[
\frac{dE_N}{dt} = \frac{E^B}{V_{LN}} \cdot \left(E^F \cdot F\right), \quad \frac{dR_A}{dt} = \frac{R^B}{V_{LN}} \cdot \left(R^F \cdot F\right), \quad \frac{dM_C}{dt} = \frac{M^B}{V_{LN}} \cdot \left(M^F \cdot F\right),
\]

Equations for the dynamics of molecules in the lymph node compartment

The dynamics of the number of molecules circulating in the lymph node compartment (IL2, IL2r, and complex molecules) and the number of complexes IL2-IL2r and IL2-mAb-IL2r formed in a single cell membrane is modeled as follows:

\[
\frac{dIL2}{dt} = K_{IL2} \cdot \left(\text{IL2} \cdot \text{IL2r} - \text{K}_{IL2} \cdot \text{IL2} \cdot \text{IL2r}\right) + \sum_{j} K_{IL2} \cdot \left(\text{C}^E \cdot \text{E}_j + \sum_{j} (C^R \cdot R) + \sum_{j} (C^M \cdot M)\right) + \sum_{j} K_{IL2} \cdot \left(\sum_{j} (P^E \cdot E) + \sum_{j} (P^R \cdot R) + \sum_{j} (P^M \cdot M)\right) + K_{IL2} \cdot \left(\text{IL2} \cdot \text{IL2r}\right) \left(1 - \frac{R^F}{s \cdot A}\right)^{\left(s-1\right)}.
\]
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\[
\frac{d\text{IL}2}{dt} = K_{\text{off}} \cdot \text{IL}2 - K_{\text{on}} \cdot \text{IL}2 \cdot \text{Ab} + \left(D_{\text{off}} \cdot \bar{b}_{\text{on}} - D_{\text{off}} \cdot \bar{b}_{\text{off}} \right)
+ \sum_i \left(1 - N_i \right) \cdot \left( K^i_{\text{off}} \cdot \sum_j \left(C_{\text{Ab}^i}^j \cdot E_j + C_{\text{Ab}^i}^j \cdot R_j + C_{\text{Ab}^i}^j \cdot M_j \right) - \right)
- \sum_i \left(1 - N_i \right) \cdot \left( K^i_{\text{on}} \cdot \text{Ab} \cdot \sum_j \left(C_{\text{Ab}^i}^j \cdot E_j + C_{\text{Ab}^i}^j \cdot R_j + C_{\text{Ab}^i}^j \cdot M_j \right) \right),
\]

(C2)

\[
\frac{d\text{P}^E}{dt} = K_{\text{off}}^E \cdot \text{IL}2 + K_{\text{on}}^E \cdot \text{IL}2 \cdot \text{Ab} + \left( D_{\text{off}}^E \cdot \bar{b}_{\text{on}} - D_{\text{off}}^E \cdot \bar{b}_{\text{off}} \right)
+ \sum_i (1 - N_i) \cdot K^i_{\text{off}}^E \cdot \sum_j \left( C_{\text{Ab}^i}^j \cdot E_j + \sum_j \left( C_{\text{Ab}^i}^j \cdot R_j + \sum_j \left( C_{\text{Ab}^i}^j \cdot M_j \right) \right) \right)
- \sum_i (1 - N_i) \cdot K^i_{\text{on}}^E \cdot \sum_j \left( C_{\text{Ab}^i}^j \cdot E_j + \sum_j \left( C_{\text{Ab}^i}^j \cdot R_j + \sum_j \left( C_{\text{Ab}^i}^j \cdot M_j \right) \right) \right).
\]

(C3)

The dynamics of the number of IL2 (IL2), mAbs (Ab) and immune complexes (IL2Ab) in the lymph node is modeled using equations (C1–C3), while the dynamics of the number of IL2-IL2R complexes (C_{\text{SigM}}^E, C_{\text{SigE}}^E, T^E) and IL2-IL2R-mAbs complexes (C_{\text{SigM}}^E, C_{\text{SigE}}^E) per cell is modeled following equations (C4–C6) and (C7) and (C8), respectively. Note that, to simplify, we only present here the equations corresponding to the IL2 complexes formed at the E cell membrane. Equivalent equations are obtaining for R and M cells.

Algebraic relations are provided in (C9) and (C10), for the amount of free alpha (P^E_{\alpha}) and beta chains (P^E_{\beta}) of the IL2 receptor per E cell, and the mean number of bound cytokines signaling receptors per activated E (SigE), R (SigR) and M (SigM) cell. Note that, the terms SigE, SigR and SigM in (C10) are the one used in the equations for the dynamics of T cells, related with the process of cells receiving cytokine-derived signals from IL2 or alternative cytokines (see Appendix B). The variables and parameters used in (C1–C10) are defined in Tables 1–3.

In equations (C1–C3), the processes of dissociation (first term in equations) and formation (second term in equations) of immune complexes, and the exchange of molecules between blood and peripheral lymph nodes (third term in equations), are modeled analogously as was done previously for IL2, mAbs and immune complexes in the blood compartment (see Appendix A). Additionally, due to the presence of T cells in the lymph node, we consider in these equations the dissociation and association processes of IL2-IL2R and IL2-mAb-IL2R complexes, formed by free IL2 alone or as immune complexes with the alpha or beta IL2R chains in the cell membranes. In this sense, is taken into account the increase in the amount of free IL2 and immune complexes, due to the dissociation process of IL2-IL2R and IL2-mAb-IL2R complexes, respectively (fourth terms in (C1) and (C3)). On the other hand, the association of free IL2 and immune complexes to free alpha or beta IL2R chains is considered to increase the amount of IL2-IL2R and IL2-mAb-IL2R complexes (fifth terms in (C1) and (C3)). Additionally, is modeled the processes where mAbs can be dissociated from IL2-mAb-IL2R complexes, increasing the number of free mAbs (fourth terms in (C2)); and the process where free mAbs associate to IL2-IL2R complexes in the cell membrane (fifth terms in (C2)).

The symbolic labels \(l\) and \(j\), appearing in the fourth and fifth terms in equations (C1–C3), denote, respectively, the functional state of the cell (\(l = N\): resting, \(l = A\): activated, \(l = C\): cycling) and the different IL2R chains (\(j = \alpha\) alpha chain and \(j = \beta\) beta dimer chain). Finally, the production of IL2 endogenous by activated E cells, which can be inhibited during cell activation by the presence of R cells coconjugated in the same APC, is considered to increase the amount of this cytokine in the lymph node (last term in (C1)).

The formation of high affinity IL2-IL2R complexes in a cell membrane is modeled as a two step process, using equations (C4–C6). First, free IL2 binds to the available free alpha or beta chains of the IL2R, forming the intermediate or low affinity IL2-IL2R complexes, respectively (first term in (C4) and (C5)), as mentioned above for the dynamics of IL2. By the unbound process, is recovered free molecules and receptor chains (second term in (C4) and (C5)). The
The formation of IL2-mAb-IL2R complexes in the cell membrane is modeled in equations (C7) and (C8) and in the fifth term in (C4) and (C5). In this sense, we consider the association and dissociation processes of free immune complexes with the alpha or beta IL2R chains (first term in (C7) and (C8)) and so that for free mAbs with IL2-IL2R complexes in the cell membrane (first term in (C4) and (C5) and second term in (C7) and (C8)). The possibility of formation of intermediate or low affinity IL2-mAb-IL2R complexes depends on the IL2 interface that mAbs recognize (controlled in simulations by the parameter \(N\), see Table 3). We don’t consider the formation of high affinity IL2-mAb-IL2R complexes, due to association of antibodies with the high affinity IL2-IL2R complexes or the association of intermediate or low affinity IL2-mAb-IL2R complexes with the remaining IL2 receptor chain because we are studying mAbs that bind to the alpha or beta interface of the IL2 which will block the formation of these complexes. Finally, the internalization of IL2 as immune complexes bound to the beta chains of IL2Rs in the cell membrane is also modeled (last term in (C8)).

Appendix D.

Parameter estimation criteria

The mathematical model developed contains 69 parameters (see Tables 1–3), which are fixed in the model simulations. The values of 40 of these parameters were taken directly from data reported in literature or obtained in our group. Other 16 parameters were indirectly estimated from experimental data available in literature. For the remaining 13 parameters, we simulate a wide range of possible values and studied for which are preserved the qualitative predictions of the model (see the range of values reported in Tables 1–3). Below, we explain how each parameter values were selected.

Parameter values taken directly from existing data

The typical lymph node compartment is set to represent an inguinal lymph node of a mouse with a volume (\(V_{LN}\)) of 1 \(\mu l\) and containing about 10^5 APCs (A). This lymph node sustains about 10^7 CD4 T cells in normal, non-autoimmune and non-infected conditions. Therefore, 10 identical lymph nodes compartments (\(N_{LN}\)) are included in the simulations, to guarantee a total of about 10^8 CD4+ T cell in the system, just as estimated for a normal, non-autoimmune and non-infected mouse. The source terms for new CD4+ T cells is known to be about 10^6 new cells per day, as estimated in normal mice (58). From the new CD4+ T cells, a small fraction (5%) is assumed to be significantly autoreactive. Half of this new autoreactive T cells are assumed to be of the regulatory phenotype (\(\Gamma_a = \Gamma_r = 2.5 \times 10^5\) cells/day).

The volume of the blood compartment (\(V_s\)) is set to 2.5 ml, which corresponds to the volume of blood in a young 20 g mouse. The degradation rates in blood for IL2 (\(Kda\)) and anti-IL2 mAbs (\(Kda\)) are set following biodistribution data obtained in mice (\(Kdi = \text{Ln}(2)/10\) min for IL2 (38) and \(Kda = \text{Ln}(2)/3\) days for mAbs (39)).

The number of IL2R beta chains (\(\Gamma_{IL2R}^\beta\)) in CD4+ T cells is determined to be 10^3 receptors/cell (44) (\(\Gamma_{IL2R}^\beta = 10^3\)). By contrast, in memory CD8+ T cells, the expression of IL2Rβ is assumed to be 0-fold higher than on CD4+ T cells, following experimental results for expression of IL2R in memory phenotype CD8+ T cells (45) (\(\Gamma_{IL2R}^\beta = 10^5\)). The levels of IL2R alpha (IL2Rα) in CD8+ memory T cells and also in resting CD4+ helper T cells are low or undetectable (45) (\(\Gamma_{IL2R}^\alpha = 10^3\)). On the contrary, in resting CD4+ regulatory T cells, the number of IL2Rα is high, being estimated experimentally by different groups to be around 10^3 (46) or 10^4 receptors per cell (44), while under activation this number can increase by about one order of magnitude (47) (\(\Gamma_{IL2R}^\alpha = 10^4\)). Activated CD4+ helper T cells also express high amount of IL2Rα, however it remain below the peak value seen on regulatory T cells (35) (\(\Gamma_{IL2R}^\alpha = 10^5\)). For cycling, CD4+ T cells are assumed a decrease in the amount of IL2Rα (35), fixing the related parameters values in \(\Gamma_{IL2R}^\alpha = 10^3\), \(\Gamma_{IL2R}^\alpha = 10^4\).

The affinity of IL2 binding to the different chains of the IL2R has been determined experimentally using different methods. The dissociation constant of IL2 to IL2Rα (\(K_{d}^{\alpha}\)) has been estimated to be of the order of 10^{-1} s^{-1} (59, 60), using Biacore’s SPR technology or experiments of dissociation kinetics of cells that express only this chain; although, some other authors have also determined values of the order of 10^{-2} s^{-1} (61, 62). The association constant of IL2 to IL2Rα (\(K_{a}^{\alpha}\)) has been estimated between 10^{7}–10^{9} M^{-1} s^{-1} (59–62). The disso-

CD4+ helper and regulatory T cells exhibit the same internalization kinetics.

The dissociation and association constant of anti-IL2 mAb to IL2 are fixed, in treatment simulations, to the values measured by our group (in preparation). Using Biacore’s SPR technology were determined these constants for mAbs usually in experiments reported in the literature: phase alpha anti-IL2 mAb (JES6-5H4: $K_{\text{off}} = 1.8 \times 10^6 \text{M}^{-1} \text{s}^{-1}$, $K_{\text{on}} = 2.6 \times 10^{-4} \text{s}^{-1}$; S46B: $K_{\text{off}} = 1.5 \times 10^5 \text{M}^{-1} \text{s}^{-1}$, $K_{\text{on}} = 1.4 \times 10^{-4} \text{s}^{-1}$) and phase beta anti-IL2 mAb (JES6-1A12: $K_{\text{off}} = 2.3 \times 10^4 \text{M}^{-1} \text{s}^{-1}$, $K_{\text{on}} = 6.6 \times 10^{-5} \text{s}^{-1}$). These are mAbs of intermediate affinity ($10^{-9}$–$10^{-10}$ M), although in the model simulations were also probed the effect of mAbs with low ($10^{-14}$ M) or high affinity ($10^{-11}$ M), as appears in Figs 7 and 8 in the main text.

Parameter values estimated indirectly from experimental data available in literature

The division rates of cycling E and R cells ($K_1^E$, $K_1^R$) are assumed equals, corresponding with a mean time of the process between 4 and 6 h ($K_1^E = K_1^R = \ln(2)/4 \text h$), as estimated in (40). For the activation ($K_2^E$, $K_2^R$) and IL2 signaling-waiting ($K_3^S$, $K_3^S$) rates we found no data reported. Although we indirectly estimate these values considering certain restrictions for them, as explained below. We assume that the IL2 signaling-waiting rate is greater than the other two rates, considering for it a range of values that correspond to a mean time of $1 - 2 \text h (K_3^E = K_3^S = \ln(2)/2 \text h)$, equal for E and R cells. The activation rate is assumed to significantly differ between E and R cells (27). To estimate a range of possible values for it in both types of cells, we consider that the division process occurs with a total doubling time from 6 to 20 h (63). Then, for E cells, we assume an activation rate corresponding to a mean time of 2 h ($K_2^E = \ln(2)/2 \text h$), to complete a total doubling time of approximately 7–10 h; while for R cells, we take 6 h ($K_2^R = \ln(2)/6 \text h$), to complete a total doubling time of 11–14 h. For M cells, we consider that the division ($K_3^M$) and IL2 signaling-waiting ($K_3^S$) rates must be in a range that allows to complete the division process with a total doubling time of 8 h (equal to that for E cells). In this sense, we fixed the value for division rate equal as for cycling E and R cells ($K_3^M = \ln(2)/4 \text h$) and calculate the corresponding IL2 signaling-waiting rate satisfying the condition for the total doubling time ($K_3^S = \ln(2)/4 \text h$).

Conjugation constants of T cells to APCs sites ($K^C$ and $K^M$) are estimated using data reported in the literature from in vivo two-photon real-time imaging of T cells and antigen-bearing dendritic cell in lymph nodes, assuming the same values for E and R cells ($K^C = K^M = K$). The dissociation rate ($K_{\text{off}}$) of T cells and APC sites is estimated using data on the distributions of the durations of effective contacts between T cell and DCs (41). This data show a mean contact time of around $t_{\text{cont}} = 20 - 30 \text{ min}$, providing an estimate of $K_{\text{off}} = \ln(2)/t_{\text{cont}} = 6 \times 10^{-4} \text s^{-1}$ (for $t_{\text{cont}} = 20 \text{ min}$). The association constant is obtained from the estimates provided in (42), once properly divided by the cell volume, $K_{\text{on}} = 10^{-13} \text L \text s^{-1} \text cell^{-1}$. This estimation of $K_{\text{on}}$ is precisely for CD8+ T cells, but here we assume this value to be similar for CD4+ T cells. Finally, the $K_{\text{on}}$ and $K_{\text{off}}$ values are combined to obtain our estimate of $K = K_{\text{on}}/K_{\text{off}} = 10^{-10} \text L \text cell^{-1}$. For the memory-phenotype CD8+ T cells, we assumed lower conjugation constant given the fact that, for the homeostasis of this population, T-cell receptor contact with self-peptide (MHC-I ligands) is weak and relative unimportant (43). In simulations, we fix the conjugation constant of this population to be one order lower than for CD4+ T cells, setting the value of the dissociation rate to $K_{\text{off}} = \ln(2)/t_{\text{cont}} = 6 \times 10^{-3} \text s^{-1}$ (for $t_{\text{cont}} = 2 \text{ min}$).

The rate of IL2 production per activated E cell ($K_{\text{pi}}$) is estimated from the experimental data provided in (37). Therefore, the amount of IL2 in culture of CD4+CD25– T cells at different times was detected by ELISA, after stimulation with DC-OVA. Using this data, the value of this parameter is estimated as $10^5 \text{ molec h}^{-1}$. This value is below, but in the order of, the maximal single-cell secretion rate estimated by Feinerman et al. (44), who used experimental measurements from (64). Also, from the study of the model behavior in a reasonable range of values for this parameter and $i_{\text{ix,E}}$ (see Fig. 5C), it was obtained that $K_{\text{pi}}$ have to be set to a sufficiently high value ($K_{\text{pi}} > 10^2 \text{ molec h}^{-1}$) to ensure bistability in model dynamics.

The sensitivity threshold for the helper T-cell response to IL2 signaling ($S_E$) is estimated from the experimental data provided in (34). There, an in vitro dose–response curve of synchronized T-cells proliferation in response to IL2 addition is provided, being appropriately matched to the study of IL2 receptors occupancy in the same cells. This study shows a proliferative response, with a typical sigmoid shape and a sensitivity which range from 1 pM to 100 pM of free IL2 concentration. For a free IL2 concentration of 10 pM, which leads to half of IL2R bound to IL2, half of maximal T-cells proliferation is obtained; for a free IL2 concentration of 100 pM, which leads to 90% of the IL2R bound to IL2, 91% percent of the maximal T-cell proliferation is scored; and for a free IL2 concentration of 1 pM, which leads to 10% of the IL2R bound to IL2, 0.1% percent of the maximal T-cell proliferation is scored. The latter relation is used to roughly adjust our sigmoid response curve for Eα cells response to bound IL2 receptor, estimating $S_E = 500$ and $h = 4$. We finally extrapolate these values for R and M cells, presuming similar sensitivity of CD4+ and CD8+ T cells to IL2 signal ($S_M = S_R = S_E$).

Parameter values simulated in a wide range

For the parameters ILα and ILm (corresponding to the number of signaling receptors bounds to an alternative cytokine (not IL2)), we haven’t found any reference value with a simple biological meaning or experimentally determined. In this case, we have set the values of these parameters to achieve biologically expected behavior ($ILα = 10^6$, $ILm = 10^7$). We note that ILα and ILm has to be set in some intermediate parameter range to guarantee bistability in model behavior. However, the precise range of values admitted strongly depends of other parameter values, particularly the value of $K_{\text{pi}}$ (see Fig. 5C in the main text).

For the remaining parameters in the model ($D_{\text{i,j}}$, $D_{\text{ab}}$, $f_{\text{ve}}$, $s$, $s_{\text{E}}, s_{\text{R}}, K^E_1$, $K^R_1$, $K^M_1$, $K_{\text{on}}$), we haven’t found any experimental values reported in the literature. For this reason, we study the behavior of the system in a reasonable and wide range of values for these parameters. The diffusion rates of IL2 ($D_i$)
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and mAbs (D_ab), between the blood and peripheral lymph nodes, are fixed assuming that this kinetic process occur in a half-time of 2 h for molecules and mAbs; although we do not get different qualitative results assuming rates 10-fold faster (which corresponds to a half-time of 12 min). We should note that this parameter control quantitative predictions of the model, increasing the minimal effective doses of treatments for slower diffusion rates. The same robust qualitative behavior is obtained assuming different values for the free volume fraction inside the lymph node (fve = 0.1–1), in which molecules and mAbs can diffuse. In the model simulations presented in the main text, this value is fixed assuming that the diffusion process occurs in a volume which is 10-fold lower than the total volume (fve = 0.1). For the number of conjugation sites on APCs (s), we obtain similar model behavior in a biological reasonable range of values varying from 2 to 8, fixing the value at 5 in the simulations article. For the fraction of activated CD4+ helper and regulatory T cells, that return to the resting state without receiving the IL2-related signal (x_a, x_b), it is found that, for any value in the possible range between 0 and 1, the model shows bistability. Simulations presented in the article were done setting this parameter to the value 0.95. For the death rate of T cells (KR), it is found that, for any value in the possible range between 0 and 1, the model shows bistability. Simulations presented in the article were done setting this parameter to the value 0.95. For the death rate of T cells (KR), it is found that, for any value in the possible range between 0 and 1, the model shows bistability. Simulations presented in the article were done setting this parameter to the value 0.95.
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