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Guillaume Churlaud, Chadi Abbara, Pierre-Axel Vinot, Gwladys Fourcade, Paul-Gydeon Ritvo, et al.. Pharmacodynamics of regulatory T cells in mice and humans treated with low-dose IL-2. Journal of Allergy and Clinical Immunology, 2018, 142 (4), pp.1344-1346.e3. 10.1016/j.jaci.2018.06.006 . hal-01957945

HAL Id: hal-01957945

https://hal.sorbonne-universite.fr/hal-01957945

Submitted on 17 Dec 2018

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Pharmacodynamics of regulatory T cells in mice and humans treated with low-dose IL-2

To the Editor:

IL-2 is the major cytokine controlling homeostasis and function of regulatory T (Treg) cells.[1] At low dose, IL-2 specifically activates Treg cells and improves autoimmunity in mice and humans.[2-4] As autoimmune diseases (AIDs) intrinsically reveal Treg-cell insufficiency,[2] IL-2 has broad potential for their treatment. Because Treg cells control all immune responses and inflammation, low-dose IL-2 (ld-IL2) also has therapeutic potential for inflammatory diseases (IDs)[2] and allergy[5] and in the field of transplantation.[2,6] However, given the pleiotropic effects of IL-2 on other immune cell types that also respond to IL-2 at higher doses, such as $CD4^+$ and $CD8^+$ effector T (Teff) cells, natural killer cells, and group 2 innate lymphoid cells,[2] and given its short half-life,[7] finding a dose and schedule of administration that maintain a proper balance of Treg/Teff cells over time is key to the therapeutic use of ld-IL2. We aimed to rationally define IL-2 doses and schemes of administration that will lead to long-term improvement of the Teff-/Treg-cell balance.

We first investigated the dynamics of immune cells in C57BL/6 mice receiving an "induction" dosage of IL-2 aimed at a brisk stimulation of Treg cells, consisting of a 5-day course of daily intraperitoneal injection of 25,000, 50,000, or 100,000 IU (Fig 1, *A*) from day 0 to day 4. The percentage of blood Treg cells (defined as $CD25^+$ $FOXP3^+$ cells among $CD4^+$ T cells[6,8]) was assessed 24 hours after each administration and results are expressed as relative variation compared with the PBS-treated group. The maximum increase in Treg cells was observed at day 5 and was dependent on the cumulative dose received. We also analyzed the effects of IL-2 on blood Treg cells at 2 hours after IL-2 injections in the same mice. Blood Treg-cell proportions were decreased at 2 hours (see Fig E1 in this article's Online Repository at www.jacionline.org). This suggested a recirculation of Treg cells immediately after IL-2 injection and prompted us to study the dynamics of Treg cells in secondary lymphoid organs. In spleen, Treg-cell proportions were increased at 2 hours (see Fig E1). Collectively, these results indicate that there is an early recirculation or margination of blood Treg cells upon ld-IL2 administration. We then assessed what would be the effects of a "maintenance" dosage aimed at sustaining a long-term effect on Treg cells, administered as repeated single IL-2 administrations given after an induction course (see Fig E1). The 50,000 IU IL-2 induction course resulted in a 1.71 ± 0.04-fold increase in blood %Treg cells ($P < .0001$) that was down to a 1.16 ± 0.03-fold increase at day 8 ($P = .02$). A single reinjection of IL-2 at day 8 increased the Treg-cell proportion 24 hours thereafter 1.17 ± 0.02-fold ($P = .004$). Further injections every 3 days sustained elevated levels of Treg cells over baseline values throughout the treatment period (from 1.16- to 1.33-fold increase). Spacing the injections every 7 or 15 days led to reduced Treg-cell increase during maintenance treatment.

We used these *in vivo* data to model the dose/scheme effects of IL-2 on Treg cells in mice during induction and maintenance phases. Data of Treg-cell expansion versus time were modeled and simulated using the NLME R package (R version 3.1.3, R development core team). A single-compartment model with first-order input and output was chosen. The evaluation of goodness-of-fit graphs showed that this model fitted the data well (Fig 1, *B*). We then used this model to simulate *in silico* reinjections every 3 days (Fig 1, *C*). The prediction fitted very well the actual *in vivo* data obtained using this treatment scheme (Fig 1, *C*).

We then aimed to adapt this model validated in mice to modeling human data. We used data from our previous study of ld-IL2 in type 1 diabetes (NCT01353833) that assessed the effects of a 5-day course of daily IL-2 injections.[8,9] The expansion of blood Treg cells for each dose is presented in Fig 2, *A*, and was modeled using the model developed for mouse studies (see Fig E2 in this article's Online Repository at www.jacionline.org). Results are expressed as delta variation of Treg-cell frequency in $CD4^+$ T cells; that is, % of Treg cells in $CD4^+$ of the day minus % of Treg cells in $CD4^+$ at day 0 and vertical dotted lines represent IL-2 injection. We then predicted *in silico* the potential effects of different doses and the periodicities of IL-2 reinjections given after an induction course

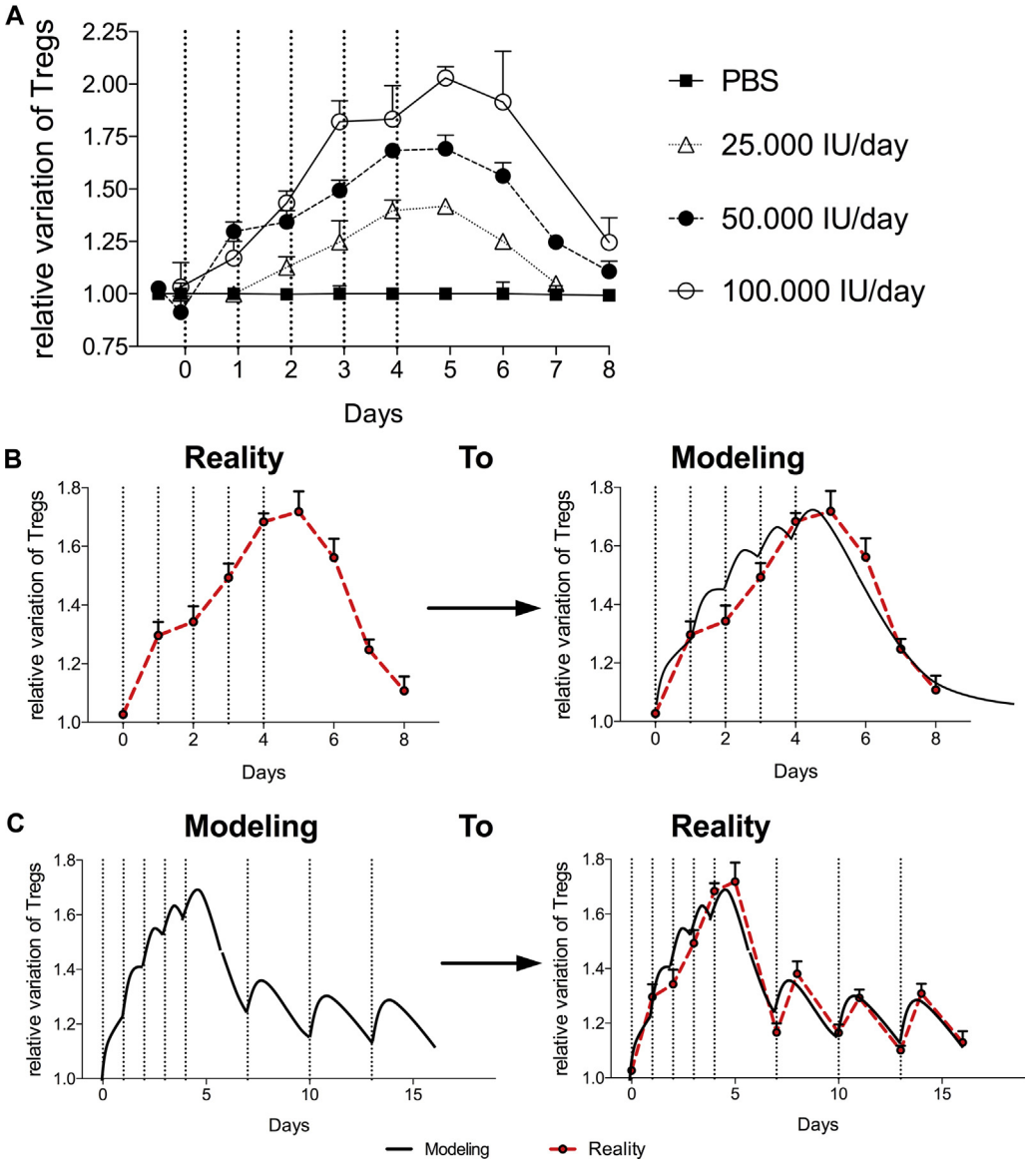

FIG 1. Dynamics and modeling of Treg cells in mice treated with IL2. **A,** Dynamics of Treg cells in blood during a 5-day course of IL-2 (pool of 3 different experiments; n = 10). Observed and *in silico* modeling of Treg-cell dynamics in blood **(B)** after a 5-day course of daily injections of 50,000 IU of IL-2, and **(C)** followed by reinjections of 50,000 IU of IL-2 every 3 days.

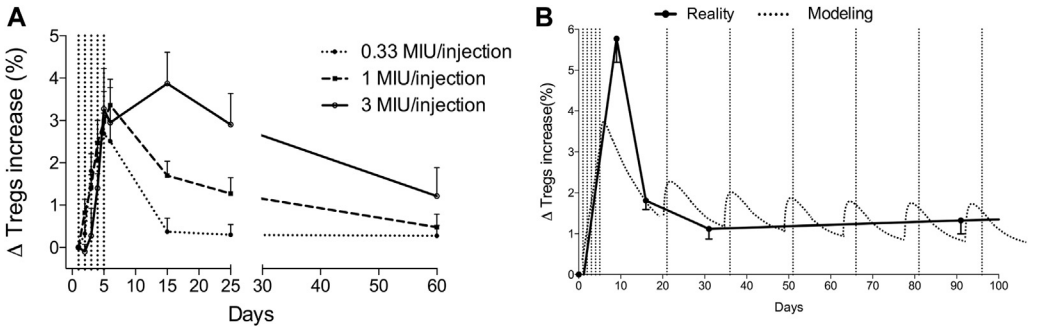

FIG 2. Dynamics and modeling of $CD4^+$ Treg cells in humans after Id-IL2. **A,** Observed Treg-cell dynamics in humans after a 5-day course of Id-IL2 (doses ranging from 0.33 MIU/d to 3 MIU/d) (n = 6 patients/treatment). **B,** Comparison of *in silico* modeling and real Treg-cell dynamics (n = 40) after reinjections of 1 MIU of IL-2 every 15 days after a 5-day course of IL-2.

(see Fig E2). For example, with 1 million international unit (MIU) IL-2 "virtual" injections every 3 days, Treg cells were expected to remain stable with a delta variation of %Treg cells ranging from 3 to 4 (ie, a 60%-80% increase); with 3 MIU every 3 days, %Treg cells were predicted to increase continuously; with reinjections every 15 days, Treg cells were expected to (1) return to their baseline approximately at day 20 with 0.33 MIU; (2) be stable with a delta variation of %Treg cells ranging from 0.5 to 1.5 (ie, 10%-30% increase) with 1 MIU; and (3) to be stable with a delta variation of % of Treg cells ranging from 2.5 to 3.5 (ie, 50%-70% increase) using 3 MIU.

On the basis of safety data from our previous clinical trials and our modeling, we chose the dose of 1 MIU/injection, with once-a-fortnight injections during the maintenance course to perform a novel clinical trial of ld-IL2 in patients with mild forms of various AIDs (rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus, psoriasis, Behcet disease, Wegener granulomatosis, Takayasu disease, Crohn disease, ulcerative colitis, autoimmune hepatitis, sclerosing cholangitis, and Gougerot-Sjögren syndrome; Transreg clinical trial: NCT01988506). Treg-cell dynamics over time in the first 40 treated patients is presented in Fig 2, *B*. Note that Treg-cell evaluations were always made just before an IL-2 injection, thus 14 days away from the last injection. These values therefore underestimate the overall effect during the 14-day period. As predicted from above, %Treg cells was increased during the maintenance phase in treated patients (delta of 1.12% ± 0.25%, $P < .0001$ at day 31, and 1.325% ± 0.33%, $P < .0001$ at day 91), representing a minimum 20% increase for a prediction that was between 10% and 30%.

Thus, we have defined and validated a model for predicting the dynamic Treg-cell response to IL-2 that should help navigate its use in numerous therapeutic targets, from various AIDs to transplantation and allergy. Our pharmacodynamic model can predict the effects of any combination of any of the treatment parameters: dose, length of the induction phase, timing of maintenance injections. Also, our results show that the response to IL-2 is predictable across multiple AIDs and that we have validated a therapeutic scheme that provides long-term improvement in the Teff-/Treg-cell balance.

We are grateful to Alice Barrateau for excellent technical assistance. We thank the animal care team members Christelle Enond, Bocar Kane, Olivier Bregerie, and Serban Morosan from the Centre d'Exploration Fonctionnelle, UPMC Paris 06. We thank the entire DF-IL2 and Transreg study groups for the performance of the clinical trials that contributed to this study (groups are listed in this article's Online Repository at www.jacionline.org).

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The project was funded by the LabEx Transimmunom (grant no. ANR-11-IDEX-0004-02) and European Research Council Advanced Grant TRiPoD (322856) to D.K. G.C. was supported by the Académie Nationale de Médecine.

Disclosure of potential conflict of interest: M. Rosenzwajg and D. Klatzmann are inventors in a patent application related to the therapeutic use of ld-IL2 for treating autoimmune-related or inflammatory disorders, which belongs to their academic institutions and has been licensed to ILTOO pharma. G. Churlaud, M. Rosenzwajg, and D. Klatzmann hold shares in ILTOO pharma. The rest of the authors declare that they have no relevant conflicts of interest.

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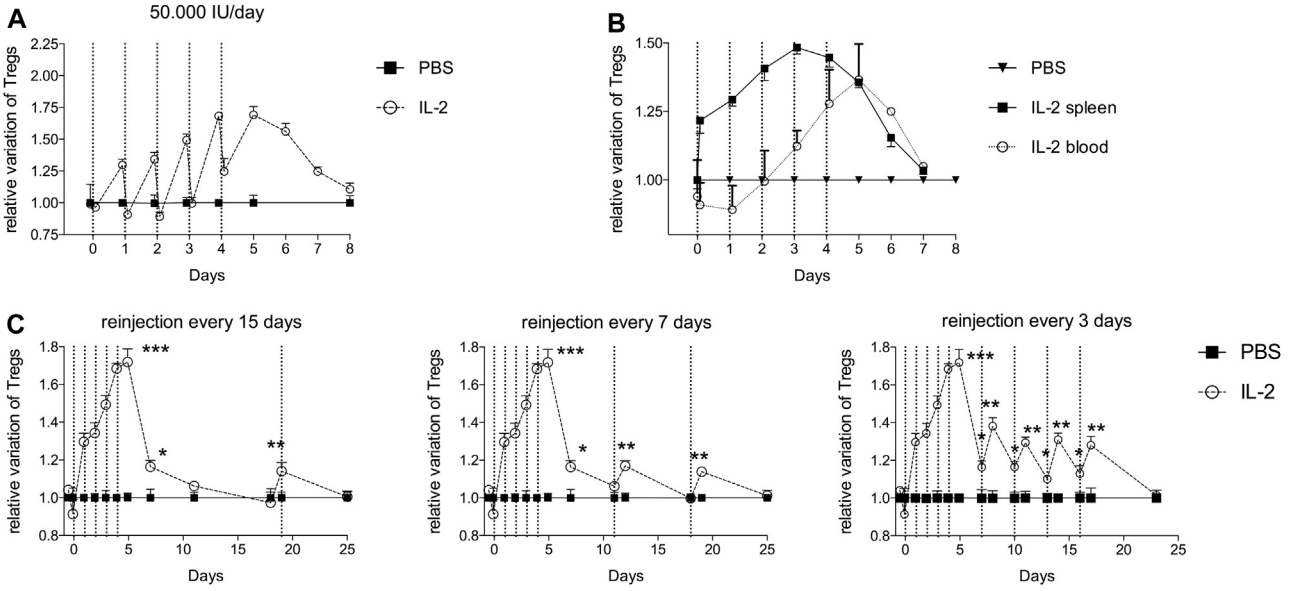

FIG E1. Dynamics and modeling of Treg cells in mice treated with IL2. Results are expressed as relative variation compared with the PBS-treated group. These data are from a pool of 3 different experiments (n = 10) using C57BL/6 mice. Treg-cell dynamics in blood at 2 and 24 hours postinjection **(A)** and Treg-cell dynamics in blood and spleen at 2 hours postinjection **(B)**, during a 5-day course of 50,000 IU/d of IL-2. **C,** Dynamics of Treg cells in blood during a 5-day course of 50,000 IU of IL-2 and then reinjection every 3, 7, or 15 days with 50,000 IU of IL-2. Comparison between IL-2 and PBS groups was done using the Mann-Whitney test (*$P \leq .05$; **$P \leq .01$; ***$P \leq .0001$).

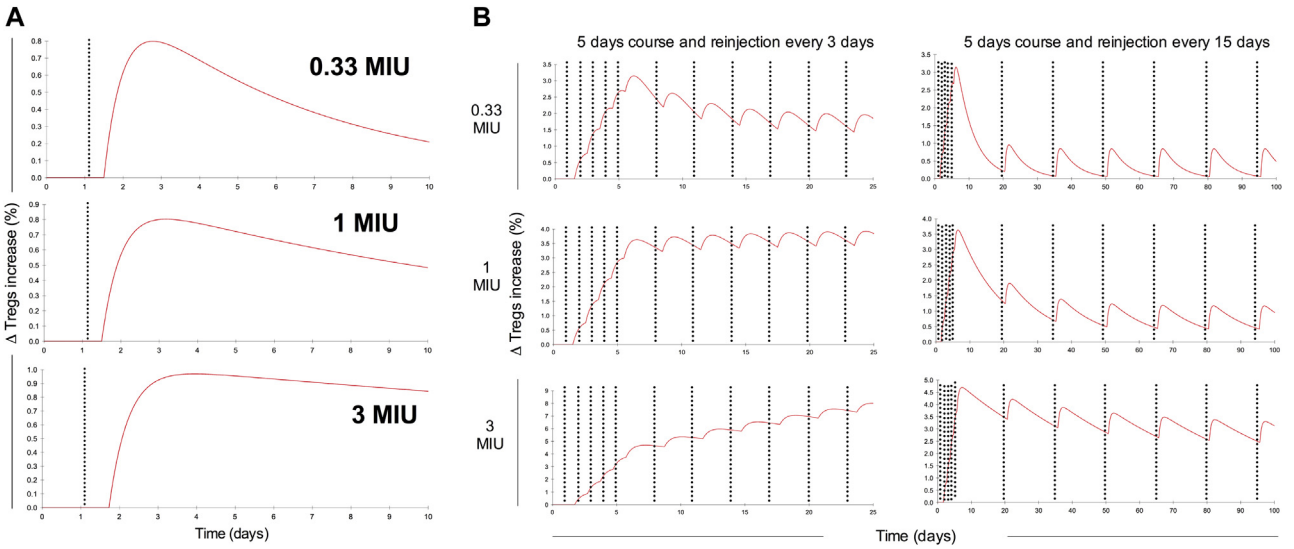

FIG E2. Dynamics and modeling of $CD4^+$ Treg cells in humans after Id-IL2. **A** and **B,** Vertical dotted lines represent IL-2 injection. Blood sampling was performed just before IL-2 injection. Results are expressed as delta variation of Treg-cell frequency in $CD4^+$ T cells, that is, % of Treg cells in $CD4^+$ of the day minus % of Treg cells in $CD4^+$ at day 0. Fig E2, *A,* Modeling of the results obtained in humans after a 5-day course of Id-IL2 (doses ranging from 0.33 MIU/d to 3 MIU/d). Fig E2, *B, In silico* modeling of Treg-cell dynamics after injections of Id-IL2 every 3 or 15 days after a 5-day course of Id-IL2, using 0.33, 1, or 3 MIU/injection.