Competition for IL-2 between regulatory and effector T cells to chisel immune responses

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INTRODUCTION
Recent studies, specifically on the role of IL-2 in the regulation of immune responses, have highlighted how cytokine competition may be a critical determinant to arbitrate the balance between tolerance and response, and/or to channel the activation of lymphocytes toward specific differentiation paths.

Cytokines are ubiquitous in immunology as mediators of cell–cell communications. Most knock-out mouse models of cytokines (with the notable exception of IFNγ) display critical and often deadly pathologies. For example, IL-2 knock-out mice are riddled with systemic autoimmune disorders (Horak et al., 1995), explained by the abrogated development and maintenance of regulatory T cells in peripheral lymphoid organs. On the other hand, there exist few clinical protocols whereby perturbations of cytokine pathways lead to clinical therapies. This review aims at presenting the need of quantitatively understanding cytokine function as a basis for more targeted therapeutic manipulation. We will discuss how the balance between cytokine secretion and consumption by multiple cell types fine-tunes the immune response.

This review is organized in four parts. First, we review recent experimental work addressing the role of cytokine consumption. Second, we present basic quantitative facts that highlight the explosiveness of cytokine secretion as well as the importance of cytokine consumption for lymphocyte–lymphocyte communication. We focus on IL-2 secretion and uptake as the best modeled case of cytokine competition, and also because it is a "self-contained" regulatory system (secreted by T cells, consumed by T cells). Third, we summarize recent theoretical studies that addressed the role of IL-2 competition as a mechanism of suppression by regulatory T cells. Finally, we discuss the biological relevance of these theoretical efforts toward better understanding immunological regulations.

THREE EXPERIMENTAL EVIDENCES AGAINST A ROLE FOR IL-2 DEPLETION AS A MECHANISM FOR T_{reg} SUPPRESSION CAN BE MITIGATED
The original identification of the CD4+CD25+ compartment as a key population to enforce peripheral tolerance (Sakaguchi et al., 1995) led to the conjecture that IL-2Rα played a critical role in T_{reg} function. Given the critical role of IL-2 for T cell survival and proliferation in vitro, many researchers originally conjectured that IL-2 depletion by T_{reg} cells would be a critical mechanism to enforce their suppressive capabilities. However, the autoimmune observed in IL-2 knock-out mice drew into question an activating function for IL-2 for T cell immune responses in vivo (Kundig et al., 1993). A partial resolution of this perceived conundrum came from the observation that the development and maintenance of T_{reg} cells depends on IL-2, so that IL-2 was attributed an immuno-suppressive – rather than an activating – function in vivo. However, careful studies that went beyond the constitutive IL-2 knock-out model have since demonstrated that the action of IL-2 on both CD4+CD25− and CD8+ T cells supports immune response in multiple ways (e.g., by sustaining different modes of proliferation of CD8+ T cells; Kundig et al., 1993; Williams et al., 2006; Cho et al., 2007), furthering the survival of CD4+ T cells (Dooms et al., 2004), and driving CD4 effector and memory cell differentiation.
(Yamane et al., 2005; Chen et al., 2011; Pandiyan et al., 2011). Thus IL-2 serves dichotomous functions in the suppression and enhancement of adaptive immunity.

While these studies open the possibility of an immunosuppressive role for IL-2 consumption by T<sub>reg</sub> cells, experiments at the turn of the millennium have further challenged this idea. For example, experiments with a knock-out genetic model for the IL-2Rβ chain (CD122) delivered negative results regarding the role of IL-2 depletion by T<sub>reg</sub> as a mechanism of suppression of autoimmune response. Specifically, Malek et al. (2002) relied on a thymic-transgenic expression of wild-type IL-2Rβ to drive the development of CD4<sup>+</sup>+CD25<sup>+</sup> T cells in the thymus then abrogate expression of IL-2Rβ in the periphery. These mice, whose peripheral lymphoid tissues contained CD4<sup>+</sup>+IL-2Rα<sup>-/−</sup>+IL-2Rβ<sup>−/−</sup> T<sub>reg</sub> cells, were devoid of autoimmune disorders, while mice from straight IL-2Rβ<sup>−/−</sup> models lacked CD4<sup>+</sup>+CD25<sup>+</sup> cells and suffered systematic autoimmune attacks, analogously with IL-2Rα<sup>−/−</sup> models. This experimental observation was further analyzed and interpreted in terms of functional suppressive capabilities among IL-2Rβ-deficient T<sub>reg</sub> cells, challenging the role of IL-2 signaling for T<sub>reg</sub> suppression. However, more recent work mitigated this conclusion with the observation that peripheral T<sub>reg</sub> cells from these IL-2Rβ thymic-transgenic knock-out mice retain their capabilities, albeit diminished, to respond to IL-2 (Bayer et al., 2007). These experimental inconsistencies may be explained by the recycling and long-term stability of the IL-2Rβ receptor, even after abrogation of its expression in the periphery. Hence, the accurate peripheral tolerance and lack of autoimmune disorders in IL-2Rβ thymic-transgenic knock-out mice can no longer be interpreted as a complete rejection of IL-2 depletion as a necessary mechanism for T<sub>reg</sub> suppression.

The discovery of FoxP3 as the transcription factor that identifies unequivocally the T<sub>reg</sub> lineage clarified the field, by offering a proprietary marker for T<sub>reg</sub> that distinguishes them from transiently expressing CD4<sup>+</sup>+CD25<sup>+</sup> effector T cells. Work by the Rudensky lab (Fontenot et al., 2005) clearly established the critical role of IL-2 signaling for T<sub>reg</sub> development and maintenance. On the other hand, this study showed that FoxP3<sup>+</sup>+CD4<sup>+</sup> cells from Il2ra<sup>−/−</sup>-mice were as suppressive as T<sub>reg</sub> from Il2ra-sufficient mice, at least in the classical in vitro proliferation assay: this observation (among others) again led to the conclusion that IL-2 signaling is dispensable for suppression. On the other hand, IL-2 signaling for Il2ra<sup>−/−</sup>-FoxP3<sup>+</sup> cells was not quantified and it is possible that compensatory mechanisms – e.g., upregulation of beta and gamma chains of the IL-2R receptors (Li et al., 2001) – would enable these IL-2Rα deficient cells to maintain their ability to respond and deplete IL-2. In particular, IL-2Rα<sup>−/−</sup>- T cells have been shown to respond to IL-2, albeit at higher concentrations (1 nM instead of the characteristics 10 pM): this could explain why IL-2Rα<sup>−/−</sup>-mice (that have FoxP3<sup>+</sup> peripheral cells but at lower frequency than IL-2Rα sufficient mice) still suffer from systemic autoimmune disorders (a hallmark of defective suppression by T<sub>reg</sub>) but with less intensity than IL-2Rα<sup>−/−</sup>-mice (these mice are completely devoid of FoxP3<sup>+</sup> cells).

A third line of experiments has previously been used to reject cytokine depletion as a mechanism for T<sub>reg</sub> suppression and have led to the dogma that cell–cell contact between T<sub>reg</sub> cells and T<sub>eff</sub> cells is absolutely required for suppression. In the past, many groups (Shevach et al., 1998; Takahashi et al., 1998; Nakamura et al., 2001; Dieckmann et al., 2002; Xu et al., 2003) have used the classical transwell assay whereby T<sub>reg</sub>-T<sub>eff</sub> contacts are forbidden by a membrane separation and found that this abolishes suppression of T<sub>eff</sub> proliferation by T<sub>reg</sub> cells. Unfortunately, this setup can potentially generate false-negative results – as originally suggested in Scheffold et al. (2005), Pandiyan et al. (2007). Indeed, as pointed out by Shevach (2009) in a recent review, “It should be emphasized that the failure to observe suppression when T<sub>reg</sub> cells are separated from the responder cells by a membrane does not rule out the possibility that T<sub>reg</sub> cells secrete an as yet uncharacterized cytokine that functions in a gradient fashion and requires proximity between suppressor and responder.” In this regard, it is noteworthy that the transwell geometry typically separate T<sub>reg</sub> and T<sub>eff</sub> cells by 4 mm when one uses the same Corning Costar transwell within a 24-well plate as described in Thornton and Shevach (1998). This is a very large distance to be bridged by diffusion. For IL-2 (a globular protein of 17kDa), the coefficient of diffusion D in solution is of the order of $1 \times 10^{-6}$ cm$^2$/s (Weidemann et al., 2011). Thus the characteristic diffusion time $\tau_D$ across $d = 4$ mm is: $\tau_D = d^2/(2D) = 40000 s = 11 h$. Hence, the physical separation imposed by the transwell geometry implies a large time-delay between the secretion of soluble molecules by T<sub>reg</sub> cells and their potential sensing (and scavenging) by T<sub>reg</sub> cells (this time-delay might in fact be even larger, $\tau_{mixing} > 20 h$, due to the low porosity of the transwell membrane). Under these conditions, paracrine and autocrine consumption of IL-2 by the T<sub>eff</sub> population, rather than competitive take-up by the very distant T<sub>reg</sub> cells will dominate (Scheffold et al., 2007; Busse et al., 2010; Feinerman et al., 2010). Thus abrogation of suppression in a T<sub>eff</sub>-T<sub>reg</sub> transwell setup (Takahashi et al., 1998; Thornton and Shevach, 1998) does not rule out cytokine competition as one of the mechanisms of T<sub>reg</sub> action (Scheffold et al., 2007). All in all, there is consensus in the field of regulatory T cells, regarding IL-2, that this cytokine is critical for the development and maintenance of this subpopulation. On the other hand, the three main lines of evidence dismiss the functional significance of IL-2 signaling in terms of the T<sub>reg</sub> cells' suppressive capacities may not be definitive.

**FUNCTIONAL EVIDENCE FOR IL-2 COMPETITION AS ONE MECHANISM FOR T<sub>reg</sub> SUPPRESSION**

The renaissance for IL-2 depletion as a mechanism for T<sub>reg</sub> suppression came with studies from the Scheffold and Stockinger groups (de la Rosa et al., 2004; Barthlott et al., 2005; Brandenburg et al., 2008). Both groups documented how IL-2 depletion or blockage phenocopied the effect of T<sub>reg</sub> cells on antigen-activated T cells. Conversely, either the exogenous addition of IL-2 or the blockage IL-2 uptake by T<sub>reg</sub> cells only – and not by T<sub>eff</sub> cells – was sufficient to abrogate suppression in vitro. Hence, IL-2 was conjectured to be a limiting factor for T<sub>reg</sub> cell expansion in vitro. These groups then demonstrated that T<sub>eff</sub> cells do produce IL-2 (despite reduced transcription of the IL-2 gene). An early predictor of suppressed T<sub>eff</sub> cell expansion in these experiments was the lack of strong IL-2Rα expression on T<sub>reg</sub> cells, accompanied in a reciprocal manner by further upregulation of IL-2Rα on T<sub>reg</sub> cells. This behavior is readily explained by competitive IL-2 consumption.
through $T_{reg}$ cells, as pSTAT5 drives IL-2Rα upregulation in both cell types. This reciprocal regulation of IL-2Rα in $T_{eff}$ and $T_{reg}$ cells has also been observed in vivo (Klein et al., 2003; Barthlott et al., 2005). Of note, IL-2 has been shown to prime $T_{reg}$ cells for later expression of the immunosuppressive cytokine IL-10 in vitro and in vivo (Barthlott et al., 2005; Brandenburg et al., 2008).

These papers were followed by the comprehensive study by the Lenardo group (Pandiyan et al., 2007) that focused on the enhanced apoptosis among activated effector cells, when common-gamma chain (γc) cytokines are missing because of their depletion by $T_{reg}$ cells. All the hallmarks of cytokine deprivation-induced apoptosis (loss of phosphorylation of AKT, phosphorylation of BAD, membrane blebbing, resistance to death in Bim−/− mutants) were observed in the suppression assay in vitro. Moreover, Pandiyan et al. (2007) reported the measurements of reduced IL-2 concentration in supernatants of $T_{eff}$-$T_{reg}$ cocultures compared to $T_{eff}$-only cultures: this was assigned to IL-2 consumption by $T_{reg}$ cells rather than to reduced IL-2 production by $T_{eff}$ cells. The measured IL-2 concentrations (around 1 unit/ml, i.e., 10 pM) were exactly in the range where maximal functional impact would be expected (see following section for details). Finally, a model of inflammatory bowel disease (IBD) was used as an in vivo assay of $T_{reg}$ function: upon adoptive co-transfer of CD4+CD25+ T cells in SCID mouse, the onset of IBD was abrogated and colitogenic $T_{eff}$ cells were shown to undergo apoptosis. Vice versa, when mice were not injected with CD4+CD25+ T cells, adoptively transferred CD45 T cells would proliferate and trigger IBD. Hence, Pandiyan et al. (2007) made a convincing case that depletion of IL-2 by $T_{reg}$ cells constitute a critical mechanism to account for $T_{reg}$ suppression.

**QUANTITATIVE ASPECTS OF CYTOKINE ACCUMULATION AND CONSUMPTION**

Addressing the role of cytokine depletion in enforcing suppression by $T_{reg}$ cells depends on the quantitative understanding of the dynamics of cytokine accumulation and consumption in the extracellular medium of lymphoid organs. Of note, most cytokines are functional in concentration ranges (below 100 pM) that are unusual for most biological systems. Indeed, most ligand-receptor interactions, most hormones and growth factors operate in 10 nM range. Hence, there are specific challenges of the biophysics of cell–cell communication in the pM range that we need to address. In this section, we summarize the numbers related to IL-2 secretion and uptake, and estimate the kinetics of IL-2 accumulation in a lymph node. We note that the experimental uncertainties for kinetic rates and receptor numbers are rather large, so the correct parameter values might be within a factor of 2–3 from the stated value. In addition the receptor numbers and secretion rates are broadly distributed within the population and depend on the experimental protocols of measurement. Accordingly, we will keep our estimations simple, favoring clarity while aiming to stay within an order of magnitude of the actual parameter values.

First, we will briefly describe the kinetic steps involved in IL-2 signaling and consumption by T cells following the model presented in Feinerman et al. (2010). In general, IL-2 consumption by T cells proceeds in three steps (Feinerman et al., 2010):

1. Free IL-2 molecules reversibly bind α chains (IL-2Rα) of IL-2 receptors with characteristic on- and off-rates of $k_{\text{weak}}(+)=1.4 \times 10^7/(M \cdot s)$, $k_{\text{weak}}(-)=0.4/s$.

$$\text{IL-2} + \text{IL-2Rα} \leftrightarrow \text{IL-2}\times\text{IL-2Rα}$$

2. IL-2*IL-2Rα locks into a tight complex with available IL-2Rβ and γc chains of the IL-2 receptor with characteristic on- and off-rates of $k_2(+)=3 \times 10^{-4}/s$, $k_2(-)=2.3 \times 10^{-3}/s$, forming a complete IL-2*IL-2R receptor.

$$\text{IL-2}\times\text{IL-2Rα} + \text{IL-2Rβ}\times\gamma_c \leftrightarrow \text{IL-2}\times\text{IL-2R}.\gamma_c$$

The assembly of the tetrameric cytokine/receptor complex triggers the phosphorylation of the transcription factor STAT5 into pSTAT5. pSTAT5 molecules dimerize and enter the cell nucleus where they regulate a variety of genes, among them many genes associated with cell survival and proliferation. Importantly within the context of IL-2 communication, pSTAT5 upregulates the expression of IL-2Rα and downregulates the secretion of IL-2.

3. IL-2*IL-R complex is internalized by the cells with the rate of $k_{\text{endocytosis}}=1.1 \times 10^{-3}/s=(15 \text{ min})^{-1}$ (Duprez and Dautry-Varsat, 1986; Duprez et al., 1988, 1991; Hemar et al., 1995). We assume further that upon internalization the IL-2R receptor chains return to the cell membrane. This constitutes a simple way to model the conditions at the cell membrane as quasi-stationary. In experiments, the quasi-stationary conditions are supported by the fact that cytokine consumption dynamics happens at the time scales of several minutes, while the numbers of receptor chains remain stable over the course of several hours.

For the kinetic rates and numbers of receptors chains typical for T cells, the two-step model for IL-2 binding and uptake gives the number $N_R$ of assembled IL-2*IL-R complexes per cells as:

$$N_R \approx \frac{N_{\gamma c}}{1 + \frac{K}{N_{\gamma c}[\text{IL}-2]}} \quad (1)$$

and the IL-2 consumption rate by the cell of

$$J_{\text{cons}} = k_{\text{endocytosis}}N_R \approx \frac{k_{\text{endocytosis}}N_{\gamma c}}{1 + \frac{K}{N_{\gamma c}[\text{IL}-2]}} \quad (2)$$

where $K = \frac{(k_{\text{weak}})_{\gamma c}}{k_{\text{weak}}(+)k_{\text{weak}}(-)}$ and $N_{\alpha}, N_{\gamma c}$ are the numbers of IL-2Rα and of IL-2Rβ*γc complexes per cell respectively.

The first main consequence of this model is that the EC50 for IL-2 signaling (defined as the concentration of IL-2 that yields 50% of the pSTAT5 response) is inversely proportional to the number of IL-2Rα chains per cell. In particular, for a moderately activated T cell with $N_{\alpha} \approx 10^5$, EC50~10 pM, while for a strongly activated T cell with $N_{\alpha} \approx 10^3$, EC50~1 pM. The second consequence of the model is that at high IL-2 concentrations ([IL-2] ≫ EC50), IL-2 signaling and consumption reach saturation and are limited by the number of IL-2Rβ*γc complexes that has been estimated.
to be $\sim 300$ in the naïve T cell and rises to $\sim 1000$ upon activation (Feinerman et al., 2010). In general, this number appears to be limited by the number of available $\gamma_c$ chains: although there typically $\sim 5000$–8000 of them per cell, they are shared between many types of different cytokine receptors; the best estimates for the number of available $\gamma_c$ came from fitting IL-2 consumption by $T_{reg}$ cells (Feinerman et al., 2010). Notice that the consumption rate being rather low $k_{endocytosis} = 1.1 \times 10^{-3}$/s, a strongly activated cell exposed to saturating concentrations of IL-2 would be able to consume at most one molecule of IL-2/s.

IL-2 secretion starts only upon activation of naïve T cells with foreign antigens. Within hours of activation through their TCR signaling pathways, effector T cells start secreting IL-2 at an average rate of $\sim 10$ molecules/s (our unpublished data). As IL-2 is secreted in the extracellular medium, it diffuses away from the secreting cell and the IL-2 field around the cell is established with the maximal concentration near the cell with a characteristic decay length of about cell radius $R$. The IL-2 concentration near the cell $c_0$ is set by the balance between IL-2 secretion and its diffusion:

$$J_{sec} = 4 \pi R^2 D c \approx 4 \pi R^2 \frac{c_0}{R}$$

where $D$ is the diffusion coefficient for IL-2. Its value in an aqueous buffer is $\sim 100 \mu m^2$/s, but the diffusion coefficient within a lymph node is not known. Assuming that the viscosity of the extracellular matrix is similar to that of the cell cytoplasm ($\sim 6$ times higher than that of aqueous solution), we could estimate $D \approx 16 \mu m^2$/s. Then using $R \approx 5 \mu m$, we arrive at $c_0 \approx 20 pM$, which is larger than EC50 of even moderately activated T cells. Thus an activated secreting cell should be able to sense IL-2 if it produces in an autocrine manner. However, at the onset of activation T cells lack IL-2R$\alpha$ chain and would need concentrations of IL-2 larger than 1 nM to respond to it. The expression of IL-2R$\alpha$ is initiated upon cell activation, and within 24–48 h the number of IL-2R$\alpha$ reaches $\sim 10^2$–$10^3$ per cell lowering EC50 to $\sim 1$–10 pM of IL-2 and allowing for cell sensing its own field of IL-2.

However, even a strongly activated cell would consume only a small fraction of secreted IL-2 in an autocrine manner. Indeed, as discussed above a strongly activated T cell can consume at most $\sim 1$ IL-2 molecule/s while producing 10 molecules/s on average. The rest of the molecules would in principle accumulate within the lymph node and contribute to communication between different T cells and to coordination and strengthening of the system immune response. Assuming there are $\sim 200$ activated T cells in a draining lymph node $\sim 48$ h after immunization (there are typically few hundreds T cells in a body tuned to activate to a particular peptide, not all of them will reach the lymph node and those activated in the node will experience 1–2 divisions during that time) and taking the volume of a lymph node to be 1 $\mu l$, we estimate that the IL-2 concentration in the node would reach $48 h \times (3600 s/h) \times (10 \text{ molecules/s/cell}) \times (200 \text{ cells})/1 \mu l/ N_{avgadr} \sim 600 pM$ within 48 h of secretion. Such IL-2 concentration should indeed allow for strong signaling and cross communication between activated T cells. Specifically, even weakly activated cells (i.e., cells with lower levels of IL-2R$\alpha$ would be able to phosphorylate STAT5: cell–cell communication through IL-2 would be rather unspecific and universal for all T cells in a lymphoid organ.

However, such accumulation of IL-2 is prevented by regulatory T cells. Unlike naïve T cells, $T_{reg}$ cells express constitutively all of the chains of IL-2R even in homeostasis ($\sim 10^4$ of IL-2R$\alpha$ and $\sim 300$ of IL-2R$\beta^\gamma_c$) and can consume IL-2 from the onset of an immune response. Thus concentration of IL-2 will be established by the balance of overall secretion rate by $T_{eff}$ cells and the consumption rate by $T_{reg}$ cells. There are $\sim 5 \times 10^9$ T cells in a lymph node and $\sim 5\%$ among them are $T_{reg}$ cells, i.e., $\sim 2.5 \times 10^8$ cells. Comparing their consumption rate from Eq. 2 to the secretion rate of 200 activated T cells (i.e., $2000 \text{ molecules/s}$), we estimate that in first 24–48 h, IL-2 concentration does not exceed 0.15 pM. This level is too low for signaling to occur, so there will be almost no cross talk between different $T_{eff}$ cells. Similarly, with the exception of cells in the immediate vicinity of a secreting $T_{eff}$ cell, the majority of $T_{reg}$ cells do not activate their pSTAT5 and downstream genes.

For an efficient cross talk to occur, the IL-2 concentration has to rise about tenfold to $\sim 1$ pM, which is achieved when the number of activated T cells reaches $\sim 2000$, which could take another 24–48 h. Well activated $T_{eff}$ cells (with EC50 $\sim 1$ pM) signal much more efficiently than the majority of $T_{reg}$ cells. As described above, IL-2 signaling leads to upregulation of IL-2R$\alpha$ expression and therefore to yet stronger signaling by $T_{eff}$ cells allowing them from that stage onward to win over $T_{reg}$ in the competition for IL-2. Only a minority of $T_{reg}$ cells, those in the immediate vicinity of a secreting cell, have chances to keep up with $T_{eff}$ cells. The number of such $T_{reg}$ cells can be estimated from the probabilistic argument: since in a close packed situation each cell has $\sim 12$ neighbors and 5% of those are $T_{reg}$ cells, the number of strongly signaling $T_{reg}$ cells should be $\sim 60\%$ of activated T helper cells. However, as $T_{eff}$ further proliferate the importance of IL-2 consumption by $T_{reg}$ becomes negligible (Sojka et al., 2005, 2008). IL-2 secreted by the weakly activated (e.g., autoimmune) $T_{eff}$ cells would be consumed mostly by $T_{reg}$ cells; the $T_{eff}$ cells would not be able to cross-communicate and their response would be suppressed. Yet $T_{eff}$ cells strongly activated by the foreign proteins will be able to eventually overcome the suppression, and to exchange IL-2 cytokines and coordinate their response.

In addition to the competition between $T_{eff}$ and $T_{reg}$ cells, there exists competition for IL-2 within the $T_{eff}$ system. As noted above, IL-2 signaling pathway leads to both upregulation of the expression of IL-2R$\alpha$ and downregulation of IL-2 secretion. This means that $T_{eff}$ cells that got more IL-2R$\alpha$ signal stronger than others, which leads them to express more IL-2R$\alpha$ and achieve yet stronger signaling capabilities. At the same time, they produce less and less of IL-2. Thus these two feedbacks are two mechanisms that bring about the split of $T_{eff}$ population into two subpopulations: “consumers” and “producers.” “Consumers” have shut down their IL-2 secretion, but have plenty of IL-2R$\alpha$ that allows them to “steal” IL-2 from “producers.” The “producers” are stuck in IL-2 secretion state since they have few IL-2R$\alpha$ and so little chances of starting signaling and changing their state as their IL-2 is being stolen by the “consumers.” This consideration implies that a continuum of IL-2R$\alpha$ levels within the $T_{eff}$ cell population may be...
A conspicuous feature of IL-2 and other T cell cytokines is their and activation of IL-2R receptors, downstream signal transduction via the Stat5 pathway during antigen-activated, IL-2 secreting CD4 T cells and incorporate in a systematic way the kind of arguments made mathematical models provide an appropriate tool. Such models of cytokine signaling shape the biological action of a cytokine, genes themselves as well as cytokine receptor genes. Dynamic in time and space since cytokines regulate the expression travel in space? Moreover, cytokine signaling is likely to be highly question in understanding the physiological effects of cytokines is: which cells actually receive the cytokine signal? Does the secreted cytokine consume most of it in an autocline manner, or is it quickly distributed to neighboring cells – and if so, how far does the signal in a suppressive mode of T cell priming. IL-2 primes Treg cells for later IL-10 expression in vivo (Brandenburg et al., 2008), this sharing of IL-2 can both support an immune response and initiate a delayed negative feedback loop (Scheffold et al., 2005; Yamaguchi et al., 2011). Taken together, these findings provide a mechanistic underpinning for IL-2 competition as a suppressive mode of Treg cells that depends on the strength of the antigen stimulus (de la Rosa et al., 2004; Barthlott et al., 2005; Pandiyian et al., 2007; McNally et al., 2011). The positive feedback regulation of IL-2Rα chain expression by IL-2/Stat5 signaling plays a critical role in this regulatory network. In responder T cells, full IL-2Rα expression and formation of high-affinity IL-2 receptors requires, in addition to the antigen stimulus, phosphorylation of Stat5. Hence Treg cells, which constitutively express IL-2Rα, will deprive of IL-2 weakly stimulated responder T cells (here, cells that fail to upregulate IL-2Rα sufficiently) and keep them from expressing high-affinity IL-2 receptors, thus inflicting a “double hit” (Feinerman et al., 2010). However, if IL-2 is abundant (strongly stimulated) responder T cells upregulate their own IL-2Rα expression due to positive feedback. These activated cells do no longer suffer from IL-2 deprivation by Treg cells, because they have themselves become efficient sensors and consumers of the cytokine. Under conditions where IL-2Rα is limiting for the formation of high-affinity IL-2 receptors, the IL-2–IL-2Rα positive feedback can function as a digital switch that converts graded changes in the antigen stimulus into an all-or-nothing decision for cell proliferation at the single-cell level (Busse et al., 2010). Quantitation of the IL-2R subunit expression and resulting Stat5 phosphorylation in responder T cells shows that both IL-2Rα and IL-2Rβ levels control the responsiveness of a cell to IL-2. Moreover, cell-to-cell variability in the expression of both receptor subunits results in a broad distribution of IL-2 sensitivities in a cell population (Feinerman et al., 2010).

Interestingly, a recent systems-biology study of an unrelated cytokine pathway, Epo signaling in erythroid progenitors, has shown a key role for signal processing of cytokine consumption by rapid receptor turnover (Becker et al., 2010). However, what makes the IL-2–IL-2R system ideally suited for cytokine competition is the positive feedback regulation of receptor (IL-2Rα) expression in both responder T cells and Treg cells. Through this self-amplification of IL-2 signaling and consumption, rather subtle initial differences in strength and timing of antigen stimulation can lead to clear-cut biological outcomes (Feinerman et al., 2010). An important question that has already been introduced in the discussion of the transwell assay is how far a cytokine signal can
Antigenic engagement of T\textsubscript{eff} cells leads to their activation, with IL-2R\textsubscript{α} upregulation and IL-2 production (A). Studies by our groups (Busse et al., 2010; Feinerman et al., 2010) quantified the strength of feedback regulations on IL-2 signaling and secretion. In particular, the role of T\textsubscript{reg} cells (whose constitutive expression of IL-2R\textsubscript{α} allows early IL-2 depletion) in regulating STAT5 phosphorylation in T\textsubscript{eff} cells was modeled in silico. Three main predictions from our models were validated experimentally: (1) T\textsubscript{reg} can impact a double suppressive hits on T\textsubscript{eff} cells by blocking IL-2R\textsubscript{α} upregulation and IL-2 accumulation hence abrogating STAT5 phosphorylation (B); (2) This suppression is highly dynamic and variable: in particular, T\textsubscript{reg} cells can rely on the competition for IL-2 to block the proliferation of weakly activated T\textsubscript{eff} cells, while allowing strongly activated T\textsubscript{eff} cells to mount an immune response (C); note how the presence of T\textsubscript{reg} cells reduce the overall proliferation of T\textsubscript{eff} cells but, as well, sharpen the dose responsive curve for proliferation vs. antigen strength; (3) Complex spatio-temporal coupling allow T\textsubscript{reg} cells to modulate and regulate the extent of suppression in crowded environments (e.g., in vivo).
travel in space – Section “Three Experimental Evidences Against a Role for IL-2 Depletion as a Mechanism for T_{reg} Suppression can be Mitigated.” Clearly, diffusion over mm-range distances, with diffusion times of several hours (as in the transwell setup), is prohibitive for efficient communication. However, a biologically more relevant question is whether cytokine gradients occur on a smaller scale that could compartmentalize cell-to-cell signaling in lymphoid organs. For in vitro experiments, Feinerman et al. (2010) estimated that diffusion through the supernatant would not allow steep concentration gradients to develop (and moreover, convection, which is much faster than diffusion over larger distances, is also likely to occur in typical in vitro setups). By contrast, the explicit modeling of diffusion in rather dense cell assemblies (where extracellular space and total cell volume are of comparable magnitude) show that competitive IL-2 uptake under conditions of limited supply can cause strong concentration gradients (Busse et al., 2010). This is particularly evident for T_{reg} cells, which due to their high constitutive IL-2-Ru expression function as potent sinks of the cytokine. As a consequence, T_{reg} cells can absorb the IL-2 secreted by localized, weakly stimulated responder T cells and thus prevent the paracrine spread of this signal to other responder T cells in the neighborhood. On the other hand, when IL-2 secretion is strong (owing to a high fraction of secreting responder T cells and/or high secretion rates), T_{reg} cells will become saturated and the IL-2 signal could pervade an entire lymph node [as shown previously for IL-4 (Perona-Wright et al., 2010)]. In summary, the modeling suggests that T_{reg} cells also control the spatial propagation of IL-2 signals in lymphoid organs.

By iterating between modeling and experiments, the studies by Busse et al. (2010) and Feinerman et al. (2010) have revealed an unexpected plasticity of the IL-2 cytokine network, where quantitative parameters (secretion rate, diffusion, and competitive uptake) shape the biological outcome. Further theoretical studies have modeled T cell population dynamics and IL-2 signaling (Burroughs et al., 2006), making experimentally testable predictions. As several other cytokines of the adaptive immunity share principle features of the IL-2 system, especially competitive uptake by different cell populations and feedback regulation of signaling, we expect that similar “behavioral” plasticity will be found also in other cytokine networks.

PERTURBING THE IL-2 TUG-OF-WAR TO MAXIMIZE IMMUNOTHERAPEUTIC IMPACT

At the same time as the issue of cytokine competition was being revisited, pre-clinical and clinical studies have put forward the possibility of applying IL-2 treatments to manipulate the T_{reg} compartment and impact on clinical outcomes (Murphy et al., 2012). Specifically, using antibody to cross-link IL-2 and to increase its lifetime in vivo (bare IL-2, because of its low molecular weight, gets filtered out of the system, mostly in the kidneys), researchers discovered that the T_{reg} compartment could be expanded. For example, Boyman and colleagues achieved proliferation of CD4+FoxP3+ lymphocytes in mice, increasing the frequency of T_{reg} cells by 10-fold, 3 days post-injection of the cytokine/antibody complex (Webster et al., 2009). The functional significance of this observation was immediately tested on a model of experimental autoimmune encephalomyelitis (EAE) whose induction could be abrogated after such robust T_{reg} cell expansion.

Similar observations (whereby the T_{reg} compartment is expanded with high levels of IL-2Rα and enhanced suppressive capacities) have been reported in other models. The Bluestone group applied a low-dose regimen of IL-2 to NOD mice, and obtained a strong delay or complete abrogation of diabetes (Tang et al., 2008): concomitantly, they reported that T_{reg} cells harvested from the pancreatic islet were more abundant (27% instead of 7% among CD4+ T cells) and expressed higher levels of IL-2Rα (20-fold higher). Similar results were obtained in a clinical setting, whereby low-dose regimen of IL-2 was demonstrated to be sufficient to delay the onset of Graft-vs.-Host-disease in allogeneic bone marrow transplant settings (Koreth et al., 2011). Again, these results should be analyzed quantitatively to test whether the subtle balance between immune response (autoimmune attack of the pancreatic islet or allogeneic activation of grafted T cells) and immune tolerance (suppression of activation and proliferation) could result from the boosted ability of T_{reg} cells to compete for cytokines. A model for such immunotherapeutic intervention has already been proposed and makes further experimental testing critical. IL-2 regimen clearly upregulates IL-2Rα levels (with enhanced ability to bind and deplete IL-2), but it could also trigger other suppression mechanisms (e.g., secretion of IL-10 or upregulation of CTLA4).

Further quantitative modeling of this cytokine competition within clinical settings will thus be necessary to test and optimize cytokine competition, mostly to block autoimmune disorders using natural suppressive capabilities, but also to boost cytotoxic impact in cancer immunotherapies (2012). Although, this may prove difficult as direct and repeated probing of the tissue of interest (skin in GVHD, pancreatic islets in diabetes), we conjecture that mathematical models will become more and more critical to interpret functional changes as measured among accessible peripheral blood mononuclear cell (e.g., IL-2Rα upregulation in an expanded T_{reg} compartment) and extrapolate them to the tissue of relevance. Note that accurate measurements of in vivo concentrations of cytokines would go a long way toward resolving issues about cytokine communications in the immune system: this remains a challenging task given the low levels of free cytokine within tissues, but technical developments (ELISA miniaturization and the use of physiological reporters for cytokines) will solve this problem in the coming years. Ultimately, mouse pre-clinical models will be particularly useful to fine-tune the blood-to-tissue quantitative interpolation of immune responses.

CONCLUSION

We presented a review of recent efforts in Systems Immunology that aim at addressing the role of cytokine competition as one mechanism of immune suppression by T_{reg} cells. Modeling quantitatively how cytokine is secreted by effector T cells undergoing activation, and how much gets scavenged by regulatory T cells remains challenging because of the dynamic complexity of the system. However, computational models have already highlighted the spatio-temporal intricacies of IL-2 competition: depending on the speed of IL-2Rα upregulation among T_{eff} cells, there exists a time window when T_{reg} cells deplete the extracellular medium of
the secreted IL-2 and “snuff” this critical cytokine for differentiation. Spatially, the tight-packed space of lymphoid organs as well as the high density of polyonal Treg cells with high tonic level of IL-2Rα expression can limit the spatial extent at which T eff cells can communicate through IL-2 sharing. This in turns, can act as a differential regulatory mechanisms to discriminate between activation of T eff cells with low or high concentrations of antigens. Future work will need to extend the modeling framework introduced for IL-2 to other cytokines (e.g., IL-4, IL-10, TGFβ) as well as other costimulatory signals (e.g., B7/CTLA4). Further modeling effort will need to deal with cell proliferation and homeostasis, as proposed in a recent study (Burroughs et al., 2012). These quantitative approaches will contribute greatly to assess the relevance of these varied mechanisms of Treg cell suppression in vitro and in vivo. Beyond fundamental immunology, such quantitative insight may open new avenues of cytokine perturbation, to maximize immunotherapeutic impact in clinical settings.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 23 April 2012; paper pending publication: 08 May 2012; accepted: 05 August 2012; published online: 05 September 2012.

Citation: Hofer, T., Krichesky O and Alban-Bonnet G (2012) Competition for IL-2 between regulatory and effector T cells to chisel immune responses. Front. Immunol. 3:268. doi: 10.3389/fimmu.2012.00268

This article was submitted to Frontiers in T Cell Biology, a specialty of Frontiers in Immunology.

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