JCI The Journal of Clinical Investigation

Can TNF-α boost regulatory T cells?

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J Clin Invest. 2010;120(12):4190-4192. https://doi.org/10.1172/JCI45262.

Commentary

Deleterious immune responses that cause autoimmune diseases such as type 1 diabetes are normally kept in check by a myriad of mechanisms. Among these, protection mediated by $CD4^+Foxp3^+$ Tregs constitutes an essential pathway. Much work over the past decade aimed to understand how Tregs affect immune responses triggered by effector T cells (Teffs), but less is known about how Teffs affect Tregs. In this issue of the *JCI*, Grinberg-Bleyer et al. report the clearest example thus far regarding this important aspect of Treg biology. They find that in mice, sustained protection from diabetes by Tregs is dependent on Teffs and partially dependent on TNF-α, a cytokine traditionally considered proinflammatory.

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Can TNF-α boost regulatory T cells?

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Deleterious immune responses that cause autoimmune diseases such as type 1 diabetes are normally kept in check by a myriad of mechanisms. Among these, protection mediated by CD4$^+$Foxp3$^+$ Tregs constitutes an essential pathway. Much work over the past decade aimed to understand how Tregs affect immune responses triggered by effector T cells (Teffs), but less is known about how Teffs affect Tregs. In this issue of the *JCI*, Grinberg-Bleyer et al. report the clearest example thus far regarding this important aspect of Treg biology. They find that in mice, sustained protection from diabetes by Tregs is dependent on Teffs and partially dependent on TNF-α, a cytokine traditionally considered proinflammatory.

Chronic inflammatory diseases such as asthma and autoimmune diseases such as type 1 diabetes arise from the breakdown of the mechanisms that normally restrain immune responses. Key among those mechanisms is a subset of CD4$^+$ T cells called Tregs. Tregs are characterized by expression of the transcription factor forkhead box P3 (Foxp3). Foxp3 is not only important for the development and maintenance of Tregs but also for their suppressive function (1, 2). Perhaps the best evidence for the indispensable role of Tregs in preventing autoimmunity and limiting chronic inflammatory diseases comes from the fact that defective development of Tregs in humans with *FOXP3* mutations leads to the life-threatening autoimmune condition immune dysregulation, polyendocrinopathy, enteropathy, and X-linked (IPEX) syndrome (3, 4). A similar lethal disease is observed in scurfy mice, which lack Tregs due to mutations in the *Foxp3* gene (5). In this issue of the *JCI*, Grinberg-Bleyer et al. (6) provide new insight into how mouse CD4$^+$Foxp3$^+$ Treg numbers and suppressive activities are regulated in the autoimmune setting of type 1 diabetes. Their data support the hypothesis that the very cells that the Tregs are suppressing (diabetogenic effector T cells [Teffs]) themselves act in a feedback loop to help islet-specific Tregs, providing sustained protection from diabetes, a hypothesis with far-reaching implications.

Tregs depend on Teffs

The vast majority of studies on Tregs have focused on the mechanisms by which they affect the responses mediated by Teffs. However, it has been noted for some time that there is substantial bidirectionality in the interactions between the two cell populations. As Tregs require IL-2 for their survival and function but do not produce it, it was thought that Teffs would be important providers of IL-2 to Tregs. In vivo mixing experiments with IL-2–deficient and –sufficient Tregs and Teffs confirmed this IL-2–based interdependence of Tregs and Teffs and led to the suggestion that Teffs were required to help maintain a functional Treg compartment (7, 8). Other studies provided additional support for the existence of a feedback loop between Tregs and Teffs, a loop that is important for preventing autoimmune and lymphoproliferative disease (9–11). Thus, it has been established that there is interplay between Tregs and Teffs, and, at least in some cases, this interplay has been shown to be mediated by IL-2 produced by the Teffs.

A feedback loop between Tregs and Teffs in type 1 diabetes

Despite the precedents in the literature (7–11), few reports of the influence of Teffs on Tregs are as clear and informative as the one presented by Grinberg-Bleyer et al. in this issue of the *JCI* (6). In their study, the authors investigated the effect of Teffs on Tregs using mouse models of autoimmune diabetes.

The authors initially found that, in vivo, Tregs proliferated significantly more when coinjected into mice with activated T cells, both in pancreata and draining pancreatic LNs (PLNs) (6). These results led to the con-

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J Clin Invest.* 2010; 120(12):4190–4192. doi:10.1172/JCI45262.

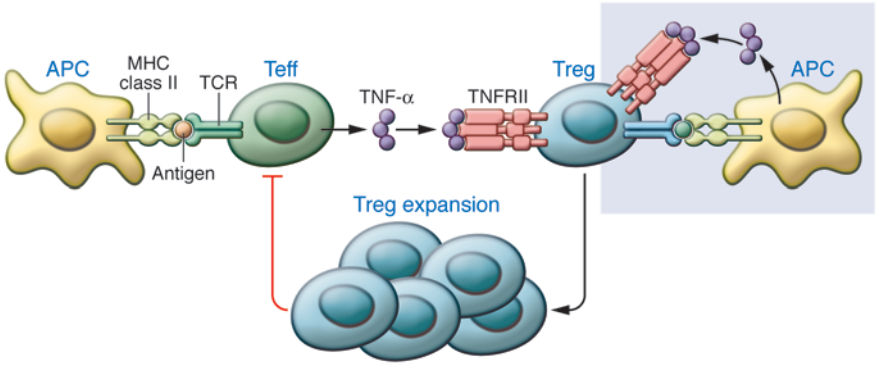

Figure 1
Feedback control of Tregs by Teffs. The drawing illustrates how Teffs help Tregs control themselves, as suggested by the data generated by Grinberg-Bleyer et al. (6). Pathogenic T cells infiltrate and inflame the tissue. Teffs produce inflammatory cytokines such as TNF-α (which can also be produced by activated dendritic cells and macrophages) that can act on Tregs to promote their proliferation and expansion. Tregs can now outcompete the Teffs and also secrete antiinflammatory cytokines that will hamper the proliferation of Teffs. Decreased proliferation of Teffs will limit ongoing inflammation. An alternative pathway in which antigen presentation directly to Tregs may lead to their expansion and subsequent control of inflammation is highlighted in the gray box.

clusion that Teffs induced the proliferation ("boosting") of Tregs, directly or indirectly. Mice injected with Tregs alone or Tregs plus Teffs did not develop diabetes. However, upon challenge with a second injection of activated Teffs three weeks after the first injection, mice that had received a first injection of Tregs alone developed diabetes, while mice that had been originally injected simultaneously with Tregs and Teffs were protected from diabetes. Presumably, the boosted Tregs in the latter recipients mediated suppression of diabetes, although other scenarios were not ruled out. In the future, it will be important to determine why the Tregs injected alone three weeks prior to the injection of the Teffs were not "boostable," despite the fact they were present at the time of the second injection.

In addition, it would be interesting to evaluate whether Teffs affect Treg expansion in a model with ongoing inflammation, for instance, transfer of Tregs shortly after the onset of diabetes induced by activated Teffs. Would Tregs be able to robustly proliferate in an adverse environment such as the one provided by the diabetogenic Teffs? Such information would be of particular relevance for clinical settings, aiding in the development of therapeutic approaches to treating rather than preventing autoimmune conditions (12).

Like other papers addressing the influence of Teff activation on the expansion and function of Tregs, the findings of Grinberg-Bleyer and colleagues (6) have strong implications for the understanding of the hygiene hypothesis — the notion that a lack of early childhood exposure to infectious agents increases susceptibility to allergic and autoimmune diseases by suppressing natural development of the immune system, including a general weakness of the Treg compartment. Specifically, these data indicate that activation of the Teff compartment could boost the Treg compartment early in life, making it more effective in subsequent challenges. Furthermore, before the contribution by Grinberg-Bleyer et al. (6), few mechanisms besides IL-2, long known to be critical for the homeostasis and function of Treg cells, had been shown to be involved in this crosstalk.

Does TNF-α help Treg expansion?

One of the biggest surprises of the data presented by Grinberg-Bleyer et al. (6) is the role of the cytokine TNF-α, and not IL-2, in the boosting of Tregs by Teffs. For reasons mentioned above, it would have been expected that IL-2 would have been the key Teff-produced cytokine to trigger Treg expansion. While Grinberg-Bleyer and colleagues confirmed that IL-2 is critical for the survival of Tregs, their data with IL-2–deficient Teffs clearly showed that the boost effect mediated by Teffs was independent of IL-2 (6).

In contrast to IL-2, which was shown not to play a role in the expansion of Tregs, the cytokine TNF-α was the one involved in Treg boosting (6). TNF-α blockade reduced, although it did not eliminate, the Treg expansion mediated by Teffs. Given the fact that TNF-α blockade constitutes one of the major therapeutic options in the treatment of some chronic inflammatory diseases in humans, such as rheumatoid arthritis and inflammatory bowel disease, the result described by Griberg-Bleyer et al. makes the role of TNF-α in Teff/Treg crosstalk even more important to understand (Figure 1). In patients with rheumatoid arthritis, TNF-α inactivates Treg function, an effect mediated through TNF receptor type II (TNFRII), which is constitutively expressed by Tregs (13–15). However, it has been shown in mouse models that TNF-α promotes the expansion and function of Tregs via TNFRII (16). Could TNF-α induce opposite outcomes in human and mouse Tregs? This is not very likely, considering that TNF-α is an evolutionarily conserved innate and adaptive cytokine with pleiotropic effects. More probable is that the way the effect of TNF-α was assessed in each of the cases described above was driving the effect of TNF-α in opposite directions. When proliferation of Tregs was the major readout, TNF-α induced proliferation, as shown by Grinberg-Bleyer et al. (6) and Chen and coworkers (16); in contrast, when the premium was placed on in vitro suppressive function of Tregs, TNF-α reduced that function (13–15), and this short-term inhibition of suppressive function was also observed by Chen et al. using mouse Tregs (16).

Looking forward

Many open questions remain for future studies. For instance, what is the source of TNF-α, and does it act directly or indirectly on Tregs to "boost" them? While the higher constitutive expression of TNFRII on Tregs than Teffs makes it more likely that TNF-α acts directly on Tregs, experiments determining whether Teffs can boost TNFRII-deficient Tregs have not been carried out, and TNFRII-deficient mice do not have fewer Tregs than wild-type mice (16). The source of TNF-α in vivo is also not clear. While activated Teffs, in particular Th1 cells, are perfectly capable of producing large amounts of TNF-α, many other cells of the innate and adaptive immune system also produce TNF-α. Why would only TNF-α produced by Teffs be effective in boosting Treg expansion in vivo? Finally, given the conflicting data between mouse and human experiments on the effect of TNF-α on Tregs, it is important to clarify whether or not mouse and human Tregs

respond differently to TNF-α or whether the experimental readouts were such that they placed in evidence one or the other outcome. Chances are that we have not heard the last word on the connection between TNF-α and Tregs.

Acknowledgments

A.M. Bilate was a recipient of a postdoctoral fellowship from the Pew Latin American Fellows Program.

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Can we build it better? Using BAC genetics to engineer more effective cytomegalovirus vaccines

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The magnitude and durability of immunity to human cytomegalovirus (HCMV) following natural infection is compromised by the presence of immune modulation genes that appear to promote evasion of host clearance mechanisms. Since immunity to HCMV offers limited protection, rational design of effective vaccines has been challenging. In this issue of the *JCI*, Slavuljica and colleagues employ techniques to genetically modify the highly related mouse CMV (MCMV), in the process generating a virus that was rapidly cleared by NK cells. The virus functioned as a safe and highly effective vaccine. Demonstration of the ability to engineer a safe and highly effective live virus vaccine in a relevant rodent model of CMV infection may open the door to clinical trials of safer and more immunogenic HCMV vaccines.

The urgent need for a vaccine against human CMV

Human CMV (HCMV) is an important cause of disease in immunologically compromised individuals, including recipients of solid organ and hematopoietic stem cell transplants and patients with advanced HIV disease. At greatest risk for HCMV-associated injury, however, is the developing fetus. HCMV is the most common agent of congenital viral infection in the United States, and among all infectious diseases is the most common cause of childhood neurological disability, including deafness, in the developed world (1, 2). Since severe and symptomatic congenital HCMV infections can be associated with a lifetime of disability, the economic burden associated with this infection is striking. When the Institute of Medicine was commissioned to prioritize vaccine development for the new millennium based on, among other factors, quality-adjusted life years (a marker of economic benefit), a vaccine for HCMV was ranked "head-and-shoulders" above all other potential new vaccines with respect to overall cost-effectiveness (3).

Although the need for an HCMV vaccine is compelling, it is less clear to whom such a vaccine should be administered, and what the constituents of such a vaccine should be. The correlates of protective immunity remain undefined, both for the nonpregnant individual and for the developing fetus. Subunit vaccines, typically based on recombinant expression of key targets of humoral and cellular immune responses to HCMV infection, have been evaluated in clinical trials, as have live-attenuated vaccines (4). Until recently, clinical trials have yielded little information about the potential for protective efficacy, largely because most studies have focused on the

Conflict of interest: The author has declared that no conflict of interest exists.

Citation for this article: *J Clin Invest.* 2010; 120(12):4192–4197. doi:10.1172/JCI45250.