commentaries

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PD-1 tempers Tregs in chronic HCV infection

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Adaptive T cell responses are critical for controlling infections with viruses such as HIV, HBV, and HCV. However, these responses must be carefully regulated because overly vigorous T cell activation can lead to excessive host tissue damage. T cell expression of the inhibitory receptor programmed death–1 (PD-1) and inhibition of effector T cells (Teffs) by CD4$^+$Foxp3$^+$ Tregs are among the many described mechanisms for achieving a balanced immune response. Although the signals that contribute to Teff function are well understood, less is known about the signals controlling Tregs. In this issue of the *JCI*, Franceschini et al. extend our understanding of how Tregs are modulated during chronic HCV infection by demonstrating that Treg proliferation is inhibited by PD-1 and that this inhibition is mediated by a potentially novel mechanism involving the prevention of IL-2–driven STAT-5 phosphorylation (see the related article beginning on page 551).

Tregs represent a subset of CD4$^+$ T cells that can inhibit the proliferation and/or cytokine production of responding effector T cells (Teffs). There are many proposed mechanisms by which this can occur, including production of IL-10 or TGF-β or by acting as an "IL-2 sink" and depleting Teffs of available IL-2 (1). Tregs can be identified by the expression of the transcription factor Foxp3 and by high-level expression of CD25, the high-affinity chain of the IL-2 receptor complex. Tregs play a critical role in the maintenance of immune tolerance (1), and accumulating evidence suggests that they also play a central role in balancing the immune response to infection (2–4). They may be particularly important in preventing tissue injury in the setting of chronic viral infections such as HBV and HCV (4). Prior studies of patients with HCV infection have reported higher Treg levels in blood during chronic infection compared with resolved infection, Treg accumulation at the site of infection, and the ability of Tregs from the peripheral blood to suppress CD4$^+$ and CD8$^+$ T cell IFN-γ production as well as CD4$^+$CD25$^-$ and HCV-specific CD8$^+$ T cell proliferation (4). Despite these findings, there has been an incomplete understanding of whether Tregs can suppress highly activated liver-infiltrating Teffs, how Tregs are regulated in the liver, and whether Tregs alter clinical outcomes of patients with HCV infection.
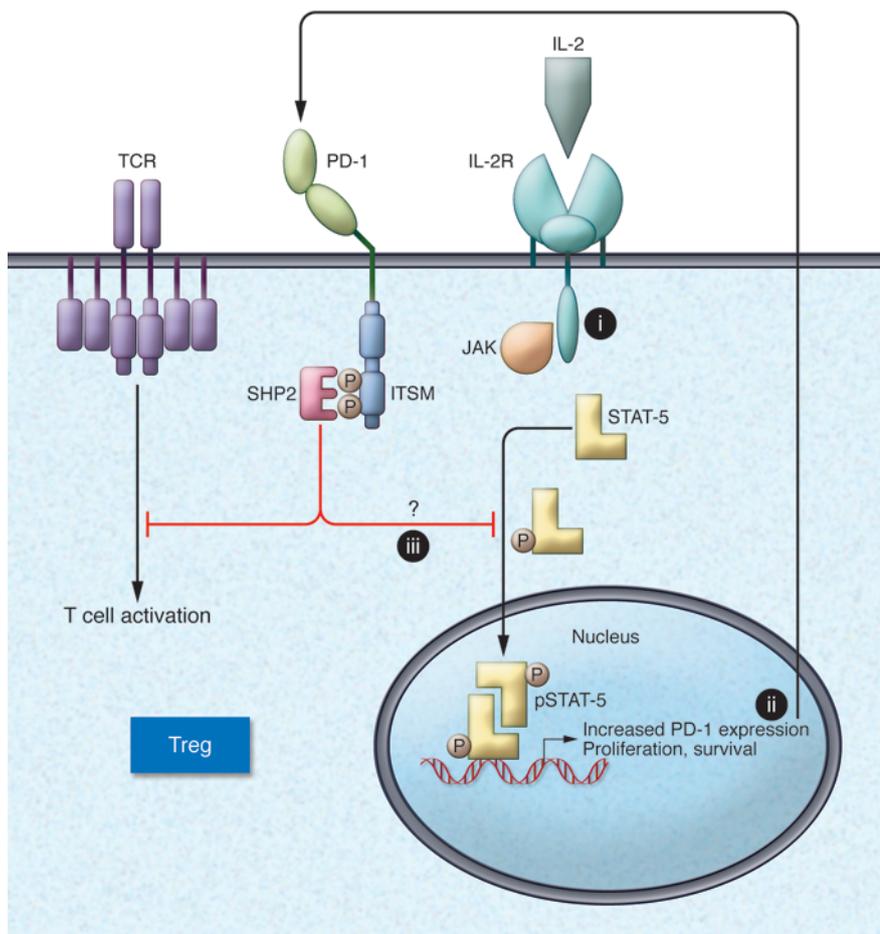
Liver-infiltrating Tregs inhibit Teffs in chronic HCV infection

In order to fully understand the immune deficits seen in HCV infection and the mechanism of immune failure, it is critical to study immune cells and signaling at the

Figure 1
Pathway for inhibition of IL-2 signaling in Tregs by PD-1. PD-1 signaling leads to dephosphorylation of molecules in the T cell activation cascade previously phosphorylated in response to TCR ligation. In this issue of the *JCI*, Franceschini et al. show that PD-1 signaling leads to inhibition of IL-2 signals in Tregs (5). (i) IL-2 signaling via the IL-2 receptor (IL-2R) induces STAT-5 phosphorylation (pSTAT-5). (ii) Dimerization and nuclear translocation of phosphorylated STAT-5 leads to increased proliferation of Tregs but also to PD-1 upregulation. (iii) Increased surface PD-1 expression inhibits STAT-5 phosphorylation (5), possibly via SHP2. ITSM, immunoreceptor tyrosine-based switch motif.

site of infection, since responses measured in the blood may not always overlap with those measured in the liver, particularly in the chronic phase of infection. In this issue of the *JCI*, Franceschini et al. (5) compared blood- and liver-infiltrating Tregs from patients with chronic HCV infection and observed an increased frequency of Foxp3+ Tregs in the liver versus peripheral blood as well as that liver-derived Foxp3+ Tregs from pooled liver biopsies suppressed the proliferation of CD4+ Teffs. Moreover, this inhibition required cell-cell contact, since separation of Tregs and Teffs in a transwell plate system prevented this inhibition. The frequencies of intrahepatic Foxp3+ Tregs correlated directly with plasma HCV viral load and inversely with histological injury, indicating that these liver-infiltrating Tregs were actively modulating the overall effector response to HCV infection in the liver.

Liver-infiltrating Tregs express high levels of PD-1

While Tregs have been shown to be prevalent in the livers of HCV-infected patients,

little is known about how these cells are regulated at the site of infection. Franceschini et al. addressed this question by measuring the level of programmed death–1 (PD-1) expression on intrahepatic Tregs (5). PD-1 is a 288–amino acid type I transmembrane protein in the CD28 family of receptors, and in humans is expressed on T cells, B cells, monocytes, and myeloid DCs (6). Ligation of PD-1 by its receptors programmed death ligand–1 (PD-L1) and PD-L2 imparts a negative signal to the responding cell via an immunoreceptor tyrosine-based switch motif associated with Src homology region 2–containing protein tyrosine phosphatase–1 (SHP1) and SHP2, which dephosphorylate molecules in the TCR signaling cascade (Figure 1). This inhibitory signaling leads to decreased cytokine production and proliferation of Teffs (6). Given the overall lack of vigorous T cell responses in patients with chronic HBV and HCV infection, it is hypothesized that high expression of PD-1 on Teffs contributes to the immune deficits in these patients. We and others have demonstrated

higher PD-1 expression on HCV-specific CD8+ Teffs in liver compared with blood (7, 8). Franceschini et al. also report high expression of PD-1 on Tregs, particularly those infiltrating the liver; in fact, the frequency of PD-1+ Tregs was greater than the frequency of PD-1+ CD4+ Teffs (5).

What impact, then, does PD-1 expression have on Treg function? Studies in mice have documented PD-1 expression on Tregs (9), but the function of this inhibitory molecule on these cells is not well understood. Franceschini et al. evaluated the function of PD-1 expression on Tregs in humans during chronic HCV infection and demonstrated that PD-1 ligation likely provides an overall inhibitory signal to Tregs in this setting (5). Specifically, the authors showed that PD-1 blockade enhanced IL-2–dependent proliferation of intrahepatic Tregs in response to HCV antigens and enhanced the overall ability of Tregs to inhibit Teffs. This effect was linked to an increase in Treg proliferation with PD-1 blockade and not to an enhancement of suppressive function on a per-cell basis. In further support
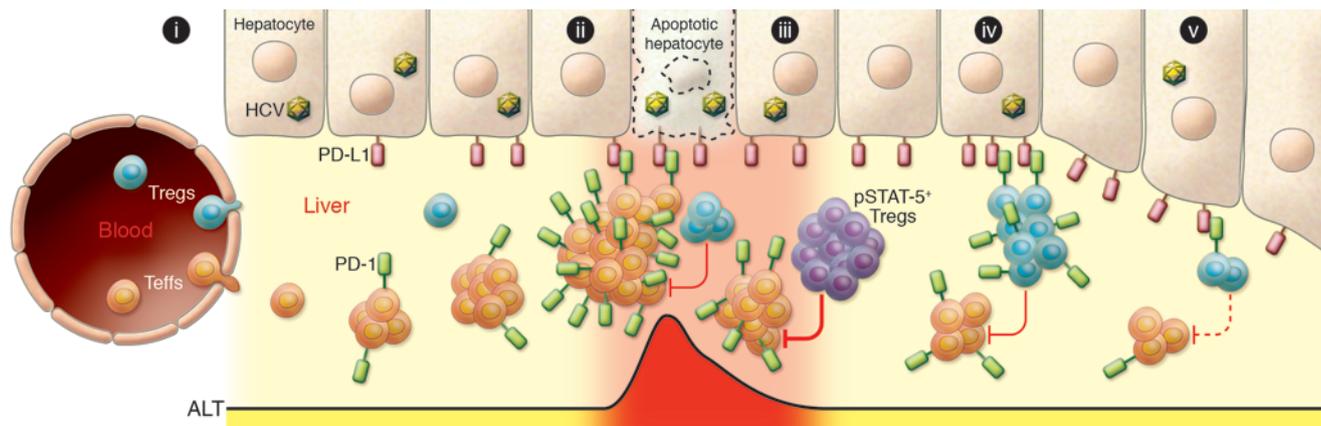
Figure 2
Model for balancing intrahepatic immune responses to HCV. (i) Teffs and Tregs extravasate from the blood vessel to the site of infection. (ii) Vigorous Teff responses induced by HCV infection promote liver injury, leading to ALT elevation. Increased PD-1 expression on Teffs, and PD-L1 expression on hepatocytes, counter this liver injury by providing an inhibitory signal to the Teffs. (iii) In addition, Tregs proliferate and act to decrease Teff-induced liver injury. Franceschini et al. demonstrate that STAT-5 is phosphorylated in the Tregs at this time (5). (iv) Thereafter, Treg responses are tempered by upregulation of PD-1 (5). (v) After Treg responses wane, PD-1 expression decreases and homeostasis is achieved, preventing further liver injury while maintaining a capacity for future Teff responses if needed.

of an inhibitory role for PD-1 on Tregs, the authors also demonstrated a direct correlation of PD-1 expression on Foxp3+CD4+ T cells with greater liver injury.

Treg PD-1 signaling prevents IL-2–driven STAT-5 phosphorylation
In addition to providing strong evidence for the regulation of Tregs by PD-1, Franceschini et al. also demonstrated a potential mechanism for this effect by evaluating the impact of PD-1 on IL-2 signaling (5). Binding of IL-2 to its receptor on T cells results in STAT-5 phosphorylation, which likely has a critical role in Treg development and function (10). In the current study, PD-1 blockade led to greater STAT-5 phosphorylation in Tregs in response to stimulation with anti-CD3/CD28 plus IL-2 (5). Thus, the impairment of intrahepatic Treg proliferation by PD-1 may be directly related to inhibition of IL-2 signaling. A potential model of the impairment of STAT-5 phosphorylation by PD-1 ligation involves SHP2, the same phosphatase responsible for the dephosphorylation of molecules in the TCR activation cascade (Figure 1). While some studies have demonstrated that SHP2 can directly dephosphorylate STAT-5 (11, 12), others indicated that SHP2 enhances STAT-5 signal transduction (13). Therefore, the relationship between SHPs and JAK/STAT signaling may be complex, and future studies will need to address the activity of PD-1–associated SHP1 and SHP2 on STAT-5

phosphorylation to confirm and advance the current findings. In addition, because IL-2 itself has been shown to induce PD-1 expression on T cells (14), a negative feedback mechanism for Treg proliferation might involve the inhibition of IL-2 signaling by IL-2–induced upregulation of PD-1 expression (Figure 1).

A model for balancing liver immune responses
PD-L1 is highly expressed in the liver during viral infection (15, 16). Given the high-level expression of its receptor, PD-1, on both liver-infiltrating Teffs (7, 8) and now Tregs (5) in chronic HCV infection, the PD-1/PD-L1 system likely plays a central role in regulating liver-specific immune responses in this setting. The finding that PD-1 expression is elevated on liver Tregs at the same time as it is elevated on Teffs, and that this expression inhibits Treg proliferation, is somewhat surprising, since one might expect a need for *enhancing* the Treg population at sites of intense inflammation in order to prevent tissue injury by infiltrating Teffs. Franceschini et al. provided some insight into this issue by investigating STAT-5 phosphorylation and PD-1 expression on Tregs prospectively in 2 patients with a severe flare of chronic HCV-induced liver injury (5). In so doing, they may have uncovered an important mechanism for balancing intrahepatic immune responses that requires eventual Treg inhibition by PD-1 in order to maintain homeostasis.

The authors demonstrated that liver injury, as evidenced by high alanine transaminase (ALT) levels in 2 patients with reactivation of hepatitis, was followed first by increased STAT-5 phosphorylation in Tregs, then by decreased ALT, and finally by increased PD-1 expression on Tregs. Once Treg responses waned, PD-1 expression decreased (Figure 2). In a sense, Treg activity increased in response to liver injury, as measured by increased STAT-5 phosphorylation. Once liver injury was under control, increased PD-1 expression — potentially induced by IL-2 (14) — served to inhibit Tregs and maintain homeostasis. Hence, the interplay of Treg activation and relative expression of PD-1 may be a critical factor in controlling the intrahepatic immune response. Recent compelling evidence has suggested that a "dynamic coevolution" of memory and regulatory CD4+ T cells occurs at the site of infections (17–19), and the current findings suggest that PD-1 may be essential to this process.

Impact of Treg control on clinical outcome
The findings of Franceschini et al. demonstrate the importance of PD-1 expression, not only on Teffs, but also on Tregs, in modulating the balance between liver injury and viral control in HCV infection (5). Evaluation of their findings longitudinally in acute HCV infection and in larger cohorts of chronically infected patients will be important in determin-

ing the role of PD-1 expression on Tregs in resolution of infection and liver disease progression. These observations have particular relevance for the potential use of PD-1 blockade in the clinical setting. In the mouse model of lymphocytic choriomeningitis virus and a nonhuman primate model of HIV, improved viral control and survival of infected animals was seen after PD-1 blockade (20, 21) and was linked to enhanced Teff responses. This observation lends hope for therapeutic application in humans, but a major risk of PD-1 blockade is unwanted autoimmunity induced by overenhancement of Teff activity. However, if PD-1 blockade also enhances the function of Tregs, as Franceschini et al. demonstrate, this may counter Teff responses and help to achieve an effective yet balanced therapeutic antiviral response.

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