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'Hardcore' OX40+ immunosuppressive regulatory T cells in hepatic cirrhosis and cancer

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Abbreviations: CHC, chronic hepatitis C; CXCR3, chemokine (C-X-C motif) receptor 3; FOXP3, forkhead box P3; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; Helios (IKZF2), IKAROS family zinc finger 2; IFNγ, interferon gamma; IL-12, interleukin 12; IL-12Rβ2, interleukin 12 receptor beta 2; MF, macrophages; OX40 (TNFRSF4), tumor necrosis factor receptor superfamily member 4; OX40L (TNFSF4), tumor necrosis factor (ligand) superfamily member 4; T-bet (TBX21), T-box 21; TGFβ, transforming growth factor beta 1; Th1, T helper 1; TNFα, tumor necrosis factor; Treg, regulatory T cell; TSDR, Treg cells-specific demethylated region

Human regulatory T cells (Tregs) comprise an array of distinct subsets displaying diverse functions in response to microenvironmental signals. Here, we review our recent findings demonstrating the preferential accumulation of uncommitted, Th1-like and OX40- Tregs in non-cirrhotic tissues in contrast to the presence of committed, Th1-suppressing and OX40+ Tregs in cirrhotic and tumor contexts in human liver affected by chronic hepatitis C.

More and more data have accumulated in the past 15 y evidently demonstrating the pivotal role played by forkhead box P3 (FOXP3)-positive regulatory T cells (Tregs) in limiting spontaneous antitumor immunity and hampering the effectiveness of immunotherapeutic approaches. Indeed, Tregs are inclined to accrue in the tumor microenvironment as a result of several, possibly concomitant, favorable processes, such as attraction from the circulation, local proliferation, de novo differentiation from non-regulatory precursors, preferential survival under stress conditions. Certainly, such events are finely arranged and modulated by the combination of many tissue-derived signals, including chemokines, cytokines, membrane ligands, and many others –provided not only by tumor cells but also by stromal components such as macrophages, fibroblasts, and blood vessels. However, Tregs cannot be considered anymore as passive and indistinct recipients of exogenous cues. Indeed, recent data have contributed to depict a more complex scenario in which the Treg pool may actually embrace a mixture of subpopulations, defined by variable capacity to recognize certain antigens, undergo proliferation, exert immune suppression, stably maintain their phenotype (under the epigenetic control at the Treg cells-specific demethylated region, or TSDR, in the Foxp3 locus), respond to cytokine and/or chemokine signals, acquire specialized suppressive functions, or even be deprogramed into cytokine-producing (so-called Th1-like or Th17-like) cells.

Chronic hepatitis C (CHC) is a condition presumably dominated by type-1 responses, which can ultimately evolve into cirrhosis and cancer, characterized instead by immune suppression. In CHC, Tregs are induced/expanded following acute infection and contribute to establish an immunological 'compromise,' allowing control of immunopathology and avoiding excessive suppression of the HCV-specific immunity.[1] However, in the long run, Tregs may play a relevant role in the progression to cirrhosis and cancer. Cirrhosis may present peculiar immunological features favoring local Treg accumulation: for instance, hepatic stellate cells, which play major roles in fibrogenesis in some contexts, possess well-known tolerogenic functions including the induction of Tregs; furthermore, transforming growth factor β (TGFβ), abundant in fibrotic microenvironments, has well-recognized roles in Treg differentiation, expansion and function. In line with this hypothesis, we could detect a significant increase of Treg frequency in both peripheral blood and liver specimens of cirrhotic compared with non-cirrhotic CHC patients, suggesting that Treg accumulation may occur not only in tumor but also in pre-tumor conditions such as cirrhosis, and that Treg-mediated immune suppression in the cirrhotic microenvironment may contribute to the oncogenic transformation.[2] This hypothesis is supported by the observation of an increased Treg infiltration in liver fibrotic areas in a mouse model of fibrosis and hepatoma.[3]

We performed an extensive analysis of Treg heterogeneity and plasticity within human liver tissues affected by non-cirrhotic chronic inflammation,

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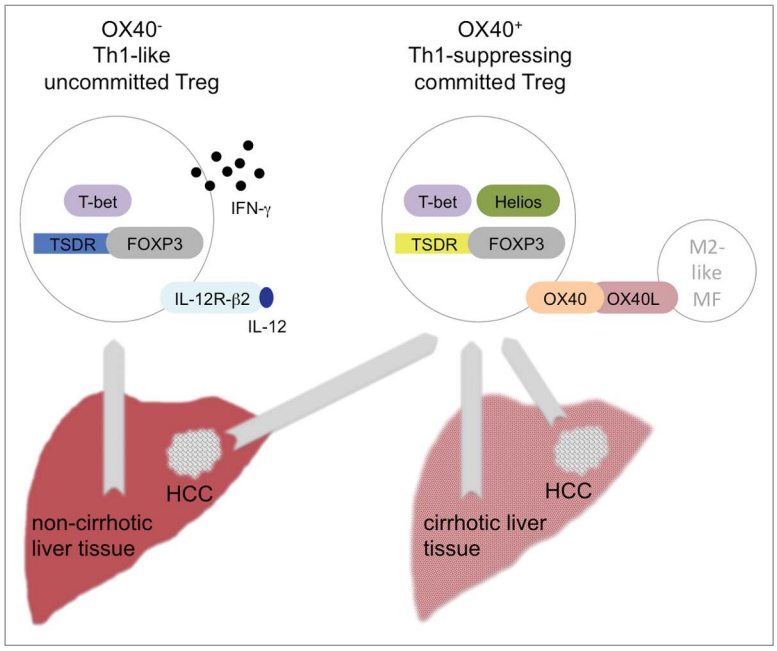

Figure 1. Distinct Treg subsets preferentially populate human liver tissues affected by different pathological conditions. In non-cirrhotic liver tissue of chronic hepatitis C (CHC) patients, regulatory T cells (Tregs) arise with a T helper type-1 (Th1)-like profile, [*i.e.*, interferon γ (IFNγ)$^+$ T-box 21 (TBX21, or T-bet)$^+$] and uncommitted with a methylated Treg cells-specific demethylated region (TSDR, *blue*) profile, as a consequence of local exposure to interleukin 12 (IL-12) sensed by interleukin 12 receptor β 2 (IL-12R-β2). Conversely, in cirrhosis and hepatocellular carcinoma (HCC), Tregs accumulate that express high levels of IKAROS family zinc finger 2 (IKZF2, or Helios) and tumor necrosis factor receptor superfamily member 4 (TNFRSF4, or OX40), and show a committed (demethylated TSDR, *yellow*) and Th1-suppressing (IFN-γT-bet^{high}) phenotype, as a result of the interaction with M2-like macrophages (MF) expressing tumor necrosis factor (ligand) superfamily member 4 (TNFSF4, or OX40L).

cirrhosis, or tumor (hepatocellular carcinoma) related to CHC. Our data revealed a dichotomy in Treg accrual and phenotype in non-cirrhotic vs. cirrhotic/tumor contexts. Indeed, the non-cirrhotic liver microenvironment was infiltrated by low frequency of Tregs, expressing low levels of the tumor necrosis factor receptor superfamily member 4 (TNFRSF4, or OX40) and displaying an interferon γ (IFNγ)$^+$ T-box 21 (TBX21, or T-bet)$^+$ Th1-like phenotype; conversely, cirrhotic and tumor tissues contained high proportions of Tregs, expressing OX40 at a high extent and including a relevant fraction of Tregs expressing T-bet but not producing IFNγ[2] (**Fig. 1**).

Th1-like Tregs have been described in a variety of mouse models of transplantation, viral or parasite infection, and also in human autoimmune diseases, such as diabetes and multiple sclerosis.[4] The development of Th1-like Tregs was dependent on interleukin 12 (IL12, or IL-12), and their immunosuppressive functions appeared to be reduced.[4] Tregs expressing the Th1-related transcription factor T-bet, but not producing IFNγ, have been observed in experimental models of type-1 inflammation and recognized as Tregs 'specialized' in the suppression of Th1 immunity. Interestingly, such 'Th1-suppressing' Tregs have been found also in human ovarian cancer, expressing chemokine (C-X-C motif) receptor 3 (CXCR3) to gain access to the tumor site, and inhibiting IFN-γ response by effector cells.[5]

Our study unraveled a role for the transcription factor IKAROS family zinc finger 2 (IKZF2, or Helios) in the segregation between OX40$^-$ Th1-like (enriched in the Helios^{low} subset) and OX40$^+$ Th1-suppressing (mostly Helios^{high}) Tregs.[2] Helios has been initially considered as a marker of so-called natural or thymus-derived Tregs, but it can also be induced at low extent in vitro and in vivo in de novo induced Tregs. Rather, Helios seems to be involved in sustaining Treg suppressive function and stability. An increased frequency of Helios^{high} Tregs has been reported in tumor-bearing mice and in cancer patients, suggesting Helios^{high} Tregs as chief actors in tumor-related immune suppression.[6] We found that Helios^{high} Tregs were enriched in TSDR-demethylated, epigenetically 'committed' cells, resistant to Th1-like plasticity, thus supporting the notion of Helios primarily as an indicator of Treg commitment, and consequently as a preferential, even though not exclusive, marker of thymic origin and suppressive function. Expansion of Helios^{high} vs. Helios^{low} Tregs may be alternatively regulated by classical or nonclassical monocytes, with the former inhibiting Helios$^-$ development via tumor necrosis factor (TNFα), and the latter constraining Helios$^+$ proliferation via IL-12.[7] Accordingly, we found expansion of M2-like, TNFα-producing, classical macrophages in cirrhosis and tumor contexts, in which IL-12 was poorly represented and Helios^{high} Tregs were abundant.[2]

The correspondence between epigenetic commitment (and the consequent susceptibility to Th1-suppressing or Th1-like differentiation) and developmental origin (and the consequent specificity for self or nonself antigens) is still under debate. Previous evidences have proposed that Th1-suppressing, committed, Tregs may be mostly thymus-derived,[5] while Th1-like, uncommitted, Tregs may preferentially originate in the periphery upon encounter with exogenous antigens.[8] However, HCV-specific Treg in CHC patients display a highly demethylated TSDR,[9] suggesting that 'committed' (possibly Helios^{high}) Treg specific for a viral antigen can locally expand from pre-existing thymus-derived Tregs, or can be peripherally induced following antigen encounter. Future studies will unravel whether, in CHC, HCV- vs. self-specific Tregs are differentially oriented to distinct functional programs by virtue of their origin and commitment.

Our study revealed many important roles for the receptor OX40 in shaping the pool of cirrhosis- and tumor-associated Tregs, promoting local Treg expansion, stability, and functions.[2] Indeed, OX40 expression identified Tregs with a stronger

suppressive activity and a Helios^{high} Th1-suppressing profile. OX40 engagement by tumor necrosis factor (ligand) superfamily member 4 (TNFSF4, or OX40L), expressed by M2-like hepatic macrophages, promoted Treg proliferation and constrained Th1-like plasticity. Of note, and contrary to many evidences emerging from murine studies, we could not observe any inhibition of Treg suppressive function upon OX40 triggering. Therefore, in human cancer, immunotherapies with OX40 agonists may be hindered by the concomitant expansion of OX40$^+$ Tregs. Supporting this possibility, Curti et al. have observed OX40 expression on tumor-infiltrating Tregs in a Phase I clinical trial using a mouse monoclonal anti-OX40 antibody in advanced cancer patients,[10] an event possibly antagonizing the protective actions exerted by OX40 stimulation on the antitumor memory response. Instead, our results indicate that OX40-directed immunotherapies may preferentially target not memory effectors but Tregs, highly expressing OX40 in both cancer and premalignant contexts, and that such approaches may be designed to eliminate or paralyze the OX40$^+$ 'hardcore' Tregs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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