Immunometabolic checkpoints of Treg dynamics in cancer

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Regulatory T cells (Tregs) represent a CD4 T cell subset physiologically devoted to the suppression of unwanted immunity and inflammation, and pathologically involved in the inhibition of protective anti-tumor responses. Tregs constitutively express a variety of cytokine and costimulatory receptors: therefore, Tregs are particularly sensitive to external signals driving their proliferation, contraction and/or functional modulation. Tregs constitutively express OX40, a member of TNF receptor superfamily. In mice, we have characterized multiple roles played by OX40 in shaping Treg suppressive function, fitness and interaction with other cells (i.e. mast cells). We could demonstrate that OX40 supported the expansion of stable and suppressive Tregs also in human cancers, namely hepatocellular carcinoma and colorectal cancer. Tregs are also particularly susceptible to the signal conveyed by type-I interferons (IFNs). However, these cytokines induce Treg apoptosis both in vitro and also in vivo: indeed, we could show that pegylated-IFN/ribavirin therapy rapidly restrains Tregs in chronic hepatitis C patients.

Lymphocytes make rapid and dynamic waves of contraction or expansion depending on the evolution of immune responses and microenvironmental signals. To comply with such plastic scenario, T cells can adapt their energetic routes, shifting from oxidative to glycolytic metabolism in resting/memory or proliferating conditions, respectively. Controversial data in the literature indicate that Treg induction de novo may rely on lipid oxidation as well as on glycolysis, while very little is known about the metabolic pathways sustaining the expansion of so-called natural Tregs, especially in cancer.

We have investigated Treg-intrinsic lipid metabolism in vitro and in vivo in contexts of active Treg proliferation, such as chronic inflammation and cancer. We would like to explore the hypothesis that Treg critically rely on a liposynthesis-fatty acid oxidation circuitry, possibly sustained by glucose uptake, for their proliferation and suppressive function, and that such immunometabolic axis may be targeted to fine-tune Treg-mediated immune suppression in pathological conditions.

To this aim, we have tested whether tumor-infiltrating Tregs display a lipogenic profile, taking advantage of a short-term experimental model of tumor growth. We observed that tumor-infiltrating Tregs accumulated higher levels of intracellular neutral lipids, compared to tumor-infiltrating conventional T cells (Tconvs) and to peripheral counterparts, as revealed by incorporation of the lipophilic dye Bodipy. A similar phenotype was detected in Tregs expanded in a context of hepatic inflammation (i.e. cholangitis in Mdr2 knock-out mice), suggesting that proliferating Tregs may accumulate intracellular lipids in different contexts. Multicolor flow cytometry, combined with the Primeflow technology, allowed visualizing, in tumor-infiltrating Tregs, a higher mRNA content of genes involved in fatty acid synthesis, uptake or usage. While Tconvs showed defective glucose uptake in the tumor microenvironment, Tregs were not impaired and also expressed slightly higher levels of GLUT1, compared to the periphery: these data indicate that, in the tumor microenvironment with low glucose availability, Tregs may prevail over Tconvs for glucose consumption, and may also resist to such a hostile microenvironment by arranging alternative metabolic routes, i.e. glucose-fueled fatty acid synthesis and usage. In line with this hypothesis, we observed that, in vitro, Treg proliferation, compared to Tconv, was associated to higher lipid accumulation and more dependent on fatty acid synthesis and oxidation.

In conclusion, our data suggest that the targeting of metabolic pathways may result in the selective block of Treg proliferation to the advantage of effective antitumor response.