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Review

Controlling the frontier: Regulatory T-cells and intestinal homeostasis

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ABSTRACT

The intestine represents one of the most challenging sites for the immune system as immune cells must be able to mount an efficient response to invading pathogens while tolerating the large number and diverse array of resident commensal bacteria. Foxp3$^+$ regulatory T-cells (Tregs) play a non-redundant role at maintaining this balance. At the same time Treg cell differentiation and function can be modulated by the intestinal microbiota. In this review, we will discuss effector mechanisms of Treg cells in the intestine and how these cells can be influenced by the intestinal microbiota.

1. Introduction

Our body surfaces are colonized with a diverse community of microorganisms composed of archaeal, bacterial, viral and fungal species. The bacterial load is particularly dense in the intestinal tract where trillions of bacteria composed of hundreds of different species reside separated from the underlying immune system by a monolayer of epithelial cells. Such an intimate relationship facilitates the dynamic cross-talk between host and microbe that underlies the delicate balance between tolerance and immunity in the intestine.

There are multiple adaptations that ensure intestinal homeostasis. For example, intestinal epithelial cells (IEC) express a thick layer of mucus containing bactericidal proteins that separate the luminal contents from the host while bacterial metabolites such as short-chain fatty acids enhance barrier function. On the one hand IEC down-regulate Toll-like receptor (TLR) expression to remain unresponsive to the vast bacterial load, while on the other hand, commensals enhance innate immunity to pathogens by providing tonic signals that are required for optimal immune stimulation [1]. In addition to activating innate immune pathways, commensal bacteria also induce antigen-specific T- and B-cell responses, which contribute to host defense to infection and compartmentalization of the intestinal microbiota [2]. Alongside these effector responses, the intestine also harbors a large population of regulatory T-cells (Treg) that play an important role in keeping deleterious inflammatory responses in check. In this review we discuss how microbial factors influence regulatory T-cell function, and how this in turn promotes immune homeostasis in the intestine and beyond.

2. Regulatory T-cell development: thymic and peripheral

Early studies indicated the presence of immune suppressive T-cells in mice and rats that could control autoimmune and inflammatory responses including intestinal inflammation [3–5]. The immune suppressive activity was found to enrich within the CD25-positive subpopulation of CD4 T-cells [6] providing a handle with which to study these cells. However there were limitations as CD25, the IL-2Rα chain, is expressed on activated T-cells, and is therefore not a unique marker of regulatory T-cells. This issue was resolved by the finding that expression of the forkhead box P3 (Foxp3) transcription factor defines the regulatory T-cell lineage [7–9]. Patients with mutations in the FOXP3 gene develop a complex autoimmune syndrome (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX)) [10], manifesting in early-onset insulin-dependent diabetes mellitus, enteropathy, eczema and severe infections, which frequently leads to a life-threatening condition at an early age. The only treatment options are chronic immunosuppression or hematopoietic stem cell transplantation, demonstrating the non-redundant role of regulatory T-cells in controlling immune responses to self and environmental antigens.

By contrast with effector T-cell subsets, a high proportion of the Treg cell pool differentiates and acquires its functional capacity in the thymus, these cells are termed thymic-derived Treg cells (tTreg). Fate determination of T-cell precursors in the thymus depends on the strength of the TCR signal received. Too strong an interaction between the selected TCR and self–peptide/MHCII is thought to lead to clonal deletion. However, Treg cell selection is favored by a stronger TCR self peptide/MHC interaction leading to a TCR repertoire with higher self-reactivity than conventional T-cells [11]. Other factors necessary for commitment to the regulatory T-cell lineage in the thymus include efficient co-stimulation via CD28 [12], as well as signaling through the common γ-chain

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receptor [13], which can be elicited by Interleukin-2, -7 as well as -15 [14,15]. In the thymus Treg cell development does not crucially depend on TGF-β1 as TGF-β1$^{-/-}$ mice show a normal frequency of regulatory T-cells [16], but signaling through the TGF-βR seems to be crucial for survival of pre-differentiated regulatory T-cells, protecting them from negative selection [17].

Importantly, regulatory T-cells can also differentiate from naïve T-cells and acquire Foxp3 expression in the presence of TGF-β [18] outside the thymus, termed peripherally derived Treg cells (pTreg). The requirements for the induction of pTregs are quite different from those required for thymic induction of tTregs, apart from an absolute requirement for TGF-β1 in the periphery [18,19], efficient CD28 cross-linking in the periphery prevents Foxp3 induction [20], while CTLA-4 ligation-dispensable for tTreg cell induction- is required for pTreg cell induction and accumulation of these cells in the colonic lamina propria [21,22].

The question of the origin of intestinal and especially colonic Treg cells is an area of active investigation. Ablation of the CNS1 site in the Foxp3 locus, which has a prominent role in pTreg cell generation [23], leads to a marked reduction in the frequency of Foxp3 positive cells in the small intestinal as well as large intestinal lamina propria, revealing that pTreg cells constitute a substantial part of these populations. The contribution of pTreg cells to intestinal homeostasis is further underlined by the finding that CNS1$^{-/-}$ mice develop a Th2-type driven inflammation in the gastrointestinal tract [24]. Recent studies suggest that intestinal bacteria may provide an important stimulus for local development of pTregs in the intestine as a reduced frequency of Treg cells was observed in the colonic lamina propria of germ-free mice [25,26]. Surprisingly the small intestinal Treg cell frequencies remained unaltered in this setting, suggesting a bacterial-antigen independent mode of pTreg induction in the small intestine. This compartmentalization between the large and small intestinal lamina propria is further strengthened by the finding that GPR15, a G protein-coupled receptor, is important for Treg cell recruitment to the large but not small intestinal lamina propria [27].

In the colonic lamina propria members of the genus *Clostridium*, particularly of cluster IV and XIVa, have now been identified as potent inducers of pTreg cells [25]. Mechanistically this induction depends on an increased expression of TGF-β1, which might reflect the induction of matrix-metalloproteinases (MMP)-2 and -9 expression, which are capable of converting latent TGF-β1 into its active form. In that study it was claimed that pTreg induction was independent of pattern-recognition receptor activation as it remained unimpaired in MyD88, Rip2 and Card-9 deficient mice. Similarly others found normal colonic Treg cell frequencies in MyD88 or Trif single knock-out mice, however pTreg induction in response to bacterial colonization was abrogated in MyD88Trif double knock-out mice [26]. This finding can be interpreted in two ways; either pTreg induction is completely dependent on the TLR-4 signaling pathway as this is the only known pathway to date which requires both the adaptor proteins MyD88 and Trif [28] or there is redundancy amongst different TLRs and/or other molecules signaling through MyD88 (such as IL-1 and IL-18) for bacteria-driven intestinal pTreg cell development. It would be of great interest to determine the cellular source of MyD88Trif dependent signaling particularly as CD103^{+} dendritic cells resident in the MLN and colonic lamina propria have previously been shown to be capable of inducing pTreg cells [29].

A further analysis of the contribution of thymic or peripherally-derived Treg cell subsets to the Treg pool has been hampered by the lack of a reliable marker to distinguish them in vivo. Recently Neuropilin-1 (Nrp1), a receptor for semaphorins as well as VEGF, has been suggested independently by two research groups as a genuine marker for tTregs [30,31]. Functionally the semaphorin-Nrp1 axis seems to play a role in maintaining Treg cell stability after T-cell receptor activation by restraining Akt activation via recruitment of phosphatase and tensin homolog (PTEN) [32]. Interestingly, Nrp1 deficiency only impairs Treg cell stability under certain inflammatory conditions but does not lead to spontaneous development of autoimmune disease. Nrp1 is expressed by approximately 50% of Treg cells in the colon and 65% in the small intestinal lamina propria [30], indicating an equal contribution of pTreg and tTreg cells to the intestinal Treg cell pool. These results are in line with two publications that carried out TCR sequencing on colonic Treg cell repertoires, demonstrating that pTregs [33] as well as tTregs [34] mediate tolerance to antigens derived from intestinal commensal bacteria.

These results point to a "division of labor" between these two developmentally distinct Treg subpopulations, which has been further strengthened by findings that neither tTreg cells nor pTreg cells are capable of fully rescuing Foxp3$^{-/-}$ mice from autoimmune pathology [35]. Rather both populations have to act in concert to offer full protection from disease development. This has been attributed to a mutual complementation of TCR repertoires between the two subpopulations as gene expression signatures were similar between both populations (Fig. 1).

3. Effector mechanisms of Treg cells

Regulatory T-cells mediate their suppressive function via a variety of effector molecules or modules of activity. These include cytokine-mediated suppression (IL-10, TGF-β, IL-35), direct cytolysis of antigen-presenting or effector cells, metabolic disruption (IL-2 expression, cAMP) or direct inhibition of dendritic cell maturation (CTLA-4, LAG3) [36]. The relative contribution of these mechanisms to Treg function remains somewhat obscure. Here we highlight some of the suppressive mechanisms that are of particular importance for intestinal homeostasis [37].

One important mechanism through which Treg cells contribute to immune homeostasis is their capacity to secrete and activate TGF-β1. TGF-β1 knock-out mice develop a fatal wasting syndrome leading to early death at around 20 days of age, similar to Foxp3 deficient mice [38]. TGF-β signaling into T-cells plays a non-redundant role in controlling autoimmune pathology as mice with a T-cell specific ablation of the TGF-βRII develop a pathology mirroring the whole body knock-out [39]. TGF-β signaling in T-cells acts in multiple ways to prevent immune pathology including deviation toward a Treg cell fate and suppression of T-cell effector functions [40,41]. Expression of TGF-β by CD4 T-cells is especially important at mucosal sites as mice with a T-cell specific deletion of the TGF-β1 gene show a more attenuated disease phenotype with a later onset at 6 months of age and confinement of pathology to the colon, lung and liver [42]. Bioavailability of TGF-β is tightly regulated by its secretion as an inactive precursor molecule that has to be cleaved and thereby activated. Dendritic cells in the intestinal lamina propria are capable of activating pro-TGF-β via their expression of integrin αvβ8, a crucial mechanism to preserve intestinal homeostasis [43]. Additionally regulatory T-cells themselves are capable of cleaving TGF-β, as specific ablation of furin, a proprotein convertase that cleaves pro-TGF-β, in CD4 T-cells ablated their protective capacity in T-cell transfer colitis [44].

A second effector cytokine essential for proper regulatory T-cell function, Interleukin-10 (IL-10), is an important component of intestinal homeostasis [45]. IL-10 knock-out mice develop intestinal inflammation if colonized with colitogenic bacteria such as *Helicobacter hepaticus*, a commensal microaerophilic gram-negative bacterium commonly found in most animal facilities, in the context of a "normal" bacterial community [46]. In the latter case, inflammation is confined to the lower intestine and subsequently leads to the development of adenocarcinoma. IL-10