DISEASE PATHOGENESIS

Wnt–β-catenin as an epigenetic switcher in colonic T_{reg} cells

In the colonic environment, sustained Wnt–β-catenin activation in regulatory T cells promotes epigenetic rewiring toward proinflammatory RORγt$^+$ T_{reg} cells, whose expansion parallels the disease progression from inflammatory bowel disease (IBD) to manifest colorectal cancer (CRC).

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Regulatory T (T_{reg}) cells are known suppressors of adaptive immune responses. In this issue of *Nature Immunology*, Quandt et al.[1] show that sustained Wnt signaling induces β-catenin binding to the transcription factor TCF-1, which displaces it from enhancer sites of genes encoding proinflammatory molecules in colonic T_{reg} cells and results in the accumulation of proinflammatory RORγt$^+$ T_{reg} cells expressing interferon (IFN)-γ and interleukin (IL)-17A.

In addition to its known role in carcinogenesis (including CRC progression), the Wnt–β-catenin pathway is emerging as a regulator of various aspects of both adaptive and innate immune response to cancers. An inverse correlation between Wnt–β-catenin pathway activation and a T cell–inflamed gene expression signature in different human tumors[2] points to Wnt signaling in the tumor microenvironment as a suppressor of T cell infiltration and/or function. Mouse melanoma cells engineered to express β-catenin were incapable of releasing the chemokine CCL4, which led to defective recruitment of dendritic cells (DCs) and decreased cytotoxic T lymphocyte infiltration and activation[3]. The infiltration of T_{reg} cells into the tumor microenvironment can also result in the inhibition of antitumor immunity[4]. In this context, β-catenin activation in tumor-infiltrating DCs has been associated with the accrual and enhanced activity of T_{reg} cells[5]. Nevertheless, Wnt–β-catenin signaling limits the immunosuppressive function of T_{reg} cells by modulating TCF-1-dependent inhibition of Foxp3 transcriptional activity[6].

While it is known that T_{reg} cells plastically adapt to their environment and inflammatory stimuli by differentiating into various subsets, the mechanisms regulating this diversity remain largely unknown. In fact, T_{reg} cells expressing other T cell–lineage transcription factors (for example, T-bet, GATA3 and RORγt) are often found in several pathophysiological conditions[7–9]. In particular, RORγt$^+$ T_{reg} cells are detected in the colon and small intestine, where they actively contribute to balancing inflammatory responses[8,9]. Interestingly, Quandt et al. show that a progressive accumulation of β-catenin^{hi}RORγt$^+$ T_{reg} cells occurs alongside the inflammatory escalation from IBD to CRC, highlighting these cells as a potential diagnostic marker for early CRC detection[1]. This T_{reg} cell subset expressed high amounts of

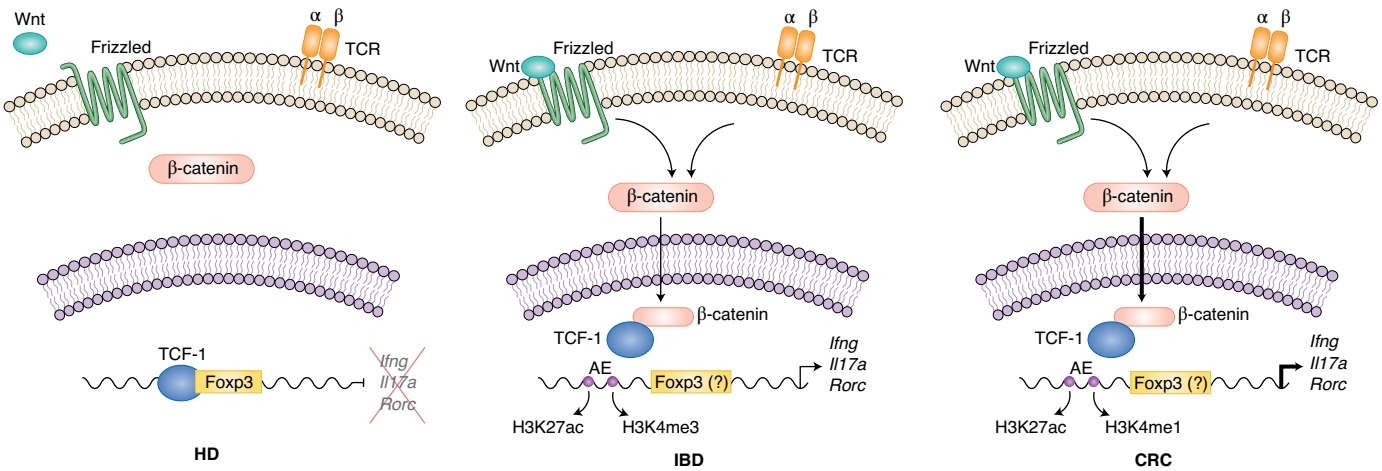

Fig. 1 | β-catenin-dependent epigenetic changes in the proinflammatory profile of T_{reg} cells. Left, in resting T_{reg} cells, TCF-1 and Foxp3 bind to overlapping DNA domains, resulting in transcriptional repression of proinflammatory genes (for example, *Ifng*, *Il17a* and *Rorc*). Middle, in inflammatory bowel disease (IBD), direct activation of the Wnt–β-catenin signaling pathway and/or sustained TCR stimulation lead to β-catenin nuclear translocation and displacement of TCF-1 from its DNA binding sites, located in the activating enhancers (AE) of proinflammatory genes. As a result, epigenetic modifications that promote open chromatin (H3K27ac and H3K4me1) are added. Whether Foxp3 remains bound to the DNA domain or is also displaced is yet to be determined (indicated by the question mark). Right, in colorectal cancer (CRC), sustained Wnt–β-catenin and/or TCR signaling are responsible for enhanced proinflammatory gene transcription. Therefore, the transition from IBD to CRC is defined by an increase in RORγt$^+$ T_{reg} cells, a decrease in T_{reg} cell–associated immunosuppression and enhanced inflammation (lower panel). HD, healthy donors.

the proinflammatory cytokines IL-17A, IFN-γ and tumor necrosis factor (TNF), and their expansion was associated with reduced immunosuppressive activity. In human primary T cells, the expression of RORγt and the gut-homing receptor CCR9 increased following Wnt–β-catenin activation and T cell antigen receptor (TCR) engagement (namely, stimulation with anti-CD3 and anti-CD28), suggesting that β-catenin stabilization alone is sufficient to stimulate the differentiation of proinflammatory T_{reg} cells[1].

In a mouse model of polyposis (that is, mice lacking the oncosuppressor gene adenomatous polyposis coli (APC^{Δ})), which recapitulates the progression from IBD to CRC in humans, the authors identified a more complex scenario[1]. They had previously demonstrated that ablation of the *Rorc* gene in Foxp3$^+$ cells in APC^{Δ} mice stabilized T_{reg} cell anti-inflammatory functions and attenuated polyposis development[10]. In these mice, the landscape of RORγt$^+$ T_{reg} cells comprised several unique populations, suggesting that antigen specificity could play a major role in expanding and driving the function of specific RORγt$^+$ T_{reg} cell subsets during the progression to CRC[1]. In a series of elegant experiments using $Foxp3^{YFP\text{-}Cre}Ctnnb1^{fl(ex3)}$ mice, in which the Cre-mediated excision of exon 3, encoding the degradation domain of β-catenin, allows β-catenin accumulation, the authors demonstrated that stabilization of β-catenin in vivo was responsible for RORγt$^+$ T_{reg} cell commitment, which was accompanied by the presence of unrestrained, highly activated effector T cells, a hallmark of lymphoproliferative disorders[1]. The number of peripheral T_{reg} cells was greatly reduced, as was their immunosuppressive ability, while their thymic generation was unaltered[1], indicating that sustained Wnt–β-catenin signaling does not influence T_{reg} cell central maturation and selection.

Taking advantage of heterozygous female $Foxp3^{YFP\text{-}Cre}$ mice, which are chimeric for β-catenin^{hi}-YFP$^+$ cells, the authors showed that β-catenin^{hi}RORγt$^+$ T_{reg} cells experience a competitive disadvantage in a non-inflamed environment, and, while they still produce proinflammatory cytokines, they do not cause overt disease[1]. Inflammatory stimuli are thus necessary for the expansion and optimal function of β-catenin^{hi}RORγt^{hi} T_{reg} cells.

Next, Quandt et al. asked how sustained β-catenin signaling resulted in the acquisition of proinflammatory traits in T_{reg} cells. Combining chromatin immunoprecipitation with sequencing (ChIP–seq) and assay for transposase-accessible chromatin using sequencing (ATAC–seq) analyses, they provided evidence that TCF-1- and Foxp3-binding sites partially overlap at loci that are crucial for T_{reg} cell function[1]. Particularly, the authors unveiled how both TCF-1 and Foxp3 bound open and poised chromatin, which was enriched in monomethylated histone H3 K4 (H3K4me1) and acetylated histone H3 K27 (H3K27ac) marks[1]. Pathway-enrichment analysis revealed that cobinding of TCF-1 and Foxp3 occurred at active enhancers (AE) of loci involved in type 17 helper T (T_H17) cell differentiation, T cell activation and cytokine production; therefore, the cobinding of TCF-1 and Foxp3 operated as a transcriptional repressor at genes that are silent in T_{reg} cells. In the case of sustained Wnt–β-catenin signaling, the binding of β-catenin to TCF-1 resulted in increased accessibility of the TCF-1–Foxp3 cobound genes (for example, *Il17a* and *Ifng*) but unaltered expression of the core T_{reg} cell gene program (Fig. 1).

With the discovery of the mechanism by which T_{reg} cells can express proinflammatory genes that have been traditionally associated with other effector T cell lineages, the question now emerges as to whether the functions of these T_{reg} cells is driven by engagement of TCRs by microbiota antigens. The greater complexity of RORγt$^+$ T_{reg} cells in the colon as compared to those in the spleen of polyposis-prone mice described by Quandt et al. hints at their local expansion in the colon followed by systemic domination of the gut-homing T_{reg} cells. In healthy individuals, the microbiota likely shapes the ratio between proinflammatory RORγt$^+$ T_{reg} and immunosuppressive T_{reg} cells in an attempt to maintain a homeostatic environment. However, starting with IBD and further progressing in dysplasia and CRC, a change in the bacterial flora combined with modifications in the antigen load and chronic mucosal damage could result in sustained TCR stimulation and possibly the release of Wnt–β-catenin inducers, providing the ideal conditions for the addition of the inflammatory gene profile to the core T_{reg} cell program through epigenetic rewiring (Fig. 1). Future studies will have to establish the exact ontology and diversity of the disease-associated β-catenin^{hi}RORγt$^+$ T_{reg} cells, as well as how they contribute to the progression from IBD to CRC. In this regard, the evidence for a negative correlation between the combined T_{reg}–T_H17 gene signature and reduced survival of patients with CRC in TCGA cohorts support the contribution of β-catenin^{hi}RORγt$^+$ T_{reg} cells to later stages of cancer progression in human patients[1]. ❐

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Competing interests

The authors declare no competing interests.