Activation of β-Catenin in Dendritic Cells Regulates Immunity Versus Tolerance in the Intestine

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Dendritic cells (DCs) play a vital role in initiating robust immunity against pathogens as well as maintaining immunological tolerance to self antigens. However, the intracellular signaling networks that program DCs to become tolerogenic remain unknown. We report here that the Wnt–β-catenin signaling in intestinal dendritic cells regulates the balance between inflammatory versus regulatory responses in the gut. β-catenin in intestinal dendritic cells was required for the expression of anti-inflammatory mediators such as retinoic acid–metabolizing enzymes, interleukin-10, and transforming growth factor–β, and the stimulation of regulatory T cell induction while suppressing inflammatory effector T cells. Furthermore, ablation of β-catenin expression in DCs enhanced inflammatory responses and disease in a mouse model of inflammatory bowel disease. Thus, β-catenin signaling programs DCs to a tolerogenic state, limiting the inflammatory response.

A central question in immunology is how the immune system launches robust immunity against invading pathogens while maintaining tolerance to self antigens. This is of particular importance in the intestine because of the trillions of commensal microorganisms and food antigens that confront the intestinal immune system every day. Antigen-presenting cells (APCs) such as dendritic cells (DCs) and macrophages are specialized immune cells that play a vital role in stimulating immune responses (1, 2). Recent evidence suggests that DCs are also critical in suppressing immune responses, through the generation of regulatory T cells (Treg cells) (2, 3). DCs express several pathogen-recognition receptors (PRRs), which are capable of sensing highly conserved structural motifs in pathogens (4–6) and initiating downstream signaling cascades that are important for controlling the innate and adaptive immune responses (2, 7). Triggering PRRs on DCs can activate DCs and determine the types of cytokines they produce. Such cytokines regulate the differentiation fate of naïve CD4+ T cells into inflammatory cells (Th1 or Th17) or Treg cells, regulating the balance between immunity and tolerance. In the intestine, emerging evidence indicates that DCs and macrophages play a pivotal role in mediating mucosal tolerance against commensals and dietary antigens while mounting inflammatory responses against harmful pathogens (8–12). The signaling pathways that program DCs into a tolerogenic or inflammatory state, however, are poorly understood.

To address this, we analyzed gene expression profiles using microarray analysis of purified intestinal lamina propria DCs (LP-DCs) and compared it with those of splenic DCs (spl-DCs) from mice (13). Several Wnt-ligands were up-regulated (1 ng/ml). Numbers in FACS plots represent percentage of cells positive for the indicated protein. Data are from one experiment representative of three.

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**Fig. 2.** β-catenin signaling in intestinal DCs promotes the expression of Raldh and suppresses the expression of proinflammatory cytokines.  
(A) Expression of Aldh1a1 and Aldh1a2 mRNA in CD11c+CD11b- and CD11c+CD11b+ DCs and CD11c-CD11b- macrophages isolated from SI-LP of β-catenin-/- or β-catenin-/- mice.  
(B) Expression of Raldh protein by CD11c+CD11b- and CD11c+CD11b+ DCs and CD11c-CD11b- macrophages isolated from SI-LP of β-catenin-/- or β-catenin-/- mice, as assessed by intracellular staining and flow cytometry.  
(C) Expression of Il-10, Tgfβ1, Il-23a, and Il-6 mRNAs in CD11c+CD11b- and CD11c+CD11b+ DCs and CD11c-CD11b- macrophages isolated from SI-LP of β-catenin-/- or β-catenin-/- mice after 24 hours. *P < 0.05; **P < 0.005. Error bars indicate mean ± SEM. Data are representative of three experiments.

**Fig. 3.** β-catenin activation in intestinal DCs is independent of commensals and induced by Wnt-signaling.  
(A and B) FACS plots representing the percentage of CD4+ T cells that express (A) FoxP3, IL-17, and (B) IFN-γ isolated from SI-LP of β-catenin-/- or β-catenin-/- mice treated with (Atx) or without (None) antibiotics.  
(C) FACS plots showing the intracellular expression levels of β-galactosidase or β-catenin protein in CD11c+CD11b- and CD11c+CD11b+ DCs and CD11c-CD11b- macrophages isolated from SI-LP of Tcf-7 reporter mice treated with or without antibiotics.  
(D and E) mRNA expression of (D) Fzd receptors and (E) Wnt ligands in CD11c+CD11b- and CD11c+CD11b+ DCs and CD11c-CD11b- macrophages isolated from SI-LP of β-catenin-/- mice.  
(F) mRNA expression levels of Wnt–β-catenin target genes Wisp1, Wisp2, and Axin1 in CD11c+CD11b- and CD11c+CD11b+ DCs and CD11c-CD11b- macrophages isolated from SI-LP of β-catenin-/- mice. Error bars indicate mean ± SEM. Data are from one experiment representative of three.
in LP-DCs as compared with spl-DCs. Consistent with this, reverse transcriptase polymerase chain reaction (RT-PCR) analysis showed that LP-DCs constitutively expressed several Wnt-ligands as compared with that of spl-DCs in the steady state (fig. S1). β-catenin, a central component in Wnt-signaling, is widely expressed in hematopoietic stem cells, macrophages, DCs, and lymphocytes (14). Furthermore, the Wnt-β-catenin pathway has been implicated in the differentiation of DCs from hematopoietic stem cells (15, 16).

Activation of Wnt-Frizzled (fzd) receptor signaling causes translocation of β-catenin from the cytoplasm to the nucleus, where it interacts with T cell factor/lymphoid enhancement factor (TCF/LEF) family members and regulates transcription of several target genes (14, 17). Thus, we assessed whether the β-catenin pathway was active in LP-DCs using the TCF/LEF-lacZ reporter mice (18). In contrast to spl-DCs, LP-DCs from TCF-reporter mice showed strong β-galactosidase expression, suggesting that β-catenin signaling pathway is constitutively active in intestinal DCs (fig. S2). Apart from Wnt signaling, disruption of homotypic E-cadherin interactions in DCs results in β-catenin activation and impairs their immune stimulatory capacity (19). Whether the constitutive β-catenin signaling in intestinal DCs programs them to induce tolerogenic responses, however, is unknown.

To directly assess the role of β-catenin specifically in DC function, we crossed floxed β-catenin allele mice (β-catfl/fl) (20) with transgenic mice (CD11c-cre) expressing the cre enzyme under the control of the CD11c promoter (21). This specifically abrogated β-catenin expression in DCs (fig. S3A). Distinct subsets of intestinal DCs and macrophages can be distinguished by the expression patterns of CD11c and CD11b (CD11 c-CD11b+ and CD11c-CD11b+ DCs and CD11c-CD11b+ macrophages) (12). β-catenin was highly expressed and constitutively active in LP-DC subsets and LP-macrophages in the small and large intestine (Fig. 1A and fig. S3B). In β-catDC−/− mice, β-catenin expression was abrogated in the CD11c-CD11b+ and CD11c-CD11b+ DCs but only partially abrogated in CD11c-CD11b+ macrophages (fig S3B).

We next determined whether β-catenin signaling in DCs is critical for intestinal homeostasis. We compared the frequencies of Treg cells and TH17/TH1 cells in the intestine of β-catDC−/− and β-catfl/fl mice because intestinal DCs and macrophages play an important role in the induction of these subsets (10–12, 22). β-catDC−/− mice had lower frequencies of Treg cells and effector CD4 cells in the small intestine and periphery. We observed higher frequencies of TH17 and TH1 cells in the SI-LP and LILP but not in the spleen of β-catDC−/− mice (fig. 1C and fig. S4, B and C). Consistent with this, CD4+ T cells isolated from SI-LP and LI-LP of β-catDC−/− mice showed elevated expression of the TH17 cell-associated mRNAs interleukin (IL)-17, IL-21, Rorγt, and the TH1 cell–associated mRNA interferon (IFN)-γ (23, 24) as compared with the CD4+ T cells isolated from β-catfl/fl mice (fig. S5). Thus, β-catenin signaling in intestinal DCs is critical in maintaining the balance between Treg cells and CD4+ T effector populations.

Intestinal DCs and macrophages can convert naïve T cells into Treg cells that express the transcription factor Foxp3 and induce effector T cell differentiation (10–12, 22, 25). We thus assessed whether β-catenin signaling in LP-DCs and LP-macrophages is critical for inducing Treg TH1, and TH17 differentiation of naïve CD4+ T cells in vitro using intestinal APCs (CD11c-CD11b+ and CD11c-CD11b+ DCs and CD11b+ macrophages) isolated from β-catfl/fl or β-catDC−/− mice. In the absence or presence of TGF-β, a cytokine important for both Treg and TH17 differentiation, LP-DC subsets from β-catDC−/− were less potent in inducing Treg differentiation as measured with Foxp3 expression, but enhanced TH17 and TH1 cell differentiation as compared with that of DCs from β-catfl/fl mice (fig. 1, D and E, and figs. S6 and S7). The ability of LP-DCs to induce Treg cells is chiefly mediated by a specialized subset of CD103+ DCs (fig. S8 and S9). Similarly, LP-macrophages of β-catDC−/− mice were somewhat less potent in inducing Treg cells as compared with the β-catfl/fl LP-macrophages (Fig. 1D), which is consistent with the partial deletion of β-catenin.

**Fig. 4.** β-catenin signaling in LP-DCs regulates intestinal homeostasis. (A) Percent body weight of β-catfl/fl or β-catDC−/− mice treated with 2% DSS for 7 days. Error bars indicate mean ± SD. (B) FACS plot representing percentage of CD4+ cells positive for IL-17 and IFN-γ isolated on day 8, from Ceacum and LI-LP of β-catfl/fl or β-catDC−/− mice treated with DSS for 6 days. Data are from one experiment representative of three. (C) Histopathological changes in colon tissue from β-catfl/fl or β-catDC−/− mice treated with or without 2% DSS treatment for 7 days. Areas of interest are infiltrations, edema (yellow arrows), goblet cells (black arrows), and basement membrane (green arrows). Images are 10× original magnification.
in macrophages. The absence of β-catenin in DCs did not completely abrogate their ability to induce T<sub>reg</sub> cells, suggesting that additional pathways are involved in programming DCs to the tolerogenic state. Thus, β-catenin signaling in DCs is required to induce T<sub>reg</sub> cells and suppress TH1/TH17 responses.

We next explored the molecular mechanisms by which β-catenin signaling in DCs promoted T<sub>reg</sub> differentiation and suppressed TH1/TH17 cell differentiation. Retinoic acid (RA, a vitamin A metabolite), IL-10, and TGF-β produced by the intestinal APCs and intestinal epithelial cells are critical for the induction of T<sub>reg</sub> cells (8, 10, 12, 26). Aldh1a1 and Aldh1a2 encode enzymes that catalyze the conversion of retinal to RA. Furthermore, Toll-like receptor (TLR)-mediated signaling in DCs up-regulates Raldh enzymes and promotes RA production (27).

Thus, we assessed the expression levels of vitamin A-metabolizing genes by means of RT-PCR and flow cytometry in APC subsets purified from the LP of β<sup>−/−</sup> and β<sup>−/−</sup> mice. We observed that LP-DCs and LP-macrophages of β<sup>−/−</sup> mice expressed much lower amounts of Aldh1a1 and Aldh1a2 mRNA in the SI (Fig. 2A) and LI (Fig S10A) compared with those of β<sup>+/+</sup> LP-DCs and LP-macrophages. The relative expression of Raldh protein was similar to that observed with mRNA (Fig. 2B and Fig S10B). Because β<sup>−/−</sup>-LP-DCs had reduced levels of vitamin A-metabolizing enzymes, we assessed whether addition of exogenous RA could rescue their defect in T<sub>reg</sub> induction. Addition of exogenous RA to β<sup>−/−</sup>-LP-DCs did indeed enhance their capacity to induce T<sub>reg</sub> cells as well as β<sup>+/+</sup> DCs (Fig S11). Thus, β-catenin-mediated signaling promotes T<sub>reg</sub> induction through the expression of vitamin A-metabolizing enzymes in DCs.

Cytokines such as IL-6 and TGF-β promote the differentiation of TH17 cells, whereas IL-23 is thought to regulate the expansion and survival of TH17 cells (24). Thus, we assessed the expression of various pro-inflammatory and anti-inflammatory cytokines in LP-DC subsets isolated from β<sup>−/−</sup> and β<sup>−/−</sup> mice. β<sup>−/−</sup>-LP-DCs had higher mRNA levels of the pro-inflammatory cytokines IL-23a and IL-6 and lower mRNA levels of the anti-inflammatory cytokines IL-10 and TGF-β, as compared with that of β<sup>+/+</sup> LP-DCs, from the SI and LI (Fig. 2C and Fig S10C). Consistent with these results, β<sup>−/−</sup>-LP-DCs produced higher amounts of IL-6 and IL-23 and lower amounts of IL-10 as compared with that of β<sup>+/+</sup> LP-DCs under steady state in both the SI and LI (Fig. 2D and Fig S10D). Thus, β-catenin signaling in LP-DCs is critical for the induction of anti-inflammatory cytokines, which are crucial for the induction of T<sub>reg</sub> cells and suppression of TH1 and TH17 responses.

Intestinal commensals play an important role in the maintenance of TH17 cells (25, 28, 29), and commensal DNA limits the induction of T<sub>reg</sub> cells in the gut (30). Moreover, intestinal DCs can directly take up bacteria and are thus constantly exposed to various pathogen-associated molecular patterns. Thus, we determined whether the enhanced frequencies of TH17 and TH1 cells in the β<sup>−/−</sup>-mice were dependent on the microbiota by treating the mice with an antibiotic cocktail. In β<sup>−/−</sup> mice, antibiotic treatment resulted in modestly enhanced frequencies of T<sub>reg</sub> cells in the LI-LP (Fig. S12A). In contrast, antibiotic treatment of β<sup>−/−</sup>-mice failed to enhance T<sub>reg</sub> cells in the LI-LP (Fig. S12A) but did enhance twofold their frequency in the SI-LP (Fig. 3A). Even with antibiotic treatment, there was a marked reduction in the frequency of T<sub>reg</sub> cells in β<sup>−/−</sup>-mice, relative to β<sup>+/+</sup>-mice, demonstrating that commensals were not essential for β-catenin activity (Fig. 3C and Fig S12C) and for the β-catenin-mediated programming of tolerogenic DCs. We also observed a decrease in the frequency of TH17 cells in the LP of β<sup>−/−</sup>- and β<sup>−/−</sup>-LP-DCs (Fig. 3B). Again, even with antibiotic treatment there was a markedly enhanced frequency of TH17 cells in β<sup>−/−</sup>-mice relative to β<sup>+/+</sup>-mice (Fig. 3B and Fig S12B), suggesting that commensals are not essential for β-catenin signaling-mediated programming of DCs to induce T<sub>reg</sub> cells and suppress TH17 responses. Furthermore, in the absence of β-catenin antibiotic treatment resulted in slightly enhanced T<sub>reg</sub> cells and diminished TH17 responses (Fig. 3, A and B), suggesting that additional β-catenin-independent pathways might be involved in regulating induction of T<sub>reg</sub> cells and TH17 by DCs.

We next assessed by means of RT-PCR the expression of Wnt ligands and Fzd receptors in intestinal APCs because Wnt-signaling activates β-catenin (17). DCs and macrophage subsets from the SI-LP and LI-LP expressed high amounts of various Fzd receptors and Wnt ligands (Fig. 3, D and E, and Fig. S12, D and E). To determine whether Fzd-Wnt receptor signaling is active in intestinal DCs and macrophages, we evaluated the expression levels of several Wnt target genes that can activate β-catenin signaling specifically in DCs may be attractive means of controlling autoimmune diseases such as IBD via the induction of T<sub>reg</sub> cells and concomitant suppression of inflammatory effector T cells.

References and Notes
13. Materials and methods are available as supporting material on Science Online.
Stability of Ecological Communities and the Architecture of Mutualistic and Trophic Networks

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Research on the relationship between the architecture of ecological networks and community stability has mainly focused on one type of interaction at a time, making difficult any comparison between different network types. We used a theoretical approach to show that the network architecture favoring stability fundamentally differs between trophic and mutualistic networks. A highly connected and nested architecture promotes community stability in mutualistic networks, whereas the stability of trophic networks is enhanced in compartmented and weakly connected architectures. These theoretical predictions are supported by a meta-analysis on the architecture of a large series of real pollination (mutualistic) and herbivory (trophic) networks. We conclude that strong variations in the stability of architectural patterns constrain ecological networks toward different architectures, depending on the type of interaction.

Robert May (1) showed that ecological complexity (defined in terms of the number of interacting species and the frequency of their interactions) constrains the stability of randomly assembled interaction networks. Although May’s model included different types of interactions, most theoretical studies since then have focused mainly on trophic interactions. These studies have revealed that many architectural patterns found in real food webs, such as patterns of interaction strength in omnivory loops (2, 3) and allometric degree distributions (4), tend to enhance community stability and species coexistence (5). During the past decade, studies have also investigated with success the particular architecture of mutualistic interactions (6, 7). For those mutualistic networks, asymmetry in interaction strength (8) and nestedness (9, 10) appear to stabilize the community. The variety of approaches used, as well as the focus on a single interaction type at a time, make the differences between trophic and mutualistic networks still unclear and very speculative, especially because food webs are traditionally represented as unipartite networks, whereas mutualistic networks are bipartite (7). We made a systematic comparison between trophic and mutualistic bipartite networks, at different levels of complexity, to test whether the type of interaction affects the relationship between network architecture and stability. First, we followed a model approach in which we analyzed the relations between community stability and a wide range of major network architectural patterns in mutualistic and trophic networks. Then we compared the model predictions with observed network architectures in a large collection of real pollination (mutualistic) and herbivory (trophic) networks.

We built a population dynamics model that can simulate the changes in species densities over time in either mutualistic or trophic networks (11). We used it to simulate the dynamic of \( 7.2 \times 10^4 \) mutualistic and trophic networks, which varied in architecture regarding four main architectural patterns: diversity, connectance, nestedness, and modularity. The values of these indices respectively describe the number of species, the relative number of interactions, the level of sharing of interaction partners among species, and the degree of compartmentalization of species. These architectural patterns have been historically described in ecological networks (7, 12), and despite the relationships between them, they can provide complementary information on how interactions are organized in communities (13). During the simulations, some species could become extinct before equilibrium was reached, thus altering the initial architecture of the networks. These extinctions led mutualistic networks to become more connected, more nested, and less modular. In contrast, species extinctions led to trophic networks that were less connected, less nested, and more modular (Fig. 1, A to D, F, and G). Because nestedness and modularity values are related to network diversity and connectance, we also calculated the relative nestedness and the relative modularity. These indices measure how nested and modular a network is as compared with the mean expected nestedness and modularity under a given null model. Following the approach developed for the study of nestedness in mutualistic networks, we used a null model that assumes that the probability of interaction between a plant and an animal depends on the observed number of interactions of both species (7, 11). Changes in relative nestedness and relative modularity follow qualitatively the same trend as changes in nestedness and modularity. Mutualistic networks increase in relative nestedness whereas trophic networks increase in relative modularity and decrease in relative nestedness. These results indicate that the alterations in network architecture are linked not only with modifications of diversity and connectance but also with complex arrangements of interactions (Fig. 1, E and H). The dynamic and stability of the networks thus constrain the existing structure of mutualistic and trophic networks toward opposite network patterns.

Because the links between community architecture and stability can depend on the stability metric used (14), we measured two distinct indices of stability: persistence (the proportion of persisting species once equilibrium is reached) and resilience (the speed at which the community returns to the equilibrium after a perturbation). We analyzed the results of our simulations by using structural equation modeling (path analysis) to disentangle the direct effects of network diversity and connectance on community stability, as well as their indirect effects mediated through changes in nestedness and modularity. As illustrated in Fig. 2...