Response to Comment on "Activation of β-Catenin in Dendritic Cells Regulates Immunity Versus Tolerance in the Intestine"

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Murphy argues that deletion of β-catenin in macrophages is a caveat to our interpretation that Wnt signaling programs dendritic cells (DCs) into a tolerogenic state in the gut. However, our data demonstrate that β-catenin–deficient DCs are greatly impaired in inducing regulatory T cells, and induce enhanced T helper 17 (T_H17)/T_H1 responses. Assessing the relative importance of DCs versus macrophages in intestinal tolerance must await tools that permit the genetic deletion of the numerous DC and macrophage subsets in the intestine.

The central message of our study (*1*) was that intestinal dendritic cells (DCs) express constitutively active β-catenin, which programs them to induce regulatory T (T_{reg}) cells. Furthermore, conditional ablation of β-catenin in DCs impairs their ability to induce T_{reg} cells and enhances their capacity to induce T helper 17 (T_H17)/T_H1 responses. Consistent with this, mice in which β-catenin expression is deleted in DCs contain reduced frequencies of T_{reg} cells and enhanced frequencies of T_H17/T_H1 cells in the intestine and are more susceptible to intestinal inflammation. Taken together, these results demonstrate a key role for β-catenin signaling in programming DCs to a tolerogenic state. Murphy (*2*) argues that the deletion of β-catenin in macrophages "remains a caveat to their interpretation that Wnt signaling programs dendritic cells into a tolerogenic state." The source of this concern is the use of CD11c-Cre mice (*3*) to specifically delete β-catenin in intestinal DCs. It is important to highlight that numerous previous studies have used the same strain of CD11c-Cre mice used in our study to selectively delete genes from DCs (*3*–*6*), including intestinal DCs (*4*, *7*, *8*).

Figure S3, A and B, in (*1*) demonstrates that expression of β-catenin was abrogated in $CD11c^+CD11b^-$ and $CD11c^+CD11b^+$ DCs, but only partially abrogated in $CD11c^-CD11b^+$ macrophages. Murphy contends that this partial abrogation "may warrant further consideration." He further questions the validity of the gating strategy used to define the various subsets of antigen-presenting cells (APCs) and states that CD11c versus CD11b staining is not shown. To address this last issue, in fact, the histograms were generated by a sequential gating strategy involving gating on forward scatter versus side scatter, followed by gating on $CD45^+$ IA^{b+} cells, followed by gating on $CD11c^+CD11b^-$ versus $CD11c^+CD11b^+$ versus $CD11c^-CD11b^+$ cells. This is a strategy that we have described previously (*7*), that we use routinely in the lab, and that is presented in figure S8 in (*1*). Whether the deletion in $CD11c^+CD11b^+$ macrophages was only "partial" or as Murphy puts it "substantial," we feel that the real test is in the functional consequences of such deletions. In fact, $CD11c^-CD11b^+$ intestinal macrophages isolated from CD11c-Cre mice crossed with floxed β-catenin mice displayed a rather modest phenotype, relative to the phenotype of DCs. For example, there was only a modest impairment in their ability to induce T_{reg} cells [figure 1D in (*1*)], and there was no effect at all in their ability to induce T_H17 or T_H1 responses [figure 1E and figure S7 in (*1*)]. This is in striking contrast to the effect of β-catenin deletion on DCs and demonstrates a partial phenotype in macrophages. Thus, we contend that Murphy's assertion that deletion of β-catenin in macrophages is a caveat to our interpretation is unjustified. The data clearly demonstrate that DCs isolated from CD11c-Cre mice crossed with floxed β-catenin mice are (i) severely impaired in their ability to induce T_{reg} cells, (ii) induce greatly enhanced T_H17 and T_H1 responses [figure 1, D and E, and figures S4 to S7 in (*1*)], and (iii) produce greatly reduced amounts of Raldh enzymes and interleukin-10 and enhanced levels of proinflammatory cytokines [figure 2 and figure S10 in (*1*)]. These data demonstrate that β-catenin signaling programs DCs to a tolerogenic state.

Another concern noted by Murphy (*2*) is the seemingly opposite conclusion of the present study to our earlier report (*9*) that macrophages, and not DCs, maintain tolerance in the intestine. It should be noted that nowhere in (*1*) did we exclude a role for macrophages in tolerance. In fact, it is very likely that multiple subsets of APCs contribute to this process. Furthermore, our recent study, in which we used a 10-color flow cytometry panel to delineate all the DC and macrophage subsets in the intestine (*10*), demonstrates that the ability of intestinal DC and macrophage subsets to induce $Foxp3^+$ T_{reg} versus T_H17 cells is critically dependent on multiple factors, such as the ratio of T cells to APCs, the regional localization of these subsets in the intestine, and, importantly, the source of the mouse strain. Thus, DCs from C57BL/6 mice from Charles River Laboratories [that have segmented filamentous bacteria, which induce robust levels of T_H17 cells in situ (*11*, *12*)], were more efficient at inducing T_H17 cells and less efficient at inducing $FoxP3^+$ T_{reg} cells than DCs from C57BL/6 mice from Jackson Laboratories (*10*), which is the source of the β-catenin floxed mice.

In conclusion, our study (*1*) demonstrates that β-catenin is constitutively active in intestinal DCs and exerts a potent role in programming such cells to a tolerogenic state. The issue raised by Murphy on the relative importance of β-catenin expression in DCs versus macrophages in maintaining intestinal tolerance is indeed an important one that needs to be directly addressed using mice in which β-catenin is deleted in macrophages. However, to the best of our knowledge, there are currently no strains that can be used to reliably delete genes from macrophages in a highly specific and nonleaky manner. These issues notwithstanding, it is important to appreciate the complexities of distinguishing between DCs and macrophages based on phenotype alone (*10*, *13*). Indeed, our recent work (*10*) demonstrates that nearly half the cells in the $CD11c^+CD11b^+$ subset in the small intestine, which phenotypically resemble DCs, in fact displayed a typical macrophage morphology characterized by abundant phagocytic vacuoles. Furthermore, the so-called "$CD103^+$ DCs" do not simply represent one subset; in fact, both the $CD11c^+CD11b^-$ subset and the $CD11c^+CD11b^+$ subsets can be subdivided into $CD103^+$ versus $CD103^-$ cells. Given this complexity, one should avoid the temptation to get mired in semantic details on whether a given function is attributable to a particular subset and whether that subset is more like a DC or a macrophage. Instead, our efforts should focus on unraveling the molecular mechanisms and transcription factors that determine the balance between immunity and nonresponsiveness. Ultimately, it is this that will be of real value in offering new insights that guide the development of strategies for the therapeutic modulation of immunity in inflammatory disorders.

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