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

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Manicassamy *et al*. (Reports, 13 August 2010, p. 849) deleted β-catenin in intestinal immune cells using a CD11c-driven Cre recombinase, which decreased anti-inflammatory mediators and increased inflammatory bowel disease. However, the deletion of β-catenin in macrophages remains a caveat to their interpretation that Wnt signaling programs dendritic cells into a tolerogenic state. Development of strains expressing Cre in a more finely lineage-restricted pattern may help resolve this issue.

Conditional gene deletion using Cre-mediated deletion of floxed genes is a powerful approach to bypass unintended lethality and restrict gene deletion to specific cell lineages. When Cre recombinase is driven by a locus that is expressed in multiple cell lineages, however, proper data interpretation requires careful determination of the extent and specificity of target deletion. In general, the promoters of different genes used to drive the expression of Cre recombinase may achieve very different absolute levels of Cre activity, even when these genes share a similar tissue-specific pattern of expression. This is true because a similar pattern of tissue expression does not ensure that genes are transcribed at equal absolute levels. The efficiency of Cre-mediated recombination depends on the absolute level of transcription and translation of the Cre recombinase, and perhaps on the accessibility of the floxed target allele in each tissue, and not simply on the relative expression levels of the driver-gene between tissues. Specifically, Cre recombinase driven by the CD11c gene may not only induce deletion of target alleles in dendritic cells (DCs), but also induce deletion of target genes in other cells that express CD11c at low to intermediate levels, such as macrophages. The extent of deletion required to ablate function in target cells may vary between different assays and cell types.

The study by Manicassamy *et al*. (*1*) elegantly demonstrated the requirement of β-catenin signaling in maintaining immune tolerance in the intestine using conditional deletion induced by crossing transgenic mice that express the Cre recombinase enzyme under the control of the CD11c promoter (CD11-Cre) with mice that express floxed β-catenin alleles. The importance of β-catenin was clear, but the interpretation that it is the activation of β-catenin in DCs that regulates tolerance—a conclusion stated in the title—might warrant additional consideration. An unusual feature of this study was its opposite conclusion to the authors' earlier report that macrophages, and not DCs maintain tolerance in the intestine (*2*). Thus, the extent and specificity of Cre-mediated deletion of β-catenin is central in judging the validity of reversing the earlier interpretation.

Pulendran and colleagues previously used a CD11c-DTR (diphtheria toxin receptor) system with oral diphtheria toxin (DT) treatment to selectively deplete lamina propria DCs (*2*). This reduced interleukin-17 production by T cells but had no effect on regulatory T (T_{reg}) cells, which was interpreted as showing that DCs were inflammatory rather than tolerogenic in the intestine. On the basis of additional in vitro co-culture analysis, the authors concluded it was macrophages that regulated tolerance. The extent of DC depletion appeared incomplete, because $CD11c^+$ cells were reduced only from 4% in controls to 1.6% in mice after DT treatment, as shown in supplementary figure 7a in (*3*). The deletion of other cells, such as $CD11b^+CD11c^-$ macrophages, was not analyzed. Still, the authors concluded that macrophages, and not DCs, maintained tolerance, even though depletion of $CD11c^+$ DCs was only 60% complete.

In (*1*), Manicassamy *et al*. claim that expression of CD11c-Cre "specifically abrogated β-catenin expression in DCs" on the basis of data presented in their figure S3, A and B. This figure shows flow cytometric analysis of intracellular β-catenin expression in cells gated as $CD11c^+CD11b^-$, $CD11c^+CD11b^+$, and $CD11c^-CD11b^+$. DCs were defined as $CD11c^+$, whereas macrophages were defined as $CD11c^-CD11b^+$. The CD11c and CD11b staining itself is not shown but may be similar to figure S8 in (*1*), the only figure that presents CD11c and CD11b staining. Although macrophages are known to express CD11c at levels significant enough to cause deletion in a DTR system (*3*), and in some cases as highly as DCs (*4*), the study refers to macrophages as "$CD11c^-$," and CD11c staining controls are not provided. Nonetheless, the authors state that "β-catenin expression was abrogated in the $CD11c^+CD11b^-$ and $CD11c^+CD11b^+$ DCs but only partially abrogated in $CD11c^-CD11b^+$ macrophages." It is this last phrase—the "only partially abrogated" part—that may warrant further consideration.

Unfortunately, raw fluorescence-activated cell sorting data are not archived with publications—unlike microarray data—preventing subsequent analysis by readers. To my eye, however, figure S3B in (*1*) shows substantial deletion of β-catenin in $CD11c^-CD11b^+$ cells. The absence of gates or percentages in the histograms makes it difficult to quantify the extent of deletion, and showing flow cytometric data without gates or percentages may be a suboptimal form of analysis (*5*). On rough visual inspection, however, a large percentage of macrophages do not express β-catenin at all and the rest express β-catenin at roughly half the level of wild-type macrophages. To be specific, in the lower right panel of figure S3B (*1*), a jagged red line directly overlays the shaded-gray isotype negative control population; these are macrophages that are negative for β-catenin. To the right, the red line forms a peak positioned well to the left of the solid-black positive control ($\beta\text{-cat}^{fl/fl}$); these are macrophages that express approximately half the amount of β-catenin found in control mice. Presenting the histograms as normalized to equal peak heights, rather than equal event numbers, tends to draw attention away from these differences between the samples. Presenting the data as equal numbers of events would have reduced the height of the red peak and drawn attention to clear differences between the level of β-catenin expression in the macrophage populations from the control $\beta\text{-cat}^{fl/fl}$ sample and the Cre-deleted experimental sample. Despite substantial deletion of β-catenin in macrophages, the study refers to these mice as "$\beta\text{-cat}^{DC-/-}$" throughout.

The $CD11c^-CD11b^+$ macrophages that express reduced amounts of β-catenin may also function abnormally because a heterozygous phenotype has been reported for β-catenin (*6*). This would be consistent with the authors' statement that "LP-macrophages of $\beta\text{-cat}^{DC-/-}$ mice were somewhat less potent in inducing T_{reg} cells as compared with $\beta\text{-cat}^{fl/fl}$ LP-macrophages." In any case, the unintended deletion of β-catenin in macrophages in the present study would appear more complete that the intended depletion of DCs in their previous study.

Distinguishing DCs and macrophages on the basis of positive and/or negative expression of CD11c and CD11b may be too simplistic. Macrophages express relevant amounts of CD11c, as shown by their deletion of β-catenin by a CD11c-driven Cre. DCs and macrophages share many overlapping markers whose expression can change under various conditions, locations, and times. All these issues suggest that ascribing functions to lineages based on Cre-mediated deletion can sometimes be a tricky business, and it may still be unresolved which cells contribute to intestinal tolerance.

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