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Cutting Edge: β-Catenin Is Dispensable for T Cell Effector Differentiation, Memory Formation, and Recall Responses

Martin Prlic and Michael J. Bevan

The molecular mechanisms that regulate mature T cell fate and enable cells to differentiate into memory T cells are largely unknown. Memory T cells share certain key features with stem cells: they both have the ability to self-renew and are long-lived. The Wnt–β-catenin signaling pathway is a key player in regulating stem cell self-renewal and differentiation. We generated a conditional knockout mouse that specifically lacks β-catenin in mature T cells and report in this article that β-catenin is not involved in regulating effector versus memory T cell differentiation. β-catenin–deficient memory T cells were phenotypically and functionally indistinguishable from control cells and made normal recall responses. β-catenin deficiency does not affect T cell migration, T cell function in a model of chronic infection, or lymphopenia-induced proliferation. Together, our data suggest that self-renewal and differentiation are regulated differently in memory T cells compared with epithelial and hematopoietic stem cells. The Journal of Immunology, 2011, 187: 1542–1546.

Mature T cells harbor an incredible proliferative potential that is displayed when a naive T cell is activated and acquires an effector phenotype during the course of ≥15 rounds of cell division (1). While T cells with an effector phenotype are rather short-lived, a population of long-lived memory T cells emerges after the peak of the expansion phase. These memory T cells have the capacity to self-renew by slow homeostatic turnover (2) and share certain transcriptional patterns with hematopoietic stem cells (HSCs) (3). The Wnt–β-catenin pathway was identified as a key pathway in regulating self-renewal and differentiation in epithelial cells (4) and has since been proposed to play the same role in HSCs (5). In the absence of Wnt signals, β-catenin is continuously marked for degradation by GSK3β. Binding of a member of the Wnt ligand family to its receptor leads to inhibition of GSK3β activity and allows β-catenin accumulation and translocation to the nucleus, where it interacts with TCF/LEF transcription factors. In addition to the Wnt signaling pathway, β-catenin release to the nucleus is affected by E-cadherin (6).

Wnt signaling has been studied in several gain-of-function studies and in a very limited number of loss-of-function studies examining HSC function, as well as lymphocyte development (7). Although there are some discrepancies in these gain-of-function studies (7), the data tend to confirm the original notion of a key role for β-catenin in epithelial cell self-renewal (4) to be true in HSCs, as well (5, 8). However, because data from loss-of-function studies are limited and conflicting, this conclusion remains disputed (9, 10). The role of β-catenin in T cell development is still controversial, as well (11, 12). The reason for the different outcome is still unclear; although the different timing of deletion might play a role, a compensatory role for γ-catenin when β-catenin is deleted early in HSCs was not found (13).

The role of β-catenin in mature T cell differentiation has not been addressed by deleting the gene in T cells after thymic selection in vivo, although one study used an elegant in vitro approach to delete β-catenin in mature T cells (14). Two groups reported that TCF-1–deficient T cells have an impaired ability to generate memory T cells (15, 16), but because TCF-1 plays a crucial role in thymic selection (17), and TCF-1–deficient T cells in the periphery have an altered phenotype (18), it is not possible to pinpoint the defect as a result of improper development in the thymus or as a mature T cell differentiation defect. Ultimately, loss-of-function studies that exclude secondary effects caused by changes in thymic selection are necessary to confirm these conclusions but are still missing.

We asked whether β-catenin expression is required for the generation of functional memory cells in vivo. To circumvent a possible effect of β-catenin deficiency during thymic selection on mature T cell function, we bred β-catenin–flox mice (19) to mice that express Cre under the control of the distal lck promoter that is turned on after positive selection and a YFP reporter (20). We tested T cell memory generation in various conditions, including after viral and bacterial infection and in lymphopenic conditions. We found that β-catenin was not necessary for memory T cell generation and function and...
conclude that β-catenin function is not congruent in epithelial cells and mature T cells.

Materials and Methods

Mice

C57BL/6 mice and RAG1-deficient mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and housed in specific pathogen-free conditions in the animal facilities at the University of Washington. β-catenin–ﬂox mice were provided by Kris Hogquist (University of Minnesota) and originally came from Rolf Kemler (Max Planck Institute, Freiburg, Germany), dlck-cre transgenic mice originated from Nigel Killeen (University of California, San Francisco) and were bred to Rosa-YFP reporter mice and provided by Pam Fink (University of Washington). Data shown are representative of results from at least two independent experiments. All experiments were done in accordance with Institutional Animal Care and Use Committee guidelines.

Infections

Listeria monocytogenes expressing a secreted form of OVA (LM-OVA) was grown as previously described (21). For primary infections, mice were injected i.v. with 2 × 10^5 CFU LM-OVA i.v. or intranasally with 1 × 10^5 actA-deficient LM-OVA. For rechallenge experiments, mice received 2 × 10^5 CFU LM-OVA i.v. and were euthanized 5 d later. For priming with lymphocytic choriomeningitis virus (LCMV) Armstrong and LCMV clone 13, mice were injected i.p. with 2 × 10^6 PFU and i.v. with 2 × 10^5 PFU, respectively.

In vitro experiments

TWS119 (Merck/EMD) experiments were performed, as previously described (22). Cells were stained with LIVE/DEAD cell stain kit (Invitrogen) prior to FACS analysis. Wnt3α experiments and Eomes expression analysis were performed as previously described (15). Data shown are combined from two experiments and were analyzed with Prism software using the t test.

Flow cytometry

Cells and tissues were prepared for staining and assays and analyzed as previously described (21). In some experiments, cells were labeled with Cell-Tracker Violet (Invitrogen), according to the manufacturer’s instructions. When applicable, YFP+ cells were sorted using a FACSAria.

Results and Discussion

Three experimental groups of mice were used: the first group lacked expression of dlck-cre (wild-type [WT]). The other two groups expressed both the dlck-cre transgene and the YFP reporter. Of these latter two groups of mice, one group had one WT and one deleted (floxed) allele (het), and the other group had β-catenin deleted on both (floxed) alleles (knockout [KO]). This setup allowed for proper control of possible Cre-mediated toxic effects that are independent of the deletion of the target gene. Our YFP-gating strategy, conﬁrmation of β-catenin deletion, and abrogation of the Wnt signaling pathway in β-catenin–deficient cells are shown in Supplementary Fig. 1. Data from the WT group are shown once and omitted for brevity in ensuing experiments. We infected all three groups with a recombinant L. monocytogenes strain (LM-OVA) that allowed us to track a specific CD8 T cell epitope. We examined T cell numbers and phenotype at the peak of the response on day 7. All experimental groups generated an equivalent primary CD8 T cell response to OVA, as measured by tetramer staining (Fig. 1A), with indistinguishable surface marker (CD62L, KLRG1) phenotypes (Supplemental Fig. 1D, data not shown). Contrary to what was reported for epithelial cells (23), CD44 expression was not regulated by β-catenin activity in T cells, because CD44 expression on Ag-specific T cells increased in all experimental groups (Fig. 1).

A recent study proposed a role for Wnt signals in mediating effector cell migration (24). We speculated that a systemic infection could mask a potential role for β-catenin in T cell migration. We infected mice intranasally with LM-OVA and

![FIGURE 1](http://www.jimmunol.org/) Normal CD8 effector differentiation in β-catenin–deficient T cells. A, WT, het, and KO mice were infected i.v. with LM-OVA, and the frequency of Ag-specific CD8 effector T cells in the blood was determined 7 d later. B, WT, het, and KO mice were infected intranasally with an actA-deficient strain of LM-OVA, and the frequency of Ag-specific CD8 effector T cells in spleen and lung was determined 6 d later. Cells shown are gated on CD8+ cells for WT cells and YFP+ CD8+ cells for het and KO cells. Data shown are representative of at least two independent experiments with three animals/group.

![FIGURE 2](http://www.jimmunol.org/) Normal memory development in β-catenin–deficient T cells. A, Frequency and function of Ag-specific memory cells in the blood were determined ≈30 d after i.v. infection with LM-OVA. Frequency and function of Ag-specific CD8 memory cells in the spleen (B) and brain and lung (C) were determined 5 d after a rechallenge with LM-OVA. Cells shown are gated on YFP+ CD8+ cells for het and KO cells. Data shown are representative of at least two independent experiments with three animals/group.
examined T cell numbers in spleen and lung. T cells of all three groups showed equivalent expansion and migration (Fig. 1B). Thus, we excluded a general role for β-catenin–mediated migration of effector cells, although this does not exclude the possibility that β-catenin signaling might be involved in migration under certain situations or in specific tissues.

It is not known whether memory cells give rise to effector cells in a true stem cell fashion, where one daughter cell differentiates into an effector cell, and the other daughter retains a memory phenotype. We hypothesized that if β-catenin was required to balance effector versus memory differentiation, a rechallenge would reveal any defects in β-catenin–deficient T cells. We determined the number of Ag-specific memory cells during the memory phase (≥30 d postinfection) and found equivalent CD8 memory T cell levels in all experimental groups (Fig. 2A, data not shown). To examine the ability of β-catenin–deficient memory cells to expand after rechallenge, we infected mice with a rechallenge dose of LM-OVA. CD8 memory T cells expanded equally well 5 d after the rechallenge and had identical functional properties, independent of β-catenin expression (Fig. 2B). WT, het, and KO CD8 T cells expressed identical levels of granzyme B and showed no evidence of any statistically significant changes in function, migration, or phenotype caused by β-catenin deficiency (Fig. 2C, data not shown). Similarly, we did not observe any differences in the re-expansion of the CD4 memory T cell compartment (data not shown). A loss of regulation mediated by regulatory T cells (Tregs) could theoretically mask a potential β-catenin–dependent phenotype; however, we could not find any evidence for an impaired Treg population. Generation of Foxp3+ cells in vitro polarization assays by β-catenin–deficient cells was normal (data not shown), but even the 50% of CD4 T cells that do not delete β-catenin (Supplemental Fig. 1A) would be sufficient for normal Treg function (25).

We went on to test the ability of β-catenin–deficient T cells to proliferate in a lymphopenic environment. We speculated that the different stimuli that drive T cell proliferation and acquisition of a memory phenotype in a lymphopenic environment might more closely resemble the turnover of epithelial cells and could reveal a dependency on β-catenin. Proliferation in a lymphopenic host is largely driven by noninflammatory cytokines and availability of self-ligands and is independent of costimulation (26). We transferred CellTracker-labeled lymph node cells into RAG-deficient recipient hosts and analyzed them 12 d later. Again, we found that the lack of β-catenin did not impair the ability of T cells to proliferate and acquire a memory phenotype (Fig. 3, data not shown). The majority of cells were CellTracker negative after 12 d, whereas another population had divided less than seven times and still contained the dye. Both populations were present in all three groups at similar numbers. Together, these data suggested that β-catenin is not involved in memory
T cell generation and maintenance, regardless of the signals that mediated T cell activation.

Although β-catenin is dispensable for the generation of memory cells, we speculated that T cell exhaustion caused by chronic infection, such as LCMV clone 13, might be more profound in β-catenin–deficient mice, if β-catenin expression is required for self-renewal of a T cell. We infected mice from all three experimental groups with LCMV Armstrong or LCMV clone 13. T cells from LCMV Armstrong-infected mice mounted a normal primary response, regardless of β-catenin expression (Fig. 4A, left panels), consistent with our data using Listeria immunization (Fig. 1). In addition, β-catenin–sufficient and -deficient T cells showed a similar response and phenotype on day 8 after LCMV clone 13 infection (Fig. 4A, right panels). Mice that were infected with LCMV Armstrong generated a functional, stable T cell memory pool (Fig. 4B, left panel). T cells from mice infected with LCMV clone 13 showed impaired cytokine production and signs of functional exhaustion, regardless of their genotype (Fig. 4B, right panels). Furthermore, Ag-specific KO and het CD8 T cells expressed an equivalent amount of PD-1 on memory stem cells by a drug that inhibits GSK3β exhaustion.

Liferative changes in a case of chronic T cell stimulation and signs of functional exhaustion, regardless of their genotype (Fig. 4B, right panels). Together, these data suggest that the lack of β-catenin does not result in any functional or proliferative changes in a case of chronic T cell stimulation and exhaustion.

Finally, we addressed whether the reported generation of memory stem cells by a drug that inhibits GSK3β is truly β-catenin dependent (22). Regardless of the genotype of cells, we found that TWS119 blocked in vitro-stimulated T cell proliferation starting at a dose that exceeded the specific target IC50 (30 nM, according to the manufacturer) by 100-fold (3 μM; Fig. 5, Supplemental Fig. 2); however, it had little to no effect if used at a lower dose (Supplemental Fig. 2), an effect also apparent in the original paper (22). Treatment of T cells with a high dose of TWS119 prevented clustering of stimulated T cells so that only a fraction of cells was activated and divided (Fig. 5, Supplemental Fig. 2). The previously reported “stem cell phenotype” of CD44low, CD62L high TWS119-treated cells was found in the undivided-cell population but not in the divided-cell population (Fig. 5). Importantly, this was the case, regardless of β-catenin expression (Supplemental Fig. 2). The reported stem cell-like features of these treated cells can be explained by the fact that the TWS119-treated cells contained a population that had been at least partially activated and, thus, are superior to the naive control cells used in these experiments (22). Thus, our data support and extend the conclusions and concerns raised by Gajewsky and colleagues (14) regarding TWS119 treatment and suggest that the data from Restifo’s group (22) need to be reevaluated.

In summary, we used an in vivo loss-of-function system to address the role of β-catenin in various differentiation and self-renewal processes of mature T cells. We showed that effector and memory T cell differentiation occurs unperturbed in the absence of β-catenin and argue against a model in which β-catenin stabilization and translocation to the nucleus are required to direct memory T cell self-renewal and differentiation. Although WT T cells responded to Wnt3α stimulation in vitro (Supplemental Fig. 1C), the response of β-catenin–deficient T cells to Wnt3α was abrogated, suggesting that there is no compensation mechanism by other catenin family members in place. Why would memory T cells have the signaling machinery in place but not use it to regulate self-renewal? HSCs are located in specialized niches, whereas effector and memory T cells can be found in lymphoid and nonlymphoid tissues, including fat tissue. Recent data suggested that HSC renewal is specifically regulated by one Wnt family member, Wnt3α, in a nonredundant manner (27). A Wnt-independent mechanism of self-renewal might allow for equal memory T cell development in all tissues, regardless of Wnt availability, a feature not necessary for HSCs that are located in specialized niches of the bone marrow. Although it is clear that T cells can respond to Wnt-mediated signals in vitro (15, 24) (Supplemental Fig. 1C), further experiments will be required to determine whether there are physiological settings in which Wnt signaling participates in regulating aspects of T cell fate or function in vivo, similarly to what was reported recently for dendritic cells (28).

Disclosures

The authors have no financial conflicts of interest.

References


Supplemental Figure 1: Gating strategy and deletion efficiency.
(a) Approximately 90% of CD8⁺ cells and 50% of CD4⁺ T cells were YFP⁺ in β-catenin KO and het (data not shown) mice. (b) We confirmed deletion efficiency in WT and YFP⁺ het and KO CD8 T cells by PCR. A WT β-catenin allele results in a 200bp product, a floxed β-catenin allele yields a 324bp product and deletion of β-catenin yields a 500bp product. The WT cells (left band) yield a 200bp and 324bp band (one allele is floxed, but not deleted due to the lack of the dlck-cre transgene), KO cells only have a 500bp product indicating close to 100% efficient deletion and het cells have one 500bp and one 200bp product, indicating the presence of one WT allele. (c) naïve WT, but not β-catenin KO cells respond significantly to a 3hr incubation with Wnt3a conditioned media (15) in vitro as measured by an increase in expression of the target gene Eomesodermin. Data shown are combined from two experiments. (d) Antigen specific CD8 effector T cells of all three experimental groups expressed little CD62L on the cell surface consistent with an effector phenotype.
Supplemental Figure 2: TWS119 inhibits cell proliferation at a dose that exceeds the target specific IC50 by 100fold. (a) YFP+ CD8+ T cells were labeled with CellTracker Violet, purified by FACS and stimulated in vitro with αCD3 and αCD28 antibodies in the presence of titrated doses of TWS119 and analyzed 5 days later. (b) Starting at a dose of 3μM we observed an inhibition of cell proliferation in β-catenin deficient and sufficient cells as shown by the lack of cluster formation 48hrs after in vitro stimulation.