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TLR2-Dependent Activation of β-Catenin Pathway in Dendritic Cells Induces Regulatory Responses and Attenuates Autoimmune Inflammation

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Dendritic cells (DCs) sense microbes via multiple innate receptors. Signals from different innate receptors are coordinated and integrated by DCs to generate specific innate and adaptive immune responses against pathogens. Previously, we have shown that two pathogen recognition receptors, TLR2 and dectin-1, which recognize the same microbial stimulus (zymosan) on DCs, induce mutually antagonistic regulatory or inflammatory responses, respectively. How diametric signals from these two receptors are coordinated in DCs to regulate or incite immunity is not known. In this study, we show that TLR2 signaling via AKT activates the β-catenin/T cell factor 4 pathway in DCs and programs them to drive T regulatory cell differentiation. Activation of β-catenin/T cell factor 4 was critical to induce regulatory molecules IL-10 (*Il-10*) and vitamin A metabolizing enzyme retinaldehyde dehydrogenase 2 *(Aldh1a2)* and to suppress proinflammatory cytokines. Deletion of β-catenin in DCs programmed them to drive Th17/Th1 cell differentiation in response to zymosan. Consistent with these findings, activation of the β-catenin pathway in DCs suppressed chronic inflammation and protected mice from Th17/Th1-mediated autoimmune neuroinflammation. Thus, activation of β-catenin in DCs via the TLR2 receptor is a novel mechanism in DCs that regulates autoimmune inflammation. *The Journal of Immunology*, **2014, 193: 4203–4213.**

Innate immune cells sense microbes with a combination of several pattern recognition receptors. Dendritic cells (DCs) play a vital role in initiating robust immune responses against pathogens (1–5). Emerging studies now show that DCs are also critical in promoting regulatory responses (6, 7). Therefore, DCs are critical for regulating the delicate balance between tolerance versus immunity that underlies disease progression in many autoimmune disorders, cancer, and chronic infection. DCs express several TLRs and the C-type lectins, which are critical in initiating immune response against pathogens (8–10). Engagement of such pattern recognition receptors promotes DC maturation and cytokine production (2, 8, 9). Consequently, types of cytokines produced by DCs dictate the outcome of adaptive immune responses (2). For example, activation of most TLRs on DCs induces strong production of IL-12(p70) that promotes IFN-γ–producing Th1 cells. Other microbial stimuli that activate TLR2 on DCs induce IL-10 production and promote Th2 or regulatory Foxp3 T (Treg) responses, whereas dectin-1–mediated signals in DCs that induce strong production of TGF-β, IL-6, and IL-23, which promote Th17 differentiation. However, the receptors and signaling networks that are critical for programming DCs in inducing inflammatory versus regulatory responses are still being elucidated.

Zymosan, a yeast cell wall derivative, is recognized by many innate immune receptors, including TLR2 and dectin-1, a C-type lectin receptor for β-gulcans (11–15). Combined activation of TLR2 and dectin-1 results in the induction of robust IL-10 production in DCs (16–19), as well as proinflammatory cytokines in macrophages and DCs (14, 20). Consistent with this, our previous work has shown that TLR2 signaling induced splenic DCs (SPDCs) to express the retinoic acid (RA) metabolizing enzyme Aldh1a2 and IL-10, and promoted T regulatory response (21). Furthermore, zymosan is also known to induce macrophages to secrete TGF-β (18, 19), a cytokine critical for the generation of regulatory T cells, as well as Th17 cells (13, 22–24). Thus, microbial activation of TLR2 signaling pathway in general promotes T regulatory/Th2 responses and suppresses inflammatory responses (7, 25). In contrast, dectin-1–mediated signaling in DCs induces proinflammatory cytokines and promote Th1 and Th17 cell differentiation (21, 26, 27). How signaling networks in DCs via TLR2 and dectin-1 are integrated and influence divergent innate and adaptive immune responses is poorly understood.

β-catenin, an essential component of canonical wnt pathway, is widely expressed in immune cells including DCs and macrophages (28). β-catenin signaling has been implicated in the differentiation of myeloid DCs and plasmactyoid DC differentiation from hemopoietic stem cells (29, 30). Our previous work has shown that unlike in SPDCs, β-catenin signaling is active constitutively in intestinal DCs and macrophages and is critical for regulating intestinal homeostasis (31). However, its role in peripheral tolerance is not known. In this study, we show that TLR2-mediated signals activate β-catenin/T cell factor 4 (TCF4) pathway resulting in programming DCs to induce regulatory responses to zymosan. We also show that

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Abbreviations used in this article: ALDH, aldehyde dehydrogenase; DC, dendritic cell; EAE, experimental allergic encephalitis; β-gal, β-galactosidase; MOG, myelin oligodendrocyte glycoprotein; qRT-PCR, quantitative real-time PCR; RA, retinoic acid; SPDC, splenic DC; TCF4, T cell factor 4; Treg, regulatory Foxp3 T; WT, wild-type.

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activation of β-catenin/TCF4 is dependent on PI3K/AKT-mediated signals and programs DCs to a regulatory state, which produce RA and IL-10. Consistent with this, the β-catenin/TCF4 pathway was critical for zymosan-mediated induction of Treg cells, and suppression of Th1 and Th17 responses mediated autoimmunity in vivo. Accordingly, in the absence of β-catenin, zymosan induced potent Th1 and Th17 responses and exacerbated autoimmunity.

Materials and Methods

Mice

C57BL/6, TCF/LEF-reporter mice (32), Axin2 (LacZ) reporter mice (33), CD11c-Cre, Akt1$^{-/-}$ (34), and TLR2$^{-/-}$ were originally obtained from The Jackson Laboratory and were bred on-site. OT-II (Rag 2$^{-/-}$) mice were obtained from Taconic Farms. β-catenin$^{flox/flox}$ or TCF4$^{flox/flox}$ mice (35) were bred with transgenic mice (DC-cre) expressing cre enzyme under the control of CD11c promoter (36) to generate mice lacking β-catenin or TCF4 in DCs. Successful cre-mediated deletion was confirmed as described in our previous study (31). All mice were maintained in specific pathogen-free conditions in the Georgia Regents University vivarium. All animal protocols were reviewed and approved by the Institute Animal Care and Use Committee of Georgia Regents University.

Reagents and Abs

CD4 (RM4-5), IL-17 (TC11-18H10), IFN-γ (XMG1.2), anti-CD3 (145.2C11), anti-CD28 (37.51), and brefeldin A were obtained from BD Biosciences. Foxp3-PE (FJK-16s), CD11c (N418), and CD11b (M1/70) were purchased from eBioscience. Zymosan, curdlan, retinol, and all-trans-RA were purchased from Sigma-Aldrich. Purified *Escherichia coli* LPS, Pam-2-cys and Pam-3-cys, CpG, and depleted zymosan were obtained from InvivoGen. Abs for phospho-AKT, phospho–β-catenin, active β-catenin, β-catenin, ERK, and phospho–GSK-3β (Ser^{9}) were obtained from Cell Signaling Technology. Rabbit monoclonal β-galactosidase (β-gal) Ab was purchased from Abcam. Peptides myelin oligodendrocyte glycoprotein (MOG)35–55 (MEVGWYRSPFSRVVHLYRNGK) and OVA323–339 (ISQVHAAHAEINEAGR) were purchased from Anaspec.

Purification of SPDCs

CD11c^{+} DCs were purified from spleen as described previously (21). In brief, spleens from mice were dissected, cut into small fragments, and then digested with collagenase type 4 (1 mg/ml) in complete DMEM plus 2% FBS for 30 min at 37˚C. Cells were washed twice and the CD11c^{+} DCs were enriched using the CD11c microbeads from Miltenyi Biotec. The resulting purity of CD11c^{+} DCs was ~95%.

TLR stimulation of APCs

CD11c^{+} SPDCs (10^{6} cells/ml) were cultured with Pam-2-cys (100 ng/ml), zymosan (25 μg/ml), or curdlan (25 μg/ml) for 24 h. The supernatants were collected for cytokine analysis by ELISA, whereas cells were collected for gene expression analysis by quantitative real-time PCR (qRT-PCR).

In vitro culture of murine DCs and T cells

In vitro stimulation was performed as described previously (21). In brief, purified CD11c^{+} SPDCs (10^{6} cells/ml) were stimulated with zymsoan (25 μg/ml) or curdlan (25 μg/ml) for 8 h and washed with media three times. In some experiments, DCs were cultured with disulfiram (100 nM) or PI3K inhibitor (5 μM), ERK inhibitor (1 μM), or AKT inhibitor (5 μM) for the duration of stimulation. Activated DCs (2 × 10^{4}) were cultured together with naive CD4^{+}CD62L^{+}OT-II CD4^{+} T cells (10^{5}) and OVA (2 μg/ml) in 200 μl RPMI 1640 complete medium in 96-well round-bottom plates. Supernatants were analyzed after 90 h for cytokine production by ELISA, and cells were collected and analyzed directly for Foxp3 or were restimulated for intracellular cytokine staining. In some experiments, 500 nM retinol (σ) and/or 1 ng/ml TGF-β (R&D Systems) were added to cultures. In some experiments, intracellular Foxp3 analysis was performed before stimulation. For secondary restimulation, cells were collected after 90 h of primary culture followed by incubation for 6 h with plate-bound anti-CD3 (10 μg/ml) and anti-CD28 (2 μg/ml) in the presence of brefeldin A for intracellular cytokine detection. In some experiments, cells were restimulated with OVA (2 μg/ml) for 48 h for analysis of cytokine production in cell supernatants.

OT-II CD4^{+} T cell adoptive transfer

β-cat$^{fl/fl}$– and β-cat$^{\Delta DC}$–recipient mice were reconstituted with 2.5 × 10^{6} OT-II TCR–transgenic CD4^{+} T cells 1 d prior to receiving 25 μg MHC class II–restricted OVA peptide in PBS alone or PBS containing either 50 μg zymosan or 50 μg curdlan by iv injection. Four days later, spleens were removed and after RBC lysis, total splenocytes were counted, stained with Abs, and analyzed by flow cytometry. Data were acquired on a FACSCalibur (BD Biosciences) or LSRII (BD Biosciences) and analyzed with FlowJo software. In vitro recall responses were assayed by restimulating total splenocytes (2 × 10^{6}/ml) for 6 h with plate-bound anti-CD3 (10 μg/ml) and anti-CD28 (2 μg /ml) in the presence of brefeldin A for intracellular cytokine detection or were restimulated with OVA (2 μg/ml) for 48 h for analysis of proliferation and cytokine production in cell supernatants.

Flow cytometry

Isolated splenocytes were resuspended in PBS containing 5% FBS. After incubation for 15 min at 4˚C with the blocking Ab 2.4G2 (anti-FcγRIII/I), the cells were stained at 4˚C for 30 min with the appropriate labeled Abs. Samples were then washed twice in PBS containing 5% FBS. The samples were immediately analyzed at this point or fixed in PBS containing 2% paraformaldehyde and stored at 4˚C. Intracellular staining for β-catenin, active β-catenin, phospho–β-catenin, ERK, AKT, GSK-3β, and β-gal was performed using rabbit mAb or with appropriate isotype control in TBS containing 1% BSA, followed by incubation with Alexa Fluor 488–conjugated rabbit-anti-goat Ig or goat anti-rabbit IgG (Molecular Probes, Eugene, OR). Aldehyde dehydrogenase (ALDH) activity was determined using Aldefluor staining kit (StemCell Technologies), according to the manufacturer's protocol. Flow cytometric analysis was performed on a BD Biosciences FACSCalibur or LSR II flow cytometer at Georgia Regents University.

Experimental allergic encephalitis induction

Experimental allergic encephalitis (EAE) induction experiments were performed as described in our previous study (21). EAE was induced by s.c. immunization in the hind flanks on day 0 using 100 μg $MOG_{35–55}$ emulsified in CFA containing 2.5 mg/ml heat-inactivated *Mycobacterium tuberculosis* (Difco). Some experiments were performed using 100 μg $MOG_{35–55}$ plus 100 μg zymosan in IFA. Mice also received 250 ng pertussis toxin (List Biological Laboratories) i.p. on days 0 and 2 postimmunization. In some instances, mice received PBS or zymosan (100 μg) by i.v. at the time of immunization. Disease severity was assessed according to the following scale: 0, no disease; 1, flaccid tail; 2, hind limb weakness; 3, hind limb paralysis; 4, forelimb weakness; and 5, moribund.

CNS T cell isolation

Mice were euthanized with CO_2 and perfused through the left ventricle with PBS. The brain and spinal cord were removed from each animal and homogenized on a 0.2-μm filter. T cells were isolated over Percoll (Sigma-Aldrich) and then were stained for Foxp3 or restimulated for 6 h with plate-bound anti-CD3 (10 μg/ml) and anti-CD28 (2 μg/ml) in the presence of brefeldin A for intracellular cytokine detection.

Measurement of cytokine production

Cytokines in culture supernatants was measured by two-site sandwich ELISA. IL-17, IL-6, IL-12(p40), IL-12(p70), IL-10, IFN-γ, and IL-23 levels were measured by ELISA kits obtained from eBioscience.

qRT-PCR

Total RNA was isolated from purified SPDCs using the Qiagen RNeasy Mini Kit, according to the manufacturer's protocol (Qiagen). cDNA was generated using the superscript First-Strand Synthesis System for RT-PCR and random hexamer primers (Invitrogen), according to the manufacturer's protocol. cDNA was used as a template for qRT-PCR using SYBER Green Master Mix (Bio-Rad) and gene specific primers as described in our previous study (21) and Gene expression was calculated relative to the housekeeping gene GAPDH.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism (Software for Science). Mean clinical scores were analyzed using the Mann–Whitney nonparametric *t* test. The statistical significance of differences in the means ± SD of cytokines released by cells of various groups was calculated with the Student *t* test (one-tailed).

Results

TLR2-mediated signals activate β-catenin/TCF pathway

We first assessed whether TLR2-mediated signals activate β-catenin using Ab that recognizes the active form of β-catenin. SPDCs without any treatment showed low/undetectable levels of active β-catenin (Fig. 1A). In contrast, DCs stimulated with zymosan showed increased levels of active β-catenin compared with untreated DCs (Fig. 1A). β-catenin activation was detected in DCs as early as 1 h after zymosan stimulation (Fig. 1A). Because zymosan signals through TLR2 and dectin-1 (18), we next determined whether activation of β-catenin is dependent on TLR2 or dectin-1. DCs treated with dectin-1 ligands, curdlan or depleted zymosan (Dep zymo) failed to activate β-catenin compared with zymosan treated DCs (Fig. 1A). Consistent with this observation, TLR2-deficient DCs treated with zymosan also showed low levels of active β-catenin compared with wild-type (WT) DCs (Fig. 1B). We next examined whether other TLR ligands can activate β-catenin in DCs. Treatment with TLR2/6 ligand Pam-2-cys or TLR2/1

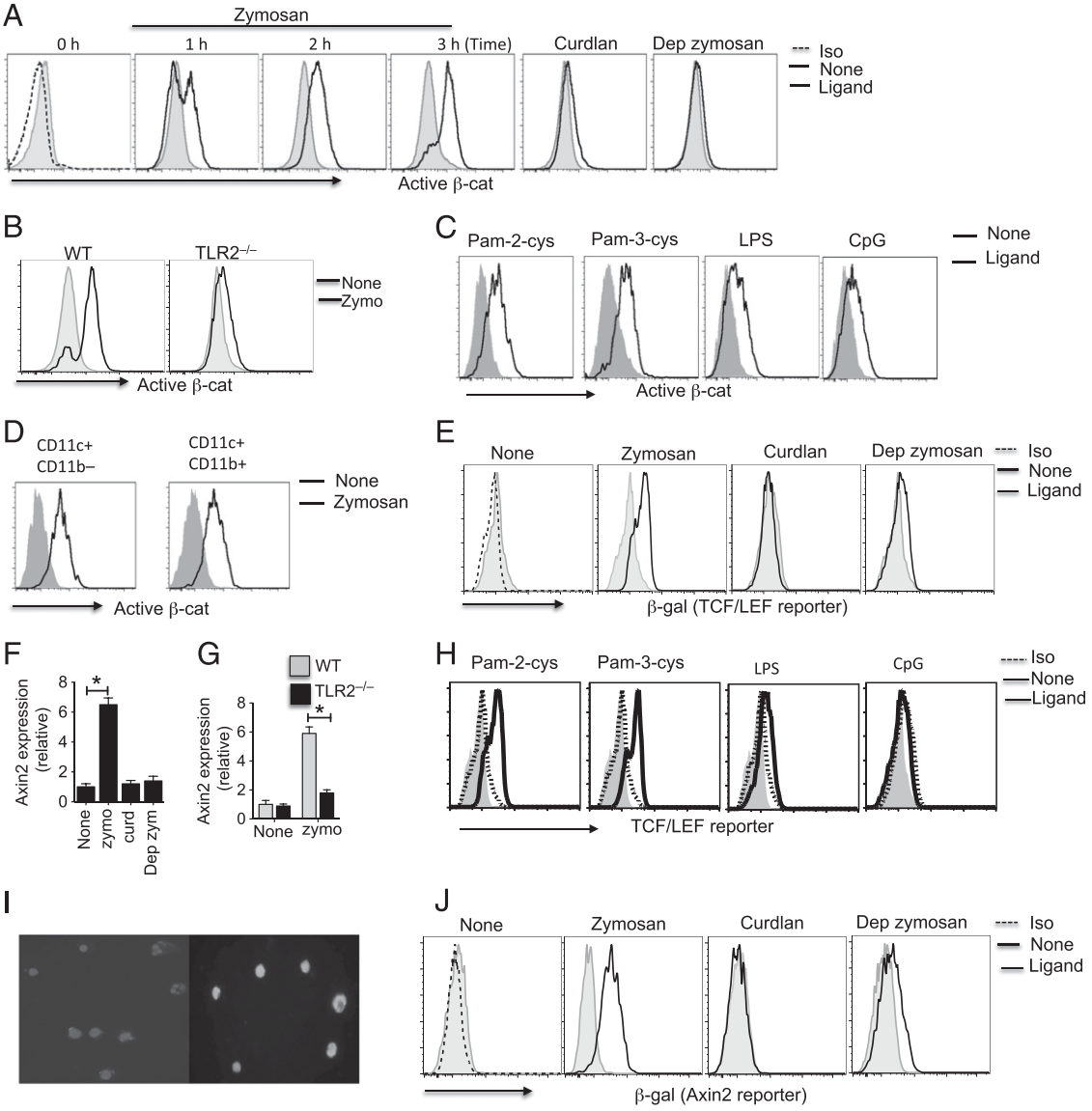

FIGURE 1. Zymosan activates β-catenin/TCF pathway via a mechanism involving TLR2. (**A** and **B**) Expression of active β-catenin by $CD11c^+$ SPDCs from WT or $TLR2^{-/-}$ stimulated with zymosan (25 μg/ml) or curdlan (25 μg/ml) or depleted zymosan (dep zymo; 25 μg/ml) at indicated time points as assessed by intracellular staining and flow cytometry. (**C**) Expression of active β-catenin by $CD11c^+$ SPDCs from WT stimulated with Pam-2-cys (2 μg/ml) or Pam-3-cys (2 μg/ml) or LPS (5 μg/ml) or CpG (5 μg/ml) for 3 h and assessed by intracellular staining and flow cytometry. (**D**) Active β-catenin expression in $CD11c^+CD11b^-$ and $CD11c^+CD11b^+$ SPDCs subsets was assessed by intracellular staining and flow cytometry. (**E**) β-Gal expression in $CD11c^+$ SPDCs isolated from TCF-reporter mice stimulated with zymosan (25 μg/ml) or curdlan (25 μg/ml) or depleted zymosan (dep zymo; 25 μg/ml) for 18 h and assessed by intracellular staining and flow cytometry. (**F** and **G**) Axin2 mRNA expression by SPDCs from WT or $TLR2^{-/-}$ stimulated with zymosan (25 μg/ml) or curdlan (25 μg/ml) or depleted zymosan (dep zymo; 25 μg/ml). (**H**) β-Gal expression in $CD11c^+$ SPDCs isolated from TCF-reporter mice stimulated with stimulated with Pam-2-cys (2 μg/ml) or Pam-3-cys (2 μg/ml) or LPS (5 μg/ml) or CpG for 18 h and assessed by intracellular staining and flow cytometry. (**I**) Intracellular expression of β-catenin (green) and nuclei (blue) in SPDCs isolated from WT mice cultured in medium alone or with zymosan for 3 h. Purified $CD11c^+$ SPDCs were stimulated with or without zymosan (25 mg/ml) for 3 h and permeabilized with BD Fix and Perm buffer. Cells were incubated with FITC-labeled β-catenin (1:100 dilution; Cell Signaling Technology) for 1 h, and nuclei were stained with DAPI and shown at original magnification ×40. Three images were acquired for each field using a Zeiss Axiovert LSM-410 confocal microscope (showing FITC and DAPI simultaneously on the cells). (**J**) β-Gal expression in $CD11c^+$ SPDCs isolated from Axin2-reporter mice stimulated with zymosan (25 μg/ml) or curdlan (25 μg/ml) or depleted zymosan and assessed by intracellular staining and flow cytometry. Data are from one experiment representative of three independent experiments. $^*p < 0.01$.

ligand Pam-3-cys resulted in increased active β-catenin, albeit at lower levels compared with zymosan (Fig. 1C). These data suggest that zymosan-induced β-catenin activation is mediated through TLR-2 in combination with other receptors. Furthermore, treatment with TLR9 ligand (CpG) and TLR4 ligand (LPS) resulted in mild β-catenin activation as compared with TLR2 ligands (Fig. 1C). With further analysis of DC subsets, we observed β-catenin activation in $CD11c^+$ $CD11b^-$ and $CD11c^+CD11b^+$ SPDCs (Fig. 1D). These data suggest that TLR2-mediated signals activate β-catenin in DCs.

Activation of β-catenin results in its translocation to the nucleus, where it interacts with T cell factor/lymphoid enhancer factor TCF/LEF family members and regulates transcription of several target genes (28, 37). As a result, we next tested whether zymosan induced β-catenin activation promoted TCF/LEF-dependent gene transcription using the TCF/LEF reporter mice and β-catenin/TCF-responsive reporter strain, $Axin2^{NLSlacZ}$. SPDCs from TCF/LEF-reporter mice cultured with zymosan showed strong β-gal expression compared with untreated DCs (Fig. 1E). Consistent with this observation, increased expression of the β-catenin/TCF target gene Axin2 was detected and profound induction of β-catenin/TCF-responsive reporter strain, $Axin2^{NLSlacZ}$ in DCs in response to zymosan (Fig. 1F). Moreover, $TLR2^{-/-}$ mice showed no induction of Axin2 in response to zymosan further confirming the role of TLR2 mediated signals in activation of β-catenin signaling pathway (Fig. 1G). Similarly, treatment with other TLR2 ligands such as Pam-2-cys or Pam-3-cys resulted in increased β-gal expression. However, TLR4 (LPS) or TLR9 (CpG) activation resulted in low levels of β-gal expression (Fig. 1H). Moreover, zymosan treatment of DCs led to a significant amount of β-catenin translocation in the nucleus, a hallmark of active signaling (Fig. 1I). In contrast, Dectin-1 ligands such as curdlan or depleted zymosan failed to induce β-gal expression in DCs compared with zymosan treated DCs (Fig. 1J). Collectively, these results suggest that TLR2-mediated signals activate β-catenin and promote downstream transcriptional activity.

β-catenin activation in DCs promotes T regulatory cell differentiation and limits Th1/Th17 cell differentiation

Zymosan conditions DCs to acquire regulatory properties that mostly induce T regulatory responses (18, 19, 21). In addition, activation of β-catenin in DCs induces T regulatory response (31, 38). Because our data showed that TLR2-mediated signals activate β-catenin in DCs, we reasoned that β-catenin pathway imparts regulatory phenotype on DCs in response to zymosan. Therefore, we tested the ability of zymosan or curdlan treated SPDCs isolated from $\beta\text{-cat}^{fl/fl}$ (WT) and $\beta\text{-cat}^{\Delta DC}$ (β-catenin deleted specifically in DCs) (31) to promote differentiation of naive OT-II $CD4^+$ T cells to induce Treg or Th1/Th17 cell differentiation. Consistent with previous studies (21, 27), zymosan-treated WT DCs induced both $Foxp3^+$ Treg cells, and IL-10–producing Tr1 cells (Fig. 2A) in the presence or absence of TGF-β (Supplemental Fig. 1A). Furthermore, zymosan-stimulated WT DCs did not induce robust Th1 or Th17 cells (Fig. 2B), whereas curdlan-stimulated WT DCs induced both Th1 and Th17 cells (Fig. 2B). Moreover, zymosan-treated DCs from $\beta\text{-cat}^{\Delta DC}$ mice induced lower frequency of both $Foxp3^+$ Treg and IL-10–producing Tr1 cells compared with WT DCs (Fig. 2A) in the presence or absence of TGF-β (Supplemental Fig. 1A). Interestingly, DCs from $\beta\text{-cat}^{\Delta DC}$ induced robust Th1 and Th17 cells in response to zymosan (Fig 2B).

We next determined whether β-catenin signaling was critical for induction of Treg cells in vivo in response to zymosan. Naive OT-II cells were adoptively transferred into WT and $\beta\text{-cat}^{\Delta DC}$ mice, followed by immunization with OVA alone, or OVA mixed with zymosan. Consistent with our previous studies (18, 21), WT mice immunized with OVA plus zymosan resulted in a robust induction of Ag specific $Foxp3^+$ Treg cells and IL-10–producing Tr1 cells, compared with mice immunized with OVA alone (Fig. 2C, Supplemental Fig. 1B). In contrast, the induction of Treg cells was significantly reduced in $\beta\text{-cat}^{\Delta DC}$ mice when compared with WT mice in response to zymosan (Fig. 2C, Supplemental Fig. 1B). Furthermore, zymosan treatment led to an enhanced Th1 and Th17 responses and reduced IL-10–producing cells in $\beta\text{-cat}^{\Delta DC}$ mice (Fig. 2C, 2D, Supplemental Fig. 1C). Collectively, these data indicate that β-catenin signaling in DCs is critical for the induction of T regulatory cells, and limiting Th1 and Th17 inflammatory responses in response to zymosan.

Activation of β-catenin/TCF4 pathway in DCs imparts anti-inflammatory phenotype on DCs

Zymosan has been shown induce immune regulatory genes IL-10, vitamin A metabolizing enzyme (Aldh1a2), and TGF-β in DCs (18, 19, 21, 39) that enable them to induce Treg cells and suppress the differentiation of Th1/Th17 cells. Furthermore, our previous study has shown that β-catenin is critical for the induction of vitamin A metabolizing enzymes and IL-10 in mucosal DCs. As a result, we hypothesized that zymosan–TLR2-mediated activation of β-catenin induces expression of IL-10 and Aldh1a2 in DCs. As observed in previous studies (18, 19, 21, 40), SPDCs from WT mice treated with zymosan showed significant increase in *Aldh1a2* and *IL-10* mRNA levels compared with the untreated DCs (Fig. 3A). In contrast, DCs lacking β-catenin showed significantly lower levels of *Il-10* and *Aldh1a2* expression in response to zymosan (Fig. 3A). Consistent with these observations, WT DCs treated with zymosan showed higher levels of RALDH activity compared with DCs lacking β-catenin (Fig. 3B). Moreover, zymosan treatment of $\beta\text{-cat}^{\Delta DC}$ DCs when compared with WT DCs produced significantly reduced levels of IL-10 and elevated levels of proinflammatory cytokines such as IL-6, IL-23, and IL-12, which induce differentiation of naive $CD4^+$ T cells into inflammatory Th17/Th1 cells (Fig. 3C). In line with these in vitro results, DCs from $\beta\text{-cat}^{\Delta DC}$ mice showed significantly reduced levels of *Aldh1a2* and *Il-10* expression upon zymosan injection (Fig. 3D). In contrast, injection of zymosan into $\beta\text{-cat}^{\Delta DC}$ mice induced robust proinflammatory cytokines compared with the WT mice (Fig. 3D). Collectively, these results show that zymosan–TLR2-mediated β-catenin activation promotes expression of IL-10 and RA by DCs that is critical for inducing T regulatory responses and limiting Th1/Th17 cell differentiation.

The downstream mediator of β-catenin signaling in DCs that is critical for inducing IL-10 and Aldh1a2 is not known. β-catenin acts as a coactivator for several transcription factors (37), and TCF family of transcription factors is among downstream mediators of β-catenin signaling. Recently, it has been shown that DCs express TCF4 isoform (41). To directly assess whether TCF4 is involved in zymosan–TLR2-mediated induction of IL-10 and Aldh1a2 in DCs, we crossed floxed TCF4 allele mice ($TCF4^{fl/fl}$) (35, 42) with transgenic mice (CD11c-Cre) expressing Cre enzyme under the control of CD11c-promoter (36). As shown in Fig. 3E, DCs lacking TCF4 showed significantly lower levels of IL-10 and Raldh2 mRNA expression in response to zymosan. In line with these observations, TCF4-deficient DCs treated with zymosan showed lower levels of RALDH activity compared with WT DCs (Fig. 3F). Collectively, these results show that zymosan–TLR2-mediated activation of β-catenin/TCF4 in DCs is critical for the induction of IL-10 and Aldh1a2.

β-catenin activation in DCs is dependent on Akt but independent of Erk

TLR2-mediated signals activate PI3K/Akt and MAPK signaling pathways. Recent biochemical and genetic studies have shown that

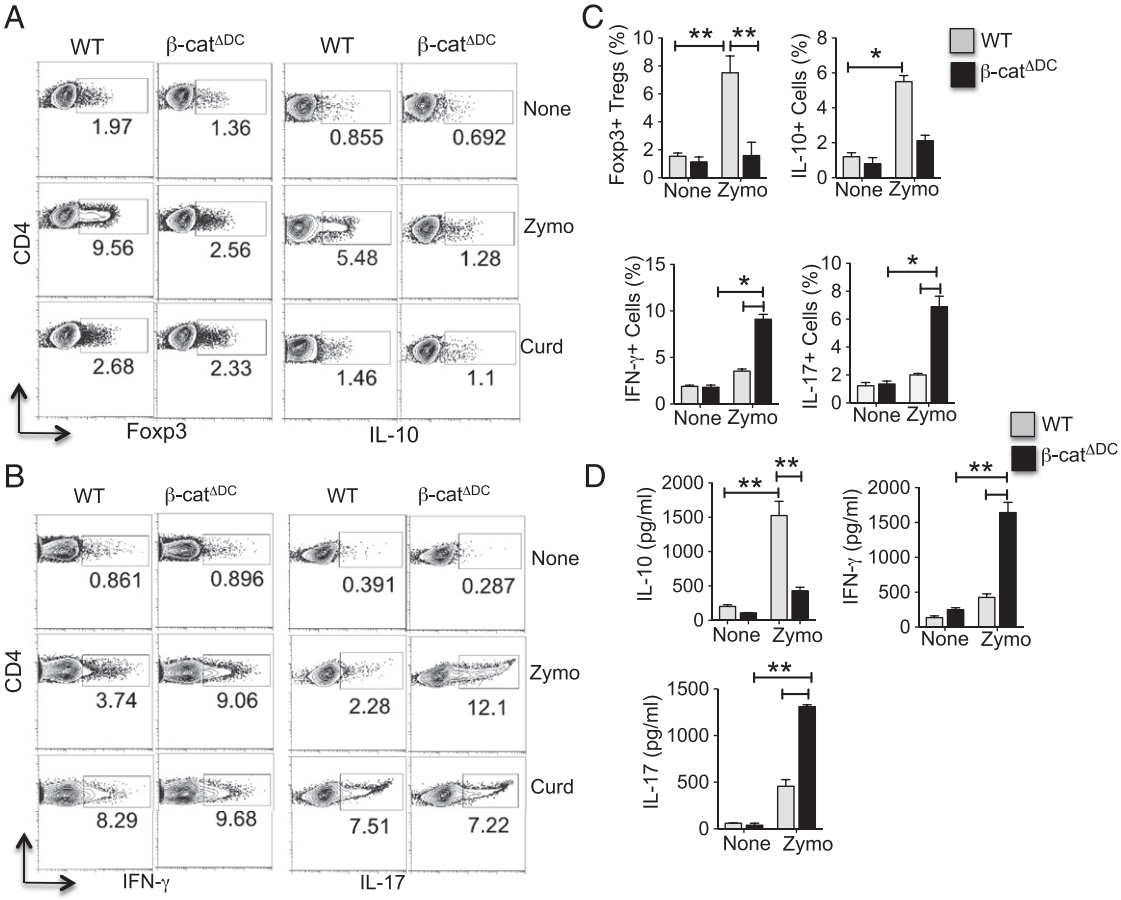

FIGURE 2. Zymosan-mediated activation of β-catenin in DCs promotes T regulatory cell differentiation and suppresses Th1/Th17 cell differentiation. (**A** and **B**) CD11c$^+$ SPDCs from WT (β-cat$^{fl/fl}$) and β-cat$^{\Delta DC}$ were stimulated with zymosan (25 μg/ml) or curdlan (25 μg/ml), and after 10 h, DCs (2×10^4) were washed and cocultured with naive CD4$^+$CD62L$^+$ OT-II T cells (1×10^5/ well) with OVA peptide (2 μg/ml) and TGF-β (1 ng/ml). After 4 d, OT-II cells were restimulated for 6 h with plated bound anti-CD3 and anti-CD28. Foxp3 expression and intracellular production of IL-17, IFN-γ, and IL-10 by CD4$^+$ T cell were assessed by intracellular staining and flow cytometry. Data are from one experiment representative of three. (**C**) WT (β-cat$^{fl/fl}$) and β-cat$^{\Delta DC}$ mice reconstituted with naive CD4$^+$CD62L$^+$ OT-II T cells and were injected i.v. with class II–restricted $OVA_{323–339}$ peptide (50 μg) plus PBS or $OVA_{323–339}$ (50 μg) plus zymosan (50 μg). Four days after challenge, unfractioned spleen cells were restimulated in vitro for 5 h with anti-CD3 and -CD28 Abs in the presence of brefeldin A. Percentage of CD4$^+$OT-II$^+$ cells positive for Foxp3, IL-17, IFN-γ, and IL-10 as assessed by intracellular staining and flow cytometry. Data are representative of two independent experiments. (**D**) Unfractioned spleen cells from immunized mice as described in (C) were restimulated with OVA peptide (1 μg/ml) in culture for 48 h, and cytokines in the supernatants were quantified by ELISA ($n = 4$, samples). *$p < 0.01$, **$p < 0.001$.

Akt phosphorylates β-catenin at Ser^{552} and promotes its transcriptional activity (43–45). Akt also was shown to phosphorylate and inactivate GSK-3β at Ser^9, thereby preventing GSK-3β–mediated β-catenin degradation (46). To elucidate the mechanisms by which zymosan–TLR2-mediated signals activate β-catenin in DCs, we first analyzed activation of Akt and MAPK ERK by flow cytometry using anti–phospho-Ab. DCs cultured with zymosan showed enhanced Akt phosphorylation at Thr^{308} relative to untreated DCs (Fig. 4A). We next tested whether Akt activation in DCs is dependent on TLR2. Accordingly, DCs from *Tlr2*$^{-/-}$ mice also showed lower levels of *phospho-Akt* compared with WT DCs in response to zymosan (Fig. 4A). Above results clearly show that zymosan via TLR2 activates both Akt and β-catenin. We hypothesized that Akt activates β-catenin and promotes downstream transcriptional activity. As a result, we tested whether Akt activates β-catenin by directly phosphorylating it at Ser^{552} in response to zymosan. DCs cultured with zymosan showed increase in phosphorylated β-catenin on Ser^{552} compared with the untreated controls (Fig. 4B). In contrast, DCs treated with Akt inhibitor completely abrogated β-catenin phosphorylation at Ser^{552} in response to zymosan (Fig. 4B). Consistent with this observation, DCs from Akt1$^{-/-}$ showed reduced levels of phospho–β-catenin in response to zymosan (Fig. 4B). Moreover, Akt also can activate β-catenin indirectly through inactivation of GSK-3β through its direct phosphorylation at Ser^9. Therefore, we quantified the phosphorylation status of GSK-3β in DCs in the presence or absence of zymosan. DCs cultured with zymosan showed marked increase in phosphorylated GSK-3β at Ser^9 compared with the untreated DCs (Fig. 4B). In contrast, Akt inhibitor treatment markedly reduced the GSK-3β phosphorylation in response to zymosan (Fig. 4B). Furthermore, DCs from Akt1$^{-/-}$ mice had reduced GSK-3β phosphorylation upon zymosan treatment (Fig. 4B). Consistent with a role for Akt in β-catenin activation, blocking Akt activity in DCs markedly decreased IL-10 and Aldh1a2 expression in response to zymosan (Fig. 4C). These results show that Akt activates β-catenin directly by phosphorylating it at Ser^{552} and indirectly preventing its degradation by inactivating GSK-3β in response to zymosan via TLR2.

TLR2-mediated activation of ERK MAPK in DCs is critical for the induction of Aldh1a2 and IL-10 in response to zymosan. As a result, we next investigated whether Erk is critical for the activation of β-catenin. Treatment of DCs with Erk inhibitor had no effect on activation of β-catenin, Akt, and GSK-3β in response to zymosan (Fig. 4D). Similarly, treatment of DCs with Akt inhibitor

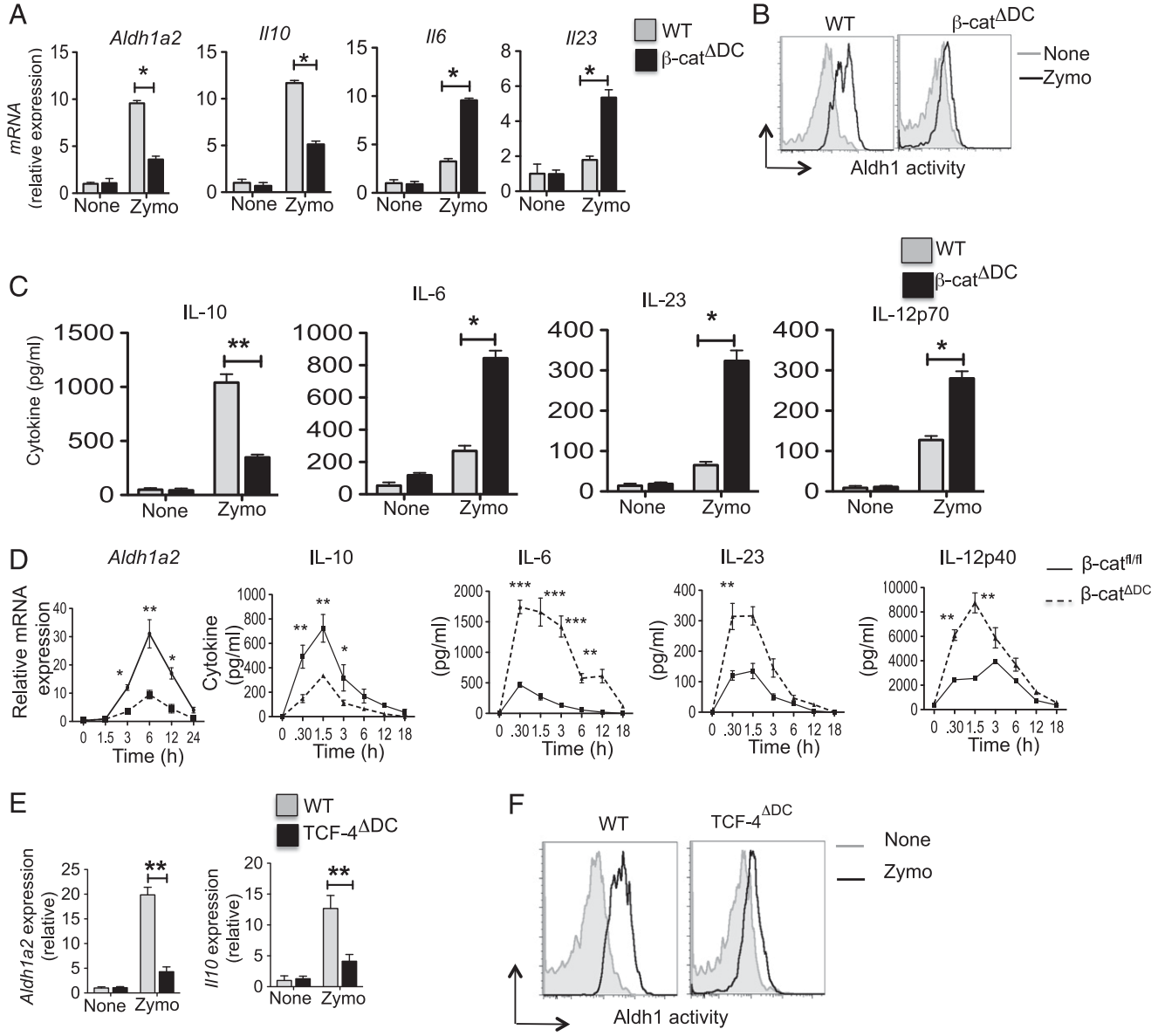

FIGURE 3. β-catenin/TCF-4 signaling pathway induces RA synthesizing enzyme (Aldh1a2) IL-10 and suppresses inflammatory cytokines in SPDCs. (**A**) Purified CD11c$^+$ SPDCs from WT (β-cat$^{fl/fl}$) and β-cat$^{\Delta DC}$ mice were cultured in media alone or with zymosan (25 μg/ml). (A) After 24 h, mRNA expression of Aldh1a2, IL-10, IL-6, and IL-23 relative to the expression GAPDH was analyzed by qRT-PCR ($n = 3$ samples). (**B**) ALDH activity on purified CD11c$^+$ SPDCs from WT (β-cat$^{fl/fl}$) and β-cat$^{\Delta DC}$ without (gray) or with (black) zymosan treatment. Data are from one experiment representative of two independent experiments. (**C**) Purified CD11c$^+$ SPDCs from WT (β-cat$^{fl/fl}$) and β-cat$^{\Delta DC}$ mice were cultured in media alone or with zymosan as described in (A). After 24 h, cytokines in the cell culture supernatants were quantified by ELISA. Data are representative of three experiments. (**D**) β-cat$^{fl/fl}$ and β-cat$^{\Delta DC}$ mice were injected with PBS or zymosan (25 mg/ml) by i.v. route. Mice were sacrificed at indicated time points, and blood samples and spleens were collected. Induction of Aldh1a2 mRNA expression in purified CD11c$^+$ SPDCs from treated mice was analyzed by RT-PCR, and serum cytokine levels were analyzed by ELISA. (**E**) Purified CD11c$^+$ SPDCs from TCF4$^{fl/fl}$ and TCF4$^{\Delta DC}$ mice were cultured in media alone or with zymosan (25 μg/ml). After 24 h, expression of Aldh1a2 and IL-10 mRNAs relative to the expression of GAPDH was analyzed by qRT-PCR ($n = 3$ samples). (**F**) ALDH activity on purified CD11c$^+$ SPDCs from WT (TCF4$^{fl/fl}$) and TCF4$^{\Delta DC}$ without (gray) or with (black) zymosan treatment. Data are from one experiment representative of two independent experiments. $*p < 0.01$, $**p < 0.001$, $***p < 0.0001$.

VII had no effect on the phosphorylation status and activation of Erk (Fig. 4B). Furthermore, we observed similar levels of Erk phosphorylation in WT and β-catenin–deficient DCs in response to zymosan (Fig. 4E). These two pieces of data demonstrate that Erk and Akt activation are independent of each other, and both are downstream of TLR2 signaling. Importantly, inhibition of Akt or Erk in DCs alone results in decreased expression of IL-10 and Aldh1a2 in response to zymosan (Fig. 4C). Furthermore, zymosan-stimulated DCs treated with inhibitors of Akt or Erk were compromised in their ability to induce Treg cells but promoted robust induction of Th1 cells (Fig. 4F). Collectively, these results demonstrate that zymosan-TLR2 mediate Akt-dependent activation of β-catenin, and Erk imparts anti-inflammatory phenotype on DCs.

β-catenin/TCF pathway activation suppresses chronic inflammation and limits EAE

We and others have shown that zymosan and other TLR2-ligand treatment can suppress inflammation and limit autoimmunity in different experimental mouse models such as EAE (21, 39), diabetes (19, 47), airway hyperresponsiveness (48), and colitis (49). As a result, we next determined the importance of zymosan–

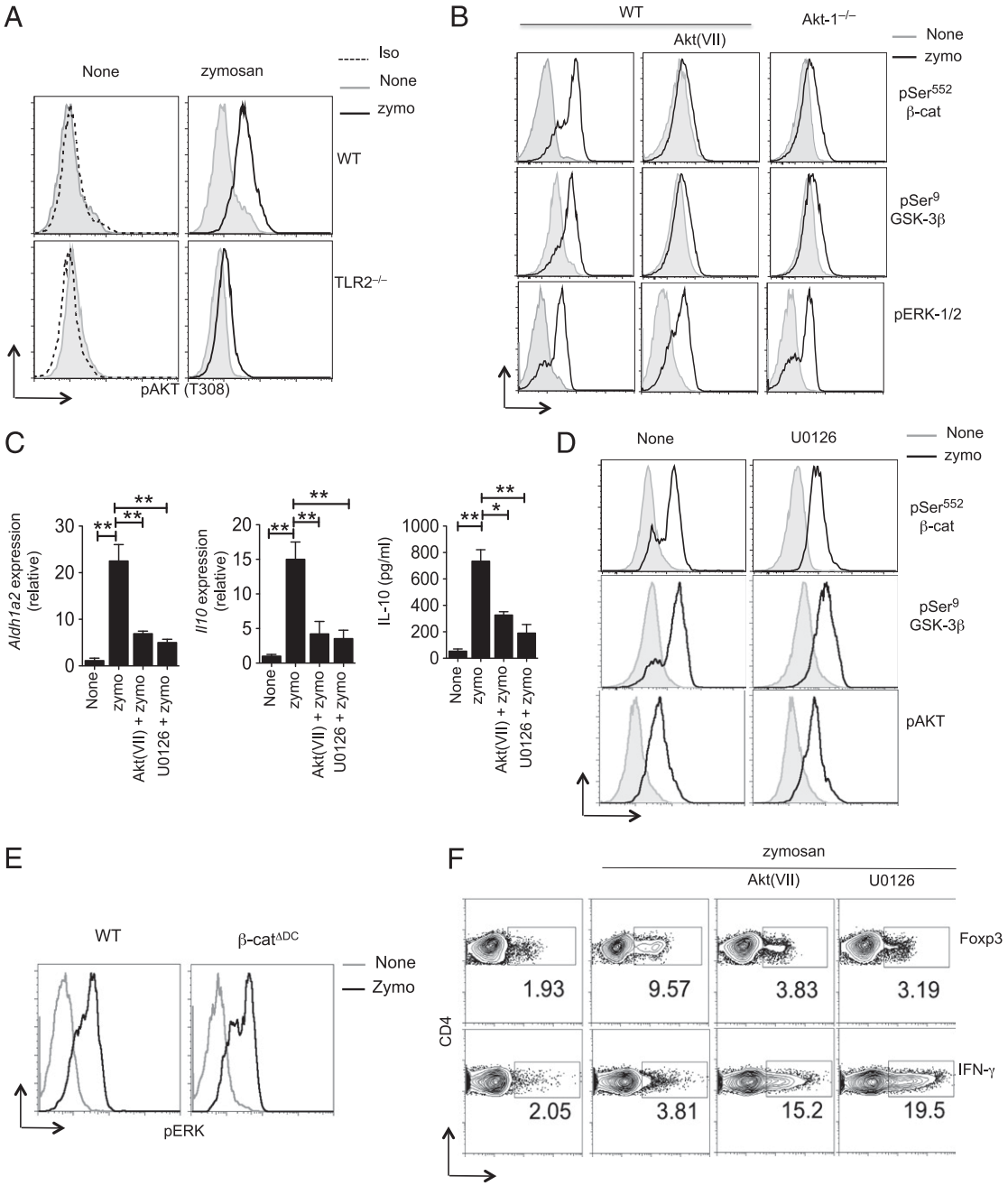

FIGURE 4. PI3K/AKT-mediated signals activate β-catenin in DCs. (**A**) Representative histograms of pThr^{308} AKT in CD11c$^+$ SPDCs from WT or TLR2$^{-/-}$ stimulated with zymosan (25 μg/ml) after 3 h as assessed by intracellular staining and flow cytometry. (**B**) Representative histograms of pSer^{552} β-cat or pSer^9 GSK-3β or pERK1/2 in CD11c$^+$ SPDCs from WT or AKT1$^{-/-}$ stimulated with zymosan (25 μg/ml) in the presence or absence of AKT inhibitor VII (5 μM) for 3 h. (**C**) Purified CD11c$^+$ SPDCs from WT mice stimulated with zymosan (25 μg/ml) in presence or absence of ERK inhibitor or AKT inhibitor. After 24-h expression of Aldh1a2, mRNA relative to the expression of mRNA encoding GAPDH was analyzed by qRT-PCR ($n = 3$ samples). IL-10 cytokine levels in the culture supernatants were quantified by ELISA. (**D**) Representative histograms of pSer^{552} β-cat or pSer^9 or pAKT in CD11c$^+$ SPDCs from WT mice stimulated with zymosan (25 μg/ml) in the presence or absence of Erk inhibitor (U0126; 1 μM) for 3 h. (**E**) Representative histograms of pERK1/2 in CD11c$^+$ SPDCs from WT or β-cat$^{\Delta DC}$ stimulated with zymosan (25 μg/ml) after 3 h as assessed by intracellular staining and flow cytometry. (**F**) CD11c$^+$ SPDCs from WT were stimulated with zymosan (25 μg/ml) or curdlan (25 μg/ml) and after 10 h DCs (2×10^4) in the presence or absence of AKT inhibitor VII (5 μM) or Erk inhibitor (U0126; 1 μM). After 10 h, DCs were washed and cocultured with naive CD4$^+$CD62L$^+$ OT-II T cells (1×10^5/ well) with OVA peptide (2 μg/ml) and TGF-β (1 ng/ml) . After 4 d, OT-II cells were restimulated for 6 h with plated bound anti-CD3 and anti-CD28. Foxp3 expression and intracellular production of IFN-γ by CD4$^+$ T cell were assessed by intracellular staining and flow cytometry. Data are from one experiment representative of three. *$p < 0.01$, **$p < 0.001$.

TLR2-mediated activation of β-catenin pathway in DCs on EAE outcome. Mice were immunized with $MOG_{35–55}$ peptide emulsified in CFA and then treated with PBS or zymosan as described previously (21). Control mice treated with PBS showed onset of neurologic impairment occurring around day 14 (Fig. 5A). We then determined whether zymosan was capable of actively suppressing the disease. Consistent with previous studies (21, 39), WT mice treated with zymosan developed significantly lower clinical scores compared with PBS-treated mice (Fig. 5A). Because zymosan activates the β-catenin pathway, we next evaluated

whether the suppressive effect of zymosan was dependent on β-catenin. β-cat$^{\Delta DC}$ mice treated with PBS showed early onset of neurologic impairment and more severe disease compared with WT mice (Fig. 5B). Consistent with the regulatory role of β-catenin in DCs, treatment of zymosan failed to actively suppress disease onset or severity in mice lacking β-catenin in DCs (Fig. 5B). Accordingly, histopathological analysis showed less inflammation in the brain of zymosan treated mice compared with the brain of untreated WT mice (Supplemental Fig. 2). In contrast, we observed increased inflammation in the brain of β-cat$^{\Delta DC}$ with or without zymosan treatment (Supplemental Fig. 2). We then determined the phenotype of CNS-infiltrated CD4$^+$ T cells at day 18. We observed that zymosan treatment resulted in enhanced induction of Tr1 cells, relative to mice treated with PBS (Fig. 5D). In contrast, control-treated mice showed a significant increase in the frequency of Th1 and Th17 cells compared with the zymosan-treated mice (Fig. 5D). Interestingly, under homeostatic conditions β-cat$^{\Delta DC}$ mice showed no difference in splenic Treg frequencies (Supplemental Fig. 1D) and total Treg numbers (data not shown). In zymosan-treated β-cat$^{\Delta DC}$ mice, we observed diminished frequency of Tr1 cells with greatly enhanced Th1 and Th17 responses in the CNS (Fig. 5D). Collectively, these results suggest that zymosan–TLR2-mediated activation of β-catenin signaling is essential for the induction of Foxp3$^+$ Treg cells and Tr1 cells and suppression of Th1 and Th17 responses. Our data also indicate that the absence of β-catenin signaling in DCs lead to an increase in the number of T effector cells (Th1 and Th17) over Treg cells during the normal course of EAE. Furthermore, zymosan treatment in these mice had no effect on disease severity because of possible lack of β-catenin activation when compared with WT mice.

Our previous studies revealed that injection of $MOG_{35–55}$ peptide plus zymosan emulsified in IFA induces a relatively attenuated and transient disease course in mice (21). In the absence of TLR2 signaling, injection of $MOG_{35–55}$ peptide plus zymosan emulsified in IFA results in severe and sustained disease (21). Next, we immunized WT and β-cat$^{\Delta DC}$ mice with $MOG_{35–55}$ peptide and zymosan emulsified in IFA. Consistent with previous studies, $MOG_{35–55}$ peptide plus zymosan induced a greatly attenuated and transient disease (Fig. 5C). In contrast, β-cat$^{\Delta DC}$ mice developed more severe and sustained disease compared with WT-immunized mice (Fig. 5C). Accordingly, β-cat$^{\Delta DC}$ mice showed diminished frequency of Treg and Tr1 cells and greatly enhanced frequencies of Th1 and Th17 cells in the CNS (Fig. 5E–G).

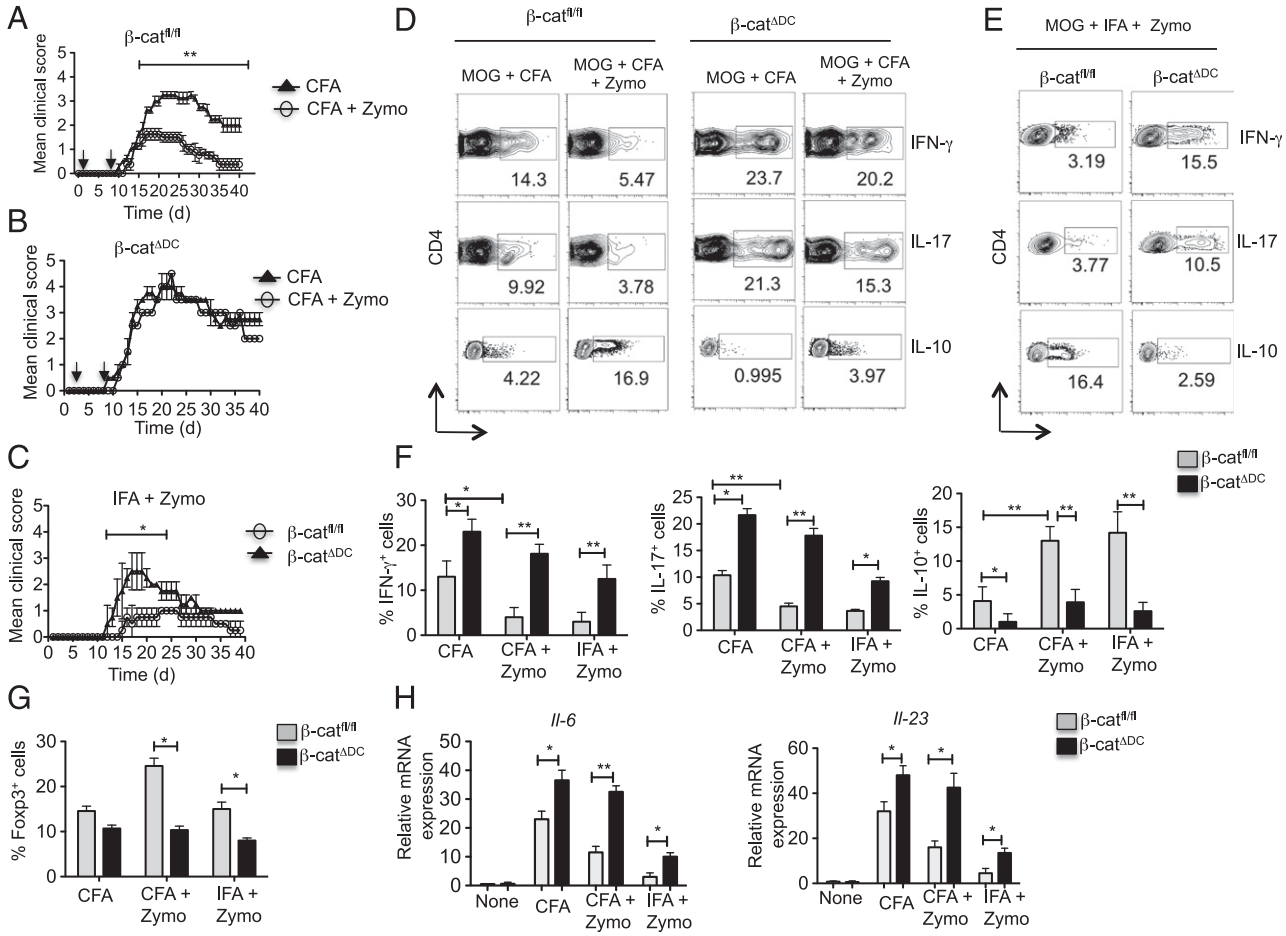

FIGURE 5. Activation of β-catenin suppresses the development of clinical EAE and chronic inflammation. (**A**–**C**) β-cat$^{fl/fl}$ and β-cat$^{\Delta DC}$ were immunized with 100 μg $MOG_{35–55}$ + CFA or $MOG_{35–55}$ + CFA + zymosan or MOG + IFA + zymosan on days 0. Mice also received 250 ng pertussis toxin on days 0 and 2. The progression of EAE disease severity in different group of mice was monitored on various days postimmunization. (**D** and **E**) Mononuclear cells were isolated from CNS tissue on day 18 after immunization and restimulated in vitro for 5 h with anti-CD3 and -CD28 Abs in the presence of brefeldin A. Induction of IFN-γ, IL-17, and IL-10 was assessed by intracellular staining and flow cytometry by gating on CD4$^+$ T cells. Numbers in FACS plots represent percentage of cells positive for the indicated protein. Data are from one experiment representative of two. (**F** and **G**) Percentage of Foxp3$^+$ Tregs, Tr1$^+$, IFN-γ$^+$ (Th1), and IL-17$^+$ (Th17) cells assessed in the CNS of β-cat$^{fl/fl}$– and β-cat$^{\Delta DC}$–immunized mice at day 18. Data represent mean (± SD) of six mice per group. (**H**) Total RNA was isolated from purified CD11c$^+$ SPDCs of β-cat$^{fl/fl}$– and β-cat$^{\Delta DC}$–immunized mice at day 18 as described above, and expression of IL23p19 and IL-6 mRNA was analyzed qRT-PCR (n = 3 samples). Data are representative of two independent experiments. *$p < 0.01$, **$p < 0.001$.

Thus, activation of the β-catenin pathway in DCs can suppress Th1 and Th17 responses and limit EAE in response to zymosan.

IL-6 and IL-23 play pivotal roles in the differentiation and expansion of Th17 cells and in the pathogenesis of EAE (50, 51). Thus, we evaluated the IL-6 and IL-23p19 mRNA expression in SPDCs isolated ex vivo from WT versus β-cat$^{\Delta DC}$ mice at day 18 in response to zymosan (Fig. 5H). We also measured the serum cytokine levels of IL-23 in WT and β-cat$^{\Delta DC}$ mice (Fig. 5H) upon zymosan injection. Zymosan treatment markedly decreased expression of IL-6 and IL-23 in DCs relative to control WT mice DCs (Fig. 5H). In contrast, zymosan treatment of β-cat$^{\Delta DC}$ mice resulted in increased expression of IL-6 and IL-23 in DCs compared with zymosan-treated WT mice (Fig. 5H). Consistent with these results, we also observed increased levels of serum IL-6 and IL-23 in β-cat$^{\Delta DC}$ mice compared with WT mice treated with zymosan (data not shown). Collectively, these results demonstrate that activation of β-catenin pathway in DCs signaling suppresses IL-6 and IL-23 production in DCs and limits chronic inflammation. Our overall results are summarized in Fig. 6, showing how TLR-2 mediated signals in DCs promote regulatory responses.

Discussion

The signaling networks and transcription factors that program DCs to induce regulatory versus inflammatory responses remain poorly defined. In this study, we show that TLR2-mediated signals activate the β-catenin/TCF pathway in DCs, resulting in the acquisition of potent regulatory phenotypes that induces T regulatory responses, both in vitro and in vivo. In addition, the current study also shows that activation of β-catenin/TCF4 pathway in DCs is critical for the induction of IL-10 and vitamin A metabolizing enzyme (Aldh1a2) in response to zymosan. In contrast, loss of β-catenin signaling in DCs programs them to an inflammatory state that promotes Th1 and Th17 responses. We further demonstrate that activation of the β-catenin/TCF pathway in DCs suppressed chronic inflammation and disease severity of EAE. Accordingly, β-cat$^{\Delta DC}$ mice showed marked increase in the frequency of Th1/Th17 cells and a concomitant increase in disease severity. Collectively, these findings support the hypothesis that the β-catenin/TCF4 pathway regulates the functions downstream of TLR2 signaling in DCs and is critical for programming them to regulatory state. Thus, activating this pathway is an effective strategy to modulate autoimmune disease. Several aspects of these findings deserve further comment.

First, a role for the β-catenin pathway in intestinal DCs and in the regulation of intestinal homeostasis is well established (31, 52, 53); however, its role in systemic immune responses is not known. Furthermore, it is not known whether the β-catenin pathway can be activated in peripheral DCs, and if so, the receptors and signaling pathways that activate β-catenin in DCs are not known. To our knowledge, the current study demonstrates for the first time that β-catenin/TCF4 pathway can be activated in SPDCs via TLR2 signaling in response to zymosan. Our study also defines an essential role for β-catenin/TCF4 pathway in programming DCs to a regulatory state. Recent studies by others and ours have shown the Wnt- and E-cadherin–mediated signaling also can activate β-catenin and program DCs to regulatory state (31, 38). However, zymosan-mediated early activation of β-catenin (1–3 h) in DCs was dependent on TLR2 but independent of Wnt.

Second, DC-derived signals and cytokines dictate the T cell activation and differentiation. The capacity of zymosan-treated DCs to suppress inflammatory responses and to convert naive T cells to Treg cells was dependent on IL-10 and RA (21). There is also increasing evidence that RA and IL-10 regulate the immune response by acting directly on DCs (54–57). However, downstream signaling pathways and transcription factors in DCs critical for the induction of these two genes are unknown. In the current study, we report that TLR2-dependent expression of IL-10 and Aldh1a2 is dependent on β-catenin and its downstream

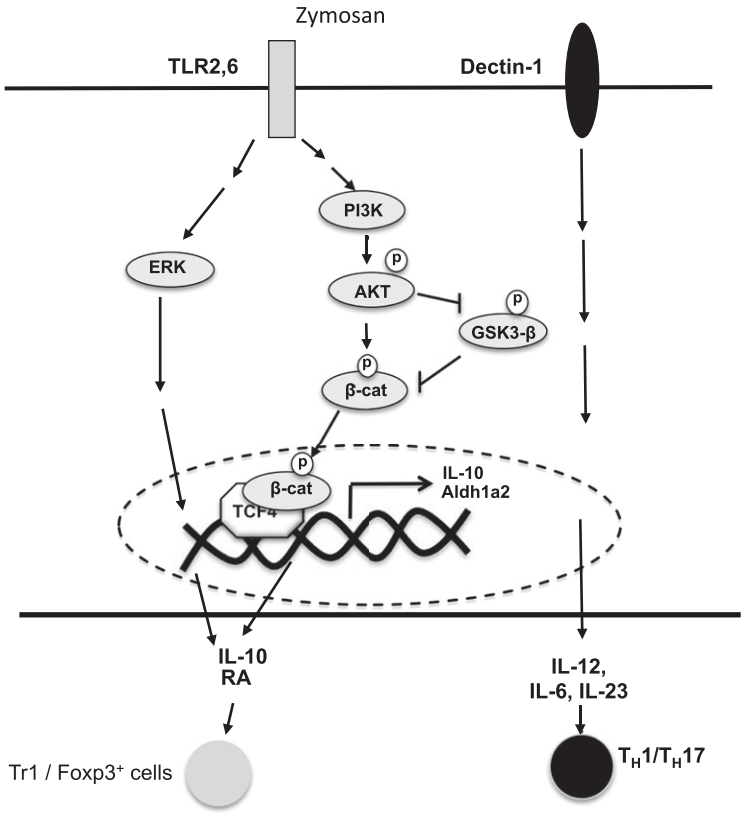

FIGURE 6. Mechanism of regulation of IL-10 and Aldh1a2 by β-catenin/TCF4 pathway in DCs. Innate sensing of zymosan via TLR2 efficiently induces Akt and Erk activation. Akt then activates β-catenin by directly phosphorylating it at Ser^{552} and indirectly by inactivating GSK-3β activity. Activated β-catenin interacts with TCF4 in the nucleus and transcriptionally induces IL-10 and Aldh1a2 gene expression in DCs, which are critical for inducing T regulatory responses and limiting Th1/Th17 cell differentiation. Activation of Erk and Akt/β-catenin pathway work synergistically to induce IL-10 and Aldh1a2. Dectin-1 signaling does not play a major role in β-catenin activation but promotes induction of proinflammatory cytokines that are critical of Th1/Th17 cell differentiation.

mediator TCF4. Deletion of β-catenin or TCF-4 in DCs resulted in significant decrease in IL-10 and Raldh expression and concurrent increase in the expression of proinflammatory cytokines. Thus, DCs from β-cat$^{\Delta DC}$ or TCF-4$^{\Delta DC}$ mice were less potent in inducing Treg cells and more potent in inducing Th1/Th17 cells. Collectively, these results strongly support the hypothesis that β-catenin/TCF4 play a pivotal role in inducing IL-10 and Aldh1a2 gene transcription. In addition, TLR2-dependent activation β-catenin in DCs is critical for expression of IL-10 and Raldh2, thereby limiting proinflammatory genes expression and reducing potential negative consequences of inflammatory gene expression.

Third, the mechanisms by which TLR2-mediated signals activate β-catenin in DCs are not known. Recent studies have shown that PI3K/Akt pathway is an important regulator of innate immune responses in response to TLR-mediated signaling (58–61). Our data indicated that TLR2-mediated signals in DCs activated Akt and Erk in response to zymosan. Our data also show that TLR2-mediated signaling via Akt pathway activated β-catenin by two different mechanisms. First, Akt directly phosphorylated β-catenin at Ser^{552} and promoted its activation in response to zymosan. Second, our data also demonstrated that Akt activated β-catenin by directly phosphorylating GSK-3β at Ser^{9} to inhibit GSK-3β–driven β-catenin degradation. Blocking Akt activation in DCs or deletion of Akt1 in DCs completely abrogated the GSK-3β phosphorylation at Ser^{9} and β-catenin phosphorylation at Ser^{552} in response to zymosan. Recent studies have highlighted the PI3K/Akt pathway as an important inducer of anti-inflammatory responses to TLR-mediated signaling (60–63). Furthermore, the PI3K/Akt pathway was shown to negatively regulate TLR-mediated proinflammatory cytokines (58). Finally, our study shows that the TLR-mediated signaling activates both Erk and Akt/β-catenin pathway, and these pathways regulate the expression of IL-10 and RA in DCs. In our study, we show that blocking the Erk or Akt pathway in DCs resulted in a marked decrease in IL-10 and Raldh2 gene expression and an increase in proinflammatory cytokines (data not shown). Consistent with this, we also observed marked decrease in IL-10 and Raldh2 expression in Akt1 DCs. These observations correlated with the marked defect in β-catenin activation and transcriptional activity in the absence of PI3K/Akt signals in response to zymosan. Thus, Akt-dependent β-catenin activation limits proinflammatory gene expression and reduces the potential negative consequences of inflammatory gene expression (Fig. 6).

Consistent with a regulatory role for the TLR2 signaling pathway, recent studies have shown TLR2 ligands or microbial activation of TLR2 can suppress inflammation and limit autoimmunity in mice in different disease settings (19, 21, 39, 47–49). Furthermore, treatment of mice with zymosan at low doses limits EAE and type 1 diabetes, and these responses were dependent on TLR2. The present study also highlights the functional significance of β-catenin/TCF pathway in DCs in active suppression of EAE disease progression. Most importantly, activation of this pathway resulted in striking reductions in the frequencies of Th1/Th17 cells and enhanced frequencies of Tregs and Tr1 cells. In addition, the current study also reveals that activation of the β-catenin/TCF4 pathway suppresses IL-23 and IL-6 production by DCs, thereby limiting Th17 cell–mediated chronic inflammation.

DCs are vital in regulating the balance between immunity versus tolerance. Recently, emerging studies have shown that multiple factors control DC responses against microbes. These include innate receptors on DCs that recognize microbial and nonmicrobial stimuli, intercellular signaling networks within DCs, intercellular interaction between DCs and multiple cell types, and inductive signals from the local microenvironment. Paradoxical to its well-established role in promoting immunity against pathogens (2, 64), recent studies have shown that signaling via innate receptors also can result in tolerogenic responses (7, 25). In this study, we have demonstrated that the TLR2-AKT-β-catenin/TCF4 signaling network is critical in programming DCs into regulatory states and promoting T regulatory responses. What evolutionary benefit might accrue to the microbe or to the host from activating the β-catenin/TCF pathway? Recent studies have shown that sensing of commensals via TLR2 induces regulatory responses and limits inflammatory responses in the gut (49, 65–67) and skin (68). In the face of a persistent immune response against a persistent microbe such as yeasts, viruses, and commensal bacteria, activating β-catenin pathway could represent a mechanism to dampen excessive inflammation and limit collateral damage to host.

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Disclosures

The authors have no financial conflicts of interest.

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