

# Generation of tolerogenic dendritic cells via the E-cadherin/ $\beta$ -catenin-signaling pathway

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**Abstract** Besides their well-characterized role as the initiator of adaptive immune responses, dendritic cells (DCs) play a critical role in the induction and maintenance of self-tolerance, the failure of which could lead to autoimmune/inflammatory diseases. Although it is clear that tolerance is a property of DCs at the steady state, the molecular mechanisms governing their generation, function and regulation remain elusive. Our recent studies have uncovered the E-cadherin/ $\beta$ -catenin-signaling pathway as a novel maturation pathway that achieves DC maturation without inflammatory cytokines. As a result, E-cadherin-stimulated DCs elicited an entirely different T cell response *in vivo*, generating T cells with a regulatory as opposed to an effector phenotype. These DCs induced tolerance *in vivo* and more importantly, immunization with these DCs provided complete protection against autoimmune diseases in experimental autoimmune encephalomyelitis (EAE). Interestingly, while DCs matured upon disruption of E-cadherin-mediated clusters were functional tolerogenic, upon further TLR ligation, they displayed a strong Th1 cytokine profile and much enhanced antigen presentation capacity consistent with enhanced immunity. Thus, E-cadherin/ $\beta$ -catenin-signaling might serve as a novel signal that contributes to the elusive steady-state “tolerogenic DCs”. Targeting E-cadherin/ $\beta$ -catenin signaling to either enhance or reduce DC-mediated tolerance might represent an attractive new strategy to achieve antigen-specific immunotherapy for cancers and autoimmune/inflammatory diseases.

**Keywords** E-Cadherin ·  $\beta$ -Catenin · DC maturation · Tolerance · Inflammatory cytokines · IL-10 · Regulatory T cells

## Introduction

Dendritic cells (DCs) play a pivotal role in the immune response, being unique among antigen-presenting cells in their ability to present a vast array of antigens with great

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efficiency and to stimulate naïve T lymphocytes [1]. DCs are further distinguished by their ability to stimulate other effector cells such as B lymphocytes and NK cells. In addition to initiating immunity, there is increasing evidence that DCs are charged with maintaining tolerance to “self” or innocuous antigens, the failure of which could lead to autoimmune and/or inflammatory diseases [2]. How DCs accomplish these seemingly contradictory functions in immunity vs. tolerance is unclear. While TLR-mediated signaling has been extensively studied for their role in inducing DC-mediated immunity, little is known about what signals lead to tolerogenic DCs.

### **DC maturation and function**

As the sentinels of the immune system, DCs commence a complex and heterogeneous transformation process termed “maturation”, which greatly enhances their capacity for antigen processing and presentation upon encountering pathogens or a variety of pro-inflammatory mediators [1, 3, 4]. Maturation may occur prior to, during, or after migration to secondary lymphoid organs where the DCs serve to prime naïve T cells [1]. The general features of DC maturation are well understood [3] and involve the translocation of MHC class II molecules (MHCII) from lysosomal compartments to the plasma membrane, the upregulation of costimulatory molecules such as CD80 and CD86, the activation of lysosomal antigen processing, and the release of a host of immunostimulatory cytokines [4]. There is also a marked increase in the expression of lymphoid chemokine receptors such as CCR7, required for directed migration of DCs to lymph nodes [5]. Although the phenotypic correlates of DC maturation are clear, their relationship to DC function is complex. For example, depending on the type of microbial stimulus, DCs can prime qualitatively different types of effector T cell responses [6]. DC maturation and migration to lymph nodes can be independently regulated [7, 8], although the underlying mechanisms have not been elucidated. In DCs lacking the TLR adaptor MyD88, the phenotypic maturation of DCs can occur without inflammatory cytokine production [9]. Such DCs cannot activate naïve CD4<sup>+</sup> T cells *in vivo* suggesting that this phenotype, should it occur physiologically, might play a role in tolerance [10].

### **DCs in tolerance and tolerogenic DCs**

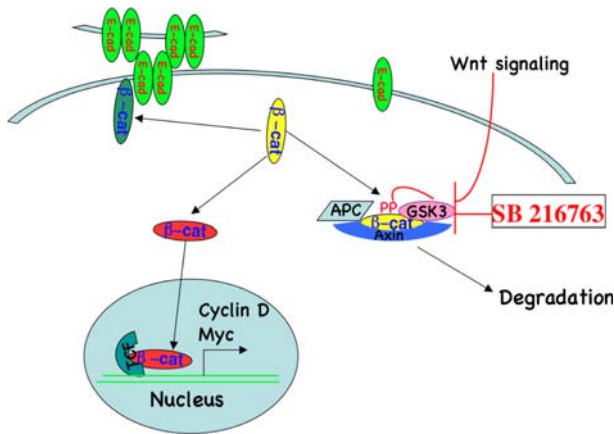
Another poorly understood but essential feature of DC function is tolerance. There is increasing evidence that in addition to initiating immunity, DCs are charged with maintaining tolerance to “self” or innocuous antigens, the failure of which could lead to autoimmune and/or inflammatory diseases [2]. How DCs accomplish these seemingly contradictory functions is unclear. It seems likely that “tolerogenic” DCs are those that present antigens captured in the absence of the microbial products or inflammatory mediators that signal the presence of conditions requiring of an immune response [2, 11]. The maturation state, origin, phenotype, and regulation of these “tolerogenic DCs” remain poorly understood, thus a confusing and often contradictory array of descriptions is available from the literature [12]. Tolerogenic DCs have often been described or predicted to be immature, since early concepts held that antigen presentation in the absence of costimulation leads to T cell anergy or death. However, immature DCs neither present antigen efficiently [13, 14] nor interact effectively with T cells [15, 16]. When immature DCs leave their site of epithelial or parenchymal residence and enter the lymphatics, one

thing is certain: they must alter their interactions with surrounding cells. Conceivably, this change may itself signal aspects of the maturation program, producing mature DCs capable of producing T cell tolerance: inducing either T cell deletion or the production of Tregs. Indeed, it has been elegantly demonstrated that targeting antigens to steady-state DCs using antibody to DEC-205 in the absence of overt inflammatory or immunostimulatory mediators led to tolerance [17–19]. These steady-state “tolerogenic” DCs were phenotypically mature, suggesting tolerance is a property of DCs at the steady state instead of being immature. Thus, DCs matured by non-inflammatory signals with enhanced antigen processing and presentation capacity are more likely to mediate and maintain peripheral tolerance to self-antigens.

### **The role of E-cadherin/ $\beta$ -catenin-signaling pathway in the generation of mature tolerogenic DCs**

In seeking non-inflammatory signals that might activate DCs to mature, we came across E-cadherin, a component of epithelial cell junctions that was also expressed in langerhans cells (LCs), specialized DCs in the skin [20, 21]. LCs form networks anchored to neighboring keratinocytes through homotypic binding mediated by E-cadherin [20, 22]. Although these networks are quite stable, LCs appear to traffic to lymph nodes, with their rate of emigration being enhanced by UV exposure or mechanical trauma [23, 24]. How this occurs is unknown, but seems likely to require the disruption of E-cadherin interactions. In epithelial cells, E-cadherin forms a complex with members of the catenin family, which control interactions with the actin cytoskeleton and (after translocation to the nucleus) act as cofactors for T cell factor (TCF; and lymphoid enhancing factor, LEF) transcriptional activators [25]. Given these functions, the amount of free cytosolic catenins, especially  $\beta$ -catenin, is carefully regulated. Under resting conditions, the bulk of  $\beta$ -catenin is sequestered to the E-cadherin cytoplasmic domain, with the cytosolic pool further attenuated by its phosphorylation by glycogen synthase kinase-3 $\beta$  (GSK-3 $\beta$ ) and subsequent proteasomal degradation [26, 27]. Activation of Wnt signaling activates TCF-dependent transcription by increasing free  $\beta$ -catenin due in part to an inhibition of GSK-3 $\beta$  (Fig. 1).

While it is unclear that whether E-cadherin-mediated cell–cell adhesion is linked to activation of  $\beta$ -catenin signaling, early works have demonstrated that disruption of LC–LC interactions in vitro could trigger phenotypic maturation [21, 28, 29]. We have found that BMDCs as well as some primary DC subsets, not just epidermal Langerhans cells, express the adhesion protein E-cadherin, and we demonstrated that cluster disruption (CD) of E-cadherin-mediated cell–cell adhesion is linked to the activation of  $\beta$ -catenin signaling [30], suggesting an intriguing possibility that E-cadherin/ $\beta$ -catenin might serve as a signal for the generation of steady-state tolerogenic DCs. Indeed, we showed that CD of E-cadherin-mediated adhesion efficiently stimulated DC maturation, triggering the dramatic upregulation of MHC class II, costimulatory molecules, and chemokine receptors [30]. Results thus far indicated that activating the  $\beta$ -catenin-TCF/LEF pathway induced maturation. However, in contrast to DCs conventionally matured by microbial products, E-cadherin-stimulated DCs displayed a dramatically different transcriptional profile, and most importantly, they failed to produce immunostimulatory cytokines, suggesting E-cadherin/ $\beta$ -catenin signaling is a novel mechanism for DC maturation [30]. DCs emigrating from tissues at the steady state in principle would contain antigens derived only from self-proteins and thus are expected to be responsible for maintaining peripheral

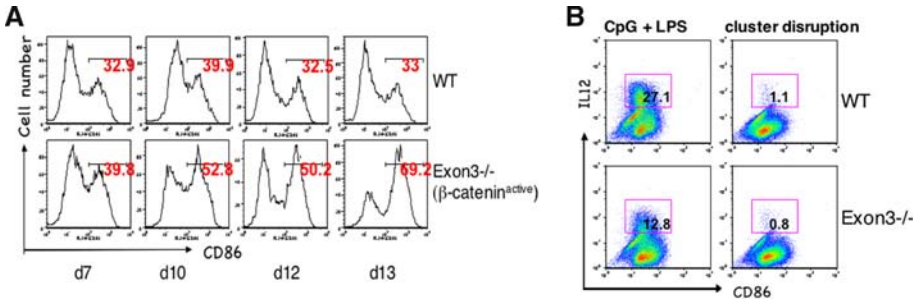


**Fig. 1** A simplified model of E-cadherin/ $\beta$ -catenin-signaling pathway. In resting cells, E-cadherin helps to control the level of the cytosolic  $\beta$ -catenin.  $\beta$ -Catenin normally forms a complex with tumor suppressor proteins APC and Axin and undergoes phosphorylation by GSK-3 and subsequent ubiquitination and degradation. Wnt signaling leads to nuclear accumulation of  $\beta$ -catenin, where it complexes with the TCF family transcription factors, resulting in transactivation of target genes includes myc and cyclin D. SB 216763 is an inhibitor to GSK-3 that also leads to DC maturation

tolerance [2, 11]. Since exit from epithelial tissues might also alter E-cadherin-mediated adhesion, we further examined the functional implications of these observations. Indeed, these CD-matured DCs elicited an entirely different T cell response in vivo, generating T cells with a regulatory as opposed to an effector phenotype. Upon adoptive transfer, these DCs induced tolerance in vivo and could even provide complete protection against subsequent induction of autoimmune diseases in a murine model of experimental autoimmune encephalomyelitis (EAE) [30]. Taken together, our results suggested an intriguing possibility that E-cadherin/ $\beta$ -catenin signaling might serve as a signal for the generation of steady-state tolerogenic DCs.

### E-cadherin/ $\beta$ -catenin signaling in regulating DC function in vivo

While our findings strongly suggested a role of E-cadherin/ $\beta$ -catenin signaling in regulating DC maturation and function in both cultured murine and human DCs, it remains an open question whether E-cadherin/ $\beta$ -catenin signaling functions similarly under physiological conditions. The embryo lethality of mice deficient in E-cadherin and  $\beta$ -catenin, however, presents a major hurdle to directly address this question. Recognizing this challenge, we have generated a series of CD11c-specific conditional knockout mice that either inactivate  $\beta$ -catenin signaling by deletion ( $\beta$ -catenin<sup>-/-</sup> and E-cadherin<sup>-/-</sup>) or activate the pathway by making  $\beta$ -catenin constitutively active ( $\beta$ -catenin Exon3<sup>-/-</sup>) [31–34], in collaboration with Bjoern Clausen in Amsterdam. These mice will allow us to directly address the role of E-cadherin/ $\beta$ -catenin signaling in a variety of settings. Our preliminary results showed a time-dependent increase in percentage of spontaneously mature DCs with constitutively active  $\beta$ -catenin than that of wild-type DCs (Fig. 2a), consistent with the activation of  $\beta$ -catenin as a tolerizing signal for DCs. Not surprisingly, DCs with constitutively active  $\beta$ -catenin exhibited diminished IL-12p40 response, with



**Fig. 2** E-cadherin/ $\beta$ -catenin signaling regulated DC maturation and cytokine production. **a** BMDCs with active  $\beta$ -catenin showed increased maturation. BMDCs from either wild-type or active  $\beta$ -catenin (CD11c-Cre  $\beta$ -catenin-Exon3<sup>Fl/Fl</sup>) mice were monitored for their expression of CD86 by FACS. **b** Activation of  $\beta$ -catenin in DCs diminished their capacity to produce IL-12p40 upon TLR signaling. BMDCs from either wild-type or active  $\beta$ -catenin mice were matured either with CpG plus LPS or CD. IL-12p40 production was assessed by intracellular staining

only 12.8% became IL-12p40-producing cells compared to 27% for wild-type DCs (Fig. 2b left panels). Further, consistent with our previous results, CD failed to induce IL-12p40 production from wild-type and mutant DCs (Fig. 2b right panels). Current efforts are made to confirm the role of E-cadherin/ $\beta$ -catenin signaling in mediating DC tolerance, especially under autoimmune disease settings.

## Summary and perspectives

While our work strongly suggested that E-cadherin/ $\beta$ -catenin pathway might serve as a novel signal to induce DC-mediated tolerance, the availability of a series of DC-specific conditional knockout mice with altered  $\beta$ -catenin signaling provide the right tools to further establish the molecular and cellular mechanisms and functional significance of the E-cadherin/ $\beta$ -catenin-signaling pathway in immune responses and autoimmunity.

Due to their pivotal role in controlling both immune and tolerance responses, DCs have emerged as good candidates to modulate the immune system in an antigen-specific manner with immunotherapy for both cancers and autoimmune/inflammatory diseases [35–38]. However, the underlying mechanisms for the regulation of DC function, especially tolerance, remained poorly understood, critically hindering successful application of these approaches. Our studies suggested E-cadherin/ $\beta$ -catenin-signaling pathway as a novel mechanism to generate tolerogenic DCs and showed that these tolerogenic DCs could be converted into highly immunogenic DCs upon certain treatments [30]. Thus, a therapeutic strategy that enhances the tolerance or immunity function of DCs by targeting E-cadherin/ $\beta$ -catenin-signaling pathway may represent an attractive approach for treatment of autoimmune/allergic diseases and cancer, respectively. We are currently focusing on investigating therapeutic effects of activating the E-cadherin/ $\beta$ -catenin-signaling pathway in DCs with preclinical animal models for melanoma, multiple sclerosis, and asthma. As E-cadherin/ $\beta$ -catenin functions similarly in human CD34<sup>+</sup>-derived DCs widely used in immunotherapy trials [30, 37], and great efforts have already been made to develop specific GSK-3 and  $\beta$ -catenin inhibitors for drug development [39, 40], our research could have immediate clinical impact.

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