Who controls the controllers? β-catenin and E-cadherin signaling in dendritic cell function

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1. From development to cell function: the role of β-catenin and E-cadherin signaling in context

The awareness of the involvement of E-cadherin and β-catenin in tumorigenesis has brought them into the focus of research ever since (1). β-catenin-controlled transcription of WNT-dependent genes represses differentiation in favor of proliferation, for instance in embryonic stem cells (2, 3). In addition to its key role in WNT signaling, β-catenin contributes to cell adhesion in a molecular complex with E-cadherin in various cell types (4, 5). E-cadherin-mediated cell-cell contacts on the other hand regulate the migratory potential of cells and guarantee tissue integrity (6). Consequently, mutations either resulting in a loss of E-cadherin-mediated cell adhesion or the prevention of cytosolic β-catenin degradation were identified to promote cancer pathogenesis by enhanced cell motility and/or proliferation. Being involved in such fundamental processes as cell differentiation, morphogenesis and gene transcription, β-catenin and E-cadherin both fulfill non-redundant tasks during embryogenesis.

Owing to the essential role of β-catenin and E-cadherin in embryogenesis, a null mutation of either β-catenin (7) or E-cadherin (8) results in embryonic lethality. This has long hampered the in vivo analysis of the role of β-catenin and E-cadherin signaling in cell specific processes that are active at later stages of development such as the regulation of the function of dendritic cells (DC), the target cells of this thesis. Hence, most insights about the participation and function of β-catenin and E-cadherin in signaling pathways and cellular mechanisms like WNT signaling and adherens junctions are based on in vitro experiments. To date it is not possible to create culture conditions that can fully imitate the complexity of the immune system, which makes it difficult to predict whether an effect observed in vitro is of relevance in vivo. The exposure of DC to tissue-derived signals such as cytokines and the cellular matrix may influence the phenotype and responsiveness of these DC. For instance, while E-cadherin is uniformly expressed by bone marrow-derived DC (BMDC) in vitro (9), most DC (except for LC) lack E-cadherin expression (10, 11). Most importantly, conditional mouse mutants based on the Cre/loxP system (12) nowadays allow to by-pass embryonic lethality of a total knockout by restricting the mutation of β-catenin (13) or E-cadherin (14) to DC.

Earlier in vitro studies suggested that β-catenin and E-cadherin play a crucial role in the regulation of DC function, in particular regarding their maturation and migratory potential (9, 15-17). While DC maturation is generally associated with the ability to induce a protective immune response, immature DC are considered to induce immune tolerance. However, recent work has challenged this paradigm showing that phenotypic DC maturation is not necessarily equivalent to DC immunogenicity but may also be associated with tolerogenic DC function (18). In particular, Jiang et al demonstrated that stabilization of β-catenin or disruption of E-cadherin binding in BMDC cultures results in phenotypically mature DC with a tolerogenic phenotype (9). In light of these
findings the objective of this thesis was to determine the role of β-catenin and E-cadherin signaling in DC function in vivo using conditional gene targeting.

1.1 E-cadherin function in skin dendritic cells

1.1.1 LC migration

E-cadherin has been shown to be necessary for cluster formation of BMDC in culture (9) while in vivo it is expressed by epidermal LC (10, 11) but not by other skin DC (chapter 2). LC were described to downregulate E-cadherin when migrating out of the epidermis (11, 17). Since migratory potential is closely associated with DC maturation, this implied a role for E-cadherin in the regulation of DC function in terms of migration and maturation. In accordance with this assumption, mechanical disruption of E-cadherin mediated adhesion in BMDC cultures resulted in phenotypically mature DC (9), while ligation of E-cadherin on BMDC-derived LC-like cells with E-cadherin antibody prevented DC maturation (15). Nevertheless, despite the conditional lack of E-cadherin in LC in vivo, neither enhanced maturation nor migration of LC to the draining LN was observed (chapter 2). Considering the essential role of E-cadherin to maintain cell adhesion in BMDC clusters, this finding was quite unexpected. However, Capaldo et al. have demonstrated in Madin-Darby canine kidney cell (MDCK) cultures that the establishment but not the maintenance of cellular junctions is dependent on E-cadherin mediated adherens junctions (19). Cellular adhesion mediated by tight junctions was not affected by a decrease of E-cadherin, though it should be noted that adherens junctions are required for the formation of tight junctions (20). Thus, the downregulation of E-cadherin prior to LC mobilization in the epidermis is more likely to be interpreted as one of several steps that are necessary to allow LC migration instead of being the trigger. In fact, LC express other cell adhesion molecules such as the tight junction protein claudin-1 (21). Tight junctions between LC and the surrounding keratinocytes allow LC to sample Ag from upper epidermal layers without destroying epidermal skin integrity (22). Within the DC located to the skin the expression of claudin-1, similar to E-cadherin, is mainly restricted to LC and depends on the presence (21) or responsiveness to TGF-β, respectively. Interestingly, mice that lack the TGFβ-receptor on LC rapidly loose epidermal LC starting within one week after birth demonstrating that TGF-β signaling is necessary for LC homeostasis (23). Thus, it is tempting to speculate that E-cadherin and claudin-1 cooperate to anchor LC in the epidermis as long as LC can sense and process a TGF-β signal. However, claudin-1 is still expressed in LC under inflammatory conditions, which suggests that additional cell adhesion molecules or tissue-derived signals in the epidermis are involved in the regulation of LC traffic to the sLN.
1.1.2 Regulation of β-catenin signaling

Beside its role in cell adhesion, E-cadherin is associated with the regulation of canonical WNT signaling due to its interaction with β-catenin. Binding to E-cadherin not only protects β-catenin from degradation, it also restricts the availability of β-catenin for WNT-dependent gene transcription (6, 24, 25). This is probably accomplished by blocking the binding sites for the protein adenomatous polyposis coli or members of the TCF/LEF-1 transcription factor family, which either target free β-catenin for destruction or form a complex with β-catenin to start gene transcription, respectively (25, 26). β-catenin recruitment to the cell membrane by E-cadherin may thus control β-catenin dependent gene transcription in two ways. For one, removing free β-catenin from the cytoplasmic pool prevents erroneous gene transcription in the absence of a WNT signal, which is associated with the development of human cancer (6). On the other hand, the protection of β-catenin from degradation generates a pool of β-catenin that can be quickly made available for gene transcription upon dissociation of E-cadherin binding (27). Notably, in murine ES cells (28) and epithelial cells (29) the transcription of E-cadherin itself is suppressed by WNT signaling. As a consequence, β-catenin is no longer sequestered to the plasma membrane by E-cadherin, which promotes further accumulation of signaling competent β-catenin to contribute to a robust WNT signaling response. Jiang et al. observed that disruption of E-cadherin in BMDC resulted in a mature DC phenotype characterized by enhanced levels of MHC II, CD86 and CCR7 (9). Moreover, disruption of E-cadherin binding promoted a tolerogenic rather than an immunogenic DC function. When BMDC were co-cultured with T cells or used to immunize mice in experimental encephalomyelitis (EAE) they induced IL-10 rather than IFN-γ producing T cells and protected recipient mice from EAE. As similar results were obtained by overexpression of β-catenin, it was suggested that the tolerogenic effect of E-cadherin disruption relies on cluster disruption induced nuclear translocation of β-catenin. In contrast, I found that LC from mice with a conditional deletion of E-cadherin did not differ in their expression of maturation markers (chapter 2). In addition, in two different delayed hypersensitivitiy (DTH) models the inflammatory response of E-cadherin deficient LC was similar to non-transgenic littermates, apart from a moderately delayed resolution of the inflammatory reaction (chapter 2).

Conditional deletion of E-cadherin is mediated by Cre recombinase expressed under the control of the CD11c promotor (30). E-cadherin produced before CD11c-Cre transgene expression and target gene deletion is probably not lost immediately but progressively as E-cadherin in adherens junctions is rather stable with a half-life of 15 h (31). In contrast to mechanical disruption of E-cadherin binding, the amount of β-catenin released by E-cadherin turnover may be insufficient to induce β-catenin-signaling and therefore a tolerogenic DC phenotype.

Alternatively, the different effect of E-cadherin on DC phenotype and function in vitro...
and in vivo may be explained by results from Kuphal et al. who knocked down E-cadherin with siRNA (32). A knockdown of E-cadherin in a colorectal cancer cell line with active WNT signaling allowed β-catenin-mediated gene transcription. In contrast, no β-catenin-dependent gene transcription was detected following E-cadherin knockdown in L929 fibroblasts lacking WNT signaling. This means that β-catenin release from E-cadherin without active WNT signaling is not sufficient to induce gene transcription. Interestingly, Jiang et al. have reported an increase of WNT10b by BMDC after cluster disruption, which may have contributed to stabilize β-catenin released from E-cadherin and thereby enhanced β-catenin signaling.

In both scenarios the tolerogenic signal by which β-catenin is supposed to regulate DC function is not provided anymore which might explain the delayed downregulation of the DTH response. In contrast, the expression of different WNT proteins in the skin (33) and the role of WNT signaling in the tolerogenic function of gut DC (34) do not support a lack of WNT signaling. Active WNT signaling has recently been demonstrated in steady state LC as blocking of WNT signaling with the antagonist dickkopf-related protein 1 decreased LC proliferation and number in the epidermis (35). However, it is not clear whether LC remain responsive to WNT signaling under inflammatory conditions. Recently, a small subset of inflammatory gut DC has been described which expresses E-cadherin (36). It is therefore tempting to speculate that, besides binding to cells that express the E-cadherin ligand CD103, an inducible expression of E-cadherin under inflammatory conditions might regulate β-catenin signaling in DC other than LC for example to support an inflammatory response. However, if and how WNT signaling does account for the release of β-catenin from E-cadherin in LC/DC is not clear. Nevertheless, in addition to the downregulation of E-cadherin expression by WNT signaling, secretases or matrix metalloproteases (MMP) can break up E-cadherin-mediated cell contacts (37). MMP expression can be induced by different pathways, including WNT signaling (5) and inflammatory signals such as IL-6, and has been reported to promote migration of T cells (38), dermal DC (39) and LC (40). Interestingly, metalloprotease ADAM10 promotes β-catenin transcriptional activity in keratinocytes (41) by proteolytic cleavage of E-cadherin. β-catenin remains attached to the c-terminal fragment (CTF) of E-cadherin, which protects β-catenin from degradation and shuttles it to the nucleus where the β-catenin-CTF complex contributes to gene transcription (42). Notably, there is evidence that isoforms of cadherin CTF can inhibit β-catenin-mediated transcription (25). As downregulation of E-cadherin prior to LC migration is observed in both, steady state (11) and inflammation (17), modulation of the transcriptional activity of β-catenin by E-cadherin CTF may direct DC function either towards a tolerogenic or an inflammatory phenotype. In contrast to proteolytic cleavage, WNT-mediated suppression of E-cadherin most likely results in a gradual loss of E-cadherin mediated cell-cell contacts, which is unlikely to result in a sufficient accumulation of cytosolic β-catenin...
to induce gene transcription. If one assumes that mechanical disruption reflects MMP mediated breakdown of E-cadherin and conditional deletion of E-cadherin corresponds to β-catenin mediated transcriptional downregulation of E-cadherin, this might at least in part explain the different outcome of E-cadherin disruption and conditional knockout of E-cadherin in LC/DC.

1.2 β-catenin function in DC and immunological tolerance

1.2.1 DC phenotype

β-catenin, in its function as the central component of canonical WNT signaling, participates in regulating the development and function of diverse cell types of the immune system (43). In agreement with the observation that β-catenin is expressed by LC where it is mainly located at the cell membrane in a complex with E-cadherin (9, 16), my data confirm expression of β-catenin in LC (chapter 3). Furthermore, I detected β-catenin expression in DC populations of skin, lung, draining LN and spleen (chapter 2 and 4). Ligation of E-cadherin by anti-E-cadherin antibodies was described earlier to suppress phenotypic maturation of BMDC (15). Moreover, mechanical disruption of E-cadherin interaction (cluster disruption) between BMDC or overexpression of β-catenin resulted in a phenotypic but not functional maturation of DC characterized by high expression of DC maturation markers like MHC II, CD86 and CD40 in the absence of inflammatory cytokines (9). The cluster-disrupted DC have a regulatory function as they promoted Treg development and were able to prevent induction of EAE following adoptive transfer into susceptible mice. Notably, the participation of β-catenin in E-cadherin containing adherens junctions removes β-catenin from the cytosol while mechanical disruption probably leads to an instant increase of cytosolic β-catenin levels (44). However, in contrast to β-catenin, most DC, except for LC, do not express E-cadherin. This suggests β-catenin as a possible master regulator of the tolerogenic DC phenotype. To elucidate the contribution of β-catenin to DC function in vivo, I generated conditional mouse mutants with a DC-specific deletion or stabilization of β-catenin. Mice whose DC specifically lack or express a stabilized version of β-catenin displayed an intact LC network, similar to mice whose LC are deficient for E-cadherin (chapter 2). As β-catenin is considered to be vital for the stabilization of E-cadherin-mediated adherens junctions (45), this supports the conclusion that adherens junctions may play a role but are dispensable for retaining LC in the epidermis. Moreover, in vivo neither DC-specific lack nor stabilization of β-catenin altered the expression of DC maturation markers as opposed to the upregulation of MHC II, CD86 and CCR7 following overexpression of β-catenin in BMDC (9). In compliance with my own observation, work by Manicassamy et al. shows that conditional deletion of β-catenin in intestinal DC does not affect the expression of maturation markers either (34). The reason for the discrepancy between in vitro and in vivo data remains elusive but may in part be explained by the induction of regulating tissue-derived signals that LC/DC receive in situ, which are absent in BMDC cultures. On the other hand, BMDC
from mutant mice expressing stabilized β-catenin did not display enhanced maturation. Therefore, one could speculate that cellular stress caused by cluster disruption or forced expression of β-catenin in vector-transfected BMDC in combination with β-catenin signaling contributes to enhanced expression of maturation markers.

### 1.2.2 DC function

Despite the fact that stabilization of β-catenin did not affect the phenotypic maturation of DC, I found a systemic increase of Foxp3⁺ regulatory T (Treg) cells in steady state. Consequently, *DC-βcat⁺* mice did not develop contact hypersensitivity (CHS) and showed a strongly ameliorated inflammatory response in asthma and EAE indicating a tolerogenic function of the mutant DC (chapters 3-5). In contrast, a DC-specific lack of β-catenin resulted in a mild effect on DC function in CHS reflected by a delayed resolution of the inflammatory response and aggravated EAE pathology. Similarly, selective depletion of β-catenin in DC exacerbated inflammatory bowel disease (34). The same group described decreased Treg numbers in the gut and found that β-catenin expression by DC is necessary to generate an anti-inflammatory microenvironment in the intestine by producing IL-10, TGF-β and retinoic acid (RA)-metabolizing enzymes. In contrast, I did not detect altered production of inflammatory or anti-inflammatory cytokines upon stimulating splenic DC expressing stabilized β-catenin with either LPS, CD40L or TGFβ (chapter 3). Considering that expression of inflammatory cytokines was suppressed in cluster-disrupted BMDC and wild-type intestinal DC, this result may indicate differences in the regulation of local DC populations. To assess whether this assumption holds true, DC from different tissues should be compared side by side with intestinal DC regarding their cytokine expression.

Manicassamy et al. described decreased frequencies of Treg in intestine and cecum but not in spleen of *DC-βcat⁻¹⁶⁴* mice, which they could link to metabolization of vitamin A to RA by DC (34). In contrast, I found an increased frequency of Treg in *DC-βcat⁺* mice not only in skin and lung draining LN but also in the spleen (chapters 3, 4), which indicates that the role of β-catenin signaling in the induction of a tolerogenic DC function is not restricted to DC in the lamina propria (LP) of the intestine. On the other hand, while splenic DC in general are capable to generate RA (46), the frequency of Treg in the spleen was not reduced in DC-specific β-catenin knockout mice (34). While β-catenin is constantly active in LP DC but not in splenic DC (34), DC transporting commensal bacteria are found in MLN but not in the spleen. The discrepancy in Treg frequencies could on the one hand be explained by a general lack of systemic immunity and tolerance directed against gut commensal flora due to the compartmentalization of intestinal immunity, which restricts homing of gut mucosal DC to mesenteric LN (47). On the other hand, the spleen might not provide an environment that supports constant stabilization of β-catenin levels. It remains to be determined whether β-catenin-mediated RA production by DC plays an equally central role in tolerance induction in organs such as the skin and
the lung (48). In the light of these findings it may be interesting to determine whether supplementation of vitamin A alone is sufficient to induce β-catenin stabilization in DC derived from other tissues. As vitamin A is also metabolized to RA by epithelial cells from the intestine (49, 50), extrinsic RA might be required to trigger a positive feedback loop in DC that leads to β-catenin stabilization, which in turn enables DC to produce RA themselves and exert a tolerogenic function. In particular, the correlation between administration of RA and diminished airway inflammation through the development of Treg in experimental asthma indicates a role for this pathway (51). Moreover, the frequency of TH17 cells producing the inflammatory cytokine IL-17 was enhanced in the intestine of DC-βcatdel mice (34) and it has been demonstrated that RA is crucial in maintaining the balance between Treg and TH17 in mice (52). Thus, inflammatory TH17 cells in EAE (53) may account for the aggravated EAE response in DC-βcatdel mice and the attenuation of EAE in DC-βcatex3 mice, respectively (chapter 6).

1.2.3 Taming the armadillo: WNT or … else?

Though active WNT signaling was detected in LP DC and macrophages (34), it is not clear whether this is the only trigger that sets off β-catenin signaling in DC. Canonical WNT signaling is the best characterized WNT-dependent signaling cascade and relies vitally on the stabilization of β-catenin, the vertebrate homologue to armadillo in drosophilia. The ligands that trigger the highly conserved WNT pathway can be found in metazoans from C. elegans to H. sapiens and are collectively referred to as WNT proteins. Binding of a WNT ligand to its receptor frizzled launches a sequence of signaling events that ultimately result in the transcription of a defined set of target genes dependent on the tissue context, the cell type and the isoform of the WNT protein (54). In absence of a WNT signal, free cytosolic β-catenin is sequestered by a degradation complex which targets β-catenin for rapid proteosomal break down by site specific phosphorylation and ubiquitination in exon 3. In contrast, binding of WNT-protein to the frizzled receptor complex leads to disassembly of the degradation complex. As a consequence, β-catenin accumulates in the cytoplasm and can translocate to the nucleus to initiate WNT-dependent gene transcription by binding to members of the TCF/LEF transcription factor family. Target genes of the WNT/β-catenin pathway, including c-myc, CD44, and BMP4 (55-57), are involved in the regulation of proliferation, differentiation, migration and adhesion of cells. E-cadherin levels are downregulated by canonical WNT signaling (29). Inversely, typical WNT target genes like CD44 have been shown to be upregulated upon disruption of E-cadherin binding (9). Assuming that mechanical severance of intercellular E-cadherin cell contacts results in an abrupt release of β-catenin into the cytoplasm, this suggests that β-catenin bound to E-cadherin represents a WNT-independent way of β-catenin mediated gene transcription (44). However, while LC express E-cadherin most other DC populations do not. Moreover, while WNT signaling seems to actively
stabilize β-catenin in LP DC it is not known to what extent WNT signaling plays a role in stabilizing β-catenin in other DC populations. Lately, alternative pathways (58-60) have been described to control β-catenin signaling which are not linked to WNT signaling and should be taken into consideration as potential regulators of β-catenin signaling in DC. Furthermore, alternative interaction partners in the control of β-catenin mediated gene transcription such as FOXO (61) and Prop1 (62) have been described that compete with TCF/LEF for β-catenin in the nucleus, most likely resulting in different gene expression profiles.

With regard to the development of therapeutic approaches involving the manipulation of β-catenin to either induce selective tolerance or immunity, it is thus vital to explore the different possibilities that can regulate β-catenin levels and to identify its cell specific interaction partners and downstream targets in DC.

2. Conditional gene targeting of LC and Langerin positive dermal DC

The most powerful way to characterize the function of a gene of interest in a particular cell type in vivo is by Cre recombinase-mediated conditional mutagenesis (63). While DC were long considered as potent inducers of a protective immune response, in recent years it became more and more clear that they may play a similarly critical role in the induction and maintenance of immune tolerance (64). The discovery of phenotypically different DC subpopulations (65-67) raised the question to what extent functional specialization and plasticity of DC contribute to the regulation of immunity versus tolerance and whether communication between DC subsets plays a role (68). As part of this thesis, a Langerin-Cre mouse was generated to specifically address the function of Langerin expressing DC. Langerin is the only known DC marker to date that is exclusively expressed by certain DC subtypes in e.g. skin and lung but not shared with other cell types. In chapter 6 we show that Langerin-Cre mice specifically delete loxP-flanked genes in LC and Langerin+ DC but not in Langerin negative cells. Moreover, the function of LC in regulating skin immunity and tolerance has been a matter of debate since experiments by independent labs depleting Langerin expressing DC prior to CHS induction yielded conflicting results (69-72). In particular, Bennett et al. describe reduced CHS, while Kaplan et al. report an enhanced ear swelling reaction when LC are absent in the epidermis. While in the Langerin-DTR mice used by Bennett et al. all Langerin expressing DC are depleted, the transgenic mouse generated by Kaplan et al. lacks only LC. In contrast to dermal DC (including dermal Langerin+ DC), LC homeostasis is dependent on TGF-β and the epidermis of mice in which LC are unable to sense TGF is devoid of LC. In chapter 6 we used mice in which Langerin-expressing DC selectively lack TGF-βR1 to demonstrate that CHS is reduced in the absence of LC while dermal Langerin-expressing DC are still present. In line with our own results and the data from Bennett et al. (72), experiments
using bone marrow chimeras by Honda et al. (73) suggest that LC and dermal Langerin' DC have a redundant function in the induction of CHS rather than a tolerogenic function. Experiments by Noordegraaf et al. in which diphtheria toxin doses were administered to Langerin-DTR mice, resulting in long term depletion of LC but not dermal Langerin' DC, further support a redundant function of dermal Langerin' DC and LC in CHS (74). In conclusion, we not only show that LC and dermal DC co-operate in the induction of skin inflammation but also demonstrate that Langerin-Cre mice provide a powerful tool to study the function of Langerin' DC. Langerin-Cre mice will be helpful in the future to dissect the role of β-catenin signaling in Langerin-expressing DC subtypes.

3. Concluding remarks

While in the past 5 years some mechanisms have emerged that may be involved in the adoption of a tolerogenic DC phenotype (75, 76), β-catenin signaling has lately taken center stage (9, 34). One of the two most urgent questions to be answered regarding the role of β-catenin signaling in DC is to unravel which signals are responsible to induce β-catenin signaling in the first place. Regarding the distinct requirements for either a highly tolerogenic microenvironment in the gastrointestinal tract or a rapid immune response upon detection of bacteria in comparatively sterile organs such as the spleen, it would not be surprising to find different signals that regulate β-catenin signaling in the local DC populations. Secondly, it needs to be determined if and which other pathways beside RA, are of importance in communicating the tolerogenic DC function to T cells. In addition, one of the major problems in developing DC-specific immunotherapies involving β-catenin or any other molecule to date is to target selectively DC in humans. As β-catenin is ubiquitously expressed, unspecific manipulation of β-catenin would probably be fatal especially with respect to its role in tumorigenesis. Unless these issues have been solved, manipulation of β-catenin signaling for therapeutic approaches remains difficult. Nevertheless, investigating the downstream targets of DC-specific β-catenin signaling provides a promising perspective to tailor DC targeted immunotherapies in the future to induce or suppress DC-mediated tolerance.
REFERENCES


