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REVIEW

Dendritic cells as gatekeepers of tolerance

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Abstract Dendritic cells (DC) are unique hematopoietic cells, linking innate and adaptive immune responses. In particular, they are considered as the most potent antigen presenting cells, governing both T cell immunity and tolerance. In view of their exceptional ability to present antigen and to interact with T cells, DC play distinct roles in shaping T cell development, differentiation and function. The outcome of the DC-T cell interaction is determined by the state of DC maturation, the type of DC subset, the cytokine microenvironment and the tissue location. Both regulatory T cells (Tregs) and DC are indispensable for maintaining central and peripheral tolerance. Over the past decade, accumulating data indicate that DC critically contribute to Treg differentiation and homeostasis.

Keywords Tolerance induction · Dendritic cells · Regulatory T cells

Dendritic cells (DC) serve as unique sentinels of the immune system, continuously sampling their environment and exerting different properties that in turn determine immunological outcomes. Although DC do not serve as effector cells that fight against pathogens, they control adaptive immunity by providing essential signals that are mandatory for directing the desired immune response. Apart from antigen presentation, DC deliver co-stimulatory signals and produce cytokines, which are necessary for instructing appropriate effector or regulatory T cell responses. In this review, we will examine different aspects of DC-derived tolerance.

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DC lineage and subsets

All DC develop from a common macrophage/dendritic cell progenitor (MDP) found in the bone marrow (BM) [1], which further proceeds to differentiate to the monocyte/macrophage lineage or to the common dendritic cell progenitor (CDP) [2, 3]. CDP in the BM give rise to both plasmacytoid DC (pDC) and pre-DC progenitors [4–6]. pDC complete their last step of maturation in the BM, before they egress into the blood stream as mature functional cells. Pre-DC, on the other hand, migrate through the vascular system to their final residence in the tissues or lymphoid organs, where they finish their differentiation into distinct conventional DC subsets, namely, $CD8\alpha^+$/$CD103^+$ DC or $CD11b^+$ DC [7]. DC differentiation, lineage and subsets are summarized in Fig. 1.

DC represent a heterogeneous family of myeloid antigen presenting cells (APCs) composed of several different subsets. They are found throughout the entire body, and although 2–4 % of all leukocytes are DC in any given tissue, the composition of the different subsets varies between organs. These subsets are defined by their localization, expression of surface proteins and functionality. Typically, DC are categorized into two distinct classes: pDC, which produce large quantities of type I interferon in response to viral infection and conventional DC, which are highly potent APCs, in particular, for activating naïve T cells. Among the conventional DC, $CD8\alpha^+$ DC

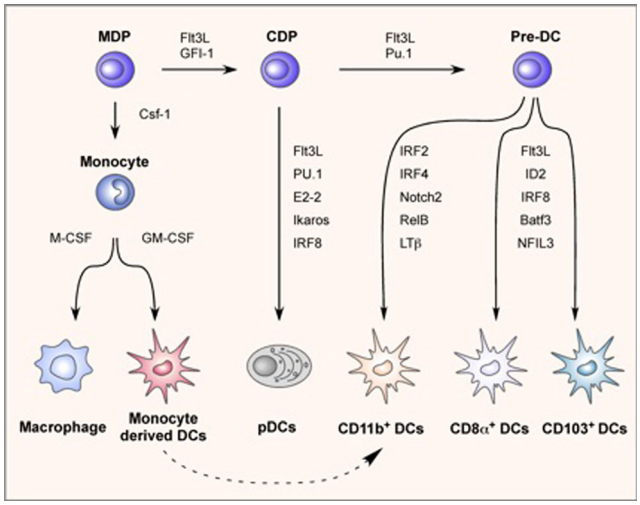

Fig. 1 Dendritic cell development. Macrophage dendritic cell progenitors (MDP) give rise to either monocytes or common dendritic cell progenitors (CDP). Dependent on macrophage colony stimulating factor (M-CSF) or granulocyte macrophage colony stimulating factor (GM-CSF), monocytes develop into macrophages or monocyte-derived DC, respectively. Monocyte-derived DC give rise to CD11b$^+$ ESAM^{low} cells. CDP are developing into pDC in a manner dependent on Flt3L, PU.1, E2–2, Ikaros and IRF8. CDP also give rise to pre-DC, which are the progenitors of the conventional CD11b$^+$ ESAM^{high}, CD8α^+ and CD103$^+$ dendritic cells. The development of CD11b$^+$ ESAM^{high} DC is dependent on IRF2, IRF4, Notch2, RelB and LTβ, while the development of both CD8α^+ and CD103$^+$ DC is dependent on Flt3L, ID2, IRF8, Batf3 and NFIL3

are important for orchestrating immune responses against intracellular pathogens, whereas CD11b$^+$ DC are thought to be more relevant for fighting extracellular pathogens.

pDC

pDC are defined as CD11c^{low} MHC-II^{low} B220$^+$ PDCA-1$^+$ Siglec-H$^+$ cells. Their development is dependent on E2-2 and IRF8 [8, 9]. pDC are specialized in high type I interferon secretion [10–12]. Steady state pDC are poor APCs, although following their activation by pathogens they are fully capable of priming naïve T cells [9]. Freshly isolated human pDC induce anergy of human CD4$^+$ T cell clones [13, 14]. Similarly, freshly isolated mouse antigen-pulsed splenic pDC induce antigen-specific T cell anergy [15, 16]. Naive T cell stimulation using CpG oligonucleotide-stimulated mesenteric LN-derived pDC gave rise to Tregs with suppressive activity [17]. In vitro human pDC induce T cell differentiation into IL-10-producing Tregs [14, 18]. Taken together, these data indicate that both mouse and human pDC induce CD4$^+$CD25$^+$ FOXP3$^+$ regulatory T cells (Tregs).

CD8α^+ and CD103$^+$ DC

Development of both lymphoid organ-resident CD8α^+ DC and their tissue-resident CD103$^+$ equivalents was shown to be dependent on tyrosine kinase receptor fms-like tyrosine kinase 3 ligand (Flt3L) [19, 20], inhibitor of DNA binding protein 2 (ID2) [5, 21, 22], the transcription factor interferon regulatory factor 8 (IRF8) [23, 24] and the basic leucine zipper transcription factor ATF-like3 (Batf3) [25, 26]. The CD8α^+ DC participate in central as well as peripheral tolerance induction due to their ability to produce high amounts of TGFβ in the steady state [27]. Furthermore, they can induce peripheral tolerance towards tissue-associated self-antigens also via direct contact with self-reactive T cells, which lead to the death of the latter [28, 29]. Their tolerogenic potential also becomes apparent when targeting antigen to CD205 (DEC205), which induces clonal deletion [30, 31] and Treg differentiation [32]. Moreover, a recent publication using conditional ablation of DEC205$^+$ DC established the importance of these cells in the generation of thymic Tregs as well as the maintenance of Tregs in the periphery, i.e. mucosal tissues [33].

CD11b$^+$ DC

CD11b$^+$ DC development is dependent on Flt3L, Notch2, IRF2, IRF4, RelB, LTβ and GM-CSF [34, 35]. These cells are found in both lymphoid organs and peripheral tissues. They are the dominant conventional DC population of the spleen, but only a minor population in the thymus. Based on the expression of endothelial cell-specific adhesion molecule (ESAM), two distinct splenic CD11b$^+$ populations can be distinguished [34]. ESAM^{high} DC represent cells derived from DC progenitors while the ESAM^{low} population stems from circulating monocytes. In the periphery, CD8α^-CD11b$^+$ DC but not CD8α^+ DC mediate cross-tolerance toward intestinal antigens [36]. In autoimmune diabetes, CD11b$^+$ DC were recently shown to play a tolerogenic role [37]. A distinct tolerogenic subset of splenic CD11b$^+$IDO$^+$ DC from orally tolerized mice is responsible for induction of systemic immune tolerance and suppression of collagen-induced arthritis [38]. Antigen-induced tolerogenic CD11b$^+$ DC are abundant in Peyer's patches during the induction of oral tolerance to type II collagen and suppress experimental collagen-induced arthritis [39].

DC promote central tolerance

Unlike their established role as prototypic APCs initiating immune responses, the role of DC in steady state immune tolerance is less understood. Several studies have highlighted the role of DC in thymic T cell development. Targeted expression of major histocompatibility complex (MHC)-II molecules demonstrated that DC can induce negative but not positive selection of thymocytes in vivo [40] and hence mice with residual expression of MHC-II on mature DC fail to promote positive selection of CD4$^+$ T cells [41]. Negative selection occurs when thymocytes are presented with and respond to

self-antigens. Medullary thymic epithelial cells (mTEC) express many tissue-restricted self-antigens (TSA) in an autoimmune regulator (AIRE)-dependent manner [42]. Thymic DC can either directly present or cross-present self-antigens shed by mTEC. Thus, self-antigen presentation by thymic DC can either result in negative selection [43–45] or lead to the generation of thymus-derived Tregs (nTregs) [46]. As DC are highly migratory cells, thymic clonal deletion and/or nTreg development can also be driven by circulating DC presenting peripheral TSA [47]. Nevertheless, constitutive deletion of DC does not result in breakdown of central tolerance, as mice lacking DC exhibit a relatively normal T cell repertoire [48], indicating that DC are capable of, yet not mandatory for induction of central tolerance. Similarly, DC-deficient mice were found to contain normal nTreg numbers in the steady state without developing spontaneous autoimmunity [49]. However, a third study described a strong autoimmune lymphoproliferative infiltration followed by spontaneous fatal autoimmunity in mice that lack DC [50]. Our mice [48, 49] also show lymphoproliferative disease that is evident at around 4 months of age, but unlike the mice described by Ohnmacht et al. neither show signs of autoimmunity nor die from such disease. As both systems are genetically similar and depend on the same Cre-expressing mouse line, we have no good explanation for this discrepancy. One may speculate that differences in the gut microbiota are responsible for the generation of pathogenic responses in one mouse colony but not in the other, thereby contributing to development of fatal inflammatory bowel disease (IBD).

DC-mediated T cell homeostasis

Two photon imaging and live intravital microscopy had uncovered frequent and dynamic interactions between DC and T cells, even in the absence of infectious stimuli [51, 52]. T cell homeostasis and optimal functionality was found to be controlled by steady state DC, delivering tonic signals through MHC-T cell receptor (TCR) complex interactions [53, 54]. Similarly, Scheinecker et al. show that under steady state conditions, DC present self-antigen to T cells in the draining lymph nodes [29]. Thus, DC play a key role in maintaining immune homeostasis and promoting peripheral T cell tolerance, by continuously presenting self- or harmless foreign antigens to T cells in the absence of infection and inflammation, that is co-stimulation and/or activating cytokines [55, 56].

DC establish peripheral tolerance

Peripheral tolerance mechanisms necessitate from the limitations of central tolerance ‘avoiding horror autotoxicus’ [55]: (i) self-reactive lymphocytes escape negative selection, (ii) many innocuous environmental antigens, including commensal microbiota, are not expressed in the thymus and (iii) lymphocyte receptors for foreign antigens can cross-react with self. Early studies showing tolerance induction by DC used antigen-targeting in vivo by antibodies targeting receptors expressed specifically by DC [30, 57]. In this approach, one conjugates the self-antigen to a DC-specific antibody, for example anti-DEC205. Following injection of the antibody conjugate, it will bind to the specific receptor on DC, be internalized and the antigen will be processed and presented by the (immature) DC, which leads to tolerance in the absence of an adjuvants, e.g. TLR ligand or CD40L [56]. Despite this proof-of-concept, limitations of these early experiments using anti-DEC205–antigen conjugates to target DC were low-level expression of DEC205 and Fc receptor-mediated uptake by other cell types (macrophages, B cells). Thus, ongoing research aims to improve this strategy, for example, by identifying novel DC (subset)-specific endocytic receptors and generating better antibodies for improved DC-based immunotherapy of human disease.

By utilizing the genetic approach of Cre-recombinase, the group of Maries van den Broek demonstrated that induced expression of an immunodominant virus-derived antigen by DC results in strong $CD8^+$ T cell unresponsiveness that could not be broken in wild type mice [58]. Follow-up studies shed light on the mechanism of DC-induced T cell tolerance, involving the expression of PD-1 and CTLA4 on $CD8^+$ T cells and Treg induction [59, 60]. Using a similar approach, we have recently shown that induced expression of self-antigen by steady state DC induces robust $CD4^+$ T cell tolerance [49]. Our data suggested that the mechanism instructing such tolerance involves the interaction of PD-L1 expressing DC with PD-1 expressing T cells, resulting in the generation of de novo antigen-specific peripheral iTregs. At the same time, constitutive DC-ablation results in enhanced autoimmunity following immunization with self-antigen [49].

In view of this tolerogenic role of DC, it was assumed that the loss of DC would result in a breakdown of peripheral tolerance. However, constitutive DC ablation did not lead to spontaneous autoimmunity but rather a myeloproliferative disorder [48]. Further studies identified an elevated Flt3L serum concentration as the driving force of this myeloproliferative syndrome due to the loss of DC [48, 61]. A similar scenario was reported in human patients with hereditary monocyte or DC deficiency [62]. Likewise, constitutive DC depletion in lupus prone MRL/lpr mice resulted in ameliorated autoimmunity. DC were shown to be crucial for the expansion and differentiation of T cells but were not required for their initial activation. In line with the above, kidney-interstitial infiltrates developed in the absence of DC, but failed to progress to an inflammatory disease [63]. In this model, systemic lupus erythematosus (SLE) becomes apparent at the age of 4 months. At this age, the mice are already partly immunodeficient (loss of tonic signals required for T cell homeostasis), harbouring reduced levels of peripheral T cells (both effector and regulatory) and to lesser extent B cells, which may explain the reduced autoimmunity.

Taken together, these studies suggest that DC can induce peripheral T cell tolerance, yet they may not be essential for it. On the other hand, steady state DC appear to be mandatory for the generation of antigen-specific peripheral iTregs, by a mechanism that involves PD-L1/PD-1 interaction.

DC and Tregs

As indicated above, DC are important for maintaining peripheral T cell homeostasis and preventing inappropriate T cell activation. Extra-thymic or peripherally induced Treg cells (iTregs) are no exception. Several in vitro studies have shown that DC can induce induced Treg (iTreg) formation, particularly when Treg-promoting cytokines are added [3, 64, 65]. Yet, the absence of DC led only to a mild reduction of total Treg numbers at best [48, 66], although Treg homeostatic proliferation was shown to be dependent on the presence of DC [67]. Conversely, Treg ablation results in accelerated DC maturation and expansion [68], a process that was dependent on Flt3 [6]. In line with the above findings, several studies uncovered a direct correlation between DC and Treg numbers, as part of a feedback-control mechanism directed by Flt3L [66, 69]. In another report, Collins et al. identified a lamina propria $CD103^+$ DC subset as the one subset that expands in response to Flt3L treatment [70]. $CD103^+$ DC represent the tissue-resident DC subset equivalent to $CD8^+$ DC in the lymph nodes and spleen, and Flt3L treatment skews DC toward the tolerogenic $CD8^+$ lineage [71]. These studies suggest that there is a bidirectional feedback loop involving Flt3/Flt3L that regulates both $CD8\alpha^+/CD103^+$ DC and Tregs. However, the development of CDP and subsequently the different DC progenies are also Flt3/Flt3L-dependent [1, 72], and Flt3L-dependent DC control immune responses to protein vaccine [73]. In addition, Flt3L might cause the expansion of other myeloid cells. Furthermore, the functionality of the DC that respond and expand in response to Flt3L might be different due to different pathways downstream of Flt3, e.g. PI3K, Akt and mTOR.

The tool kit of DC to generate Tregs

It is evident that DC support the differentiation and maintenance of different types of Treg cells, including the IL-10-producing Tr1, TGFβ-producing Th3, thymic-derived (nTregs) and peripherally induced (iTregs) $Foxp3^+$ T cells. This is achieved by various mechanisms, including direct cell-cell contact-dependant signalling through surface molecules, as well as by affecting Treg cell fate through secretory proteins. These mechanisms are described in detail below and summarized in Fig. 2.

Co-stimulatory signals delivered by cell-cell contact

CD80/86

Like all other T cell subtypes, Tregs also express CD28 and the interaction of CD28 with CD80/CD86 is required for normal thymic and peripheral Treg development and maintenance [74]. A recent study revealed that DC are an important source of CD80/86 to enhance Treg numbers [75]. Intriguingly, the CD80/86 signal delivered by DC was dispensable for the development of thymus-derived nTregs, but the loss of these molecules specifically on DC resulted in about 40 % reduction of peripherally induced iTregs.

CD70

CD70 is a TNF family member expressed on DC and mTECs (both $AIRE^+$ and $AIRE^-$), whereas its receptor CD27 is expressed on developing thymocytes. Recently, it has been discovered that the CD70–CD27 pathway plays an important role in thymic-derived nTreg development. Using CD70-deficient BM chimeric mice, Coquet et al. demonstrated that CD70-expressing $CD8\alpha^+$ DC (but not $CD11b^+$ DC or pDC) contributed to nTreg development in the thymus. In particular, CD70–CD27 signalling drives the positive selection of nTregs and prevents them from undergoing apoptosis [76]. In the periphery, CD70 signalling promotes Th1 [77] and suppresses Th17 differentiation, which in turn results in reduced autoimmunity [78]. In contrast, in vitro experiments indicated that CD70-overexpression was not sufficient to drive Th17 differentiation, nor did it affect the generation of Tregs [79].

ICOS-L

In a model of airway inflammation, ICOS-L-expressing semimature DC promoted the induction of TGFβ-producing, antigen-specific iTregs [80]. Similarly, pDC stimulate the induction of iTregs in an ICOS-L-dependent manner [18]. In agreement, adoptive transfer of WT but not ICOS-L-deficient pDC protected mice against asthma [81].

PD-ligands

In contrast to WT APCs, when PD-L1-deficient APCs were used to generate iTregs in vitro, only a minimal iTreg conversion of naive $CD4^+$ T cells was observed [82–84]. Recently, it has been reported that PD-1 ligation affects the stability of DC-T cell contacts [85]. Using a diabetes mouse model, T cells tolerized for self-antigen failed to form stable interactions with antigen-specific DC, but rather moved freely in the draining lymph nodes. Following administration of PD-L1 or PD-1 blocking antibodies, continued PD-L1 and PD-1 interactions inhibited TCR-mediated signal transduction [85].

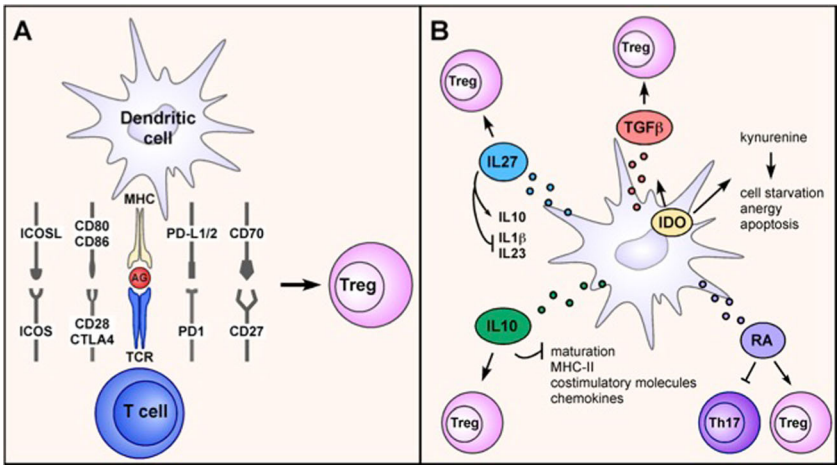

Fig. 2 Dendritic cells mediate tolerance. **a** DC can mediate Treg generation via several surface molecules, including CD70, CD80/CD86, ICOS-L and PD-L1 or PD-L2. The expression of CD70 on DC induces a survival signal in thymic autoreactive Treg cells expressing CD27 and prevents them from undergoing negative selection. MHC-II antigen presentation by DC without an additional co-stimulatory signal (e.g. CD80/CD86), or in combination with a co-inhibitory signal (e.g. PD-L1/2) can lead to tolerance induction. Similarly, ligation of CD80/CD86 by cytotoxic T lymphocyte antigen 4 (CTLA4) drives Treg differentiation while insufficient ligation of CD80/CD86 by CD28 induces tolerance. The inducible T cell co-stimulator ligand (ICOS-L) expressed by DC binds to its receptor on T cells and maintains Treg homeostasis. **b** DC secrete many factors that are known to induce tolerance and Treg generation. Both TGFβ and IL-10 production by DC mediates Treg differentiation. At the same time, IL-10 also inhibits DC maturation, as well as MHC-II, co-stimulatory molecules and chemokine expression by DC. DC-derived IL-27 induces Treg generation by increasing IL-10 expression and repressing IL-1β and IL-23 production. DC are also an important source of retinoic acid (RA), which is involved in the generation of Tregs, while simultaneously inhibiting Th17 cells. Indoleamine 2,3-deoxygenase (IDO), giving rise to kynurenines and other tryptophan metabolites, is a strong inducer of T cell anergy, apoptosis and Treg differentiation. IDO may also induce TGFβ expression

Moreover, DC stimulation with soluble PD-1 suppresses DC maturation and promotes IL-10 secretion [86]. Recently, we have shown that upon transient or constitutive DC depletion, mice developed encephalomyelitis (EAE) after active immunization with the MOG peptide p35-55. Furthermore, PD-L1 expressing DC were crucial for in vivo generation of antigen-specific iTregs, which in turn dampens disease severity. De novo generation of antigen-specific iTregs was markedly elevated in cDC-proficient but not in cDC-deficient mice, whereas de novo generation of antigen-specific PD-1-deficient iTregs was comparable irrespective of the presence or absence of DC [49].

Signals conveyed by secreted molecules

Il-10

IL-10 is a regulatory cytokine produced by and acting on different immune cells, including T cells and DC [87–89]. While the effects of IL-10 on (regulatory) T cells are well established, its broad anti-inflammatory properties may also result from the ability to inhibit APC function, including their maturation, expression of MHC-II, co-stimulatory molecules, chemokines (both CC and CXC) and production of pro-inflammatory cytokines [90, 91]. IL-10 conditions human DC to acquire a tolerogenic phenotype favouring the induction of T cell anergy and suppressive function [92, 93]. Moreover, transfer of IL-10–treated DC limits effector T cell responses [94], protects mice from experimental autoimmune encephalomyelitis (EAE) and inhibits graft rejection in transplanted hosts [95, 96]. More recently, the in vivo role of IL-10 to restrain APC function is beginning to unfold. IL-10 controls DC in the skin to limit contact hypersensitivity and attenuate anti-*Leishmania major* immunity [97, 98]. In addition, IL-10 control of $CD11c^+$ APCs and, in particular, CX_3CR1^+ macrophages is essential to maintain immune homeostasis in the intestine [99, 100]. Intriguingly, in the absence of IL-10 signalling in $CD11c^+$ myeloid cells oral tolerance remains intact [100].

On the other hand, early in vitro studies have identified IL-10 production by DC to be an important mechanism in driving T cell anergy and suppression [101]. In vivo experiments had shown that following respiratory antigen challenge, IL-10 producing mature pulmonary DC induce tolerance via the generation of Tr1 cells [102]. BM-derived DC, differentiated in the presence of IL-10, GM-CSF and TNFα, developed into semi-mature DC, which following LPS-challenge produced high levels of IL-10 and promoted the differentiation of IL-10 producing suppressive T cells [103]. Similarly, Langerhans cells suppress contact hypersensitivity through IL-10 secretion, which in turn promotes Tr1 cell differentiation [104]. While IL-10–deficient mice develop spontaneous IBD by the age of 4–6 weeks [105], DC-specific deletion of IL-10 results in a mild spontaneous colitis, developing at a much older age (6–8 months) and with significantly lower incidence (Yogev and Waisman, unpublished observation). On the other hand,

CX_3CR1-specific and CD11c-specific deletion of IL-10 had no effect on, respectively, the development of colitis and EAE induction, severity or resolution [99] (Yogev and Waisman, unpublished observation).

Il-27

Co-culturing Tregs and DC results in the secretion of IL-10, IL-27 and TGFβ by DC, which further drives the differentiation of Tr1 cells [106]. DC-derived IL-27 suppresses the production of IL-1β and IL-23 and induces IL-10 secretion, thus blocking immunogenic Th17 differentiation and consequently autoimmunity [107]. At the same time, IL-27 drives the expression of c-Maf, IL-21 and ICOS in naïve T cells, which renders them to become Tr1 cells [108, 109]. DC-derived IL-27, via STAT-1 and STAT-3 activation, drives IL-10 transcription and activation of the IL-10 promoter, thus inducing Tr1 differentiation [109]. Importantly, it has been recently shown that the stimulation of human DC with IL-27 led to upregulation of PD-L1 surface expression, without leading to DC maturation [110]. In addition, it was shown recently that stimulation of DC with IL-27 might induce other regulatory pathways, such as the upregulation of CD39 and suppression of the inflammasome pathway [111].

TGFβ

Another well-known regulatory cytokine with pleiotropic effects on T cells as well as APCs is TGFβ [88, 90]. TGFβ promotes conversion of peripheral naive T cells into $CD4^+CD25^+$ Tregs by inducing Foxp3 expression [112–114]. Following LPS stimulation, a subset of splenic DC secretes high levels of TGFβ and drives Tr1 cell differentiation [115]. Similarly, several in vitro studies demonstrated that DC promote extra-thymic iTreg differentiation in a TGFβ-dependent manner [3, 64, 65, 92, 116, 117]. In vivo delivery of low-dose agonistic peptide to DC induces iTreg cell differentiation from antigen-specific naïve T cells. Inhibition of T cell-specific TGFβ signalling, by expression of a dominant-negative TGFβRII, blocked iTreg differentiation [32]. In line, CD11c-specific deletion of the TGFβRII leads to spontaneous multi-organ autoimmunity due to altered Treg cell function [118], although this phenotype may be partially mediated by TGFβ receptor-deficient T cells (Kel and Clausen, unpublished observation). The integrin α4β8 induces TGFβ activation via metalloproteinase-mediated degradation of latency-associated protein (LAP), resulting in the release of active TGFβ into the extracellular space [119]. DC-specific ablation of the α4β8 integrin results in activation and expansion of T cells, reduced levels of colonic Tregs and consequently the development of colitis [120]. In addition, TGFβ is required to maintain the pool of epidermal immature Langerhans cells, and hence, CD11c-specific TGFβRI-deficient mice develop reduced contact hypersensitivity due to the lack of these APCs [121].

Retinoic acid and β-catenin

DC-derived retinoic acid (RA) is a well-established mediator of oral tolerance induction via the generation of iTregs. Mucosal DC direct T cell homing into the gut, in a molecular mechanism that is attributed to DC-derived RA [122]. Moreover, Mucida et al. demonstrated that DC-derived RA has a reciprocal activity, inhibiting TGFβ-dependent Th17 cell generation while promoting $Foxp3^+$ Treg cell differentiation [123]. Other studies revealed that RA promotes iTreg differentiation by blocking the generation of IFNγ- or IL-21–producing effector memory T cells [124]. Several publications reported that LP $CD103^+$ DC induce peripheral Tregs by expressing aldehyde dehydrogenase (ALDH), an enzyme that metabolizes vitamin A into RA [125, 126]. Likewise, dermis-derived $CD103^-$ DC constitutively produce RA and induce Tregs [127].

A second signalling pathway that can induce RA-producing enzymes involves β-catenin, the central component of the canonical Wingless-Int (Wnt) signalling pathway [128], which is constitutively expressed in DC. Early studies had revealed a crucial role for β-catenin in the regulation of BM-DC maturation and function. In vitro disruption of E-cadherin/β-catenin binding in BM-DC results in their *phenotypic maturation*, i.e. upregulation of MHC-II and co-stimulatory molecules without activation of pro-inflammatory cytokine secretion, leading to a tolerogenic DC phenotype that promotes the induction of IL-10–producing Tregs [129]. In line, mice with a CD11c-specific deletion of β-catenin are more susceptible to DSS-induced colitis and EAE, which is accompanied by increased Th1/Th17 and reduced $Foxp3^+$ Treg responses [130, 131]. Moreover, Wnt/β-catenin signalling contributes to tumour-induced immunosuppression by inhibiting DC cross-priming of CD8 T cells [132] via upregulation of IL-10 [133]. On the other hand, β-catenin signalling in DC supports the maintenance of CD8 T cells after clonal expansion [133] and drives the differentiation and pro-inflammatory function of IRF8-dependent DC subpopulations [134]. Our ongoing work indicates that mice with stabilized β-catenin in DC mount attenuated Th2 responses with reduced airway hyperresponsitivity and harbor more $CD4^+Foxp3^+$ Tregs after induction of allergic asthma (Ober-Blöbaum and Clausen, unpublished observation). Together, these observations led to the concept that β-catenin promotes a tolerogenic DC phenotype in vivo [135]. However, despite a lower frequency of Tregs, CD11c-specific β-

catenin deficiency did not affect the severity or course of autoimmune collagen-induced arthritis [136].

Indoleamine-2,3-dioxygenase

Indoleamine-2,3-dioxygenase (IDO) catabolizes the essential amino acid tryptophan into the stable metabolite kynurenine. The latter results in cell starvation due to physical depletion of tryptophan from the local environment and activation of the general control nonderepressible 2 (GCN2) kinase that phosphorylates the eukaryotic initiation factor 2 (eIF2) (which activates the amino acid stress response) pathway that promotes Treg generation and expansion in an IDO-rich environment [137]. Furthermore, kynurenine can directly generate an immunosuppressive environment by binding and interacting with aryl hydrocarbon receptor (AhR) on $CD4^+$ T cells, thus promoting their polarization into Tregs [138, 139]. In addition, AhR can also upregulate the expression of IDO and RA-producing enzymes directly in the DC [139, 140].

DC production of IDO plays a critical role in tolerance induction by promoting iTreg generation [141]. In turn, iTregs can induce the production of IDO through reverse signalling in pDC [142]. IDO production differs between different DC subsets, with $CD8\alpha^+$ DC producing higher levels of IDO compared to $CD8\alpha^-$ DC [143]. Intestinal $CD103^+$ DC also produce high levels of IDO and play a central role in maintaining gut homeostasis and oral tolerance [144]. Furthermore, ligation of CD80/86 on DC with CTLA4 induces IDO expression [145–147]. IDO-deficient mice developed exacerbated EAE, which could be prevented by treatment with the tryptophan metabolite 3-HAA. 3-HAA instructs DC to produce TGFβ and blocks IL-6 synthesis, thus promoting Treg and at the same time inhibiting Th17 differentiation [148].

Outlook: treatment of autoimmune diseases with DC

DC hold a great promise for the therapy of human diseases. On the one hand, DC may enhance anti-tumour immunity when attempting to fight cancer. On the other hand, they may induce tolerance, which is essential in case of transplantation and autoimmunity. Nonetheless, here lies their main danger: the potential threat that the transferred cells may change once within the patients, and thus cause tolerance instead of immunity, and vice versa.

In recent years, much effort was put into establishing protocols to induce tolerogenic DC and retain them as such also after transfer [149, 150]. To obtain tolerogenic DC, one can either culture them using mixtures of cytokines and stimuli that results in their generation, or genetically manipulate DC so that they express less immunogenic molecules, leading to DC that induce tolerance rather than immunity. As indicated above, deletion of co-stimulatory molecules, e.g. deficiency of B7-H1 (PD-L1), lead to the generation of tolerogenic DC in the mouse system [151]. But obviously, this is not possible to achieve in humans, so other protocols were established by using cytokine cocktails, including for example IL-10 or TNFα [152–154]. These protocols led to the generation of DC that were able to induce profound tolerance in mouse models of different autoimmune diseases, including graft-versus-host disease [155], collagen-induced arthritis [156], autoimmune thyroiditis [157] and EAE [154].

In humans, it is obviously not possible to generate DC from genetically manipulated individuals; however, it is possible to manipulate human DC gene expression using siRNA. Using such methods, it is possible to block the expression of co-stimulatory molecules or enhance the expression of IDO or TGFβ by DC (for review see [150]). One can also generate, similarly to what was done before in the mouse system, tolerogenic DC from the patients using cytokine cocktails. Such protocols were rather successful in initial experiments [158–160]. Once tolerogenic DC are established, they must be put to test in clinical trials. There were not many clinical trials performed to date with DC, and most of the ones that were performed were done with the aim to boost immunity in cancer therapy and not using tolerogenic DC to treat autoimmunity. A comprehensive review of the up-to-date clinical trials was written by Galluzzi et al. [161, 162]. It is evident that although many phase I and a few phase II experiments were initiated, these trials were found to be safe for the patients, but on the other hand, no clear advantage was reported so far. More trials should be performed to establish beneficial effects for the patients. The lack of side effects in these trials is therefore helpful also in the new attempts to treat autoimmune diseases with DC, such as the trial reported by Giannoukakis et al. for type I diabetes [45, 163].

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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