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Review

Role and therapeutic value of dendritic cells in central nervous system autoimmunity

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Dendritic cells (DCs) are professional antigen-presenting cells that control the generation of adaptive immunity. Consequently, DCs have a central role in the induction of protective immunity to pathogens and also in the pathogenic immune response responsible for the development and progression of autoimmune disorders. Thus the study of the molecular pathways that control DC development and function is likely to result in new strategies for the therapeutic manipulation of the immune response. In this review, we discuss the role and therapeutic value of DCs in autoimmune diseases, with a special focus on multiple sclerosis.
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Facts

- Dendritic cells (DCs) control central and peripheral tolerance through their effects on effector and regulatory T cells.
- Specific signaling pathways regulate the ability of different DC populations to promote effector and regulatory T-cell responses.
- Abnormalities in DC numbers, recruitment and function contribute to the pathology of multiple sclerosis (MS) and can be partially overcome by the use of disease-modifying therapies and the targeting of specific molecular pathways.
- Nanotechnology provides new tools for the modulation of DC activity *in vivo* and the therapeutic induction of antigen-specific tolerance in immune-mediated disorders.

Open Questions

- Although DC populations that promote the development of forkhead box P3-positive (FoxP3$^+$) regulatory T cells (Tregs) have been identified, it is not yet clear whether these populations constitute separate tolerogenic DC lineages or represent alternative activation or maturation states of other DC populations.
- What are the different molecular pathways that control the DC's ability to prime effector or tolerogenic T-cell responses?
- There is an unmet clinical need for the development of methods for the efficient and consistent generation of tolerogenic DCs *in vitro* and *in vivo* that can be implemented in large-scale clinical setups.

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that control the activation and polarization of T cells into specific lineages and, consequently, the generation of antigen-specific antibody and T-cell responses.[1] In the context of an infectious challenge, the induction of pathogen-specific immune responses provides protective immunity to fight the infection. However, in the context of autoimmune diseases DCs regulate the balance between pathogenic and regulatory immune mechanisms, controlling disease onset and progression. Thus, DCs have a central role in the control of the adaptive immune response to pathogens and self-tissues and therefore constitute potential targets for the therapeutic modulation of the immune response. In this review, we discus the role of DCs in autoimmune diseases, with a special emphasis on their role in the modulation of central nervous system (CNS) inflammation in MS.

Classes of DCs

Two major classes of DCs have been identified based on their morphological and functional characteristics (Figure 1): conventional or classical DCs (cDCs) and plasmacytoid DCs

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Abbreviations: AHR, aryl hydrocarbon receptor; AP-1, activator protein 1; APC, antigen-presenting cell; Batf3, basic leucine zipper transcription factor, ATF-like 3; CCL3, chemokine (C-C motif) ligand 3; CCR5, chemokine (C-C motif) receptor 5; CD, cluster of differentiation; cDC, conventional or classical dendritic cell; CNS, central nervous system; DC, dendritic cell; EAE, experimental autoimmune encephalomyelitis; FDA, US Food and Drug Administration; Flt3L, FMS-like tyrosine kinase 3 ligand; FoxP3, forkhead box P3; GA, glatiramer acetate; gp130, glycoprotein 130; IDO, indoleamine 2,3-dioxygenase; IFN, interferon; IFNAR, interferon alpha/beta receptor; IL-27Rα, interleukin 27 receptor alpha; IRF8, interferon regulatory factor 8; ITE, 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester; KLF4, Kruppel-like factor 4; MAPK, mitogen-activated protein kinase; MHC class II, major histocompatibility complex class II; MS, multiple sclerosis; mTEC, medullary thymic epithelial cell; NF-κB, nuclear factor κB; NLRP3, NLR (NOD-like receptor) family, pyrin domain containing 3; NP, nanoparticle; pDC, plasmacytoid dendritic cell; RelB, v-rel avian reticuloendotheliosis viral oncogene homolog B; RIPgp/B6 model, rat insulin promoter expressing glycoprotein of LCMV virus, in C57BL/6 mice; RRMS, relapsing remitting MS; Spi-B, Spi-B transcription factor (Spi-1/PU.1 related); SPMS, secondary progressive MS; STAT1, signal transducer and activator of transcription 1; Th, T helper; TLR, Toll-like receptor; Tr1, FoxP3$^-$ IL-10$^+$ type 1 regulatory T cells; Treg, FoxP3$^+$ regulatory T cell; Zbtb46, zinc finger and BTB domain containing 46
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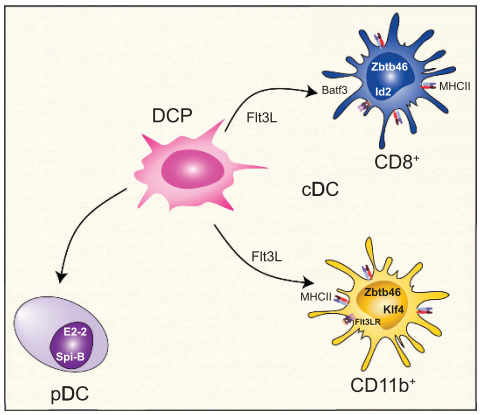

Figure 1 Classes of DCs. Two major classes of DCs have been identified based on their morphological and functional characteristics: conventional or classical DCs (cDCs) and plasmacytoid DCs (pDCs). They are differentiated from a common dendritic cell precursor (DCP) in a process controlled by lineage-specific transcriptional programs

(pDCs).[2–4] The differentiation and function of cDCs and pDCs is associated with specific transcriptional programs that show some overlap, such as their dependency on interferon regulatory factor 8 (IRF8),[5,6] but are mainly controlled by lineage-specific elements.

cDCs present a characteristic dendritic morphology and are highly phagocytic cells that express high levels of major histocompatibility complex class II (MHC class II) and are endowed with potent APC function. cDCs have a short half-life and are constantly replaced from bone marrow precursors generated in a FMS-like tyrosine kinase 3 ligand (Flt3L)-dependent manner.[7] At the molecular level, the generation of cDCs is controlled by transcription factor Zbtb46 (zinc finger and BTB domain containing 46)[8,9] and also by the transcription factors B-cell lymphoma 6 proteinv-rel avian reticuloendotheliosis viral oncogene homolog B and IRF4.[10–13]

Two major subsets of cDCs have been identified: cluster of differentiation 8α^+ (CD8α^+) cDCs and CD11b$^+$ cDCs.[1,2,4,14] CD8α^+ cDCs efficiently present exogenous antigens to CD8$^+$ T cells; these cells also require Id2 and basic leucine zipper transcription factor, ATF-like 3 (Batf3) for their differentiation,[15,16] but a Batf3-independent pathway has also been described for their generation.[17] CD11b$^+$ cDCs preferentially activate CD4$^+$ T cells and require Kruppel-like factor 4 for their differentiation.[18]

pDCs present a spherical shape that resembles plasma cells and produce high amounts of type I interferons following Toll-like receptor 7 (TLR7) or TLR9 activation.[3,19] pDCs are not phagocytic and are considered inefficient inducers of CD4$^+$ T-cell responses. Similarly to cDCs, pDCs are also derived from bone marrow progenitors in an Flt3L-dependent manner.[20,21] The development of pDCs is promoted by the transcription factor basic helix-loop-helix transcription factor (E protein)[22,23] with the contribution of Spi-B transcription factor (Spi-1/PU.1 related).[24]

Function of DCs in CNS Autoimmunity

In the context of MS and its model experimental autoimmune encephalomyelitis (EAE), DCs have important roles related to the generation of the T-cell repertoire and the activation and polarization of myelin-specific T cells in the periphery and the CNS (Figure 2).

Role of DCs in central tolerance. Central tolerance is enforced by the thymic expression of tissue-specific antigens such as myelin proteins in medullary thymic epithelial cells (mTECs) driven by the transcription factor autoimmune regulator.[25–27] The expression of peripheral antigens in mTECs results in the depletion of high affinity self-reactive clones and the differentiation of natural Tregs.[27–29] Thymic DCs cross-present tissue-specific antigens expressed by mTECs.[30,31] Moreover, peripheral DCs migrate to the thymus where they present peripheral antigens.[32,33] Taken together, these observations suggest that DCs participate in the maturation of T cells and the generation of FoxP3$^+$ Tregs in the thymus. However, the depletion of DCs does not affect thymic T-cell maturation and FoxP3$^+$ Treg generation, suggesting that the contribution of DCs to these processes is minimal.[34] Nevertheless, it is possible that DCs participate in the enforcement of tolerance to a specific subset of antigens or under specific conditions.

Role of DCs in the peripheral activation of T cells. Dendritic cells also have a significant role in peripheral tolerance. The delivery of antigen to DCs using antibody or transgene-based strategies induces profound CD4$^+$ and CD8$^+$ T-cell tolerance.[35–37] The induction of immune tolerance as a result of antigen delivery or expression in DCs is associated with the induction of CD4$^+$ FoxP3$^+$ Tregs. Indeed, DCs promote the differentiation of FoxP3$^+$ Tregs via the production of TGFβ1, retinoic acid and kynurenine.[38–41] DCs can also promote the differentiation of FoxP3$^-$ interleukin (IL)-10$^+$ type 1 regulatory T cells (Tr1 cells) through several mechanisms, including the production of IL-27.[42–44] DCs contribute not only to the differentiation but also to the maintenance of FoxP3$^+$ Tregs in the periphery through CD80 and CD86-dependent interactions.[45] Conversely, although the removal of cDCs and pDCs does not result in spontaneous autoimmunity, their removal results in the worsening of EAE and decreased levels of FoxP3$^+$ Tregs.[46] Taken together, these data suggest that DCs can control peripheral tolerance through their effects on the generation and maintenance of Treg populations under homeostatic and inflammatory conditions.

Please note that although it has been postulated that specific DC populations promote the development of FoxP3$^+$ Tregs[38,40] and Tr1[43] cells *in vivo*, it is not yet clear whether these populations constitute specific tolerogenic DC lineages or represent alternative activation or maturation states of DCs.[47–49]

Following activation, DCs can promote the differentiation of effector T cells that drive CNS autoimmunity. Several APC populations can promote T-cell activation and polarization. However, the transgenic expression of MHC class II in DCs is sufficient to recover the susceptibility to EAE of otherwise disease-resistant MHC class II-deficient mice.[50] It should also be noted that EAE can be induced in the absence of DCs,[46] indicating that although DCs are sufficient to induce CNS autoimmunity, other APCs can also promote the

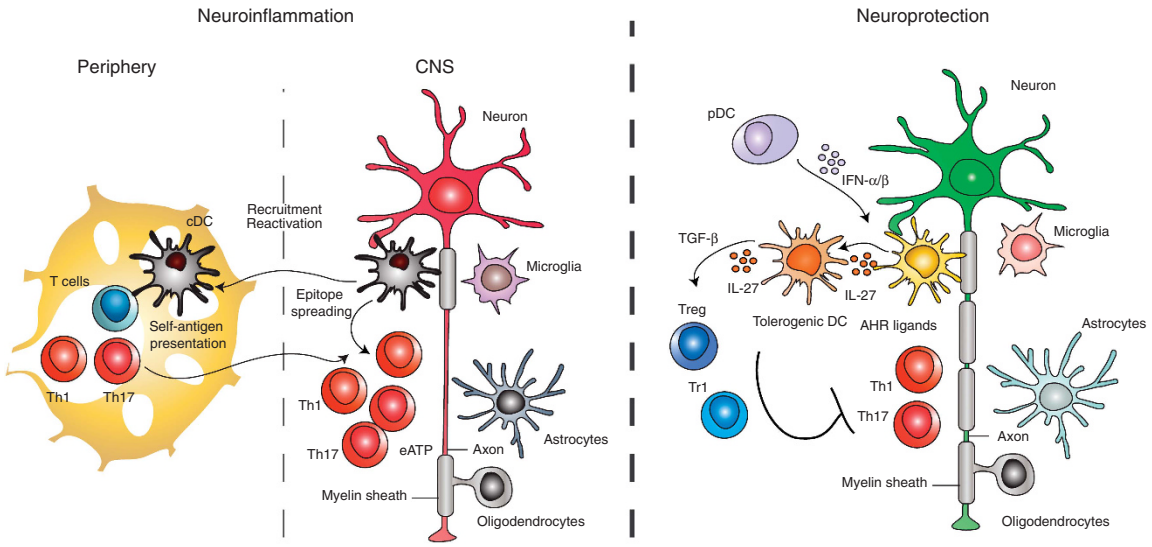

Figure 2 Function of DCs in CNS autoimmunity. In the context of CNS autoimmunity DCs have pro- and anti-inflammatory roles. DCs boost neuroinflammation by promoting the activation of effector T cells, their recruitment and reactivation in the CNS, resulting in the spreading of the pathogenic immune response. DCs also promote the differentiation of FoxP3$^+$ Tregs via the production of TGFβ1, retinoic acid and kynurenine; DCs can also promote the differentiation of Tr1 cells through the production of IL-27

differentiation of pathogenic T cells. Taken together, these data suggest that different signaling pathways in DCs control their ability to promote the differentiation of regulatory and effector T-cell responses. For example, the mitogen-activated protein kinase p38α is needed for the differentiation of T helper type 17 (Th17) cells by DCs.[51] The identification of these pathways has the potential to lead to new therapeutic approaches for MS and other immune-mediated diseases.

Role of DCs in the activation of T cells in the CNS. The role of DCs in CNS autoimmunity is not restricted to the polarization of T cells in peripheral immune organs. DCs secrete chemokines that promote T-cell recruitment and reactivation in the CNS.[52] Epitope spreading, the diversification of epitope specificity from the initial epitope-specific immune response to additional epitopes on the same or different antigens, is thought to have an important role in CNS autoimmunity.[53] Miller and coworkers identified a subpopulation of F4/80$^-$ CD11c$^+$ CD45^{hi} DCs that infiltrate the CNS and promote epitope spreading in mouse models of MS.[54] They also reported that CNS-infiltrating CD11b$^+$ DCs preferentially polarize T cells into the Th17 lineage.[28,29] However, not all CNS-infiltrating DCs promote inflammation. pDCs were found to limit the differentiation of effector Th1 and Th17 cells in the EAE model in an indoleamine 2,3-dioxygenase (IDO)-dependent manner; these effects are not associated with the differentiation of FoxP3$^+$ Tregs.[55] Taken together, these data show that, both in the periphery and the CNS, DCs control the balance between effector and regulatory T cells and consequently the development of autoimmune disorders.

DC Phenotype and Function in MS

MS is a chronic demyelinating autoimmune disease of the CNS.[56] In most patients, MS initially presents a relapsing-remitting clinical course (relapsing-remitting MS (RRMS)) that is followed by a progressive phase (secondary progressive MS (SPMS)) characterized by the continued and irreversible accumulation of disability.[57] High numbers of cDCs and pDCs accumulate in the cerebrospinal fluid and white matter of MS patients,[58,59] and DC abnormalities have been associated with different stages of the disease.

Circulating cDCs in RRMS and SPMS present an activated phenotype and produce increased levels IL-12p70 and IL-23p19.[60–62] Moreover, circulating cDCs in RRMS show increased expression of activation markers and chemokine (C-C motif) receptor 5 (CCR5) than healthy controls.[63,64] Interestingly, the expression of the CCR5 ligands chemokine (C-C motif) ligand 3 (CCL3) and CCL5 is increased in MS CNS lesions,[65] and CCR5 polymorphisms have been linked to changes in disease onset and activity,[66,67] suggesting that this pathway contributes to the recruitment of cDCs to the inflamed CNS of MS patients.

Following TLR9 activation, circulating pDCs from RRMS patients show diminished production of interferon (IFN)-α.[68] Moreover, pDCs from MS patients show a decreased ability to influence Tregs.[68] Thus, it is possible that deficits in pDCs contribute to disease pathology. Of note, however, several findings on pDC in MS have not always been replicated by independent groups, and it has been suggested that specific pDC subpopulations (e.g., pDC1 *versus* pDC2) are affected in MS.[69]

Effect of MS Disease-Modifying Therapies on DCs

The data shown in the previous section highlights the abnormalities in DC numbers, recruitment and function associated with MS. These abnormalities are likely to contribute to MS pathology and its response to therapy. Thus the study of the effects of therapy on DCs might provide information regarding mechanisms of disease pathogenesis in MS and potential therapeutic interventions.

Type I interferon signaling through IFN alpha/beta receptor has profound effects on DCs, diminishing their ability to promote the differentiation of effector T cells[70–72] and limiting CNS inflammation during EAE.[73–75] IFN-β administration, a first-line therapy for MS, affects both pDCs and cDCs. IFN-β treatment decreases the numbers of circulating cDC in RRMS, without affecting pDCs.[69] At the functional level, monocyte-derived DCs show decreased IL12p70 production and produced increased amounts of IL-10 following treatment with IFN-β.[76] In addition, IFN-β treatment induces an anti-inflammatory phenotype in pDCs, characterized by the upregulation of PDL1 and IL-10 expression.[76] Finally, IFN-β also induces the production of IL-27 by DCs, a cytokine with broad anti-inflammatory effects.[77]

Glatiramer acetate (GA) is an immunomodulatory drug currently used to treat MS. Treatment with GA is associated with a decreased production of TNF-α and IL-12p70 by monocyte-derived DCs, reduced CD40 expression and reduced pro-inflammatory activity in pDCs.[78–81] GA has also been shown to induce type II anti-inflammatory monocytes that share some functional and phenotypic characteristics with anti-inflammatory DCs and have an important role in the therapeutic effects of GA in MS.[82] Thus it is possible that similar molecular mechanisms operate in the induction of anti-inflammatory DCs and type II monocytes by GA.

Laquinimod is a new oral immumodoulatory agent that is under development for the treatment of RRMS.[83–86] Among other effects, Laquinimod has been shown to modulate the T-cell response in rodents and humans as a result of its effects on signal transducer and activator of transcription 1 (STAT1), mitogen-activated protein kinase and NF-κB signaling in DCs.[87,88] Thus, by modulating DC function, Laquinimod might alter the balance between effector and regulatory T cells and therefore suppress the pathogenic T-cell response that drives RRMS.

Taken together, these data suggest that the modulation of DC activity is a potential therapeutic approach for MS and other immune-mediated diseases, particularly for the re-establishment of antigen-specific tolerance. However, these drugs were not specifically designed to target DCs and obviously affect many cell types and biological processes *in vivo*, potentially leading to unwanted side effects. Thus the study of the pathways that control DC activity might lead to the development of new and more effective therapies for autoimmunity.

Regulation of DC Activity

Newly generated immature cDCs have strong phagocytic activity but express relatively low levels of MHC and costimulatory molecules, resulting in a limited capacity to activate T cells. The activation of cDCs by microbial or inflammatory signals triggers a cascade of signaling pathways that boost the expression of MHC and costimulatory molecules and, consequently, the ability of cDCs to induce adaptive immune responses. Interestingly, the analysis of the transcriptional response to stimulation suggest that most of the chromatin marks in DCs are established during the development of DCs and are not affected by their activation, while a limited number of transcription factors and signaling pathways mediate the maturation of DCs and potentially their ability to activate T cells.[89,90] The pathways that regulate DC activation have been extensively discussed elsewhere; in the following sections, we will focus on a handful of pathways of interest.

Nuclear factor κB (NF-κB). The transcription NF-κB signaling has a central role in DC activation and the transcriptional programs that regulate their ability to activate and polarize T cells.[89,91–93] Because of its important role in the immune response, NF-κB activity is tightly regulated. One important regulator of NF-κB is the A20 ubiquitin-editing enzyme.[94] A20 catalyzes the removal of K63 linked poly ubiquitin chains (which activate signaling) and the addition of K48 poly ubiquitin chains, which promote proteasomal degradation. A20 controls the ubiquitination of several proteins involved in NF-κB signaling and regulation, actively limiting NF-κB-dependent signaling. Consequently, A20-deficient DCs are hyper-responsive to stimulation, and mice with a specific deletion of A20 in DCs show an accumulation of activated T cells and develop spontaneous autoimmunity.[95,96] Notably, A20 polymorphisms are also associated with human autoimmune disease,[94] highlighting the physiological relevance of NF-κB regulatory pathways for DC function.

NF-κB activation is an important component of DC activation; however, recent data by Ohashi and coworkers suggest that different members of the NF-κB family of transcription factors have different roles in DC maturation and function.[92] Using bone marrow-derived DCs and the RIPgp/B6 model (rat insulin promoter expressing glycoprotein of LCMV virus, in C57BL/6 mice) of diabetes they found that *nfkb1*-deficient DCs promote the differentiation of effector $CD4^+$ and $CD8^+$ T cells that drive autoimmune inflammation in the absence of activation by microbial signals.[92] These data suggest that the immature/quiescent state of DCs is actively maintained by a specific transcriptional program controlled by *nfkb1*. However, it is not yet known whether this interpretation model also applies to DCs *in vivo*.

STAT3. The transcription factor STAT3 mediates the effects of several cytokines that regulate DC differentiation and function. STAT3 participates in the signaling cascade triggered by FLT3L, a molecule important not only for the differentiation of pDCs and cDCs but also for their anti-inflammatory effects.[97–99] In addition, it has been recently shown that a long noncoding RNA targeting STAT3 regulates the activity of mouse and human DCs.[100] The importance of STAT3 for the regulation of DC function is further highlighted by the effects of STAT3 deficiency. Mice harboring STAT3-deficient DCs show enhanced cytokine production following activation, resistance to IL-10–mediated suppression and increased APC function.[101] Moreover, naive mice with a specific deficiency of STAT3 in DCs develop spontaneous peribronchial and gut inflammation, suggesting that STAT3-dependent signaling controls DC function under homeostatic conditions. In addition, these findings suggest that STAT3 signaling in DCs contributes to the anti-inflammatory effects of other STAT3-activating cytokines, such as IL-27.

Aryl hydrocarbon receptor (AHR). The AHR is a ligand-activated transcription factor that regulates several biological processes, including development[102,103] and the immune

response.[104] The AHR has an important role in the control of the adaptive immune response, through mechanisms that involve the epigenetic remodeling and direct transactivation of target genes.[104–113] For example, we have found that the AHR controls the differentiation of Tr1 cells in response to IL-27.[44,114] Recent data suggest that AHR also controls DC activation *in vitro* and *in vivo*, affecting the course of CNS inflammation. Although the specific molecular mechanisms involved in the effects of AHR signaling in DCs are mostly unknown, AHR is known to regulate the activity and degradation of NF-κB and activator protein 1,[115,116] molecules known to control the response of DCs to stimulation.[89,91] Regardless of the molecular mechanisms involved, AHR has profound effects on DC function. AHR activation decreases the expression of MHC class II and costimulatory molecules and also the production of Th1- and Th17-polarizing cytokines by DCs.[41,117–123] Indeed, AHR activation boosts the ability of DCs to promote the differentiation and expansion of FoxP3$^+$ Tregs.[41,118–120] These effects involve at least two types of tolerogenic metabolites: (1) Kynurenins. AHR activation upregulates the expression of IDO in DCs,[119,120] which catalyzes the production of kynurenine. (2) RA. AHR activation in DCs induces the enzymatic machinery that controls the production of RA,[112] a metabolite that promotes the differentiation of FoxP3$^+$ Tregs.[124] These observations suggest that AHR in DCs constitutes a potential target or therapeutic immunomodulation.

Regulation of DC Function by IL-27

IL-27 is composed of Ebi3 and IL-27p28.[125] IL-27 suppresses Th1/Th2 and Th17 responses.[126–128] In addition, IL-27 promotes the differentiation of Tr1 cells through mechanisms that involve the activation of STAT3- and AHR-dependent signaling.[42,44,114,129] The anti-inflammatory effects of IL-27 are highlighted by the development of exaggerated Th17 immunity and severe EAE by IL-27 receptor alpha (IL-27Rα)-deficient mice.[130] IL-27 is produced by innate cells in response to TLR activation, by a mechanism that involves the autocrine effects of IFN-β.[131,132] As exogenous IFN-β triggers IL-27 production by cells of the innate immune system including DCs,[133] it has been proposed that the beneficial effects of IFN-β treatment in RRMS involve the induction of IL-27 synthesis and its effects on Tr1 and Th17 cell differentiation.[77] Recent data suggests that, in addition to its activities on T cells, the immunoregulatory effects of IL-27 involve its effects on DCs.

The receptor for IL-27, constituted by the glycoprotein 130 subunit of the IL-6 receptor plus a unique IL-27Rα chain,[134] is expressed by T cells and also by cells of the innate immune system including DCs.[135,136] However, the role of IL-27 signaling in DCs during CNS autoimmunity is unknown. We recently found increased IL-27Rα expression in cDCs than in pDCs.[137] Moreover, we found that mice carrying IL-27Rα-deficient DCs develop exacerbated CNS inflammation following EAE induction. The activation of IL-27R signaling in DCs results in a decreased ability to promote the differentiation of effector Th1 and Th17 cells, concomitant with an increased differentiation of FoxP3$^+$ Tregs and Tr1 cells *in vivo* and *in vitro*. Similar anti-inflammatory effects of IL-27 have been recently reported on pDCs and human DCs.[138,139]

A genome-wide analysis of the effects of IL-27 on DCs found that it modulates NF-κB and A20 signaling and upregulates the expression of molecules with anti-inflammatory activity such as TGFβ1 and IDO; IL-27 also induces the expression of the ectonucleotidase CD39 (ectonucleoside triphosphate diphosphohydrolase 1) in DCs in a STAT3-dependent manner.[137] Indeed, the anti-inflammatory effects of IL-27 signaling in DCs on EAE were mediated by the upregulation of CD39 and the degradation of extracellular adenosine triphosphate, which activates the NLRP3 (NLR (NOD-like receptor) family, pyrin domain containing 3) inflammasome in DCs[137] (Figure 3).

Therapeutic Effects of Vaccination with IL-27 Conditioned DCs

The anti-inflammatory effects of IL-27 on T cells and DCs support its therapeutic use in MS and other autoimmune diseases. IL-27, however, has been reported to act directly on T cells to boost CD8$^+$ T-cell responses,[140–144] suggesting that IL-27 administration could potentially have undesired detrimental side effects in immune-mediated disorders.

DC vaccination induces immunity to tumors and pathogens[145] and has been recently approved by the US Food and Drug Administration (FDA) for the treatment of advanced prostate cancer.[146] Conversely, vaccination with tolerogenic DCs induces antigen-specific tolerance.[147,148] Thus, based on the tolerogenic effects of IL-27 signaling in DCs, and to avoid the potential pathogenic effects of IL-27 administration, we investigated the therapeutic effects of vaccination with IL-27-conditioned DCs on EAE. We found that IL-27 conditioned DCs loaded with myelin antigens arrest CNS inflammation and EAE development in preventive and therapeutic paradigms in a CD39-dependent manner.[137] Interestingly, DC vaccination with IL-27-conditioned DCs arrests the immune response directed against the antigen use to induce EAE and also the subsequent spreading of the immune response against additional CNS antigens as measured with antigen arrays.[110,149,150] Taken together, these data demonstrate that IL-27 signaling in DCs limits inflammation in the CNS through CD39-dependent mechanisms. It is still unknown, however, whether this immunoregulatory axis is relevant for the regulation of inflammation in other tissues and whether CD39 is involved in the anti-inflammatory effects of other STAT3-activating cytokines that act on DCs, such as IL-10.

Nanoparticles (NPs) for the Induction of Antigen-Specific Tolerance

Our DC vaccination experiments suggested that IL-27 signaling in DCs might provide targets for the therapeutic modulation of the immune response. However, the use of cell-based therapies in the clinical practice is limited by the logistical issues associated with the preparation of DC vaccines from each patient under highly controlled conditions.

In order to address these limitations, we investigated the signaling pathways controlled by IL-27 in DCs. Our transcriptional analysis identified AHR as a potential mediator of the modulatory effects of IL-27 in DCs.[137] Indeed, this finding is

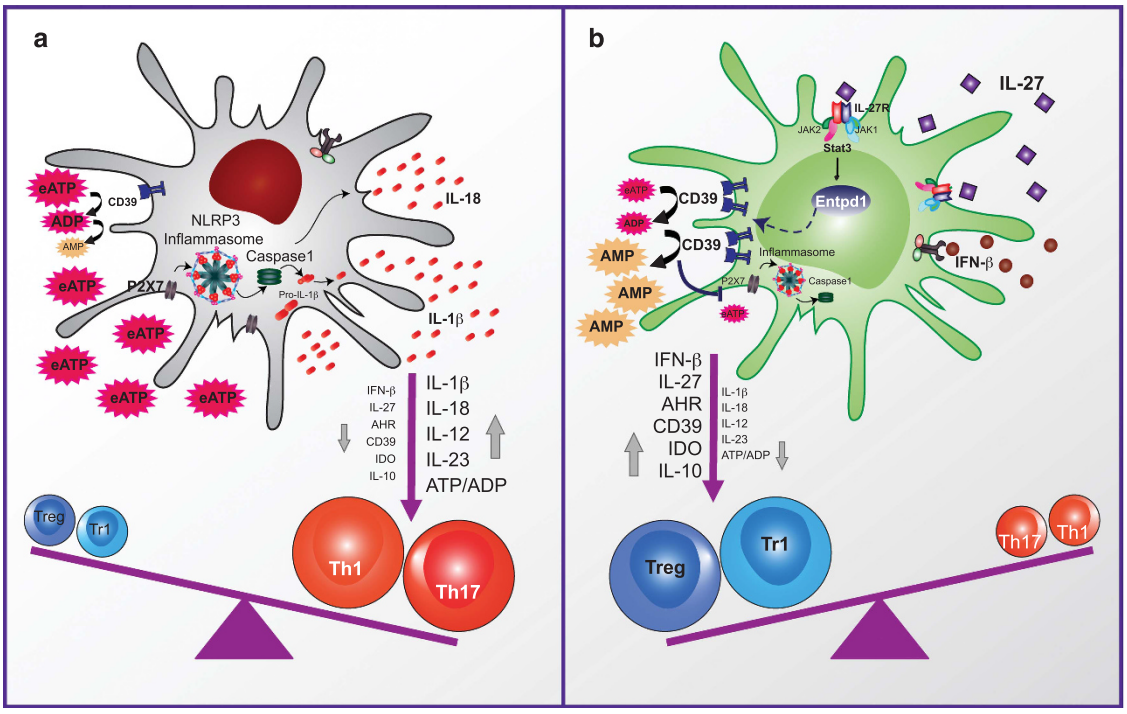

Figure 3 IL-27 acts on DCs to control Treg and Teff differentiation via ENTPD1 (CD39) upregulation. (**a**) Extracellular ATP (eATP) activates the NLRP3 inflammasome in DCs and promotes the differentiation of effector T cells. (**b**) ENTPD1 (CD39) induced by IL-27 in a STAT3-dependent manner degrades eATP, limits effector T-cell differentiation and promotes the generation of regulatory T cells

not surprising, because AHR also mediates the effects of IL-27 in T cells during Tr1 differentiation.[44,114]

The broad expression pattern of AHR, however, constitutes a challenge for the therapeutic exploitation of its immunomodulatory effects. However, recent developments in nanotechnology provide new approaches for the cell-specific delivery of one or several compounds *in vivo*.[151] Based on the tolerogenic effects of AHR activation in DCs, we engineered NPs to co-deliver the endogenous non-toxic AHR ligand 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) together with myelin-specific antigens to APCs *in vivo*[113] (Figure 4).

We found that ITE-loaded NPs activate AHR signaling in DCs, inducing a tolerogenic phenotype characterized by the reduced ability to generate Th1 and Th17 cells, and an increased ability to promote FoxP3$^+$ Treg differentiation *in vitro* and *in vivo*.[113] Accordingly, NPs loaded with ITE and myelin antigens suppress the development of EAE both in preventive and therapeutic paradigms. These effects were mediated by the activation of AHR signaling in DCs, because they were not observed in mice carrying a specific deletion of AHR in DCs. Taken together, these data show that the activation of tolerogenic signaling pathways in DCs with NPs offers a new avenue for the selective regulation of the immune response in immune-mediated disorders.[108]

Conclusion and Future Directions

Considering the central role of DCs in the regulation of the immune response, it is important to identify the molecular pathways that regulate their activity, because these pathways might provide new targets for therapeutic immunomodulation. Furthermore, candidate pathways should be examined in DCs from patients affected by immune-mediated disorders, to determine their relevance for the regulation of human DCs and also their potential to revert DC abnormalities associated with disease pathology.

A related issue is whether tolerogenic DC lineages exist or whether they represent alternative stages of DC maturation or activation. The analysis of transcriptional programs associated with tolerogenic DCs will certainly address this point and provide additional tools for their characterization and manipulation.

Finally, it is important to develop methods for the efficient generation of immunogenic or tolerogenic DCs that can be implemented in a clinical setup for the treatment of human diseases by DC vaccination. The approval by the FDA of DC vaccination as a therapy for prostate cancer is an encouraging step, but much more has to be done to efficiently translate these findings to the clinic. An alternative to this approach is the development of NP-based strategies for the specific modulation of signaling pathways in DCs. However, these approaches have to be optimized to include the production of biocompatible materials in large scale and also to reach specific DC populations in the periphery and target organs. Nevertheless, considering the recent advances in our understanding of DC function and regulation in autoimmunity, a new generation of DC-based immunomodulators can be envisioned in the near future for the therapeutic manipulation of antigen-specific immunity.

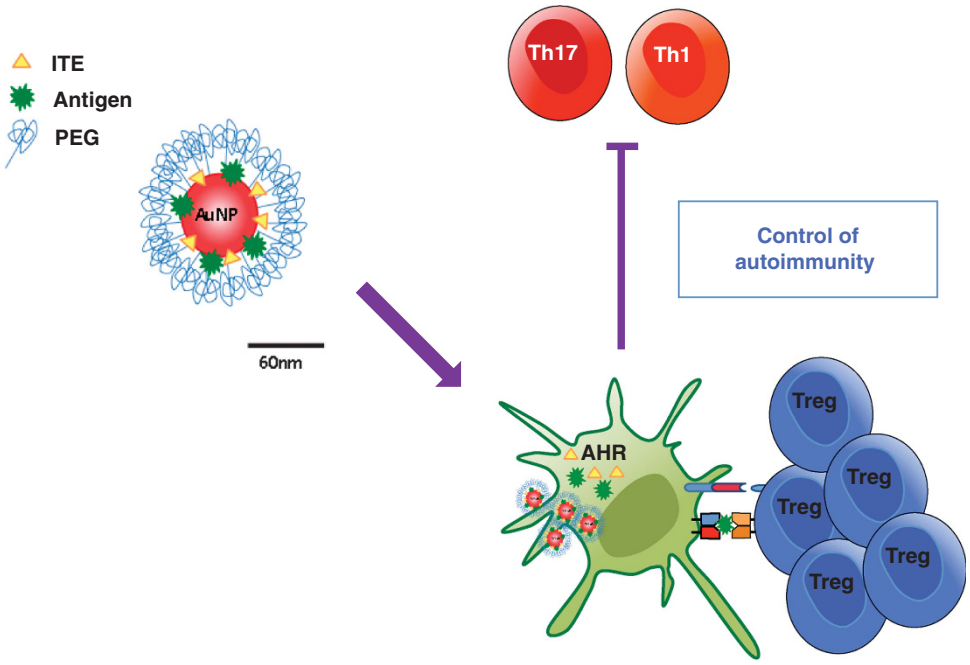

Figure 4 Nanoparticles for drug delivery and induction of antigen-specific tolerance. NPs are engineered to co-delivery the non-toxic AHR ligand ITE and the myelin-specific antigen MOG_{35-55} to DCs. Targeting of AHR in DCs induces a tolerogenic phenotype and the ability to promote the generation of antigen-specific $FoxP3^+$ Tregs *in vitro* and *in vivo* that suppress the encephalitogenic T-cell response (Th1 and Th17)

Conflict of Interest

The authors declare no conflict of interest.

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