



Review

Role of dendritic cells in the initiation, progress and modulation of systemic autoimmune diseases



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ABSTRACT

Dendritic cells (DCs) play a key role in the activation of the immune response against pathogens, as well as in the modulation of peripheral tolerance to self-antigens (Ags). Furthermore, an imbalance in the activating/inhibitory receptors expressed on the surface of DCs has been linked to increased susceptibility to develop autoimmune diseases underscoring their immunogenicity potential. It has been described that modulation of activating or inhibitory molecules expressed by DCs, such as CD86, TLRs, PDL-1 and FcγRs, can define the immunogenic phenotype. On the other hand, T cell tolerance can be achieved by tolerogenic DCs, which have the capacity of blocking undesired autoimmune responses in several experimental models, mainly by inducing T cell anergy, expansion of regulatory T cells and limiting B cell responses. Due to the lack of specific therapies to treat autoimmune disorders and the tolerogenic capacity of DCs shown in experimental autoimmune disease models, autologous tolDCs are a potential therapeutic strategy for fine-tuning the immune system and reestablishing tolerance in human autoimmune diseases. New advances in the role of DCs in systemic lupus erythematosus (SLE) pathogenesis and the identification of pathogenic self-Ags may favor the development of novel tolDC based therapies with a major clinical impact. In this review, we discuss recent data relative to the role of DCs in systemic autoimmune pathogenesis and their use as a therapy to restore tolerance.

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Contents

| | | |
|-----|--|-----|
| 1. | Introduction | 128 |
| 2. | Contribution of endogenous TLR ligands to autoimmune diseases | 128 |
| 3. | Modulation of DC function by ICs | 129 |
| 4. | DC costimulation contribution to immune and autoimmune responses | 130 |
| 5. | DC subsets and their role in autoimmune diseases | 131 |
| 6. | Interactions between DCs and B cells | 132 |
| 7. | DC–Treg interactions | 133 |
| 8. | TolDCs in autoimmune diseases | 133 |
| 9. | Experimental approaches targeting tolDCs in lupus-prone mice | 134 |
| 10. | Clinical approaches targeting tolDCs in autoimmune diseases | 135 |
| | 10.1. Type 1 diabetes | 135 |
| | 10.2. Rheumatoid arthritis | 135 |
| | 10.3. Systemic lupus erythematosus | 135 |
| 11. | Conclusion and future perspective | 135 |

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| | |
|---------------------------------|-----|
| Take-home messages | 135 |
| List of abbreviations | 136 |
| Acknowledgments | 136 |
| References | 136 |

1. Introduction

The major function of the immune system is to detect and eliminate pathogens and detrimental Ags to protect the integrity of the host [1]. Recognition of self-Ags plays a crucial role in shaping the repertoire of T and B cell receptors (TCR and BCR), preventing the occurrence of harmful autoreactive cells and generating tolerance mechanisms that reduce the susceptibility to autoimmune diseases [2]. However, in susceptible hosts immune tolerance may fail, leading to autoimmunity. Although the etiology for autoimmunity remains unknown, environmental factors and genetic determinants are the most important contributors [3].

Dendritic cells (DCs) play a crucial role in both initiation of immune responses and induction of T cell tolerance, mostly by fine tuning the signaling of activating and inhibitory receptors [4]. The relevance of the inhibitory receptors expressed by DCs has been highlighted by the observation that PDL-1 blocking modulates T cell activation during experimental autoimmune encephalomyelitis [5,6]. However, the mechanisms responsible for peripheral T cell anergy mediated by DCs are not well understood.

Autoimmunity may involve any tissue or cell types of the body and the severity and duration of the disease vary widely [7]. Patients suffering from autoimmune diseases may have more than one concurrent disorder. Depending on involved tissues, autoimmune diseases are classified as organ-specific or systemic. The complexity of autoimmune diseases and the side effects associated with unspecific immunosuppression approaches have dampened the development of new and more Ag-specific therapies, particularly in systemic autoimmune diseases. One of the most injurious autoimmune diseases is systemic lupus erythematosus (SLE). SLE is a chronic disease that preferentially affects women and is clinically characterized by an extensive range of heterogeneous symptoms that may affect the blood vessels, kidneys, the peripheral and central nervous systems, skin and mucosa.

It has been widely demonstrated that both innate and adaptive immune cells contribute to SLE pathogenesis [8–10]. Antigen presenting cells (APCs) such as DCs and monocytes from SLE patients have been shown to present phenotypic and functional abnormalities. It has been postulated that self-reactive T cells specific for nuclear self-Ags may arise from an impaired clearance of apoptotic fragments by monocytes [11]. Moreover, we and others have shown that DCs from SLE patients display increased expression of the co-stimulatory molecules CD40 and CD86, as well as a higher ratio of activating to inhibitory Fc gamma receptors (FcγRs) as compared to DCs from healthy controls [9]. These observations suggest that DC maturation may be involved in an inefficient peripheral tolerance in these patients [9].

Although much progress has been made in autoimmunity research, specific and effective therapies for systemic inflammatory disorders have not yet been developed. Nevertheless, some progress has been made in the development of therapy of different autoimmune disorders with the use of biological agents that mainly prevent signaling of pro-inflammatory cytokines [12]. However, current medications for treating SLE have not been significantly effective because they induce systemic immunosuppression that can lead to a wide spectrum of adverse effects [13]. Recently, it has been proved that the use of a biological agent that blocks the B-lymphocyte stimulator (BLyS) (Belimumab) in SLE patients ameliorates clinical symptoms [14].

Due to their capacity to modulate autoreactive responses by inducing T cell anergy and regulatory T helper (Th) polarization profiles, the use of DCs for immunotherapy has become an attractive possibility for autoimmune disease treatment, where reestablishing immune

tolerance is essential [15]. Currently, most work is focused on developing “tolerogenic” DCs (tolDCs) with the capacity of blocking undesired specific autoimmune responses [16,17]. In fact, several approaches based on pharmacological and genetic modifications of DCs that intend to enhance their tolerogenic capacity are currently in progress [17,18]. Herein, we discuss current knowledge relative to the understanding of DCs in systemic autoimmune pathogenesis, focusing on the role of activating and inhibitory receptors expressed on DCs which interact with T cells. In addition, we discuss recent data on tolDCs in autoimmunity and new technical approaches related to carry out tolDC immunotherapy with a special focus on systemic autoimmunity, such as SLE.

2. Contribution of endogenous TLR ligands to autoimmune diseases

DCs sense pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) through membrane and cytosolic receptors known as pattern-recognition receptors (PRRs), such as Toll like receptors (TLRs) and NOD-like receptors [19]. Pro-inflammatory stimuli, such as PAMPs and DAMPs promote functional, morphological and phenotypic changes in immature DCs that lead to maturation and increase their immunogenicity. Mature DCs show strong co-stimulatory and T cell activating capacity, with high MHC-II and CD80/CD86 expression [20–22]. In addition, mature DCs can produce large amounts of pro-inflammatory cytokines such as IL-12, IL-23, IL-6 and others that will define the T helper (Th) profile and subsequently the nature of the effector immune response triggered by a particular antigen [23–26].

During the past decades, evidence has accumulated for associations between PRR polymorphisms and pro-inflammatory cytokine production with the pathogenesis mechanisms underlying immune-mediated diseases in humans [27,28]. For instance, Crohn's disease patients usually show polymorphisms in the NOD2 receptor, which may lead to an imbalance in IL-1β, TNF-α and GM-CSF production that results in mucosal inflammation [28,29].

Several TLRs (TLR1, TLR2, TLR3, TLR4, TLR7, TLR8 and TLR9) also recognize endogenous molecules such as DAMPs as HMGB1, HSP60-70, surfactant protein A, fibronectin, fibrinogen, lactoferrin, serum amyloid A, hyaluronic acid fragments, heparan sulfate, mRNA, ssRNA and immunocomplexes (ICs) containing chromatin [30–32]. Recognition of these endogenous molecules by TLRs can contribute to tissue damage due to inflammation. For example, it is well known that circulating ICs containing self nucleic acids can promote immune-inflammatory activation and tissue injury in SLE. Interestingly, HMGB1 present in circulating DNA-containing ICs was crucial for anti-dsDNA development in SLE by a mechanism likely to be driven by a TLR2/MyD88/microRNA-155 dependent pathway [31]. Also, the effective activation of rheumatoid factor-specific B cells is mediated by IgG2a-chromatin ICs and requires engagement of the BCR and TLR9, highlighting an important role for receptors of the innate and adaptive immune responses in the development of systemic autoimmunity [32]. Furthermore, some extracellular DAMPs, such as self-DNA, need to be transported into PRR containing endosomes in plasmacytoid DCs (pDCs) in order to trigger autoimmunity. The association of the antimicrobial peptide LL-37, a cathelicidin polypeptide, with self-DNA targets this DAMPs to intracellular compartments to be recognized by TLR9 leading to cellular activation and IFN-α production [33–35]. During psoriasis, a common immune mediated disease, LL-37 can be overexpressed and turns quiescent self-DNA into a potent IFN-α inducer by changing DNA structures and targeting this DAMP to early endocytic compartments, where it is recognized by TLR9 in pDCs [35] (Fig. 1).

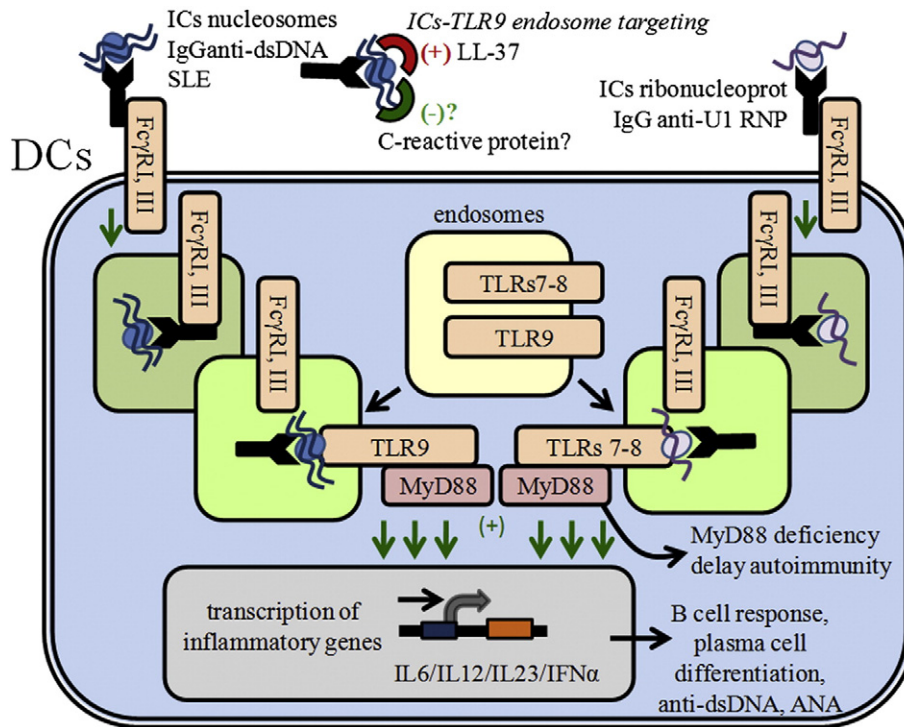


Fig. 1. ICs stimulate pDCs by TLR9 ligation after endocytosis mediated by FcγRs. Extracellular DAMPs such as self-DNA/self-RNA needs to be transported into PRRs (pathogen recognition receptors)/TLR3, 7–9 containing endosomes to fully activate pDCs leading to IFN- α production. The association of the antimicrobial peptide LL-37 with self-DNA (present in SLE patients as IgG anti-dsDNA ICs) prevents DNA degradation and targets this DAMPs to intracellular compartment to be recognized by TLR9 and subsequently leading to cellular activation and IFN- α production. Similar mechanism is thought to occur with self-RNA ICs (present in SLE patients as IgG anti-ribonucleoprot bound to U1 RNP), where DAMP recognition is due to TLR7, 8 binding. The activation of DCs by these mechanisms leads to cellular activation which may modulate B cell response and plasma cell differentiation and would play a role in breaking tolerance to self-nucleic acids and the generation of anti-nuclear antibodies (ANAs) in SLE.

Similar observations were made after LL-37 binding to self-RNA released due to apoptosis, preventing DAMPs degradation and transporting the complex into pDC endosomes to trigger TLR7 signaling. Clinically important, this DAMP has been found in psoriatic skin lesions [36]. As observed for self-DNA-TLR9 ligation, self-RNA recognition by TLR7 induces potent IFN- α production. Furthermore, as conventional DCs (cDCs) express TLR8, a RNA recognition receptor, self-RNA-LL37 also induces TNF- α and IL-6, promoting cDC maturation [36]. Extracellular nucleases rapidly degrade self-DNA released by dying cells, preventing recognition by TLR9. When self-DNA forms multimeric aggregates by binding to LL-37, self-DNA enters endosomes, binds TLR9 and may cause disruption of tolerance to self-DNA and subsequently to an inflammatory response leading to the onset of autoimmune conditions, such as psoriasis, arthritis and SLE [37,38] (Fig. 1).

In SLE-prone mouse strains, transfer of DCs loaded with dying cells showed an increased immunogenicity that was sufficient to initiate systemic autoimmunity in vivo consisting on the development of anti-nuclear antibodies (ANAs), anti-dsDNA antibodies and diffuse proliferative glomerulonephritis with mesangial and capillary hypercellularity and fibrinoid necrosis [39,40]. This type of immune response is similar to that observed in human SLE diseases. However, in resistant strains of mice, the transfer of DCs loaded with dying cells just produced transient autoimmune symptoms [41]. Although DCs have been implicated in the pathogenesis of several autoimmune diseases, a primary defect in DC function has not been reported to be crucial in initiating an autoimmune disorder.

In SLE patients, hydroxychloroquine (HCQ) treatment, a drug that increases cytoplasmic pH preventing acidification and maturation of endosomes, decreases IFN- α and TNF- α production upon TLR7 and TLR9 ligation in pDCs [42]. Of note, HCQ treatment has demonstrated to improve overall survival and decrease flares of disease in lupus patients [43]. It is thought that HCQ inhibits IFN- α activity by preventing

endosome maturation and the binding of TLR7 and TLR9 to internalized ICs containing self-DNA/RNA [44].

3. Modulation of DC function by ICs

ICs contribute significantly to inflammatory responses and are involved in several pathogenic processes during lupus progression [45, 46]. In a FcγR-dependent fashion, IC deposition contributes to organ damage during autoimmune conditions caused by autoantibodies, such as SLE [45].

IgG-ICs bind to FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa and FcγRIIIb in humans, and to FcγRI, FcγRIIb, FcγRIII and FcγRIV in mice, which are expressed on the membrane of various types of immune cells [47–49]. The balance of activating/inhibitory signals on DCs may define the nature of the immune response leading to antigen-specific immunity or tolerance [50], thus ICs binding to activating receptors FcγRI and FcγRIII on DCs lead to cellular activation and maturation, while binding to inhibitory FcγRIIb, render DCs immature [24,25,47,48,51,52]. Activating FcγRs promote cellular responses via immunoreceptor tyrosine-based activating motifs (ITAM). Cellular activation by activating FcγRs can be counteracted by the inhibitory FcγRIIb, which signals through immunoreceptor tyrosine-based inhibitory motifs (ITIM) [47,51] (Fig. 2). The inhibitory FcγRIIb is expressed by APCs, such as B cells, monocytes and DCs, while activating FcγRs are expressed in APCs and several other non-immune cells [51]. Engagement of activating FcγRs on DCs by ICs induces maturation and improves immunogenicity to prime antigen-specific T cells. Studies in a mouse model for melanoma showed that transfer of DCs lacking the inhibitory FcγRIIb led to protective tumor immunity, probably due to IC signaling restricted only to the activating FcγRs [53]. Even more, FcγRIII KO mice are more resistant to EAE induction than WT mice, while contrarily FcγRIIb KO mice showed an accelerated and more severe form of this autoimmune disease [50]

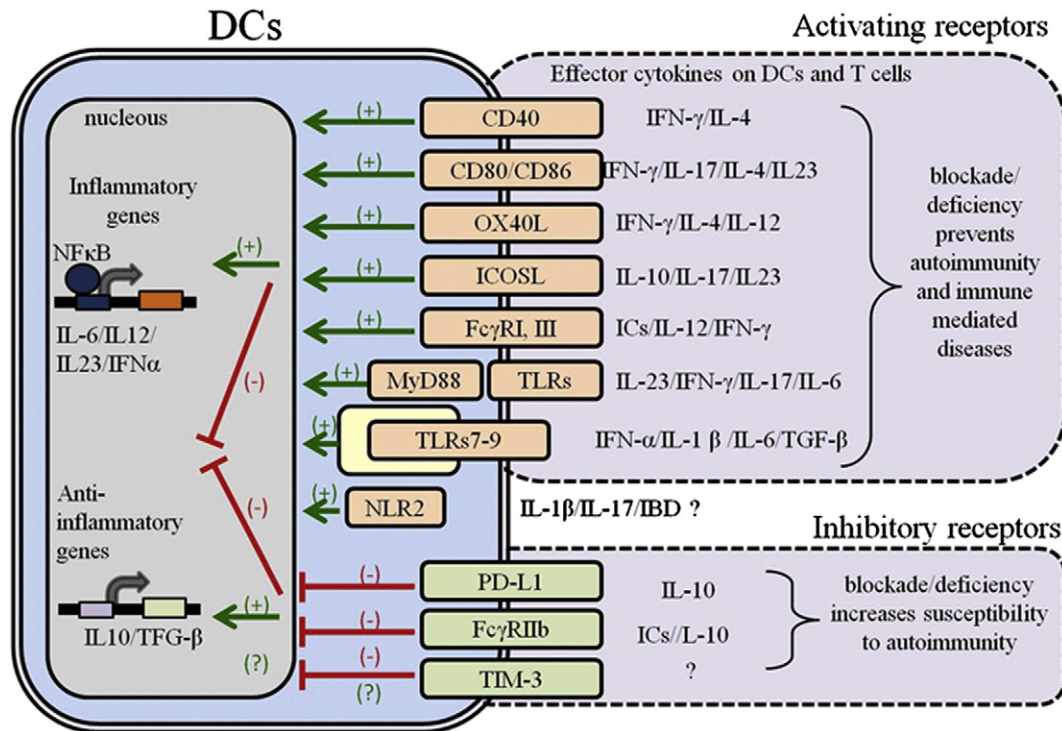


Fig. 2. Co-stimulation is crucial for DCs to modulate both immune and tolerance responses. Modulation of DCs is mediated by different interacting signals that may lead to DC activation and initiation of the immune response or may lead to immunoregulation. Binding of surface molecules expressed on DCs such as CD40, CD80, CD86, OX40L, ICOSL, TLRs, FcγRI and III, leads to cell activation and maturation, inducing NFκB nuclear translocation (between other signaling pathways) and transcription of pro-inflammatory cytokines such as IL-6, IL-12, IL-23 and IFN-α. Their role in the onset of autoimmunity has been extensively studied in different approaches such as the use of blocking antibodies and targeted gene deletion (knock-out mice). The blockade or deficiency of these activation receptors prevents (or decrease) susceptibility to autoimmune and immune mediated diseases. In contrast, ligation of inhibitory receptors such as PD-L1, FcγRIIb and TIM-3 confer a regulatory phenotype inducing production of IL-10. The blockade or deficiency of PD-L1 or FcγRIIb leads to a lupus like syndrome as well as an increased susceptibility to develop autoimmune diseases.

(Fig. 1). Also, FcγRIIb KO mice show increased susceptibility to other autoimmune diseases, such as collagen-induced arthritis (CIA) [54]. Remarkably, FcγRIIb deficiency in mice can lead to the spontaneous development of an SLE-like disease, characterized by overproduction of anti-DNA antibodies, IC deposition in kidneys leading to glomerulonephritis, systemic inflammation and lymphoproliferation [55]. It is likely that an imbalanced activating/inhibitory FcγR signaling in SLE patients may significantly influence DC immunogenicity due to the presence of ICs containing apoptotic cells and the deficiency in apoptotic body clearance [11].

As compared to healthy donors, cDCs from SLE patients show higher expression of activating FcγRs and lower expression of the inhibitory FcγRIIb, resulting in an altered ratio of activating/inhibitory FcγRs [9]. This alteration on the ratio of activating/inhibitory FcγRs in mature DCs correlates with the activity of SLE, as determined by the SLE Disease Activity Index (SLEDAI) score [56]. An altered balance of activating/inhibitory FcγRs in SLE patients may impact DC immunogenicity increasing T cell priming and promoting the escape/expansion of self-reactive T cells.

It has been shown that TLRs expressed by pDCs recognize RNA and DNA when added along with IgGs from lupus patients after FcγRIIIa-mediated internalization. No such response could be observed when IgGs from healthy controls were internalized as ICs by FcγRIIIa [44,57,58]. A possible explanation for this phenomenon is that ICs from lupus patients contain DNA that stimulates pDCs to produce pro-inflammatory cytokines via TLR9/FcγRIIIa [44]. These observations suggested a relevant interaction between FcγRs and TLRs, which target Ags to intracellular endosomes/lysosomes containing TLRs leading to pDC activation and contributing to SLE pathogenesis [44]. Recently, it was shown that C-reactive protein (CRP), a serum molecule that binds to apoptotic cells, was capable of inhibiting the IFN-α response to ICs

containing anti-U1 RNP-snRNP and anti-DNA-DNA [59]. Also, CRP may modify trafficking of auto-Ags to prevent TLR engagement in pDCs [59].

The importance of IC recognition and delivering/trafficking by FcγRs is also evidenced in immune evasion mechanism of some bacteria. Targeting *Salmonella typhimurium* to FcγRs on DCs prevents *Salmonella* immune evasion and drives presentation of bacterial Ags leading to T cell priming [60,61]. Further research must be done to understand the precise role of the activation/inhibitory FcγR balance in DCs and its potential selective engagement to modulate DC function to promote either Ag-specific tolerance or immunity [62].

4. DC costimulation contribution to immune and autoimmune responses

DCs are sentinels of the immune system that are located in almost all tissues of the body. DCs capture self- and non-self-Ags, degrade them and present them as peptide-MHC complexes on their surface to naive or effector T cells (Teff) in lymphoid organs. DCs are the most efficient APCs [63], which constantly sense and respond to Ags and danger signals. In autoimmune susceptible hosts, it is hypothesized that the onset of autoimmune diseases is triggered when APCs capture self-Ags and present them to self-reactive T cells that have avoided central and peripheral tolerance [2]. Besides the expression of MHC-I and MHC-II, DCs can also present lipid Ags to T cells through non-classical MHC-like molecules, such as CD1d [1,64,65]. The expression of co-stimulatory molecules by DCs constitutes a major aspect in T cell activation [63]. DCs express co-stimulatory molecules, including CD80 (or B7-1) and CD86 (or B7-2), which are essential at inducing naive T cell activation [1,23,24,66]. Ligation of CD28 expressed by T cells with CD80/CD86 molecules expressed by DCs confers optimal mRNA

stabilization and secretion of the proliferating cytokine IL-2, promoting clonal expansion and survival of specific T cells [67,68]. To modulate T cell activation, CTLA-4 functions as a co-inhibitor molecule expressed on T cells to compete with CD28 for binding to CD80/CD86 expressed on DCs, disrupting the immunological synapse and targeting cell signaling molecules [69,70]. Also, the interaction between CD40-L and CD40, expressed by T cells and APCs respectively, is crucial for T–B cell cooperation and T–DC interactions and results in the initiation of a full immune response [71]. CD40/CD40-L blockade by the administration of anti-CD40L mAb not only prevents different autoimmune diseases – including experimental autoimmune encephalitis (EAE) and uveoretinitis – but also ameliorates an established autoimmune disease [72,73]. Furthermore, cDCs obtained from lupus patients show high expression of co-stimulatory molecules, such as CD86 and CD40, suggesting a highly immunogenic state for these APCs [9,74].

OX40 ligand (OX40L) is another co-stimulatory molecule widely expressed on APCs and non-immune cells, such as endothelial and smooth muscle cells [75]. OX40L expression on DCs is induced by CD40/CD40L ligation and the presence of pro-inflammatory cytokines. OX40L on DCs binds to OX40 on T cells and promotes T cell survival and a Th2 polarization [76]. The role of OX40L/OX40 interaction in autoimmunity has been studied in FoxP3-deficient mice, in which a deficiency (or blockade) of OX40 delays the onset of autoimmune symptoms by inhibiting the expression of the effector cytokines IFN- γ and IL-4. These data support the notion that this strategy could be considered as a potential immunotherapy in immune-mediated diseases (Fig. 2) [77].

APCs also express ICOS-ligand (ICOS-L; B7-H2) that specifically interacts with the protein ICOS, which is expressed by CD4⁺T cells after TCR-pMHC-II ligation. This molecular interaction modulates T cell proliferation, survival and polarization [78,79]. Noteworthy, blockade of ICOS/ICOS-L interaction in co-culture experiments with naïve (CD45RO⁺) T cells and DCs, selectively inhibits IL-10 release, without affecting IL-2 secretion [80]. This observation suggests an active role for the ICOS/ICOSL interaction in peripheral tolerance [80] while early CD28/B7 interaction remains the dominant pathway leading to the production of many pro-inflammatory cytokines, such as IL-2, IL-6, IL-13 and IFN- γ . ICOS/ICOS-L interaction in later phases of T cell activation could down modulate IL-10 production [80]. Moreover, a subset of regulatory T cells (Tregs) expressing ICOS are actively suppressive cells, indicating that the ICOS/ICOS-L axis can influence effector T cell responses [81]. IL-10 may negatively regulate ICOS-L expression on DCs and the presence or absence of IL-10 regulates ICOS expression in Th17 and Treg subsets, affecting effector functions [82]. Also, ICOS-L deficiency can impair Th2 differentiation by affecting IL-4 and IL-10-producing T cells, without affecting IFN- γ production [83].

Flt3L is an important cytokine in development, differentiation, regulation and apoptosis of DCs [84]. In the Flt3L deficient mice, DC numbers were severely affected which is reversed with the administration of recombinant Flt3L [84,85]. In contrast, it has been reported that Flt3L levels in synovial fluid from rheumatoid arthritis (RA) patients are higher than in non-inflammatory synovial fluid samples [84,86]. Some evidence also suggests that an increase in Flt3L levels in synovial fluid is associated with disease progression and joint erosion underscoring its role in DC modulation during systemic autoimmune pathogenesis [87].

One of the most important interactions between T cells and DCs is mediated by the programmed cell death-1 (PD-1) molecule and its ligand PD-L1 [6,88]. PD-1 is expressed on the surface of T cells and works as an inhibitory receptor that binds to PD-ligand-1 (PD-L1), which is expressed on DCs [89]. PD-1 also binds to PD-L2, another surface protein only expressed by APCs, such as DCs, B cells and monocytes. PD-L2 expression can be induced on macrophages and DCs upon stimulation with either IFN- γ , GM-CSF or IL-4 [89,90]. Expression of PD-L1 on DCs inhibits T cell activation [91,92] and by modulating crucial signaling molecules promotes Treg development [93]. In the mouse model for multiple sclerosis, EAE, it was shown that tolDCs prevent myelin

oligodendrocyte glycoprotein-specific autoimmune response by the induction of PD-1⁺ Treg cells [88]. In addition, PD-1/PD-L1 but not PD-1/PD-L2 interaction can suppress the IFN- γ /Th1 polarization without altering the IL-17/Th17 response in the autoimmune proteolipid protein-induced EAE in mice [5]. A deficient expression of PD-1 axis molecules by T cells or APCs can lead to an enhancement on the IFN- γ production by CD4⁺ T cells and the activation of CD8⁺ T cell responses, which can cause susceptibility to autoimmunity in mouse strains that normally are resistant to disease [94]. Furthermore, PD-1 deficiency leads to the development of a lupus-like syndrome in B6-PD-1 KO mice, which showed glomerulonephritis and lymphoproliferative disorders [95] (Fig. 2). Recently, TIM-3 has been reported to display an inhibitory function on DCs [96]. TIM-3 ligation by mAbs on DCs leads to a phenotype that is resistant to maturation by inhibiting NF- κ B activation and upregulating CD40, CD80 and CD86 co-stimulatory molecules [97]. Furthermore, DCs deficient in TIM-3 produce higher amounts of proinflammatory cytokines upon TLR ligand stimulation [96]. Because no one unique co-stimulatory molecule is fully immunogenic or tolerogenic, further research is needed to elucidate the complex molecular networks that modulate DC immunogenicity (Fig. 2).

Understanding the activation/inhibitory signaling network controlling DC tolerogenicity/immunogenicity is essential to develop new therapies for autoimmune diseases, that would reduce immunogenicity and enhance the tolerogenic capacity of DCs. Strategies based on interfering TLR signaling and the subsequent maturation of pDCs are of clinical interest mainly in autoimmune diseases.

5. DC subsets and their role in autoimmune diseases

DCs are classified into two major subsets, plasmacytoid (pDCs) and conventional DCs (cDCs), which show different phenotypes and functions [1]. Both subsets can display abnormalities during autoimmune diseases, such as SLE and multiple sclerosis (MS) [98,99]. cDCs are efficient APCs that express TLRs 3, 4, 5, 6, and 8, and produce TNF- α , IL-1, IL-6, IL-12, and IL-10 upon stimulation with TLR ligands [1,26]. SLE patients show lower numbers of peripheral blood cDCs as compared to control patients with other autoimmune diseases receiving equivalent pharmacological therapies [100,101]. MS patients showed increased numbers of cDCs expressing CD80 and CD40, with a decreased expression of PD-L1 [99]. In addition, cDCs from MS patients show an increased secretion of the pro-inflammatory cytokines IL-12 and TNF-alpha as compared to healthy controls. Consistent with these observations, DCs from MS patients preferentially induce Th1-skewed T cell responses [99].

Similar to cDCs, pDCs can detect PAMPs by TLRs, such as TLR7 and TLR9, contributing to protective immunity against viral and bacterial infections [102–105].

pDCs differ from cDCs in important functional capacities, including antigen (Ag) uptake, Ag presentation and priming of CD4⁺ T cells [106,107]. In mice, pDCs differentially express the surface markers B220, BST-2 or SiglecH and human pDCs differentially express CD123, BDCA-2 (also known as CD303) and neuropilin-1 (also known as CD304 or BDCA-4) [108]. pDCs circulate in the bloodstream and constitute about 0.2%–0.8% of the peripheral blood mononuclear cells in both humans and mice [109]. pDCs show expression of MHC-II, CD80 and CD86 but the precise role of antigen presentation and T cell priming of pDCs in adaptive immunity has yet to be assessed [25,109]. During the development of autoimmunity, pDCs have been shown to play both immunogenic and tolerogenic functions depending on disease, progression and the experimental condition. It has been shown that human pDCs induce anergy of antigen-specific CD4⁺T cell lines [110]. Poly (I:C) and CpG ligation of TLR7 and TLR9 on pDCs endosomes produces high amounts of type I IFN via IRF7 signaling pathway [109,111,112]. Because purified pDCs challenged with virus induce CD40-activated B cells to differentiate into plasma cells through type I IFN and IL-6 stimulation, pDCs can directly induce class switching in B cells [113–115]. pDC–B

cell interactions could be targeted to improve autoantibody-mediated diseases. Strikingly, it has been reported that 4% to 19% of patients receiving IFN- α therapy for hepatitis C virus infection or malignant tumors developed anti-dsDNA and ANA, lupus-like disease, arthritis, serositis, glomerulonephritis and vasculitis, which supports the notion that pDCs, the major IFN- α producing cell, might be involved in the loss of tolerance and autoimmune development [116–118]. Autoimmune symptoms develop between 2 weeks and 7 years after initiation of IFN- α therapy and after discontinuance of IFN- α administration clinical features of autoimmunity generally remit [117].

DCs control the initiation of different effector functions of adaptive immunity, which are suitable for clearance of a wide range of pathogens. Thus it is likely that different subsets of DCs would promote the appropriate T helper response for the clearance of each type of pathogen [26,119]. Interestingly, DCs from female mice lacking Blimp-1, a master regulator of plasma cell commitment in B cells and effector/memory functions in T cells, show enhanced expansion of follicular helper T cell through the production of IL-6 associated with an enhanced germinal center response and the development of autoreactivity [120]. These alterations in T cell priming and differentiation due to a deficiency of Blimp-1 in DCs led to the production of autoantibodies and a lupus-like phenotype. These data agree with human genome-wide association studies in which a polymorphism of Blimp-1 is associated to SLE susceptibility [121]. Furthermore, IFN- α -treated pDCs showed a restricted pattern of miRNAs regulating Blimp-1 expression, suggesting that this molecule could act as an IFN- α -induced mediator that may be involved in autoimmune pathogenesis [122].

In addition, it has been shown that pDCs activated by CpG can suppress Treg function, while inducing the expansion of Th17 cells, thereby promoting a pro-inflammatory response [123–126]. Furthermore, CpG-mediated activation of pDCs promotes the differentiation of Th17 cells by secreting IL-6, TGF- β and IFN- α [127]. However, a different study showed that pDCs stimulated with the TLR9 ligand CpG can positively impact Treg function [128]. TLR7- or TLR9-matured pDCs induced the proliferation of Tregs [128]. In addition, TGF- β -treated pDCs favor Th17 but not Treg differentiation, contributing to enhancing disease in collagen induced arthritis mouse model for RA. Even more, injection of TGF- β -treated pDCs enhanced disease severity and promoted pathogenic Th17 cell accumulation [129]. Similarly, human pDCs stimulated with the TLR7 ligand Imiquimod or with inactivated RNA virus produce high amounts of pro-inflammatory cytokines, such as IL-1 β and IL-23 leading to a Th17 differentiation in an allogeneic leukocyte reaction producing IL17, IL-22, IL-26, and CCL20 [105]. All these data underscore the important role played by pDCs at modulating immunity and tolerance.

6. Interactions between DCs and B cells

The main effector function of B cells is to produce antibodies after BCR engagement by the specific antigen in either a T cell-dependent or independent manner [130]. The importance of B cells in autoimmune pathogenesis was underscored by the observation that B cell depletion by the biological agent rituximab can be beneficial for RA patients and anti-neutrophil cytoplasmic antibody-associated vasculitis. Paradoxically, this therapy has failed as treatment of SLE, a disease in which B cells play a critical role in pathogenesis [131]. Due to the presence of autoantibodies such as ANA, anti-dsDNA and others, lupus has been traditionally defined as a disease dependent of B cells. The inhibitory receptor Fc γ RIIb expressed on B cells regulates their activation and contributes to peripheral B cell tolerance and, in susceptible hosts, Fc γ RIIb deficiency can lead to lupus-like syndromes [49,55]. Activated B cells produce cytokines, and process and present Ags on MHC-II molecules to T cells, which contribute to their function as professional APCs. Ag presentation during B cell–T cell interactions has been shown to be important for the onset of SLE pathogenesis, specially as this process involved self-Ags contained by circulating nuclear Ag-IgG ICs [132,134].

However, regulated B cell function is required for the maintenance of tolerance, as they can produce large amounts of anti-inflammatory cytokines, such as IL-10 and induce T cell apoptosis via FasL (CD178) ligation [134,135]. LPS-activated B cells express FasL and TGF- β and co-incubation with T cells leads to apoptosis of these latter cells [134]. Adoptive transfer of immunosuppressive B cells delayed Th1 autoimmunity type 1 diabetes in pre-diabetic NOD mice and showed mononuclear cell apoptosis in the spleen and transiently impaired function of APCs [134]. Furthermore, regulatory CD19⁺CD24^{high}CD38^{high} B cells from SLE patients showed an impaired function, as they are refractory to CD40 stimulation, produce lower levels of IL-10 and fail to suppress T cell activation. These altered features of B cells could cause a deficiency on immune tolerance and lead to autoimmune pathogenesis in SLE patients [135]. As a result of CD40 stimulation, regulatory B cells suppress Th1 cell priming, via IL-10 but not TGF- β , and their suppressive capacity is prevented by interfering CD80 and CD86 ligation [135].

In addition, it has been shown that regulatory B cells stimulated with IL-5 and CD40L induce antigen-specific T cell apoptosis in a FasL/Fas-dependent fashion. Interestingly, it was demonstrated that killer B cell activity induced by IL-5 was completely abrogated by IL-4. Interfering the balance of IL-5/IL-4 cytokines may be an interesting strategy for the development of therapies exploiting the suppressive capacity of B cells as well, which could also contribute to prevention of autoantibody production [136].

Besides T–B cell cooperation, B cells could also interact with other APCs such as DCs and macrophages. DCs and macrophages efficiently transfer large Ags such as particulates and ICs and present them to naïve B cells in lymphoid organs [133]. In addition, it has been shown that DCs may modulate Ig class switch. Fayette et al. demonstrated that DCs induced surface IgA expression on CD40-activated naïve B cells and this effect is potentiated in the presence of IL-10 and TGF- β suggesting that human DCs directly modulate T cell dependent B cell activation and differentiation [137].

Interactions between DCs and B cells may also have a crucial role in autoimmune pathogenesis. Wan et al. showed that activated LPS- or anti-CD40-stimulated DCs in lupus-prone mice enhanced B cell proliferation and antibody production [138]. Also, DCs from lupus-prone mice induced chemokine receptor expression on B cells that target them to initiate germinal center responses with subsequent IgG production [138]. Anti-CD40 stimulation induced IL-6, IL-10, and TNF- α in DCs from lupus-prone mice but the specific mechanism underlying B cell modulation by DCs is still unknown [138]. Furthermore, studies have established that pDCs can induce plasma cell differentiation and promote antibody secretion through type 1 IFN and CD70 expression [139]. Also, it has been shown that DCs are stimulated with IFN- α , IFN- γ or CD40-L upregulate BlyS and APRIL and in the presence of IL-10 or TGF- β , BlyS and APRIL induce immunoglobulin class-switch DNA recombination [140].

The intracellular tyrosine kinase Lyn mediates inhibitory receptor function in B cells and myeloid cells and a Lyn deficiency develops a lupus-like syndrome [141]. Using conditional allele of MyD88 in Lyn deficiency mice, authors showed that the production of ANA and germinal center response were fully prevented in MyD88-deficient B cells while in MyD88-deficient DC mice, ANAs were delayed (Fig. 1). Thus, the autoimmunity of Lyn deficient mice was dependent on TLR/MyD88 signaling in B cells as well as DCs, highlighting that DC immunogenicity together with a failure in B cell tolerance may result in a T cell-dependent lupus-like syndrome [141].

Also, Poeck et al. showed that the interaction of CpG-stimulated pDCs and BCR activated B cells induced CD86 expression, IL-6, IL-10 and TNF- α and plasma cell differentiation of human B cells [115]. This effect of pDCs is mediated by IFN- α secretion suggesting a crucial role of pDCs in T cell-independent plasma cell differentiation [115]. Teichmann and coworkers showed that deleting DCs in lupus-prone MRL.Fas^{lpr} mice ameliorates disease, highlighting the crucial role of DCs in the modulation of autoantibody development [142]. As expected,

DC deletion decreased inflammation and prevented the progression of kidney infiltration resulting in less proteinuria and a milder form of renal damage compared to untreated MRL.Fas^{lpr} mice [142]. These data endorse DCs as a potential therapeutic target and supports the development of DC based vaccines for treating Ig-mediated diseases such as SLE [142]. Reports also indicate that a subset of splenic regulatory CD11b^{hi}CD11a^{low} DCs induce B cell differentiation into IL-10 producing regulatory CD19^{hi}FcγRIIb^{hi} B cells by IFN-β and CD40L signaling, which could abrogate T cell responses via IL-10 in vitro and in vivo [143]. Also, regulatory CD19^{hi}FcγRIIb^{hi} B cells have an enhanced phagocytic capacity, which may be involved in IC clearance to prevent immune cell activation by FcγR and TLR ligation [143]. All these data have helped our understanding of the interplay of several pro- and anti-inflammatory mediators in DC modulation of B cell response and highlights the need to be considered in selecting the DC approach to develop a therapy for Ig-mediated diseases, such as lupus.

7. DC-Treg interactions

Efforts in understanding the mechanisms responsible for peripheral DC-induced tolerance have led to studies showing the crucial role of DCs in Treg induction. Also, Tregs could modulate T cell priming by DCs. The role of Tregs in maintaining peripheral tolerance is essential because their depletion leads to autoimmunity [144,145]. It has been reported that DCs activated by PAMPs impair Treg function [146]. LPS and CpG stimulation of DCs bypasses Treg suppression leading to T cell priming [146]. The capacity of DCs to impair Treg suppression is independent of co-stimulatory molecules because DCs deficient in MyD88, which still up-regulate co-stimulatory molecules in response to LPS to normal levels, fail to prevent Treg-mediated suppression [146]. Most importantly, cytokines produced by DCs, such as IL-6 play, a critical role in DC-induced T cell activation by limiting Treg suppression [146,147]. On the other hand, studies in CD11c⁺-OVA transgenic mice that were transferred with OVA-specific CD8⁺ T cells showed that Tregs can control T cell proliferation when DC priming occurs during immunogenic conditions, such as the stimulation with a TLR ligand [148]. In contrast, Tregs have only marginal effects on CD8⁺ T cells that are primed by immature DCs, suggesting that Tregs accomplish their most important function under pro-inflammatory conditions [148]. In SLE, pro-inflammatory cytokines such as IFN-α, IL-6 and IL-18 are elevated, which might affect the capacity of Tregs to suppress DC-mediated priming of T cells, thus promoting extensive lymphoproliferation [149–151]. It has been reported that blocking IL-6 by monoclonal antibodies in different lupus mice models improves clinical symptoms, highlighting the role of this pro-inflammatory cytokine in systemic autoimmunity (reviewed in [152]).

8. TolDCs in autoimmune diseases

Since DCs are key players in initiating and regulating immune responses, DCs are potential targets for the treatment of diseases mediated by the immune system. During the last years, approaches based on DC transfer to promote tolerance by either Treg promotion or induction of anergy, apoptosis and phenotype skewing of autoantigen-specific T-cell responses have been developed [17,153]. Although, the ability of tolDCs to induce T cell immunosuppression by Treg expansion or T cell anergy makes them particularly attractive as therapeutic targets for T cell dependent organ-specific autoimmune diseases, the therapeutic potential in systemic autoimmunity could also be beneficial as T cells also play a role in these illnesses [154]. In order to function as an efficient and specific strategy for autoimmune disease treatment, it is crucial for tolDCs to maintain the capacity of changing the pathogenic antigen-specific T cell response, without general immune suppression.

Antigen presentation and co-stimulatory molecules in immature or tolDCs are crucial for T cell immunosuppression. Currently, tolDCs are characterized by a low expression of MHC-II as well as co-stimulatory

molecules such as CD40, CD80, CD86, and a reduced production of pro-inflammatory IL-12 and increased secretion of anti-inflammatory IL-10 [26,153,155]. Most of current knowledge about tolDCs to treat autoimmunity is derived from mice models of autoimmune diseases such as EAE and type 1 diabetes (T1D), conditions where pathogenesis is highly dependent on T cells [155]. Currently, it is possible to generate tolDCs by several methods, such as in vitro modulation by chemicals, biological agents and gene therapy. Among the pharmacological agents used to manipulate DC function and induce tolDCs, maturation inhibitors have been successfully employed [17,25,156]. DC maturation can be pharmacologically prevented in vitro, resulting in DCs that do not respond to PAMPs/DAMPs and pro-inflammatory stimulation [157–163].

DCs treated with dexamethasone (Dex) or 1α,25-dihydroxyvitamin D3 (VD3) show a stable, semi-mature phenotype with intermediate expression of molecules involved in T cell activation such as MHC-II and CD86 [158,164]. Dex or VD3 treated DCs are resistant to maturation by pro-inflammatory stimulation without affecting IL-10 production (Fig. 3). Also Dex- or VD3-treated DCs produce IL-10, differentiate Tregs from CD4⁺ T cells and suppress the proliferation of T cell priming [164]. Furthermore, Dex modulates the NF-κB pathway, inflammatory cytokines, chemokines, and Ag-presenting molecules [165].

Acetylsalicylic acid (aspirin) inhibits CD40, CD80, CD86, and MHC class II expression on murine DCs and decreases NF-κB nuclear translocation [161]. Aspirin-treated DCs showed an immature phenotype and failed to stimulate T cells in mixed lymphocyte reactions [161]. Inhibition of mTOR by rapamycin (RAPA) promotes tolDCs that induce Treg expansion in vivo and in vitro [163]. Hackstein et al. showed that RAPA binds to FKBP12 inhibiting mTOR, which exerts different cellular functions, including modulation of activation and proliferation [163]. In DCs, rapamycin suppresses IL-4-dependent maturation by post-transcriptional down-regulation of both subunits of the IL-4 receptor [162,163] (Fig. 3). Also, rapamycin prevents IL-18 production by DCs after LPS stimulation [166].

Blockade of the NF-κB pathway has been extensively used to enhance the tolerogenic potential of DCs. Inhibition of NFκB promotes a tolerogenic phenotype in DCs that prevents LPS-maturation and reduce the ability to prime effector T cells [167] (Fig. 3). BAY-117085 and andrographolide induce Treg expansion and ameliorate CIA and EAE respectively [155].

Hemeoxygenase-1 (HO-1) is an antioxidant enzyme induced under oxidative conditions and currently being considered as a target molecule for tolDC induction [168]. Although HO-1 expression can be induced by cobalt protoporphyrin (CoPP) preventing DC maturation, the production of the anti-inflammatory cytokine IL-10 is not altered [169] (Fig. 3). Interestingly, DCs treated with CO, a byproduct generated by HO-1 enzymatic activity, prevent autoimmune development in a diabetic transgenic model by blocking β1-integrin expression in T cells thus impairing homing to specific tissues [168].

One of the most studied cytokines that induce tolDCs is IL-10 that regulates several anti-inflammatory genes [170–173]. IL-10-treated DCs induce antigen-specific T cell anergy, preventing proliferation and proinflammatory cytokine production [174–176]. Also, IL-10 prevents DC maturation and increases the expression of immunoregulatory receptors like ILTs, which are inhibitory receptors, improving the tolerogenic phenotype [177]. Additionally, we showed that the use of a synthetic peptide homologous to the functional domain of IL-10 was also capable of inducing human monocyte differentiation to TGF-β-producing tolDCs [178]. Less studied, TGF-β has been also demonstrated to induce tolDCs. Thomas et al. showed in mice that DCs treated with TGF-β prevent the induction of CD80/CD86, IL-12 production and the capacity to prime T cells [179] (Fig. 3). Most importantly, TGF-β-induced tolDC transfer to grafted β-cell islets highly improved survival of the graft suggesting an acquired tolerogenic phenotype which may ameliorate the autoimmune disease by restoring self-tolerance [179] (Fig. 3).

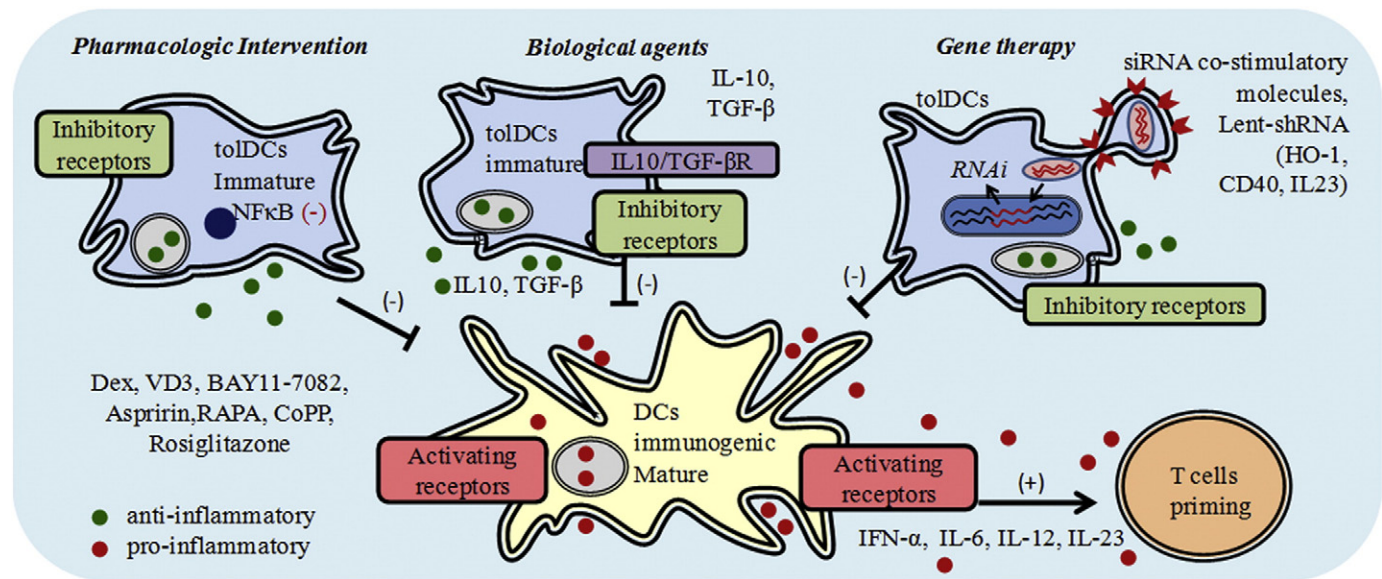


Fig. 3. Most used strategies to induce tolDCs. Pharmacologic intervention to generate tolDCs could be achieved mainly by NF κ B inhibition (Dex—dexamethasone, VD3—vitamin D3, aspirin, RAPA—rapamycin, BAY11-7082, CoPP—cobalt protoporphyrin, rosiglitazone) which interfere in the maturation process. Biological agents such as IL-10 and TGF- β family factors also induce tolDCs which produce high amounts of anti-inflammatory cytokines. Recently, advances in iRNA technology leads to the generation of tolDCs by targeting mRNA of pro-inflammatory molecules such as co-stimulatory CD40, CD80 and CD86, and cytokines such as IL-12 and IL-23. Also, the overexpression of anti-inflammatory enzymes such as hemoxygenase-1 (HO-1) by adenovirus vectors could induce tolDCs. Generally, all these strategies prevent DC maturation process including pro-inflammatory cytokine production and up-regulation of co-stimulatory molecules.

Biological agents that can also modulate immune responses are components derived from pathogens. D'Ambrosio et al. showed that cholera toxin B subunit induces tolDCs, which produce high amounts of IL-10 and reduces the ability of stimulating T cells in a mixed lymphocyte reaction [180] (Fig. 3). Furthermore, excretory/secretory products from the helminth *F. hepatica* can modulate DC function and T cell priming [181] (Fig. 3). DCs treated with total extracts of *F. hepatica* increase the production of the anti-inflammatory cytokines IL-10 and TGF- β and impair the secretion of pro-inflammatory cytokines, such as IL-12p70, TNF- α , IL-6, and IL-23 after CpG stimulation, suggesting a tolerogenic phenotype [181]. Moreover, when CIA mice were administered with these cells, the clinical score of arthritis diminished mainly by TGF- β -induced Tregs, promoting this biological compound as an in vivo tolerogenic agent [181]. Moreover, it has also been demonstrated that *F. hepatica* extracts impaired the production of TNF- α , IL-6 and IFN- γ by innate immune cells after LPS stimulation thus affecting their ability to induce a Th1 differentiation [182]. This mechanism may be associated with a decreased activation of TLR signaling molecules such as MAPK and NF- κ B induced by *F. hepatica* extracts [182].

Recent advances in the interference RNA (iRNA) technology have provided researchers new strategies for autoimmune therapy design [183,184]. It has been demonstrated that gene silencing of different pro-inflammatory molecules, such as CD40, CD80, CD86 and IL-12 promotes a tolerogenic phenotype to DCs with the capacity to ameliorate the clinical score of arthritis in the CIA mice model after tolDC transfer mainly by the suppression of T and B cell immune responses and expanding Treg subset [185–187]. In the EAE model, Kalantari et al. demonstrated that the transfer of tolDCs induced by lentiviral transduction of CD40 and IL-23 specific shRNA decreases disease symptoms [188]. All these data highlight the potential use of lentivirus transduction expressing iRNA for key pro-inflammatory factors in autoimmune disease therapy.

The insertion of anti-inflammatory genes by genetic manipulation technology may also induce a tolerogenic phenotype [18,189]. Bone marrow-derived macrophages transduced with an adenovirus overexpressing hemoxygenase-1 showed a reduction of nitric oxide production and a decrease of pro-inflammatory cytokines after TLR ligation [189].

Hematopoietic stem cell transplantation (HSCT) has been considered as a potential therapy for autoimmune diseases [190]. Interestingly, it was reported from preclinical studies in MS, T1D, RA and SLE patients that HSCT ameliorates clinical scores and improves immunological parameters [190–194]. In addition, the transduction with genes encoding for immunodominant autoantigen to HSC by retroviral vectors induces specific tolerance in experimental autoimmune diseases, such as EAE and T1D [195,196]. These data suggest that the HSCT approach would be an alternative therapy for the treatment of autoimmune diseases.

9. Experimental approaches targeting tolDCs in lupus-prone mice

Although self-Ags have been reported in SLE, the lack of a well-defined pathogenic T cell-dependent self-Ags is still a major obstacle in developing antigen-specific tolDCs for lupus treatment [197]. Our group has reported that pharmacologic NF κ B inhibition by andrographolide and rosiglitazone to Fc γ RIIb KO lupus prone mice prevented clinical symptoms, such as reduced IC deposition in glomeruli due to a decrease in ANA levels [198]. Furthermore, NF κ B inhibition maintained splenic DCs with low CD40/CD86 expression, as well as an increase in the I κ B- α expression suggesting an induction of tolDCs [198].

Interestingly, Kang et al. showed that the administration of low dose of nucleosomal histone peptide induced specific tolDCs with low IL-6 and high TGF- β production [199]. The transfer of peptide-loaded tolDCs to lupus-prone SNF1 hybrid mice ameliorated lupus symptoms and decreased ANA levels [199]. The mechanism of restoring immune tolerance with the histone-loaded tolDC transfer may be due to an increased TGF- β and a decreased IL-17 production by lupus T cells, as well as an expansion of functional specific Tregs [199]. Although it is known that several self-Ags are involved in lupus autoimmunity, the same group showed that a single peptide ameliorated the autoimmune response more effectively than a mixture of self-Ags, suggesting an expansion of the tolerogenic response [200]. These results highly promote the use of tolDCs loaded with self-Ags to restore immune tolerance in systemic autoimmunity [199].

Similarly, Sela et al. showed that administration of the tolerogenic peptide based on the complementarity determining region-1 (hCDR1) of an anti-DNA antibody decreased the expression of co-stimulatory signals CD80/CD86 and the production of IL-12 and IFN- α [201,212]. Moreover, DCs from hCDR1 peptide-treated mice failed to induce T cell proliferation and IFN- γ secretion while inducing TGF- β production by T cells, which is consistent with an immature/tolerogenic phenotype [201]. Although few works have been done in evaluating the use of autoantigen loaded tolDCs to ameliorate systemic autoimmunity, this strategy is a promising cell therapy for the treatment of immune mediated diseases.

10. Clinical approaches targeting tolDCs in autoimmune diseases

Most of current knowledge in DC based vaccines to treat autoimmune diseases, tumors, or infectious diseases is derived from experimental studies in mice [17]. Advances in immune intervention with human tolDCs as well as the identification of the immunodominant T cell self-antigen in systemic autoimmunity are crucial for developing a novel therapy based on autologous tolDC transfer. Achieving this goal would skip systemic immunosuppression without compromising the ability to initiate the immune response against pathogens and preventing the increased susceptibility to opportunistic infections, which are common complications linked to current pharmacological therapies [203]. The use of biologic agents such as TNF- α blockers, IL-1 antagonist, IL-6 antagonist, anti-CD28, and anti-B cell may confer an increased risk of developing adverse events such as infections and malignancies [204–206]. In contrast, the tolDC based therapy will not affect the immune response to infections and malignancies mediated by the escape of infectious or tumor Ags.

Due to systemic autoimmunity such as in lupus, self-Ags are present in several tissues and cell types, tolDCs might suppress systemic T and B cell responses making it more difficult to achieve tolerance induction. The efficacy of systemic tolDC administration may be assessed by evaluating changes in the balance of blood Th17/Treg subsets based on the report of Treg induction by tolDCs [146].

10.1. Type 1 diabetes

The low amount of self-Ags involved and the damage restricted to a single target tissue leads to the first approach of tolDC therapy being assessed in T1D, an organ-specific autoimmune disease. Giannoukakis et al. conducted a Phase I randomized placebo-controlled trial (ClinicalTrials.gov identifier NCT00445913) based on the administration of tolDCs induced ex vivo in patients with T1D [207,208]. TolDCs were generated in GM-CSF/IL-4 conditions with anti-sense oligonucleotides for the co-stimulatory molecules CD40, CD80, and CD86. The administration consisted in an intradermal injection of 1×10^7 autologous tolDCs once every two weeks for two months, while the placebo group received unmanipulated DCs. These researchers reported that the tolDC transfer was well-tolerated, with no adverse effects after one year [207]. Immune cell counts in blood as well as the immune response in allogeneic mixed lymphocyte reaction were unaffected, suggesting the absence of systemic immunosuppression [207]. Although there are several reports demonstrating that tolDCs generated from relapsing-remitting MS patient induce hyporesponsiveness in myelin-specific autologous T cells, the development of clinical trials with tolDC therapy in MS is still remaining [209–211].

10.2. Rheumatoid arthritis

Interestingly, two clinical trials based on tolDCs in RA are being conducted. Thomas et al. initiated a Phase I clinical trial in RA patients evaluating the feasibility and safety of autologous tolDC therapy describing its clinical and immune effects. TolDCs were generated by the tolerogenic agent BAY11-7082, an NF κ B inhibitor, and loaded with

citrullinated peptides (cit-vimentin, cit-fibrinogen, cit-fibrinogen, cit-collagen type II). RA patients received only one intradermic dose of 1×10^7 tolDCs. Only mild adverse effects such as headache and minimal changes in hematology parameters were reported while the expected therapeutic effect could already be observed in some patients [212]. In addition, another randomized, placebo-controlled Phase I clinical trial based on tolDC therapy in RA patients is currently recruiting participants [Autologous Tolerogenic Dendritic Cells for Rheumatoid Arthritis (AutoDECRA); ClinicalTrials.gov Identifier: NCT01352858]. As a Phase I trial, this study is evaluating the safety and feasibility of autologous tolDC therapy. A single dose of 1×10^6 tolDCs from blood monocytes generated with Dex and VD3 are administered arthroscopically into an inflamed knee following saline irrigation. Patients will be monitored daily and subjected to arthroscopy studies to evaluate safety and effectiveness. Results are not available yet.

10.3. Systemic lupus erythematosus

Following clinical trial approaches performed in AR and T1D, tolDC therapy will also be technologically feasible in SLE patients. Between self-Ags described in SLE pathogenesis, the use of tolDCs loaded with a restricted mix of nucleoproteins such as nucleosomes, Ro, La and Sm would be a good choice to start developing a specific tolDC therapy for lupus. To achieve this goal, tolDCs must restore immune tolerance of long-term established autoreactive T and B cell responses [211,213,214]. Although, generation of tolDCs from lupus patients has already been reported, its use in clinical trials must be extremely controlled due to reports showing an increased immunogenicity of lupus DCs [9,74,156,215,216]. Crispin et al. have shown that monocyte-derived DCs from SLE patients exhibit a more immunogenic phenotype, which can be reversed with the administration of IL-10, leading to the induction of lupus tolDCs [74]. These data support the feasibility of using tolDCs in therapeutic clinical trials for SLE patients. However, there are still some issues to be resolved such as the complexity of tracking injected tolDCs to evaluate the in vivo lifespan and homing pattern, which may impact in the frequency and length of tolDC administration. Most importantly, the selection of the time point for tolDC administration during the active/inactive phases may impact on the therapeutic efficacy [211]. As autoimmunity is a chronic disease, tolerance-inducing therapies based on autologous tolDCs must be long-lasting reducing the administration frequency and then decreasing its expensive cost.

11. Conclusion and future perspective

Although much progress has been made in understanding the mechanism of autoimmune diseases, the improvement of current therapies is still the major goal of rheumatologists. The immunomodulatory effects of tolDCs such as the induction of T cell anergy, inhibition of B cell response and Treg induction underlie the role of DCs in maintaining peripheral tolerance promoting its potential use in clinical interventions. Furthermore, the therapeutic efficacy of tolDC administration in experimental autoimmune models such as EAE, T1D and RA highlights the value of testing this approach in human autoimmune diseases. One of the most important features of tolDC intervention as a therapeutic approach includes specificity without the adverse effects associated with the systemic immunosuppression observed in current treatments. Advances in the identification of the immunodominant T cell dependent self-antigen as well as the understanding of the precise role of DCs in SLE pathogenesis are crucial for developing a novel therapy based on autologous tolDC transfer, which may have a major clinical impact.

Take-home messages

- Activating and inhibitory receptors expressed on DCs are crucial for maintaining the immunological tolerance.
- Breaking down the interaction between DCs and effector T cells,

regulatory T cells and B cells may increase the susceptibility to systemic autoimmunity.

- tolDCs could be induced by different strategies including modulation by chemicals, biological agents and gene therapy.
- tolDCs are a promising tool for the generation of antigen-specific tolerance in systemic autoimmune diseases avoiding systemic immunosuppression.

List of abbreviations

| | |
|-------|--|
| APCs | antigen presenting cells |
| ANAs | anti-nuclear antibodies |
| cDCs | conventional dendritic cells |
| CIA | collagen induced arthritis |
| CoPP | cobalt protoporphyrin |
| DAMPs | danger-associated molecular patterns |
| DCs | dendritic cells |
| Dex | dexamethasone |
| EAE | experimental autoimmune encephalitis |
| HSC | Hematopoietic stem cell |
| iRNA | interference RNA |
| MS | multiple sclerosis |
| PAMPs | pathogen-associated molecular patterns |
| pDCs | plasmacytoid dendritic cells |
| PRR | pattern-recognition receptor |
| RA | rheumatoid arthritis |
| SLE | systemic lupus erythematosus |
| T1D | type 1 diabetes |
| Teff | effector T cells |
| Th | T helper |
| TLRs | Toll like receptors |
| Tregs | regulatory T cells |
| VD3 | 1 α ,25-dihydroxyvitamin D3 |

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