Skewing dendritic cell differentiation towards a tolerogenic state for recovery of tolerance in rheumatoid arthritis

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A R T I C L E  I N F O

A B S T R A C T

To date, the available options to treat autoimmune diseases such as rheumatoid arthritis (RA) include traditional corticoids and biological drugs, which are not exempt of adverse effects. The development of cellular therapies based on dendritic cells with tolerogenic functions (TolDCs) has opened a new possibility to eradicate symptoms and control the immune response in the field of autoimmunity. TolDCs are an attractive tool for antigen-specific immunotherapy to restore self-tolerance in RA and other autoimmune disorders. A promising strategy is to inject autologous self-antigen-loaded TolDCs, which are able to delete or reprogram autoreactive T cells. Different protocols for the generation of stable human TolDCs have been established and the therapeutic effect of TolDCs has been investigated in multiple rodent models of arthritis. Pilot studies in humans confirmed that TolDC application is safe, encouraging clinical trials using self-antigen-loaded TolDCs in RA patients. Although an abundance of molecular regulators of DC functions has been discovered in the last decade, no master regulator of tolerogenicity has been identified yet. Further research is required to define biomarkers or key regulators of tolerogenicity that might facilitate the induction and monitoring of TolDCs.

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1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease, resulting from an autoimmune response to synovial antigens, and leading to cartilage and bone destruction that causes pain and disability [1]. The treatment for RA is based on a wide variety of therapeutic tools that include non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids (GCs), disease-modifying anti-rheumatic drugs (DMARDs) and biologic agents [2]. While NSAIDs constitute only a symptomatic relief and therefore they are not recommended as monotherapy, GC and DMARDs are immunosuppressive drugs with a wide spectrum of action, which are able to arrest the disease progression, but causing severe long-term adverse effects [3]. To overcome this issue, biologic agents intended to block specific pathways or targets involved in RA pathology have been introduced in the last decade. At present, biologic drugs approved for use in RA include cytokines- and cytokine receptor-blocking antibodies or cytokine soluble receptors, chimeric molecules that interfere with T-cell activation [4], B cell-depleting antibodies, and biologic inhibitors of cell signaling [5]. Although these therapies have a lower toxicity profile than DMARDs, they can occasionally cause severe complications, such as infections, autoimmunity or cancer [2]. Moreover, a considerable amount of patients still remain refractory to single or combined therapy with DMARDs and biologic agents, compelling the pharmaceutical industry to develop new members of both families of drugs, which are currently under evaluation in multiple clinical trials [6]. These drugs do not restore self-tolerance and therefore accomplish only a temporary disease remission requiring life-long treatment. Emerging therapeutic approaches focus on strategies to interfere with the generation and amplification of autoimmune responses, to achieve permanent restoration of self-tolerance without affecting protective immune functions [7,8].

Dendritic cells (DCs) are an attractive target of immunotherapy since they efficiently present antigens to T cells and govern the induction of immunity and tolerance dependent on their expression level of stimulatory and inhibitory ligands, receptors and soluble mediators [9]. A promising strategy is to modulate DCs in such a way, that they are able to silence or reprogram autoreactive T cells to a regulatory phenotype in vivo.

This article discusses the role of DCs in immune homeostasis and RA pathogenesis, the strategies for their modulation to a tolerogenic state (TolDCs), as well as the effects that TolDCs exert in pre-clinical models of autoimmune diseases and clinical trials in patients. Additionally, putative molecular regulators of DC tolerogenicity are reviewed.

2. Dendritic cells command T cell immunity and tolerance

2.1. Dendritic cell biology

Under steady state conditions, different subtypes of immature DCs residing in peripheral and lymphoid tissues or circulating in the blood, act as sentinels for incoming antigens. DCs become activated after recognition of pathogen-associated molecular patterns (PAMPs) or damage-associated molecular pattern molecules (DAMPs), either directly through pattern recognition receptors, such as toll-like receptors (TLRs), cell surface C-type lectin receptors (CLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), and the retinoic acid-inducible gene (RIG) I-like receptors (RLRs) [10], or indirectly by capturing apoptotic or necrotic cells through a DAMP-mediated TLR activation mechanism [11,12]. Alternatively, DCs can be activated through inflammatory cytokines secreted by cells of the innate immune system, epithelial cells, or fibroblasts, among others [13]. Activation by such “danger signals” induces a complex and coordinated process of maturation and migration in DCs. This differentiation process comprises: morphologic changes, endorsing high cellular motility [14]; loss of phagocytic receptors while endocytic receptors are retained [15]; secretion of specific chemokines, depending on the immune cells that need to be recruited [16]; upregulation of costimulatory (CD80, and CD86) and functional activator (CD40) molecules [17], and chemokine receptors CXCR4 and CCR7, among others [18,19]; synthesis of MHC molecules and translocation of peptide-MHC class II complexes to the cell surface [20]; and finally, the secretion of a specific cytokine profile that promote differentiation and polarization of effector immune cells [21].

DCs are a heterogeneous group of cells, comprising BDCA2 + CD123 + plasmacytoid DCs, CD1c + and CD141 + myeloid DCs, as well as CD14 + CD1c + inflammatory DCs [22]. Plasmacytoid DCs produce large amounts of type I interferons upon activation [23], and induce B cell differentiation into antibody-producing cells [24]. Initially, myeloid DCs were characterized by CD11c expression and subdivided into CD1c +, CD141 + and CD16 + subsets, however, assignment of the latter subset to DCs or monocytes is controversial [25,26]. Upon activation, myeloid CD1c + DCs secrete T lymphocyte-recruiting chemokines [27], and are potent stimulators of allogeneic T cells [25]. Myeloid CD141 + DCs ingest necrotic cells via CLEC9A, and are able to efficiently crosspresent antigen to CD8 + T cells [28]. Inflammatory DCs have been found in murine models of inflammatory diseases [29] and affected tissues from patients with atopic dermatitis, psoriasis, and RA [30,31]. In contrast to myeloid and plasmacytoid DCs which originate from a common DC precursor, inflammatory DCs differentiate from CD14 + monocytes recruited from the blood to sites of inflammation [30]. The ability of monocytes to differentiate into DCs was first described by Sallusto and Lanzavecchia, who reported the generation of DCs from human peripheral blood monocytes after in vitro culture with granulocyte macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4 for 7 days [32]. The close relationship between in vitro generated monocyte-derived DCs and inflammatory DCs found in vivo was confirmed by transcriptome analyses [30]. During the past two decades, the generation of monocyte-derived DCs has enabled numerous functional studies on human DCs that were previously hampered because of the small number of DCs present in human peripheral blood, and has henceforth become a promising tool for cell-based immunotherapies [33].
2.3. Dendritic cells as key players in central tolerance and peripheral tolerance

A pivotal role of DCs in the control of central (thymic) tolerance and peripheral tolerance was underscored by the fact that the absence of DCs leads to the development of spontaneous autoimmunity in mice [34]. In the thymus, DCs are involved in negative selection and clonal deletion of autoreactive thymocytes with high affinity T cell antigen receptors (TCR) [35,36]. Epithelial cells in the Hassall’s corpuscles of the thymus express thyminic stromal lymphopoietin which instructs thymic DCs to convert self-reactive thymocytes into natural occurring CD4 + CD25 + FOXP3 + regulatory T cells (Treg) [37], which constitutively express the cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), an essential protein for the prevention of aberrant activation and expansion of conventional T cells [4]. However, autoreactive T cells with low-affinity TCRs are still released to the periphery, thus relying on well-organized mechanisms to prevent autoimmunity. In this sense, both Treg and CD8 + suppressor T cells play crucial roles in hindering the appearance of autoimmune diseases [38].

Several studies provide evidence on the tolerance-inducing properties of plasmacytoid DCs. Plasmacytoid DCs stimulated through CD40 have been shown to induce IL-10-secreting type 1 CD4 + regulatory T cells (Tr1) [39] as well as CD8 + Treg [40]. Moreover, the depletion of plasmacytoid DCs exacerbated airway hypersensitivity in a mouse asthma model [41].

Under steady state conditions, tissue-resident immature and migratory semi-mature DCs of the myeloid lineage continuously present self-antigens to autoreactive T cells and, due to deficient costimulation, induce anergy or deletion of potentially harmful T cells [42]. Steady-state migratory DCs acquire antigens in non-inflammatory tissues and carry these antigens to the draining lymph nodes where they convert naïve T cells into Treg or autoantigen-specific Tr1 [43]. In the guts and lungs, CD103 + DCs are responsible for retinoic acid and transforming growth factor beta (TGFβ)-dependent induction of Treg [44,45]. Dermsis-derived DCs that trigger the generation of Treg are CD103 +, including the langerin-CD11b + retinoic acid-producing DC subset in mice [46], and the CD141 + CD14 + IL-10-producing DC population in human skin [47]. Dermal RelB + langerin + DCs use TGFβ/latency associated protein (LAP) complexes on their surface to convert CD4 + T cells into Treg [48]. Epidermal Langerhans cells of the skin are also potent inducers of T cell hyporesponsiveness and Treg conversion [49]. In human liver, an IL-10-secreting CD1c + DC subset has been shown to induce the differentiation of Treg and IL-4-producing type 2 T helper (Th2) cells in hyporesponsive CD4 + T cells, contributing to hepatic tolerance [50]. Recently, a population of IL-10-producing CD1c + CD141 + CD14 + DCs has been detected in human peripheral blood, and functional assays using their in vitro analogues point to their immunoregulatory capabilities [51]. Nevertheless, it is likely that tolerogenic features of naturally occurring steady-state DC subsets can be easily overcome by inflammatory signals [52].

3. Dual role of dendritic cells in rheumatoid arthritis

3.1. Dendritic cells in the pathogenesis of rheumatoid arthritis

Synovial fluid and tissue of RA patients are infiltrated by immature and mature DCs that accumulate in perivascular regions closely associated with T cells and B cell follicles [53,54]. It has been suggested that DCs might either migrate to the joint in response to cytokines and chemokines, or differentiate locally from myeloid progenitors in response to growth factors present in synovial fluid [55]. There is strong evidence that DCs contribute to the initiation and/or perpetuation of inflammation through the presentation of synovial autoantigens and activation of T cells [56,57]. Synovial DCs are highly activated in RA patients, including enhanced expression of MHC and costimulatory molecules, RelB, receptor activator of nuclear factor κB (RANK) and its ligand, RANKL [54,57,58].

Both myeloid and plasmacytoid DCs populations are increased in synovial fluid from RA patients compared with peripheral blood [59]. Myeloid CD1c + DCs appear to play a key role in promoting synovial inflammation by producing large amounts of T cell-attracting chemokines and the pro-inflammatory cytokines IL-12 and IL-23, which promote T cell differentiation towards IFNγ-producing type 1 (Th1) and IL-17-producing type 17 (Th17) cells, respectively [60,61]. Plasmacytoid DCs recruited to RA synovial tissue and capable of producing interferon (IFN)α, IFNγ, IL-18, and IL-23 could contribute to the local immunomodulatory environment [61]. Furthermore, plasmacytoid DCs expressing B-cell-activating factor of the tumor necrosis factor (TNF) family (BAFF) might promote B cell survival within RA synovial tissue [61]. This is supported by the particular increase of synovial plasmacytoid DCs in RA patients who display anti-citrullinated protein antibodies, and the positive correlation between autoantibody levels in serum and plasmacytoid DC numbers in synovium [61]. It has been reported that TGFβ-treated plasmacytoid DCs promote the differentiation of naïve T cells into Th17 cells with arthritogenic features [62]. In contrast, some investigators attributed plasmacytoid DCs a protective role in RA. For instance, a population of plasmacytoid DCs expressing indoleamine 2,3-dioxygenase (IDO) and capable of generating Tr1 cells in vivo, increase in peripheral blood of RA patients after therapy-induced remission [63]. In an animal model of RA, selective depletion of this plasmacytoid DC population aggravated severity of disease and enhanced autoreactive responses [64].

A population of monocyte-derived inflammatory DCs has been recently identified in synovial fluid of RA patients [30,65]. These inflammatory DCs were characterized by the production of the pro-inflammatory cytokines TNF, IL-6 and IL-1β, and the capacity to drive Th17 responses [30]. Human monocyte-derived DCs have been previously shown to promote cartilage destruction in vitro through a TNF-dependent mechanism [66]. The prominent role of DC-derived TNF in early autoimmune lesions has previously been shown in the mouse model [67], and is supported by the effective suppression of joint damage in RA patients who receive anti-TNF therapy [68].

3.2. Tolerogenic dendritic cells as therapeutics for rheumatoid arthritis

In the last two decades, researchers have made intensive efforts in developing innovative therapies based on conditioned DCs for the treatment of RA [69–72] and other autoimmune diseases [7,8]. The main therapeutic approach is to differentiate patient’s precursor cells (such as monocytes and bone marrow-derived stem cells) ex vivo into DCs with tolerogenic properties, which are then loaded with appropriate autoantigens and re-infused in the patient with the goal of restoring T cell tolerance to specific autoantigens and achieving long-term remission (Fig. 1).

3.3. Experimental approaches for in vitro generation of tolerogenic dendritic cells

There is a multitude of experimental procedures to induce stable tolerogenic features in DCs derived from monocytes [69,72] or bone marrow-derived stem cells [73]. These include DC modulation with cytokines, such as IL-10 [74], TGFβ [75], IL-21 [76], hepatocyte growth factor [77], IL-6 [78], TNF [79], and combination of IL-10 with TGFβ or IL-6 [80]; short stimulation with microbial products like lipopolysaccharide (LPS) [81] or Aspergillus oryzae protease [82]; immunosuppressive drugs, such as dexamethasone (Dex) [83], rapamycin [84], aspirin [85], cyclosporine A [86], mycophenolic acid [87], and the Janus kinase inhibitor tofacitinib [88]; natural compounds like resveratrol [89], curcumin [90], sulforaphane [91], 1α,25-dihydroxyvitamin D3, the active form of vitamin D3 (VD3), either alone [92], or in combination with Dex [93]; the hormone vasoactive intestinal peptide [94]; protein kinase...
C inhibitors [95]; modulators of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activity, such as BAY11-7082 [96], rosiglitazone and andrographolide [97]; and the CTLA4-Ig fusion protein abatacept [98], among others. Alternatively, TolDCs have been obtained through genetic modifications such as transduction of the IL-10 or IL-4 genes [99,100], and knockdown of costimulatory molecules using RNA interference [101]. Partial maturation of TolDCs, induced by additional stimulation with LPS [102], the non-toxic LPS analogue monophosphoryl lipid A (MPLA) [103], or a cytokine cocktail, containing TNF, IL-1β and prostaglandin E2 [104], enhances antigen presentation, association of lymph node homing capacity, and stability of the tolerogenic phenotype [105,106], features which are all desirable when thinking in a potential therapeutic use of TolDCs.

Though the properties of TolDCs may vary dependent on the protocol used for their generation, there are fundamental similarities, such as (i) reduced expression of co-stimulatory molecules, (ii) low production of pro-inflammatory cytokines, i.e. IL-12, and high levels of anti-inflammatory cytokines IL-10 and/or TGF-β, and (iii) the ability to induce T cell hyporesponsiveness or IL-10-producing Tr1 cells. In recent years, protocols have adapted Good Manufacturing Practice (GMP) requirements for therapeutic application in patients [103–105].

3.4. Tolerogenic dendritic cells in pre-clinical models of rheumatoid arthritis

Several studies in mice suggest that TolDCs are able to modulate arthritic autoimmune responses in vivo. In a mouse model of adjuvant-induced arthritis, provoked by the intra-articular injection of methylated bovine serum albumin, BAY 11-7082-treated bone-marrow-derived DCs induced antigen-specific immune suppression, associated with increased IL-10 production and antibody isotype switching from IgG2b to IgG1 and IgA [96]. Collagen-induced arthritis (CIA) is the most studied mouse model of RA, which develops in susceptible strains after immunization with type II collagen (CII), the major component of articular cartilage, and shares many pathophysiological features with human RA, such as mononuclear cell infiltration, pannus development, and cartilage and bone erosion [107]. The development of CIA can be prevented by administration of DCs modulated with TNF, Dex, or IL-10 [108]. A recent study demonstrates that CII-pulsed DCs treated with the calcineurin inhibitor FK506/Tacrolimus act as cellular drug delivery system that targets antigen-specific T cells and inhibits CIA [109]. Interestingly, FK506-treated DCs exhibited a mature phenotype and continuously released the drug over several days, causing activation induced cell death in encountered T cells [109].

Established CIA can be reduced by CII-pulsed TolDCs treated with Dex and VD3 [110]. The decline in disease severity and progression is accompanied by a decreased proportion of Th17 cells and an increase in IL-10-producing T cells [110]. Recently, our group demonstrated that short-term LPS-modulated TolDCs loaded with CII inhibit CIA in an IL-10 and TGF-β dependent manner [111]. Another report demonstrates that allogeneic murine bone-marrow-derived DCs, modulated with IL-10 plus TGF-β and loaded with CII, reduce Th17-driven inflammation and CIA progression [112]. Even human monocyte-derived TolDCs, modulated by FK506/Tacrolimus, exhibited a therapeutic effect in CIA mice [113].

Despite similarities between RA-like disease in mice and human RA, studies in mice are limited by differences between murine and human immune systems [114], the diversity of genetic and environmental factors predisposing to human RA [115], and the multitude of T cell autoantigens involved in the development and perpetuation of chronic RA, requiring more sophisticated preclinical models, as humanized mice, to validate the therapeutic effect of TolDCs before their application in patients.

3.5. Tolerogenic dendritic cells in clinical trials of rheumatoid arthritis and other autoimmune diseases

The use of TolDCs as tolerance-inducing therapeutics was inspired by a visionary study from Dhodapkar and colleagues, who injected autologous immature DCs pulsed with influenza matrix peptide into healthy individuals [116]. As a result, the effector function of antigen-specific CD8+ T cells was inhibited, while antigen-specific IL-10-secreting T cells emerged, pointing to a tolerizing effect of immature DCs [116]. More than ten years passed from this pioneer study until Giannoukakis and colleagues published the first clinical trial using TolDCs in type 1 diabetic patients [117]. In a randomized, double-blind, phase I study, seven insulin-requiring type 1 diabetic patients received four intradermal administrations of autologous TolDCs, generated from monocytes and treated with a mixture of antisense oligonucleotides targeting the primary transcripts of CD40, CD80 and CD86 [117]. The trial proved that administration of TolDCs was not only safe and well tolerated, but also increased the frequency of a B220+ CD11-B cell population [117]. Further in vitro studies and in vivo experiments in non-obese diabetic mice indicated that the TolDCs used in the clinical trial convert and expand IL-10-producing regulatory B cells with suppressive properties through the production of retinoic acid [118,119].

At present, two phase I clinical trials with TolDCs are conducted in RA patients. An approach published by J. Isaacs and C. Hilkens uses autologous DCs modulated with Dex, VD3, and MPLA, and pulsed with synovial fluid, which were injected into inflamed joints [69]. The setup of Thomas and co-workers is based on the intradermal administration of autologous DCs, modulated by the NF-κB inhibitor BAY11-7082 and pulsed with a mixture of four different citrullinated peptides, to patients displaying “shared epitope” alleles and anti-citrullinated protein antibodies [120]. Both strategies, using different protocols for DC generation and antigen loading, highlight the emergence of TolDC therapy as a new approach to treat autoimmune diseases.
4. Molecular regulators of tolerogeneity in dendritic cells

An important point to consider when translating TolDCs to clinical application is the definition of specific tolerance-inducing features as quality control parameters of the ex vivo differentiated cells. The multitude of modulation strategies, interfering with different signaling pathways and evoking a particular DC phenotype, makes it a challenging task to identify unique intrinsic factors that confer tolerogenic properties to DCs (Table 1).

4.1. Transcription factors and adaptor proteins

To date, no master regulator of DC tolerogeneity is known. Approaches to identify biomarkers for TolDCs, either by transcriptional profiling [80,91] or 2D gel electrophoresis and mass spectrometry [121], showed inconsistent results and failed to identify a universal molecular regulator of tolerogenic function. However, studies in knockout mice revealed several transcriptional regulators, which exert tolerizing effects [122].

Table 1 Intrinsic regulators of dendritic cell tolerogenicity.

<table>
<thead>
<tr>
<th>Regulator</th>
<th>Targets or affected signaling pathways in DCs</th>
<th>Inducing stimuli or agonist</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF-κB p50 (homodimer)</td>
<td>Suppression of TLR-induced inflammatory cytokines; induction of IL-10 transcription and expression of TGF-β and retinoic acid</td>
<td>IL-10, TLR signals, commensals?</td>
<td>[124,125,183]</td>
</tr>
<tr>
<td>PPAR-γ</td>
<td>Inhibition of NF-κB nuclear localization</td>
<td>TGD, e.g. rosiglitazone</td>
<td>[97,137]</td>
</tr>
<tr>
<td>BLIMP-1</td>
<td>Suppression of Il6 and Ccl2 transcription</td>
<td>TLR ligands</td>
<td>[144,145]</td>
</tr>
<tr>
<td>STAT3</td>
<td>Inhibition of NF-κB activation; induction of SOCS3 expression; reduction of LPS-induced maturation and pro-inflammatory cytokine production</td>
<td>IL-10, IL-6, CTLA-4</td>
<td>[132,133,184]</td>
</tr>
<tr>
<td>GILZ</td>
<td>Blockade of NF-κB, MAPK, and AP-1 signal transduction pathways; induction of Tr1-promoting features</td>
<td>GCs, IL-10, TGF-β, VD3</td>
<td>[82,139,140]</td>
</tr>
<tr>
<td>AhR</td>
<td>Promotion of Il8, Bcl, Ilf3 and Cyp1a expression when partnering with NF-κB subunit RelB; induction of indoleamine 2,3-dioxogense expression</td>
<td>Toxins (e.g. TCDD), tryptophan metabolites (IFC2, kynurenine)</td>
<td>[143,185,186]</td>
</tr>
<tr>
<td>SOCS-1</td>
<td>Blockage of TLR-INDUCED STAT-1 activation; impairment of Tnf-1-inducing capacity</td>
<td>GCs, TLR ligands</td>
<td>[128,130]</td>
</tr>
<tr>
<td>SOCS-3</td>
<td>Blockage of p38/MAPK activation; overexpression inhibits Th2 polarizing features</td>
<td>Retinoic acid, IL-6, TLR ligands</td>
<td>[135,152]</td>
</tr>
<tr>
<td>ERK</td>
<td>Decrease of NF-κB DNA-binding activity and inhibitor of βGal levels</td>
<td>Mitogens, growth factors</td>
<td>[169]</td>
</tr>
<tr>
<td>SHP-1</td>
<td>Recruitment to cytoplasmic domains of inhibitory receptors; inhibition of NF-κB, AP-1, ERK, and JNK activity, while enhancing p38 activity; inhibition of pro-survival signals through AKT activation; inhibition of CCR7 expression</td>
<td>Cytokines, growth factors, binding to inhibitory receptors (e.g. ILT3, FcγRIIB)</td>
<td>[171,172]</td>
</tr>
<tr>
<td>Lyn</td>
<td>Src family tyrosin kinase; phosphorylating of ITIM motifs (e.g. FcγRIIB); negative regulation of MyD88 signaling pathway; recruitment of inhibitory phosphatases SHP1/2</td>
<td>Integrins, growth factors, binding to FcγRI</td>
<td>[173,187]</td>
</tr>
<tr>
<td>HO-1</td>
<td>Heme oxygenase, degradation of heme into biliverdin, CO and free iron; inhibition of NF-κB p65 and IκB-α activation</td>
<td>IL-10, cobalt protoporphyrin</td>
<td>[165–167]</td>
</tr>
<tr>
<td>A2O/TFNAP3</td>
<td>Deubiquitination, degradation of intermediate NF-κB signaling molecules, e.g. RIP1, TRAF6; interference with TLR, NOD2, CD40, IL-1R and TNF signals</td>
<td>TNF</td>
<td>[162,163]</td>
</tr>
<tr>
<td>TAM</td>
<td>Action via type I interferon receptor (IFNAR)-STAT1 pathway; induction of SOCS1 and SOCS3, inhibition of TRAF3/6</td>
<td>Gas6/ProS</td>
<td>[160]</td>
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<tr>
<td>RALDH2</td>
<td>Retinaldehyde dehydrogenase, conversion of retinal to retinoic acid which induces SOCS3 expression; suppression MAPK p38 activation and prevents expression of proinflammatory cytokines</td>
<td>Dex</td>
<td>[82,152]</td>
</tr>
<tr>
<td>IDO</td>
<td>Indoleamine 2,3-dioxogense: degradation of tryptophan to kynurenic acid; tryptophan deprivation induces stress response pathway mediated by the GCN2 kinase</td>
<td>TGF-β, IL-12</td>
<td>[80,174]</td>
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<td>CD39</td>
<td>Ecto-ATPDase, hydrolysis of ATP and ADP to AMP reduces extracellular concentration of pro-inflammatory ATP and decreased ATP-triggered activation of NLRP3 in inflammasome</td>
<td>VD3 + LPS</td>
<td>[102]</td>
</tr>
<tr>
<td>PD-L1</td>
<td>Interaction with PD-1 on T cells favors induction of Treg responses and triggers IL-10 production</td>
<td>Resveratrol, IL-10, IFN-α, Dex, VD3, rapamycin</td>
<td>[89,148,149]</td>
</tr>
<tr>
<td>ILT3</td>
<td>Inhibition of cell activation by transmitting negative signals through cytoplasmic ITIM, recruitment of SHP-1 and inhibition of NF-κB signaling</td>
<td>Resveratrol, IL-10, IFN-α, Dex, VD3, rapamycin</td>
<td>[51,89,148,149]</td>
</tr>
<tr>
<td>ILT4</td>
<td>Inhibition of cell activation by transmitting negative signals through cytoplasmic ITIM, recruitment of SHP-1 and inhibition of NF-κB signaling</td>
<td>HLA-G</td>
<td>[51,89,148]</td>
</tr>
<tr>
<td>TLR2</td>
<td>Induction of RALDH2 expression via activation of ERK</td>
<td>Dex, VD3, IL-10 + LPS</td>
<td>[152,188]</td>
</tr>
<tr>
<td>FcγRIIB (CD32b)</td>
<td>Inhibition of cell activation by transmitting negative signals through cytoplasmic ITIM, recruitment of SHP-1 and inhibition of NF-κB signaling</td>
<td>IgG immune complexes</td>
<td>[157,159]</td>
</tr>
<tr>
<td>DEC-205</td>
<td>Delivery of endocytosed protein for MHC class II presentation and MHC class I crosspresentation, leading to induction of regulatory CD4+ and CD8+ T cells, expression of B7-H1 molecules, and TGF-β secretion</td>
<td>Modified extracellular proteins (of apoptotic cells)</td>
<td>[153,154,189]</td>
</tr>
<tr>
<td>DCIR</td>
<td>C-type lectin antigen uptake and signaling receptor; inhibition of cell activation by transmitting negative signals through cytoplasmic ITIM, recruitment of SHP-1 and SHP-2, leading to downregulation of TRLR-induced IL-12 and TNF production</td>
<td>Glycoproteins from pathogenic and endogenous origin</td>
<td>[155,190,191]</td>
</tr>
<tr>
<td>DC-SIGN</td>
<td>C-type lectin antigen uptake and signaling receptor; modulates TLR signals through ERK1/2 and Akt phosphorylation, thereby favoring IL-10 production and Th2/Th1 responses</td>
<td>Mannan-containing glycoconjugates from pathogenic and endogenous origin</td>
<td>[192–194]</td>
</tr>
<tr>
<td>Wnt5a</td>
<td>Stimulation of intracellular Ca2+ mobilization and triggering of Calmodulin-dependent protein kinase II, leading to upregulation of inhibitor of DNA binding 3 (ID3) and SOCS3 expression; induction of STAT3 and IL-6 production</td>
<td>Inflammation, sepsis</td>
<td>[176,177,195]</td>
</tr>
<tr>
<td>C1q</td>
<td>Complex formation with cT1r and DC-SIGN; increase of ERK, p38 and p70S6 kinase activity</td>
<td>TLR ligands, IL-6, Dex</td>
<td>[181,182]</td>
</tr>
<tr>
<td>MFG-E8</td>
<td>Phosphatidylethanolamine-binding protein; suppression of ISRE and NF-κB activity; activation of STAT-3 and A2O</td>
<td>GM-CSF</td>
<td>[178,179]</td>
</tr>
</tbody>
</table>

Abbreviations used: BLIMP-1: B lymphocyte-induced maturation protein 1; CO: carbon monoxide; DCIR: DC immunoreceptor; DC-SIGN: DC-specific intercellular adhesion molecule-3-grabbing non-integrin; Dex: dexamethasone; ERK: extracellular signal-regulated kinase; FcγRIIB: Low affinity immunoglobulin gamma Fc region receptor II-B; FICZ: 6-formylindolo[3,2-b] carbazole; Gas6: growth arrest-specific gene 6; HO-1: heme oxygenase 1; IκB-α: interferon regulatory factor 3; ISRE: interferon-stimulated response element; ITIM: immunoreceptor tyrosine-based inhibitory motifs; MFG-E8: milk fat globule-epidermal growth factor 8; PD-L1: programmed death ligand 1; PPAR: peroxisome-proliferator activated receptor; RALDH2: retinaldehyde dehydrogenase type 2; RIP: receptor interacting protein; SHP-1: SH2-containing protein tyrosine phosphatase 1; SOCS: suppressor of cytokine signaling; STAT: signal transducer and activator of transcription; TLR: Toll-like receptor; VD3: Vitamin D3.
NF-κB is known to play a central role in DC activation. A detailed analysis of the different NF-κB family members revealed that heterodimers of NFκB1 p50 and RelA or NFκB2 p52 and RelB promote inflammatory responses, while NFκB1 p50 homodimers act as transcriptional repressors [123–125]. Self-antigen-pulsed unstimulated DCs deficient for NFκB1 induced CD8+ T cell responses and triggered autoimmunity in vivo [126]. Moreover, DCs lacking NFκB1 have shown an increased secretion of pro-inflammatory cytokines and an extended lifespan [127].

Differentiation, maturation and functions of DCs are tightly controlled by the counterregulation of the signal transducer and activator of transcription (STAT) and suppressor of cytokine signaling (SOCS) family protein members. TLR-mediated STAT1 activation is attenuated by GCs through the induction of SOCS1 [128]. SOCS1-deficient DCs induce hyperactivation of Th1, promote B cell proliferation, autoantibody production and the development of lupus-like autoimmune disease [129,130]. STAT3 directs the anti-inflammatory effects of IL-10, IL-6 and CTLA-4 [131–133], and the activation of STAT3 reduces LPS-induced DC maturation [131]. STAT3-deficiency in DCs leads to enhanced pro-inflammatory cytokine production, antigen-dependent T-cell activation and resistance to IL-10-mediated suppression [132]. Mice with conditional knockout of STAT3 in CD11c+ DCs develop intestinal inflammation [132]; SOCS3-deficiency in DCs resulted in constitutive activation of STAT3, and decreased expression of MHC class II, costimulatory molecules, and IL-12 [134]. SOCS3-deficient DCs promoted TGFβ-mediated Treg expansion, and reduced the severity of experimental autoimmune encephalomyelitis (EAE) [134]. Interestingly, transcription with SOCS3 also decreased expression of MHC class II, CD86, IL-12 and IL-23, while enhancing IL-10 production [135]. Adap- tive transfer of DCs overexpressing SOCS3 suppressed EAE and skewed differentiation of myelin antigen-specific T cells towards Th2 [135].

Peroxisome–proliferator activated receptor (PPARγ) is highly expressed in immature human monocyte-derived DCs [136]. Activation of PPARγ by thiazolidinediones/glitazones hampers nuclear localization of the RelB subunit of NFκB, thereby affecting LPS-induced maturation, IL-12 secretion, expression of CCR7 and its ligand CCL19, and the capacity to prime antigen-specific CD4+ and cytotoxic T cell responses [136,137]. Moreover, PPARγ ligation induces the expression of TGFβ and retinoic acid in murine bone-marrow-derived DCs and endows them with the capacity to generate Treg [138]. DCs that were treated with the PPARγ agonist rosiglitazone and pulsed with myelin antigens prevented the development of EAE in mice [97].

The transcription inhibitor GC-induced leucine zipper (GILZ) is a key mediator of the anti-inflammatory effects exerted by GCs, IL-10, and TGFβ [139]. GILZ interacts with and inhibits NFκB, STAT, AP-1, Ras and Raf-1, thereby preventing the upregulation of costimulatory molecules, inflammatory cytokines and chemokines in DCs [140]. Additionally, GILZ stimulates immunoglobulin–like transcript 3 (ILT3) and programmed death ligand 1 (PD-L1) expression, IL-10 production, and induction of antigen-specific Tr1 cells [139,141]. In the EAE model, GILZ expression in transferred TolDCs was indispensable for the inhibition of Th1 and Th17 responses, Treg induction and reduction of disease severity [142].

The expression of the ligand-activated transcription factor aryl hydrocarbon receptor (AhR) equips DCs with the capacity to express IL-10 and IDO [143]. IDO degrades tryptophan to kynurenine, which inhibits T cell proliferation due to amino acid starvation, and promotes the generation of FOXP3+ Treg [143].

An important role of the transcriptional repressor B lymphocyte-induced maturation protein–1 (BLIMP-1) in the regulation of DC function has been discovered recently [144,145]. The expression of BLIMP-1 is upregulated upon DC activation and negatively regulates the expression of IL-6 and CCL2 genes [144]. BLIMP-1-deficient DCs exhibit elevated expression of MHC II and increased secretion of pro-inflammatory cytokines upon exposure to TLR agonists, and conditional knockout of BLIMP-1 in DCs induced a lupus-like disease in mice [145].

4.2. Membrane inhibitory receptors

Several surface molecules have been suggested as TolDC markers. Inhibitory receptors ILT3 and ILT4 transmit negative signals through cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (ITIM) [146]. While ILT4 binds to classical MHC class I and non-classical MHC human leukocyte antigen (HLA)-G, the ligand of ILT3 is unknown [147]. Expression of ILT3 has been shown to be required for the induction of Treg by TolDCs [148]. TolDCs derived from ILT3–transduced human hematopoietic stem/progenitor cells exhibited decreased expression of costimulatory molecules, downregulation of NFκB, and the capacity to induce alloogenic T cell hyporesponsiveness and functional Treg [149]. Gregori and co-workers found that both, ILT4 and HLA-G are required for the induction of T1 cells by IL-10–derived TolDCs [51].

The inhibitory receptor PD-L1, constitutively expressed on murine and human T and B cells, macrophages and DCs, upon interaction with PD-1 on T cells, blocks the CD28/CD80/CD86-mediated T cell activation [150]. This CD80/CD86 co-inhibitory molecule, linked to the development and function of Tregs, could be considered as an alternative biomarker for cytokine- or LPS-activated VD3–induced DCs given its high expression on this type of TolDCs [102,151]; on the contrary, LPS-activated Dex–induced DCs displayed a low PD-L1 expression [102]. Our recent study, with partial matured TolDCs, induced by additional stimulation with MPLA, did not find a differential expression of PD-L1 when compared to mature DCs [103].

Interestingly, TolDCs that were modulated with GCs, VD3, or IL-10 plus LPS showed a markedly elevated TLR2 expression in comparison to immature and mature DCs [93,103]. Activation of TLR2 has been shown to induce the expression of retinaldehyde dehydrogenase type 2 (RALDH2) in DCs via activation of extracellular signal–regulated kinase (ERK) [152]. RALDH2 converts retinal to retinoic acid, which promotes the de novo generation of Treg [44], but also acts in an autocrine manner on DCs, inducing the expression of SOCS3, which suppresses mitogen-activated protein kinase (MAPK) p38 activation and the production of proinflammatory cytokines [152].

C-type lectin receptors (CLRs), such as DEC-205, DC immunoreceptor (DCIR), and DC–SIGN, recognize glycosylated endogenous tissue antigens and play an important role as negative regulators of DCs. In vivo targeting of antigen to DEC–205 generates tolerance against the antibody–coupled antigen [153,154]. Mice deficient for DCIR spontaneously developed autoimmune sialadenitis and enthesitis, accompanied by elevated serum autoantibodies [155]. Activation of SIGN–R1, a murine DC–SIGN homolog, has been shown to induce IL–10 expression in lamina propria DCs promoting the generation of Tr1 cells [156].

Engagement of the inhibitory FcγRIIb (CD32B) by IgG–bearing immune complexes modulates DC function increasing IL–10 production, induces CD4+ and CD8+ T cell tolerance and promotes the generation of antigen–specific Tr1 cells [157–159]. TLR- and cytokine-induced DC maturation can also be inhibited by the Tyro3/Axl/ Mer (TAM) receptor tyrosine kinases family, which forms a complex with type I interferon receptor (IFNAR), and uses the associated transcription factor STAT1 to trigger the production of SOCS1 and SOCS3 [160]. TAM triple gene–deficient mice exhibited DC hyperactivation and developed systemic autoimmunity [161].

4.3. Enzymes and signaling pathways–associated molecules

Recent studies have identified the ubiquitin-editing enzyme A20, the product of the TNF alpha–induced protein 3 gene (TNFAIP3), as a molecular checkpoint for the control of DC activation [162,163]. A20 deubiquitinate intermediate NFκB–signaling molecules, such as receptor interacting protein–1 (RIP1) and TNF receptor associated factor 6 (TRAF6) and thereby interferes with TLR, NOD2, CD40, IL–1R and TNFR signals in DCs [123,163]. DCs lacking A20 were resistant to apoptosis and show increased NFκB–signaling which leads to elevated expression of costimulatory molecules, MHC class II, and inflammatory cytokines
The stress-inducible heme oxygenase-1 enzyme (HO-1) is also exclusively expressed in immature DCs [165] and catalyzes the degradation of heme into biliverdin, carbon monoxide (CO) and free iron [166]. The expression of HO-1 can be induced by IL-10 or cobalt protoporphyrin, and prevents LPS-induced maturation and secretion of pro-inflammatory cytokines in DCs through inhibition of NF-κB p65, while preserving IL-10 production [165]. Both induction of HO-1 expression and treatment with CO inhibit the IFN regulatory factor 3 pathway, thereby affecting maturation and T cell stimulatory capacity of DCs [165,167]. Gaseous CO-treated DCs pulsed with pancreatic β cell peptides showed reduced IL-12 production and protected mice from autoimmune diabetes [168].

MAPK p38 and ERK signaling pathways differentially regulate the maturation of monocyte-derived DCs [169]. Opposite to MAPK p38, ERK signal transduction negatively regulates LPS- and TNF-induced DC maturation by decreasing the NF-κB DNA binding activity and levels of inhibitor of NF-κB [169]. Lentineral transfer of an ERK activator to DCs induced TGFβ production and conveyed the ability to induce functional Treg [170].

The Src homology region 2 domain-containing tyrosine phosphatase-1 (SHP-1) has been suggested as another intrinsic regulator of DC function. Specific inhibition of or deletion of SHP-1 in DCs enhanced production of the pro-inflammatory cytokines IL-6, IL-12, and IL-1β, promoted survival, migration to draining lymph nodes, and Th1 activation resulting in glomerulonephritis and autoantibody production in aged mice [171,172].

A recent report demonstrated that DC-specific deletion of the Src family tyrosine kinase Lyn, a negative regulator of the MyD88 pathway, induced spontaneous T and B cell activation leading to lupus-like autoimmune disease [173].

Ecto-ATPase CD39, which reduces the extracellular concentration of pro-inflammatory ATP, has been shown to be upregulated in TolDCs generated with TGFβ or IL-27 [80,174]. In vivo, CD39 expression by TolDCs prevented pathogenic Th1 and Th17 responses and the development of EAE [174].

4.4. Soluble regulators

The activation of β catenin signaling pathways by the binding of Wnt ligands to its Frizzled (Fzd) receptor programs tolerogenic functions in DCs [175]. The Wnt proteins Wnt5a and Wnt3a induce the secretion of IL-10 and TGFβ, respectively, and inhibit TLR-induced production of pro-inflammatory cytokines while suppressing the generation of Treg [176]. Exogenous Wnt5a reduces the expression of HLA-DR and CD86, and increases DC-SIGN, PD-L1, and PD-L2 levels as well as IL-10 secretion in DCs [177]. Blocking experiments and transcriptional profiling revealed that Wnt5a acts through a non-canonical pathway, stimulating intracellular Ca2+ mobilization and triggering calmodulin-dependent protein kinase II, leading to an upregulation of inhibitor of DNA binding 3 (ID3) and SOCS3 expression [177].

Milk fat globule EGF VIII (MFG-E8) has also been shown to be linked to DC tolerogenicity [178,179]. The expression of MFG-E8 can be induced by GM-CSF, and confers the ability to suppress Th1 and Th17 responses and to induce Treg upon engulfment of apoptotic cells [178]. Immature, but not mature DCs, express high levels of MFG-E8, which restrains the co-stimulatory capabilities and pro-inflammatory cytokine production in response to necrotic cells via activation of STAT-3 and A20 [179].

Immature DCs, derived from monocytes or CD34+ stem cells, produce early complement component 1q (C1q), whose expression is completely downregulated following maturation [180]. C1q renders DCs tolerogenic, inducing IL-10, while reducing IL-12 and IL-23 production, and impairing the capacity to stimulate Th1 and Th17 responses [181]. C1q and its receptor C1qR were suggested to associate with DC-SIGN and might thereby regulate DC differentiation and function through DC-SIGN-mediated intracellular signaling pathways [182]. In clinical responders to allergen immunotherapy, levels of C1q were shown to be associated with the in vivo generation of TolDC [82].

5. Conclusions

The therapeutic effect of ex vivo generated TolDCs has been confirmed in animal models of RA and other autoimmune diseases, and the development of GMP-compliant protocols for the generation of stable human TolDCs has made a considerable progress in the recent years. Pilot studies on safety of the administration of TolDCs demonstrated promising results, which led to the conduction of the first phase I/I clinical trials using autologous-loaded TolDCs.

Further research is required to: (i) define immunodominant autoantigen peptide candidates for loading of TolDCs; (ii) establish preclinical humanized models for the characterization of TolDC effects on multiple cell types in vivo, and determination of optimal dose, time interval and route of TolDC application; and (iii) identify biomarkers or key regulators of tolerogenicity that would facilitate the quality control of TolDC preparations before administration to the patient, and enable monitoring of therapeutic success. Finally, the knowledge about proteins that control the differentiation and maintenance of the tolerogenic state could provide novel therapeutic targets for the development and application of reagents that specifically modulate DC functions.

Take-home messages

- DCs contribute substantially to the pathogenesis of RA and are thus target of novel therapeutic approaches.
- DCs with tolerance-inducing features can be generated in vitro from RA patient’s peripheral blood monocytes.
- Hallmarks of TolDCs are (i) reduced expression of costimulatory molecules, (ii) an anti-inflammatory cytokine profile, and (iii) modulation of T cell responses.
- The efficacy of TolDCs has been demonstrated in mouse models of autoimmunity.
- First studies in humans confirmed safety of TolDCs administration; clinical phase I/I trials using autologous TolDCs in RA are in progress.
- A master regulator of TolDC function has not been yet identified.

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References


[163]. Mice with specific deletion of A20 in DCs are prone to develop autoimmune disease [162]. Single-nucleotide polymorphisms in the human TNFAIP3 locus are associated with increased susceptibility to multiple autoimmune disorders including RA, multiple sclerosis, and systemic lupus erythematosus [164].

The stress-inducible heme oxygenase-1 enzyme (HO-1) is also exclusively expressed in immature DCs [165] and catalyzes the degradation of heme into biliverdin, carbon monoxide (CO) and free iron [166]. The expression of HO-1 can be induced by IL-10 or cobalt protoporphyrin, and prevents LPS-induced maturation and secretion of pro-inflammatory cytokines in DCs through inhibition of NF-κB p65, while preserving IL-10 production [165]. Both induction of HO-1 expression and treatment with CO inhibit the IFN regulatory factor 3 pathway, thereby affecting maturation and T cell stimulatory capacity of DCs [165,167]. Gaseous CO-treated DCs pulsed with pancreatic β cell peptides showed reduced IL-12 production and protected mice from autoimmune diabetes [168].
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