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REVIEW ARTICLE

Dendritic Cell-based Immunotherapy for Rheumatoid Arthritis: from Bench to Bedside

Md. Selim Ahmed[#] and Yong-Soo Bae*
Department of Biological Science, Sungkyunkwan University, Suwon 16419, Korea

Dendritic cells (DCs) are professional antigen presenting cells, and play an important role in the induction of antigen-specific adaptive immunity. However, some DC populations are involved in immune regulation and immune tolerance. These DC populations are believed to take part in the control of immune exaggeration and immune disorder, and maintain immune homeostasis in the body. Tolerogenic DCs (tolDCs) can be generated *in vitro* by genetic or pharmacological modification or by controlling the maturation stages of cytokine-derived DCs. These tolDCs have been investigated for the treatment of rheumatoid arthritis (RA) in experimental animal models. In the last decade, several *in vitro* and *in vivo* approaches have been translated into clinical trials. As of 2015, three tolDC trials for RA are on the list of ClinicalTrial.gov (www.clinicaltrials.gov). Other trials for RA are in progress and will be listed soon. In this review, we discuss the evolution of tolDC-based immunotherapy for RA and its limitations and future prospects.
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Keywords: Tolerogenic dendritic cell (tolDC), Immunotherapy, Tolerance, Rheumatoid arthritis (RA), Mouse collagen-induced arthritis (CIA), Clinical study, Treg, Limitation, Future

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease, characterized by persistent joint inflammation leading to breakdown of articular cartilage and bone damage (1). Although the exact disease etiology is not known yet, it has been reported that RA arises from breakdown of immune tolerance. The autoreactive T cell ($CD4^+$ T cell) and impaired regulatory T cell (Treg) play an important role in the pathogenesis of RA. T cells infiltrate into the synovial joint, and increase the level of pro-inflammatory cytokines (interferon-γ and IL-17), causing synovial cartilage and bone destruction (2). Although some immunosuppressive drugs [cytokine antagonists, cytotoxic T lymphocyte antigen 4 (CTLA4) immunoglobulin blockades] can reduce disease symptoms, they do not have long-lasting efficacy in the body, but rather increased risk of side effects (3,4). Novel therapeutic biologics, which will be able to restore the immune tolerance, are eagerly awaited in the field of autoimmune arthritis. Tolerogenic DC (tolDC)-based immunotherapy would be one of the most attractive approaches among the novel cell-based treatments for RA (1,4).

Dendritic cells (DCs) are the most professional antigen presenting cells, which play an important role in maintain-

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*Corresponding Author. Yong-Soo Bae, Department of Biological Science, Sungkyunkwan University, Seobu-ro 2066, Jangan-gu, Suwon 16419, Korea. Tel: 82-31-290-5911; Fax: 82-31-290-7087; E-mail: ysbae04@skku.edu
[#]Present address: Department of Medicine, Surgery and Obstetrics, Faculty of Animal Science and Veterinary Medicine, Patuakhali Science and Technology University, Khanpura, Babugonj-8210, Barisal, Bangladesh

Abbreviations: RA, rheumatoid arthritis; DC, dendritic cells; Treg, regulatory T cells; CIA, collagen-induced arthritis; smDC, semi-mature dendritic cells; MoDC, monocytes-derived dendritic cells; BMDC, bone marrow derived dendritic cells; EAE, experimental autoimmune encephalitis

ing immune homeostasis in the body. Mature DCs (mDCs) are immunogenic. They have the ability to present antigens on their surface, and initiate and activate adaptive immune responses (5). On the other hand, immature DCs (imDCs) have weak antigen presenting and T cell priming abilities, thus can induce T cell tolerance (6,7). The imDC-mediated peripheral tolerance is known to be induced by silencing of differentiated antigen-specific T cells, activation and expansion of naturally occurring Tregs, and conversion of naïve $CD4^+$ T cells into Tregs (8,9). Some reports demonstrated that partial or semi-matured DCs (smDCs) also have the ability to induce tolerance (10,11). For example, DCs pulsed with self-antigen induced tolerance when partially matured (12). The tolerogenic potential of smDCs is thought to be associated with the release of immunosuppressive cytokines like IL-10 and TGF-β, surface expression of programmed death ligands such as PD-L1 and PD-L2, and expansion of Tregs (13). Sometimes, immunogenic mDCs also generate tolerogenic peptides due to processing of self-antigen, such as thyroid peroxidase (14). smDCs have greater tolerogenic potential than imDCs, because of their lymph node homing property by which DCs can reach T cell zone at their anatomical locations (15).

TolDCs can be generated *in vitro* by genetic or pharmacological modification or by controlling the maturation of DCs (4). Genetically modified tolDCs can be produced by ectopic expression of IL-4, Fas ligand (FasL), indoleamine 2, 3-dioxygenase (IDO), or CTLA4 (16,17). Pharmacologically modified tolDCs, BAY 11-7085, and LF15-0195 DC vaccines are prepared by treatment of monocytes-derived DCs (MoDC) with NF-κB signaling inhibitors, such as dexamethasone (Dex) or vitamin D3 alone, or both synergistically, in order to induce a tolerogenic/regulatory phenotype of DCs (4,18). It has also been shown that TNF-α-treated bone-marrow-derived smDCs (TNF/DCs) have tolerogenic potential in mouse collagen-induced arthritis (CIA) model (19,20).

smDCs induce Treg population and Th2 cytokines (20). $CD4^+$ $CD25^+$ Tregs play an important role in peripheral immune tolerance and the prevention of autoimmunity (21,22). Foxp3 transcription factor is essential for the development and immune suppressive function of $CD4^+$ $CD25^+$ Tregs (23,24). $CD4^+$ $CD25^+$ $Foxp3^+$ T cells are actively involved in the negative control of various physiological and pathological immune responses, and induction of transplant tolerance. It has been reported that smDCs were very effective in inhibiting arthritis progression in CIA mice probably by induction of Tregs (15,20). It is well established that $CD25^+$ $Foxp3^+$ Tregs are a critical player in the treatment of RA (4,25).

In this review, we will discuss the evolution of DC-based immunotherapy for RA, and its future implications, with an emphasis on its efficacy and limitations. In specific, we will review literatures addressing tolDC biology, manipulation techniques, animal experiments, and recent clinical approaches. This will provide a better understanding of present and future DC-based immunotherapy for RA.

GENERATION OF tolDCs

Unlike the immunogenic DCs, which are involved in the activation of adaptive immunity against invading pathogens and tumors, tolDCs in the body play an essential role in central and peripheral tolerance to self-antigens (15). TolDCs present self-antigens to T cells with inadequate co-stimulation and expression of immunosuppressive cytokines, leading to silencing of autoreactive T cells, and induction of Tregs (7,26). Three different approaches have been addressed for the generation of tolDCs *in vitro*:

Genetically modified tolDCs

It has been shown that transduction of DCs with FasL promoted immune tolerance by depletion of autoreactive T cells (17). Moreover, IDO or CTLA4 immunoglobin-transduced DCs were effective to induce $Foxp3^+$ Tregs (16). In addition, transduction of microRNA-23b promoted tolerogenic properties of DCs through the inhibition of Notch1/NF-κB signaling pathway (27). Recently, microRNA clusters comprising miR-17, miR-133b and miR-203 were detected in tolDCs (28), suggesting that these microRNAs could be used for generation of tolDCs. Furthermore, Wnt signaling plays an important role in immune balance; and thus, can be used as a direct target for tolerance induction. It was reported that the increase in Wnt5a signaling during the differentiation of human MoDCs reduced the expression of IL-12p70 and TNF-α but increased the level of IL-10, leading to differentiation of DCs with tolerogenic features (29).

Pharmacologically modified tolDCs

When treated with IL-4 and retinoic acid synergistically,

DCs were shown to display tolerogenic/regulatory phenotype, providing a potential treatment for autoimmune diseases (30). Other studies reported that DCs treated with IL-10 and TGF-β1 became tolerogenic in mice (31-33). In addition, rapamycin-treated DCs could induce $CD4^+$ $CD25^+$ $Foxp3^+$ Tregs in the absence of TGF-β1 when inoculated in mice (34,35). Moreover, DCs treated with glucocorticoid Dex or vitamin D3 or both inhibited Th17 activity, but enhanced the activity of IL-10 producing Tregs, leading to immune tolerance *in vivo* (4).

Controlling DC maturation for generation of tolDCs

When treated with TNF-α, imDCs differentiated into smDCs, which expressed less amounts of co-stimulatory molecules and increased Th2 cytokine (IL-4, IL-5, IL-13 and IL-10) production (20). Sometimes, short-term treatment with LPS (lipopolysaccharide) could induce DCs to become tolerogenic (36). Maturation of DCs in the presence of certain PAMP (Pathogen Associated Molecular Pattern), which are derived from invasive parasites, produces marked amounts of anti-inflammatory cytokine IL-10 and induces IL-10 producing Tregs (37).

ROLES OF tolDCs IN THE IMMUNE SYSTEM

TolDCs maintain a steady state characterized by antigen presentation without T cell activation. TolDCs function to silence autoreactive T cells by inducing T cell anergy, apoptosis and/or clonal deletion probably due to a consequence of inadequate antigen presentation to T cells (1,38), or by promoting Tregs and suppressor T cells (15,39). $CD4^+$ Treg cells are broadly classified into natural (nTreg) and induced Tregs (iTreg): nTregs arise in the thymus to maintain peripheral immune homeostasis, whereas iTregs are generated in the periphery following $CD4^+$ T cell activation under the immunosuppressive environments (40). $Helios^+$ nTregs suppress immune responses via contact-dependent inhibition mechanisms (41), whereas $Helios^-$ iTregs control T cell responses via secretion of immunosuppressive cytokines (42). IDO^+ splenic DC subset completely blocked clonal expansion of T cells following adoptive transfer from TCR transgenic mice (43). Mucosal $CD103^+$ DC populations are involved in oral tolerance by inducing $FoxP3^+$ Treg cells via TGF-β and retinoic acid-dependent mechanism (44,45), IDO-dependent mechanism (46) and/or integrin $\alpha5\beta8$-dependent-maner (47). It was also demonstrated that transfer of antigen (Ag)-loaded liver plasmacytoid DCs (pDCs) to naive recipient mice induced Ag-specific oral tolerance by inducing anergy or deletion of Ag-specific T cells via a $CD4^+$ T cell-independent mechanism (48). In addition, FasL-transduced tolDC vaccine was shown to be effective in the treatment of established mouse collagen-induced arthritis (CIA) via depletion of autoreactive T cells (17), and adenovirally transduced CTLA-4Ig-expressing bone marrow-derived DCs (BMDCs) reduced the inflammation in CIA mice in an IDO-dependent immunosuppressive manner (16). Moreover, Dex/vitamin D3-modulated DCs showed a strong tolerogenic potential, which enhanced the activity of IL-10-producing Tregs (4).

TolDCs AND AUTOIMMUNITY

The results from preclinical animal experiments provide strong evidence that tolDCs can be used as an immunotherapeutic agent for the treatment of various autoimmune diseases (1). Menges et al. demonstrated that TNF-α-stimulated smDCs expressed IL-10 and induced increased antigen-specific Treg populations in mouse models of experimental autoimmune encephalitis (EAE), leading to inhibition of disease progression (19). Faunce et al. showed that myelin basic protein (MBP)-pulsed, TGF-β2-treated tolDCs reduced both the severity and incidence of ongoing EAE. The authors suggested that this was due to the promotion of peripheral antigen-specific tolerance via the induction of $CD8^+$ Tregs that are capable of suppressing Th1 and Th2 immunity (49). It has been shown that treatment by an optimal dose of smDC vaccine protected mice from CIA (20). Clinical-grade monocyte-derived tolDCs generated from the PBMC of RA patients, using immunosuppressive drugs Dex and vitamin D_3, and an immunomodulator, monophosphoryl lipid A, exhibited a typical tolerogenic phenotype of reduced costimulatory molecules, low production of proinflammatory cytokines, and impaired stimulation of autologous antigen-specific T cells. These tolDCs were effective to suppress T cell proliferation, interferon (IFN)-γ, and IL-17 production, and rendered T cells hyporesponsive to further stimulation (50). TolDCs stimulated by IL-10, TGF-β, and GM-CSF also exhibited reduced the levels of pro-inflammatory cytokines, but induced $CD25^+$ and IL-10-secreting Tregs, which were protective against lethal GVHD in murine

models (51). Thyroglobulin-primed smDCs also induced IL-10-secreting $CD4^+CD25^+$ Tregs, which efficiently suppressed experimental autoimmune thyroiditis (EAT) (52). The 1,25- dihydroxy/vitamin D3-stimualted mDCs generated from bone marrow cells of both non-obese diabetes (NOD) mice and C57BL/6 mice inhibited the proliferation and activation of autoreactive T cells due to the expansion of $CD25^+$ $Foxp3^+$ Tregs and intracellular IL-10 production by T cells (53). It has been reported that CD40 engagement on DCs activates multiple genes required for dendritic cell function. Vitamin D can modulate the expression of surface CD40 on DCs, which induce Tregs and increase IL-10 production, causing increased tolerance to transplant and ameliorated diabetes (54). On the other hand, infiltration of pDCs into the islet of NOD mice caused local inflammatory reaction, leading to the development of diabetes (55). It is well known that pDCs produce high amounts of type I IFN, which induces type 1 diabetes in NOD mice (56).

TolDC IMMUNOTHERAPY IN CIA MICE

RA is a systemic inflammatory autoimmune disease characterized by leukocyte invasion and synoviocyte activation followed by cartilage and bone destruction. TolDCs generated from naive DCs can induce T cell tolerance, thus, they represent a promising strategy for specific cellular therapy for autoimmune diseases. Even though high doses (2×10^6) of TNF-α-treated smDCs uniformly accelerated arthritic symptoms, low doses (2×10^5) of smDCs showed excellent anti-arthritic activity in CIA animals, with markedly increased production of $FoxP3^+$ Treg, Th2 cytokines (IL-4/IL-10) and TGF-β (20,57). Mouse bone marrow-derived $CD11b^+F4/80^+$ DCs generated under the stimulation of GM-CSF and IL-4 significantly reduced the pathologic scores in the joints and spleen, which correlated significantly with reduced T cell proliferation and number of Th17 cells, and with increased number of Tregs (58). It was also reported that treatment of CIA mice with tolDCs modified by tacrolimus significantly inhibited the severity and progression of the disease with the alteration of the proportion of the Th1 and Th17 in the spleen (59). Apigenin-treated DCs retarded the maturation and migration of DCs and protect mice from CIA (60).

Another important question is whether allogeneic tolDCs (allo-tolDCs) can be used to induce tolerance in RA, and if yes, whether the numbers of adoptively transferred allo-tolDCs, or the RA-related auto-antigens are important. Adoptive transfer of low doses of CII-loaded allo-tolDCs generated from C57BL/B6 mice showed a remarkable anti-arthritic activity in CIA mice, along with improved clinical scores and histological end-points, reduced levels of inflammatory cytokines, anti-CII antibodies and decreased proportion of Th17/Treg cells (61). These data strongly suggest that patients with RA can be treated with the allo-tolDC vaccine generated from any healthy donor, and such approaches provide a better understanding in moving forward with tolDC-based immunotherapy.

CLINICAL TRIALS OF tolDC IMMUNOTHERAPY FOR PATIENTS WITH RA

Animal experiments have shown the potential use of collagen-pulsed tolDCs to suppress CIA in an antigen-specific manner. However, translation of these results to human clinical trials has been problematic, as a number of unanswered questions remain, such as the safety of this approach and choosing the most suitable technique. The basic concept of tolDC therapy is to target the pathogenic autoreactive T cells without damaging the protective immunity, and this concept holds true when used as a therapeutic option for RA in humans. Clinical grade tolDCs have been designed and generated for the treatment of RA patients based on murine studies (1). The tolDCs for clinical grade showed typical features including intermediate expression of co-stimulatory molecules and anti-inflammatory cytokines, and inducing antigen-specific T cell hyporesponsiveness *in vitro* (1,50). A group at the University of Queensland, Australia has performed a Phase 1 clinical study with autologous tolDC in patients with RA, and addressed their preliminary data at a Meeting in 2011 (1,62). This study is followed by many other clinical trials that are currently in progress with some of them close to completion (www.clinicaltrials.gov). The Queensland group generated tolDCs by treating human MoDCs with NF-kB signaling inhibitor, BAY 11-7082. This clinical grade tolDC, pulsed with four citrullinated peptide antigens, was named 'Rheumavax'; lack CD40 expression but express high levels of CD86, thus phenotypically different from other tolDCs (62). The phase 1 study of Rheumavax was completed and published recently in *Science Translational Medicine* (63). This was the first-in-human trial of modi-

fied tolDCs in RA patients (64). Rheumavax was well tolerated to 18 patients with early RA (disease duration < 1 year), and revealed promising efficacy data with the reduction of effector T cell population and increase of Treg cells in a month after single injection. They concluded that the single intradermal injection of autologous tolDCs exposed to citrullinated peptides was safe and effective against human leucocyte antigen (HLA) risk-positive RA patients (63). Another approach for manufacturing human monocyte-derived clinical grade tolDCs from RA patients was done by Harry et al. at Newcastle University (50). To generate tolDCs, they used Dex and vitamin D3, and the cGMP-grade immunomodulator, monophosphoryl lipid A (MPLA), in cGMP-compliant medium. Their clinical-grade tolDCs were phenotypically stable in the inflammatory circumstances and exhibited a typical tolerogenic phenotype of reduced costimulatory molecules, low production of proinflammatory cytokines, IFN-γ and IL-17, and impaired stimulation of autologous antigen-specific T cells (50). The autologous tolDCs were exposed to autologous synovial fluid as a source of autoantigen, and named 'AutoDECRA' in a randomized, unblinded, placebo-controlled, dose-escalation Phase I clinical study (1). AutoDECRA was designed to be administered intra-articularly under arthroscopic guidance and assumed to have promising clinical results as compared with systemic administration (1).

LIMITATIONS OF tolDC-based RA IMMUNOTHERAPY

TolDC therapy is thought to target pathogenic autoreactive T cells. That is the reason why an antigen-pulsing step is essential for tolDC immunotherapy using smDCs (65), Bay-treated tolDCs (66) and Dex/vitamin D3-treated DCs (67). A pragmatic approach is to load tolDCs with recombinant proteins or peptides derived from different candidates of autoantigens or with autologous synovial fluid. Several RA-associated autoantigens have been identified and reported (4), and the selection of autoantigens for the generation of autologous tolDCs is thought critical for the efficacy of tolDC therapy in RA patients. However, these autoantigens are not always detected in all RA patients, and little is known about the immunodominant profile of these autoantigens in association with RA pathogenesis and disease progression. Our previous results clearly demonstrated that even identical batches of smDCs could be immunogenic when treated at high dosages (20). This means that smDCs cannot be applied to the treatment of autoimmune arthritis until the optimal dosage is determined. Careful consideration should be given to the amount of inoculum of smDCs when considering smDC-based RA immunotherapy.

Another important issue is the timing of tolDC treatment in RA patients as described previously (1). Unlike patients in the transplant setting, tolDCs would be administered in RA patients during the phase of disease progression. It takes many years before onset of RA symptoms from the tolerance break (68). During this period, the autoreactive immune system is being established in RA patients, which is then augmented further after disease onset. It is generally thought that tolDC therapy would be more successful when applied as early as possible (69). Supporting this, the first-in-human trial of modified tolDCs revealed promising efficacy in early RA patients (64). However, for safety and ethical reasons, tolDC therapy was only offered to patients with far advanced disease and who have failed other treatments. When used in such conditions, it is very likely that tolDC therapy would fail to provide desired outcomes and thus discourage further advancement in the field. Also, tolerance induction should be monitored before and after therapy. However, lack of a readily available biomarker of tolerance induction is another obstacle in tolDC-based RA immunotherapy. Finally, tolDC therapy is a highly customized therapy, which makes it both expensive and limits its application to institutions that have appropriate facilities.

FUTURE PROSPECTS OF tolDC-based RA IMMUNOTHERAPY

TolDC immunotherapy is one of the most attractive approaches for the treatment of autoimmune diseases including RA. Over decades, many groups have established several methods for the generation of tolDCs from mouse bone marrow cells. The characteristics of these tolDCs were defined from *in vitro* studies, and their therapeutic potential has been demonstrated in mouse CIA models. CIA models have been used for the development of anti-rheumatic drugs, and are instrumental in the development of tolDC therapy. TolDC therapy in CIA models has provided important proof-of-concept data, suggesting that

even established RA can be controlled by tolDC therapy. Encouraged by the exciting results from mouse CIA models, tolDC therapy has currently been translated into clinical studies in patients with RA. Recently, the first-in-human phase I trial of modified tolDCs in RA patients was successfully completed by the Queensland group, revealing that the tolDC therapy was well tolerated and effective for the treatment of RA. However, several important issues remain to be resolved for further clinical studies. First, the question of which autoantigens should be used for pulsing tolDCs. One important advantage of tolDC therapy over currently available systemic anti-inflammatory drugs is targeting of autoantigen-specific autoreactive T cells. Thus, the efficacy of tolDC therapy in RA patients is believed to be largely contingent upon the autoantigens loaded to tolDCs. However, little is understood about the immunodominant autoantigens among the several reported autoantigens in RA patients. The next issue is the *in vivo* stability of the tolerogenicity of tolDCs after vaccination in the systemic inflammatory environment of RA patients. Another concern is how to measure efficacy of tolDC therapy. TolDC therapy may take time to induce immune tolerance, thus may not result in an immediate reduction of inflammation. It has been observed that some immunotherapies were not effective in the short term, but were appeared to give clinical benefits to patients in the long term. Therefore, special attention should be paid to determine the timing of the end-points. Appropriate biomarkers are urgently required to monitor tolerance induction after tolDC therapy. It is also important that the optimal dose, route, and frequency of administration are determined for each type of tolDCs. Although these issues have been studied in animal experiments, they should be reestablished in human tolDCs to facilitate future development of tolDC therapy in patients with RA. Combination of tolDC therapy with currently available short-term anti-rheumatic drugs and other immunosuppressive drugs is expected to be synergistically effective in RA treatment. It is also conceivable to develop a preventive vaccine with tolDCs for potential RA prevention.

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CONFLICTS OF INTEREST

The authors have no financial conflict of interest.

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