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## REVIEW ARTICLE

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# Tolerogenic dendritic cells: role and therapeutic implications in systemic lupus erythematosus

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### Abstract

Dendritic cells (DCs) are antigen presenting cells that activate T cells and determine the outcome of immune response. In addition to their important function in defense against pathogens, DCs are increasingly recognized as playing a crucial role in the regulation of immune tolerance. Plasticity of DCs with different maturity status and functions enable them to be exploited as potential cell-based therapy to restore immune tolerance in auto-immune diseases. Various *ex vivo* methods have been developed to generate stable tolerogenic DCs that are able to induce and maintain regulatory T cell homeostasis. The beneficial effect of tolerogenic DCs have been studied in murine autoimmune models with promising results. Systemic lupus erythematosus (SLE) is a prototypic multi-systemic autoimmune disease characterized by autoantibody production and deposition of immune complexes in organs. There are evidences that dysregulated DCs play a pivotal role in the initiation and perpetuation of lupus disease. Peripheral blood monocytes in SLE patients were found to have active phenotype with accelerated differentiation into DCs efficient in antigen presentation. Plasmacytoid DCs in SLE patients produce high levels of interferon-alpha, the signature cytokine of this disease, that cause a positive feedback loop in the amplification of activation of innate and adaptive immunity. Furthermore, manipulation of DCs via toll-like receptor knockout in a murine lupus model leads to alteration in disease severity and survival. Thus, tolerogenic DCs may appear as a potential cell-based therapeutic option in SLE.

**Key words:** disease aetiology and pathogenesis – animal models, disease aetiology and pathogenesis – human, innate immunity, systemic lupus erythematosus.

### INTRODUCTION

Dendritic cells (DCs) are innate cells involved in the first line of defense against pathogens. They are highly efficient in antigen presentation to T lymphocytes, resulting in elicitation of the second line of immune defense. The past decades of intensive research in DC biology have revealed a key function of DCs in bridging innate and adaptive immunity, as well as a pivotal role in the regulation of immunity and immune tolerance.<sup>1</sup> DCs exhibit plasticity in their maturity status and func-

tions and have been exploited in clinical application as cell-based vaccine and immunotherapy in cancer, allergy and organ transplantation, and emerge as appealing targets in the treatment of autoimmune diseases.<sup>2</sup>

Systemic lupus erythematosus (SLE) is a multi-systemic autoimmune disease that is characterized by a plethora of circulating autoantibodies<sup>3</sup> with immune complexes deposition in organs leading to inflammation and damage.<sup>4</sup> The mainstay of treatment of SLE involves high-dose corticosteroids and immunosuppressive agents that are associated with significant adverse effects. Extensive research effort has been made in the elucidation of the pathogenesis of lupus and to facilitate identification of key soluble or cellular immune targets that have potential therapeutic

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implications. Recent development of biologic therapies focuses chiefly on B cell-targeted treatment as plasma cells, the terminal effector cells of B cell lineage, and produce autoantibodies and enhanced formation of immune complexes.<sup>5</sup> On the other hand, DCs at the upstream of the dysregulated innate and adaptive immune responses in SLE, have been shown to be involved in the initiation and perpetuation of this disease that form the basis for potential DC-targeted treatment in this condition.

## DCs ARE CONTROLLERS OF IMMUNITY

DCs are distributed ubiquitously in the body, particularly in barrier tissues such as the respiratory and gastrointestinal tracts that are kept under constant surveillance for microbial invasion. DCs respond quickly to environmental stimuli. They are specialized with expression of pattern-recognition receptors (PRRs) including Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors and retinoic acid-inducible gene 1 (RIG-I)-like receptors, that enable them to sense and recognize pathogen-associated molecular patterns (PAMP) on cell surfaces of microbes.<sup>6</sup> Some TLRs are expressed on DC cell surfaces, whereas some are found in the intracytoplasmic compartment that allow DCs to recognize microbial antigens, DNA or RNA,<sup>7</sup> as well as endogenous signals such as damage-associated molecular patterns (DAMPs) which are intracellular factors released by dying cells in the context of tissue injury or inflammation.<sup>8</sup>

DCs are specific antigen-presenting cells and they capture, process and present antigens to T cells leading to activation of antigen-specific T cells, and shaping the immune response by priming and polarizing the differentiation of naïve T cells into different T effector cells.<sup>9</sup> Stimulation of DCs via TLRs leads to their activation and maturation.<sup>10</sup> Activated DCs upregulate expression of chemokine receptor CCR7 and migrate to T-cell areas in secondary lymphoid tissue. Exogenous antigens taken up by DCs are processed and loaded on major histocompatibility complex (MHC) Class II molecules on DC cell surface for T cell recognition. Communication between DCs and T cells occurs upon cognate interaction in the immunological synapse. Activated DCs acquire further maturation upon DC-T cell interaction, and via activation of antigen-specific T cells, adaptive immune response is initiated and is mediated by concerted signals at the DC-T cell encounter.<sup>11</sup> Recognition and binding of peptide-loaded MHC molecules by T cell receptor complex on T cells contributes the first

signal to T cell activation. Induction of DC to maturation leads to expression of high levels of MHC Class II and upregulation of cell surface expression of co-stimulatory molecules, including CD80 and CD86 that binds to CD28 on T cells, and CD40 that binds to CD40L expressed by activated T cells.<sup>12</sup> The second signal generated by these co-stimulatory molecules synergises to augment the strength for T cell activation. Antigen-loaded activated DCs also secrete cytokines which comprises the third signal that drives differentiation of naïve T cells into T cells with distinct effector functions and determines the outcome of the induced immune response. Production of high levels of interleukin (IL)-12 and IL-4 leads to differentiation into T helper (Th)1 and Th2 cells that are involved in cellular immunity and humoral immunity, respectively. Both Th17 and regulatory T cells (Tregs) require transforming growth factor (TGF)- $\beta$  for their induction, whereas high levels of IL-6 favor differentiation toward Th17 cells.<sup>13</sup> Tregs are T effector cells that possess suppressive function on other immune cells and play an important role in the regulation and maintenance of peripheral tolerance. Natural Tregs (nTreg) are derived in the thymus, whereas inducible Tregs (iTreg) are induced by antigens and cytokines in the periphery.<sup>14</sup> Th17 cells produce IL-17, an inflammatory cytokine that mediates the pathophysiology of inflammatory diseases such as rheumatoid arthritis (RA)<sup>15</sup> and SLE.<sup>16</sup>

## DCs ARE REGULATORS OF PERIPHERAL TOLERANCE

Not only are DCs important in immunity, DCs have also been found to play a crucial role in immune tolerance,<sup>17</sup> the breakdown of which leads to emergence of autoimmunity. During T cell development, circulating T lymphocytes that are reactive to self-antigens are eliminated under the process of central tolerance in the thymus. However, some autoreactive T lymphocytes escape elimination and persist in the periphery. Peripheral tolerance acts as another checkpoint in peripheral tissue where autoreactive T cells are curbed from activation and contribution to autoimmunity. At the steady state where there is absence of pathogen or inflammation, DCs exist in immature status and possess high capability of antigen uptake.<sup>18</sup> Immature DCs that have taken up endogenously expressed antigens in the periphery migrate to lymph nodes, interact with T cells and induce peripheral tolerance.<sup>19</sup> Presentation of endogenous antigen to T cells by immature DCs in the absence of co-stimulatory signals results in T cell anergy, a state

of T cell hyporesponsiveness.<sup>20</sup> Induction of peripheral tolerance can be achieved by DC-T cell interaction via mechanisms including T cell deletion, induction of T cell energy, cytokine deviation and induction of Tregs.<sup>21</sup>

## REGULATION OF IMMUNE TOLERANCE IS NOT DC SUBSET-RESTRICTED

DCs are derived from bone marrow precursors and comprise heterogeneous populations. In a simplistic view, there are generally two major subsets, namely, myeloid DCs (mDCs) and plasmacytoid DCs (pDCs), in peripheral blood in humans. These DC subsets express different PRRs and both can elicit immune response depending on the inciting stimuli encountered.<sup>22</sup> Compared to mDCs, pDCs are less capable of antigen uptake and presentation but they are high producers of type I interferon which is essential for antiviral immunity. Homologous counterparts of these two subsets can be found in mice.

The contribution of DCs to immunity and immune tolerance can be illustrated by manipulating these cells in animal models. Experimental ablation in mice of conventional DCs (cDCs) that are homologous to human mDCs, were not found to affect T cell or Treg homeostasis.<sup>23</sup> However, constitutional ablation of all DC subsets in mice is associated with fatal autoimmune manifestations, including circulating anti-nuclear antibodies, increased Th1 and Th17 infiltration into organs and development of inflammatory colitis.<sup>24</sup> On the other hand, exogenous administration of Flt3-ligand that boost DC populations was accompanied by expansion of nTregs and resulted in alleviation of inflammatory colitis<sup>25</sup> and prevention of death in the graft-versus-host model.<sup>26</sup> These findings suggest that DCs are controllers at the interface of immunity and immune tolerance and their regulatory function is not restricted to a particular DC subset.

## THE ROLE OF DCs IN THE PATHOGENESIS OF SLE

Aberrant apoptosis<sup>27</sup> with inefficient clearance of apoptotic cells is an important feature in lupus pathogenesis and provides continual supply of autoantigens. Our group had previously shown that macrophages in SLE patients were deficient in swift removal of apoptotic cells<sup>28</sup> which was related to soluble serum factors,<sup>29</sup> resulting in accumulation of apoptotic materials. Although uptake of apoptotic DCs by immature DCs

has been shown to render DCs tolerogenic,<sup>30</sup> excessive apoptosis leads to secondary necrosis, allowing DC activation and self-antigen presentation to T cells in an immunogenic context.<sup>31</sup> Challenge by necrotic cell- but not apoptotic cell-loaded DCs was found to induce SLE disease in a murine model with susceptible genetic background,<sup>31</sup> suggesting that DCs are key immune cells maintaining immune tolerance in SLE. The role of DCs as regulators of immunity and immune tolerance was further demonstrated by constitutional deletion of both cDCs and pDCs in the MRL/lpr murine lupus model. Although ablation of DCs did not fully abolish T cell activation and inflammatory kidney infiltrate, adaptive immune responses, including expansion and differentiation of autoreactive T cells, plasmablast differentiation and production of serum anti-double-stranded DNA (anti-dsDNA) antibodies were found to be DC-dependent.<sup>32</sup>

Experiments on TLR signaling in DCs and manipulation of TLR expression in a murine lupus model also support a key role of DCs bridging innate and adaptive immunity in the pathogenesis of SLE. Human mDCs express TLR 1, 2, 4, 5 and 8 and are potent inducers of Th1 response. They produce pro-inflammatory cytokines, including tumor necrosis factor (TNF)- $\alpha$  and IL-12 upon stimulation by various ligands.<sup>33</sup> On the other hand, TLR7 and TLR9 are constitutively expressed by pDCs and are involved in immune response toward self DNA and RNA in SLE patients.<sup>34</sup> Gene knockout models of various TLRs have been shown to have different effects on lupus phenotype and disease severity. TLR9 knockout in lupus mice resulted in increased number of activated pDCs and T lymphocytes, accelerated glomerulonephritis and reduced survival.<sup>35</sup> While TLR9 was found to play a protective role in murine lupus, TLR7 signaling was shown to mediate autoantibody production, interferon-alpha (IFN- $\alpha$ ) production and lupus disease progression.

There is increasing evidence revealing the important contribution of dysregulated DCs to the initiation and perpetuation of SLE. Peripheral blood monocytes in SLE patients have a propensity to develop into DCs which have more mature phenotype with expression of higher levels of co-stimulatory molecules and possess higher antigen presentation and T cell stimulatory capacity,<sup>36</sup> an effect mediated by IFN- $\alpha$ . Circulating pDCs in lupus patients have upregulated expression of chemokine receptors that facilitate their infiltration into inflammatory organs such as the kidney in active lupus nephritis.<sup>37</sup> Furthermore, pDCs activated by endogenous ligands, particularly nucleic acid that binds to

immune complexes, produce high levels of IFN- $\alpha$  which represents a characteristic cytokine signature in SLE.<sup>38</sup> IFN- $\alpha$  produced by pDCs causes maturation of mDCs, thus promoting downstream T and B cell activation with formation of immune complexes that in turn, bind to self nucleic acid and stimulate pDCs, forming a positive feedback loop amplifying the dysregulated innate and adaptive immune responses (Fig. 1). IFN- $\alpha$  also has a direct effect on B cells inducing plasma cell differentiation,<sup>39</sup> leading to production of autoantibodies and immune complexes perpetuating the lupus disease. All these evidences suggest that SLE patients may benefit from DC-based therapy in the restoration of immune tolerance.

### EXPLOITATION OF TOLEROGENTIC DCs IN THERAPEUTICS

Plasticity of DCs with different maturity status and functions involved in the regulation of immunity and immune tolerance allows them to be exploited as tolerogenic DCs for their clinical applications in organ transplantation<sup>40</sup> and autoimmune diseases<sup>41</sup> with the goal of maintenance of peripheral tolerance. Antigen uptake and presentation in the context of low co-stimulatory molecules expressed by immature DCs is associated with immune tolerance. On the other hand, mature DCs express high-level MHC and co-stimulatory molecules, have potent antigen presen-

tation and T cell activation capability and produce high levels of IL-12.<sup>42</sup> As safe and efficacious cell-based therapy in autoimmune diseases, it is crucial that tolerogenic DCs remain maturation-resistant without losing their immunomodulatory effect or exacerbating the underlying inflammatory condition. Immature and semi-mature DCs were shown to be unstable and acquire maturity upon encounter with the pro-inflammatory environment after administration to the recipient.<sup>43,44</sup>

In recent years, extensive research has established a variety of *ex vivo* methods which can generate DCs with stable tolerogenic functions to be applied clinically as autologous cell-based therapy. While these DCs may express low to intermediate levels of co-stimulatory molecules, these tolerogenic DCs are characterized by their tolerogenic function with suppressive effects on allogeneic antigen-specific T cells, distinct cytokine profiles with production of low levels of IL-12 but high levels of IL-10, and expression of CCR7 that allows them to migrate to lymph nodes for T cell interaction.<sup>45</sup> Generally, tolerogenic DCs can be derived by intervening DCs at their checkpoints of differentiation, maturation or activation (reviewed in Morelli and Thomson<sup>40</sup>). In brief, DCs can be derived from peripheral blood monocytes and manipulated to acquire tolerogenic function by treatment using immunosuppressive cytokines such as IL-10 and TGF- $\beta$ , or immunosuppressive drugs including cyclosporine, rapamycin, mycophenolate

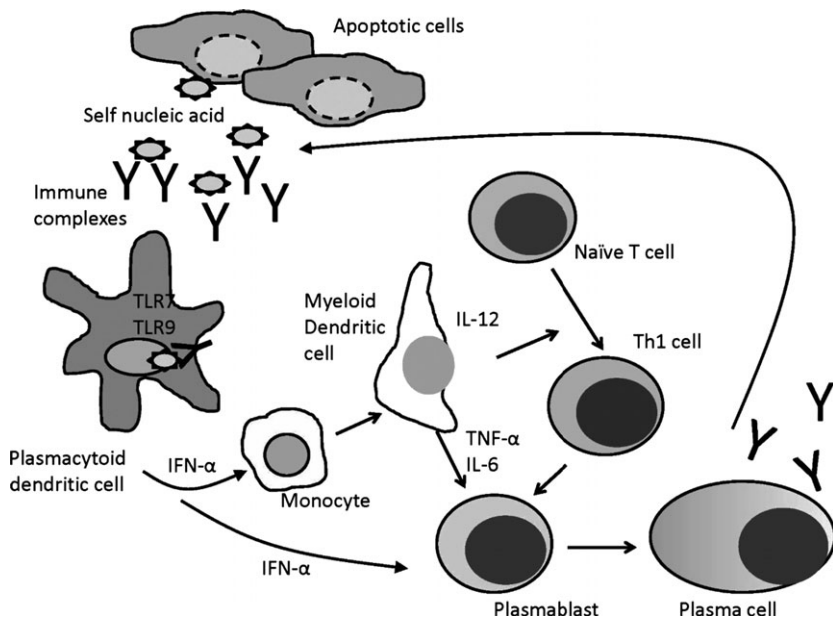
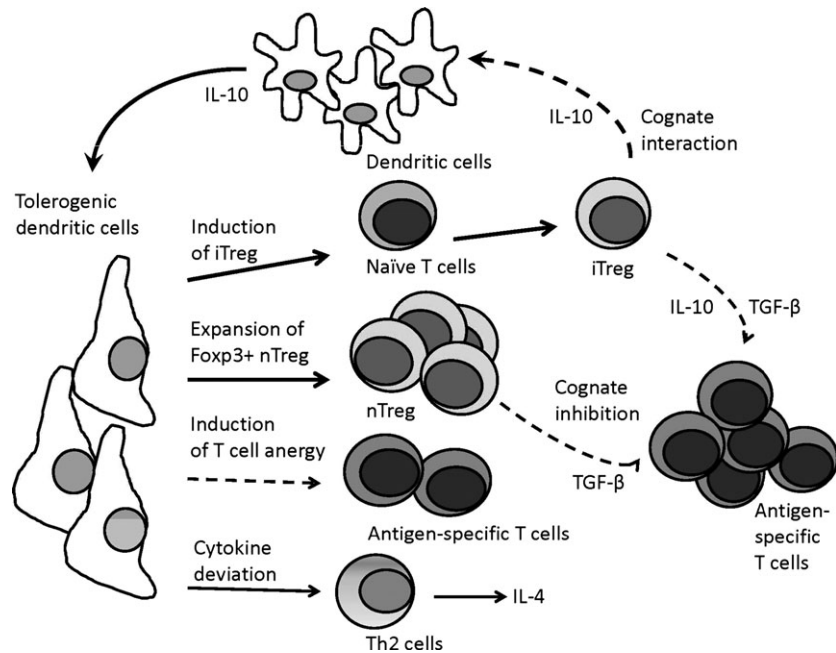


Figure 1 Simplified diagram illustrating the role of myeloid and plasmacytoid dendritic cells mediating innate and adaptive immune response in lupus pathogenesis.



**Figure 2** Positive feedback loop for tolerogenic dendritic cells and regulatory T cells in the maintenance of peripheral tolerance.

mofetil (MMF), vitamin D3, dexamethasone or other agents like N-acetyl-cysteine, glucosamine, human leukocyte antigen G (HLA-G) or cyclic adenosine 3',5'-monophosphate (AMP) inducers such as prostaglandin E2 (PGE2). Methods of genetic engineering can also be applied to generate tolerogenic DCs, involving induction of expression of immunosuppressive cytokines such as IL-10 and TGF- $\beta$  in DCs, and insertion of oligodeoxynucleotide that silences NF- $\kappa$ B pathway-related signaling molecules which are involved in DC maturation.

The effect of tolerogenic DCs in the maintenance of peripheral tolerance are mediated by different mechanisms, including induction of T cell anergy, T cell apoptosis, cytokine deviation to Th2 and expansion of nTreg, Foxp3+ Treg as well as induction of IL-10-producing Treg, depending on the method adopted to generate these tolerogenic DCs. For instance, manipulation by rapamycin<sup>46</sup> was shown to expand Foxp3+ Tregs, whereas vitamin D3 or MMF can induce Treg from naïve T cells.<sup>47</sup> In addition, tolerogenic DCs may also induce peripheral tolerance mediated by induction of expression of indoleamine 2,3 deoxygenase (IDO) or programmed death ligand 1 (PDL-1) on DCs. Combination of PGE2 and TNF- $\alpha$  is an example of generating tolerogenic DCs that express IDO and IL-10.<sup>48</sup> IDO catalyses degradation of tryptophan into kynurenines<sup>49</sup> which leads to suppression of allogeneic T cell proliferation.<sup>50</sup> IDO activity can also lead to enrichment of

nTreg.<sup>51</sup> PDL-1 is an inhibitory molecule on some DCs that are involved in the maintenance of peripheral tolerance.<sup>52</sup> Furthermore, some tolerogenic DCs also express heme oxygenase-1 (HO-1)<sup>53</sup> or Epstein-Barr virus-induced gene 3 (EBI3)<sup>54</sup> that mediates tolerogenicity via inhibition of allogeneic T cell proliferation or induction of IFN- $\gamma$  production by double negative T cells, respectively.

A previous experiment revealed that monocyte-derived DCs in direct cell contact with CD4+ CD25+ Treg acquired a less mature phenotype with impaired antigen presentation capability.<sup>55</sup> These DCs in coculture with Treg express high levels of IL-10 that act on the DCs in an autocrine fashion with induction of negative regulatory molecules such as B7-H4 which have a suppressive effect on proliferation of allogeneic T cells.<sup>56</sup> Indeed, the induced tolerogenic DCs and Tregs isolated *ex vivo*, after induction of tolerance by anti-CD45RB monoclonal antibody and an analogue of immunosuppressive drugs in a transplant model, have been shown to promote further generation of functional Tregs from naïve T cells and generation of tolerogenic DCs from bone marrow progenitors, respectively.<sup>57</sup> Thus, tolerogenic DCs regulate induction and maintenance of homeostasis of Tregs, which in turn inhibit DC maturation and promote generation of tolerogenic DCs, contributing to a feedback loop of tolerogenic DC-Treg interaction in sustaining the maintenance of peripheral tolerance (Fig. 2).

## TOLEROGENIC DCs AS CELL-BASED THERAPY IN ANIMAL STUDIES AND CLINICAL TRIALS

Adoptive transfer of tolerogenic DCs generated by different methods have been studied in a murine model of autoimmune diseases, particularly in collagen-induced arthritis, with promising results.<sup>58</sup> DCs are believed to play a key role in the initiation and perpetuation of RA.<sup>59</sup> In RA, chemokine receptor profile expression suggested that immature DCs were recruited into the rheumatoid joint which functioned as ectopic lymphoid tissue and underwent further development into mature DCs.<sup>60</sup> DCs in rheumatoid synovium and synovial fluid had a more mature phenotype expressing high levels of MHC Class II and co-stimulatory molecules compared to DCs in peripheral blood and these DCs strongly induced T cell activation and proliferation in response to type II collagen.<sup>61</sup> Administration of type II collagen pulsed tolerogenic DCs were shown to alleviate disease severity, induce IL-10-producing T cells and reduce the number of Th17 cells in collagen-induced arthritis.<sup>62</sup> Tolerogenic DCs have also been studied in other murine autoimmune models with alleviating effect on disease severity, such as the administration of  $\beta$ 2-glycoprotein-I loaded tolerogenic DCs in mice with anti-phospholipid syndrome<sup>63</sup> and tolerogenic DCs with silenced expression of CD40 and IL-23 in experimental autoimmune encephalomyelitis.<sup>64</sup> Tolerogenic DCs generated by the plant extract curcumin from bone marrow-derived DCs, were found to induce Tregs that mediated inhibition of antigen-specific colitis in a mouse model.<sup>65</sup>

In humans, IL-10 and TGF- $\beta$ 1 generated tolerogenic DCs from monocyte-derived DCs, that were pulsed with pancreatic islet antigens, and were found to induce antigen-specific T cell hyporesponsiveness and were associated with better glycemic control in patients with type 1 diabetes.<sup>66</sup> Indeed, the first clinical trial applying tolerogenic DCs as cell-based therapy was carried out in 2009 in patients with insulin-dependent diabetes mellitus.<sup>67</sup> These patients were given three doses of bi-weekly subcutaneous injection of tolerogenic DCs which had silenced gene expression of various co-stimulatory molecules. The treatment was not associated with adverse effects or emergence of disease-associated autoantibodies, although no difference in clinical outcome was observed compared to the control group over 12 months. The effect of tolerogenic DCs derived by *ex vivo* treatment using NF- $\kappa$ B inhibitor and pulsed with citrullinated peptides was examined in patients with

active RA. A single dose of these DCs was injected subcutaneously and the treatment was well tolerated with modest improvement in disease activity 3 and 6 months after injection.<sup>68</sup> There is another on-going clinical trial applying intra-articular injection of tolerogenic DCs generated by treatment of vitamin D3 and dexamethasone.<sup>69</sup>

## DC TARGETED THERAPY IN SLE

*In vitro* experiments showed that monocyte-derived DCs from SLE patients in co-culture with iC3b-opsinized apoptotic cells demonstrated tolerogenic properties.<sup>70</sup> Our group applied combinational treatment of vitamin D3 and dexamethasone to monocyte-derived DCs and we found that tolerogenic DCs generated using these pharmacological agents can induce IL-10-producing T cells with regulatory function and downregulate IFN- $\gamma$  and IL-17 production from mature T cells.<sup>71</sup> Tolerogenic DCs as cell-based therapy have not been examined *in vivo* in a murine model or human lupus so far. As SLE involves a diversity of autoantigens, generation of tolerogenic DCs with broader antigen specificities warrants further studies. Indeed, administration of an immunomodulatory peptide p140 derived from small nuclear ribonucleoprotein (snRNP), a spliceosomal autoantigen, to MRL/lpr mice demonstrated a phenomenon of tolerance spreading with broadened immunoreactivity and resultant downregulation of autoreactive T and B cell responses to other spliceosomal proteins and self-antigens associated with SLE.<sup>72</sup> On the other hand, the application of non-antigen loaded tolerogenic DCs together with suboptimal immunosuppression was shown to induce antigen-specific allograft tolerance in an organ transplantation model and was likely related to *in vivo* uptake of endogenous antigens by the injected DCs.<sup>73</sup> Another peptide derived from nucleosomal histone was shown to delay onset of disease, reduce anti-dsDNA antibody levels, suppress Th17 infiltration into the kidneys and prolong lifespan in lupus-prone mice, the effect of which was DC-dependent and involved increased production of TGF- $\beta$ , reduced IL-6 and induction of Tregs.<sup>74</sup> These studies provide evidences to support DC-targeted treatment as an appealing tool in the treatment of SLE.

## TARGETING DCs BEYOND CELL-BASED THERAPY

Recent understanding on the regulatory effects of exogenously administered tolerogenic DCs reveals an

important role of conventional DCs in the lymphoid organs of the recipient.<sup>75</sup> There was evidence to suggest that after the infused tolerogenic DCs migrate to secondary lymphoid tissue, they were taken up by the local quiescent conventional DCs which mediate tolerogenicity via deletion of antigen-specific T effector cells and enhanced numbers of Tregs.<sup>76</sup> These quiescent conventional DCs may also be targeted *in vivo* by administration of immunomodulatory peptides,<sup>72,74</sup> autoantigen-coupled antibodies specific to DC surface receptors such as DEC205<sup>77</sup> and tolerogenic DC-derived microparticles.<sup>78</sup>

## CONCLUSIONS

DCs are immune cells that bridge innate and adaptive immune responses and are critical players at the interface of immunity and immune tolerance. The regulatory function of DCs is not confined to a particular DC subset but is recognized by their functional capability in the induction and maintenance of Treg homeostasis. Plasticity of DCs with different maturity status and functions enable them to be engineered into stable tolerogenic DCs *ex vivo* as potential autologous cell-based therapy in the restoration of immune tolerance in autoimmune diseases. As dysregulated DCs have been shown to be involved in the initiation and perpetuation of SLE, cell-based therapy using tolerogenic DCs may appear as a potential therapeutic option for this disease. Before the goal of DC-targeted therapy becomes a reality in the treatment of SLE, more research is needed to study the DC biology in this disease, the feasibility and clinical applicability of cell-based therapy,<sup>79</sup> such as the best methods of induction of tolerogenicity, dosing, frequency, route of administration and definition of achievement of immune tolerance, as well as issues regarding good manufacturing practice such as generation of clinical-grade immune cells and quality control of these cell-based therapies.

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