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Alternatively activated dendritic cells derived from systemic lupus erythematosus patients have tolerogenic phenotype and function

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KEYWORDS
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Abstract Tolerogenic dendritic cells (DCs) are potential cell-based therapy in autoimmune diseases. In this study, we generated alternatively activated DCs (aaDCs) by treating monocyte-derived DCs from patients with systemic lupus erythematosus (SLE) and healthy subjects with combination of 1,25 dihydroxyvitamin D(3) (vitD3) and dexamethasone followed by lipopolysaccharide-induced maturation. Lupus aaDCs were found to acquire semi-mature phenotype that remained maturation-resistant to immunostimulants. They produced low level of IL-12 but high level of IL-10. They had attenuated allostimulatory effects on T cell activation and proliferation comparable to normal aaDCs and demonstrated differential immunomodulatory effects on naïve and memory T cells. These aaDCs were capable of inducing IL-10 producing regulatory T effectors from naïve T cells whereas they modulated cytokine profile with suppressed production of IFN-γ and IL-17 by co-cultured memory T cells with attenuated proliferation. These aaDCs were shown to be superior to those generated using vitD3 alone in lupus patients.

Abbreviations: aaDCs, alternatively activated dendritic cells; matDCs, mature dendritic cells; Taa, T cells primed by alternatively activated dendritic cells; Tmat, T cells primed by mature dendritic cells; Tvd, T cells primed by vitD3-treated dendritic cells; TolDCs, tolerogenic dendritic cells; vdDCs, vitD3-treated dendritic cells

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1. Introduction

Tolerogenic dendritic cells (TolDCs) have increasingly been studied as cell-based therapeutic regimen in murine models of autoimmune diseases such as diabetes [1], experimental autoimmune encephalitis [2] and inflammatory arthritis [3] with promising results. TolDCs can induce and maintain peripheral T cell tolerance by various mechanisms including induction of T cell deletion, anergy, cytokine deviation, and induction of regulatory T cells (Treg) [4]. TolDCs can be induced by cytokines such as interleukin (IL)-10 [5], tumour necrosis factor (TNF)-α [6] and pharmacological agents [7]. They are characterised by semi-mature phenotype with high expression of co-stimulatory molecules and MHC class II and production of low level pro-inflammatory cytokines such as IL-12, IL-6 and TNF-α [8]. Treatment with active metabolite of Vitamin D, 1,25 dihydroxyvitamin D_3 [1,25$(OH_2)D_3$] (vitD3), and dexamethasone followed by lipopolysaccharide (LPS) maturation has previously been adopted to generate alternatively activated DCs (aaDCs) that are maturation-resistant with tolerogenic function [9,10].

Systemic lupus erythematosus (SLE) is an autoimmune disease that is characterised by dysregulated innate and adaptive immune responses with immune-complexes formation leading to tissue inflammation and organ failure. Current treatment strategies for SLE involve high dose corticosteroid and immunosuppressants that are associated with significant adverse effects. Thus, induction of immune tolerance by tolDCs appears to be an appealing therapeutic tool for this disease. One issue unresolved in SLE patients in this regard was the altered phenotype of peripheral monocytes with accelerated differentiation and maturation into DCs with T cell activating capability [11–13]. It remains controversial if this over-stimulated phenotype of lupus monocyte arises as a result of inherent cellular abnormalities or secondary to an aberrant cytokine and chemokine environment [14]. In this study, we generated aaDCs by vitD3 and dexamethasone from SLE patients with quiescent disease and examined their phenotype and function as tolDCs. We found that aaDCs derived from lupus patients and normal subjects have comparable suppressive effects on allogeneic T cells, can polarise naïve T cells into IL-10 producing Treg and attenuate pro-inflammatory phenotype of memory T cells.

2. Material and methods

2.1. Patients and controls

The study was approved by the ethics committee of the Institutional Review Board of The University of Hong Kong/Hong Kong West Cluster with informed consent from recruited subjects. Patients who satisfied the American College of Rheumatology revised classification criteria for SLE [15] were recruited from the University affiliated lupus clinic. Disease activity was determined according to SLE disease activity index (SLEDAI) [16]. Monocytes and T cells were isolated from patients with quiescent disease for DC derivation and functional DC-T co-culture experiments. Sera was obtained from patients with inactive and active disease (SLEDAI >6) for DC challenge experiments. Age- and sex-matched healthy controls were recruited from staff clinic.

2.2. Generation of mature DCs, vitD3-treated DCs and alternatively activated DCs

Peripheral blood mononuclear cells were isolated from fresh venous blood by density centrifugation on Ficoll-Paque (GE Healthcare, Sweden). $CD14^+$ monocytes were isolated using magnetic microbeads (Miltenyi Biotec, Germany). Monocyte-derived DCs (MDDCs) were generated by culture of monocytes at 1×10^6 cells/ml in the presence of IL-4 and GM-CSF (20 ng/ml each, PeproTech, USA) for 9 days with refreshed medium and cytokines on day 5. Mature DCs (matDCs) were generated on day 8 by addition of LPS (50 ng/ml, Sigma, USA) for 24 h. To generate vitD3-treated DCs (vdDCs), 1,25$(OH_2)D_3$ (1×10^{-10} M, Sigma) was added on day 8 for 24 h in the presence of LPS (50 ng/ml). Alternatively activated DCs (aaDCs) were generated by treating DCs with dexamethasone (1×10^{-6} M, Sigma) on day 5, followed by dexamethasone (1×10^{-6} M), vitD3 (1×10^{-10} M) and LPS (50 ng/ml) on day 8 for 24 h. Cells were cultured in RPMI 1640 supplemented with 10% foetal calf serum, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C with 5% CO_2. In some experiments, MDDCs were challenged by soluble CD40L (sCD40L, 3 μg/ml, PeproTech), CpG-B DNA (10 μg/ml, Hycult Biotech, USA), R848 (1 μg/ml, InvivoGen, USA), interferon (IFN)-α (1000 U/ml, Millipore, USA), 25% inactive or active SLE serum.

2.3. DC-T co-culture experiments

$CD45RA^+/RO^-$ naïve and $CD45RA^-/RO^+$ memory $CD4^+$ T cells were isolated by negative isolation kits (Miltenyi Biotec). MDDCs were co-cultured with allogeneic naïve or memory T cells (1:10) for 6 days. T cell proliferation was assessed by incorporation of ^{3}H-thymidine for the last 18 h of culture measured by scintillation counting (Microbeta TriLux, USA). Recombinant human IL-12 (rIL-12) (5 ng/ml PeproTech), neutralizing anti-IL-10 (10 μg/ml) and isotypic antibody (R&D, USA) were used in some experiments.

2.4. T cell suppression assay

$CD4^+CD25^-$ responder T cells were isolated by depleting $CD25^{hi}$ cells using anti-CD25 beads (Miltenyi Biotec). Allogeneic naïve or memory T cells were primed by DCs (10:1) for 6 days and rested for 4 days in 50 ng/ml rIL-2 (PeproTech). Irradiated (50 Gy) MDDC-primed T cells were co-cultured with allogeneic $CD4^+CD25^-$ responder T cells (1:2) for 5 days in the presence of matDCs (DC:responder T cells 1:10, 1:40, 1:80). $CD4^+CD25^{hi}$ Treg enriched by Treg isolation kit (Miltenyi Biotec) were used as positive control.

2.5. Flow cytometry

To examine the expression of surface markers and intracellular molecules, DCs or T cells were incubated with FcR blocking reagent (Miltenyi Biotec) for 10 min followed by primary antibodies on ice for 30 min. Antibodies used for