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Active Systemic Lupus Erythematosus is associated with decreased blood conventional dendritic cells

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Abstract

Objective—To determine the frequency and functionality of blood conventional dendritic cells (cDC) in relation to disease activity in Systemic Lupus Erythematosus.

Methods—Blood cDC were enumerated for 34 SLE patients, defined as “active” (SLEDAI ≥ 4) or “inactive” (SLEDAI < 4), 26 RA and 8 healthy subjects by FACS. cDC activation was measured by IL-12p40/70 staining following resiquimod stimulation.

Results—The frequency of blood cDC were significantly lower in active compared to inactive patients, however, with comparable cDC functionality.

Conclusion—cDC frequency in active SLE is decreased with no perturbation in cDC function, possibly due to enhanced turnover and/or tissue-specific migration.

Keywords

Systemic Lupus Erythematosus; dendritic cells; disease activity; autoimmunity

INTRODUCTION

Dendritic cells (DC) are professional antigen-presenting cells (APC), mediating both immunity and tolerance. Two major subsets, conventional (cDC) and plasmacytoid DC (pDC), have been described in humans, differing in their expression of surface markers, antigen-presenting capabilities as well as cytokine secretion. cDC (CD11c+/CD123-) are the most potent APC in humans, priming naive T cells to their cognate antigens upon antigen acquisition and activation. pDC (CD11c-/CD123+) are less efficient activators of T cells,

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and are characterized by the secretion of Type-I-interferons (Kis-Toth and Tsokos, 2010). It is postulated that both cDC and pDC significantly contribute to the pathogenesis of SLE, candidate mechanisms being dysregulation in the clearance, sampling and presentation of self-antigens, and cytokine secretion. Reduced numbers of circulating pDC in active SLE subjects have been reported, with cell migration to the periphery and retention of the ability to secrete IFN α (Blanco et al., 2001). Studies have also shown decreased cDC in SLE, however, often without consideration of disease activity or assessment of function during analysis (Blanco et al., 2001; Scheinecker et al., 2001; Crispin and Alcocer-Varela, 2007; Crispin et al., 2012; Nie et al, 2010; Jin et al., 2008; Henriques et al., 2012). Moreover, functional data has relied often on monocyte-derived DC (moDC), a cell type with non-identical, albeit cDC-resembling phenotypic and functional properties (Crispin and Alcocer-Varela, 2007, Nie et al, 2010). This is surprising, considering the role of cDC in the maintenance of immune tolerance, a process that is aberrant in the prototypical autoimmune-disease SLE.

In this study, therefore, we aimed to elucidate blood cDC frequency and activation state in relation to the overall disease activity in SLE patients.

MATERIALS AND METHODS

Study population

Subjects met the ACR criteria for the diagnosis of SLE (Tan et al., 1982). SELENA-SLEDAI scores were determined at the time of each sample collection and all medications were recorded (Petri et al., 2005).

Patients were categorized as active (≥ 4) or inactive (< 4) by SLEDAI score.

All except 3 subjects received immunosuppressive treatment at the time of inclusion into the study. About half of the subjects received “minor” immunosuppression (defined as antimalarials and/or prednisone equivalent of no more than 10 mg/day) and half “major” immunosuppression (defined as more than 10 mg Prednisone equivalent, Methotrexate, Azathioprine and/or Cellcept). No study subject was taking cyclophosphamide or pulse steroids or required hospitalization at the time of a study-encounter.

Healthy human donors and RA subjects fulfilling the 1998 ACR criteria were recruited as disease controls.

The study was approved by the Institutional Review Board of NYU School of Medicine, and informed consent obtained from all study participants in accordance with the Declaration of Helsinki.

Enumeration of DC subsets in Blood

100 μ l of blood were stained with the following antibodies from BD Pharmingen: Lin1-FITC, CD123-PE, HLA-DR-PerCP, and CD11c-APC. Erythrocytes were lysed with ACK lysis buffer (Lanzo) and cells were fixed in 1% paraformaldehyde. The cells were acquired on BD FACSCalibur (BD Biosciences), and analyzed using FlowJo 8.8.3 (TreeStar).

Stimulation of PBMC with TLR7/8 agonist, R848

PBMCs were isolated by gradient using Ficoll-Hypaque (Amersham Pharmacia Biotech) from whole blood. PBMC were stimulated with 1.25 μ M R848 (Resiquimod, 3M Corporation) for 16-20h. Cells were stained with CD11c PE-CY7, HLA-DR APC-CY7, CD123 PercP/Cy5.5, and Lineage cocktail Alexa-Fluor 700 (BioLegend) for identification of DC subsets. Cells were additionally stained with IL-12p40/p70 PE (BD Biosciences). The

cells were acquired by BD LSRII FACSCalibur (BD Biosciences), and analyzed using FlowJo.

Statistical analysis

The unpaired t test was used for the comparison of DC subset frequency in peripheral blood and cytokine production in the groups of SLE patients and control subjects using GraphPad Prism 4. In all cases, *P* values < .05 were considered statistically significant.

RESULTS

Data for enumeration and intracellular cytokine staining were obtained in 56 samples from 34 SLE patients. Of the 56 encounters, 28 were associated with “active” disease and 26 with “inactive” disease with no patients in both groups and multiple visits averaged for analysis.

The percentage of cDC in PBMC was significantly decreased in patients with active compared to inactive disease (.10 $\pm$ SD vs .26 SD respectively, *p* < .008, Figure 1). The frequencies in RA patients (N=26) and healthy controls (N=8) were similar to the values obtained for the active SLE patients (data not shown).

In contrast to the influence of disease activity on the circulating levels of cDC, functional differences were not demonstrated based on IL-12 data available from 21 patients who provided 22 visits. Specifically, following stimulation of PBMC with R848 (TLR7/8 agonist), intracellular staining of IL-12p40/70, a pro-inflammatory cytokine produced by cDC, was equivalent between the groups (Figure 2). As expected, expression of CD40 and CD80 increased after incubation with R848 stimulation (data not shown), and percentages of blood cDC in SLE patients were similar to those obtained from either patients with RA or healthy controls (Figure 3).

DISCUSSION

A significant decrease in the percent of cDC in SLE patients with active compared to inactive disease was demonstrated. However, stimulation of PBMC with R848 led to increased cytokine production per ICS, and upregulation of maturation markers independent of disease activity. Accordingly, whereas frequency of cDC is influenced by or a reflection of SLE activity, the functional capacity of blood cDC is independent of activity and retained. These findings suggest that factors related to disease activity play a role in the subsequent reduction of cDC in the PBMC compartment of active SLE patients, such as increased inflammation which accompanies active disease in addition to secondary necrosis due to failure of APC to clear apoptotic cells. These factors may contribute to enhanced migration of mature blood cDC to peripheral tissues during active disease. This view is further supported by DC migration studies in SLE (Gerl et al., 2010), as well as published observations of Blanco et al. and others that certain SLE-intrinsic *in vivo* factors (e.g. high interferon states, increased High-mobility group box 1), facilitate the *in vivo* differentiation of DC in SLE (Kis-Toth and Tsokos, 2010; Urbonaviciute et al., 2008).

In summary, we suggest SLE-associated factors (and factors related to SLE disease activity states), such as decreased clearance of apoptotic material and inflammation, to be responsible for decreased blood cDC frequency with no perturbation in their function. These findings can partially reconcile contradictory reports on cDC frequencies and activation stages in the literature and may form the basis for a more thorough understanding of their dynamic regulation in human SLE.

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Figure 1. Percentage of blood cDC in Peripheral blood mononuclear cells (PBMC) in “inactive” and “active” SLE patients

Figure 2.

Fold increase in IL-12p40/70 in cDC by intracellular cytokine staining (ICS) after 16-20h stimulation of PBMC with TLR7/8 agonist, R848. IL-12p40/70 is a pro-inflammatory cytokine produced by cDCs. ICS is a flow cytometry-based method for the detection of cytokines in a cell-specific way.

Figure 3.
Percentage of blood cDC in Peripheral blood mononuclear cells (PBMC) in SLE, RA and Healthy controls