Peripheral Blood Dendritic Cells Secrete Lower Levels of Pro-Inflammatory Cytokines in Multiple Sclerosis

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INTRODUCTION

Generation of an immune response requires presentation of antigen to immune cells. Dendritic cells (DCs) are considered professional antigen presenting cells and may be important in the presentation of self-antigens during an auto-immune response. Recent work suggests that DCs are present and activated in the brain and cerebrospinal fluid (CSF) of MS patients, and that circulating DCs are activated and prime an inflammatory immune response. Data from mouse experiments indicates they are key mediators of the immune response in experimental allergic encephalomyelitis (EAE). We postulated that during periods of relative disease stability pro-inflammatory responses of DCs may be dampened.

METHODS

Cell Separation and Culture: Peripheral blood was obtained from healthy donors (HD) and MS patients with stable disease (with or without treatment). Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation (BD vacutainer CPT tubes) and myeloid DCs (mDCs) purified by magnetic separation using CD1c-microbeads (Miltenyi Biotec). All patient samples were obtained by informed consent and IRB approved.

Table 1. Patient Population Demographics

	#	Treatment	Disease	EDSS± SD	Active
MS	20	8 natalizumab, 6 Avonex, 2 rituximab, 3 methotrexate	10 SP, 5 PP, 5 RR	4.6 ± 2.1	5/20
HD	9	N/A	N/A	N/A	N/A

* EDSS = Expanded disability status scale

DC purity was measured by flow cytometry after staining with the lineage-1 markers: anti-human CD3-, 14-, 16-, 19-, 20-, and 56-FITC (lin-1) and the DC marker CD11c-PE (Figure 1). Populations with greater than 90% purity were plated in RPMI-10 medium -/+ 1 μgmL^{-1} human CD40L (R&D Systems).

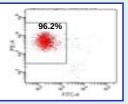

Figure 1. DC Purity Analyzed by Flow Cytometry. MACS purified cells were stained with lin-1-FITC and CD11c-PE markers to asses population purity. Gating was determined using isotype control antibodies. Red = mDCs

Dendritic Cell Analysis: 24 hours later supernatants were taken for cytometric bead array (CBA) kits on a FACSAria™ (BD Biosciences) for quantification of cytokine secretion. DC activation was measured by flow cytometry of surface markers HLA-DR-APC, CD40-APC, CD80-FITC, CD83-FITC (all antibodies from BD Biosciences).

RESULTS

Recovery of Dendritic Cells

During isolation initial numbers of PBMCs and numbers of recovered mDCs were noted to determine any difference between healthy donors and MS patients.

Although lower numbers of mDCs were recovered from MS patients compared to healthy donors this difference was not found to be significantly different (Table 2).

Table 2. Recovery of Dendritic Cells.

	Total PBMCs	Ave. # DCs	% DC/PBMC
MS	$4.5x10^7 \pm 3.0x10^7$	$1.9x10^5 \pm 1.7x10^5$	0.47 ± 0.30
HD	$6.0x10^7 \pm 4.5x10^7$	$2.0x10^5 \pm 7.9x10^4$	0.54 ± 0.31

Activation Status of mDCs

Peripheral blood mDCs were analyzed 24 hours post-stimulation -/+ hCD40L by flow cytometry after staining for surface marker expression (Figure 2).

- HLA-DR expression was high -/+ hCD40L, CD40, CD80 and CD83 was low on unstimulated mDCs in HD and MS patients but increased on hCD40L stimulation. There was no significant difference between HD or MS (Figure 2A and B).
- The staining intensity was higher for HLA-DR in MS patients compared to HDs -/+ hCD40L stimulation, indicating increased HLA-DR expression in MS patients (Figure 2A).

Cytokine Secretion by mDCs

Cytokine secretion was measured 24 hours post-stimulation -/+ hCD40L (Figure 3). IFNγ, IL-12p70, IL-2, IL-5 levels were below the limit of detection for this assay (20 $pgmL^{-1}$).

- IL-4 levels were not significantly different between HD and MS mDCs (data not shown).
- IL-1β, IL-6 (below the limit of detection in the MS -hCD40L sample) and IL-10 levels were found to be significantly lower from unstimulated MS patient mDCs compared to HD mDCs (Figure 3. p = 0.01, 0.0005, and 0.006 respectively).
- TNFα levels were found to be significantly lower from hCD40L stimulated MS mDCs compared to HD mDCs (Figure 3. p = 0.032).
- IL-8 levels were significantly lower from MS mDCs -/+ hCD40L stimulation (Figure 3. p = 0.001 and p = 0.027 respectively).

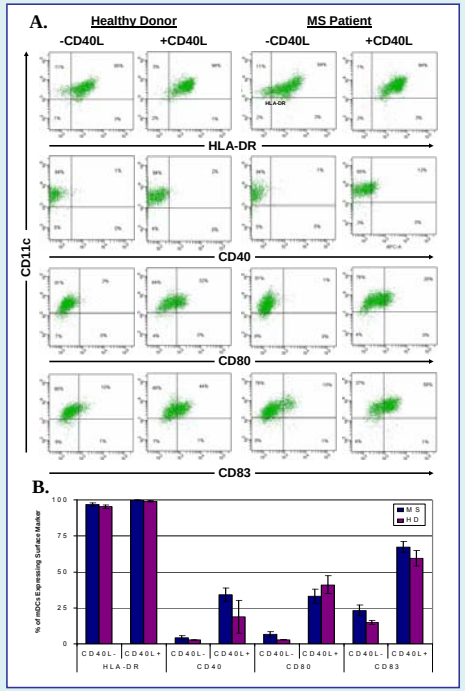

Figure 2. Activation Status of Dendritic Cells. A. Example of surface marker expression for one healthy donor and one MS patient. B. Mean surface marker expression in healthy donors and MS patients. Surface marker expression was analyzed by antibody staining 24 hours post-stimulation -/+ hCD40L on a FACSAria flow cytometer.

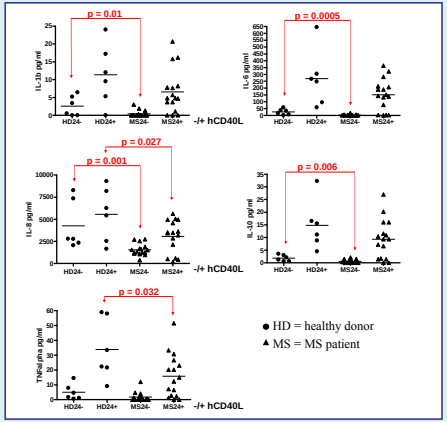

Figure 3. Cytokine Secretion from Dendritic Cells. Cytokine secretion from mDCs was measured 24 hours post-stimulation -/+ hCD40L using CBA.

CONCLUSIONS

- mDCs from MS patients are activated and express surface activation markers, including CD83. However they secrete lower levels of pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, IL-10 and TNFα.
- Despite their activation, the responses of peripheral mDCs during stable MS are suppressed.
- No significant differences were found between disease type or whether patients were on treatment or not.
- Further studies ongoing: Analysis of mDC stimulated T cell responses, studying treated vs treatment naïve mDC responses during active and inactive disease.

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