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Siglec-1-positive plasmacytoid dendritic cells (pDCs) in human peripheral blood: A semi-mature and myeloid-like subset imbalanced during protective and autoimmune responses

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ABSTRACT

Plasmacytoid dendritic cells (pDCs) play a central role in the pathogenesis of systemic lupus erythematosus (SLE) as IFN-α producers and promoters of T-cell activation or tolerance. Here, we demonstrated by flow-cytometry and confocal microscopy that Siglec-1, a molecule involved in the regulation of adaptive immunoresponses, is expressed in a subset of semi-mature, myeloid-like pDCs in human blood. These pDCs express lower BDCA-2 and CD123 and higher HLA-DR and CD11c than Siglec-1-negative pDCs and do not produce IFN-α via TLR7/TLR9 engagement. In vitro, Siglec-1 expression was induced in Siglec-1-negative pDCs by influenza virus. Proportions of Siglec-1-positive/Siglec-1-negative pDCs were higher in SLE than in healthy controls and correlated with disease activity. Healthy donors immunized with yellow fever vaccine YFV-17D displayed different kinetics of the two pDC subsets during protective immune response. PDCs can be subdivided into two subsets according to Siglec-1 expression. These subsets may play specific roles in (auto)immune responses.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease caused by the loss of tolerance to self antigens and the development of autoantibodies. It is characterized by multiple organ manifestations, which may be quite severe, culminating in organ failure. SLE is associated with high serum levels of IFN-α [1], which were found to correlate with several biological and clinical manifestations [2]. Plasmacytoid dendritic cells (pDCs) are suggested to play a key role in the pathogenesis of SLE mainly because they produce high levels of IFN-α [3]. Moreover, SLE patients have low numbers of blood pDCs [4–6], and SLE pDCs exhibit altered migratory behavior [7].

Plasmacytoid dendritic cells in human peripheral blood were identified as lineage −/CD11c −/CD123 ++/BDCA-2 + cells [8] with CD11c used as a classical marker for myeloid DCs, or simply as lin −/MHCII +/BDCA-2 + [9]. However, pDCs display high phenotypic and functional plasticity and are described as unique professional effector cells of the immune system, that is, they are not terminally differentiated and may respond to the inflammatory milieu, orchestrating an immune response [10]. Besides their innate ability to produce IFN-α, pDCs can further differentiate into professional APCs or pDC-derived DCs, thus losing innate characteristics, such as their ability to express BDCA-2 and IFN-α; [8,10,11] the maturation process is also characterized by the upregulation of co-stimulatory factors (CD86, CD80, CD40) and of maturation marker CD83 [12], resulting in an increased T-cell stimulatory capacity [10]. Alternatively, pDCs may assume an adaptive function that promotes peripheral T-cell tolerance [13–15].

Siglec-1 (sialoadhesin, CD169) is an adhesion molecule first characterized on cells of macrophage-monocyte lineage [16]. Siglec-1 is suggested to play a primary role in cell–cell interactions (e.g. with T cells via CD43) [17] and adhesion events [18,19]. It has been proposed that these features may be important in the regulation of adaptive immune responses [18]. It was indeed shown that Siglec-1 expression on monocytes promotes T-cell activation and pro-inflammatory cytokine secretion [17,18,20]. Moreover, Siglec-1 expression on monocytes was identified as an IFN-α-regulated marker for active disease in SLE [21] and can be used as indirect marker for IFN-α serum levels [22]. These findings support the idea that Siglec-1 might play a role in adaptive

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immune regulation of SLE. Conventional dendritic cells do normally not express Siglec-1. However, its expression can be induced on human monocyte-derived dendritic cells following exposure to rhinovirus in vitro, and this expression partly correlates with the acquisition of an immunosuppressive phenotype by dendritic cells [23]. Therefore, it is possible that Siglec-1 is immunostimulatory when expressed by macrophages and/or monocytes and immunosuppressive on dendritic cells [18]. This hypothesis has remarkable importance in the biology of dendritic cells, particularly pDCs, considering their central role in the orchestration of immune responses.

The expression of Siglec molecules in human pDCs has not been described until now, and studies investigating the role of Siglec molecules in the context of autoimmune diseases in which Siglecs might participate in the promotion of autoimmunity or tolerance are scarce. Considering the impact of Siglec-1 on adaptive immunity and its nature as an IFN-α regulated molecule, we therefore conducted the present study to analyze the expression of Siglec-1 in pDCs with focus on SLE pathogenesis.

We found a Siglec-1-expressing subset of pDCs exhibiting a myeloid-like, semi-mature phenotype in the peripheral blood of both healthy donors and SLE patients. Siglec-1 expression could be induced in vitro by flu antigen. Homeostasis of Siglec-1 expressing pDCs appears to be imbalanced in SLE patients correlating with disease activity. Using a model of yellow fever vaccination for monitoring Siglec-1-expressing pDCs, we showed that this pDC subset may also play an important role in protective immune response.

2. Materials and methods

2.1. Study participants

SLE patients who fulfilled the American College of Rheumatology Criteria [24,25] were recruited from the Charité Universitätsmedizin Berlin Department of Rheumatology and Clinical Immunology. Healthy gender-matched volunteers were included as controls. Patients and controls with acute infections, controls suffering from an autoimmune disease, and patients with another autoimmune disease in addition to SLE were excluded. Disease activity was evaluated using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [26].

Peripheral blood from 22 SLE patients (100% female, 100% Caucasian, mean age 41 years, range 21–61) and 25 healthy control volunteers (100% female, 96% Caucasian, 4% Asian, mean age 33 years, range 20–63) was collected in heparinized tubes by venipuncture. At the time of venipuncture, five of the patients were not on SLE medications, one was on hydroxychloroquine, and the remaining 16 were on prednisolone (average 12.5 mg/d, range 3.5–100 mg/d). Thirteen of the latter sixteen additionally received azathioprine (n = 7), mycophenolate mofetil (n = 5), belimumab (n = 2), or hydroxychloroquine (n = 11).

The study was approved by the Ethics Committee of the Medical Faculty of Charité Universitätsmedizin Berlin. Written informed consent was obtained from all participants.

2.2. Immunophenotyping of blood DCs

Dendritic cells were immunophenotyped by incubating 200 μl whole blood with anti-CD14-PacB IgG1 (clone TM1, German Rheumatism Research Centre, DRFZ), anti-CD19-PacB IgG1 (DAKO/Biozol, Eching, Germany), anti-BDCA-2-APC IgG1, anti-BDCA-1-FITC IgG2a (Miltenyi-Biotec, Bergisch-Gladbach, Germany), anti-CD3-PacB IgG2a, anti-CD11c-APC/Cy7 IgG1, anti-CD123-PerCP/Cy5.5 IgG1, anti-CD16-PECy7 IgG1, anti-CD83-APC IgG1 (all Biolegend, London, UK), anti-HLA-DR-V500 IgG2a, anti-CD56-PE/Cy7 IgG2b, anti-CD86-FITC IgG1 (all BD Biosciences, Heidelberg, Germany), anti-Siglec-1-RPE IgG1 (AbD Serotec, Kidlington, UK) in the presence of 1 mg/ml Beriglobin (CSL Behring, Germany). Afterwards, erythrocyte lysis was performed using FACS Lysing Solution as recommended by the manufacturer (Becton Dickinson, Heidelberg, Germany). Cells were then washed twice with PBS supplemented with 0.5% (*w*/*v*) bovine serum albumin and 2 μM EDTA. Immunostainings were analyzed by acquisition of the whole sample on a FACSCanto flow cytometer (Becton Dickinson, Heidelberg, Germany) equipped with FACSDiva software, and were further analyzed by FlowJo 7.6.5. (TreeStar, Ashland, OR, USA). Specific staining results were expressed as relative fluorescence intensity (RFI) by calculating the median fluorescence intensity ratio of a specifically stained probe to the respective isotype control.

2.3. In vitro stimulation of pDCs

Intracellular IFN-α expression in pDCs in the blood of healthy donors was studied on the basis of a protocol by Della Bella et al. [27] One milliliter of whole blood was cultured in 2 ml of serum-free RPMI 1640 (GibcoBRL/Invitrogen, Karlsruhe, Germany) with 10 μg/ml TLR7 ligand (Imiquimod-R837) (InvivoGen, San Diego, CA, USA) for 5 h at 37 °C with 5% CO_2. 10 μg/ml Brefeldin A (Sigma-Aldrich, St. Louis, MO, USA) was added after 1 h. Cells were harvested, subjected to common erythrocyte lysis in ammonium chloride solution, and then washed with PBS and stained for surface molecules (see above). The cells were then fixed and permeabilized for 30 min with the Leucoperm kit (AbD Serotec, Kidlington, UK) in the presence of anti-IFN-α-FITC IgG1 (pbl, Piscataway, NJ, USA) or isotype antibody. They were then washed again and analyzed by flow cytometry.

Alternatively, TLR9-dependent IFN-α expression in pDCs was studied in peripheral blood mononuclear cells (PBMCs) obtained by density gradient separation using Ficoll-Paque (Amersham Pharmacia Biotech, Freiburg, Germany). Cells were seeded in 12-well plates at a density of 6×10^6/2 ml RPMI 1640 (GibcoBRL/Invitrogen, Karlsruhe, Germany) and cultivated with 10 μg/ml CpG-A (ODN 2216, Hycult Biotech, Beutelsbach, Germany) in the presence of human AB serum (GemCell™, Gemini Bioproducts, West Sacramento, CA, USA) for 10 h. After 2 h, 5 μg/ml Brefeldin A (Sigma-Aldrich, St. Louis, MO, USA) was added. The cells were harvested and the plates washed with PBS/BSA/EDTA to detach all cells. After PBS washing, the cells were stained with the LIVE/DEAD Fixable Violet Dead Cell Stain Kit (Molecular Probes/Invitrogen, Paisley, UK) for 10 min. The surface molecule antibodies (see above) were then added and the cells were washed 10 min later. Intracellular IFN-α staining was then performed as described above.

In vitro induction of Siglec-1 expression in pDCs was studied in whole blood and sorted pDCs by stimulation with influenza virus vaccine (Begripal 2011/2012, Novartis) 1:10 in serum-free RPMI 1640 or 120 U/ml human interferon alpha sampler (PBL interferon source, Piscataway, NJ) for 18–20 h at 37 °C and 5% CO_2, in the presence of 10 ng/ml IL-3 (R&D Systems, Minneapolis, MN, USA). Siglec-1-negative pDCs were sorted from PBMCs in two steps. First, cells were incubated with PE-labeled antibodies against CD3, CD14, CD19 (all in-house antibodies from DRFZ) and Siglec-1 (AbD Serotec, Kidlington, UK) following incubation with anti-PE-magnetic beads (Miltenyi Biotec, Bergisch-Gladbach). Next, they were passed through two LS columns by collecting the negative fraction, which was further sorted with the anti-BDCA-4 MACS kit (Miltenyi Biotec, Bergisch-Gladbach). In case of negligible amounts of Siglec-1-positive pDCs, cells were sorted solely via anti-BDCA-4 MACS. Sorted pDCs were seeded in 96-well-plate at a density of 4×10^4 cells/200 μl medium. For whole blood stimulations, 1 ml of heparinized blood was cultured in 1 ml serum-free RPMI 1640. After culture, cells were harvested, washed with PBS, and stained for FACS analysis with anti-CD123-PerCP, anti-HLA-DR-V500, anti-BDCA-1-FITC and anti-CD11c-APC/Cy7. Anti-Siglec-1-A647 (AbD Serotec, Kidlington, UK) was used in Siglec-1 negatively sorted pDCs to discriminate new generated Siglec-1-positive pDCs from Siglec-1-PE labeled residues from MACS sort. For BDCA-4 sorted as well as for whole blood pDCs, Siglec-1-RPE (AbD Serotec, Kidlington, UK) was used to detect Siglec-1 expression. All stimulations were performed in duplicate. IFN-α