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Identification of novel dendritic cell subset markers in human blood

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ABSTRACT

Human dendritic cells (DC) are key regulators of innate and adaptive immunity that can be divided in at least three major subpopulations: plasmacytoid DC (pDC), myeloid type 1 DC (mDC1) and myeloid type 2 DC (mDC2) exhibiting different functions. However, research, diagnostic and cell therapeutic studies on human DC subsets are limited because only few DC subset markers have been identified so far. Especially mDC2 representing the rarest blood DC subset are difficult to be separated from mDC1 and pDC due to a paucity of mDC2 markers. We have combined multiparameter flow cytometry analysis of human blood DC subsets with systematic expression analysis of 332 surface antigens in magnetic bead-enriched blood DC samples. The initial analysis revealed eight novel putative DC subset markers CD26, CD85a, CD109, CD172a, CD200, CD200R, CD275 and CD301 that were subsequently tested in bulk peripheral blood mononuclear cell (PBMC) samples from healthy blood donors. Secondary analysis of PBMC samples confirmed three novel DC subset markers CD26 (dipeptidyl peptidase IV), CD85a (Leukocyte immunoglobulin-like receptor B3) and CD275 (inducible costimulator ligand). CD85a is specifically expressed in mDC1 and CD26 and CD275 represent novel mDC2 markers. These markers will facilitate human DC subset discrimination and additionally provide insight into potentially novel DC subset-specific functions.

1. Introduction

Dendritic cells (DCs) are a heterogeneous group of professional antigen presenting cells playing essential roles in the induction and regulation of immune responses to allo- and autoantigens in infectious diseases, cancer, autoimmunity, transplantation and vaccination [1–4]. Human blood DC are of special interest for both research and clinical application because they are easily accessible. However, human blood DC can be separated in functionally distinct subsets, exhibiting a complex phenotype and are present in peripheral blood of healthy individuals at very low frequencies ranging from 0.0% to 0.59% of all leukocytes [5]. Based on a consensus report, human blood DCs are divided in three major subsets that are plasmacytoid DCs (pDC) and 2 types of myeloid DCs (mDC1 and mDC2) according to the expression of CD303, CD1c and CD141, respectively [6]. Importantly, for proper DC subset discrimination additional staining of lineage marker, at least CD19+ and CD14+ has been recommended to exclude B cells and monocytes co-expressing CD1c and CD141 [6,7].

Accurate separation of blood DC subsets is critical because an increasing number of publications have reported unique functional features not only for pDC and mDC but additionally for mDC1 and mDC2 subsets. MDC1 have been reported to phagocytose Escherichia coli and to suppress T cell proliferation in an IL-10 dependent manner in contrast to mDC2 [8]. MDC2 have been revealed to represent a unique myeloid DC subset cross-presenting antigens to CD8α+ cytotoxic T cells indicating that mDC2 may be particularly effective in DC vaccination trials [9–12]. However, mDC2 represent the rarest DC subset in peripheral blood with mean absolute numbers of only one mDC2/μl whole blood in healthy individuals [5,13]. In contrast, mDC1 and pDC have been reported to be >10–15-fold more frequent in healthy blood donors [5,13]. With request to mDC2, only very few DC subset markers have been described so far [7].

Here we have systematically analyzed surface markers that are broadly available to the research community for their capacity to serve as human blood DC subset markers. We have performed a stepwise approach, where we first identified novel candidate markers in DC-enriched blood samples and subsequently tested the discriminatory capacity and usability of these markers in bulk peripheral blood mononuclear leukocyte (PBMC) samples of healthy blood donors. We have identified three novel human DC subset markers that will facilitate discrimination of mDC1 (CD85a) and the rare mDC2 subset (CD26, CD275).

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2. Materials and methods

2.1. Cell separation and enrichment of blood DC

Human PBMC were isolated from buffy coats of healthy blood donors after informed consent by Ficoll-Paque (Pharmacia, Germany) density gradient centrifugation. The in vitro studies of human blood samples were approved by the ethic study board of the University Hospital Gießen (File Nr 05/00). Blood DC were enriched in some experiments in a two-step immunomagenetic purification process using the Blood Dendritic Cell Isolation Kit II (Miltenyi Biotec) according to the manufacturer's instructions.

2.2. Multiparameter flow cytometry

Flow Cytometry analyses were performed on a FACS Canto II cytometer equipped with FACS Diva software (Becton Dickinson, San Jose, CA, USA). Monoclonal antibodies were from Biolegend, Germany, unless otherwise indicated. DC-enriched or bulk PBMC sample were incubated for 30 min at 4 °C in PBS ph 7.2 (PAA laboratories, Germany), 2 mM EDTA (Sigma, Germany) and 2% BSA (Serva, Germany) with consensus monoclonal antibodies for mDC1 (CD1c), mDC2 (CD141) and pDC (CD303, Miltenyi Biotec, Germany) [6] and a lineage cocktail (CD14, CD16, CD19, CD20, CD56, TCR αβ) to unambiguously exclude monocytes, B cells, NK cells and T cells. In some experiments with bulk PBMC samples, a reduced lineage cocktail (CD14, CD19) excluding only monocytes and B cells was used. Unspecific Fc-receptor mediated antibody binding was inhibited through pre-incubation with IgG (Gammunex, Talecris, Germany) at a concentration of 0.15% for 15 min at 4 °C. Multiparameter DC subset staining was combined with a panel of PE-labelled mAbs (20 min, 4 °C) targeting 332 surface antigens (http://www.biolegend.com/legendscreen) and 10 isotype controls (Legendscreen™ Human Cell Screening, Biolegend, Germany). Fluorescence minus one controls (FMO) were used consistently to control for specificity of surface antigen staining. 25,000–40,000 events were analyzed in surface antigen screening experiments with DC-enriched samples. 1.5–2 million events were analyzed in confirmatory experiments with bulk PBMC samples. Sytox dye was used according to the manufacturer instructions as a vital dye to exclude non-specific staining of dead cells (Biolegend, Germany).

2.3. Generation of DC

Monocyte-derived DC were generated as described [14,15], with minor modifications. Briefly, CD14+ monocytes were purified (>95%) by flow cytometry sorting on a BD FACS Aria III cells sorter (Becton Dickinson, San Jose, CA, USA) using fluorochrome-labelled CD14 monoclonal antibody (Biolegend, Germany) and 1×10^6 cells were cultured for 6–7 days in 1 ml DC medium in 24 well flat bottom plates (Greiner, Frickenhausen, Germany). DC medium contained RPMI 1640 (PAA Laboratories, Linz, Austria), L-Glutamine (PAA Laboratories), penicillin/streptomycin (PAN Biotech, Aidenbach, Germany), sodium-pyruvate (Gibco, Carlsbad, USA), nonessential aminoacids (Sigma), Hepes buffer (Gibco), 10% heat inactivated FCS (PAA Laboratories), 1000 IU/ml recombinant human GM-CSF (Promocell, Heidelberg, Germany) and 1000 IU/ml recombinant human IL-4 (Strathmann, Hamburg, Germany). After 3 days, 50% supernatant was replaced with fresh DC medium. Monocyte-derived DC differentiation (CD1a+ $CD14^{neg}$) was controlled by flow cytometry [15]. In some experiments, DC were stimulated at day 6 with 1 μg/ml LPS (Sigma, Germany) for 16 h.

3. Results and discussion

3.1. Identification of novel human DC subset marker candidates

In order to acquire a sufficient high number of human DC, including the rare mDC2 subset we first systematically screened DC-enriched PBMC preparations from buffy coats of healthy blood donors. Human DC-enriched PBMC samples were subsequently stained with consensus DC subset surface markers (CD1c, CD141, BDCA-2) and a lineage cocktail to unambiguously exclude contam-

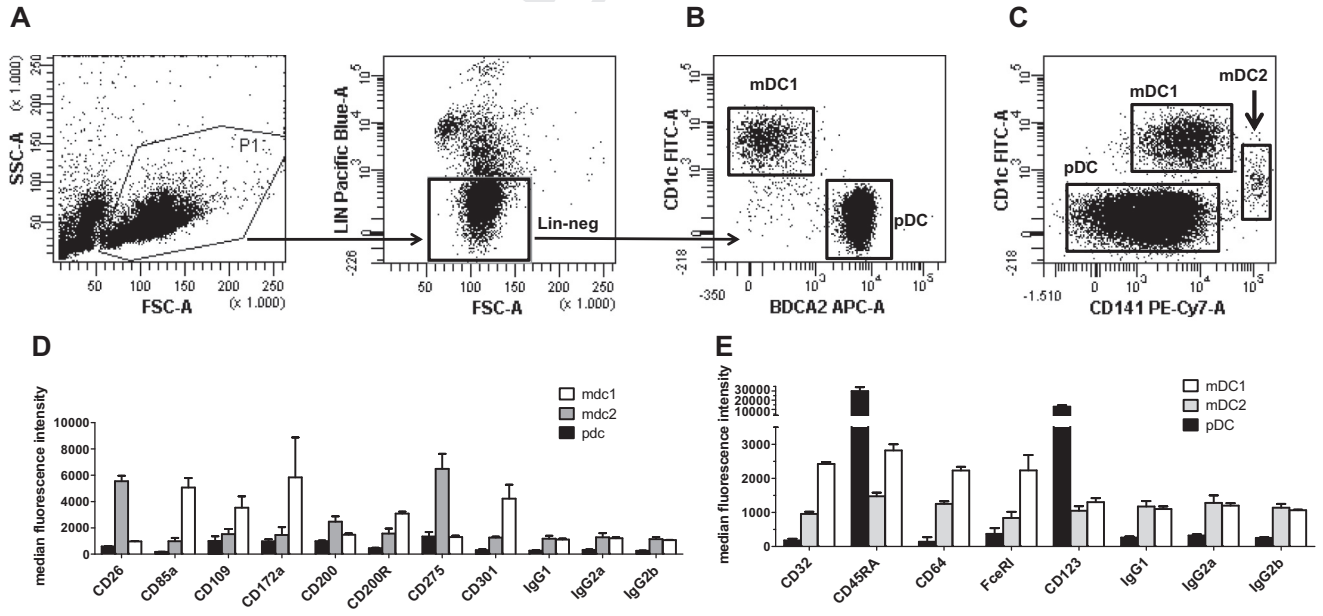

Fig. 1. Identification of human DC subset marker candidates by flow cytometry. DC-enriched blood samples were dissected into mDC1, mDC2 and pDC according to consensus markers CD1c, CD303 (BDCA-2) and CD141 (A–C) and screened for 332 PE-labelled surface markers. Analysis revealed eight DC-subset marker candidates according to signal–noise ratio in comparison to isotype-matched FMO controls (D). Previously reported differentially expressed surface markers CD32 (mDC1), CD45RA (pDC), CD64 (mDC1), FcεRI (mDC1), and CD123 (pDC) served as internal controls (E).

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