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Maturation and cytokine production potential of dendritic cells isolated from rheumatoid arthritis patients peripheral blood and induced *in vitro*

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ABSTRACT

Background: Since dendritic cells (DC) are involved in the development of autoimmune inflammation, researchers consider DC both as target cells for specific therapy of rheumatoid arthritis (RA) and as candidate cells for the development of cell-based methods to treat autoimmune diseases. The development of treatment strategies requires comprehensive research into the quantitative and qualitative characteristics of DC subtypes both ex vivo from RA patients and *in vitro*, to determine the possibility of inducing functionally mature DC in RA.

Objective: To study the phenotypic and functional properties of myeloid (mDC) and plasmacytoid (pDC) DC isolated from the peripheral blood of patients with RA and induced *in vitro*.

Materials and methods: Blood samples were obtained from RA patients and healthy donors. Immature DC in the whole blood and *in vitro* induced DC were characterized by the positive expression of CD80, CD83, CCR7, IL-10, IL-4, IL-12 and IFN-α. R848 and lipopolysaccharide were used to determine DC maturation ability. From PBMCs of RA patients and health donors DCs with myeloid (imDC) and plasmacytoid (ipDC) phenotype were induced.

Results: The relative count of mDC in the peripheral blood between studied groups did not differ. pDC count was significantly lower for RA patients. DC from RA patients were characterized by low expression levels of CD80 and CD83 on both populations cells and high expression of CCR7 only on pDC. An increase in pDC producing IL-12 and IFN-α and a decrease in mDC and pDC producing IL-4 and IL-10 were shown in RA. imDC and ipDC obtained from RA patients according to their phenotype and cytokine profile did not differ from those obtained from healthy donors.

Conclusions: There is an imbalance between subpopulations of DC in the peripheral blood of RA patients. DC of RA patients are less mature. The data suggest the involvement of DC in RA pathogenesis and confirm DC participation in balance shift towards Th1-type immune responses. At the same time, *in vitro* induced RA DC are phenotypically and functionally competent.

1. Introduction

Dendritic cells (DC) are antigen-presenting cells originating from bone marrow, which localize in lymphoid organs and initiate and regulate primary and secondary immune responses [1]. DC possess the unique ability to activate naïve T lymphocytes during their first contact with antigen [2].

There are two main subtypes of DC, myeloid (mDC) and plasmacytoid (pDC), in the peripheral blood defined by the expression of specific markers on the cell surface. Immature DC, irrespective of their subtype, have low expression of CD80, CD83 and CD86 [3]. mDC are highly potent in antigen capture and presentation and therefore can effectively stimulate T-cells [4]. pDC possess the unique property to produce large amounts of IFN-α/β following exposure to viral components [5].

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DC are the subject of intensive research in several diseases. Most studies of DC in patients with rheumatoid arthritis (RA) have shown the important role played by DC in initiation and maintenance of inflammation by presenting antigens to autoreactive T cells [6,7]. One mechanism allowing DC to regulate autoimmune processes in RA is the production of several cytokines required for the differentiation of T-helper cells. In RA, different DC subtypes produce both inflammatory and anti-inflammatory cytokines that influence autoimmune disease progression in different ways, defining the character and extent of impaired immune regulation [8].

Since DC are involved in the development of autoimmune inflammation, researchers consider DC both as target cells for specific therapy of RA and as candidate cells for the development of cell-based methods to treat autoimmune diseases [9]. The development of treatment strategies requires comprehensive research into the quantitative and qualitative characteristics of DC subtypes both *ex vivo* from RA patients and *in vitro*, to determine the possibility of inducing functionally mature DC in RA.

2. Materials and methods

2.1. Donors

We enrolled a group of patients with RA consisting of 35 individuals with a mean age of 54.7 (range 34–75) years (3 men and 32 women), who were hospitalized at the Clinic of Immunopathology under the Federal State Budgetary Scientific Institution "Research Institute of Fundamental and Clinical Immunology", in Novosibirsk. Diagnosis of RA was verified in accordance with the American College of Rheumatology criteria (2010). The severity of RA was determined by counting the number of painful and swollen joints among 28 specified joints, determination of the erythrocyte sedimentation rate, assessment of each patient's general well-being according to the visual analogous scale (VAS; range between 0 and 100 mm), and subsequent calculation of the DAS28 index. All patients had a high disease activity (DAS28 > 5.9) at the time of their admission to the clinic. Peripheral blood samples for blood DC (BDC) evaluation were collected by venipuncture during routine laboratory investigations before the starting of therapy. Before prescription of treatment in hospital all patients received individual supportive therapy with either NSAID or glucocorticoid; no statistically significant differences between patients who received different types of therapy were found, so presented data were not divided by therapy. Human peripheral blood from 22 healthy donors (HD) with a mean age of 46.7 (range 30–59) years (3 men and 19 women) was obtained from the Novosibirsk Center of Blood. The status of donors was determined through a questionnaire and C-reactive protein latex test (Olvex Diagnosticum Ltd, St. Petersburg Russia). Research was performed in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the local ethics committee of the FSBI "Research Institute of Clinical Immunology". All individuals provided informed consent before the study was carried out.

2.2. Phenotypic analysis of DC

BDC were identified from whole blood. Heparinized blood (100 μl) was incubated for 20 min at room temperature with monoclonal antibodies. DC immunophenotype was assessed by five-color flow cytometry Fig. 1 (BD FacsAria, USA) and was determined using a panel of monoclonal antibodies: anti-CD14-FITC, anti-CD19-FITC, anti-CD3-FITC (Sorbent, Moscow Russia), anti-HLA-DR-PerCP (BD Biosciences, San Jose, CA) and anti-CD123-APC, anti-CD11c-PeCy7 (BD Biosciences). Monoclonal antibodies against

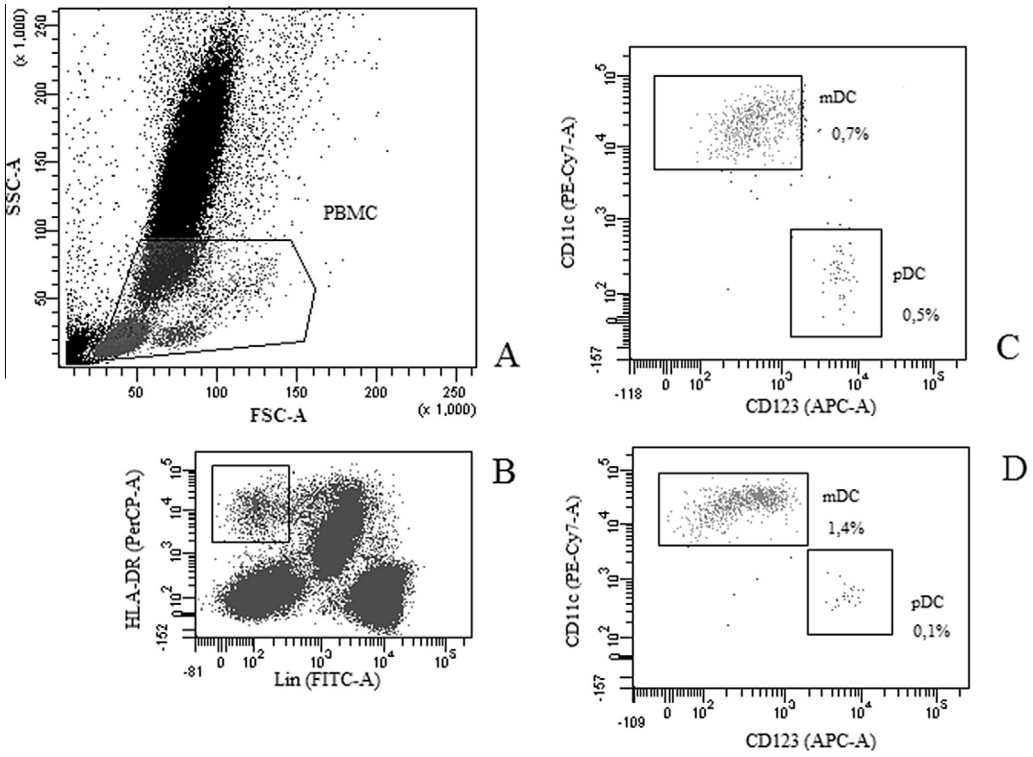

Fig. 1. Gating strategy and typical dot-plots for evaluation of percentage pDC and mDC subsets: (A) selection of PBMC fraction, (B) selection of linear-negative (CD3-CD14-CD19-FITC) HLA-DR-positive (PerCP) gate from lymphocyte-monocyte cells, from which subtypes of myeloid DC (CD11c+PE-Cy7) and plasmacytoid DC (CD123 APC) were chosen in health (C) or RA (D).