

# The number and distribution of blood dendritic cells in the epidermis and dermis of healthy human subjects

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**Abstract:** Human blood dendritic cells (BDC) can be divided into three subsets: plasmacytoid DC (PDC) and two myeloid subsets - MDC1 and MDC2. Several studies revealed the presence of both MDC and PDC in blood of healthy subjects, however no precise literature data exist on the number and distribution of BDC in the skin. The aim of our study was to assess the number and distribution of BDC and their subtypes in the healthy skin. The study included 30 healthy volunteers (age 18-51). Punch biopsies were taken from the buttock skin from each subject, and immunofluorescent staining was performed using monoclonal mouse IgG1 antibodies directed against BDCA-1, BDCA-2, BDCA-3 and BDC-4. The BDC were present both in the epidermis and dermis. PDC were detected mainly in the dermis (mean 1.2 cells per field). Myeloid subtypes were observed mainly in the middle layers of the epidermis and in the upper part of the dermis (mean 1.8 cells per field). The detection of blood dendritic cells in the skin proves their role in immune cutaneous surveillance. ([www.cm-uj.krakow.pl/FHC](http://www.cm-uj.krakow.pl/FHC))

**Key words:** Dendritic cells - Blood - Skin - Immunofluorescence

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## Introduction

Human blood dendritic cells (BDC) constitute small subpopulation of leukocytes and they represent less than 1% of peripheral blood mononuclear cells. According to recent findings, BDC can be divided into three subsets: plasmacytoid DC (PDC) and two myeloid subsets - MDC1 and MDC2 [3]. Myeloid DC recognize several bacterial components while PDC play an important role in anti-viral defense. MDC produce high levels of IL-12 and PDC have the capacity to produce high levels of interferons (IFN- $\alpha$  and IFN- $\beta$ ) [4]. The new panel of monoclonal antibodies directed against BDCA (blood dendritic cell antigen), gives an unique opportunity to exactly define BDC subtypes. PDC express specific BDCA-2 and BDCA-4 antigens, and are CD123+, CD11c-, CD4+, CD2- and CD45RO+. MDC1 are CD1c (BDCA-1)+, Lin-, HLA-DR+, CD11c<sup>bright</sup>, CD123<sup>dim</sup>, CD4+, CD45RO+, CD2+ and express the myeloid lineage markers CD13, and CD33, as well as Fc receptors (CD32, CD64, Fc RI). The second myeloid population,

MDC2, is BDCA-3 positive and expresses similar markers to the MDC1 population. However, these cells are CD11c<sup>low</sup>, CD123<sup>dim</sup> and CD2-, and lack expression of the Fc receptors: CD32, CD64 and Fc RI [2].

Several studies revealed the presence of both MDC and PDC in blood [2, 5] however, no precise literature data exist on the number and distribution of BDC in the skin of healthy subjects. Some authors revealed the presence of PDC in the lesional skin in the course of various dermatoses [9] while no similar experiments on the presence of MDC in any conditions were performed.

The aim of our study was to assess the number and distribution of BDC and its plasmacytoid and myeloid subsets, defined by anti-BDCA monoclonal antibodies, in the normally appearing skin in healthy volunteers.

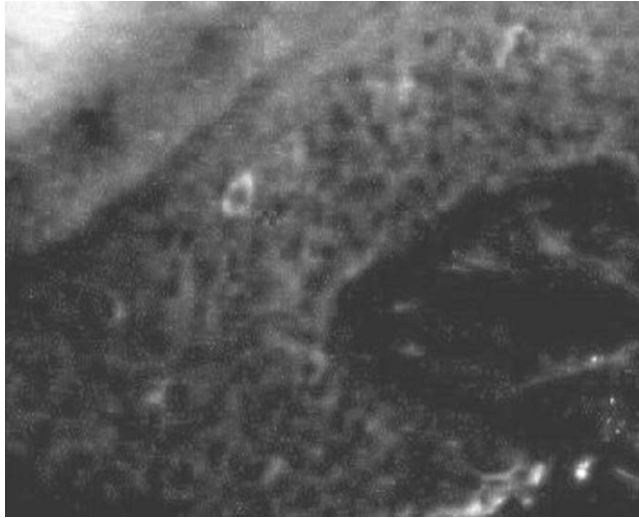
## Materials and methods

The study included 30 healthy volunteers (age 18-51) with either skin phototype II or III. They were without any diseases and were not receiving any medications. Each volunteer gave a written informed consent before entry into the study, and the experimental plan was approved by the local ethics committee of Medical University of Łódź.

Three mm punch biopsies were taken from the buttock skin from each subject immediately placed in liquid nitrogen and stored at -80°C until analysis. Immunofluorescent staining of cryostat sections was performed using monoclonal mouse IgG1 antibodies directed

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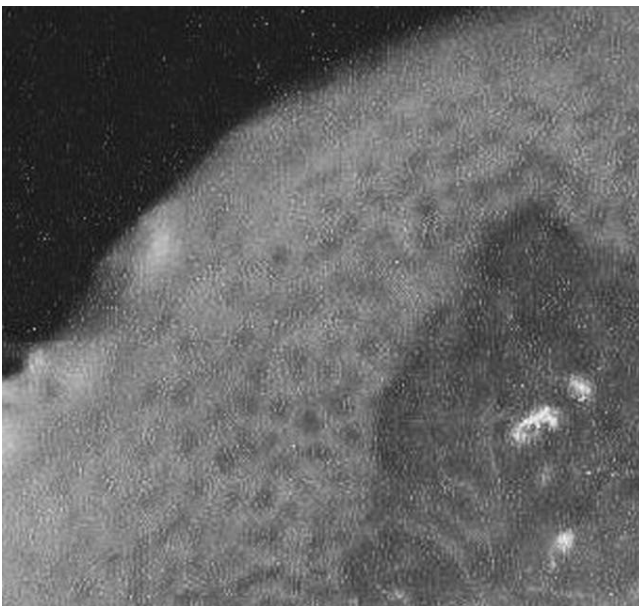
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**Fig. 1.** Myeloid blood dendritic cell in the epidermis, stained with antibodies directed against BDCA-3 antigen. Direct immunofluorescence,  $\times 400$ .



**Fig. 3.** Plasmacytoid blood dendritic cell in dermis, stained with antibodies directed against BDCA-2 antigen. Direct immunofluorescence,  $\times 400$ .



**Fig. 2.** Myeloid blood dendritic cell in dermis, stained with antibodies directed against BDCA-3 antigen. Direct immunofluorescence,  $\times 400$ .

against BDCA-1 (clone AD5-8E7), BDCA-2 (clone AC144), BDCA-3 (clone AD5-14H12), all labelled with FITC, and BDC-4 (clone AD-17F6), labelled with PE (all Miltenyi Biotec, Bergish Gladback, Germany). Skin samples were incubated with a 1:9 dilution of the antibodies for 60 min at room temperature in the dark. After washing in phosphate buffered saline, the tissue specimens were dried and mounted in glycerol. To avoid nonspecific antibody binding with Fc receptors on other populations of dendritic cells we used FcR blocking reagent according to manufacturer's advice.

Sections were analyzed in a fluorescence microscope (Olympus Bx40, Olympus Optical Co., Ltd., Tokyo, Japan) coupled to a digital camera (Camedia, Olympus) and DPX Olympus Software program. Three sections from each biopsy were examined. The skin specimens

were evaluated in five  $\times 400$  high power fields per specimen and the mean count of each BDC subtype per field was calculated.

## Results and discussion

Some data showed the presence of blood dendritic cells, especially plasmacytoid subset in different skin diseases. They were observed within skin lesions taken from patients with lupus erythematosus, contact dermatitis or psoriasis vulgaris and as well in cutaneous Jessner's lymphocytic infiltration [9].

We do not know any other report showing the presence of MDC1 and 2 in normal or inflamed skin. In our study, myeloid subtypes (BDCA-1 and -3) were observed mainly in the middle layers of the epidermis and also in the upper part of the dermis (Fig. 1, 2). The mean number of detected MDC was 1.8 cells per field (range 1-3 cells). As Langerhans cell (LC) precursors are myeloid ones, it may also be suggested that under specific conditions BDCA-1 or BDCA-3 cells become classic LC with expression of CD1a or langerin [8].

Dzionic *et al.* [2] revealed BDCA-2 plasmacytoid cells in lymphoid (inflamed tonsils, lymph nodes and thymus) and nonlymphoid (testis) tissues. Bangert *et al.* [1] found 1.4 PDC/mm<sup>2</sup> in the normal human dermis and none in the epidermis. We also revealed PDC mainly in the dermis (Fig. 3), however, occasional cells were also noted in the epidermis. The mean number of detected PDC (BDCA-2 and BDCA-4) was 1.2 cells per field (range 0-2 cells).

Our results proved the presence of MDC1 and 2 cells in human skin, with more prominent number in the epidermis. The presence of BDC and their subtypes in the skin under normal conditions may suggest that they

play a role in immune surveillance and together with LC are likely to initiate cellular immune response in the skin.

To our knowledge, this is the first study to show the number and distribution of BDC and their subtypes in the healthy human skin by direct immunofluorescence.

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