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Abstract

The brain and dendritic cell (BAD)-associated lysosome-associated membrane protein (LAMP)-like molecule, also known as BAD-LAMP, c20orf103, UNC-46, is a newly identified LAMP family member, which has been recently officially designated as LAMP-5. It is a transmembrane glycosylated protein localized in the ER-Golgi intermediate compartment (ERGIC). LAMP-5 shares sequence and structural homology with other LAMP family members. In mouse, LAMP-5 is expressed in postnatal cortical neurons of particular layers, where it is enriched in defined zones along neuronal projections. In human, like its murine homolog, LAMP-5 is principally expressed in brain, and it was recently found in primary plasmacytoid dendritic cells (pDCs). However, LAMP-5 expression profile and function have not been extensively studied in pDCs. By using anti-human LAMP-5 monoclonal antibody (clone 124-40B), we report that LAMP-5 is specifically expressed in peripheral blood pDCs which are characterized by surface expression of CD303+/CD304+/CD123+/HLA-DR+/CXCR3+/CD11c-/CD14-/CD209-, but is not expressed by GM-CSF plus FL3 or IL-4 induced monocyte-derived CD303+/CD123+/HLA-DR+/CD209+/CD14+/CXCR3dim pDCs or CD209+/CD123dim/CXCR3dim/HLA-DR+/CD11c+/CD14+ myeloid dendritic cells (mDCs). Like other LAMP members, LAMP-5 is rapidly relocated on the cell surface of peripheral blood pDCs after 24 hours stimulation by IL-3. These data suggest that LAMP-5 may be a useful marker for studying pDC development and activation.

Introduction

LAMP-3 and LAMP-5 are the two most recently found LAMP family members. LAMP3, also known as DC-LAMP, was originally described as a molecule specifically expressed in mature dendritic cells (DCs). It appears transiently on DC activation at the limiting membrane of the MHC class II-containing intracellular compartments (MIIC) involved in MHC class II peptide loading and transport to the cell surface. It may play an important role during the processing of exogenous antigens, and might also participate in the functional remodeling of the MIIC by facilitating the translocation of MHC class II molecules to the cell surface. LAMP-5 is expressed in postnatal cortical neurons of particular layers, where it is enriched in defined zones along neuronal projections. Human LAMP-5 is principally expressed in brain; it is also specifically found in primary CD303 (BDCA-2)-positive pDCs. It may represent a novel marker of human primary and transformed pDCs.

Materials and Methods

Materials

Human PBMCs were isolated from healthy donor peripheral blood. All the antibodies and recombinant proteins are from BioLegend.

Cell culture and stimulation

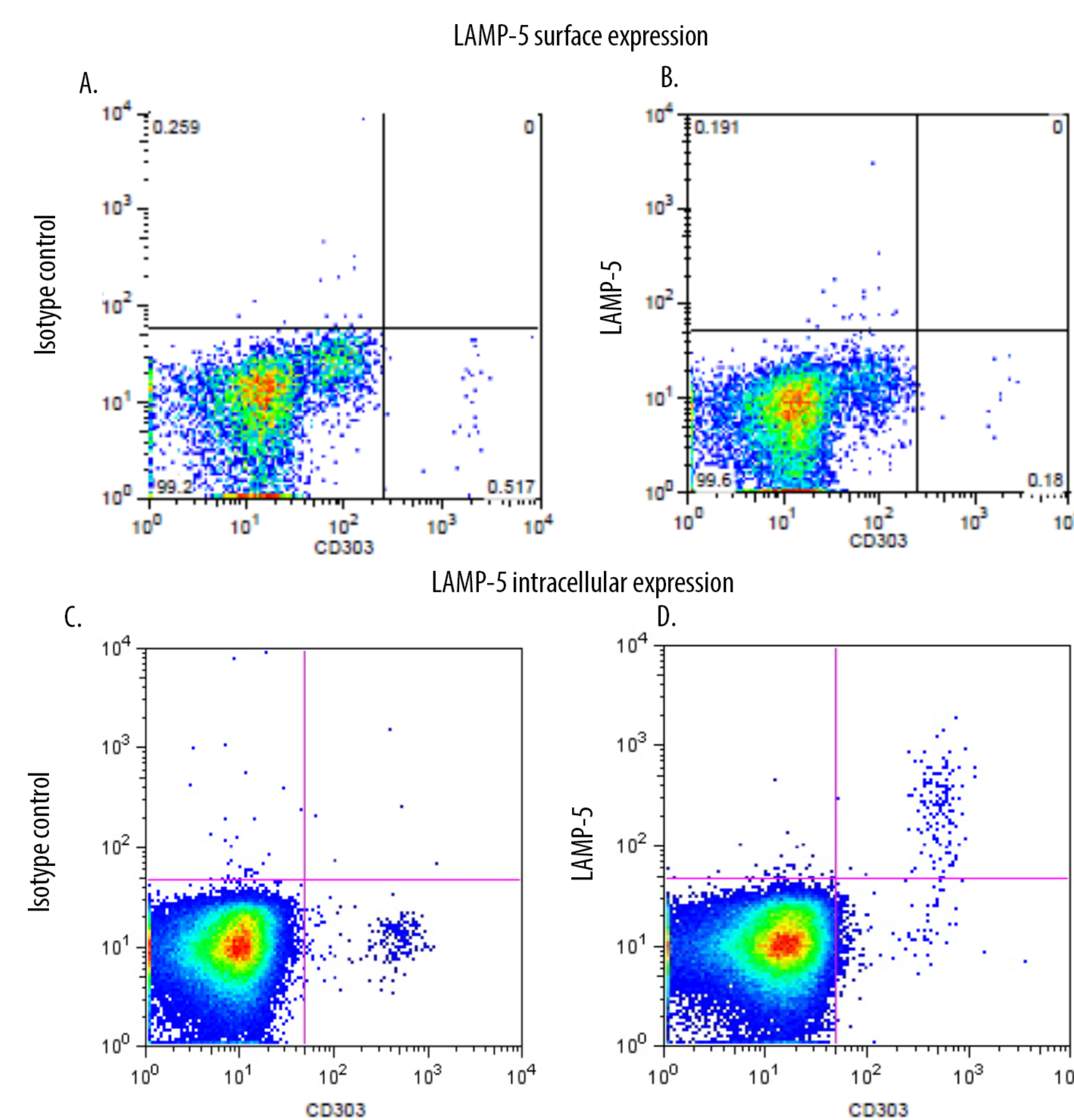
Human PBMCs were cultured with 100ng/ml of GM-CSF plus 50 ng/ml IL-4 for 6 days. On day 7, the cells from the cocultures were incubated with 1ug/ml LPS for additional 6 hours to induce DC maturation.

Immunofluorescence staining:

Cells were stained with indicated antibodies according to BioLegend staining procedures. Stained cells were analyzed by flow cytometry with appropriate instrument settings.

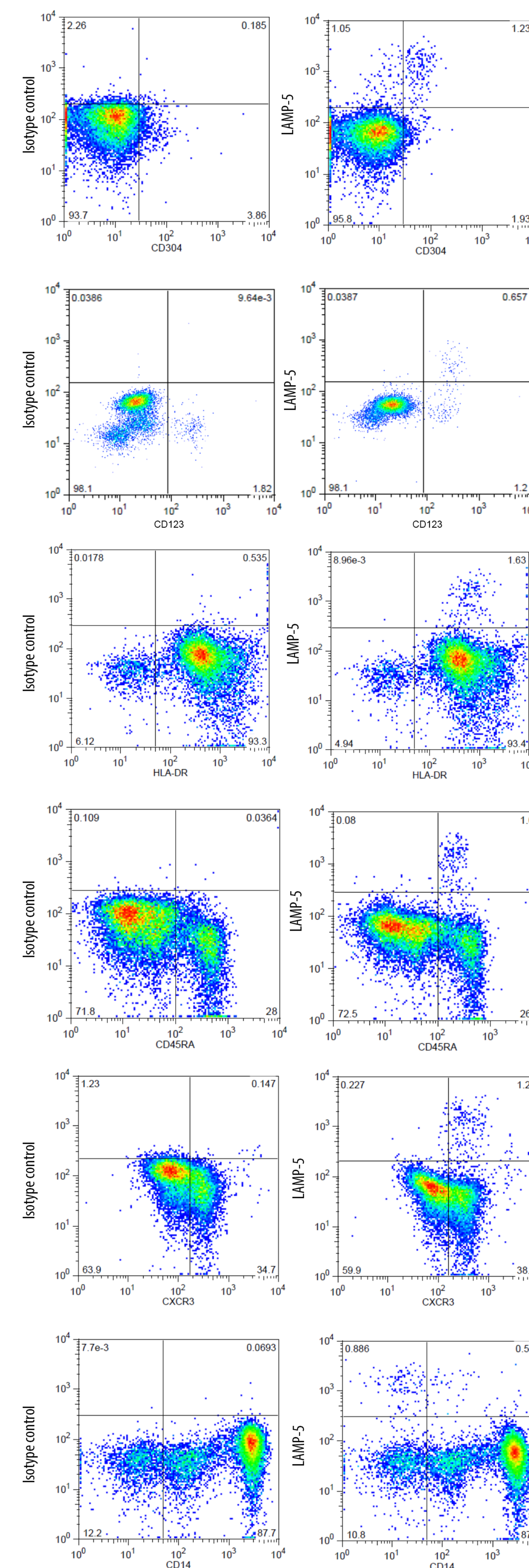
Results

Figure 1. LAMP-5 is mainly expressed intracellularly



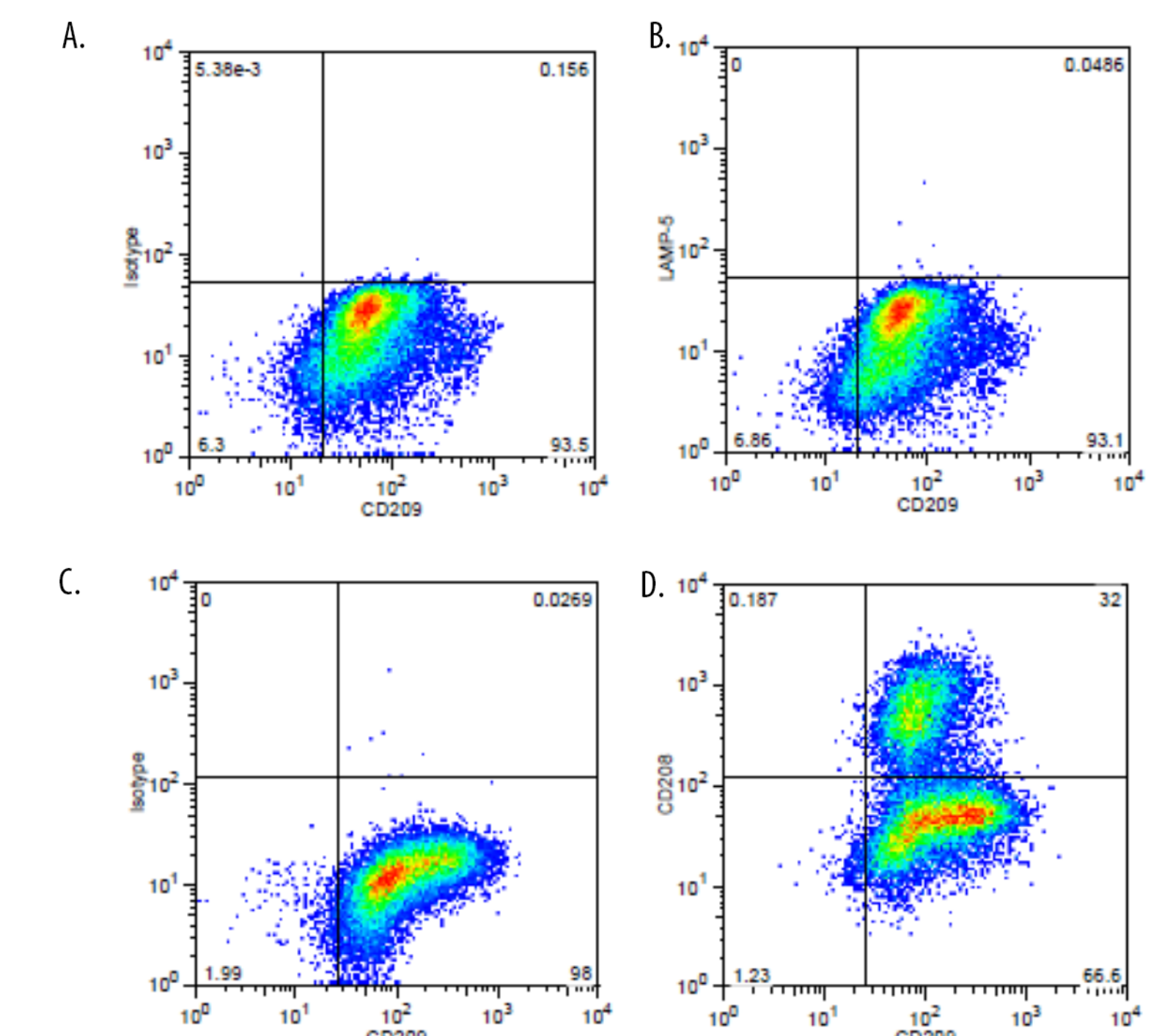
(A and B). Human PBMCs were surface stained with CD303 and LAMP-5 or isotype control. (C and D) Human PBMCs were surface stained with CD303, fixed and permeabilized followed by intracellular staining with LAMP-5.

Figure 2. Characterization of LAMP-5-positive cells



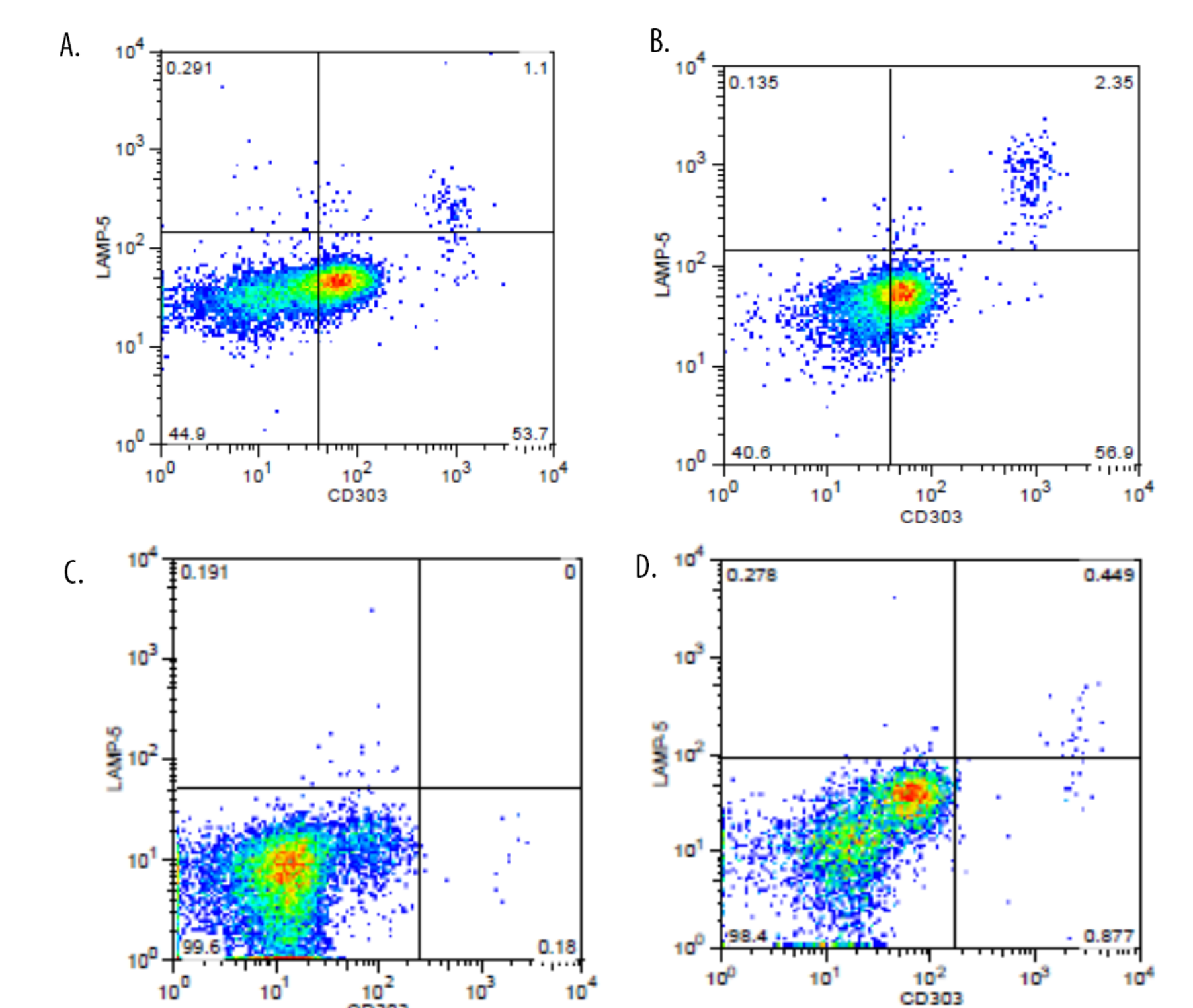
Human PBMCs were surface stained with indicated markers then fixed and permeabilized. Then the cells were intracellularly stained with anti-human LAMP-5 (clone: 124-40B) or isotype control.

Figure 3. LAMP-5 is not expressed in PBMC derived mDCs



Human PBMCs derived DCs were surface stained with CD209, fixed and permeabilized and then stained intracellularly with isotype/LAMP-5 (A and B) or isotype/CD208 (C and D).

Figure 4. LAMP-5 is relocated on peripheral blood pDCs surface after IL-3 stimulation



Human PBMCs were overnight stimulated with IL-3 (C and D) or without IL-3 (A and B). The cells were surface stained with CD303, fixed and permeabilized and then intracellularly stained with LAMP-5, clone 124-40B (A and B). The cells were surface stained with CD303 and LAMP-5, clone 124-40B (C and D).

Conclusions

1. LAMP-5 is constitutively expressed in CD303+/CD304+/CD123+/HLA-DR+/CXCR3+/CD11c-/CD14-/CD209- cells.
2. LAMP-5 is relocated on the cell surface of peripheral blood pDCs after 24 hours stimulation by IL-3.