Differential Expression of the Transcription Factor SPI1 (PU.1) in Dendritic Cell Subsets

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Abstract

SPI1 belongs to the E26-transformation-specific (Ets) family of transcription factors and is exclusively expressed in hematopoietic cells. Originally identified as an oncogene that blocks erythroblast differentiation, SPI1 plays a critical role in the development and lineage commitment of both myeloid and lymphoid cells. Gene knockout studies show that *spi1-/-* mice exhibit a multi-lineage defect in the generation of monocytes, granulocytes, B, T, and dendritic cells. While SPI1 expression is required for normal hematopoietic cell development, the level of SPI1 expression influences specific lineage commitment. Using in-house generated species-specific clones, human peripheral blood mononuclear cells (PBMCs) and mouse splenocytes were evaluated for SPI1 expression by flow cytometric analysis. In human PBMCs, Lin$^-$ HLA-DR$^+$ CD11c$^+$ CD303$^-$ monocyte-derived dendritic cells (mDCs) exhibit high levels of expression while Lin$^-$ HLA-DR$^+$ CD11cint CD303$^+$ plasmacytoid dendritic cells (pDCs) do not express SPI1 protein. These data suggest SPI1 expression is required for the development and function of human mDCs, while SPI1 expression is not necessary for terminally differentiated human pDCs. Interestingly, SPI1 expression is detected in both murine DC subsets: Lin- I-A/I-E$^+$ CD11c$^+$ CD317$^-$ mDCs (high levels) and Lin$^-$ I-A/I-E$^+$ CD11c^{int} CD317$^+$ pDCs (low levels). These observations indicate expression patterns differ between mouse and human cells, and demonstrate that DC subsets exhibit differential expression of SPI1 protein.

Methods

PBMC Isolation

Human PBMCs from healthy volunteers were isolated using Ficoll-Paque (GE Healthcare). Cells were then washed in Cell Staining Buffer (Cat. No. 420201), resuspended at 20 x 10^6 cells, and 2 x 10^6 cells (or 100 mL) were aliquoted for staining.

Splenocyte Isolation

C57BL/6 spleens were digested with Collagenase from Clostridium hystoloyticum (Sigma-Aldrich) to create a single cell suspension. Red cells are then lysed using 1X RBC Lysis Buffer (BioLegend Cat. No. 420301). Cells were then washed in Cell Staining Buffer (Cat. No. 420201), resuspended at 20 x 10^6 cells, and 2 x 10^6 cells (or 100 mL) were aliquoted for staining.

Viability Staining

Human PBMCs and murine splenocytes were stained first with Zombie Aqua™ (Cat. No. 423102) for viability determination.

Surface Staining Staining

Human PBMCs were stained with a cocktail of surface anti-human antibodies: CD303-BV421™ (Cat. No. 354211) , Lineage-FITC [CD3, CD4, CD16, CD19, CD20, CD56] (Cat. No. 348801), HLA-DR-PE (Cat. No. 307605), CD14-BV605™ (Cat. No. 301833), CD11c-BV785™ (Cat. No. 301643), and CD123-PerCP/Cy5.5 (Cat. No. 306015). C57BL/6 splenocytes were stained with a cocktail of surface anti-mouse antibodies: CD11c-BV421™ (Cat. No. 117343), CD3e-Alexa Fluor® 488 (Cat. No. 100321), CD19-Alexa Fluor® 488 (Cat. No. 115521), NK1.1-Alexa Fluor® 488 (Cat. No. 108717), CD317-PE (Cat. No. 127104), and I-A/I-E-BV785 ™ (Cat. No. 107645).

Fixation/Permeabilization

After surface staining, cells are washed twice with Cell Staining Buffer (Cat. No. 420201). Cells were fixed and permeabilized with True-Nuclear™ Transcription Factor Buffer Set (Cat. No. 424401).

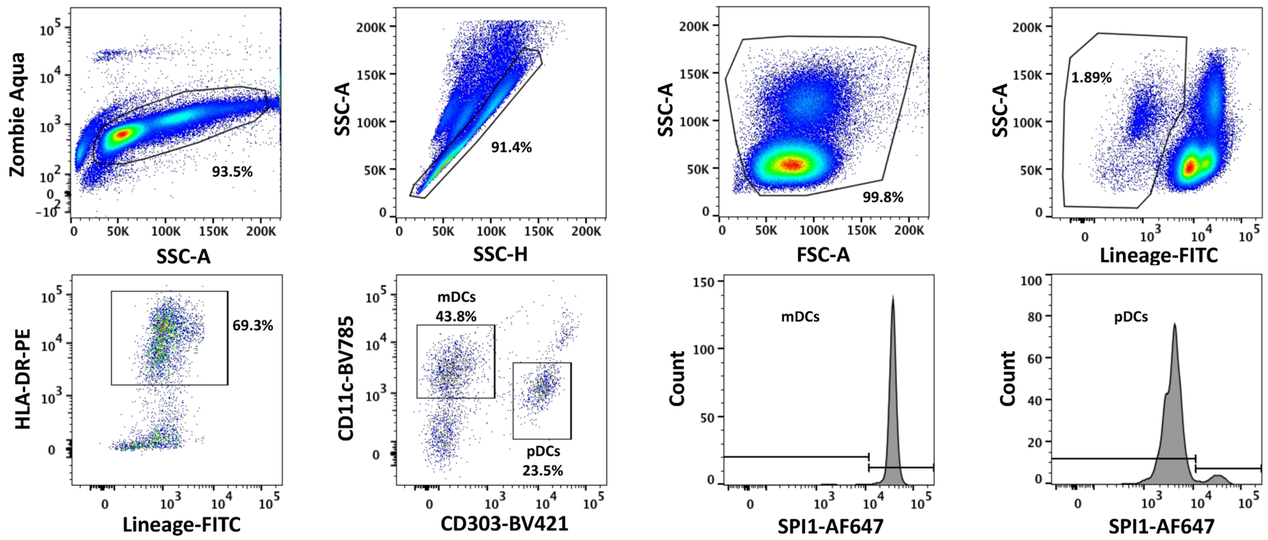
Intracellular Staining

Human PBMCs were stained with anti-Human SPI1-Alexa Fluor® 647 (Cat. No. 658004) or Isotype-Alexa Fluor® 647 (Cat. No. 400135). Mouse splenocytes were stained with anti-mouse SPI1-Alexa Fluor® 647 (Cat. No. 681304) or Isotype-Alexa Fluor® 647 (Cat. No. 400526)

Instruments

Data was collected on a BD LSRFortessa™

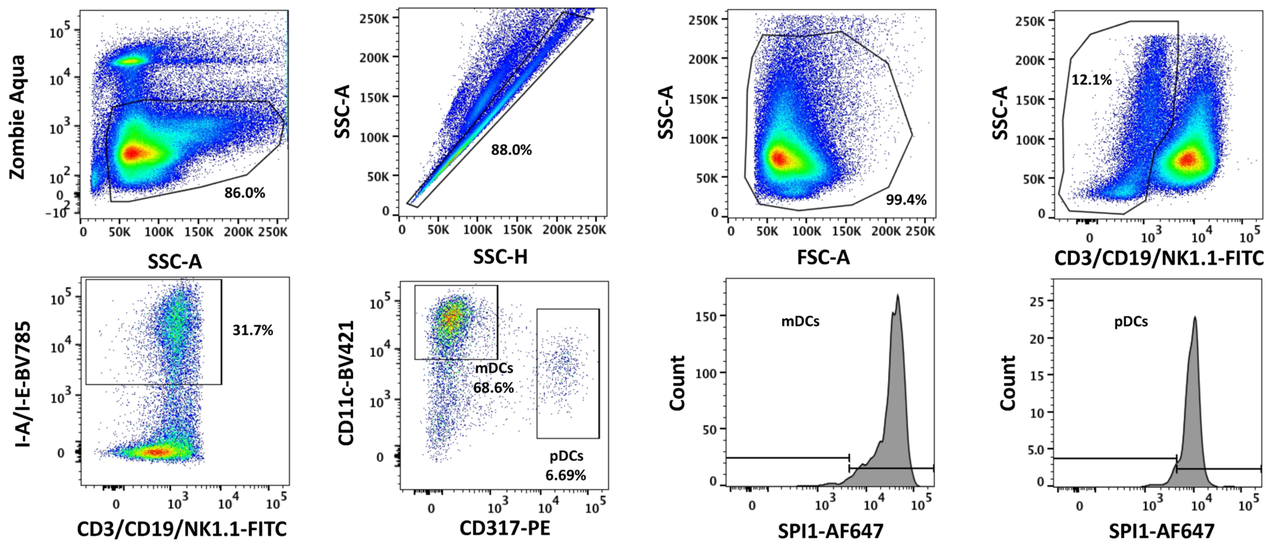
Figure 1. Human mDCs exhibit high levels of SPI1 protein while pDCs do not express SPI1 protein



Gating strategy and phenotypic identification of human dendritic cell subsets

Human PBMCs were surface stained for CD303-BV421™, Lineage-FITC [CD3, CD4, CD16, CD19, CD20, CD56], HLA-DR-PE, CD14-BV605, CD11c-BV785™, and CD123-PerCP/Cy5.5. Cells were then fixed and permeabilized with True-Nuclear™ Transcription Factor Buffer Set and intracellularly stained with anti-human SPI1-Alexa Fluor® 647 (clone 7C6B05) or Isotype-Alexa Fluor® 647 (clone MOPC-21). Histogram gates are based on isotype control staining.

Figure 2. SPI1 expression is detected in murine mDCs (high levels) and pDCs (low levels)



Gating strategy and phenotypic identification of murine dendritic cell subsets

Murine splenoocytes were surface stained for CD11c-BV421™, CD3e-Alexa Fluor® 488 , CD19-Alexa Fluor® 488, NK1.1-Alexa Fluor® 488, CD317-PE, and I-A/I-E-BV785. Cells were then fixed and permeabilized with True-Nuclear™ Transcription Factor Buffer Set and intracellularly stained with anti-mouse SPI1-Alexa Fluor® 647 (clone 7C2C34) or Isotype-Alexa Fluor® 647 (clone RTK2758). Histogram gates are based on isotype control staining.

Results

Using in-house generated species-specific clones, PBMCs and mouse splenocytes were evaluated for SPI1 expression by flow cytometric analysis. In human PBMCs, Lin$^-$ HLA-DR$^+$ CD11c$^+$ CD303$^-$ monocyte-derived dendritic cells (mDCs) exhibit high levels of expression while Lin$^-$ HLA-DR$^+$ CD11c^{int} CD303$^+$ plasmacytoid dendritic cells (pDCs) do not express SPI1 protein. Interestingly, SPI1 expression is detected in both murine DC subsets: CD3$^-$/CD19$^-$/NK1.1$^-$ I-A/I-E$^+$ CD11c$^+$ CD317$^-$ mDCs (high levels) and CD3$^-$/CD19$^-$/NK1.1$^-$ I-A/I-E$^+$ CD11c^{int} CD317$^+$ pDCs (low levels).

Conclusions

These observations suggest that SPI1 expression is required for the development and function of human mDCs, while SPI1 expression is not necessary for terminally differentiated human pDCs. In the murine system, our data indicate that expression of SPI1 is required by both mDC and pDC subsets, however the level of expression differs between populations. Similar data was observed in cell subsets of mouse whole blood (data not shown).