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Activation-induced colocalisation of SCAMP5 with IFNα in human plasmacytoid dendritic cells

Juliëtte N Pouw ,[1,2] Michel A M Olde Nordkamp,[1,2] Tom G O'Toole,[2] Timothy R D J Radstake,[1,2] Emmerik F A Leijten,[1,2,3] Marianne Boes[2,4]

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[1]Rheumatology and Clinical Immunology, University Medical Centre Utrecht, Utrecht University, Utrecht, The Netherlands
[2]Center for Translational Immunology, University Medical Centre Utrecht, Utrecht University, Utrecht, The Netherlands
[3]Rheumatology, Sint Maartenskliniek, Nijmegen, The Netherlands
[4]Pediatric Immunology, Wilhelmina Children's Hospital University Medical Centre, Utrecht University, Utrecht, The Netherlands

Correspondence to
Juliëtte N Pouw; j.n.pouw-3@umcutrecht.nl

ABSTRACT

Introduction Plasmacytoid dendritic cells (pDCs) are the main producers of type I interferon (IFN) in SLE. pDCs express high secretory carrier membrane protein 5 (SCAMP5). Recent work in transfected HEK cells connects SCAMP5 to the type I IFN secretory pathway. To further study the role of SCAMP5 in IFNα secretion by pDCs, we focused on the subcellular distribution of SCAMP5 in human pDCs freshly isolated from peripheral blood.

Methods We measured SCAMP5 expression by flow cytometry in peripheral blood mononuclear cells of healthy subjects (n=8). Next, we assessed the colocalisation of SCAMP5 with IFNα in pDCs of healthy subjects (n=4) by evaluating bright detail similarity (BDS) scores using ImageStream technology.

Results We confirm that SCAMP5 is highly expressed by pDCs derived from peripheral blood. In activated pDCs, we show that SCAMP5 colocalises with IFNα (mean BDS 2.0±0.1; BDS >2.0 in 44% of pDCs).

Conclusion SCAMP5 colocalises with IFNα in activated human pDCs, in support of a role of this trafficking protein in the secretion of type I IFN by pDCs.

Plasmacytoid dendritic cells (pDCs) are a rare immune cell type that links innate with adaptive immunity and are specialised in the production of type I interferon (IFN). In autoimmune diseases characterised by a type I IFN signature, including SLE and primary Sjögren's syndrome, pDCs are implicated in the pathophysiology.[1] A remaining question has been how type I IFN secretion by pDCs is regulated. Among the leucocytes in peripheral blood, expression of secretory carrier membrane protein 5 (SCAMP5) is highly selective for pDCs.[2] SCAMPs are involved in the regulation of membrane trafficking and SCAMP5 has been identified as a novel risk gene for SLE.[2] In human monocytes, SCAMP5 can promote calcium-regulated secretion of C-C Motif Chemokine Ligand 5 (CCL5).[3] Recently Ghanem *et al*[2] used human embryonic kidney (HEK) cells as the model system to investigate the possible connection between SCAMP5 and type I IFN secretion. Using transduction of constructs encoding for SCAMP5 and IFN fluorescent fusion proteins, SCAMP5-positive endosomal vesicles were shown to traffic between the cell surface and the Golgi apparatus, and intersect with the IFNα secretory pathway.[2] To further study the role of SCAMP5 in type I IFN secretion, we focused on the cellular distribution of SCAMP5 in human pDCs freshly isolated from peripheral blood.

SUBJECTS AND SAMPLES

We collected peripheral blood from healthy subjects, who signed informed consent for experimentation with human samples.

CELL ISOLATION

Blood was drawn into BD Vacutainer plastic blood collection tubes with lithium heparin. Peripheral blood mononuclear cells (PBMCs) were isolated using density centrifugation (Ficoll-Paque). pDCs were isolated using anti-CD304 (neuropilin-1) magnetic beads (MicroBead Kit 130-097-149, Miltenyi) for autoMACS automated cell isolation.

STAIN PROTOCOL

We stained samples by incubation with 25 µL antibody mix diluted in buffer (500 mL phosphate-buffered saline + 5 mL 10% sodium azide + 5 g bovine serum albumin) for 25 min at 4°C. Before intracellular staining, we fixed and permeabilised cells with 100 µL Fixation/Permeabilization Concentrate and Diluent (00-5123-43 and 00-5223-56; eBioscience). The antibodies are listed in online supplemental table S1.

FLOW CYTOMETRY

Acquisition was performed on BD LSRFortessa (405 nm, 488 nm, 561 nm and 635 nm lasers)

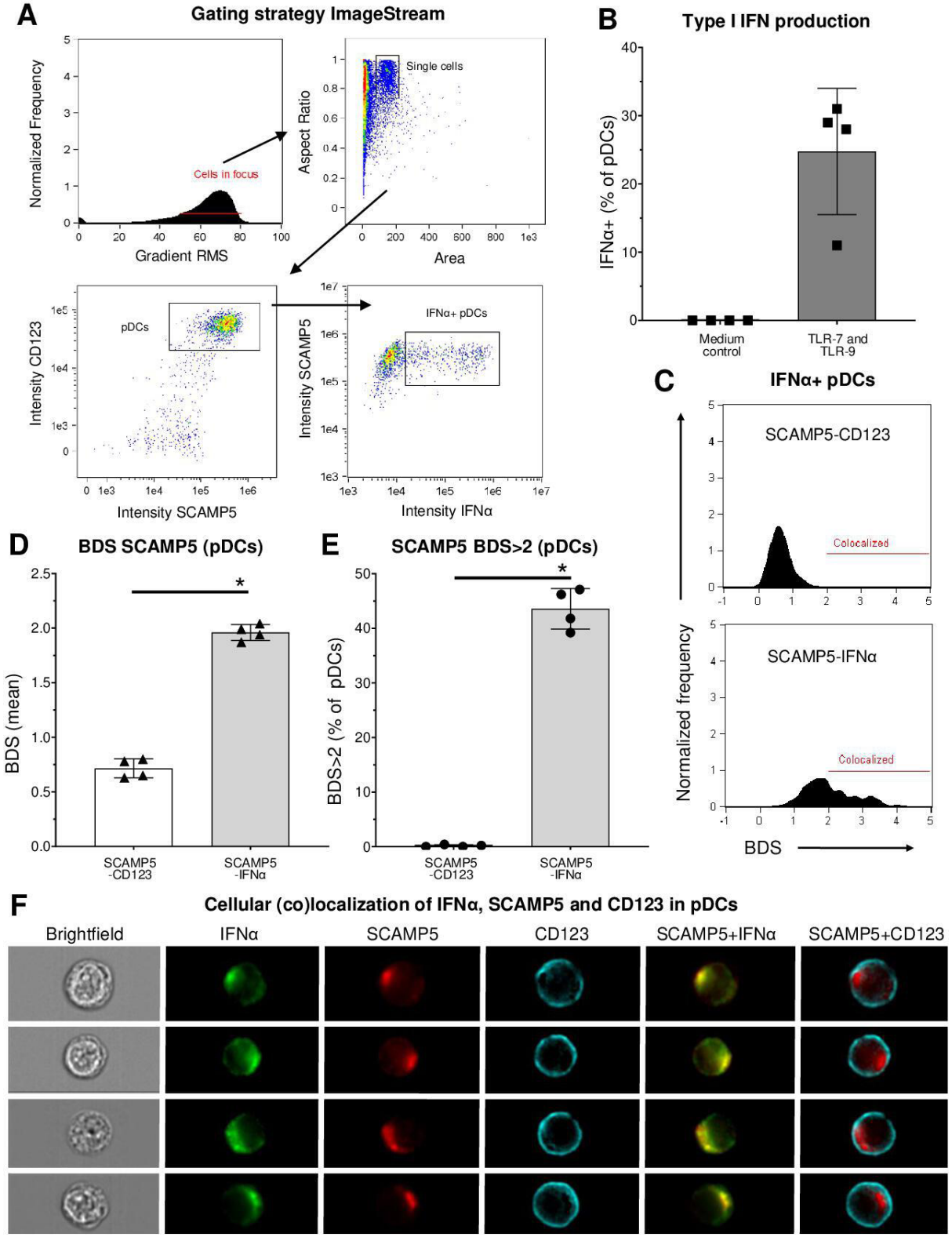

Figure 1 SCAMP5 in activated human pDCs colocalises with IFNα. ImageStream analyses of pDCs from four healthy subjects after Loxoribine and CpG oligodeoxynucleotides class A stimulation. (A) Gating strategy. Selection of single, in-focus, CD123+SCAMP5+ pDCs that produce IFNα. (B) Percentage of CD123+SCAMP5+ pDCs that produce IFNα on activation. (C) Colocalisation of SCAMP5 with CD123 and IFNα presented as histograms of BDS scores, measured in activated pDCs. (D) Mean BDS of SCAMP5-CD123 and SCAMP5-IFNα in activated pDCs. (E) Percentage of activated pDCs with a BDS score >2 of SCAMP5-CD123 and SCAMP5-IFNα. (F) Representative images of cellular location of IFNα+ (green), SCAMP5 (red) and CD123 (blue) in activated pDCs, including composite images of SCAMP5-IFNα+ (yellow: colocalised) and SCAMP5-CD123 (purple: colocalised). *P<0.05. BDS, bright detail similarity; IFN, interferon; pDCs, plasmacytoid dendritic cells; SCAMP5, secretory carrier membrane protein 5; TLR, toll-like receptor.

with FACSDiva software (V.8.0.1). Analysis was performed using FlowJo (V.10.5.3).

PDC ACTIVATION

To assess the colocalisation of SCAMP5 with IFNα, we cultured autoMACS-isolated pDCs in complete medium (RPMI 1640 GlutaMAX (61870044; Thermo Fisher Scientific), 10% fetal bovine serum, 1% penicillin-streptomycin) for 6 hours with toll-like receptor (TLR) 7 and TLR-9 ligands (1 mM Loxoribine Tlrl-lox, Invivogen; 1 mM CpG oligodeoxynucleotide class A, tlrl-2216-1, Invivogen), while inhibiting protein transport with 1:1000 BD GolgiStop during 3 hours (51-2092KZ, BD Biosciences).

IMAGESTREAM

Acquisition was performed on Amnis ImageStreamX Mark II Imaging Flow Cytometer (488 nm and 642 nm lasers) using ISX INSPIRE software. Analysis was performed using IDEAS (V.6.2). To assess colocalisation we calculated the bright detail similarity (BDS) scores using IDEAS software. We considered a BDS score ≥2 as a high degree of overlap between two fluorescent signals, indicative of colocalisation within the cell.

STATISTICAL ANALYSIS

We applied Wilcoxon signed-rank test to compare the median fluorescent intensity (MFI) of SCAMP5 and the percentage of IFNα producing pDCs. For other comparisons we used Mann-Whitney U test. P<0.05 was considered statistically significant. Statistical analyses were performed with GraphPad Prism (V.8.3.0).

First, we confirmed pDC-specific high expression of SCAMP5 by flow cytometry (MFI PBMC 17.972 vs pDC 2171, p=0.008) (online supplemental figure S1). Next, we assessed the colocalisation of SCAMP5 with IFNα in pDCs ex vivo by ImageStream. We used CD123—the alpha chain of the interleukin 3 receptor, a pDC membrane marker—as the 'negative control' in our colocalisation analyses. To identify activated pDCs, we gated CD123+SCAMP5+IFNα+ cells (figure 1A). On TLR-7 and TLR-9 stimulation, on average 25% of pDCs produced IFNα (figure 1B). To assess the colocalisation of SCAMP5 with CD123 and IFNα, we evaluated the BDS scores (figure 1C). Based on the mean BDS scores in activated pDCs, SCAMP5 and IFNα were colocalised (mean BDS 2.0±0.1), but SCAMP5 and CD123 were not (mean BDS <1) (figure 1D). Of the activated pDCs, 44% have a high degree of colocalised SCAMP5 and IFNα (BDS >2) (figure 1E). ImageStream (composite) images showed cellular localisation of SCAMP5, IFNα and CD123 (figure 1F), and visualised colocalisation of SCAMP5 and IFNα near the cell surface in activated pDCs. In non-activated pDCs, no colocalisation of SCAMP5 with IFNα was observed (online supplemental figure S2 and online supplemental table S2).

To our knowledge, we are the first to visualise cellular localisation of both SCAMP5 and type I IFNs in human pDCs ex vivo. Here we provide evidence on the colocalisation of SCAMP5 with IFNα in activated pDCs. Our data are in line with the hypothesis that SCAMP5 is implicated in the secretion of type I IFNs.[2] These results provide a basis to better understand the role of SCAMP5 in human pDCs and may have implications for type I IFN signature diseases. Future research could aim at visualising colocalisation of SCAMP5 with individual components of the IFNα secretory pathway in pDCs, possibly towards finding new targets for therapeutic intervention. In conclusion, we found evidence on the colocalisation of SCAMP5 with IFNα in activated pDCs ex vivo, supporting the hypothesis that SCAMP5 in pDCs is implicated in type I IFN secretion.

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Contributors TRDJR and MB were responsible for conception. JNP, EFAL and MB designed the experiments. JNP and MAMON performed the experiments. JNP and TGO'T were responsible for the analyses. JNP and MB primarily wrote the manuscript. All authors contributed to substantial discussion of content, and review and revision of the manuscript before submission.

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Competing interests MB reports research grants from Actuate Therapeutics, Nutricia and Argenx, unrelated to the submitted work. All other authors state no conflict of interest and have no disclosures.

Patient consent for publication Not required.

Ethics approval This study involves human participants. At University Medical Center Utrecht, a cohort of healthy subjects is used from the Mini Donor Service (MDS). The MDS aims to collect blood from healthy volunteers to support scientific research and laboratory diagnosis at UMC Utrecht. Donors are volunteers who by means of a written declaration have indicated their willingness to donate small quantities of blood (100 mL maximum) four times a year without being reimbursed by financial or other means. The work has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

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ORCID iD

Juliëtte N Pouw http://orcid.org/0000-0002-1818-7833

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