

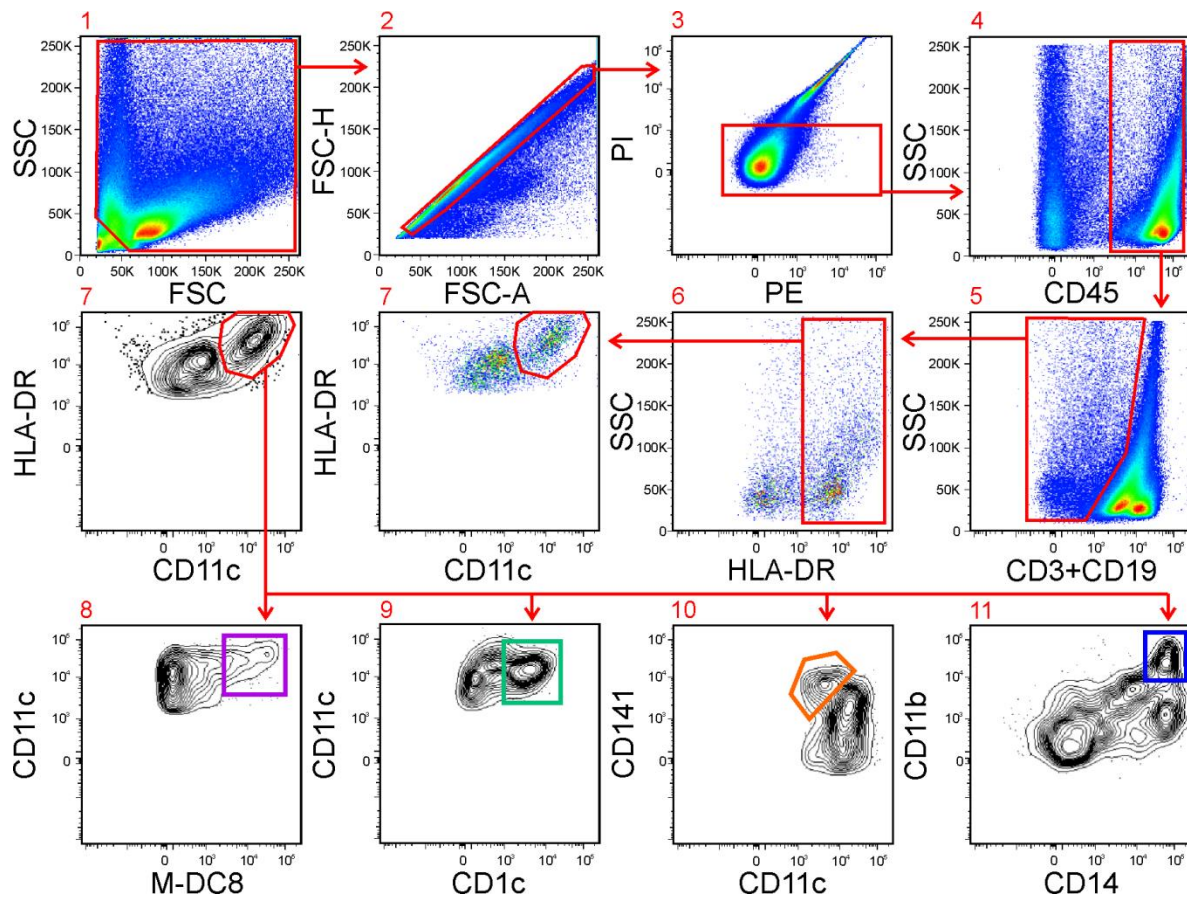
slan/M-DC8⁺ cells constitute a distinct subset of dendritic cells in human tonsil

Supplementary Table S1. List of the antibodies used for immunohistochemistry studies.

Reagent	Clone	Dilution	Isotype	Source
BCL6	IG191E/A8	1:300	mIgG1	kindly provided by G. Roncador (Centro Nacional de Investigaciones Oncológicas Madrid, Spain)
CD1a	010	1:50	mIgG1	Dako
CD3	SP7	1:100	rabbit	Thermo Scientific, Waltham, MA
CD4	4B12	1:40	mIgG1	Thermo Scientific
CD8	C8/144B	1:30	mIgG1	Dako
CD11b		1:300	rabbit polyclonal	Sigma-Aldrich Novocastra Laboratories, Newcastle upon Tyne, United Kingdom
CD14	7	1:50	mIgG2a	Newcastle upon Tyne, United Kingdom
CD66b	G10F5	1:200	mIgM	BioLegend, San Diego, CA
CD83	1H4b	1:150	mIgG1	Novocastra Laboratories
DD1	DD1	1:60	mIgM	kindly provided by Knut Schäkel (University Hospital Heidelberg, Heidelberg, Germany)
Keratin (wide spectrum-CKP)	MNF116	1:100	mIgG1	Dako
Ki-67	MM1	1:100	mIgG1	Novocastra Laboratories

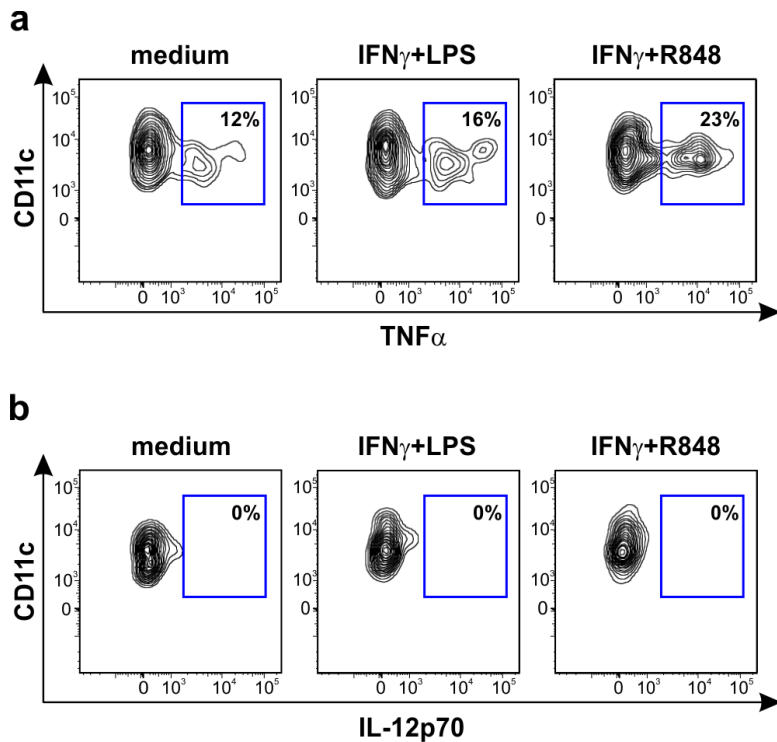
Supplementary Table S2. List of the antibodies used for flow cytometry.

Antibody	Clone	Isotype	Source
AlexaFluor488 anti-human CD1c	L161	mIgG1	BioLegend
AlexaFluor647 anti-human CX3CR1	2A9-1	rat IgG2b	BioLegend
<i>AlexaFluor647 rat IgG2b</i>	RTK4530		BioLegend
APC anti-human CD11b	ICRF44	mIgG1	BioLegend
APC anti-human CD11c	MJ4-27G12	mIgG2b	Miltenyi Biotec
APC anti-human CD14	TUK4	mIgG2a	Miltenyi Biotec
APC anti-human CD141 (BDCA-3)	AD5-14H12	mIgG1	Miltenyi Biotec
APC-Cy7 anti-human HLA-DR	L243	mIgG2a	BioLegend
Brilliant Violet 510 anti-human CD45	HI30	mIgG1	BioLegend
FITC anti-human CD14	TÜK4	mIgG2a	Miltenyi Biotec
FITC anti-human CD141	AD5-14H12	mIgG1	Miltenyi Biotec
FITC anti-human CD303	AC144	mIgG1	Miltenyi Biotec
FITC anti-human Slan (M-DC8)	DD1	mIgM	Miltenyi Biotec
PE anti-human CD1c (BDCA-1)	AD5-8E7	mIgG2a	Miltenyi Biotec
PE anti-human CD11b	ICRF44	mIgG1	BioLegend
PE anti-human CD115	9-4D2-1E4	rat IgG1	BioLegend
PE anti-human CD14	TUK4	mIgG2a	Miltenyi Biotec
PE anti-human CD16	3G8	mIgG1	BioLegend
PE anti-human CD163	GHI/61	mIgG1	BioLegend
PE anti-human CD40	HB14	mIgG1	BioLegend
PE anti-human CD80	2D10	mIgG1	BioLegend
PE anti-human CD83	HB15	mIgG1	Miltenyi Biotec
PE anti-human CD86	IT2.2	mIgG2b	BioLegend
PE anti-human FcεRI	CRA1	mIgG2b	Miltenyi Biotec
PE anti-human CD206	15-2	mIgG1	BioLegend
PE anti-human CD209 (DC-SIGN)	9E9A8	mIgG2a	BioLegend
<i>PE mouse IgG1</i>	MOPC-21	mIgG1	BioLegend
PE-Cy7 anti-human CD19	HIB19	mIgG1	BioLegend
PE-Cy7 anti-human CD3	UCHT1	mIgG1	BioLegend
PerCP-Cy5.5 anti-human CD16	3G8	mIgG1	BioLegend
Vioblue anti-human CD11c	MJ4-27G12	mIgG2b	Miltenyi Biotec



Supplementary Figure S1. Gating strategy to distinctively identify slan/M-DC8⁺ DCs, CD1c⁺ DCs, CD141⁺ DCs and CD14⁺CD11b⁺ monocytes/macrophages in human tonsils.

Single cell suspensions from tonsils were processed for flow cytometry analysis to identify slan/M-DC8⁺ DCs, CD1c⁺ DCs, CD141⁺ DCs and CD14⁺CD11b⁺ monocytes/macrophages. Steps 1-4 were sequentially used to exclude cell debris (1), doublets (2), dead cells (3), and, ultimately, to gate CD45⁺ leukocytes (4). Subsequently, in steps 5-7, analysis was performed on CD3/CD19-negative cells (5). Within the latter cells, HLA-DR-positive (6) and subsequently HLA-DR⁺CD11c⁺ cell populations (7) were gated. The latter HLA-DR⁺CD11c⁺ population includes, in fact, all myeloid DCs, macrophages and monocytes. Steps 8-11 show the specific combination of markers used to gate each myeloid population type: slan/M-DC8⁺ DCs (purple gate, 8), CD1c⁺ DCs (green gate, 9), CD141⁺ DCs (orange gate, 10) and CD14⁺/CD11b⁺ monocytes/macrophages (blue gate, 11).



Supplementary Figure S2. TNF α and IL-12p70 secretion by tonsil slan/M-DC8⁺ DCs.

Tonsil cell suspensions were incubated with or without 100 U ml⁻¹ IFN γ plus either 100 ng ml⁻¹ LPS or 5 μ M R848, either for 4 h (to detect TNF α secretion), or for 12 h, after a 6 h pre-incubation (to detect IL-12p70 secretion). Contour plots display a representative experiment illustrating the percentage of TNF α - (a) or IL-12p70- (b) secreting tonsil slan/M-DC8⁺ DCs.