Online supplement methods and figures

Human bronchial epithelium orchestrates dendritic cell activation in severe asthma.

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Materials and methods

Study subjects

Diagnosis of asthma was based on the assessment of clinical features consistent with asthma and objective criteria of variable airflow obstruction (FEV1 increase of at least 15% after inhalation of 200 µg of salbutamol). Asthma severity was evaluated according to the current GINA guidelines (www. ginaasthma.org). Patients with severe asthma also met the American Thoracic Society (ATS) criteria for refractory asthma (1). Asthmatic patients had been free of respiratory infections and asthma exacerbations for at least 6 weeks at the time of inclusion. None of the asthma patients had nasal polyps and all tolerated NSAIDs. All subjects were current non-smokers and those who had smoked in the past had a smoking history of less than 5 pack/year.

Control subjects (no asthma, no allergy, non-smokers, normal chest X-ray and pulmonary function tests), underwent bronchoscopy for various purposes (three foreign body removal, two unconfirmed haemoptysis suspicions and one peripheral nodule (< 10 mm) found on CT scan examination) and had normal macroscopic appearance of the airways.

Endobronchial biopsies

Flexible bronchoscopy was performed by the same operator and two biopsies from each donor were removed using alligator forceps (Olympus, Tokyo, Japan) on a subsegmental bronchus of the left lower lobe, as previously described (2).

Peripheral blood collection

Antecubital venepuncture of volunteers allowed blood collection (20 mL) into heparinized tubes which were gently mixed. Blood was transported and stored at 15-25°C for no longer than 20 hours prior to PBMC isolation.

Phenotype of HBEC culture

Transepithelial electrical resistance (TEER) was monitored weekly using an EVOM volt-ohmmeter (World Precision Instruments, Aston, UK) and cells with TER $\geq 400\Omega.cm^{-2}$ on day 21 were used for experiments.-After 21 days, ALI cultured epithelium was fixed for 10 min in 4% paraformaldehyde, followed by a 15 min permeabilization in 0.1% Triton X-100 in PBS, and one hour blocking buffer (Sigma-aldrich, France) incubation. Then, cells were stained with appropriated primary antibodies directed against MUC-5AC (abcam, France), β-tubulin IV (Sigma-aldrich, France) (abcam, France) or zonula-occludens 1 (ZO-1) at 4°C overnight and revealed with FITC or TRITC conjugated antibody (Jackson Immunoresearch,). Slides were mounted with the antifading agent Slowfade (Molecular Probes, Invitrogen, Carlsbad, Calif). Cells were visualized using a Nikon Eclipse NiE microscope equipped with DS-Ri2 camera (Nikon,Tokyo,Japan).

Monocyte-derived Dendritic cells

Briefly, fresh heparinized blood obtained from volunteers was used for leukocyte separation (MSL, Eurobio) and then peripheral blood mononuclear cells were allowed to adhere for 2 h

to cell culture plates. Non-adherent cells (lymphocytes) were removed and frozen in a mix of FBS 80%/DMSO 20% until use. Adherent cells (monocytes) were cultured for 6 days in RPMI 1640 supplemented with 10% fetal bovine serum, 2mM L-glutamin, 100 U/mL penicillin and 100 µg/mL streptomycin. Recombinant human IL-4 (300 U/mL; Eurobio, Courtaboeuf, France) and GM-CSF (100 U/mL; R&DSystems, Lille, France) were added at the onset of culture and every other day to obtain immature dendritic cells.

To investigate DC differentiation, moDC cytospin slide were prepared and stained with Hemacolor kit (Merck, Molsheim, France) (Figure S2C). CD11c expression was checked and reached virtually 100% of non-adherent cells. The maturation marker CD83 was virtually absent on plasma membrane of immature DC, and low levels of cell surface molecules involved in antigen presentation and costimulatory signal delivery (HLA-DR, CD80, CD86 and CD40) were detected (data not shown). Moreover, to demonstrate purity of moDC and exclude lymphocyte contamination, cells were stained with human anti CD11c-FITC (DakoCytomation, France), CD19-PE and CD3-PE (Beckman Coulter, France) and negative results were obtained (Figure S2D and 2E).

Particulate matter

Jet exhaust particles are spherical (9.9 nm diameter) carbonaceous compounds displaying physical, chemical and DC-adjuvant properties similar to those of Diesel exhaust particles. For cell culture experiments, PM were solubilized in DMSO for a stock solution of 50 mg/mL and extemporaneously diluted in culture medium at a final concentration of 100µg/mL for coculture experiments.

Transmission Electron Microscopy

PM and *ex vivo* differentiated epithelium pulsed with PM for 30 min, 2 hours and 24 hours were examined by transmission electron microscopy (TEM) using a Hitachi 7100 transmission electron microscope (IBDML, Marseille, France).

Cells were immersed in a solution of 3.5% glutaraldehyde in PBS (0.1M, pH 7.4) overnight at 4°C. They were then rinsed in phosphate buffer and post-fixed in a 1% osmic acid containing 0.8% potassium ferrocyanide for 2 hours in the dark at room temperature. After two rinses in a phosphate buffer, cells were dehydrated in a graded series of ethanol solutions (30-100%). Cells were embedded in EmBed 812 DER-736 (Electron Microscopy Sciences, Hartfield, PA). Thin sections (85 nm; Leica-Reichert Ultracut E) were collected at different levels of each block. These sections were counterstained with uranyl acetate and lead citrate.

Coculture of bronchial epithelial cells and dendritic cells

All cocultures were performed on a heterologous mode, i.e. HBEC and moDC were issued from distinct donors, except where specifically stated. In some experiments, 100 µg/mL PM or vehicle was added on top of the HBEC layer. The HBEC-moDC cocultures were incubated for 24 hours at 37°C and 5% CO2, and then HBEC-coated transwell were removed. Content of the basal compartment was centrifuged; supernatants were stored at -80°C for further cytokine and chemokine measurements. moDC in the pellet were checked for viability by trypan blue exclusion prior to labelling for flow cytometry.

Immunoassays for epithelial and dendritic cells markers

Cytokines and chemokines measurement in basal supernatants

Briefly, specific antibody-coated beads detected target cytokines or chemokines, the signature of each type of bead being unique phycoerythrin-fluorescence intensity. Experiments were

performed with a FACS Canto II flow cytometer using the FacsDIVA software (Becton Dickinson, Le Pont de Claix, France).

Conditioned-DC/lymphocyte coculture

moDC from control volunteers (n=3) were obtained as previously described. To condition DC, they were incubated in supernatant from cHBEC or saHBEC in triplicate for 24h. After centrifugation, DC were cocultured in RPMI/FBS 10% with lymphocytes (ratio 1:10) from the same donors (obtained during preparation of moDC) for 48h. Supernatant were removed and IL2, IL4, IL6, IL10 and IFNγ were measured by cytometric bead array (Becton Dickinson, Le Pont de Claix, France).

Statistical analysis

All results were expressed as median (min-max). A normality test was performed showing that the results did not follow a normal distribution. Statistical comparisons were performed using non parametric Wilcoxon's test. The significance level was set at $p \leqslant 0.05$. Variation rate was calculated using the formula (final value - initial value)/ (absolute value of initial value x100). Statistical comparisons were performed using non parametric Mann-Whitney (when 2 groups were compared) or Kruskal-Wallis's test (when 3 groups were compared) followed by post-hoc test. The significance level was set at $p \leq 0.05$.

References

1. Chanez P, Wenzel SE, Anderson GP, Anto JM, Bel EH, Boulet LP, et al. Severe asthma in adults: what are the important questions? J Allergy Clin Immunol. 2007;119(6):1337-48.

2. Wilson JW, Li X. The measurement of reticular basement membrane and submucosal collagen in the asthmatic airway. Clin Exp Allergy. 1997;27(4):363-71.

Figure Legends

Figure S1. Experimental design

(A) HBEC were obtained from control and severe asthmatic volunteers by bronchoscopy. After 21 days in air liquid interface culture, fully differentiated mucociliary epithelia were obtained. *(B)* Monocytes were purified from peripheral blood of control and severe asthmatic volunteers. Then they were cultured with rhIL4 and rhGM-CSF during 6 days to obtain moDC. *(C)* Coculture systems were generated by adding moDC at the basolateral side of epithelia (from left to right control HBEC-control moDC, asthmatic HBEC-control moDC and asthmatic HBEC-asthmatic moDC). *(D)* PM obtained from jet aircraft engine was added at the apical side of epithelia. *(E)* PM obtained from jet aircraft engine was added at the apical side of the different coculture systems.

Figure S2. Characterisation of HBEC and moDC model.

Antibodies directed against MUC5AC and β-tubulin IV (Figure S2*A*, red and green respectively) and ZO-1 (Figure S2*B*) were used to identify differentiation and pseudostratification of epithelia (Magnification x400). Cytospin of moDC were stained with Hemacolor kit (Figure S2*C*). moDC were stained for DC marker (CD11c) and T cells or B cells marker (CD3 and CD19 respectively) using flow cytometry. Representative dot plots are shown on Figure S2*D* and S2 *E* respectively.

Figure S3. Effect of autologous asthmatic HBEC-asthmatic moDC coculture on chemokine/cytokine secretion, costimulatory molecule mRNA expression and moDC phenotype.

HBEC and moDC obtained from the same severe asthmatic patient (SA) were cocultured for 24h *(A)* Levels of CXCL8, CXCL10, CCL2, TSLP and IL-33 (pg/ml) were measured in basolateral samples from ALI cultures. *(B)* Semi-quantitative analysis of PD-L1 and PD-L2 mRNA expression with 18S as a reporter gene. *(C)* Surface expression of CD11c, CD83, HLA-DR, CD40, CD80 and CD86 was measured by flow cytometry on moDC. Results represent median fluorescence intensity (MFI) of the whole DC population after isotype control subtraction. All results are expressed as the median and interquartile range, with the min and max values (n=4).

Figure S4. Effect of PM addition on autologous asthmatic HBEC-asthmatic moDC coculture on chemokines/cytokines secretion, costimulatory molecule mRNA expression and moDC phenotype.

HBEC from severe asthmatic patients were cocultured for 24h with severe asthmatic moDC obtained from the same patient. PM were added concomitantly on the apical surface of the system. *(A)* Levels of CXCL8, CXCL10, CCL2, TSLP and IL-33 (pg/ml) were measured in basolateral samples from ALI cultures. *(B)* Semi-quantitative analysis of PD-L1 and PD-L2 mRNA expression with 18S as a reporter gene. *(C)* Surface expression of CD11c, CD83, HLA-DR, CD80, CD86 and CD40 was measured by flow cytometry on moDC. Results represent median fluorescence intensity (MFI) of the whole moDC population after isotype control subtraction. All results are expressed as the median and interquartile range, with the min and max values (n=4).

Table S1: Effect of HBEC-moDC coculture on HBEC mediator release and moDC phenotype (raw data)

Target		moDC	HBEC	HBEC+moDC	p-value[1]	Variation rate[2]	p-value[3]
CXCL8	C/C	0 (0-72.4)	9425 (4640-19979)	27230 (4370-37174)	NS	62.3 (-5.8-356)	NS
	SA/C	0 (0-25.6)	14690 (3830-51520)	34058 (7123-56502)	**0.03**	80.5 (9.7-850)	
	SA/SA	0 (0-0)	8180 (1751-35048)	36419 (7385-41546)	**0.015**	142.4 (3.9-670)	
TSLP	C/C	0 (0-0)	8.75 (2.5-11.25)	11.25 (7.5-25)	NS	64.3 (0-200)	NS
	SA/C	0 (0-0)	10 (2.5-17.5)	16.3 (7.5-47.5)	**0.015**	85.7 (15.4-500)	
	SA/SA	0 (0-0)	10 (6.25-16.3)	20 (11.3-38.8)	**0.008**	113 (44.4-480)	
IL33	C/C	0 (0-0)	9.1 (4.5-14.5)	12.2 (9-17.2)	NS	25.9 (-31-177)	NS
	SA/C	0 (0-0)	10.7 (6.2-15.2)	14.1 (10.3-18.6)	**0.015**	29 (17.5-66)	
	SA/SA	0 (0-0)	10.7 (8.8-11.2)	14.7 (12.8-16.6)	**0.008**	47.7 (19-63.6)	
CXCL10	C/C	0 (0-0)	253.8 (33-2660)	628.06 (79.1-6946)	**0.03**	146.1 (67-256)	NS
	SA/C	0 (0-0)	75.66 (6.2-144.8)	78.6 (28.1-1323)	NS	39.4 (-57-1649)	
	SA/SA	3.9 (1.7-21)	44.15 (16.3-84.1)	87.9 (27.7-376.5)	NS	137.5 (-9.2-493)	
CCL2	C/C	148 (0-460)	322 (195-1083)	1046 (286.1-1397)	**0.03**	55.1 (5.2-296.8)	NS
	SA/C	127 (16-627)	1401 (124-3684)	1466 (442-2312)	NS	-7.2 (-37.2-257)	
	SA/SA	82.8 (6-1582)	3955 (223-11740)	5886 (766-10331)	NS	14.4 (-28.1-244)	
IL25 mRNA	C/C	NA	0.75 (0.43-1.2)	0.5 (0.41-0.94)	NS	-18.3 (-58.6-83)	NS
	SA/C	NA	0.59 (0.26-1.79)	0.38 (0.36-1.69)	NS	-7.4 (-78.1-42)	
	SA/SA	NA	0.59 (0.26-1.81)	0.48 (0.36-1.69)	NS	-15.1 (-78-83.2)	
PD-L1 mRNA	C/C	NA	1.32 (0.46-4.5)	3.61 (0.53-9.1)	NS	97.2 (-16.9-628)	NS
	SA/C	NA	0.46 (0.33-1.07)	2.56 (0.59-4.31)	**0.02**	315.7 (30.3-847)	
	SA/SA	NA	0.25 (0.1-0.9)	1.47 (0.55-4.85)	**0.02**	508.9 (-6.7-1335)	
PD-L2 mRNA	C/C	NA	0.7 (0.23-2.8)	2.39 (0.9-15.85)	**0.03**	187.1 (43.7-847)	NS
	SA/C	NA	0.25 (0.12-1.34)	1.61 (0.86-12.1)	**0.02**	800.3 (165-1258)	
	SA/SA	NA	0.09 (0.04-0.9)	0.53 (0.47-4.85)	**0.02**	683.8 (127-1200)	
CD11c	C/C	4.785 (3-11.1)	NA	8.11 (7.2-11.43)	NS	56.35 (-10,7-169)	NS
	SA/C	7.625	NA	7.64	NS	4.61	

		(4.2-10.9)		(4.8-8.8)		(-34.4-45)	
	SA/SA	8.66 (3.9-10)	NA	9.6 (4.6-11.6)	NS	13.28 (-8.6-99.7)	
CD83	C/C	0.1 (0-0.55)	NA	0.05 (0-0.66)	NS	-40 (-100-62.5)	NS
	SA/C	0.48 (0-0.9)	NA	0.46 (0.15-0.89)	NS	36.36 (-46.7-102)	
	SA/SA	0.18 (0.0-0.87)	NA	0.29 (0-0.62)	NS	-5 (-29-77.8)	
HLA-DR	C/C	5.94 (5.3-14.1)	NA	15.87 (10.5-35.8)	**0.03**	146.6 (30.3-209)	NS
	SA/C	6.03 (0.6-17.7)	NA	9.54 (1.87-27.86)	NS	60.5 (-23.2-234)	
	SA/SA	15.73 (0.9-49.3)	NA	29.18 (0.91-38.9)	NS	-15.6 (-26.3-108)	
CD40	C/C	13.34 (3.9-21.9)	NA	20.91 (19.8-37.3)	**0.03**	72.98 (26.1-455)	NS
	SA/C	14.26 (5.2-33.6)	NA	22.13 (9.99-44.18)	NS	23.05 (10.2-93.6)	
	SA/SA	20.95 (3.9-37.1)	NA	18.52 (10-31.75)	NS	34.85 (-23.4-155)	
CD80	C/C	1.12 (0.65-4.4)	NA	2.580 (1-6.64)	**0.03**	52.72 (13.9-272)	**0.025**
	SA/C	3.89 (2.5-5.6)	NA	4.46 (0.97-6.3)	NS	4.79 [#] (-66.2-44)	
	SA/SA	3.04 (0-9.02)	NA	3.34 (0-9.6)	NS	-7.5 [#] (-79-55.4)	
CD86	C/C	1.460 (0.34-3.8)	NA	0.71 (2.7-10.38)	**0.03**	118 (26.2-567)	NS
	SA/C	2.64 (0.05-7.5)	NA	3.9 (0.75-11.68)	**0.03**	78.19 (2.8-1400)	
	SA/SA	4.06 (0.03-19)	NA	8.52 (1.2-19.15)	**0.03**	29.29 (-0.4-590)	

Values are median (min-max)
CXCL8, TSLP, IL33, CXCL10 and CCL2 are expressed in pg/ml
IL25, PD-L1 and PD-L2 are expressed in arbitrary unit
CD11c, CD83, HLA-DR, CD80, CD86 and CD40 are expressed in MFI
[1] Wilcoxon test between HBEC and HBEC + moDC
[2] Compared to HBEC alone, values are expressed in %
[3] Kruskal-Wallis test followed by post-hoc test if necessary between the 3 groups.
[#] significant compared to C/C
NA, not applicable
NS, not significant

Table S2 : Effect of PM on HBEC mediator release (raw data)

Target		HBEC	HBEC+PM	p-value[1]	Variation rate[2]	p-value[3]
CXCL8	C	9426 (4640-19980)	15291 (3540-22585)	NS	12.99 (-23.7-62.7)	NS
	SA	8180 (1751-51520)	21560 (5371-57023)	**0.001**	99.06 (-8.9-354.7)	
TSLP	C	8.75 (2.5-11.25)	8.75 (5-12.5)	NS	11.11 (-28.57-100)	NS
	SA	10 (2.5-17.5)	10 (7.5-17.5)	NS	0 (-38.46-250)	
IL33	C	9.1 (4.5-14.5)	11.9 (5.9-15.9)	NS	18.82 (-26.2-111.1)	NS
	SA	10.7 (6.2-15.2)	11.9 (6.9-16.6)	**0.0003**	11.11 (-6.5-49.1)	
CXCL10	C	253.8 (33-2660)	273.7 (42.51-1417)	NS	2.963 (-46.7-31.7)	NS
	SA	42.87 (6.24-162.3)	58.39 (5.97-1444)	NS	22.9 (-74.65-1382)	
CCL2	C	119.2 (30.57-622.9)	175.6 (56.36-842.5)	NS	76.14 (-66.2-123.9)	NS
	SA	427.5 (36.29-2709)	539.9 (14.97-3503)	NS	31.09 (-65.44-1325)	
IL25 mRNA	C	0.745 (0.43-1.19)	0.487 (0.315-1.04)	**0.03**	-27.55 (-41.2- -12.7)	**0.04**
	SA	0.596 (0.26-1.79)	0.769 (0.28-1.76)	NS	6.101 (-35.75-63.5)	
PD-L1 mRNA	C	1.32 (0.46-4.52)	1.224 (0.575-3.69)	NS	11.53 (-30.04-37.9)	NS
	SA	0.452 (0.097-1.07)	0.366 (0.17-1.76)	NS	-1.778 (-67.8-277.1)	
PD-L2 mRNA	C	0.7044 (0.23-2.8)	0.8042 (0.06-2.53)	NS	3.738 (-73.9-20.83)	NS
	SA	0.1462 (0.04-1.34)	0.1373 (0.026-2.65)	NS	-1.546 (-89-176.4)	

Values are median (min-max)
CXCL8, TSLP, IL33, CXCL10 and CCL2 are expressed in pg/ml
IL25, PD-L1 and PD-L2 are expressed in arbitrary unit
[1] Wilcoxon test between HBEC and HBEC + PM
[2] Compared to HBEC alone, values are expressed in %
[3] Mann-Whitney test between the 2 groups
NS, not significant

Table S3: Effect of PM on HBEC/moDC coculture (raw data)

Target		HBEC+moDC	HBEC+moDC+PM	p-value[1]	Variation rate[2]	p-value[3]
CXCL8	C/C	27230 (4370-37174)	20956 (3474-44585)	NS	-17,46 (-29,43-71,65)	NS
	SA/C	34058 (7123-56502)	42453 (25772-78195)	NS	49,36 (-18,62-261,8)	
	SA/SA	36419 (7385-41546)	45557 (5712-89772)	NS	10,24 (-57,6-787,5)	
TSLP	C/C	11.25 (7.5-25)	17.50 (10-22.5)	NS	20 (-20-75)	NS
	SA/C	16.3 (7.5-47.5)	11.3 (5-45)	NS	-21,43 (-66,67-26,67)	
	SA/SA	20 (11.3-38.8)	17.55 (10-56.3)	NS	-0,717 (-62,96-55,17)	
IL33	C/C	12.2 (9-17.2)	11.05 (7.6-17.2)	NS	-14,36 (-24,14-9,76)	NS
	SA/C	14.1 (10.3-18.6)	12.1 (6.6-17.6)	NS	-14,63 (-42,55-15,15)	
	SA/SA	14.7 (12.8-16.6)	14.8 (8-17)	NS	-0,607 (-40,3-21,81)	
CXCL10	C/C	628.06 (79.1-6946)	573.8 (50.59-2832)	NS	-28,89 (-59,23-75,89)	NS
	SA/C	78.6 (28.1-1323)	118.7 (15.21-1279)	NS	29,32 (-79,94-50,96)	
	SA/SA	87.9 (27.7-376.5)	49.05 (22.97-1335)	NS	-25,09 (-59,32-254,6)	
CCL2	C/C	1046 (286.1-1397)	696.5 (314.2-1843)	NS	-13,07 (-39,77-64,82)	**0.0135**
	SA/C	1466 (442-2312)	2155 (1255-5147)	**0.015**	88,39 [#] (9,25-183,8)	
	SA/SA	5886 (766-10331)	6804 (847.8-9493)	NS	17,05 (-23,53-101,5)	
IL25 mRNA	C/C	0.5 (0.41-0.94)	0.3992 (0.289-1.06)	NS	-11,46 (-64,13-66,41)	NS
	SA/C	0.38 (0.36-1.69)	0.608 (0.348-2.289)	NS	17,31 (-38,18-482,4)	
	SA/SA	0.48 (0.36-1.69)	0.5285 (0.289-2.289)	NS	3,686 (-64,14-482,4)	
PD-L1 mRNA	C/C	3.61 (0.53-9.1)	3.977 (0.611-5.536)	NS	9,903 (-45,24-43,16)	NS
	SA/C	2.56 (0.59-4.31)	2.167 (0.575-4.579)	NS	0,22 (-22,24-39,64)	
	SA/SA	1.47 (0.55-4.85)	1.707 (0.929-7.282)	NS	48,22 (0,904-88,19)	
PD-L2 mRNA	C/C	2.39 (0.9-15.85)	2.512 (0.677-4.044)	NS	-4,108 (-74,55-148,6)	NS
	SA/C	1.61 (0.86-12.1)	1.603 (0.505-11.36)	NS	-20,41 (-40,96-414,5)	
	SA/SA	0.53 (0.47-4.85)	0.472 (0.231-7.282)	NS	-16,11 (-100-50,05)	
CD11c	C/C	8.11 (7.2-11.43)	7,26 (6,75-10,31)	NS	-13,03 (-20,28-35,3)	NS
	SA/C	7.64 (4.8-8.8)	7,065 (5,56-10,13)	NS	9,761 (-9,283-18,24)	
	SA/SA	9.6	9,95	NS	5,964	

		(4.6-11.6)	(4,83-20,02)		(-7,143-72,59)	
CD83	C/C	0.05 (0-0.66)	0,005 (0-0,84)	NS	-96,15 (-100-27,27)	NS
	SA/C	0.46 (0.15-0.89)	0,565 (0,28-1,55)	NS	46,69 (-20-86,67)	
	SA/SA	0.29 (0-0.62)	0,33 (0-0,82)	NS	31,61 (-9,091-500)	
HLA-DR	C/C	15.87 (10.5-35.8)	18,83 (10,91-171,3)	NS	6,608 (-41,49-23,82)	NS
	SA/C	9.54 (1.87-27.86)	9,917 (2,2-34,05)	NS	15,90 (-3,46-22,64)	
	SA/SA	29.18 (0.91-38.9)	17,85 (0,68-37,13)	NS	-3,055 (-39,45-166,4)	
CD40	C/C	20.91 (19.8-37.3)	21,69 (17,48-40,99)	NS	4,444 (-12,56-9,95)	NS
	SA/C	22.13 (9.99-44.18)	22,45 (9,65-52,2)	NS	3,593 (-10,17-18,15)	
	SA/SA	18.52 (10-31.75)	24,28 (7-31,35)	NS	-1,26 (-30-46,53)	
CD80	C/C	2.580 (1-6.64)	1,73 (1,16-7,03)	NS	-12,87 (-59,3-78)	NS
	SA/C	4.46 (0.97-6.3)	5,07 (2,38-12,1)	NS	23,11 (-5,464-145,4)	
	SA/SA	3.34 (0-9.6)	2,6 (0,21-12,02)	NS	-8,124 (-51,03-153,5)	
CD86	C/C	0.71 (2.7-10.38)	1,515 (0,51-13,99)	NS	-18,94 (-77,5-38,03)	NS
	SA/C	3.9 (0.75-11.68)	4,515 (1-16)	NS	25,12 (-9,32-36,99)	
	SA/SA	8.52 (1.2-19.15)	8,16 (1,59-16,95)	NS	1,383 (-24,39-166,7)	

Values are median (min-max)
CXCL8, TSLP, IL33, CXCL10 and CCL2 are expressed in pg/ml
IL25, PD-L1 and PD-L2 are expressed in arbitrary unit
CD11c, CD83, HLA-DR, CD80, CD86 and CD40 are expressed in MFI
[1] Wilcoxon test between HBEC and HBEC + moDC
[2] Compared to HBEC +moDC, values are expressed in %
[3] Kruskal-Wallis test followed by post-hoc test if necessary between the 3 groups.
[#] significant compared to C/C
NS, not significant

Table S4: Heterologous vs Autologous coculture of saHBEC (raw data)

Target		HBEC	HBEC+moDC	p-value[1]	Variation rate[2]	p-value[3]
CXCL8	Heterologous	8180 (1751-35048)	36419 (7385-41546)	**0.015**	142.4 (3.9-670)	NS
	Autologous	20978 (1620-52581)	39508 (4418-57251)	NS	89.1 (27.9-172.8	
TSLP	Heterologous	10 (6.25-16.3)	20 (11.3-38.8)	**0.008**	113 (44.4-480)	NS
	Autologous	13.42 (1.053-99.21)	153.8 (21.05-342.1)	NS	205.7 (68.99-491.7)	
IL33	Heterologous	10.7 (8.8-11.2)	14.7 (12.8-16.6)	**0.008**	47.7 (19-63.6)	NS
	Autologous	5.586 (4.03-5.77)	7.283 (6.63-8.42)	NS	44.47 (18.56-64.71)	
CXCL10	Heterologous	44.15 (16.3-84.1)	87.9 (27.7-376.5)	NS	137.5 (-9.2-493)	NS
	Autologous	123.7 (9.57-1362)	104 (16.2-2150)	NS	26.3 (-86.04-40.91)	
CCL2	Heterologous	3955 (223-11740)	5886 (766-10331)	NS	14.4 (-28.1-244)	NS
	Autologous	523.1 (169.4-876.7)	310.6 (249.5-557.9)	NS	12.16 (-63.73-135.9)	
PD-L1	Heterologous	0.25 (0.1-0.9)	1.47 (0.55-4.85)	**0.02**	508.9 (-6.7-1335)	NS
	Autologous	0.481 (0.078-0.699)	1.323 (0.64-4.28)	NS	542.5 (-4.18-1384)	
PD-L2	Heterologous	0.09 (0.04-0.9)	0.53 (0.47-4.85)	**0.02**	683.8 (127-1200)	NS
	Autologous	0.278 (0.14-0.44)	0.855 (0.35-1.65)	NS	223.8 (141.2-393.5)	
Target		moDC	HBEC+moDC	p-value[1]	Variation rate[2]	p-value[3]
CD11c	Heterologous	8.66 (3.9-10)	9.6 (4.6-11.6)	NS	13.28 (-8.6-99.7)	NS
	Autologous	10.23 (4.18-11.99)	8.615 (6.72-13.66)	NS	12.96 (-36.2-60.77)	
CD83	Heterologous	0.18 (0.0-0.87)	0.29 (0-0.62)	NS	-5 (-29-77.8)	NS
	Autologous	0.1 (0.09-0.12)	0.09 (0.03-0.18)	NS	-7.22 (-70-50)	
HLA-DR	Heterologous	15.73 (0.9-49.3)	29.18 (0.91-38.9)	NS	-15.6 (-26.3-108)	NS
	Autologous	9.45 (3.44-34.65)	10.75 (0.18-34.78)	NS	16.59 (-97.13-37.5)	
CD40	Heterologous	20.95 (3.9-37.1)	18.52 (10-31.75)	NS	34.85 (-23.4-155)	NS
	Autologous	13.78 (13.24-20.1)	13.85 (11.25-21.32)	NS	-4.48 (-18.24-19.74)	
CD80	Heterologous	3.04 (0-9.02)	3.34 (0-9.6)	NS	-7.5 (-79-55.4)	NS
	Autologous	0.56 (0-3.22)	1.48 (1-1.8)	NS	-1.067 (-44.1-41.96)	
CD86	Heterologous	4.06 (0.03-19)	8.52 (1.2-19.15)	**0.03**	29.29 (-0.4-590)	
	Autologous	2.875 (1-5.87)	3.62 (1.57-7.21)	NS	39.91 (2.51-78.98)	NS

Values are median (min-max)
CXCL8, TSLP, IL33, CXCL10 and CCL2 are expressed in pg/ml
PD-L1 and PD-L2 are expressed in arbitrary unit
CD11c, CD83, HLA-DR, CD80, CD86 and CD40 are expressed in MFI
[1] Wilcoxon test between HBEC and HBEC + moDC
[2] Compared to HBEC, values are expressed in %
[3] Mann-Whitney test
NS, not significant

Table S5: Heterologous vs Autologous coculture of saHBEC + moDC ± PM (raw data)

Target		HBEC+moDC	HBEC+moDC+PM	p-value[1]	Variation rate[2]	p-value[3]
CXCL8	Heterologous	36419 (7385-41546)	45557 (5712-89772)	NS	10.24 (-57.6-787.5)	NS
	Autologous	39508 (4418-57251)	33576 (7476-97445)	NS	22.15 (-43.09-195.5)	
TSLP	Heterologous	20 (11.3-38.8)	17.55 (10-56.3)	NS	-0.717 (-62.96-55.17)	NS
	Autologous	153.8 (21.05-342.1)	76.84 (23.16-577.9)	NS	-4.625 (-67.7-68.92)	
IL33	Heterologous	14.7 (12.8-16.6)	14.8 (8-17)	NS	-0.607 (-40.3-21.81)	NS
	Autologous	7.283 (6.63-8.42)	7.428 (6.95-8.34)	NS	1.681 (-0.94-5.56)	
CXCL10	Heterologous	87.9 (27.7-376.5)	49.05 (22.97-1335)	NS	-25.09 (-59.32-254.6)	NS
	Autologous	104 (16.2-2150)	64.31 (29.07-1637)	NS	-27.99 (-41.3-79.5)	
CCL2	Heterologous	5886 (766-10331)	6804 (847.8-9493)	NS	17.05 (-23.53-101.5)	NS
	Autologous	310.6 (249.5-557.9)	669.7 (371-1433)	NS	35.73 (4.85-156.8)	
PD-L1	Heterologous	1.47 (0.55-4.85)	1.707 (0.929-7.282)	NS	48.22 (0.904-88.19)	NS
	Autologous	1.323 (0.64-4.28)	1.907 (1.065-2.947)	NS	29.06 (-31.1-65.4)	
PD-L2	Heterologous	0.53 (0.47-4.85)	0.472 (0.231-7.282)	NS	-16.11 (-100-50.05)	NS
	Autologous	0.855 (0.35-1.65)	0.879 (0.341-2.705)	NS	-2.659 (-18.51-64.44)	
CD11c	Heterologous	9.6 (4.6-11.6)	9.95 (4.83-20.02)	NS	5.964 (-7.143-72.59)	NS
	Autologous	8.615 (6.72-13.66)	8.1 (7.37-13.05)	NS	-1.972 (-11.17-9.673)	
CD83	Heterologous	0.29 (0-0.62)	0.33 (0-0.82)	NS	31.61 (-9.091-500)	NS
	Autologous	0.09 (0.03-0.18)	0.121 (0.01-0.29)	NS	10.56 (-92.31-606.7)	
HLA-DR	Heterologous	29.18 (0.91-38.9)	17.85 (0.68-37.13)	NS	-3.055 (-39.45-166.4)	NS
	Autologous	10.75 (0.18-34.78)	12.32 (0.127-41.51)	NS	15.31 (-33.33-26.64)	
CD40	Heterologous	18.52 (10-31.75)	24.28 (7-31.35)	NS	-1.26 (-30-46.53)	NS
	Autologous	13.85 (11.25-21.32)	15.64 (10.50-29.98)	NS	16.64 (-6.67-40.62)	
CD80	Heterologous	3.34 (0-9.6)	2.6 (0.21-12.02)	NS	-8.124 (-51.03-153.5)	NS
	Autologous	1.48 (1-1.8)	1.385 (0.82-2.1)	NS	-11.78 (-21.9-32.08)	
CD86	Heterologous	8.52 (1.2-19.15)	8.16 (1.59-16.95)	NS	1.383 (-24.39-166.7)	NS
	Autologous	3.62 (1.57-7.21)	2.335 (1.62-4.89)	NS	-27.6 (-37.86-54.92)	

Values are median (min-max)
CXCL8, TSLP, IL33, CXCL10 and CCL2 are expressed in pg/ml
PD-L1 and PD-L2 are expressed in arbitrary unit
CD11c, CD83, HLA-DR, CD80, CD86 and CD40 are expressed in MFI
[1] Wilcoxon test
[2] Compared to HBEC +moDC, values are expressed in %
[3] Mann-Whitney test
NS, not significant