

# **Development of a Simple and Robust Whole Blood Assay with Dual Co-Stimulation to Quantify the Release of T-Cellular Signature Cytokines in Response to *Aspergillus Fumigatus* Antigens**

Supplement

Table S1: Antibodies used for flow cytometric studies.

Fluorochrome		Panels			
		Cell populations	T-cell activation	Granulocyte activation	NK-cell activation
VioBlue	Marker	CD3	CD3		
	Volume	2 µl	2 µl		
	Company	Miltenyi Biotec	Miltenyi Biotec		
FITC	Marker	CD66b	CD4	CD66b	CD3
	Volume	2 µl	2 µl	2 µl	10 µl
	Company	Miltenyi Biotec	Miltenyi Biotec	Miltenyi Biotec	BD
PE	Marker		IFN-γ	CD62L	IFN-γ
	Volume		2 µl	2 µl	2 µl
	Company		Miltenyi Biotec	Miltenyi Biotec	Miltenyi Biotec
PE-Vio615	Marker		CD69		CD69
	Volume		2 µl		2 µl
	Company		Miltenyi Biotec		Miltenyi Biotec
PerCP	Marker	CD14	CD8		CD16
	Volume	2 µl	2 µl		5 µl
	Company	Miltenyi Biotec	Miltenyi Biotec		Biologend
PE-Vio770	Marker		CD107a		CD107a
	Volume		10 µl		10 µl
	Company		Miltenyi Biotec		Miltenyi Biotec
APC	Marker	CD56	CD154	CD11b	CD56
	Volume	5 µl	2 µl	2 µl	5 µl
	Company	BD	Miltenyi Biotec	Miltenyi Biotec	BD
APC-Vio750	Marker	L/D fixable near-IR	L/D fixable near-IR	L/D fixable near-IR	L/D fixable near-IR
	Volume	0.1 µl	0.1 µl	0.1 µl	0.1 µl
	Company	Invitrogen	Invitrogen	Invitrogen	Invitrogen

Added directly to WB stimulation tubes as described in Materials & Methods (section 2.7)

Life/Dead staining (L/D) performed before extracellular staining in 1 ml Hank's balanced salt solution for 30 min

Extracellular staining

Intracellular staining

Abbreviations: APC = allophycocyanin, FITC = fluorescein isothiocyanate, IR = infra red, PE = phycoerythrin, PerCP = Peridinin-Chlorophyll-Protein.

**Table S2: Raw data for AfuLy-induced cytokine release (in pg/ml) in samples from patients with *Aspergillus*-associated lung pathologies and other chronic lung diseases.**

Patient →	Control	Control	Control	Control	Control	CF	CF	ABPA	ABPA	ABPA	CPA	CPA	CPA	CPA	Median	Median	
Cytokine	#1	#2	#3	#4	#5	#1	#2	#1	#2	#3	#1	#2	#3	#4	Controls	Asp-ass Dis.	MMR
IFN- $\gamma$	0.0	0.0	0.0	23.2	5.5	28.9	16.7	6.4	6.0	0.6	29.0	3.8	23.9	10.0	0.0	10.0	INF
IL-2	0.0	0.4	0.0	136.2	1.0	46.4	4.8	0.8	4.2	36.1	46.1	41.7	312.6	4.0	0.4	36.1	85.9
IL-4	0.0	0.0	0.0	0.0	0.0	36.5	4.5	7.3	0.0	2.7	19.0	2.7	11.7	2.7	0.0	4.5	INF
IL-5	0.0	0.0	0.6	5.0	0.7	22.6	4.5	3.6	1.4	4.3	22.2	0.9	22.6	1.9	0.6	4.3	7.1
IL-10	0.0	0.0	0.0	292.6	0.0	185.5	70.4	0.0	20.3	72.8	1886.9	10.8	18.7	32.4	0.0	32.4	INF
IL-13	0.5	0.0	0.0	39.3	0.0	27.4	8.7	0.0	0.0	2.8	33.5	2.2	34.8	3.9	0.0	3.9	INF
IL-17A	0.0	0.0	1.3	48.3	6.2	28.7	21.6	8.2	10.1	5.5	24.1	4.1	17.1	14.2	1.3	14.2	10.9
TNF- $\alpha$	2.1	0.0	0.0	2339.4	0.0	561.2	3324.0	0.0	1422.2	158.8	15241.4	5.4	721.4	640.6	0.0	640.6	INF
MIP-1 $\alpha$	8.6	0.0	11.6	97207.3	459.9	8137.0	28744.2	45.3	3169.3	1483.2	97207.3	171.5	97191.7	1290.6	11.6	3169.3	273.0
MIP-1 $\beta$	0.0	0.0	0.0	1593.4	0.0	622.8	1026.9	0.0	412.5	415.7	23121.0	175.7	2867.8	587.1	0.0	587.1	INF
MIP-3 $\alpha$	0.0	0.0	190.2	14286.5	1939.0	3459.0	12636.9	21.0	10155.0	9671.1	161434.3	256.4	3322.4	5507.9	190.2	5507.9	29.0
IL-1 $\beta$	0.0	0.0	0.0	649.7	4.6	0.0	7716.3	0.0	1527.9	85.9	53147.3	0.0	209.8	1070.2	0.0	209.8	INF
IL-6	0.0	0.0	0.0	25229.5	8.2	4647.9	2684.6	2.2	3077.8	3247.5	26841.8	7.0	11703.5	2294.6	0.0	3077.8	INF
IL-7	0.0	5.3	0.0	30.1	0.0	56.4	15.0	0.0	0.0	11.8	61.1	9.7	26.0	0.0	0.0	11.8	INF
IL-8	0.0	11402.0	52040.8	75918.0	77693.0	36622.0	96147.0	13346.0	57674.0	46756.0	89414.0	7642.5	98065.9	42691.6	52040.8	46756.0	0.9
IL-12p70	0.0	0.0	0.0	2.3	0.0	9.1	0.0	0.0	0.0	0.0	4.3	0.0	0.0	1.5	0.0	0.0	ND
IL-21	0.0	0.0	0.0	126.7	0.0	129.8	89.5	0.0	17.0	58.3	179.1	0.0	128.8	38.8	0.0	58.3	INF
IL-23	0.0	0.0	36.7	146.6	0.0	527.4	180.9	0.0	0.0	37.1	253.4	0.0	169.6	104.0	0.0	104.0	INF
GM-CSF	0.0	0.0	15.3	463.4	20.2	167.0	126.6	13.6	85.7	53.5	1204.5	27.1	251.0	42.8	15.3	85.7	5.6
Fractalkine	0.0	0.0	0.0	0.0	0.0	356.5	0.0	0.0	0.0	0.0	2.8	0.0	146.6	96.5	0.0	0.0	ND
ITAC	116.0	162.7	199.0	347.0	205.0	396.3	531.6	136.0	187.0	208.7	174.6	530.7	324.2	123.0	199.0	208.7	1.0

Abbreviations: ABPA = allergic bronchopulmonary aspergillosis, AfuLy = *A. fumigatus* mycelial lysate, Asp-ass. Dis. = *Aspergillus*-associated diseases, CF = cystic fibrosis, CPA = chronic pulmonary aspergillosis, INF = infinite (0 pg/ml in the control cohort), MMR = median-to-median ratio, ND = not defined (0 pg/ml in both cohorts).

**Table S3: Raw data for Asp4-induced cytokine release (in pg/ml) in samples from patients with *Aspergillus*-associated lung pathologies and other chronic lung diseases.**

Patient →	Control	Control	Control	Control	Control	CF	CF	ABPA	ABPA	ABPA	CPA	CPA	CPA	CPA	Median	Median	MMR
Cytokine	#1	#2	#3	#4	#5	#1	#2	#1	#2	#3	#1	#2	#3	#4	Controls	Asp-ass Dis.	
IFN- $\gamma$	17.9	16.8	11.7	15.0	19.1	70.2	8.2	22.7	11.3	0.0	74.9	2.0	8.2	27.1	16.8	11.3	0.7
IL-2	7.9	5.8	1.5	0.6	8.5	17.1	8.5	9.6	12.5	3.5	11.0	0.2	4.2	6.0	5.8	8.5	1.5
IL-4	3.6	7.3	0.0	0.0	3.6	21.8	2.7	22.0	10.9	0.0	0.0	0.0	0.0	2.7	3.6	2.7	0.7
IL-5	5.5	4.8	1.2	6.3	8.6	21.8	3.1	10.3	8.8	0.1	11.3	1.2	2.4	4.6	5.5	4.6	0.8
IL-10	83.8	140.9	165.1	183.7	131.1	1244.0	871.9	127.1	631.8	125.5	16510.9	0.0	16.0	1205.6	140.9	631.8	4.5
IL-13	5.8	5.1	1.7	2.2	8.8	18.3	7.7	7.8	10.9	0.0	13.8	0.0	7.0	9.8	5.1	7.8	1.5
IL-17A	20.1	17.4	1.3	1.3	16.3	31.2	24.6	20.2	21.5	0.0	32.6	0.0	7.5	18.8	16.3	20.2	1.2
TNF- $\alpha$	3821.2	1588.2	1549.4	458.8	5855.0	4319.2	1358.0	733.5	2317.2	172.1	6855.4	5.8	1349.4	3030.4	1588.2	1358.0	0.9
MIP-1 $\alpha$	16981.2	917.1	4956.8	29011.3	21490.8	17247.0	23817.2	5772.5	3619.3	2302.2	97207.3	192.1	97191.7	8383.6	16981.2	8383.6	0.5
MIP-1 $\beta$	3165.2	233.1	11608.6	1901.4	4591.6	1482.8	4757.9	4294.9	1008.3	3069.1	23121.0	377.3	7902.8	4026.1	3165.2	4026.1	1.3
MIP-3 $\alpha$	2732.5	0.0	2008.1	1435.5	6138.0	1963.0	11105.9	2249.3	5514.0	4790.1	51071.3	448.4	1423.4	4409.9	2008.1	4409.9	2.2
IL-1 $\beta$	2346.7	544.3	873.1	2833.3	4274.9	3334.8	805.8	1786.8	1531.9	189.3	30955.3	6.0	2881.3	3316.2	2346.7	1786.8	0.8
IL-6	7674.8	1403.3	4808.5	23183.5	8457.1	11960.9	4989.6	5462.0	14414.8	2043.5	26208.8	88.3	15881.5	12362.6	7674.8	11960.9	1.6
IL-7	12.7	3.9	1.4	NA	57.1	28.0	0.0	0.0	62.1	11.4	57.3	0.0	36.3	0.0	8.3	11.4	1.4
IL-8	22731.0	624.0	7935.8	24880.0	57937.0	28772.0	48113.0	17375.0	44006.0	8148.0	101520.0	289.5	74112.9	19525.6	22731.0	28772.0	1.3
IL-12p70	14.0	2.2	0.0	5.5	16.2	25.0	0.0	5.5	7.2	0.0	6.7	0.0	2.3	8.5	5.5	5.5	1.0
IL-21	34.2	44.0	35.9	81.0	65.5	97.1	49.5	0.0	136.5	19.6	172.8	0.0	133.6	66.2	44.0	66.2	1.5
IL-23	141.6	351.2	109.5	468.4	883.0	789.4	127.0	353.7	300.1	37.1	360.4	0.0	527.7	157.0	351.2	300.1	0.9
GM-CSF	79.9	85.4	53.5	168.5	137.8	311.1	87.6	159.4	184.0	33.4	759.2	0.0	204.6	97.6	85.4	159.4	1.9
Fractalkine	0.0	167.5	0.0	0.0	174.2	253.6	0.0	0.0	155.4	0.0	0.0	0.0	0.0	112.3	0.0	0.0	ND
ITAC	254.0	24.6	6.0	147.0	327.0	306.8	186.6	155.7	188.0	9.5	101.6	155.7	20.6	10.9	147.0	155.7	1.1

Abbreviations: ABPA = allergic bronchopulmonary aspergillosis, AfuLy = *A. fumigatus* mycelial lysate, Asp-ass. Dis. = *Aspergillus*-associated diseases, CF = cystic fibrosis, CPA = chronic pulmonary aspergillosis, INF = infinite (0 pg/ml in the control cohort), MMR = median-to-median ratio, NA = not available (invalid measurement), ND = not defined (0 pg/ml in both cohorts).