September 26th, 2022

Monocyte SFC panel #2

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	.3 2E+6 (sc's 2 unstained)	R10
2900c/μl NY062 #1			20E6	08/12/21		2	5.89	7.93		510μl	51μl	
2900c/μl NY062 #2			20E6	08/12/21		2	6.21	8.32		483μl	48μl	17
330c/μl NY062 #3			20E6	08/12/21		2	6.83	9.11		439μl	44μl	61
320c/μl NY062 #4			20E6	08/12/21		2	7.34	9.76		409μl	41μl	91
							52.5					

↑ relatively clean bone? relatively abans

Ctrl Backbone ○ | PPD Stim backbone ○ | TT stim backbone? ○

ctrl sc ○ unstained | ctrl stim sc ○ unstained | TT stim sc ○ unstained

PPD Stim backbone
+CD19 ○
+CD20 ○
+FcERIa ○
+CD5 ○
+CD141 ○

PPD stim backbone
+CD19
+FcERIa ○
+CD5 ○
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+FcERIa
+CD5 ○
+CD141 ○

PPD stim backbone
+CD19
+FcERIa
+CD141
+CD5 ○

14 stained backbone samples
+26 sc controls (stim) ~300k
+3-4 unstained controls (1 unstained, 2-3 stim)

~3 M cells / tube
42 million + 30 × .3 = 9.0 million = 51 million cells

Stim concentration
PPD @ 1 mg/ml → 1 μg/μl
2.5 μg/500ml → ~5 μg/ml
2.5μl
TTx - 1.925μl ~2.5 ~5
~3 hrs?

+ BV brilliant stain (to verify)

+26 sc bead stained wells (0.5μl, 1μl)
× 2
52 sc bead stained wells
differential brightness of control

+ 1 unstained bead well

Thaw 10:50 #2 + #3 melted
#1 pellet
#4 hanged on
10:50 pm thaw spin
11:16 pm stain count
Count @ 11:30 am
Stim @ 12:14 pm ✓

10 ml media × 3-4 tubes
resuspend 2-3 mls
3M or 2M range
↑ 500μl

0.5M
4μl/ml edta

Abs prepped @ 2:18 pm

2% FBS 10%
5 2.0%
4 2.5%
3 3.3%
2 5%
1 10

25ml
125
× 4
500μl

44 × .1 = 4.4ml
0.4% PFA-PBS
44 × .3 = 13.2 ml RBC lyse
Rbclyse 1.5 + 13.5ml H2O
4/27 28

Reagents prepped @ 2:54 pm
3:17 cell count
3:25 pm cells spin down
15:42 w/o
:46 3μl FcBlock
→ 16:00
w/o spin @ 4:05 pm
Vortexed sorted @ 4:24
4:30 hots in → 5:00 pm

Hot sc's @ 4:45pm → 5:15pm
Cold sc's @ 4:57pm → 5:27pm
w/o sc @ 4:58pm → 5:13pm
hot sample spin @ 5:05 pm ✓
thawed @ 5:14 pm → fridge
LD wash 2ml spin 1400 6min
Hot sc's @ 5:17 - 5:20pm
wash w/ Avg10% FBS → 2% + EDTA

15 × .800 = 12,000 ml

w/o
2μl 5ml
6μl 15ml

sc's
~~:21~~ hotwash

sc
~~5:34~~ hot out.
sc Coldout Rbc 36 → 39/40

Hot PFAed @ 5:36 pm

Cold spin @ 5:42.

return adding test tubes CD5

Started 5:14 ~~2:~~ 5:44 all indiv combos added ← (best estimate ~20 minutes alone sit for CD141, FceRIa, CD19) pre add? CD5 (indiv) & 4°c mix?

cold 4°c samples @ 5:49pm → 6:19pm (rbc lyse)

sc's organized @ 5:58pm

samples rbc lyse 6:20-21

6:44 pm

Done, moved w/ fridge to emergency power

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#	Filter	Single color (ul)	Ref ctrl type	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	L/D + FC blocker 3ul/rxn	37oC (RT) for 30min	14	4oC for 30min	14
1	UV2				BUV395	CD18	(6.7)			0.8	11.2		
22	UV7				BUV496	CD19							0
2	UV9				BUV563	CCR2	(Is132.1D9)			0.8	11.2		
	UV10												
3	UV11				BUV661	CX3CR1	(2A9-1)			1	14		
	U14												
4	UV16				BUV805	CD14	(M5E2)					1.0	14
23	V1				BV421	FcER1a							0
	V2												
	V2												
24	V3				Pacific Blue	CD5							0
5	V5				BV480	CD11a	(HI111)					0.6	8.4
25	V7				BV510 (dim)	CD20 19	(2H7)			0	0		
6	V8				BV570	CD16	(3G8)					1.5	21
	V10												
7	V10				BV605	CD56	(5.1H11)					1.0	14
8	V11				BV650	CD11c	(B-ly6)					1.0	14
9	V13				BV711	CD80	(L307.4)			1.5	21		
10	V14				BV750	CCR5	(2D7)			1.5	21		
26	V15				BV785	CD141							0
	B1												
11	B2				FITC	CD11b						0.8	11.2
	B3				Spark blue 550								
12	B4				PE	CD88	(S5/1)			0.6	8.4		
13	B6				PE-Dazzle594	CD3						0.6	8.4
14	B8				PE-Cy5	CD86	(IT2.2)			0.5	7		
	B9												
15	B10				PerCP-Cy5.5	CD123	(7G3)			2	28		
16	B13				PE-Vio770	HLA-DR	(REA805)					0.5	7
	R1												
17	R2				Alexa Fluor 647	CD163	(GHI/61)					1.0	14
	R3												
18	R4				APC-R700	CD40	(5C3)			1.0	14		
19	R6				Zombie NIR	L/D			<1:2500>				
20	R7				APC/Fire 750	CD64	(10.1)			1	14		
21	R8				APC/Fire 810	CD38	(HIT2)			1.0	14		
					True Stain FcX					0.5	7	0.5	7
And UNSTAINED CONTROLS !!!					Antibody Total					12.2	171	8.5	119
					PBS					50	700	50	700
					Pippette draw volume/sample					61.2		57.5	

APCs: ?

500 µl media ~2.5 µg PPD ~ 5 µg/ml conc

6E6 cells

Simplified Protocol 3E6 cells/tube

Aliquot cells 1.2E+6 cells/tube

Wash with 2ml PBS, 1300rpm, 8min

2500x diluted Zombie NIR, 1E+6/1ml + 3ul Fc blocker at RT for 15min

Wash with 2ml 2%FBS-PBS-2mM EDTA

Spin at 1300rpm for 8min

x ul of 37oC Ab mix, at 37oC for 30min

Remove RBC with 1ml lysing solution for 3min ?

15:42

46

Wash with 2ml 2%FBS-PBS-2mM EDTA

Spin at 1300rpm for 8min

x ul of 4oC Ab mix, at 4oC for 30min CD19 run @ 4°c not hot

Treat with 600ul of 1x Lysing solution at RT for 3min?

Wash with 2ml 2%FBS-PBS-2mM EDTA

Spin at 1300rpm for 8min

Resuspend in 0.4%PFA-PBS

Notes: TrueStain FcX (Biolegend 422301) final 0.1ug/ml for 24hr Lipopolysaccharides from Escherichia coli O111:B4

InvivoGen tlrl-3pelps **LPS stock 5mg/ml, 10ul; take one aliquot out and add 490ul R10 to make 100ug/ml working solution** Wash with 2mM EDTA

5a not quite

Not good for a pDC marker

Monocytes SFC Panel

9/26/2022

Spectrum	UV				Violet				Blue				Red			
373	UV1															
388	UV2	BUV395	[2]	CD18												
428	UV3				V1	BV421	[4]	FcER1a								
443	UV4				V2											
458	UV5				V3	PacBlue	[1]	CD5								
473	UV6				V4											
508					V5	BV480	[3]	CD11a	B1							
514	UV7	BUV496	[2]	CD19												
525					V6				B2	FITC	[1.5]	CD11b				
542	UV8				V7	BV510	[1.5]	CD20	B3	SparkBlue 550	[1]					
582	UV9	BUV563	[3]	CCR2	V8	BV570		CD16	B4	PE	[4]	CD88				
598					V9				B5							
613	UV10	BUV615	[3]		V10	BV605	[3]	CD56	B6	PE-Dazzle	[4]	CD3				
664	UV11	BUV661	[3]	CX3CR1	V11	BV650	[3.5]	CD11c	B7				R1	APC	[3.5]	
679									B8	PE-Cy5	[4.5]	CD86	R2	AF647	[3.5]	CD163
697	UV12				V12				B9	PerCP-Cy5.5	[2]	CD123	R3			
717	UV13				V13	BV711	[4]	CD80	B10				R4	APC-R700	[3]	CD40
738									B11				R5			
750	UV14	BUV737	[3]		V14	BV750	[2.5]	CCR5								
760									B12				R6	Zombie NIR	[ ]	L/D
783	UV15				V15	BV785	[3]	CD141	B13	Pe-Vio770	[3]	HLA-DR	R7	APC-Fire 750	[2]	CD64
812	UV16	BUV805	[1]	CD14	V16				B14				R8	APC-Fire 810	[3]	CD38

AF → Monocyte
Lymphocytes ~ 0.95
0.29 ← 0.49
dead dying signals

less intense

extra-extra-extra-bright
↓ concentration

0.72

September 26th, 2022

Monocyte SFC panel

Aurora-UV early AM edition: button ran on @ 5:12 AM
Shifted time 6:30 - 9:30 AM, will adjust accordingly.

Template set up done @ 5:40 AM, start @ 5:45 AM (3hr)
SC's → 30,000, medium event rate ~700 ~1 minute (lymphocytes)
remember monocytes today love. (similar)

PE CD88
¿ ↑ in pDC's → due to decoupling?
PE - dazzle
PE - cy5
PE - Vio770

→ test via expart sc as fcs and unmix in ExtraTools.

Samples start @ 6:30 am ~5min each

Playing short-term gains paying long term consequences / #covid #vaccines

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CD19, CD3, CD56 unmixed w/ Lym AF, everything else monocyte.

HLA-DR PE-Vio770 almost off scale!!

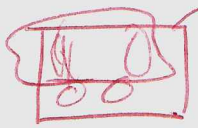
CD19 AF signal ~ brightest B cell on BUV496

BV570 CD16 brightest might be on NKs ~ monocytes ~$10^{5\wedge1}$ (few 16+ monocytes in this donor)
unmixing w/ monocyte AF

BUV11 CD80 ↑ @ 3 hrs stim Postive leading edge negative #great control

CCR5 dribbles $10^{5\wedge1}$

exagerated



for L/D

HLA-DR high expression ($10^{5\wedge}$ ~ $10^{6\wedge}$)
38 $10^{4\wedge1/2}$

CD18 ✓

CD80 minimal !!

fcERIA marginal

~CCR5 spotty?
~CD141
~CD86
~CD123

Pacific Blue most CD5 10^4 edge

64/46

- Monocyte vs Dc/PDC express on the 64/40 expression axis
- May need to draw new AF gate for L/D unmixing
- Swap out CD16 AF for CD3

Did 16 get added? ↓ or donor specific issue?

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Monocyte SFC panel

Nd CD3's:

CD8 + CD11c
CD11b

CCR2 CD11b
CD64
CD38

CX3CR1 CD11b/84
CD64
CD38

CD14 . CD11b
CD84
64
38

CD11c . CD1c
CD11b
64
38

CD16 . CX3CR1
. CD11c
CD11b
CD84