October 14th
~~September 26th~~, 2022

Monocyte SFC panel #4

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	2E+6
NY062 a1	Adult		20E6	08/12/21		3ml	4.5E6	6.28E6	13.5	.666	
NY062 a2	Adult		20E6	08/12/21		3ml	4.41E6	6.08E6	13.23	.680	
									26.73		

(margin: out 200µl; out 200µl)

Biosicion broke entirely, tree upper lights & beeps

count colorstain @ 9:05 AM

Very low c/µL ~ sth of debris in part? (yes!)

Everything TTx stimulated? [~12 wells]

.666 = .5 / 2.56µl 1.925µl TTx

2.5 ~ 5µg/ml

2.5=5µg/ml

SSC-B → SSC-V for debris removal

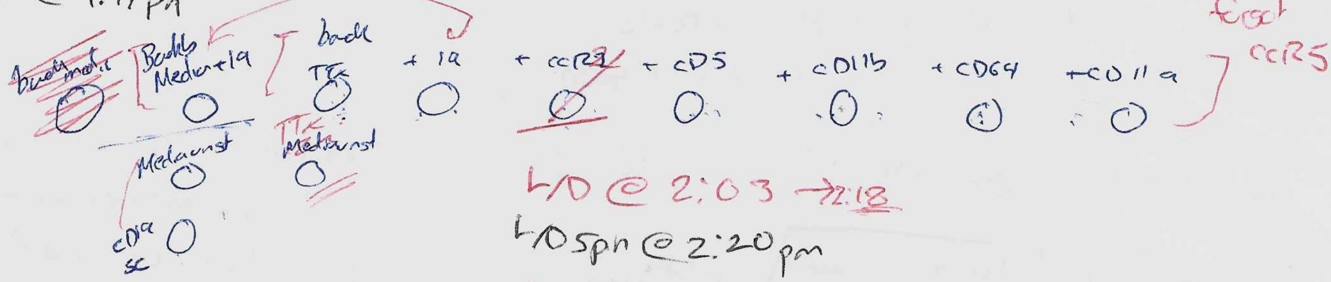
✪ Cells into incubation w/ TTx 5µg/ml conc @ 9:45 AM (+2 tubes R10 alone)

Ab prep start @ 12:51 pm ~3hr (4? hr)

9 / vs / [1-1:30]

Abs done @ 1:17 pm

9 samples
+ 2 unstaineds
+ 1 sc CD19



L/D @ 2:03 → 2:18

L/D spin @ 2:20 pm

~~37°c @ 2:38 pm → 3:08 pm out~~

3:11 37°c spin in

3:27 4°c in w/all add ons (CD11b → 0.4 instead 0.6)

→ 3:57 pm

RBC lyse @ 3:58 → 4:0 pm

Done @ 4:13 pm

12 tubes
× 85ml
60
960
1020

50 ml
× 4µl
200 µl EDTA

10 + 10 70%
40 + 40 PBS

300µl * 12 ~ 4 ml RBC lyse
.400µl 3600µl H2O

100µl × 12 → 1.2 ml 0.4% PFA-PBS

600µl × ~~9~~ 12 → ~~9.6~~ 7.2 ml L/D → 3µl 7.3ml

Reagents prepped @ 1:37 pm.

Spin down @ 1:47 pm [4 hours incubation]

☆ gating really important NK vs small gates

[spreading error of CD16 into 56.]

hematogenes

CD11c negatives ~18 Cx3CR1 ~CD11 HLA-DR - +/- CD38 - more w/ AFs.

CD14 Cx3CR1 56 ~cp

cDC's more 86 than the monocytes / HLA-DR, CD163

[14 * FcER1a] → DC's: running a PacMap on 7500 cells

Cx3CR1, CD14, 56, 163 88, 86, HLA-DR - CD38

Cx3CR1, > CD163, > CD14, > CD88, CD38

cDCs = equivalent CD18, FcER1a, CD11c, CD141

← cDC2's don't express Cx3CR1 or CD14

56 by individual subsets

higher 88 expression in 3's but light

tiny bit more 86 in cDC2's.

→ 163 3's ↑ 40CD

cMon vs cDC3's

↑ CD18

~ Cx3CR1, CD11c, 141

↑ CD14

~ 163, 40, 38

↓ FcER1a

↑ CD88

↓ 86

↓ HLA-DR

Repeating for cMon/oddballs: 18, 86, 38 (324 cells) rarely un-activated?

CD14⁻ oddball CD88+

orange

- ↓ 18
- ↑ Cx3CR1
- ↑ 11c
- ↓ 141
- ~ 88
- no 86
- ↑ 123
- ↓ HLA-DR
- ↓ CD163
- no CD38

CD14 - oddball CD88 - low

relative exclusion

blue

- ↓ 18
- ↓ Cx3CR1
- ↓ 11c
- ↓ 141
- ↓ 88
- no 86
- ↓ HLA-DR
- ↓ CD163
- intermediate CD38

No FcER1a
" CD16

cMon vs oddballs

(a subset expresses 56)

- ↓ CD18
- +/- Cx3CR1
- CD14 - (14 - Cx3CR1 -) ↓ gone
- 14 - Cx3CR1 + CD11c
- ↓ CD141
- ↑ also CD88 more
- no 86
- ↑ CD123
- ↓ HLA-DR
- no CD163
- ↓ CD40
- No/low CD38

38 * 88

[123 * CD38]

October 14th, 2022 Monocyte SFC panel

#	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	9	4oC for 30min	9
1	UV2				BUV395	CD18	(6.7)			0.6	5.4		
	UV6				AF350								
22	UV7				BUV496								
2	UV9				BUV563	CCR2	(Is132.1D9)			<0.6>	####		0
	UV10												
3	UV11				BUV661	CX3CR1	(2A9-1)			1	9		
	U14				BUV737	CD19						<1.2>	####
4	UV16				BUV805	CD14	(M5E2)					1.0	9
23	V1				BV421	FcER1a						1.0	9
	V2												
	V2												
24	V3				Pacific Blue	CD5							
5	V5				BV480	CD11a	(HI111)					<1>	####
25	V7				BV510 (dim)							<0.4>	####
6	V8				BV570	CD16	(3G8)			0	0		
	V10											1.5	13.5
7	V10				BV605	CD56	(5.1H11)						
8	V11				BV650	CD11c	(B-ly6)					1.0	9
9	V13				BV711							1.0	9
10	V14				BV750	CCR5	(2D7)						
26	V15				BV785	CD141				<1.5>	####		
	B1											1.2	10.8
11	B2				FITC	CD11b							
	B3				Spark blue 550							~~<0.6>~~ 0.4	####
12	B4				PE	CD88	(S5/1)						
13	B6				PE-Dazzle594	CD3						0.4	3.6
14	B8				PE-Cy5	CD86	(IT2.2)					0.6	5.4
	B9									0.5	4.5		
15	B10				PerCP-Cy5.5	CD123	(7G3)						
16	B13				PE-Vio770	HLA-DR	(REA805)			2	18		
	R1											0.3	2.7
17	R2				Alexa Fluor 647	CD163	(GHI/61)						
	R3											1.0	9
18	R4				APC-R700	CD40	(5C3)						
19	R6				Zombie NIR	L/D			<1:2500>	1.0	9		
20	R7				APC/Fire 750	+CD64						0.8	
21	R8				APC/Fire 810	CD38	(HIT2)			1.0	9		
					True Stain FcX					0.5	4.5	0.5	4.5
And UNSTAINED CONTROLS !!!					Antibody Total					6.6	59.4	9.5	85.5
					PBS					50	450	50	450
					Pippette draw volume/sample					55.6		58.5	

Simplified Protocol

Aliquot cells 3E+6 cells/tube

Wash with 2ml PBS, 1300rpm, 8min

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

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

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

Spin at 1400rpm for 6min

x ul of 4oC Ab mix, at 4oC for 30min

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

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

Spin at 1400rpm for 6min

Resuspend in 0.4%PFA-PBS

No CCR5 single color + backbone (If I knew I forgot someone

SM = 8.74

↑ 8.95 w/ AF tag

5000 cells for int Mon/Nc Mon

HLA-DR/CD163 (CD64's basophil on FcεRIα)

Reproducing hd clustering on 2D

CX3CR1* CD14 > 163

— cMon are 14+ x 163 + (oddballs fallout)

— oddballs CD123 vs 38

that leading edge that matters

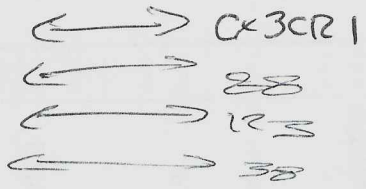
** I spit out enough to NE analysis for a paper for breakfast *

Monocytes SFC Panel

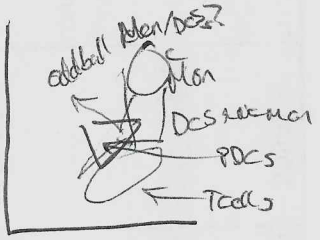
10/14/2022

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	AF350			V4											
508					V5	**BV480**	[3]	**CD11a**	B1							
514	UV7	BUV496	[2]	<AF>												
525					V6											
542	UV8				V7	BV510	[1.5]	<AF>	B2	**FITC**	[1.5]	**CD11b**				
582	UV9	**BUV563**	[3]	**CCR2**	V8	**BV570**		**CD16**	B3	SparkBlue 550	[1]					
598					V9				B4	**PE**	[4]	**CD88**				
613	UV10	BUV615	[3]		V10	**BV605**	[3]	**CD56**	B5							
664	UV11	**BUV661**	[3]	**CX3CR1**	V11	**BV650**	[3.5]	**CD11c**	B6	**PE-Dazzle**	[4]	**CD3**				
679									B7				R1	APC	[3.5]	
697	UV12				V12				B8	**PE-Cy5**	[4.5]	**CD86**	R2	**AF647**	[3.5]	**CD163**
717	UV13				V13	BV711	[4]		B9	**PerCP-Cy5.5**	[2]	**CD123**	R3			
738									B10				R4	**APC-R700**	[3]	**CD40**
750	UV14	**BUV737**	[3]	**CD19**	V14	**BV750**	[2.5]	**CCR5**	B11				R5			
760																
783	UV15				V15	**BV785**	[3]	**CD141**	B12				R6	**Zombie NIR**	[ ]	**L/D**
812	UV16	**BUV805**	[1]	**CD14**	V16				B13	**Pe-Vio770**	[3]	**HLA-DR**	R7	**APC-Fire 750**	[2]	**CD64**
									B14				R8	**APC-Fire 810**	[3]	**CD38**

The two oddballs



Mon ↑ CD18 int CX3CR1
↑ 141 ↑ 86 ↑ HLA-DR ↑ 143 ↑ 38



DN → CD8, CX3CR1, CD40, CD38,

September 26th, 2022

Monocyte SFC panel

Acquisition: unstained media 4500 cells/event rate

NY062 sample looks to be in good health.

sample → 6000/events rate (the 2 media tubes had less volume)

Sample → 9000 events rate

7600 events for sample /8300/

NY062 CD9 just hiccuped ← check it w/ PeacoQC.

8500 CCR2

//Δ brightness across samples//

AF PacMAP ~1M~ / 12:47pm / → / 1:20pm / =

~3M~ / 1:24pm / → / ——— / ≡ finished between 3:05 & 4:05pm

BUV737 works for CD19 ✓ (easily clears the SSC monocyte barrier)

BUV737 CD19 + backbone =

CD18 negative bleed   CD11c 141

~CX3CR1~

CD14 minor ~~

~ FcER1a ~

~ 16 / blowback * 56

Monocytes UV7 * V10

No Δ between media & tx well for CD86

Create a Folder and place all experimental setup files within it

You can eventually export final experiment & include in it

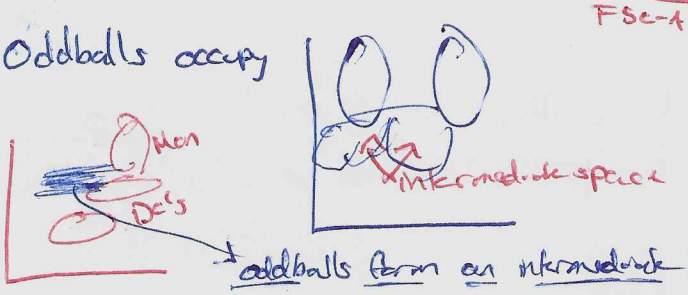
tag cleared up 56 split except CD11a

CD19 works well in its new location ✓

FcER1a move it.

Classical Monocytes + oddballs = CD14 | FSC-A

Oddballs occupy



oddballs little bit lower CD16
↑% CX3CR1 - no CD14

Dead Monocytes → intermediate FcERIa (AF?)

oddballs form an intermediate level pop (MF?)
not all oddballs are dying, Overlap w/ healthy DC's location.
(this also explains the Mon low AF, it's the DC's location)

CD88+ cMon vs oddballs vs FcERIa DCs

CD88+ cMon — no CD14

No FcERIa
N CX3CR1, CD16

The only difference in oddballs is less CD88 on the → DC side vs ← dead side.
but only barely

↓ CD141
↓ 88
↓ HLA-DR
↓ CD38

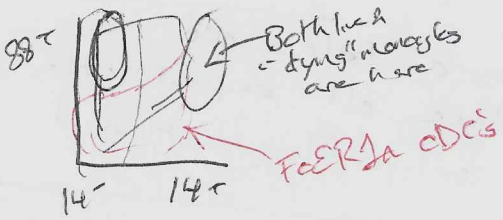
163 is higher cMon

Noticing a trend here.

cMon live vs dead
↑ CD81 ↑ FcERIa
↑ CD163

Running a PacMap w/ FSC & SSC-A on that monocyte cluster 11411 cells

The CD14 oddballs occupy non-monocyte space ←



Δ mon pops ↗ SC ↑ 163
other ↓ CD8
cMon ↑↓ FSC ~ FcERIa

~~☆~~ CD14 - CD88+ Pop = No CD38, No CD163, ↓ HLA-DR, ↑ CD123, No/Low CD86
No/Low CD141, No CD14, equ CD88, ↓ CD16
↑ CX3CR1

↗ More CX3CR1 than a cDC1; ↓ 16, SC, ↑ CD11c ↓ CD141 ↑ 88 ↓ HLA-DR ↓ 38

vs cMon no CD14

14- mid +/- 88 ↓ CX3CR1 ↓ 141 ↓ 88

September 26th, 2022 Monocyte SFC panel

A lot of the CD1c- EDI23- cells are in ~~dying monocyte gate~~ pdc/basophil hinge region ~CD11c +141s

No discernable +/- to CD88 or FcERIa, ← spread is a lot

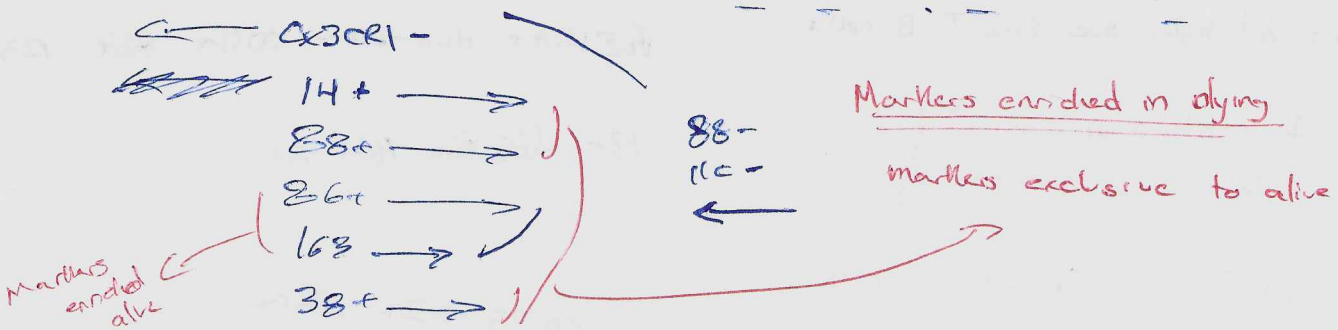
FcERIa CD14 * 163

"Files double in size once you unmix in SpectraFlo"

Backbone + CD19 → FcERIa on BV421 such spread

* backspill unmixing from CD19

After CD3, basophil removal, Nil, B cell only 3.43 oddballs on gate



Needs gentle cleanup ahead of basophil,

B-A
B-W not set

FSC-A SSC-B-A SSC-B-A
FSC-H

SSC-W * SSC-B-W → oddballs on the side doublets

-A * B-A

SSC-B-A & SSC-H

FSC-W * SSC-W edge

FSC-A * SSC-W → Monocyte debris dead

SSC-A * SSC-H → a SSC-singlet removes lower edge
* SSC-W

→ Sample had a fluctuation

PeacoQC catches oddballs. First FSC-~~A/H~~ cleans → edge
SSC-A/H cleans ↑ edge

FSC-A shows debris, but makes uncertain LiveDead at

Debris field is HLA-DR − // RBCs & platelets, ¿ a fika nele?

¿ Mast cells?

BSSC-H VSSC-H

Granulocytes 3rd wave ↗

?: Basophils by HyperFinder ¿ ?

FcERIa +

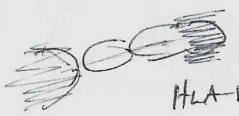
123 + stretch

88 + stretch

141 −

3, CX3CR1 −

11c −



PacMap 1 → FcERIa

4 → CD16/56

cluster 2 → 18 negative

4 → 11c 141 ~88

FcERIa, CD88, CD123, CD38+

NK's bit higher ssc than T/B cells

FcERIA+ HLA-DR − CD8+ 88+ 123+ 38+

CDC1 clusters identical

only 8 dot different

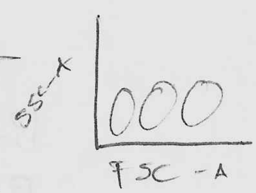
18 − FcERIa HOt 38+

Basophils? CDC1s PDCs?

18=

CD123 → IL-3Ra

Bas L, M, H



↑ CD18 high

↑ FcERIa

~ CD88

↑ 123 high

↑ CD38

These are indeed basophils

Within the CD3+ gate

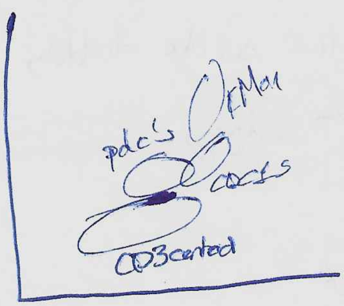
→ 22.8% pop

Subsets small differences but not distinguishable

Dying basophils ↓ 18, FcERIa, ~CD88

↓ CD123

↓ 38



CD11c remnant: HLA-DR+ 19 −

less HLA-DR less CD40

19+ coincides exactly w/ the 40+ SSC low pop

oddball HLA-DR+ 38+ (200 cells)

11c ↗ True oddball only info HLA-DR+, CD8 weak, ~200 cells

(probably a precursor pop)