January 28th, 2023

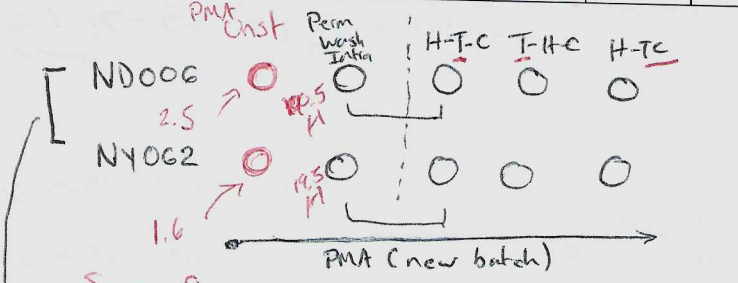
Variable Adult Responses? Or Cytokine Issue?

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	.3E+6
ND006			17.2 µl			2	5.72	7.60	11.4	432 µl	
NY062			18.4 µl			2	5.47	8.20	10.9	457 µl	
(+NY058)											

6:55 Guava cleaning
7:15 Cells thawed waterbath, into spin
7:41 Count (no CD45 stain)
8:16 Cells PMAed (new batch)
8:26 CD107a retroactively added
→ out @ 2:20 pm

2:32pm spin down
2:44pm ↳ 0 → 3:00 pm

500 * 9 * 2 = 4500 (1 µl PMA R10)



Since focus is on V52 cytokine responses, and non-specific background tetramers, everyone got 2.5 million cells

Split Unstaineds (3, 2) to track cells in the processing, reusing last weeks colors so best.

T-H-C tet stain @ 3:19 → 4:00pm.
H-T-C & H-T-C into 37°C @ 3:22pm → 3:52 pm

Also grab @ CD107a sc? (new vial?)

All spinning post FBS wash @16:00pm

4:15 pm → 4:25 pm H-T-C (then 58.5µl cold) → 4:55 (RBC Lyse!!)
→ 4:55 pm H-T-C's
37°C @16:17 for T-H-C → 4:47pm ✓ spin
→ cold @ 5:00pm → 5:30pm (RBC lyse!!)
spin down @ 5:03pm

T-HC FixPerm @ 5:13 → 23 → 03pm ✓
H-T-C's into 4°C @ 5:17pm → 5:47pm (RBC lyse!!)

H-T-C 1st perm wash & T-H-C post cold FBS @ 7:35 pm → 2nd permeab (Cloth @ BLK/8...)
FixPerm @ 5:47 → 5:57 → 6:07

RBC lysed @ 5:49 pm

Make a bead sc TNFa
No CD25 in this run
Make a bead sc CD107a
Make a bead sc CD161!!

T-H-c Fix Perm. 5:47 → 57 → 6:07 pm

H-Tc Intracellular @ 54.5µl ammc; intrac 5:56 pm → 6:36 pm out
(2 1300/8 min...)

H-T-c's Rbc lyse 5% FBS wash @

↳ Fix Perm @ 6:05 → 15 → 25

↳ 1st perm wash for T-H-c @ 6:08 pm 1460 rpm/ 6 min
2nd perm wash @ 6:16 pm

T-H-c Intrac @ 18:25 → 7:05 pm

H-T-c's First Perm @ 18:27 Final 2 ml 5 min @ 1400 6 min rpm
2nd perm wash @ @ 6:37 pm

Sc's in perm wash made @ 6:32 pm

H-T-c intrac @ 6:47 pm → 7:27 pm done

H-TC resuspended 0.4% PFA @ 6:48 pm

7:14 pm T-H-c done ✓

Done @ 7:35 pm

- CD161 old - No CD25

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#	Filter	Single color (ul)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	During stim!!!	8	L/D 15 min (RT)	Tetramer 40 min @ RT	HotStain 30min @37C	8	ColdStain 30min @4C	8	RBC Lyse, then Fix/Perm	Spiked 30 min @RT	8
1	UV2				BUV395	CD62L	(DREG-56)												
					AF	AF-UV6									1.2	9.6			
2	UV7				BUV496	CD8	(RPA-T8)												
3	UV9				BUV563	CD69	(FN50)								0.7	5.6			
4	UV10				BUV615	CCR4	(1G1)								0.5	4.0		1	8.0
5	UV11				BUV661	Vδ2	(B6)						2.0	16					
6	U14				BUV737	CXCR3	(1C6/ CXCR3)								0.7	5.6		0.5	4.0
7	UV16														1.3	10.4			
8	V1				BUV805	CD4	(SK3)												
9	V3				BV421	CD127	(A019D5)						1.5	12				0.5	4.0
	V3				Pacific Blue	CD14	(M5E2)						1.5	12					
10	V5				Pacific Blue	CD19	(HIB19)								2.0	16.0			
11	V7				BV480	CD161	(REA631)								2.0	16.0			
					BV510	CD45RA	(Hi100)						2	16					
12	V10														0.7	5.6			
13	V11				BV605	CD56													
28	V13				BV650	CCR7							1.0	8					
14	V14				BV711	CD7							1	8				1	8.0
15	V15				BV750	IFNγ	(B27)						1	8					
16	B2				BV786	CCR6	(11A9)											1.5	12.0
17	B3				AlexaFluor 488	hCD1d							1.5	12					
18	B4				Spark blue 550	CD3	(SK7)					<: >( )			<: >( )				
19	B6				PE	NKG2D									1.2	9.6		0.5	4.0
20	B8				PE-CF594	CD26	(M-A261)						1.5	12				0.5	4.0
21	B10				PE-Cy5	CD25	(M-A251)						1.2	9.6					
22	B13				PerCP-Cy5.5	TNFα	(MAB11)						1.2	9.6				0.5	4.0
29	R1				PE-vio770	PD1												2.5	20.0
23	R2				APC	CD16									1.5	12.0		0.5	4.0
24	R4				AlexaFluor647	hMR1									0.7	5.6			
25	R6				APC-R700	CD107a	(H4A3)		6.0	48		<: >( )			<: >( )				
26	R7				Zombie NIR	L/D					<1:2500>								
27	R8				APC/Fire 750	CD27	(O323)						2	16					
					APC/Fire 810	CD38	(HIT2)						1.6	12.8					
								Antibody Total	6.0	48	Antibody Total		19.0	152	12.5	100.0		9	72
And UNSTAINED CONTROLS !!!								R10 Media	14.5	116	Brilliant Stain		50	400	50.0	400		50	400
								Pippette draw volume /sample	19.5		Pippette draw volume /sample		66		59.5			56	

Notes:

Simplified Protocol

Thaw cells, DNAse, count.
Collect, count, aliquot cells 3.0E+6 Cells R1(
Bring volume upto 1 ml R10, add 2 ul PMA/C
Cap and incubate at 37°C for 6 hours

Wash with 2 ml PBS, spin down 1300rpm 8n
800 ul of LiveDead mix (1:2500) @RT for 15n
Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8mi

Add HotStain mix, incubate @37C for 30 mir
Wash 2 ml 5% PBS-FBS 1300 rpm, 8 min

Add Tetramers, incubate @RT for 10 min
Add ColdStain mix, incubate @ 4C for 30mir
Add 300-500 ul 1x RBC Lysis for 3 minutes
Wash 2 ml 5% PBS-FBS 1300 rpm, 8min

300 ul BD FixPerm, incubate @ 4C for 20mi
(vortex every

First PermWash: 1 ml PermW
Second Perm Wash: 1 ml PermW

Add Intracellular Stain, incubate @ RT for 4(
First PermWash: 2 ml PermW

Resuspend in 100 ul 0.4% PFA-PBS
Cap tubes, wrap rack in foil, store at 4°C

54.5

PMA - 8% pop AF

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Running Aurora water @ 1:41 pm (on for a bit before w/ template set up)

QC @ 01:48 pm event rate: ~100

The new batch PMA is working (by FSC/SSC Samples ~ 77000 events rate (1:58 pm) inspection)

Where are the doublets in NY062? Is it the macrophages?

✡ Can only run x 4 samples individual AEs at one time w/ our screen. < Swap in the pma etc.... ala last experiment > ✡

		TNFα	IFNγ	
ND006 H-T-c	CD3	28.19	11.27	Previous NY062 ctrl .... Vd2 → 0.28% CD3 → 67%, 47% Vd2 → 75%, 70%
	Vd2 (1.87%)	45.06	49.30	
NY062 H-T-c	CD3	19.74	8.58	Perm Wash ↓↓↓
	Vd2 (0.18%)	39.90	37.12	
Ugly smear → ND006 H-T-c	CD3	31.3%	12.56%	33.1% 12.26%
	Vd2 (1.44%)	58.60	62.34%	52.5 48.34%
NY062 H-T-c	CD3	19.01%	8.91%	20.53 5.71%
	Vd2 (0.21%)	39.56%	39.17%	22.9 18.94

✡ ✡ The issue was the other things spiked in at that volume > ✡

NY062

ILT & NK SFC Panel

1/28/2023

Spectrum	UV			Violet			Blue			Red			
373	UV1												
388	UV2	BUV395	[2] CD62L										
428	UV3			V1	BV421	[4] CD127							
443	UV4			V2									
458	UV5			V3	PacBlue	[1] CD14/19							
473	UV6	AF-UV6	AF	V4									
508				V5	BV480	[3] CD161	B1						
514	UV7	BUV496	[2] CD8										
525				V6			B2	AF488	[1.5] hCD1d/Va24Ja18				
542	UV8			V7	BV510	[1.5] CD45RA	B3	SparkBlue 550	[1] CD3				
582	UV9	BUV563	[3] CD69	V8	BV570		B4	PE	[4] NKG2D				
598				V9			B5						
613	UV10	BUV615	[3] CCR4	V10	BV605	[3] CD56	B6	PE-CF594	[4] CD26				
664	UV11	BUV661	[3] Vd2	V11	BV650	[3.5] CCR7	B7			R1	APC	[3.5]	CD16
679							B8	PE-Cy5	[4.5] CD25	R2	AF647	[3.5]	hMR1/Va7.2
697	UV12			V12			B9	PerCP-Cy5.5	[2] TNFa	R3			
717	UV13			V13	BV711	[4] CD7	B10			R4	APC-R700	[3]	CD107a
738							B11			R5			
750	UV14	BUV737	[3] CXCR3	V14	BV750	[2.5] IFNg							
760							B12			R6	Zombie NIR	[ ]	L/D
783	UV15			V15	BV786	[3] CCR6	B13	Pe-Vio770	[3] PD1	R7	APC-Fire 750	[2]	CD27
812	UV16	BUV805	[1] CD4	V16			B14			R8	APC-Fire 810	[3]	CD38

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* CCR4
* CX3CR3
* CD127
* CD161
* CD56
* CCR6
* CD3 AF488
  burst of NIH620 ....
* PD1
  > CD3 AF647
* CD38 bbb → ugh...

CD7 streamer

Swapping out AF488/AF647 both for CD3s ...

↓ ↓ ↓ ↓

moved ← those gates over a bit to the right

CD38 ≠ APC-R700?

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