50 Shades of Fluorescence to Follow Immune Response (Saffir) with spectral cytometry.

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Introduction

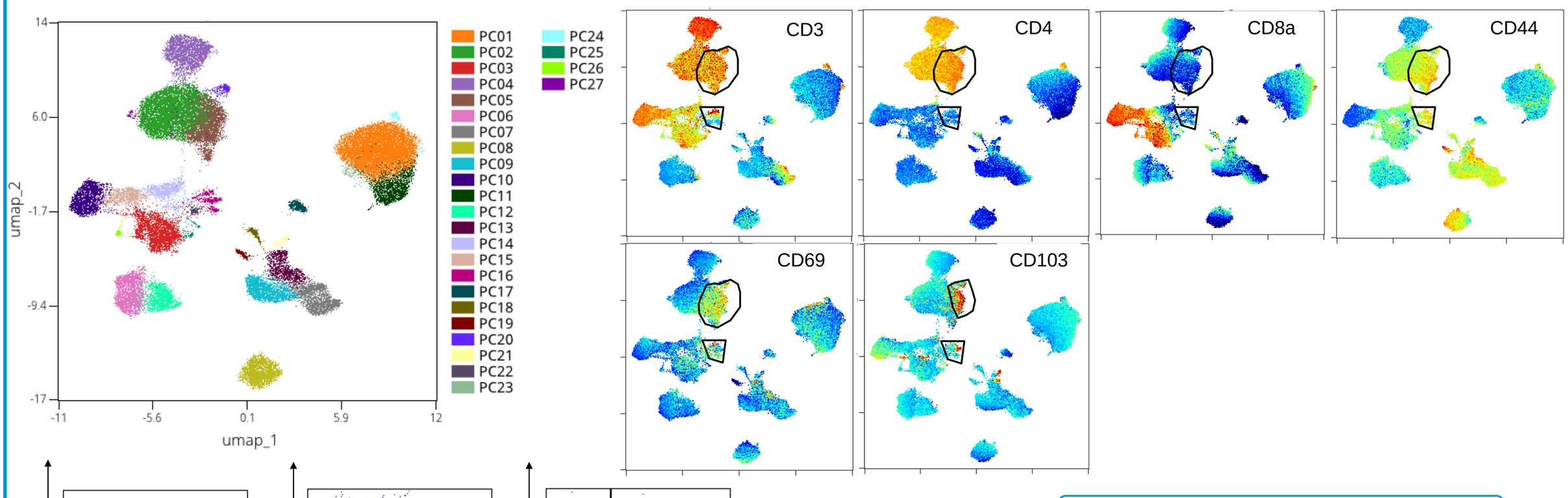
Whooping cough persists as an endemic disease (40million cases and 300000 child death per year worldwide) even in vaccinated populations. This disease is caused by the gram(-) bacterium *Bordetella pertussis*. This bacteria expresses multiple virulence factors acting in concert to facilitate its adherence, survival and proliferation in human respiratory tract. More, it has been shown, in a mouse model, that $CD4^+$ Trm are generated during the time course of infection[1]. Our objectives is to decipher the cellular mechanisms underlying the "cross-talk" between antigen presenting cells and T cells that allow the differentiation, maintenance and function of Trm at the tissue level. To do this aim we developped a 48 markers panel to follow the behavior of several immune populations in the mean time in the same tube.

Methods

- Mice were infected with a wild strain of Bordetella pertussis (Tahoma I). 15 days later they were sacrificed. After intra-cardiac perfusion with PBS, collected lungs were digested with a mix of Collagenase IV/DNAse I for 30 mn at 37°C. Lung cells were seeded at $3x10^6$ cells/well and stained in PBS, 2mM EDTA, 0,5% BSA, 40% brilliant violet stain buffer(BD Biosciences) and 10% Fc block at 37°C for 45mn. Cells washed 3 times before staining in PBS + live/dead marker (Zombie NIR) for 15mn. After 3 washes cells were resuspended in PBS for analysis on Spectral Cytometer Aurora (Cytek Biosciences).
- Data analysis were performed using OMIQ.

ANTIGEN	CLONE	ANTIGEN	CLONE	ANTIGEN	CLONE	ANTIGEN	CLONE	ANTIGEN	CLONE
CD3	17A2	**CD25**	PC61	**CD69**	H1.2F3	**CD196**	29-2L17	**Ly6G**	1A8
CD4	GK1.5	**CD26**	H194-112	**CD88**	20/70	**B220**	RA3-6B2	**MHC-II**	M5/114.15.2
CD5	FAB115U	**CD27**	LG3A10	**CD90-2**	30H12	**BST2**	927	**NK1.1**	PK136
CD8a	53-6.7	**CD44**	IM7	**CD103**	2 E7	**CX3CR1**	SA011F11	**NKp46**	29A1.4
CD8b	53-5.8	**CD45**	30F11	**CD115**	afs98	**EpCAM**	G8.8	**PD-1**	29F1A12
CD11b	M1/70	**CD49a**	HA31/8	**CD138**	281-2	**F4/80**	BM8	**Siglec F**	E50-2440
CD11c	N418	**CD49b**	HMa2	**CD154**	MR1	**IgM**	RMM-1	**XCR1**	ZET
CD19	1D8	**CD49d**	PS/2	**CD172a**	P84	**IgD**	11-26c.2a	**γδTCR**	GL3
CD23	B3B4	**CD62L**	MEL-14	**CD183**	CXCR3-183	**Klrg1**	2F1	**Live/ Dead**	Zombie NIR
CD24	M1/69	**CD64**	X54-5/7.1	**CD192**	SA203G11	**Ly6C**	HK1.4	**AutoFluo**	

Results



Isolation of $CD4^+$ and $CD8^+$Trm

Using the cytek full spectrum viewer we calculated a similarity index of 113 for our 49 colors panel.

Upper panel: Unsupervised analysis of $CD45^+$ cells using UMAP and Phenograph for dimension reduction and clusterisation leaded to isolate clusters (PC05 in brown and PC16 in magenta) representing 5% and 0,5% respectively of total $CD45^+$ cells.

Lower Panel:
Using supervised analysis (2 by 2) from $CD45^+$->$CD19^-CMH\text{-}II^-$ -> $CD90.2^+CMH\text{-}II^-$ -> $CD5^+CD3^+$-> $CD3^+$ $\gamma\delta T^-$ ->$CD4^+$ or $CD8a^+$ we finally are able to define a population of $CD4^+CD44^+CD69^+CD103^+$ (Trm) corresponding to 3,5% of total $CD45^+$ cells. Idem $CD8^+$ Trm represent 0,15% of $CD45^+$ cells

Conclusion

Using Saffir we were able to follow the behavior of the majority of immune cells in response to *Bordetella pertussis* infection. Here, we highlighted the fact that using supervised or unsupervised analysis leaded to similar results in the frequency of both $CD4^+$ and $CD8^+$ Trm in lung of infected mice.

Reference: 1- Wilk, M. M. et al. Lung CD4 Tissue-Resident Memory T Cells Mediate Adaptive Immunity Induced by Previous Infection of Mice with Bordetella pertussis. J. Immunol. Baltim. Md 1950 199, 233–243 (2017)