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Early production of IL-17A by $\gamma\delta$ T cells in the trachea promotes viral clearance during influenza infection in mice

Early production of IL-17A by V γ 4+ $\gamma\delta$ T cells in the trachea promotes neutrophil recruitment and viral clearance during influenza infection.

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Supplementary information

Supplementary Figure 1 Supplementary Movie 1

Supplementary Table 1



Supplementary Figure 1. (A) Representative scatterplots showing the reduction of $\gamma\delta$ T cell in trachea and spleen from mice treated with anti- $y\delta$ TCR antibody (α - $y\delta$ TCR) at day 3 p.i.. (**B**) Levels of secreted IL-17A, IFN-y and KC in trachea from mice treated with anti-y δ TCR antibody (α -y δ TCR ab) and with isotype control antibody (day 3 p.i.) at day 3 p.i. (n = 4-5 mice/group). (C) (Upper panels (*)) flow cytometric characterisation of neutrophils and NK cells, and (lower panels (**)) inflammatory dendritic cells (IDC), plasmacytoid DC (PDC), CD103+ conventional DC (CD103+ cDC), CD11b+ conventional DC (CD11b+ cDC) and resident respiratory tract macrophages (RTM) in mouse trachea according to [6]. (D) Flow cytometric quantification of total numbers of T cell subsets, DC subsets and macrophages in trachea from mice treated with anti- $\gamma\delta$ TCR antibody (α - $\gamma\delta$ TCR ab) and with isotype control antibody (α -Isotype ab) at day 0 and 3 p.i. (n = 3-4 mice/group). (**E**) Flow cytometric quantification of $\gamma\delta$ T cell in IL-17AFKO mice at day 3 p.i. (n=3-5 mice/group). (**F**) Virus titres in trachea determined by TCID₅₀ from mice infected with influenza virus and treated with an anti- $\gamma\delta$ TCR antibody (PR8+ α - $\gamma\delta$ TCR) or with the isotype control antibody at day 3 p.i. (n = 5 mice/group). The presented data are representative of at least two (A, D, E and F) or three (B) independent experiments. Results are given as mean ± SD. In B box plots show 25th to 75th percentiles and whiskers show minimum and maximum values. Statistical significance was determined by Two-tailed Student's t-test (B, F) or Mann-Whitney U-test (D, E). (ns p>0.05; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001).



Supplementary Movie 1. In vivo imaging of $\gamma \delta$ T cell recruitment to the trachea after influenza infection. (First part) Two videos (a,b) of 30-45 min time-lapse 3D image showing the dynamics of $\gamma \delta$ T cells (green, small and motile), SHG signal from fibrillary collagen (grey) and blood vessels (red) in trachea at day 3 p.i. (Second part) magnified representative sequences showing $\gamma \delta$ T cell extravasation from the blood vessels into the tracheal tissue. Scale bar represents 50 μ m.

Supplementary Table 1.

Antibody target	Clone	Color	Manufacturer
B220	RA3-6B2	PE/Cy7, BV605	Biolegend
CD11b	M1/70	AF488, BV785,	Biolegend
CD45	30-F11	AF700	Biolegend
CD3ɛ	eBio500A2	eFluor450	eBioscience
NK1.1	PK136	AF488, PerCP-Cy5.5	Biolegend, eBioscience
CXCR3	CXCR3-173	AF488	Biolegend
CD69	H1.2F3	FITC, PE	Biolegend
IFN-γ	XMG1.2	BV785	Biolegend
IL17-A	TC11-18H10.1	PE	Biolegend
Ly-6G	1A8	BV711	Biolegend
CD16/32	93	Pure	Biolegend
CD8α	53-6.7	PE/Cy7, BV650	Biolegend
CD4	GK1.5	APC/Cy7	Biolegend
γδΤCR	GL3	APC	Biolegend
CD27	LG.7F9	PE/Cy7	eBioscience
CCR6	29-2L17	PE/Dazzle 594	Biolegend
αLy-6C	HK1.4	PE	Biolegend
αF4/80	BM8	AF488	Biolegend
αCD103	2E7	AF647	Biolegend
αCD11b	M1/70	BV785, VioBlue	Biolegend, Miltenyi
αI-A/I-E (MHCII)	M5/114.15.2	Pacific Blue	Biolegend
αCD11c	N418	APC/Cy7	Biolegend
Bst2	927	Bv605	Biolegend
TCR Vγ4 (2)	UC3-10A6	FITC, Pure	Biolegend, BioXcell
TCR Vy1.1/Cr4	2.11	PerCP/Cy5.5	Biolegend
TCR Vy6	17D1	DyLight 800*	donated by Dr. Immo Prinz
			(U. of Hannover)
TCR γ/δ	UC7-13D5	Pure	Bioxcell
Armenian hamster IgG	(isotype control)	Pure	Bioxcell

*In-house labelled with Lightning-Link® Rapid DyLight® 800 conjugation kit (Innova Biosciences).