Novel MHC Class I Antigen Cross Priming Ability of Type 2 Innate Lymphoid Cells

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Background

- Current cancer immunotherapeutics (e.g. CAR T cell) lack efficacy in solid tumours, necessitating expansion to other immune cells[1]
- Metastatic tumor immune escape involves downregulation of interleukin-33 (IL-33), reducing (Major Histocompatibility Complex (MHC) I processing[2]
- **Type 2 innate lymphoid cells (ILC2s):** Conventionally Th2 cytokine secretors activated by IL-33, increasingly identified role in cancer and vaccine immunity[3,4]
- Lab discovery that ILC2s can promote **anti-tumour Th1 cytotoxic T lymphocyte responses**, unclear mechanism behind this process[4]
- Recently found ILC2s can **cross present antigens through MHC I** to activate CD8+ T cells (previously unique to dendritic cells)

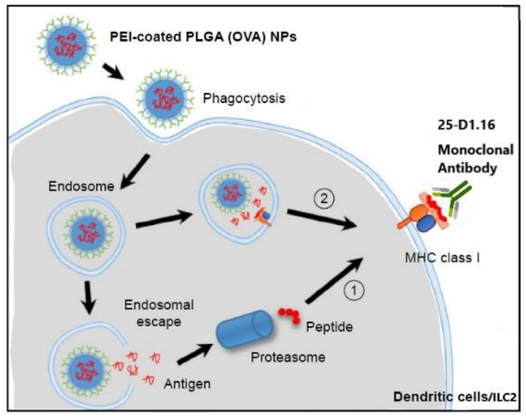


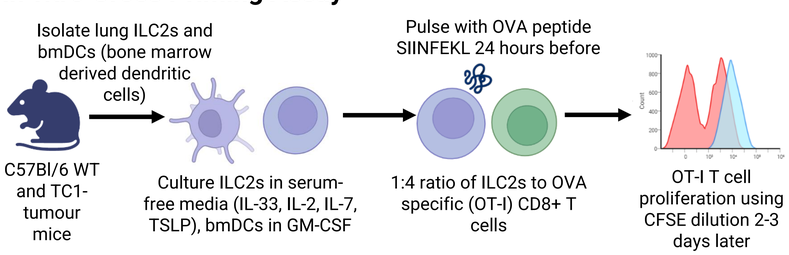
Figure 1: OVA model of cross-presentation. The 25-D1.16 monoclonal antibody recognizes the same structure as CD8+ T cells, and specifically reacts with ovalbumin-derived peptide SIINFEKL bound to H-2Kb of MHC class I, but not with unbound H-2Kb or H-2Kb bound with an irrelevant peptide to recognize the DC or ILC2.

Objectives/Hypothesis

Novel subpopulations of ILC2s are capable of MHC I cross-priming cytotoxic T lymphocytes, which is predicted to underpin anti-tumour ILC2 Th1 immune responses

Methods

In vitro Cross Priming Assay



- **Serum-free culturing of ILC2s allows for ex vivo expansion of ILC2s while maintaining phenotype[5]**
- **OT-I mice: MHC class I-restricted, CD8+ T cells recognizing ovalbumin peptide residues (OVA 257-264), SIINFEKL**

Results

ScRNAseq Shows Subpopulations of Tumour-Associated ILC2s with Pro-inflammatory Phenotypes

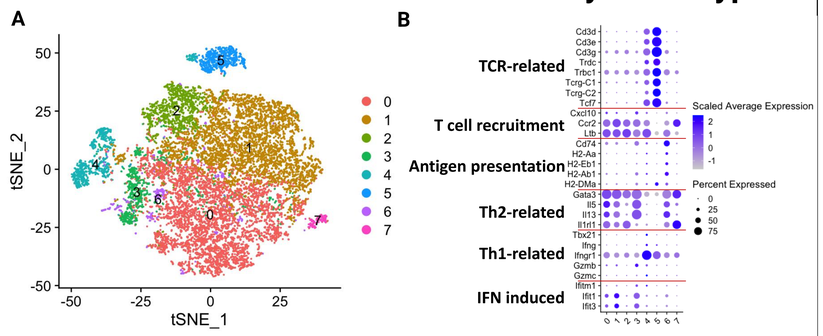
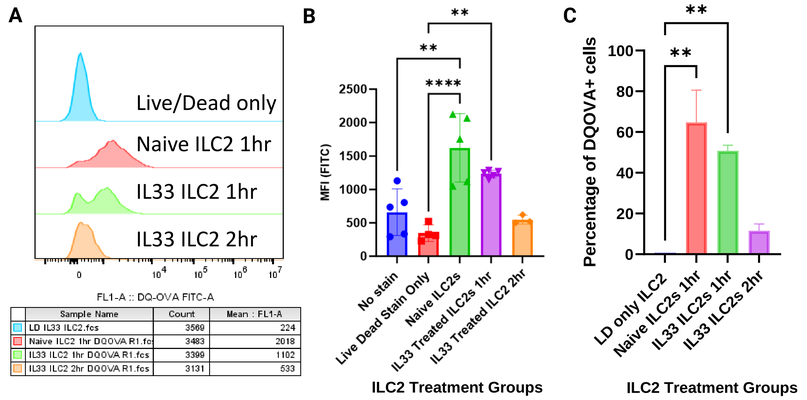


Figure 2: A.) t-distributed stochastic neighbour embedding visualization of clustering of the ta-ILC2 subsets and naïve ILC2 using Seurat. Cluster identities are assigned and labelled by important immune functions. B.) Dotplot shows marker-of-interest expression per cluster. Color intensity indicates log-scaled mean gene expression level. Dot size indicates the fraction of cells in the cluster for each gene.

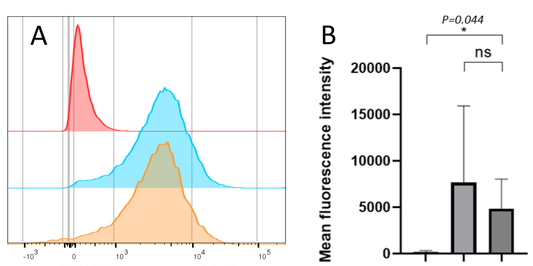
Lung ILC2s can process DQ-OVA peptide



Sample Name	Count	Mean : FL1-A
LD IL33 ILC2.fcs	3569	224
Naive ILC2 1hr DQOVA R1.fcs	3483	2018
IL33 ILC2 1hr DQOVA R1.fcs	3399	1102
IL33 ILC2 2hr DQOVA R1.fcs	3131	533

Figure 3: A.) ILC2s were isolated from saline-injected or IL-33 treated mice and incubated for 1 or 2 hours with DQ-OVA, FITC fluorescence measured via flow cytometry. B.) ILC2 MFI (n=3-6), two independent experiments, and C.) Percentage of DQOVA+ cells for ILC2s. One-way ANOVA multiple comparisons test, each bar represents 2-3 replicates, p <0.05 (mouse n=10 for each sample).

Lung ILC2s Successfully Process OVA Protein and Cross-Present the SIINFEKL Peptide

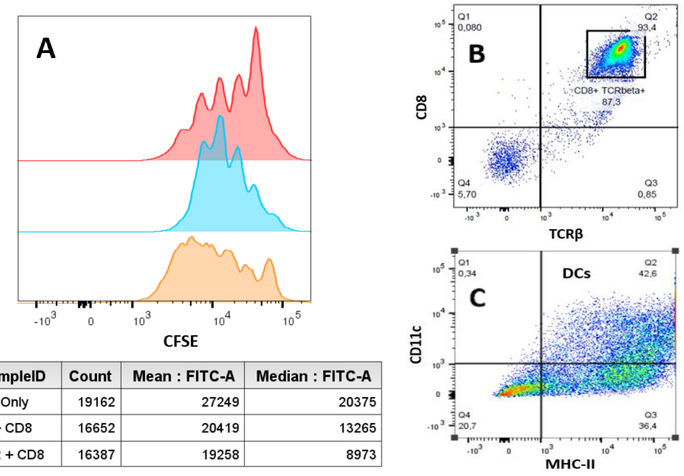


SampleID	Count	Mean : APC-A
Blank	16634	244
ILC2 + SIINFEKL	17792	5111
ILC2 + OVA	16607	4354

Figure 4: A) Pooled Lung ILC2s from mice (n=20) bearing primary tumours (TC1) treated with either whole ovalbumin protein (OVA) or OVA peptide (SIINFEKL) for 24 hrs, and then antibody 25-D1.16 was used to detect MHC-I bound to SIINFEKL on the cell surface. B) Mean fluorescence intensity of ILC2 cross presentation (n=3 replicates). Adapted from Pablo de Lucía Finkel.

Results

Lung ILC2s Can Cross Prime Naïve CD8+ T cells



SampleID	Count	Mean : FITC-A	Median : FITC-A
CD8 Only	19162	27249	20375
DC + CD8	16652	20419	13265
tILC2 + CD8	16387	19258	8973

Figure 5: TC1- tumour derived ILC2s (orange), bone marrow derived dendritic cells (bmDCs) (blue) were co-culture with CFSE labelled OT-I cells in a 1:4 ratio, proliferation rounds tracked and compared to OT-I cells only (red). B) Isolated CD8+ T-cells and C) differentiated bone marrow–derived DCs. Mouse n=50 for ILC2 isolation (25 for TC1 mice + 25 for A9 mice). Proliferation is indicated by a skewing to the left. Adapted from Pablo de Lucia Finkel

Conclusions

- Lung ILC2s are a heterogenous population, with subtypes involved in pro-inflammatory Th1 cytolytic T lymphocyte responses
- Certain subpopulations possess professional antigen presentation ability to cross prime naïve CD8+ T cells
- Overall, this work improves our understanding of cancer surveillance with eventual translation to ILC2 cancer immunotherapy

References

1.Bagley, S. J. & O'Rourke, D. M. Clinical investigation of CAR T cells for solid tumors: Lessons learned and future directions. Pharmacol Ther 205, 107419 (2020).
2. Saranchova, I. et al. Discovery of a Metastatic Immune Escape Mechanism Initiated by the Loss of Expression of the Tumour Biomarker Interleukin-33. Sci Rep-Uk 6, 30555 (2016). https://doi.org:10.1038/srep30555
3. de Lucia Finkel, P., Xia, W. & Jefferies, W. A. Beyond Unconventional: What Do We Really Know about Group 2 Innate Lymphoid Cells? J Immunol 206, 1409-1417 (2021).
4.Saranchova I, Han J, Zaman R, Arora H, Huang H, Fenninger F, et al. Type 2 Innate Lymphocytes Actuate Immunity Against Tumours and Limit Cancer Metastasis. Sci Rep. 2018 Feb 13;8:2924.
5. de Lucía Finkel P, Sherwood C, Saranchova I, Xia W, Munro L, Pfeifer CG, et al. Serum free culture for the expansion and study of type 2 innate lymphoid cells. Sci Rep. 2021 Jun 10;11:12233.

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