Dissecting the IFNγ- *versus* IL-17-specific mRNAomes of effector γδT lymphocytes

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INTRODUCTION

The ability of murine γδ T cells to rapidly produce the pro-inflammatory cytokines interleukin-17 (IL-17) or interferon-γ (IFN-γ) underlies their crucial roles in several pathophysiological contexts, from infection to cancer or autoimmunity. This functional capacity stems from a complex process of 'developmental pre-programming' in the thymus, after which a significant fraction of γδ T cells migrate to peripheral sites already committed to producing either IL-17 (γδ17) or IFN-γ (γδ1). While several studies have studied these gd T cell subtypes using surface markers that enrich for effector function, we still lack a characterisation of the mRNA transcriptomes that specifically associate with IL-17 or IFN-γ production by γδ T cells. To overcome this limitation, in this study we established a double reporter IL-17-GFP:IFN-γ-YFP mouse strain, which allowed us to isolate pure IL-17$^+$, IFN-γ$^+$ and the remaining IL-17$^-$IFN-γ$^-$ (DN) γδ T cell populations from the peripheral lymphoid organs in order to perform RNA-sequencing and identify the subset-specific mRNAomes.

METHODS

To identify molecular factors directly associated with the effector function of γδ T cells, we generated a double reporter mouse from single reporter mouse strains already established (1,2). The reporter mice co-express the green fluorescent protein (GFP) with IL-17A and the yellow fluorescent protein (eYFP) with IFN-γ, which we designate as double reporter (DR), to simultaneously study pure populations of IFN-γ or IL-17-producing γδ T cells (Fig. 1). This strategy overcomes the limitations observed in previous studies that have been relying on several extracellular markers of γδ T cells that correlate with the potential of IFN-γ or IL-17- production but that may not reflect γδ T cells actively producing these effector cytokines.

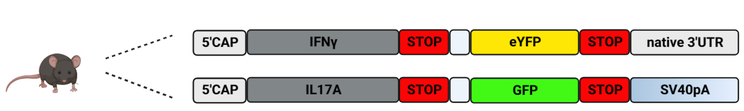

Fig. 1 - Reporter mice co-expressing the green fluorescent protein (GFP) with IL-17A and the yellow fluorescent protein (eYFP) with IFN-γ.

Highly enriched populations of γδ T cells expressing either IL-17 (GFP+), IFN-γ (YFP+), or double negative (DN) were isolated from the peripheral lymph nodes (pLN) of double reporter mice, stimulated ex vivo to allow expression of the cytokines and reporters, and separated into pure populations by fluorescence-activated cell sorting (FACS) according to the expression of the YFP and GFP markers (Fig.1). Subsequently, total RNA was purified, an mRNA library was generated for each sample, and submitted to next-generation sequencing (NGS) (Fig.2).

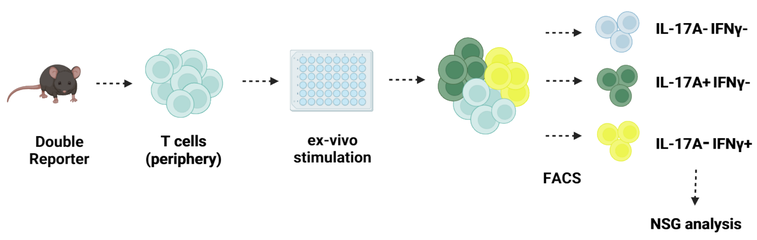

Fig. 2- The methodology used to isolate the cells of interest for NGS analysis.

REFERENCES

1. Reinhardt, R. L., Liang, H.-E. & Locksley, R. M. Nat. Immunol. 10, 385–93 (2009).
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RESULTS

Overall, we detected the expression of 12822 genes in γδ T cells, with a significant number of genes being enriched in γδ17 when compared with γδ1 and γδDN cells (Fig. 3A). Among these, 936 genes were differentially expressed between the three populations (Fig. 3B), with γδ17 and γδ1 cells displaying the most distinct mRNAomes, which highlights their functional specialization, and γδ1 being more similar to DN than γδ17 cells (Fig. 3C).

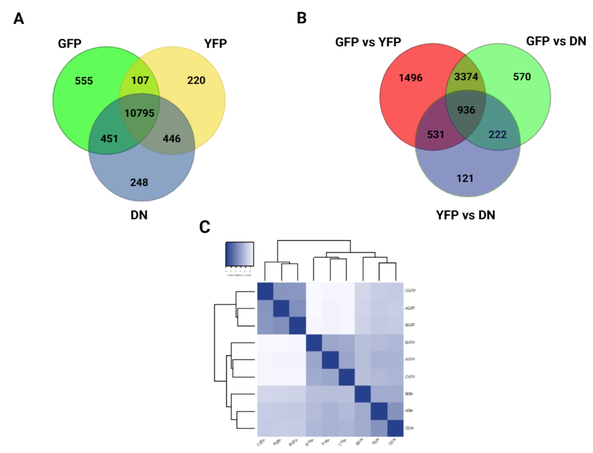

Fig. 3 - Venn diagrams of the differentially expressed genes (DEGs) within each population (A) and the shared and unique DEGs in GFP+, YFP+, and DN γδ T cell population (B). Correlation analysis of GFP+, YFP+, and DN γδ T cell population (C).

A clear segregation of IL-17A and IFN-γ expression levels is observed in GFP+ vs YFP+, GFP+ vs DN and YFP+ vs DN cells, with IL-17A and IFN-γ overexpression being associated, respectively, with the GFP+ and YFP+ γδ T cells as depicted in the respective volcano plots (Fig. 4).

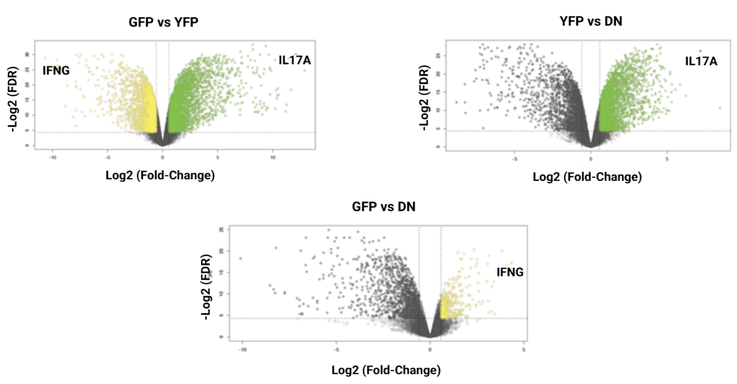

Fig.4 - Volcano plots showing differential gene expression between GFP+, YFP+, and DN γδ T cells.

Not surprisingly, the cytokines IFN-γ and IL-17A are amongst the top 50 genes with the significant fold change in the respective populations (FC=707x and 4346x). We also found other differentially expressed transcripts associated with one of the two effector subsets, such as Eomes (FC=239x) and Rorc (FC=801x), essential transcription factors, respectively, for IFN-γ and IL-17 production by γδ T cells (Fig. 5).

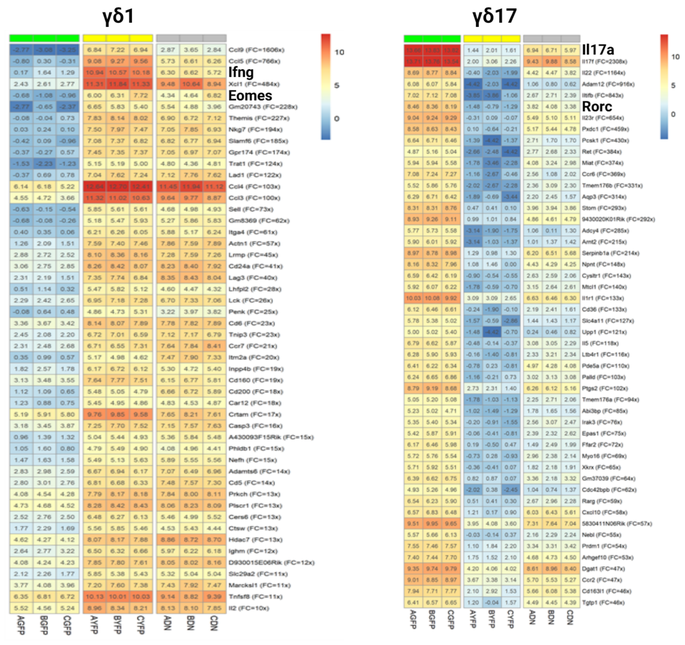

Fig.5 - Top 50 genes enriched in γδ1 and γδ17 cells.

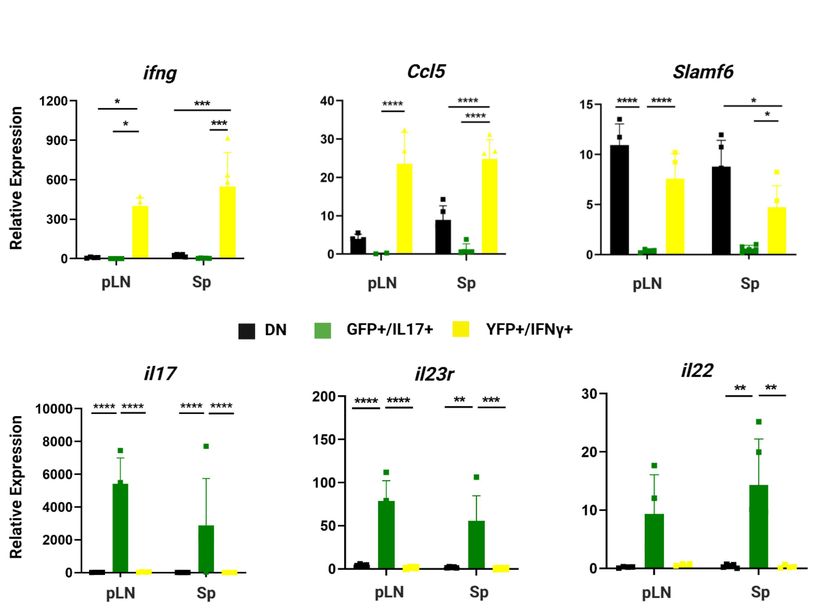

Fig.6 - Gene expression levels in YFP+/IFNγ+,GFP+/IL-17, and DN γδ population.

RESULTS

Below, we show the validation of some of these differentially expressed genes by γδ1 and γδ17 cells in lymph nodes (pLN) and spleen (Sp) (Fig. 6).

Next, we show the expression of these same genes in sorted Vγ4+, Vγ6+GFP+ γδT cells, and Vγ1+YFP+T cells from pLNs to assess heterogeneity within each population.

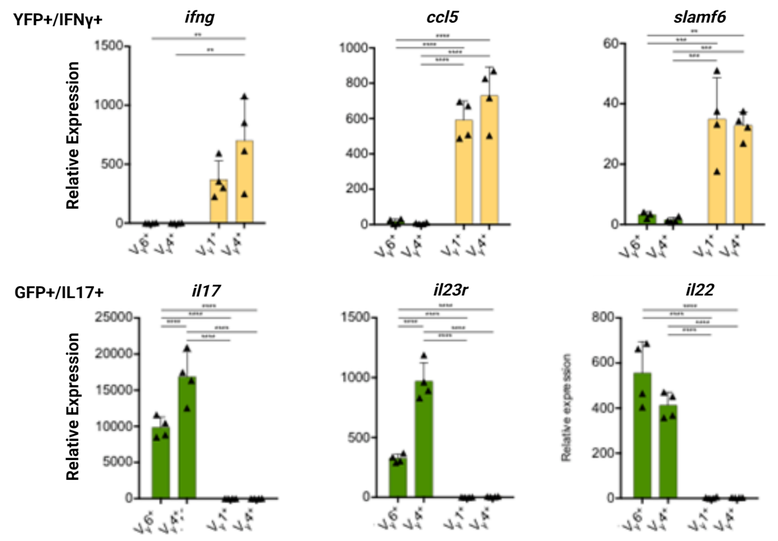

Fig. 7 - Gene expression in sorted Vγ4+, Vγ6+GFP+ γδT cells, and Vγ1+YFP+T cells.

CONCLUSIONS

Pathway and gene ontology analyses indicated that γδ17 cells differ from their IFN-γ-producing counterparts in their selective ability to sense and integrate external cues, whereas γδ1 cells stood out in replication, transcription and translation processes. A more detailed analysis of the top 30 differentially expressed genes ~~among the most expressed genes~~ by γδ17 and γδ1 cells revealed that the majority of the signature genes increase their expression levels in the periphery upon their egress from the thymus, suggesting that these effector subsets only terminate their differentiation process at peripheral sites. Collectively, our data allowed us to identify distinct mRNA signatures directly associated with cytokine expression in γδ T cells, several of which we are currently studying in disease models to identify potential new roles in pathophysiology.

ACKNOWLEDGEMENTS

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