Dissecting the IFN-γ versus IL-17-specific transcriptomes of effector γδ T lymphocytes: a new role for signalling adaptor Themis

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Introduction

The crucial role of murine γδ T cells in several (patho)physiological contexts stems from a complex process of 'developmental pre-programming' in the thymus, after which a significant fraction of γδ T cells populate peripheral sites already endowed with the capacity to secrete either IL-17 or IFN-γ[1]. However, despite the relevance of these γδ T cell effector subsets, we still lack knowledge on the transcriptomes that specifically associate with IL-17 or IFN-γ production. To address this, we established a double reporter IL-17-GFP:IFN-γ-YFP mouse strain, which allowed us to isolate pure peripheral IL-17-producing ($\gamma\delta^{17}$) or IFN-γ-producing ($\gamma\delta^{IFN}$) γδ T cells to perform RNA-sequencing.

Aims

1. Characterize the transcriptomes of pure effector $\gamma\delta^{17}$ and $\gamma\delta^{IFN}$ subsets;
2. Uncover new genes with an important role in γδ T cell differentiation or effector function.

Results

1. mRNA profiling of $\gamma\delta^{17}$ and $\gamma\delta^{IFN}$

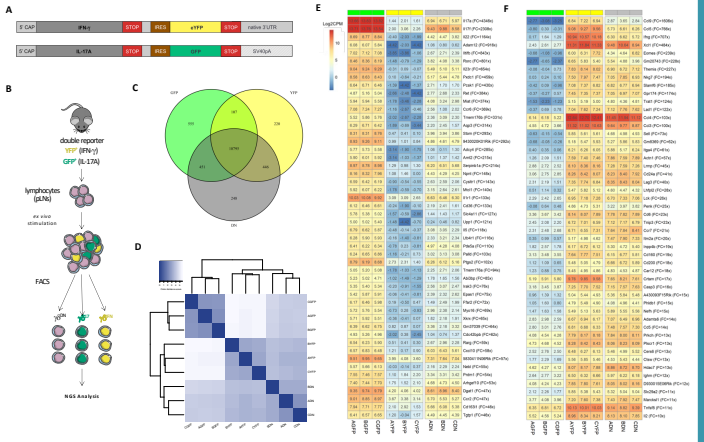


Figure 1: Differentially expressed genes between $\gamma\delta^{IFN}$ and $\gamma\delta^{17}$. (A-B) Schematic illustration of the isolation of YFP$^+$-IFN-γ- and GFP$^+$-IL-17-producing γδ T cells and and YFP$^-$GFP$^-$DN γδ T cells from the peripheral lymph nodes of double reporter IFN-γ.YFP-IL-17A.GFP mice strain in a C57BL/6 background. **(C)** Venn diagram depicting the number of genes specifically expressed by each population or shared among them. **(D)** Correlation analysis of $\gamma\delta^{DN}$, $\gamma\delta^{17}$ and $\gamma\delta^{IFN}$. **(E-F)** Heatmaps depicting overexpressed mRNAs in $\gamma\delta^{17}$ **(E)** and $\gamma\delta^{IFN}$ **(F)** cells. Color refers to Log$_2$CPM and values inside squares refer to calculated z-scores. Fold change ≥ 1.5 and FDR < 0.05.

2. Finding signature genes for $\gamma\delta^{17}$ and $\gamma\delta^{IFN}$

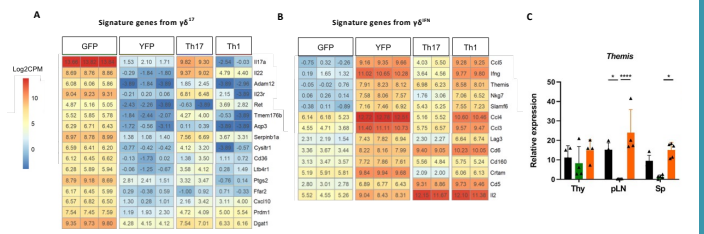


Figure 2. $\gamma\delta^{17}$ and $\gamma\delta^{IFN}$ 'signature genes'. (A-B) Heatmaps depicting a selection of the top differentially expressed genes between $\gamma\delta^{17}$and $\gamma\delta^{IFN}$and comparison with their expression in Th17 and Th1-counterparts generated in vivo during the course of experimental autoimmune encephalomyelitis model. **(C)** qPCR analysis of *Themis* expression in $\gamma\delta^{DN}$, $\gamma\delta^{17}$ and $\gamma\delta^{IFN}$isolated from the adult thymus, peripheral lymph nodes or spleen.

3. Themis: a T cell-specific gene involved in the regulation of TCR signal strength

- Themis is a critical component of the T cell developmental program, being essential for the positive selection of thymocytes[2];
- Mechanistically, albeit lacking known catalytic domains, Themis constitutively binds to the adapter Grb2[2] and the phosphatase Shp1 [6]–[8], whose catalytic activity it has been found to directly regulate. Nevertheless, it remains unclear if its effects on Shp1, and hence on TCR signalling, are mainly stimulatory or inhibitory.

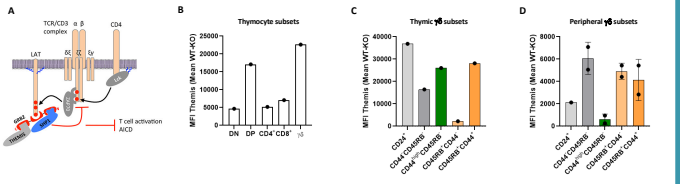


Figure 3. Themis is differentially expressed across thymocyte and γδ T cell subsets. (A) A Themis:Grb2:Shp complex regulates the strenght of TCR signaling. Adapted from [3]. (B to D) Flow cytometry analysis of THEMIS mean fluorescence intensity (MFI) in adult mouse thymocyte subsets and in thymic and peripheral lymph nodes γδ T cell subsets. Autofluorescence from each subset in Themis-knockout mice was subtracted to that of respective littermate controls.

4. Themis decreases the ratio of steady state peripheral $\gamma\delta^{IFN}/\gamma\delta^{17}$

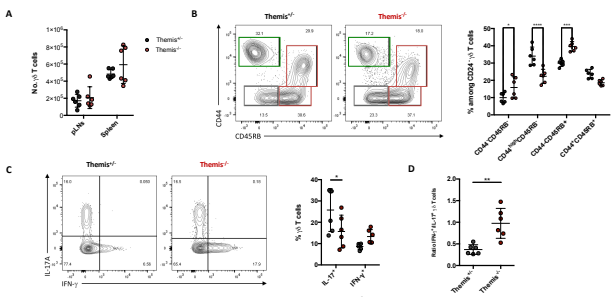


Figure 4: Themis deficiency increases IFN-γ to IL-17 commitment and production ratio by γδ T cells (A) Absolute number of peripheral lymph nodes or splenic γδ T cells in Themis-deficient and –sufficient mice. **(B)** Frequency of peripheral lymph nodes mature CD24$^-$ committed subsets γδ T cells: uncommited (CD45RB$^-$ CD44$^-$), IL-17- (CD44^{high}CD45RB$^-$) or IFN-γ-committed (CD45RB$^+$CD44$^{+/}$) in Themis-deficient and –sufficient mice. **(C)** Frequency of IL-17 and IFN-γ production by peripheral lymph nodes γδ T cells and **(D)** ratio of IFN-γ/IL-17 production in Themis-deficient and –sufficient mice. Data are representative of two independent experiments. *P < 0.05, **P < 0.01, *** P < 0.001 and ****P < 0.0001.

5. Themis leads to increased activation of $\gamma\delta^{IFN}$ upon *in vitro* TCR stimulation

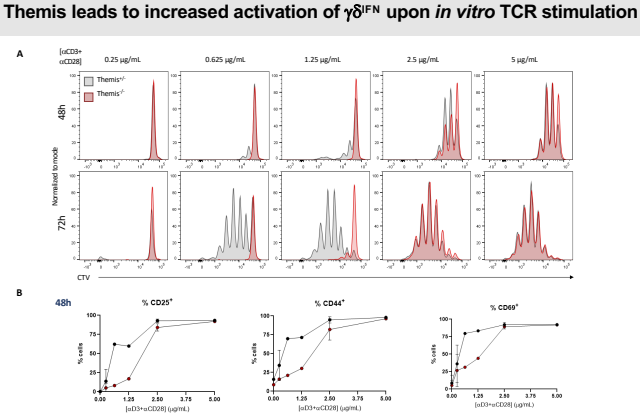


Figure 5: Themis-deficiency leads to decreased activation of gd T cells upon TCR stimulation *in vitro*. (A) CTV-labeled IFN-γ-poised CD27$^+$ γδ T cells from Themis-deficient or –sufficient mice were cultured *in vitro* for 48 or 72h hours in the presence of increasing concentrations of plate-bound anti-CD3 plus anti-CD28. **(B)** CD25, CD44 and CD69 expression in CD27$^+$ γδ T cells upon 48h of culture.

6. Themis expression contributes to the development of $\gamma\delta^{IFN}$-dependent experimental cerebral malaria

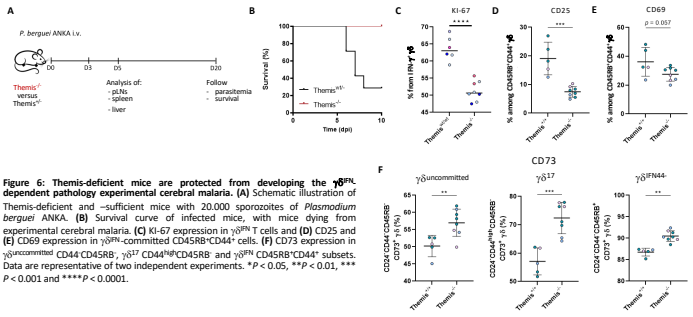


Figure 6: Themis-deficient mice are protected from developing the $\gamma\delta^{IFN}$-dependent pathology experimental cerebral malaria. (A) Schematic illustration of Themis-deficient and –sufficient mice with 20.000 sporozoites of *Plasmodium berguei* ANKA. **(B)** Survival curve of infected mice, with mice dying from experimental cerebral malaria. **(C)** KI-67 expression in $\gamma\delta^{IFN}$ T cells and **(D)** CD25 and **(E)** CD69 expression in $\gamma\delta^{IFN}$-committed CD45RB$^+$CD44$^+$ cells. **(F)** CD73 expression in $\gamma\delta^{uncommitted}$ CD44$^-$CD45RB$^-$, $\gamma\delta^{17}$ CD44^{high}CD45RB$^-$ and $\gamma\delta^{IFN}$ CD45RB$^+$CD44$^+$ subsets. Data are representative of two independent experiments. *P < 0.05, **P < 0.01, *** P < 0.001 and ****P < 0.0001.

7. Shp1/Shp2 also decrease the ratio of steady state peripheral $\gamma\delta^{IFN}/\gamma\delta^{17}$

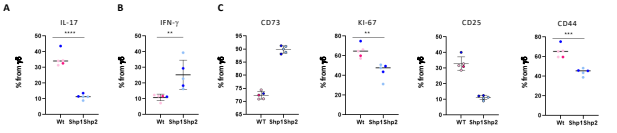
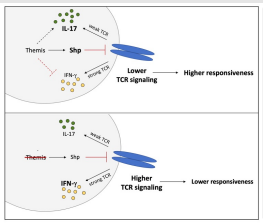


Figure 7: Shp1.Shp2-deficiency leads to increased IFN-g/IL-17 steady state ratio but decreased activation of gd T cells. Frequency of **(A)** IL-17 and **(B)** IFN-γ production by γδ T cells from Rag1^{Cre}.Shp1$^{fl/fl}$.Shp2$^{fl/fl}$-deficient and –sufficient mice and frequency of **(C)** CD73, **(D)** KI-67, **(E)** CD25 and **(F)** CD44 expression by these cells. Data are representative of two independent experiments. **P < 0.01, *** P < 0.001 and ****P < 0.0001.

Themis inhibits TCR signalling in γδ T cells to allow for higher responsiveness upon stimulation



Open questions/future work

1. How do *ex vivo* Themis-deficient γδ T cells respond *in vitro* to the cytokines IL-2 + IL-15, IL-12 + IL-18 and IL-1β + IL-23?
2. How do Rag1^{Cre}.Shp1$^{fl/fl}$.Shp2$^{fl/fl}$-deficient mice cope with malaria infection? Does the γδ phenotype match that of Themis-deficient mice infeted mice?
3. Do Themis-deficient γδ T cells present decresead pShp1, pShp2 and pErk1/2 upon *ex vivo* TCR crosslinking?

References

1. Tan L., Inácio D., Prinz I and Silva-Santos B., New insights on murine gd T cells from single-cell multi-omics, *Sci Bulletin*. 2022.
2. Lesourne R. *et al.*, Themis, a T cell-specific protein important for late thymocyte development, *Nat Immunol*. 2009.
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