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

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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. 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. This led to the identification of the distinct transcriptomes of $\gamma\delta^{17}$ and $\gamma\delta^{IFN}$ cells, which surprisingly diverged in 6337 differentially (over 1.5-fold) expressed genes. Pathway and gene ontology analyses indicated that $\gamma\delta^{17}$ cells differ from $\gamma\delta^{IFN}$ cells in their selective ability to sense and integrate external cues, whereas $\gamma\delta^{IFN}$ stand out in replication, transcription and translation processes. A detailed analysis of the top differentially expressed genes between $\gamma\delta^{17}$ and $\gamma\delta^{IFN}$ cells revealed that most of the signature genes of each subset increased their expression levels in the periphery (compared to the thymus), suggesting that $\gamma\delta^{17}$ and $\gamma\delta^{IFN}$ cells only terminate their differentiation process at peripheral sites. Among the top differentially expressed genes, we found *Themis*, a T cell-specific gene involved in the regulation of TCR signal strength, to be enriched in $\gamma\delta^{IFN}$ cells. Importantly, we found that Themis deficiency leads to a dysregulated effector γδ T cell peripheral compartment at steady state, which upon infection with *Plasmodium berguei* ANKA sporozoites confers Themis-deficient mice full protection from experimental cerebral malaria, a $\gamma\delta^{IFN}$-dependent pathology. Accordingly, we observed a less activated and less proliferative $\gamma\delta^{IFN}$ population in the peripheral lymph nodes of infected Themis-deficient mice compared to Themis-sufficient controls. This work demonstrates the relevance of the characterization of the $\gamma\delta^{IFN}$ and $\gamma\delta^{17}$ transcriptomes to uncover new players in the regulation of γδ T cell effector functions, which may open new avenues for their manipulation in disease settings.