

## Insights

### $\gamma\delta$ IL17 under control

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In the mouse,  $\gamma\delta$  IL17 cells are poised to make IL-17 and these cells have been involved in various infection and cancer models. Edwards et al (2022 JEM doi....) now report how different  $\gamma\delta$ IL17 subsets are controlled during homeostasis and cancer.

$\gamma\delta$  T cells are T cells that express a  $\gamma$  and  $\delta$  chain to form their T cell receptor (TCR) instead of an  $\alpha$  and  $\beta$  chain as in conventional CD4<sup>+</sup> and CD8<sup>+</sup>  $\alpha\beta$  T cells. They can be activated by their TCR (in an MHC-unrestricted way) but also via 'innate-like' means such as cytokine-mediated signaling. Innate  $\gamma\delta$  T cells can already be programmed during their development in the fetal/perinatal thymus towards either an IFN $\gamma$  ( $\gamma\delta$ IFN) or IL17 ( $\gamma\delta$ IL17) effector status.  $\gamma\delta$ IL17 cells can be further subdivided by the type of TCR expressed on their surface, which is usually identified by the V $\gamma$  chain used, either V $\gamma$ 6 or V $\gamma$ 4 (Fiala et al., 2020). In the periphery, including lung,  $\gamma\delta$ IL17 have been shown to contribute to protection against infections but they can also promote tumor development (Guo et al., 2018; Reis et al., 2022; Silva-Santos et al., 2019) . How these programmed  $\gamma\delta$ IL17 cells are regulated in the periphery was not clear. Edwards et al addressed this question both under homeostatic and cancer conditions (Edwards et al., 2022) (Figure 1).

First, the authors scrutinized the biology of the  $\gamma\delta$  T cell compartment in the lung of mice during homeostasis by using a single-cell RNA sequencing approach. This analysis revealed a

familiar landscape, with  $\gamma\delta$  T cells segregating in two major clusters that corresponded to cells poised towards IL17A (IL17) or IFN $\gamma$  production. The authors identified  $V\gamma6^+$  cells as the main subset from the  $\gamma\delta17$  cluster, albeit some  $V\gamma4^+$  were present, and further extended our knowledge from this population by reporting two important observations: i) the lung  $V\gamma6^+$  transcriptome possesses a pan-tissue gene signature common with its thymic, skin, uterine, adipose and lymph node counterparts; ii)  $V\gamma6^+$  cells are enriched (at transcript and protein level) for markers linked to residency in conventional T resident memory cells ( $T_{rm}$ ), such as PD-1, CXCR6, ICOS and JAML. The constitutive high expression of PD-1 observed exclusively in the lung  $V\gamma6^+ \gamma\delta17$  subset prompted the authors to further investigate the effect of this checkpoint on these cells. Functional in vitro and in vivo experiments modulating PD-1 signaling did not reveal any effect on IL-17 production capacity by  $V\gamma6^+$  cells as such, but showed that the checkpoint protein controlled their expansion, effectively keeping  $V\gamma6^+$  cells 'under control' during homeostasis (Figure 1A). Of note, all these observations were highly specific for the lung-resident  $V\gamma6^+$  compartment as no similar effects could be seen in  $V\gamma4^+ \gamma\delta17$  cells. In order to understand the intracellular mechanism involved in the PD-1-mediated control of  $V\gamma6^+ \gamma\delta17$  cells, a careful dissection of the molecular aftermath of PD-1 signaling was carried by the authors under type 3 inflammatory conditions (IL-1 $\beta$  and IL-23 cytokines). The results showed that PD-1 ligation restrained  $V\gamma6^+$  T cell expansion by modulating FOXO1 activity, a transcription factor that has been described to inhibit the master regulator of the  $\gamma\delta17$  phenotype, ROR $\gamma$ t (Fiala et al., 2020; Lainé et al., 2015).

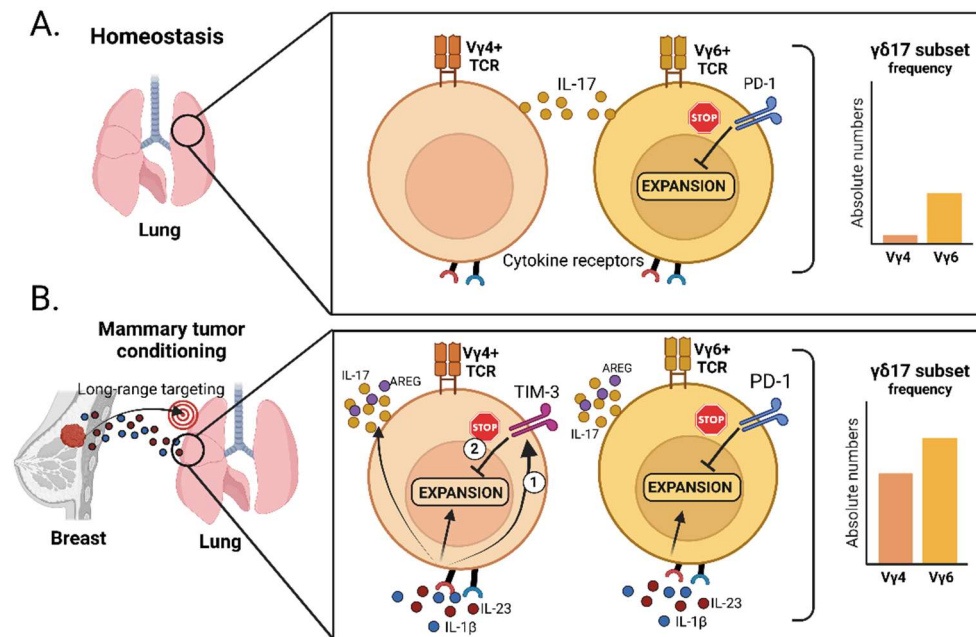
Several lines of evidence have described previously the involvement of  $V\gamma6^+$  and  $V\gamma4^+ \gamma\delta17$  cells in tumor progression at different anatomical sites (Reis et al., 2022; Silva-Santos et al., 2019; Van hede et al., 2017). Consequently, the authors decided to further explore the lung  $\gamma\delta$  ecosystem by single-cell sequencing and functional experiments under a tumor conditioning environment in a well-established mouse model of triple negative breast cancer (the KB1P tumor model). These experiments revealed that the lung  $\gamma\delta17$  population expanded under the tumor conditioning environment, functionally atomizing in several distinct clusters in the single-cell data and increasing the gene/protein expression of pro-tumoral cytokines such as IL-17A, IL-17F and amphiregulin (AREG). Additional analysis indicated that  $\gamma\delta17$  expansion was more pronounced in the  $V\gamma4^+$  subset due to an increased sensitivity towards tumor-derived factors that went along with changes in their phenotype, including an up-regulation of the co-inhibitory receptor TIM-3 (Figure 1B). The authors found that all these changes were the result of a long-range targeted action from tumor-derived IL-1 $\beta$  and IL-23. It remains to be explored

whether this conditioning can affect  $\gamma\delta 17$  populations resident in other tissues. Finally, the authors reported  $\gamma\delta 17$  subset-specific responses in KB1P tumor-bearing mice undergoing checkpoint inhibitor treatment, with anti-PD1 treatment expanding  $V\gamma 6^+$  cells and anti-TIM3 expanding the  $V\gamma 4$  subset, in the lung and also in the lymph nodes. Interestingly, genetic ablation of  $\gamma\delta$  T cells sensitized KB1P tumors towards checkpoint inhibitory therapy, indicating that  $V\gamma 6^+$  and  $V\gamma 4^+$  cells could induce resistance to anti-PD-1 or anti-TIM-3 immunotherapy respectively. Overall, these important advances highlight that  $\gamma\delta$  T cell subsets can be differentially regulated according to the type of TCR they express, despite their similar effector function. Further research may identify the (lymph node) source of expanding  $V\gamma 4^+$   $\gamma\delta 17$  cells in the lung from the breast cancer model, the (epigenetic) mechanism of their increased sensitivity (compared to the  $V\gamma 6^+$  subset) towards long-distance (breast) tumor-derived conditioning factors and the potential important role of the (lung) microbiome herein (Jin et al., 2019; Reis et al., 2022).

Thus, Edwards et al identified PD-1 signaling in lung  $V\gamma 6^+$   $\gamma\delta 17$  cells as a main regulatory mechanism, both in homeostatic and breast cancer settings, while TIM-3 came into action to regulate  $V\gamma 4^+$   $\gamma\delta 17$  cells in the mouse breast cancer model. The expansion of  $\gamma\delta 17$  cells after treatment with checkpoint inhibitors in this KB1P model may have important therapeutic implications. However, how the data presented by Edwards et al can be translated to the human setting is not clear. Indeed, mouse and human  $\gamma$  and  $\delta$  loci are not conserved; for example, there are no clear homologues in human for the mouse  $V\gamma 4$  and  $V\gamma 6$   $\gamma\delta 17$  subsets (Papadopoulou et al., 2020). Furthermore, while IL-17-producing  $\gamma\delta$  T cells have been identified in some human cancer settings (Silva-Santos et al., 2019), recent studies focusing on lung and breast cancer could not identify IL-17-producing  $\gamma\delta$  T cells, either in tumor tissue or paired non-tumor tissue (Wu et al., 2022, 2019). In addition, TRDV1 gene expression, encoding for the  $V\delta 1$  chain of the human  $V\delta 1^+$   $\gamma\delta$  T cell subset, in pre-treatment tumor biopsies of various origin, is associated with increased survival upon anti-PD1 (pembrolizumab) treatment (Wu et al., 2022). Thus it remains to be explored whether in particular human cancer settings checkpoint inhibition may promote the expansion of, possibly fetal-derived (Sanchez Sanchez et al., 2022),  $\gamma\delta 17$  cells .

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### Figure legend

**Figure 1.  $\gamma\delta 17$  subsets ( $V\gamma 4^+$  and  $V\gamma 6^+$ ) are differentially regulated during homeostasis and cancer.** Under homeostatic conditions (A), fetal-derived lung  $V\gamma 6^+$  display a resident phenotype which include the constitutive expression of PD-1 that negatively regulates the expansion of these cells. Under cancer context (B), cytokines derived from breast tumors (KB1P mouse model) expand the cells from the lung  $\gamma\delta 17$  compartment and induce subset-specific responses.  $V\gamma 4^+$  cells show increased expression of pro-tumoral cytokines (IL17-A, IL17-F and AREG) and of the co-inhibitory receptor TIM-3 (indicated by '1') that in turn inhibits the expansion of this subset (indicated by '2'). Although tumor-derived factors expand  $V\gamma 6^+$  cells, their phenotype remain relatively stable under this condition.

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