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INSIGHTS

γδIL17 under control

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In the mouse, γδ IL17 cells are poised to make IL-17, and these cells have been involved in various infection and cancer models. Edwards et al. (2022. *J. Exp. Med.* https://doi.org/10.1084/jem.20211431) now report how different γδIL17 subsets are controlled during homeostasis and cancer.

γδ T cells are T cells that express a γ and δ chain to form their TCR instead of an α and β chain as in conventional CD4⁺ and CD8⁺ αβ 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 γδ T cells can already be programmed during their development in the fetal/perinatal thymus towards either an IFN-γ (γδIFN) or IL-17 (γδIL17) effector status. γδIL17 cells can be further subdivided by the type of TCR expressed on their surface, which is usually identified by the Vγ chain used, either Vγ6 or Vγ4 (Fiala et al., 2020). In the periphery, including lung, γδIL17 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 γδIL17 cells are regulated in the periphery was not clear. Edwards et al. (2022) addressed this question both under homeostatic and cancer conditions (see figure).

First, the authors scrutinized the biology of the γδ 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 γδ T cells segregating in two major clusters that corresponded to cells poised towards IL-17A (IL-17) or IFN-γ production. The authors identified Vγ6⁺ cells as the main subset from the γδ17 cluster, albeit some Vγ4⁺ were present, and further extended our knowledge from this population by reporting two important observations: (i) the lung Vγ6⁺ transcriptome possesses a pan-tissue gene signature common with its thymic, skin, uterine, adipose, and lymph node counterparts; (ii) Vγ6⁺ cells are enriched (at transcript and protein level) for markers linked to residency in conventional T resident memory cells, such as PD-1, CXCR6, ICOS, and JAML. The constitutive high expression of PD-1 observed exclusively in the lung Vγ6⁺ γδ17 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 Vγ6⁺ cells as such, but showed that the checkpoint protein controlled their expansion, effectively keeping Vγ6⁺ cells "under control" during homeostasis (see panel A of figure). Of note, all these observations were highly specific for the lung-resident Vγ6⁺ compartment, as no similar effects could be seen in Vγ4⁺ γδ17 cells. In order to understand the intracellular mechanism involved in the PD-1–mediated control of Vγ6⁺ γδ17 cells, a careful dissection of the molecular aftermath of PD-1 signaling was carried by the authors under type 3 inflammatory conditions (IL-1β and IL-23 cytokines). The results showed that PD-1 ligation restrained Vγ6⁺ T cell expansion by modulating FOXO1 activity, a transcription factor that has been described to inhibit the master regulator of the γδ17 phenotype, RORγt (Fiala et al., 2020; Lainé et al., 2015).

Insights from Guillem Sanchez Sanchez and David Vermijlen. Photo by Thor Vermin.

Several lines of evidence have described previously the involvement of Vγ6⁺ and Vγ4⁺ γδ17 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 γδ 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 γδ17 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 γδ17

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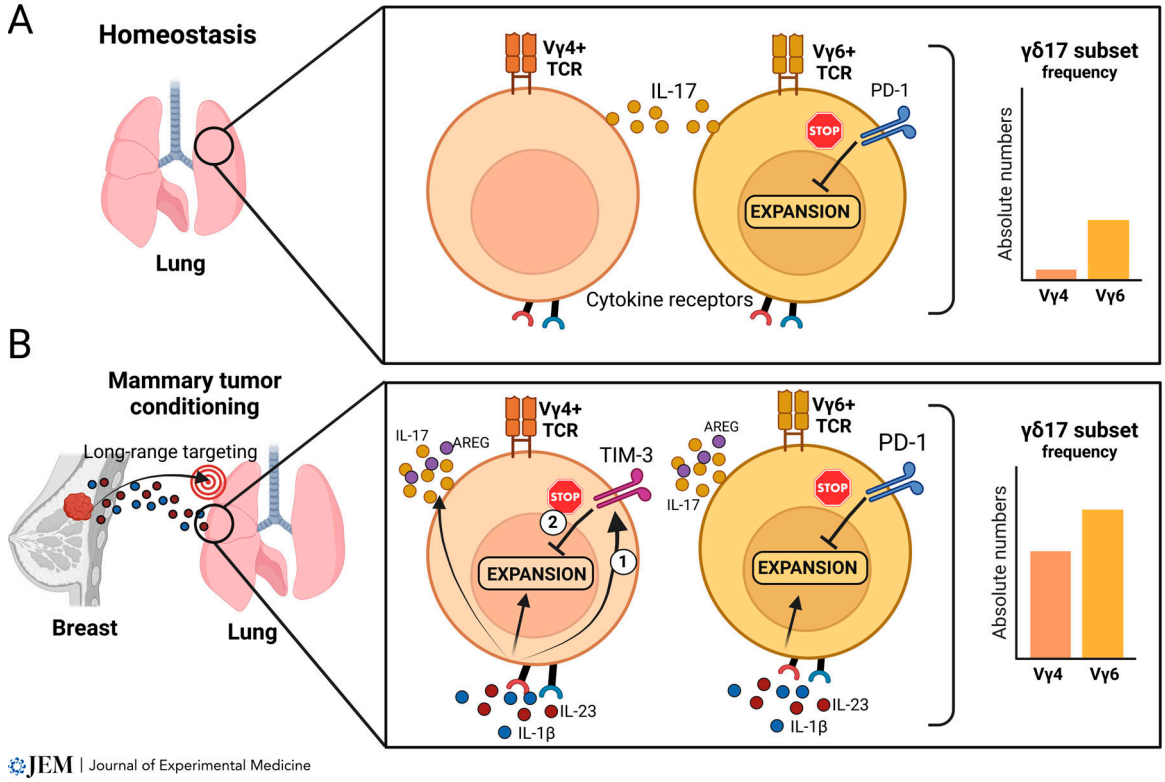

γδ17 subsets (Vγ4⁺ and Vγ6⁺) are differentially regulated during homeostasis and cancer. Under homeostatic conditions (A), fetal-derived lung Vγ6⁺ display a resident phenotype, which includes the constitutive expression of PD-1 that negatively regulates the expansion of these cells. Under cancer context (B), cytokines derived from breast tumors (KB1P mouse model) expand the cells from the lung γδ17 compartment and induce subset-specific responses. Vγ4⁺ 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 Vγ6⁺ cells, their phenotype remains relatively stable under this condition. Figure created with BioRender.com.

expansion was more pronounced in the Vγ4⁺ 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 (see panel B of figure). The authors found that all these changes were the result of a long-range targeted action from tumor-derived IL-1β and IL-23. It remains to be explored whether this conditioning can affect γδ17 populations resident in other tissues. Finally, the authors reported γδ17 subset-specific responses in KBP1 tumor-bearing mice undergoing checkpoint inhibitor treatment, with anti–PD-1 treatment expanding Vγ6⁺ cells and anti-TIM3 expanding the Vγ4 subset, in the lung and also in the lymph nodes. Interestingly, genetic ablation of γδ T cells sensitized KB1P tumors towards checkpoint inhibitory therapy, indicating that Vγ6⁺ and Vγ4⁺ cells could induce resistance to anti–PD-1 or anti–TIM-3 immunotherapy, respectively. Overall, these important advances highlight that γδ 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 Vγ4⁺ γδ17 cells in the lung from the breast cancer model, the (epigenetic) mechanism of their increased sensitivity (compared to the Vγ6⁺ 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. (2022) identified PD-1 signaling in lung Vγ6⁺ γδ17 cells as a main regulatory mechanism, both in homeostatic and breast cancer settings, while TIM-3 came into action to regulate Vγ4⁺ γδ17 cells in the mouse breast cancer model. The expansion of γδ17 cells after treatment with checkpoint inhibitors in this KB1P model may have important therapeutic implications. However, how the data presented by Edwards et al. (2022) can be translated to the human setting is not clear. Indeed, mouse and human γ and δ loci are not conserved; for example, there are no clear homologues in human for the mouse Vγ4 and Vγ6 γδ17 subsets (Papadopoulou et al., 2020). Furthermore, while IL-17–producing γδ 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 γδ T cells, either in tumor tissue or paired non-tumor tissue (Wu et al., 2022; Wu et al., 2019). In addition, TRDV1 gene expression, encoding for the Vδ1 chain of the human Vδ1⁺ γδ T cell subset, in pre-treatment tumor biopsies of various origin, is associated with increased survival upon anti–PD-1 (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), γδ17 cells.

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