Trends in Cancer

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Spotlight

Battle of the γδ T cell subsets in the gut

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In a study in *Science*, Reis *et al.* describe a temporal segregation of γδ T cell activities in colorectal cancer (CRC). Initially tumor surveillance is orchestrated by interferon-γ (IFN-γ)-producing and cytotoxic γδ T cell subsets, but once the tumor thrives, it becomes infiltrated by interleukin (IL)-17$^+$ γδ T cell subsets that promote its growth.

Current immunotherapies for cancer mostly rely on conventional αβ T cells, which recognize tumor-specific or tumor-associated peptides presented by major histocompatibility complexes (MHC). By contrast, the alternative T cell lineage, γδ T cells are independent of antigen processing or MHC presentation and thus represent a complementary strategy to mount immune responses to tumors. These have been extensively characterized over the past two decades, since the seminal study by Hayday and colleagues on murine models of cutaneous carcinogenesis [1], building the foundations for γδ T cell-based approaches that are now being tested in the clinic [2].

While analyzing the contributions of γδ T cells to tumor surveillance/progression in murine models of cancer, a striking dichotomy emerged: whereas γδ T cells making IFN-γ, a cytokine that promotes cytotoxicity and inhibits angiogenesis and tumor proliferation, displayed overt antitumor functions, those producing interleukin 17A (here simplified to IL-17) instead supported tumor growth via mobilization of immunosuppressive cells and promotion of angiogenesis [3]. The two key cytokines were expressed by different γδ T cell subsets that could be distinguished based on their expression levels of receptors associated with their activation, such as CD27 and CD122 (selectively present in IFN-γ producers) or BTLA and PD-1 (enriched in IL-17 producers) [4]. Interestingly, the variable region (V) of the T cell receptor (TCR), which characterizes 'developmental waves' of γδ T cells that are generated in the mouse thymus and populate peripheral tissues, also segregate effector functions: Vγ1$^+$ cells are enriched in IFN-γ producers, whereas Vγ4$^+$ cells are biased towards IL-17 and Vγ6$^+$ cells are almost exclusively IL-17 producers [3].

In the context of cancer development, a major gap in knowledge has been the lack of a dynamic perspective on the different γδ T cell subsets in the tumor microenvironment, especially as previous studies had either focused on the anti- or protumor contributions of γδ T cells, without a temporal/spatial integration of the two components. This caveat has been overcome by a recent study published in *Science*, where Reis *et al.* investigated the roles of γδ T cells in CRC development and progression [5].

The authors analyzed γδ T cell responses in two murine models of CRC, the chemically induced azoxymethane-dextran sodium sulfate (AOM-DSS) colitis-associated model and the genetically inducible adenomatosis polyposis coli deficiency model. Whereas in healthy murine colon, γδ T cells had a CD8αα$^+$ PD1$^-$ phenotype that associated with IFN-γ and granzyme (A and B) expression, tumor-infiltrating γδ T cells were enriched in IL-17-producing PD-1$^+$ cells with protumor potential (Figure 1). TCR sequencing analyses revealed that γδ T cells with antitumor features were composed of polyclonal Vγ7$^+$ and Vγ1$^+$ cells, whereas the IL-17-producing cells contained some Vγ4$^+$ cells, but mostly clonally expanded Vγ6Vδ1$^+$ cells, a subset that was rarely observed in non-tumor areas or in early phases of tumor development. Increased proliferation of these protumor subsets led to their accumulation, which was dependent on TCR stimulation. Moreover, consistent with what was previously shown in lung cancer [6], Vγ6$^+$, but not Vγ4$^+$ cells, depended on microbiota to sustain IL-17 production [5].

Although Vγ6$^+$ cells were the prominent protumor subset in tumors, Vγ4$^+$ cells were seemingly able to compensate for the absence of Vγ6$^+$ cells, as antibody-mediated depletion of Vγ4$^+$ cells in Vγ6$^{-/-}$ mice was necessary for reductions in tumor size and in the IL-17$^+$ γδ T cell pool in the AOM-DSS model. Similarly, the antitumor functions of Vγ7$^+$ cells, which are mostly gut-specific γδ T cells, and Vγ1$^+$ cells, which have a broad tissue distribution, also appeared redundant, as ablation of Vγ1$^+$ cells in Vγ7$^{-/-}$ mice was required to impact on tumor development. One should highlight and praise the experimental tools generated and the solid approaches used by the authors to address these complex questions. What remains uncertain is if these functional redundancies are due to the constitutive deficiency of either Vγ6$^+$ or Vγ7$^+$ cells, allowing for compensation by other subsets with a similar cytokine profile. Clarifying this issue may require the development of new inducible genetic ablation models, or combined antibody depletion strategies. Overall, Reis *et al.* propose a temporal segregation of γδ T cell activities in preclinical models of CRC, where initially tumor surveillance is orchestrated by IFN-γ-producing (and cytotoxic) Vγ7$^+$ and Vγ1$^+$ γδ T cells. Once the tumor thrives, it, instead, becomes increasingly infiltrated by IL-17$^+$ (especially Vγ6$^+$) γδ T cells that promote its growth [5].

The translation of these findings to humans is complicated at two levels. First, the evolutionary divergence in TCR gene loci between rodents and primates means that no direct counterparts of the four murine γδ T cell subsets mentioned earlier can be ascribed in humans. Second, human γδ T cells are much less prone to

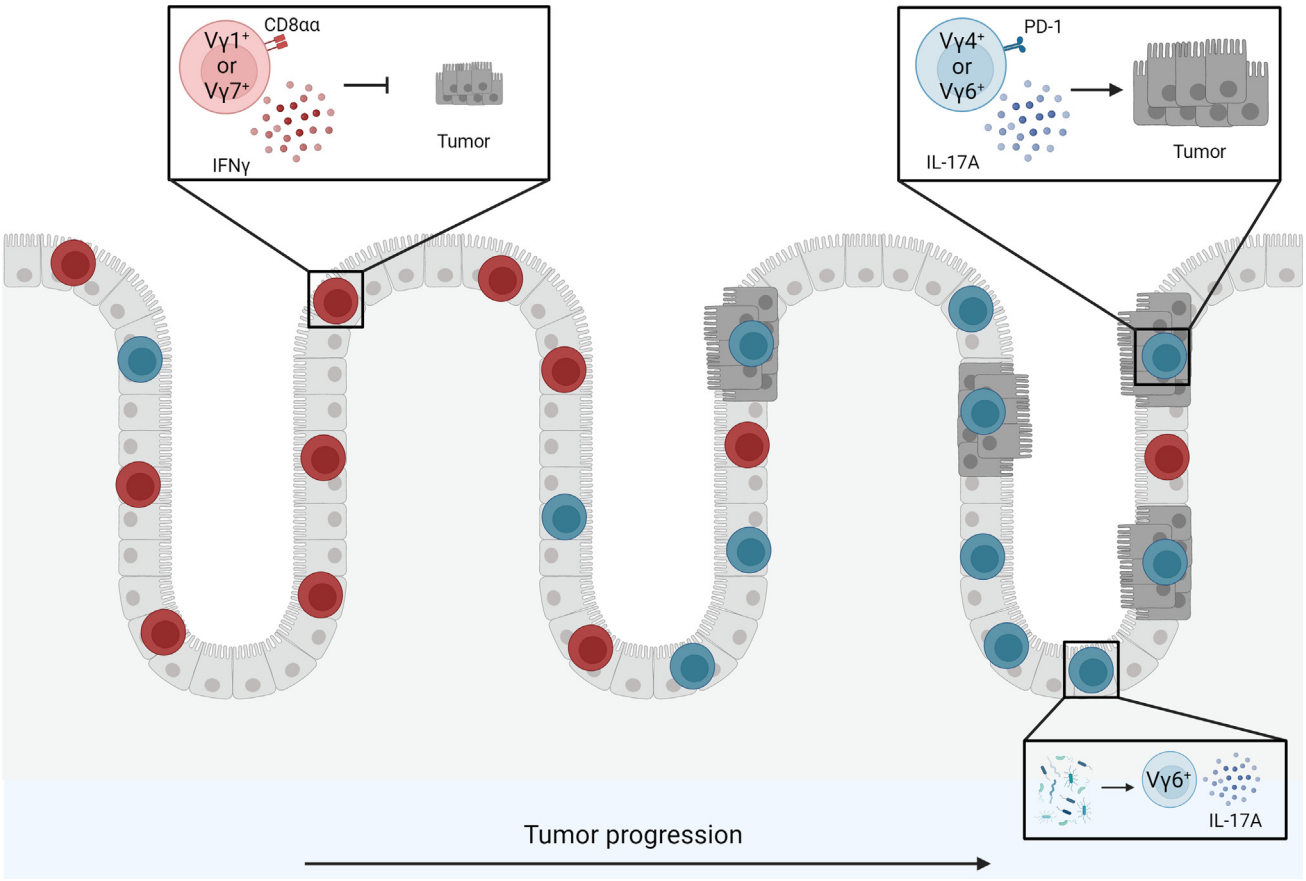

Figure 1. γδ T cell subset dynamics in colon cancer progression. In the healthy mouse colon, as well as at early stages of colorectal cancer development, γδ T cells were mainly Vγ1$^+$ or Vγ7$^+$, expressed high levels of CD8αα and interferon-γ (IFN-γ), and participated in tumor surveillance. During tumor progression, interleukin (IL)-17-producing γδ T cells, either Vγ4$^+$ or Vγ6$^+$, expressing PD-1, accumulated within tumors and promoted their growth. Of note, IL-17 production by expanding Vγ6$^+$ cells was sustained by microbiota, as shown by the impact of antibiotics treatment. Based on findings by Reis *et al.* [5].

produce IL-17 than mouse Vγ6$^+$ or Vγ4$^+$ cells [3]. This notwithstanding, Reis *et al.* analyzed sorted human γδ T cells from tumors and adjacent non-tumor areas of CRC patients by single-cell RNA sequencing and found that while the γδ T cells isolated from adjacent non-tumor areas expressed cytotoxic mediators, tumor-infiltrating γδ T cells showed an enriched cytokine signature, concomitant with the expression of some genes, like *CD9* and *LGALS3* that were previously associated with murine IL-17$^+$ γδ T cells [7]. However, IL-17 itself was not one of the upregulated genes, which is in agreement with a recent transcriptomic analysis of human blood γδ T cells [8]. This finding adds to a lasting controversy, despite an early report in CRC [9], on whether any potential protumor roles of human γδ T cells are mediated by IL-17. For example, a more recent study found no IL-17 but instead highlighted the cytotoxic and IFN-γ-producing potential of human gut-resident Vδ1$^+$ γδ T cells [10]. In any case, Reis *et al.* found a segregation (both in terms of TCR sequences and gene expression signatures) between Vδ1$^+$ γδ T cell clones expanded in tumor versus nontumor areas, which substantiates the claim of a spatial segregation of γδ T cell activities in human CRC [5]. Overall, this elegant study clearly shows the importance of the temporal and spatial dimensions in understanding the contributions of γδ T cells to cancer development and progression, which should be taken into account as these cells become the object of novel cancer immunotherapies [2].

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Declaration of interests

The authors declare no competing conflict of interest.

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