γδ T cells turn the tables on immune-evasive colon cancer

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Summary

Why is checkpoint blockade immunotherapy still effective in tumors that are unrecognizable to $CD8^+$ T cells? In a recent study published in *Nature*, de Vries *et al.* provide evidence that a lesser-known T cell population, called γδ T cells, may mediate beneficial responses to immune checkpoint blockade when cancer cells lose expression of HLA molecules.

Main body

Across all cancer types, those tumors with high mutational burden are particularly responsive to immune checkpoint blockade (ICB). These cancer types include melanoma, lung cancer, and DNA mismatch repair-deficient (MMR-d) colon cancer, among others. In these mutationally high cancer types, boosting (neo)antigen-specific $CD8^+$ T cell activity with anti-PD-1 prevents immunosuppression and increases T cell-mediated killing. However, these tumors frequently exhibit mutations in β2-microglobulin (*B2M*), a component of the major histocompatibility (MHC)-I complex, and other proteins involved in antigen processing and presentation, making them invisible to $CD8^+$ T cells. Fortunately, other lymphocytes, like γδ T cells, can recognize mutated cells lacking MHC molecules. γδ T cells are distinct from conventional T cells in many ways, including the genes that encode their T cell receptor chains and their inability to bind MHC molecules, but γδ T cells also share functions with conventional T cells, such as inducing death of cancer cells (1). What we know about the mechanisms by which γδ T cells counteract cancer progression lags far behind what is known about $CD8^+$ T cells. Therefore, the recent study by de Vries *et al.* showing that γδ T cells may participate in ICB response in MMR-d colon cancer patients is a major advance for the cancer immunotherapy field (2).

From a small cohort of 21 patients, de Vries *et al.* found that 95% of *B2M*-mutated, MMR-d tumors are still responsive to ICB despite their inability to engage $CD8^+$ T cells (2). To gain insight into this seemingly contradictory observation, the authors used gene expression data from MMR-d colon, gastric, and endometrial tumors to compare differences between *B2M* wild-type and *B2M*-mutated tumors. This analysis showed that *B2M*-mutated tumors contain increased numbers of γδ T cells. Further phenotypic examination of γδ T cells from MMR-d colon cancers revealed that some subsets of γδ T cells express PD-1 and other checkpoint molecules. The PD-1-expressing γδ T cell subsets were superior at killing B2M-deficient colon cancers than γδ T cell subsets

lacking PD-1 expression, highlighting the heterogeneity of γδ T cell capabilities. In addition, de Vries *et al.* investigated colon cancer tissue from the NICHE trial that consisted of pre- and post-ICB MMR-d samples. Those tumors with *B2M* mutations displayed increased γδ T cell numbers after ICB, whereas γδ T cell numbers remained the same before and after ICB in *B2M* wild-type tumors. Taken together, these data provide compelling evidence that ICB boosts γδ T cell activity in *B2M*-mutated tumors where they participate in tumor control.

One important aspect of the study by de Vries *et al.* is that not all tumor-associated γδ T cells are the same. Human γδ T cells are defined by the δ chain they express: Vδ1 and Vδ3 cells are mostly tissue resident, whereas Vδ2 cells circulate in blood. The majority of studies on the anti-tumor functions of γδ T cells are focused on circulating Vγ9Vδ2 cells (3); although, the killing ability of Vδ1 cells has been established for some time (4). de Vries *et al.* demonstrate that tumor-infiltrating Vδ1 and Vδ3 cells can express PD-1 (to a limited degree) and these populations are endowed with killing ability of *B2M*-mutated cancer cells, while PD-1 is mostly absent from Vδ2 cells that are inefficient at triggering cancer cell death (2).

The authors also show that PD-1-expressing Vδ1 and Vδ3 cells can use NKG2D to recognize cancer cells, but whether other molecules are involved in recognition remain unknown. Similarly, the data raise questions regarding the role (if any) of the γδTCR in cancer cell recognition and killing. Gut-resident Vδ1 cells that dimerize with the Vγ4 chain bind members of the butyrophilin family, BTNL3 and BTNL8, which regulates their development and phenotype (5, 6); however, our lab has found that expression of BTNL3 and BTNL8 is lost during cancer progression (7). Whether BTNL3 and BTNL8 are required for Vδ1 cell killing of colon cancer cells or whether expression of BTNL3 and BTNL8 are maintained specifically in *B2M*-mutated colon cancer is not clear. As BTNL3 and BTNL8 expression is restricted to gut tissue, a TCR-independent role would be advantageous to engage Vδ1 cells in other *B2M*-mutated cancer types beyond colon cancer. The TCR on Vδ3 cells can bind the MHC class I-related protein, MR1, dimerized to B2M that presents vitamin B metabolites, so cancer cells would need to express these metabolites (and B2M) for Vδ3 cell engagement (8). These obscurities on γδTCR importance further confuse the role of PD-1 in these cells. PD-1 signaling suppresses TCR activation in conventional T cells, but whether the same mechanisms occur downstream of Vδ1 and Vδ3 TCR signaling is unknown. Recently, our lab discovered that PD-1 signaling inhibits mouse IL-17A-producing γδ T cells independently of TCR (9), highlighting the possibility that PD-1 suppression of human Vδ1 and Vδ3 cells may work differently than in conventional T cells.

There are multiple implications for the de Vries *et al.* study, such as using γδ T cells as biomarkers of ICB responsiveness, expanding this work beyond colon cancer to other *B2M*-mutated cancer types, and testing cellular-based γδ T cell therapies together with ICB in patients with *B2M*-mutated tumors. In the meantime, larger cohorts of patients are necessary to verify the reported observations, especially in the context of pre- and post-ICB. To gain further insight into these mechanisms and test new immunotherapy approaches, mouse

models of cancer will be highly advantageous, given the conserved biology between humans and mice in gut-resident γδ T cell and BTNL interactions (5, 6). The interest in γδ T cells for cancer immunotherapy has increased exponentially in recent years (10), and the findings by de Vries *et al.* expand the potential of ICB beyond $CD8^+$ T cells to the underappreciated population of γδ T cells, lending credence to the notion that γδ T cells are viable and effective tools for cancer immunotherapy.

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Declaration of Interest

The authors declare no competing interests.