Commentary

Immunosuppressive role of γδ T cells in cancer: the other side of the coin

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Development of cancer is associated with evolution of tumor microenvironment which subverts the immune response for its benefit. The tumor environment promotes the production of proinflammatory cytokines, leading to the accumulation of suppressive cells that inhibit antitumor immunity. In contrast, strong lymphocyte infiltration has been reported to be associated with an antitumor response and improved clinical outcome (1). The prevalence of suppressive or anti-tumor immune cells in the tumor environment determines the course of the disease. T cells expressing γδ T cell receptor (TCR) are one of the vital players in tumor infiltrating lymphocytes (TILs). The functional significance of γδ T cells in the tumor environment is still an enigma. The disparate properties of γδ T cells to recognise antigens in MHC unrestricted manner bestow an advantage to γδ T cells over αβT cells for anti-tumor immunity. γδ T cells specifically respond to the stress-induced MHC class I-related molecules MICA, MICB, and the UL16-binding proteins (ULBP) that are upregulated on malignant or stressed cells (2).

The activation of γδ T cells induces expression of perforins and granzymes, engagement of death-inducing receptors, such as FAS and expression of TNF-related apoptosis-inducing ligand (TRAIL) (3,4). IFNγ secreted by activated γδ T cells enhances MHC class I expression by tumour cells, thus promoting $CD8^+$ T cell responses (4). The major subsets of γδ T cells, $V\delta1^+$ (predominant in tissues) and $V\delta2^+$ (predominant in the circulation) show cytolytic activity against solid and hematologic tumors (5). Meta-analysis of clinical trials using γδ T cells in patients with acute myeloid leukemia, chronic lymphocytic leukemia, non-small cell lung carcinoma, renal cell carcinoma, multiple myeloma, cervical cancer, etc. highlights the significance of γδ T cells as potential candidate for cell based immunotherapy in cancer patients (5). This suggests that it is possible to harness the antitumor properties of γδ T cells by *in vitro* and/or *in vivo* manipulation of γδ T cells. However, the functional properties of γδ T cells during progression of cancer in pre-invasive and clinically non apparent disease are not well understood.

The emerging evidences in recent past have highlighted the tumor promoting functions of γδ T cells in patients. A common mediator of such functions is shown to be the cytokine IL17 (6). Using a methylcholanthrene (MCA)-induced fibrosarcoma model, it was first shown that γδ T cells were the main source of IL17 and in the absence of IL17, reduction in tumor growth and blood vessel density were observed (7). Similarly, in lung metastasis model, Tγδ17 cells were associated with detrimental effects observed in the tumor bearing host (8). Tγδ17 cells suppress $CD8^+$ cytotoxic T lymphocytes by expansion and polarization of neutrophils in the mice bearing mammary tumours (9). It was reported that Tγδ17 cells induce mobilization of pro-tumor small peritoneal macrophages (SPM) as well as myeloid-derived suppressor cells (MDSCs) to the tumor bed which induce angiogenesis and immune suppression respectively (10,11). In human colorectal cancer Tγδ17 cells are reported to recruit MDSCs to the

tumor bed and associate with clinical stage of patients (12). Recently our group has shown that Tγδ17 cells were elevated in tumor tissue and peripheral blood of gallbladder cancer patients and were associated with poor survival by inducing angiogenesis in the gallbladder tumor cells (13). Human Vδ1^{+}γδ T cells were also reported to suppress CD4^{+} and CD8^{+} T cells and also impaired the maturation and T-cell priming capacity of dendritic cells (14). However, the molecular mechanism behind the immunosuppressive behaviour of γδ T cells is poorly understood.

Recent data from Daley and collaborators have provided compelling evidence to show the robust immunosuppressive effect of γδ T cells restraining antitumor αβT cells to support pancreatic ductal adenocarcinoma (PDA) (15). Previous reports show that in a murine model of pancreatic intraepithelial neoplasia (PanIN), mutation in Kras^{G12D} induced infiltration of IL17 producing T cells to the tumor bed. The propensity of IL17 production by γδ T cells was shown to be higher than CD4^{+} T cells (16). However, the contribution of γδ T cells in pancreatic cancer progression remains elusive. Daley *et al*. addressed the functional role of γδ T cells in pancreatic cancer using invasive [C57BL/6-Trdc^{tm1Mal} mice harbouring orthotopically implanted Pdx1^{Cre};Kras^{G12D};Tp53^{R172H} (KPC)-derived invasive PDA] and pre-invasive [p48^{Cre};Kras^{G12D} (KC) mice harbouring pre-invasive tumor] murine PDA. It was shown that γδ T cells abundantly infiltrated the invasive PDA tissue and predominantly expressed Vγ4 than Vγ1. The PDA infiltrating γδ T cells showed upregulated expression of molecules associated with immunosuppression such as FAS ligand, NK1.1, CD39, JAML, and OX40. Moreover, these γδ T cells also expressed elevated levels of IL10 and FoxP3 than their splenic counterpart. In addition, the PDA infiltrating γδ T cells also expressed IL17, TNFα, IFNγ, NKG2D receptor, TLR4, TLR7 and TLR9 at higher level compared to γδ T cells in the spleen. This suggests that the γδ T cells were distinctly activated in PDA. Similar observations were obtained in 6-month-old KC mice, a model of pre-invasive PDA. In human PDA, γδ T cells comprise up to 75% of tumor infiltrating CD3^{+} T cells and were significantly increased than CD8^{+} T cells. Similar to murine models of PDA, Vγ9^{+} γδ T cells were absent in human PDA and predominantly belonged to CD45RA^{-} CD27^{-}T$_{(effector\ memory)}$ phenotype.

Daley *et al*. reported that PDA infiltrating γδ T cells showed elevated levels of CCR2, CCR5, and CCR6. The involvement of these chemokine receptors in recruitment of γδ T cells to the tumor bed was demonstrated using CCR2$^{-/-}$, CCL2$^{-/-}$, CCR5$^{-/-}$ and CCR6$^{-/-}$ mice with orthotopic KPC-derived tumor. Deletion of CCR2, CCL2, or CCR6 significantly reduced γδ T cell infiltration to the tumor bed which was correlated with reduced tumor burden.

The PDA infiltrating γδ T cells have significant role in pancreatic oncogenesis. The genetic deletion of γδ T cells (wild type KC mice crossed with Tcrδ$^{-/-}$ mice and challenged orthotopically with PDA) were associated with reduced dysplastic ducts and slower PanIN progression. Moreover, depletion of Vγ4^{+} γδ T cells in KC mice or in orthotopic KPC model (using neutralizing monoclonal antibody UC3-10A6) protected against tumor growth and showed increased survival. Notably, absence of γδ T cells did not affect pancreatitis suggesting the tumor promoting effect of γδ T cells was specific to PDA. However, γδ T cells failed to enhance proliferation or deregulate expression of oncogenic or tumor suppressor genes in transformed epithelial cells suggesting indirect involvement of γδ T cells on PDA progression.

Daley *et al*. observed that depletion of γδ T cells resulted in pronounced infiltration of activated CD4^{+} and CD8^{+} T cells. The infiltrated αβT cells expressed higher levels of CD44, ICOS, CTLA-4, granzyme B, OX40 and PD-1 in Tcrδ$^{-/-}$ mice. Further, the co-culture studies of γδ T cells with αβT cells showed contact dependent inhibition of activation of CD4^{+} and CD8^{+} T cells highlighting immunosuppressive behaviour of γδ T cells. Signalling through PD-1/PD-L1 induces T cell exhaustion (17). Daley *et al*. have shown that PDA infiltrating γδ T cells expressed elevated levels of PD-L1 and galactin-9 but decreased levels of other activating ligands including B7-2, ICOSL, and OX40L in orthotopic KPC and KC tumors. Blockade of PD-L1 in co-culture experiment of γδ T cells with αβT cells reversed the γδ T cells mediated suppression of CD4^{+} and CD8^{+} T cells. Moreover, blockade of PD-L1 and galectin-9 using neutralizing monoclonal antibodies (mAbs) in wild type mice protected from tumor progression but were ineffective at further inducing tumor protection in KC;Tcrδ$^{-/-}$ animals. Interestingly, it was observed that expression of PD-L1 on MDSCs was not affected and exhibited PD-1/PD-L1 dependent T cell inhibition in KC;Tcrδ$^{-/-}$ mice. However, MDSCs did not promote tumor growth in KC;Tcrδ$^{-/-}$ mice treated with checkpoint inhibitors. Daley *et al*. showed that αβT cells were in proximity to γδ T cells in the PDA. Myeloid cells were separated by great distances from αβT cells, in invasive murine PDA, pre-invasive disease and in human PDA. This suggests enhanced opportunity for γδ T cells to restrain the

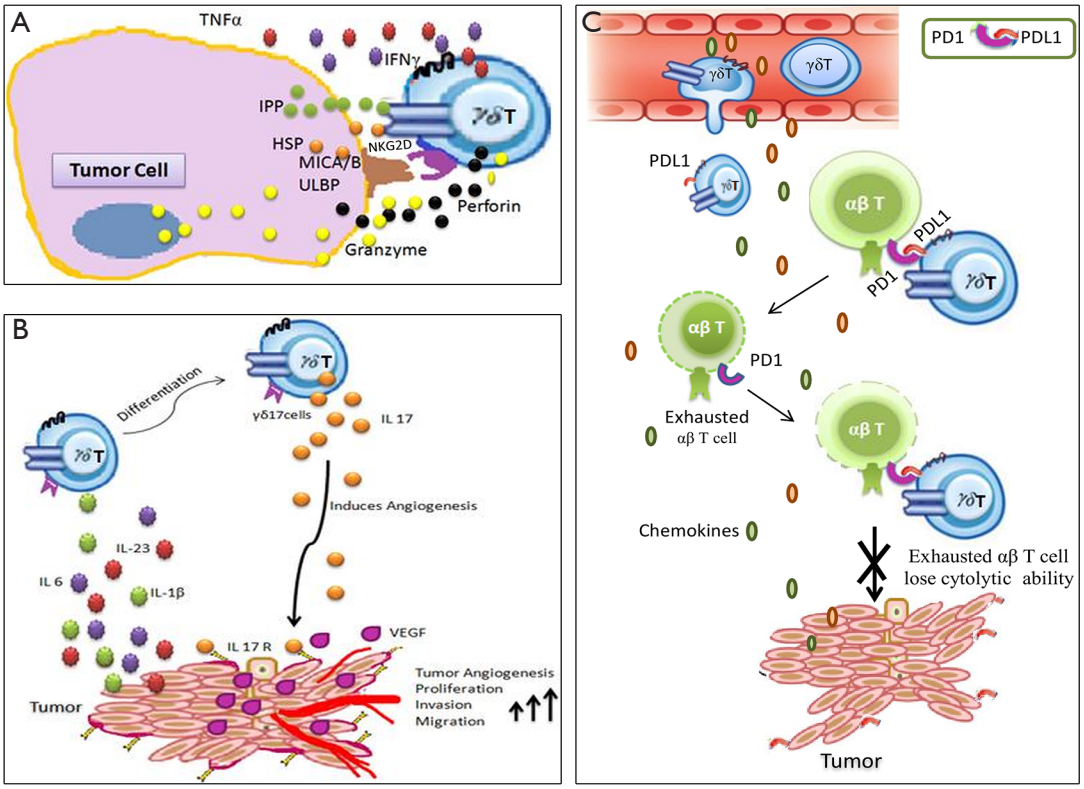

Figure 1 Anti- and pro-tumor role of γδ T cells. (A) Anti-tumor role of γδ T cells: γδ T cells respond to class I-related molecules MICA, MICB, UL16-binding proteins (ULBP), heat shock protein (HSP) that are upregulated on malignant or stressed cells and can recognise non-peptide phosphoantigens-isoprenoid pyrophosphate (IPP) (intermediate of mevalonate pathway). γδ T cells show cytolytic activity against solid and hematologic tumors and produce high amounts of IFNγ, TNFα, perforins and granzymes upon activation; (B) γδ T cells induces tumor angiogenesis: tumor secretes cytokines like IL23, IL1β and IL6 which induces differentiation of γδ T cells to form T γδ17 cells (IL17 secreting γδ T cells). IL17 interacts with IL17R and induces VEGF expression, angiogenesis, invasion, migration and proliferation of the tumor; (C) immunosuppressive role of γδ T cells: γδ T cells are recruited into the tumor following the chemokine signalling. PD-L1 expressing γδ T cells interaction with PD-1 expressing αβT cells induces exhaustion in αβT cells. Thus, γδ T cells help in tumor progression.

αβT cell activation in PDA compared to myeloid cells.

In summary, the present article by Daley *et al.* has provided the molecular mechanism of immunosuppressive behaviour of γδ T cells through PD-1/PD-L1 signalling imposing immune exhaustion of antitumor αβT cells. This study has revealed the suppressive behaviour of γδ T cells which adds new dimensions to the previously reported pro-angiogenic functions of γδ T cells in treatment naive condition (*Figure 1*). The monoclonal antibodies targeting PD-1/PD-L1 are in clinical trials in patients with melanoma, renal cancer, non-small cell lung, bladder and head and neck cancers (18). The present study has provided rationale for therapeutic use of anti-PD-1/PD-L1 antibodies in treatment of patients with PDA. Thus, for achieving therapeutic benefits of γδ T cells in clinical setting it is necessary to fine tune γδ T cells using appropriate blocking antibodies for IL17/IL17R and PD-1/PD-L1 pathway in combination with phosphoantigens and TLR agonists.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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