SUPPRESSION OF HUMAN GAMMA DELTA T CELL ACTIVATION BY SOLUBLE FACTORS PRODUCED BY PANCREATIC DUCTAL CANCER ORGANOPTIC CULTURE


Background: Pancreatic ductal adenocarcinoma (PDAC) is highly resistant to immunotherapies. Gamma delta (gdT) cells play a key role in driving immunosuppression within the tumor microenvironment. In PDAC, the tumor microenvironment is heavily infiltrated with immunosuppressive gdT cells. However, the mechanism by which circulating gd T cells acquire an immunosuppressive phenotype within the tumor microenvironment specific to PDAC remains unknown. We hypothesized that secretory cues produced by PDAC act on infiltrating gd T cells to drive immunosuppressive phenotypes.

Methods: PDAC organoids were generated from surgical specimens obtained with IRB approval (UW-Madison B00000976). Organoids were cultured in defined media in serum-free conditions. Conditioned media was collected at passaging. Whole blood was collected, and samples were enriched for T cells (RosetteSep, Stem Cell Technologies), followed by magnetic gdT cell isolation. gdT cells were cultured in conditioned media from two separate patient-derived organoid lines, versus control base media for 72 hours. Cells were then activated with anti-CD3/CD28/CD2 beads in the presence of GolgiStop cocktail. These cells were subjected to intracellular cytokine phenotyping by flow cytometry.

Results: After 72 hours of incubation in PDAC conditioned media, production of IFN-gamma and TNF-alpha, indicative of a Th1 antitumor phenotype, are significantly diminished (71% IFN-g+ in control vs 48% in PDAC1 media, 56% in PDAC2 media; p<0.002) in conditioned media cultured gdT cells versus control (figure 1B-C). In a separate series of experiments, we evaluated the specificity of this suppression. Interestingly, there is increased suppression of gdT activation relative to either CD4+ or CD8+ ab T cells, with 40% reduction in IFNg producing gdT cells versus 10% reduction in IFNg producing cells amongst CD4 and CD8+ abT cells (preliminary data).

Conclusions: Our work demonstrates for the first time a specific effect of a secreted mediator produced by PDAC that preferentially drives gdT cell immunosuppression. We demonstrate that circulating gdT cells from healthy donors produce significantly less Th1 cytokines after TCR stimulation, following incubation in conditioned media derived from multiple, independent human PDAC organoids. Our next steps include scRNAseq of conditioned gdT cells as well as bulk RNAseq of PDAC organoids to identify ligand-receptor interactions between tumor and gdT cells, aiming to use proteomics approaches to validate and modulate these candidates in the tumor microenvironment. In the broader clinical context, this may suggest a new axis that drives immunosuppression in PDAC, with our work identifying new targets for therapeutic modulation.

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REFERENCES

Ethics Approval: University of Wisconsin-Madison IRB B0000976

Abstract 1443 Figure 1  gdT cell activation is attenuated by pancreas tumor
A. Experimental schema; gdT are isolated from healthy donors, cultured for 72h in PDAC organoid media, activated with CD3/CD28/CD2 beads as described in the Methods section, and subjected to flow cytometry analysis.
B. Representative flow cytometry for gated gdT cells. Right panel= gdT cultured for 72h with PDAC conditioned media, which results in decreased IFNg and TNFa production versus gdT cultured in control media (left).
C. Quantification of flow cytometry evaluating IFNg and TNFa production using two different PDAC organoid conditioned media versus control media (gdT incubation and activation after 72h of culture).

** p<0.02