P09.19 COMPLEX PRIMARY ORGANOID CULTURES TO DISSECT IMMUNOGENIC EFFECTS OF THERAPY ON MACROPHAGES IN A PRECISION MEDICINE-LIKE APPROACH

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Background Primary co-cultures of colorectal cancer (CRC) organoids with various immune components are emerging as models for probing immunological effects of novel and established cancer treatments. Tumor associated macrophages (TAM) play a central role as regulators, directing responses of other immune cell types in the microenvironment. Cancer associated fibroblasts (CAF) were shown to polarize macrophages.[1] Therefore, we aimed to set up a complex primary co-culture assay consisting of primary organoids, CAFs and TAMs illuminating phenotypic and functional changes of TAM under therapy in CRC patients.

Materials and Methods A living biobank of primary CRC organoids and CAF was established. Organotypic co-cultures of monocytes derived from healthy volunteers and organoids were set up in presence and absence of patient matched CAF. Flow-cytometry-based phagocytosis assays were established to asses functional capacity of monocytes to phagocytose CRC organoid cells. Model treatments included oxaliplatin, 5-FU and two oncolytic influenza A virus prototypes.

Results CAF presence was necessary for monocytes to develop a TAM phenotype upon three days of CRC organoid co-culture, defined by macrophage-like motility within the gel matrix and enhanced expression of TAM associated phenotypic markers CD163 and CD206. Treatment of complex organoids with oxaliplatin, 5-FU or oncolytic virus treatment re-polarized macrophages towards a pro-inflammatory phenotype with respect to marker expression. The magnitude of the potential re-programming was patient dependent. Phagocytosis of cancer cells from intact organoids could be modeled upon treatment. Presence of CAF enhanced phagocytosis of cancer cells. Phagocytosis upon oxaliplatin treatment was abrogated when CRC cell death was inhibited, indicating the observed effect consisted of clearance of dead cells. Phagocytosis under viral treatment was not significantly altered by inhibition of cell death, indicating an immunogenic effect.

Conclusions CAF appear necessary to model the TAM phenotype and their responses to treatment in primary-CRC-organoid-based organotypic assays and functional phagocytosis assays. These systems allow to assess response to therapy on the myeloid compartment in primary organoid cultures using a precision medicine approach.

REFERENCES

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P09.20 IMPACT OF TCR INDEPENDENT, KIR-HLA-C INTERACTIONS ON KIR+CD8+ T CELLS IN HEALTH AND DISEASE

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Background While the role of Killer Ig-like receptors (KIRs) as important receptors for antigen recognition and function of Natural killer (NK) cells has long been established, their expression on a subset of CD8+ T cells has only recently been described. In healthy donors KIR+CD8+ T cells make up for 1%-38% of all CD8+ T cells, whereas increased percentages were found in a variety of autoimmune and infectious diseases, generating hypotheses assigning them with an immunoregulatory role. As such they could have a huge impact on CD8+ T cell immunity against oncologic, autoimmune or infectious diseases and the respective treatments aiming at improving or blocking CD8+ T cell function.

Materials and Methods We comprehensively analyzed a cohort of 10 cytomegalovirus (CMV)+ healthy donors for KIR expression on CD8+ T cells and the respective phenotype, specificity and function of these cells using combinatorial coding of pMHC multimers, DNA barcoded pMHC multimer screenings, multi-parametric flow cytometry and functional assays, comparing CMV-specific HLA-A and -B restricted CD8+ T cell responses with HLA-C restricted CD8+KIR+ T cell responses. We further aim to compare these findings to cancer-specific CD8+ T cell responses in a cohort of 10 melanoma patients.

Results We have demonstrated that KIR receptors can bind certain subgroups of peptide-MHC molecules. It seems to particularly be the case among peptide HLA-C complexes. We observe that certain pHLA-C multimers can bind and stain T cells via the KIR receptor. This follows the observations that HLA-C restricted CD8+ T cell responses are enriched in PBMCs from cohorts of melanoma, NSCLC, MDS, RCC and bladder cancer patients. We are currently further evaluating the role of KIR-HLA-C interaction in cancer-specific T cell responses. We found that KIR+CD8+ T cells resemble a distinct CD8+ T cell population and binding of pHLA-C multimers could be prevented by prior blocking of KIR receptors with antibodies, confirming TCR independent, KIR-mediated multimer binding. Interestingly, not all pHLA-C complexes will bind KIR receptors, and hence HLA-C binding to KIR seemed to be partially dependent on the specificity of the bound peptide.

Conclusions Here we show that KIR+CD8+ T cells are a distinct cell population identified by TCR independent HLA-C-KIR interaction. Analyses of specificity, phenotype and functionality of this cell population, elevated in the context of autoimmune, infectious and oncologic diseases, will enable us to assess their role in CD8+ T cell immunity.