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In Vitro Characterization of Antibodies Targeting the SIRPα–CD47 Phagocytosis Checkpoint using Tumor-Associated Macrophages from HuGEMM™ Mice

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Abstract No. 502

INTRODUCTION

The signal-regulatory protein SIRPα-CD47 pathway is a phagocytosis checkpoint for macrophages and other innate immune cells. Tumor cells ubiquitously express CD47 and blocking SIRPα-CD47 interactions has been shown to promote cancer cell elimination by macrophage phagocytosis. The impact of SIRPα–CD47 blockade may be further improved by targeting prophagocytic receptors (Fc receptors) to enhance phagocytosis for anti-CD47 antibodies. Tumor-associated macrophages (TAMs) are the most abundant innate immune cells in late-stage tumors, though availability of TAMs from patients is limited. Currently, drug development targeting SIRPα-CD47 is rapidly growing, requiring robust *in vitro* macrophage/tumor cell co-culture assays to evaluate new agents targeting this pathway. Here, we report on the use of macrophages from an engineered mouse model expressing human SIRPα, which may serve as a good tool to test drug candidates.

METHODS

- **Generation of tumor-bearing SIRPα-CD47 HuGEMM™ mice:** Human CD47 and SIRPα double knock-in mouse model (CD47/SIRPα HuGEMM) was generated by CRISPR/Cas9 targeting. Mouse syngeneic MC38 cells were generated to express human CD47 (MC38-hCD47 HuCELL™). MC38-hCD47 HuCELL cells were inoculated into CD47/SIRPα HuGEMM mice to develop tumors
- **Isolation of tumor-associated macrophages (TAMs) from tumor-bearing CD47/SIRPα HuGEMM mice:** 1050mm^3 MC38/hCD47 tumors were enzymatically digested into single cells and tumor-infiltrating leukocytes were isolated by Percoll gradient. Macrophages were then enriched with Anti-F4/80 MicroBeads UltraPure from Miltenyi Biotec
- **Generation of bone-marrow derived macrophages (BMDMs) from tumor-bearing CD47/SIRPα HuGEMM mice:** Mouse bone marrow (BM) cells were harvested and cultured in the presence of murine macrophage colony-stimulating factor (M-CSF) for 9 days. Differentiated macrophages were harvested for subsequent functional assays
- **Antibodies:** Anti-human CD47 IgG4 antibody Hu5F9 and isotype control IgG4 antibody were used for *in vitro* assays
- **Phagocytosis assay[1]:** TAMs or BMDMs were used as effector cells. Murine MC38-hCD47 HuCELL or human Jurkat cells were used as target cells expressing human CD47. Target cells were labeled with carboxyfluorescein succinimidyl ester (CFSE). Macrophages were co-cultured at an E:T ratio of 1:3 or 1:5, for 3 or 16 hours at 37°C in the presence of antibodies. To block macrophage FCR function, macrophages were pretreated with mouse IgG2a prior to addition of target cells. Flow cytometry was performed, followed by doublet exclusion analysis and phagocytosis index was reported as a percentage of F4/80$^+$ macrophages that were CFSE$^+$
- **Tumor cell killing measurement:** CFSE$^+$ tumor cells were stained for live/dead dye and cell death was identified by flow cytometry

REFERENCES

1. Peluso *et al.* The Fully human anti-CD47 antibody SRF231 exerts dual-mechanism antitumor activity via engagement of the activating receptor CD32a. J Immunother Cancer 2020; 8(1): e000413.

RESULTS

Fig 1. Phagocytosis and killing of murine MC38-hCD47 HuCELL tumor cells by TAMs from CD47/SIRPα HuGEMM mice. Macrophages isolated from MC38-hCD47 HuCELL tumors from CD47/SIRPα HuGEMM mice were cocultured with MC38-hCD47 HuCELL tumor cells at E:T ratios of 1:3 or 1:5 for 16 hours. **(A)** Flow cytometry gating strategy. Live singlets were gated for F4/80$^+$ macrophages and then macrophages were analyzed for the % of CFSE$^+$ cells; **(B)** Phagocytosis index following antibody treatments. Anti-CD47 Hu5F9 antibody induced CD47-dependent antibody mediated cellular phagocytosis (ADCP) of tumor cells by TAM; **(C)** After 16 hr coculture, anti-CD47 Hu5F9 antibody increased CD47-dependent tumor cell killing at E:T ratio of 1:3, but not 1:5

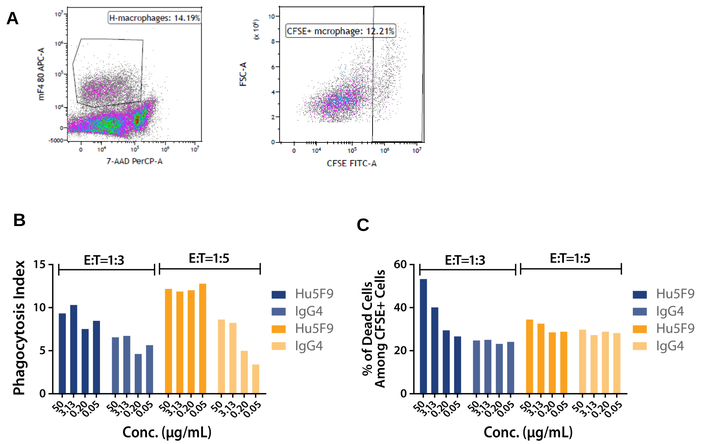


Fig 2. Phagocytosis and killing of murine MC38-hCD47 HuCELL tumor cells by BMDM from CD47/SIRPα HuGEMM mice. *In vitro* differentiated BMDM were cocultured with MC38-hCD47 HuCELL tumor cells at E:T ratios of 1:3 or 1:5 for 3 hours. **(A)** Anti-CD47 Hu5F9 antibody mediates the phagocytosis of tumor cells by BMDM; **(B)** After 3 hours coculture, anti-CD47 Hu5F9 antibody did not induce CD47-dependent tumor cell killing by BMDM

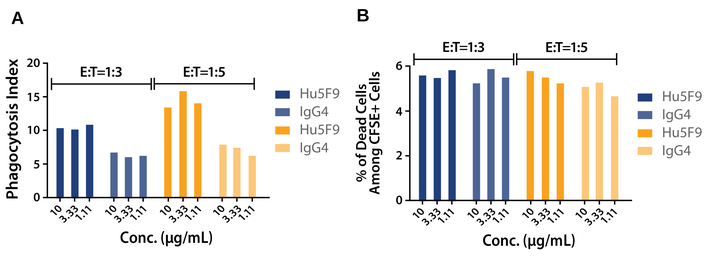


Fig 3. Phagocytosis and killing of Jurkat cells by TAMs from CD47/SIRPα HuGEMM mice. (A) Macrophages isolated from tumors were cocultured with human CD47-positive Jurkat cells at an E:T ratio of 1:3 for 3 hours (top) or 16 hours (bottom). Saturated mouse IgG2a antibody was used to block FCR function on macrophages. Anti-CD47 Hu5F9 antibody mediates CD47-dependent phagocytosis of tumor cells by TAM. By blocking FC function of Hu5F9 antibody, anti-CD47 Hu5F9 antibody still increased the phagocytosis function of TAMs via CD47-SIRPα pathway blockade; **(B)** After 3 hours (top) or 16 hours (bottom) coculture, tumor cell killing was analyzed. Anti-CD47 Hu5F9 antibody induced CD47-dependent tumor killing in the 3 hour coculture. By blocking FC function of Hu5F9 antibody, Hu5F9 antibody still increased tumor cell killing by TAMs due to blocking the CD47-SIRPα pathway

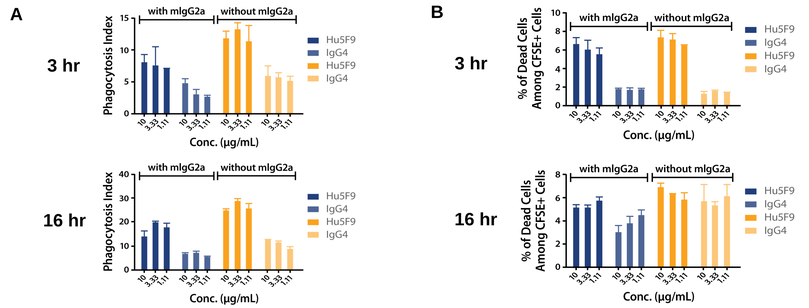
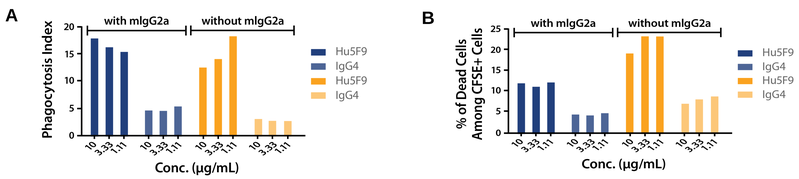


Fig 4. Phagocytosis and killing of Jurkat cells by BMDM from CD47/SIRPα HuGEMM mice. (A) *In vitro* differentiated BMDM were cocultured with Jurkat cells at an E:T ratio of 1:3 for 3 hours. Anti-CD47 Hu5F9 antibody mediates the phagocytosis of tumor cells by BMDM. By blocking FC function of Hu5F9 antibody, anti-CD47 Hu5F9 antibody still increased the phagocytosis function of BMDM, via CD47-SIRPα pathway blockade; **(B)** Anti-CD47 Hu5F9 antibody induced CD47-dependent tumor killing in the 3 hour coculture. By blocking FC function of Hu5F9 antibody, anti-CD47 Hu5F9 antibody still increased tumor cell killing by BMDM due to blocking CD47-SIRPα pathway



SUMMARY

- Murine TAMs and BMDMs from CD47/SIRPα HuGEMM mice can be used to evaluate the effect of anti-human CD47/SIRPα antibodies on phagocytosis and tumor cell killing
- Both murine tumor cells engineered to express human CD47 and human tumor cell lines expressing CD47 can be used as target cells to evaluate *in vitro* ADCP function of anti-human CD47/SIPRα antibodies using CD47/SIRPα HuGEMM macrophages as effector cells
- CD47/SIRPα HuGEMM TAMs can potentially be a powerful tool to test the effect of drugs on tumor-derived macrophages to overcome limitations in the availability of TAMs from patients
- In addition, *in vitro* function assays using CD47/SIRPα HuGEMM macrophages may provide proof of concept for subsequent *in vivo* efficacy testing in CD47/SIRPα HuGEMM mice