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IMPACT OF PSMA ANTIBODY OR CHIMERIC ANTIGEN RECEPTOR ON PHAGOCYTOSIS AND TUMOR LOCALIZATION BY WILD-TYPE AND NF-κB P50-DEFICIENT MACROPHAGES

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Background Absence of the repressive NF-κB p50 transcription factor subunit increases expression of pro-inflammatory M1 genes and reduces expression of M2 genes in myeloid cells. Adoptive transfer of immature myeloid cells lacking p50 (p50-IMC) slows the growth of syngeneic murine prostate cancer, pancreatic ductal carcinoma, or neuroblastoma when given after a dose of myelo-depleting 5-fluorouracil.1 2 p50-IMC develop into tumor macrophages and dendritic cells and induce increased total and activated tumor T cells. Depletion of T cells eliminates p50-IMC efficacy. We now evaluate whether we can enhance wild-type (WT) or p50-IMC tumor localization and/or the phagocytosis of PSMA+ prostate cancer cells by expressing a prostate-specific membrane antigen (PSMA)-specific chimeric antigen receptor (CAR) on IMC, or by combining IMC with PSMA antibody (Ab), which binds to the surface of myeloid cells via their Fc receptors.

Methods For phagocytosis assays, bone marrow-derived macrophages (BMM) from WT or p50(-/-) mice were stimulated with IFNγ or IL-4 and combined with PSMA Ab or IgG control, or transduced with empty vector (EV) or PSMA.CAR. These macrophages were incubated with CFSE-labeled parental or PSMA-expressing MyC-CaP cells, followed by flow cytometry for CFSE and CD11b. For tumor localization experiments, WT-IMC or p50-IMC were combined with PSMA Ab or IgG, or transduced with PSMA.CAR or EV, then CFSE-labeled and injected into mice bearing subcutaneous MyC-CaP/PSMA tumors. Tumors were analyzed for CFSE+CD11b+ cells ~ 20 hours later. Phagocytosis was confirmed using CFSE-labelled macrophages and pHrodo-Red-labeled MyC-CaP/PSMA cells.

Results PSMA Ab increased phagocytosis of PSMA+ MyC-CaP cells by WT or p50(-/-) BMM to an average of 14% in IFNγ and to 2.7% in IL-4, compared with 1.5% and 0.9% in IgG controls. PSMA.CAR expression increased phagocytosis to 22% in IFNγ and 11% in IL-4, compared to 1.4% and 1.9% in EV controls (n=3). PSMA Ab increased p50-IMC localization to PSMA+ MyC-CaP tumors 4-fold, on average (n=7, p= 0.03), whereas PSMA CAR was ineffective (n=6).

Conclusions Absence of NF-κB p50 does not enhance or impair low-level, basal phagocytosis of PSMA-expressing prostate cancer cells. Both PSMA Ab or CAR expression increased phagocytosis, with CAR being more effective, which could favor tumor antigen cross-presentation. PSMA Ab, but not CAR, increased p50-IMC tumor localization, although CARs might be effective in other models. Overall, these data indicate that combining p50-IMC with a tumor-specific Ab or CAR has the potential to increase the anti-tumor efficacy already observed with p50-IMC alone.

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REFERENCES

Ethics Approval Work with laboratory animals was improved by the John Hopkins University Animal Care and Use Committee (protocol MO21M390).