Nitric Oxide Metabolic Pathways in Tumor Microenvironment Induced Macrophage Phenotypic Response

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Macrophage cells can be activated along a phenotypic spectrum from M1 (acute cytotoxic) to M2 (alternate reparative). M1 macrophages are tumoricidal, while M2 are tumor supportive and can be referred to as Tumor Associated Macrophages (TAMs). Tumor cells release molecules which can change macrophage phenotype and promote TAM formation. Arginine metabolism, either via nitric oxide production or ornithine and urea formation can be used to determine macrophage phenotype. M2 macrophages primarily consume arginine in the arginase pathway, while M1 macrophages produce nitric oxide via inducible nitric oxide synthase (iNOS).
We hypothesize that the tumor cells will induce the phenotypic change of macrophages to M2 and alter nitric oxide metabolism when those macrophages are exposed to lipopolysaccharide (LPS) challenge. Raw-Blue cells are an NF-κβ reporter murine macrophage cell line which expresses secreted embryonic alkaline phosphatase (SEAP) when stimulated by LPS. Raw-blue cells were co-cultured with Lewis lung carcinoma (LLC) cells for 96 hours. They were then incubated with or without 100ng/mL LPS. Griess assay was performed to determine nitrite levels as a marker of nitric oxide production, and SEAP assay to determine NF-κβ levels. Expression levels of Arginase and iNOS proteins were determined by western blot, using GAPDH as a control protein. The cells were stained to detect the F4/80 surface marker, then analyzed by flow cytometry to determine macrophage phenotype. The Griess assay shows a 9-fold increase in nitrite levels in the LPS stimulated cells from the control level of 2.25 (s.d. 0.33) μmoles/mL to 20.10 (s.d. 4.24) μmoles/mL, but no such increase when co-cultured with LLC cells from an unstimulated level of 1.14μmoles/mL to 1.00μmoles/mL when stimulated. The SEAP assay shows a 12-fold increase in SEAP release, thus an increase in NF-κβ activity with LPS stimulation, but no increase from stimulation in the LLC co-culture. The Western blot shows that co-culture induced Arginase expression and LPS fails to induce iNOS expression during the co-culture. Raw-blue cells expressed increased levels of F4/80 with LPS treatment, indicating that LPS induced M1 macrophage phenotype, but when co-cultured with LLC cells, LPS treatment did not increase F4/80 expression. The results confirm that tumor cells induce the change of M1 macrophages to tumor promoting M2 phenotype, which can be detected by assaying for iNOS and Arginase activity. Supported by NIH R25ES020721 and P30ES005022.