The Regulatory Role of Folate Receptor Beta on Inflammasome Activation

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Background and Hypothesis: Group B *Streptococcus* (GBS) causes intrauterine infection during pregnancy. The inflammatory response of macrophages at the maternal-fetal interface to GBS may contribute to host defense. The NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome regulates macrophage responses, activated by microbial patterns and danger signals, leading to caspase-1-driven release of proinflammatory cytokines like IL-1β and cleavage of gasdermin D (GasD), which induces pyroptotic cell death. Folate receptor beta (FRβ), encoded by the *FOLR2* gene, is a GPI-anchored glycoprotein that is highly expressed by macrophages, but its function is unknown. We hypothesize that FRβ regulates NLRP3 activation.

Method of Study: The human monocytic cell line THP-1 was CRISPR/Cas9-modified to delete the *FOLR2* gene (knock-out, KO) or mock-transfected for wild-type (WT) control cells. WT and *FOLR2* KO macrophages were cultured in a physiologic amount of folate (25 nM) for 7d before being treated with phorbol 12-myristate 13-acetate (PMA) to induce macrophage differentiation. WT and *FOLR2* KO macrophages were stimulated for 4h with either GBS or FSL-1 (toll-like receptor 2/6 agonist); or for 3h with lipopolysaccharide (LPS) + 30m ATP (LPS+ATP), or LPS + 30m Nigericin (LPS+Nig). Supernatants were analyzed for IL-1β, caspase-1, and cleaved GasD release by ELISA, while lysates were analyzed for IL-1β, caspase-1, and cleaved GasD by Western Blot.

Results: FRβ KO macrophages showed a reduced ability to secrete IL-1β after FSL-1 or LPS+Nig stimulation *vs* WT macrophages, which was not observed in the cellular lysate. Also observed was a significantly reduced ratio of cleaved GasD in FRβ KO macrophages stimulated with LPS+Nig compared to WT macrophages, not seen in the cell supernatant. There was not an observed significant difference between FRβ KO and WT macrophages in the amount of caspase-1 both inside and outside the cell. Both WT and FRβ KO macrophages were weakly, and equally, stimulated with GBS or LPS+ATP.

Conclusions: FRβ KO THP-1 cells exhibit a reduced NLRP3-dependent inflammatory response to stimulation, suggesting a proinflammatory regulatory role for FRβ in immune surveillance.