00212 Intrinsic Migration Differences Between Macrophage Phenotypes Across Mesothelium in Vitro and Application to Endometriosis Pathophysiology

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Aims: Endometriosis, a gynaecological disorder affecting up to 16% of women, is characterized by endometrial-like implants outside the pelvic cavity, resulting in chronic peritoneal inflammation and infertility. This is associated with elevated numbers of activated macrophages, postulated to contribute to endometriosis establishment and progression. Macrophages are highly plastic, leaning toward M1 or M2 functional phenotypes in response to environmental stimuli, driving inflammation propagation or resolution. One mechanism is macrophage egress across the mesothelium during inflammation resolution. Thus, we hypothesized more M1 than M2 macrophages are retained in the peritoneum. This pilot study investigates the intrinsic migration differences of macrophage phenotypes across mesothelial cells in vitro.

Methodology: THP-1 monocytes were differentiated to M0 macrophages and polarized to M1 and M2 phenotypes. For phenotype characterization and validation, surface markers were analyzed by flow cytometry, and secreted cytokines by multiplex ELISA. Concentrations of sphingolipids were analyzed by mass spectrometry. Transwell migration assay was run to determine macrophage movement across a mesothelial cell (MeT-5A) membrane. Migrated cells were counted, averaged, and expressed as ratios of M1:M0, M2:M0 and M1:M2.

Result: Flow cytometry established M1 macrophages as CD80 and HLA-DR positive and ELISA analysis found they secreted established cytokines IL-1α, IL-1β, TGF-α, TNF-β and IL-12p70, and 9 other novel cytokines. Additionally, 4 sphingolipids' concentrations were higher in M1 than M2 macrophages. M2 macrophages secreted higher IL-10 (30.30-fold), VEGF-A (1.52-fold) and IL-18 (5.88-fold) concentrations than M1. By transwell migration assay, M2 macrophages migrated 2.9-folds more than M1 across the mesothelial cells in vitro, with significant difference between migrated M2:M0 vs. M1:M0 ($p<0.05$).

Conclusion: These results suggest M1 macrophages are more sessile, have higher retention in the peritoneum, and consequentially contributes to the altered peritoneal environment in endometriosis. Potentially, this discerns M1 macrophages and its

associated factors as potential targets for attenuating chronic peritoneal inflammation in endometriosis.