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"An *In Vitro* Model to Test the effects of Tryptophan Metabolites on *Chlamydia trachomatis* development"

Chlamydia trachomatis (Ct) is an obligate intracellular bacterium that is a common cause of genitourinary tract infections. Women with bacterial vaginosis (BV), a dysbiosis of the vaginal microbiota, have an elevated rate of Ct infection. Ct is a tryptophan auxotroph, but BV associated bacteria utilize and significantly lower vaginal tryptophan, produce an array of tryptophan catabolites and induce proinflammatory cytokines that can induce the tryptophan-degrading enzyme IDO1. In vitro assays have demonstrated that genital Ct strains have mechanisms to counteract this stress, but if, and how Ct development is modulated under specific BV-mediated environmental conditions remains unknown.

The present study aimed at developing a new in vitro model that would enable us to determine if, and at which time points, tryptophan metabolites could modulate the Ct developmental cycle. We first created a dual promoter reporter Ct strain, in which an early promoter was linked to the mCherry fluorescence protein and a late promoter to the green fluorescence protein GFP. Next, HeLa cells were infected with this reporter strain and exposed to tryptophan catabolites at a range of concentrations found in optimal and BV-like conditions. Untreated Ct infected cells served as a control. Live-cell imaging was undertaken at 24- and 42-hours post infection (h pi) and the mean fluorescence intensity (MFI) of mCherry and GFP for individual Ct inclusions were measured. The MFI ratio of late promoter-driven GFP to early promoter-driven mCherry (GFP:mCherry) in each inclusion was used to assess the spectrum of Ct development in HeLa cells. Additionally, a CCK8 assay was performed to exclude any potential host cell cytotoxicity.

We observed two groups of metabolites that had different consequences on Ct development. The first group significantly decreased GFP:mCherry at 42 h pi, despite no change at 24-h pi compared with the untreated control. The second group significantly increased GFP:mCherry at 24 h pi, however, decreased GFP/mCherry at 42 h pi. These results suggest that, depending on the compound and testing condition, some metabolites may stimulate Ct development, resulting in earlier expression of the late promoter (increased GFP:mCherry at 24 h pi). The other compounds may promote formation of secondary inclusions which is typified as the activation of the early, but not the late, promoter (decrease in GFP:mCherry at 42 h pi).

The methodology we established, and preliminary results generated, indicate the need for further investigation into the complex interactions between Chlamydia, tryptophan metabolites, genital microbiota and host cells. The system we established may also be useful for screening small molecule activity against Chlamydia.