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Investigating the complement-independent effects of C4BP on Gc pathogenesis

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Background

We recently reported that Gc binds human complement C4b-binding protein (C4BP) to resist killing by neutrophils in a complement-independent manner. C4BP interferes with phagocytosis of Gc that express CEACAM-binding Opa proteins. However, the fate of C4BP-bound Gc that is phagocytosed, as well as other contributions of C4BP to neutrophil activation and bacterial resistance to neutrophil antimicrobial products, has yet to be uncovered. Since C4BP is present in serum secretions at inflamed mucosal surfaces, it may also affect how Gc interacts with human epithelial cells. We seek to fully elucidate the role of C4BP binding in interactions of Gc with physiologically relevant human cells.

Aim/Methods

To determine the fate of C4BP that is bound to phagocytosed Gc, primary human neutrophils were exposed to C4BP-bound OpaD+ Gc of strain FA1090. At various times, intracellular and extracellular Gc were differentially stained using a bacteria-specific antibody with and without permeabilization. C4BP was recognized with a specific antibody and samples were analyzed by fluorescence microscopy. To determine how C4BP affects Gc susceptibility to neutrophil proteases, OpaD+ Gc with and without C4BP bound were incubated with purified neutrophil proteases (neutrophil elastase and cathepsin G), and Gc colony forming units were enumerated over time to measure bacterial survival. To test how C4BP affects Gc resistance to proteases during neutrophil infection, protease inhibitors were added to neutrophils prior to adding OpaD+ Gc with and without C4BP. C4BP fluorescence on Gc was measured by fluorescence microscopy as above, and Gc viability by live/dead fluorescence assay.

Results

C4BP-bound Gc that are phagocytosed by neutrophils lose C4BP fluorescence over time, while bacteria outside of the neutrophil remain C4BP-positive. Preliminary data suggests that C4BP binding does not change susceptibility of Gc to LL-37 cathelicidin. Similar studies are ongoing for neutrophil proteases.

Conclusions

C4BP may be degraded at the surface of Gc in neutrophil phagosomes; how this contributes to Gc survival within neutrophils is being examined. Going forward, we will use CHO cells expressing CEACAMs to determine if C4BP binding affects Opa-CEACAM interactions at cell surfaces, and human endocervical cells to examine how C4BP binding affects Gc colonization and inflammatory signaling.