Flow cytometric applications for bacterial pathogenesis

Alison Criss
Department of Microbiology, Immunology, and Cancer Biology, University of Virginia, Charlottesville, VA, United States

In order to survive and be transmitted, pathogenic microorganisms must gain access to target host tissues, establish infection of those sites, and overcome local and systemic immune defenses. A preeminent example of a highly successful pathogen of humans is *Neisseria gonorrhoeae,* the cause of gonorrhea. *N. gonorrhoeae* colonizes mucosal epithelial surfaces via phase-variable outer membrane proteins that bind human-specific receptors. Infection stimulates a potent innate immune response characterized by the local recruitment of neutrophils. Despite their abundance and numerous antimicrobial properties, neutrophils are unable to clear *N. gonorrhoeae* during human infection. Consequently, infection is not resolved and neutrophilic inflammation is sustained, leading to tissue damage that underlies pelvic inflammatory disease, sterility, ectopic pregnancy, and other debilitating health repercussions. The focus of the Criss laboratory is to define the mechanisms used by *N. gonorrhoeae* to recruit neutrophils to mucosal sites yet resist killing by them. For these studies, we use *ex vivo* model systems with primary human neutrophils, polarized epithelial cells from tissues that are naturally infected with *N. gonorrhoeae,* and infectious bacteria. We have discovered that infection by *N. gonorrhoeae* coordinates neutrophil trans-epithelial migration, allowing the neutrophils to interact with bacteria on the apical aspect of the epithelium. To investigate *N. gonorrhoeae* interactions with host cells ex vivo, we combined an immunofluorescence assay based on differential staining of internalized and surface-bound bacteria with the high throughput multi-parameter data acquisition and image analysis afforded by imaging flow cytometry to quantify bacterial binding and phagocytosis by host cells. This approach and others demonstrate that depending on the outer membrane protein repertoire, *N. gonorrhoeae* interacts with selected receptors on neutrophils that traffic the bacteria into different subcellular compartments. One receptor drives *N. gonorrhoeae* into degradative phagolysosomes that have antibacterial activity, while the other allows the bacteria to reside in an immature compartment in which they survive. We have also developed imaging flow cytometry protocols using purified receptor domains to define the receptor binding profiles of outer membrane protein variants of *N. gonorrhoeae,* and are using this information to test hypotheses about bacterial-host receptor interactions, neutrophil and epithelial cell functionality, and the long-term impact of bacterial-neutrophil interactions on *Neisserial* pathogenesis. Our studies provide crucial insights into how *N. gonorrhoeae* causes disease in its obligate human host, with the potential to identify new therapeutic targets in the host or pathogen to prevent infection and its negative consequences for human health.