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Understanding the Innate Immune System with Designer Jackets and Synthetic Treads

The bacterial cell wall, a polymer of carbohydrate and peptides, makes an excellent antibiotic target for two reasons: (1) it is essential for bacteria and (2) humans do not have bacterial cell walls – thus the drugs do not harm human cells. In addition to serving as a target for antibiotics, the human innate immune system uses the bacterial cell wall as a molecular calling card to recognize their presence and subsequently generate the appropriate immune response. We are interested in understanding how the bacterial cell wall is processed both by bacteria and the human host and propose new methods and tools for the characterization of this important polymer. Both commensal and pathogenic bacteria are believed to produce peptidoglycan fragments and misrecognition can lead to the development of inflammatory bowel disease (IBD), such as Crohn’s disease (CD), asthma and gastrointestinal (GI) cancers. Importantly, a long-standing debate around the biological relevance of the immunoactive synthetic fragment muramyl dipeptide (MDP) remains unclear due to a lack of NAM-based probes. We hypothesize that there are unidentified enzymatic targets and bacterial cell wall fragments that will be useful in the design of novel antibiotics and anti-inflammatory therapies.

We have taken a two-pronged approach towards testing this hypothesis. From the small molecule side, we have established an in vitro assay, which allows us to assess the affinity of Nod2, an innate immune receptor that binds to bacterial cell wall fragments. This assay has allowed us to tease apart binding from activation and we have begun to derive rules for molecular recognition by intracellular innate immune receptors. From the larger polymer side, we have embedded carbohydrates with small modifiable tags into the bacterial cell wall. We developed a method to label the NAM glycan backbone of *E. coli, P. putida, and B. subtillis* in whole cells. The results reveal fundamental architectural details of the glycan chains of the peptidoglycan, and further enable us to track the engulfment and breakdown of bacteria by macrophages, ultimately revealing a peptidoglycan digestion mechanism for invasive bacteria. Finally, we have combined the approached to produce peptidoglycan arrays to probe the involvement of the adaptive immune system in sensing these bacterial walls.

The field of bacterial activation of the innate immune system is limited to a few select synthetic tools derived from bacterial peptidoglycan. The bacteria are believed to communicate to the human cell through the use of specific carbohydrate polymers. The synthesis of these polymers is extremely demanding and thus the probes are scarce. My research laboratory has focused on developing a combination of chemical and enzymatic synthesis to prepare bacterial cell wall fragments from a number of different types of bacteria. These fragments are then used to investigate how different strains of bacteria initiate an immune response. Here we will discuss our progress in using these tools to probe innate immune recognition pathways.