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Grant Title: Boosting immune response against *Bordetella bronchiseptica* EPS facilitates clearance.

Abstract

Many pathogenic bacteria produce exopolysaccharide (EPS) as a shield to avoid host recognitions. Early work have shown that *Bordetella bronchiseptica* (*Bb*) EPS is important for efficient transmission between hosts. Here we show that two unique activated sugars, UDP-N-acetyl-glucosaminouronate (UDP-GlcNAcA) and UDP-N-acetyl-galactosaminouronate (UDP-GalNAcA), are involved in EPS biosynthesis. When mice are inoculated with *Bb* wild type they produced high level of IgM against EPS while *Bb* EPS mutants lack anti-EPS IgM, suggesting a faster clearance of *Bb* wild type than *Bb* EPS mutant. TLR4 mutant mice cleared both *Bb* wild type and *Bb* EPS mutant similarly, suggesting that TLR4 is involved in EPS-targeting clearance.

Objectives:

1- To determine function of selective genes involved in EPS biosynthesis;

2- To initiate and characterize EPS chemical structure; and

3- Use mice model to determine the biological role the EPS.

Methods:

- Gene encoding specific EPS enzyme were cloned and expressed in E.coli. Recombinant enzymes were purified and used for enzyme assay. Enzyme's products analyses were performed by mass spectrometry and by proton NMR.
- EPS was isolated from bacteria culture grown under different conditions and defined media. The EPS was isolated by chemical fractionation followed by ion exchange. The EPS was was proven hard to hydrolyzed and therefore subjected to various hydrolysis condition and derivatization prior being detected by GC-MS and by HPLC-PAD detection.
- Mice were infected with known amounts of bacteria (wild type and mutant) and samples were analyzed t different day post infection.

Results:

Two *Bordetella* genes bb24 and bb25 were shown to encode enzymes that sequentially convert UDP-N-acetyl-glucosamine (UDP-GlcNAc) to UDP-GlcNAcA and then 4-epimerization to UDP-GalNacA. The products of both enzymes confirmed by LC-MS, GC-MS and by NMR. These rare highly charged amino-uronic acids suggest an EPS strauctur that will resist chemical hydrolysis. Different hydrolyses condition were tested. To further evaluate EPS role, Bordetella mutats lacking the entire EPS operon or lacking gene expressing a protein predicted to be involved in EPS transloaction across membranes were generated. These bacterial wild type and mutants were used to intranasally inoculate mice and mice were subsequently exaimined.Polysaccharides are usually not very immunogenic. In order to trigger anti-EPS immune response, mice were inoculated with large amounts of *Bb* wild type or Δ*tEPS* mutant (negative control). Periodically post inoculation, bacteria colonization level was determined and mice serum were collected to test for antibody response. Compared to Δ*tEPS*, RB50 was found to trigger higher total IgM level at day 7 and day 14 post inoculation. Serum from RB50 infected mice were found to be cross-reactive to *Salmonella typhi* Vi antigen, which is also composed of GalNAcA, while serum from Δ*tEPS* infected mice were negative to Vi, proposing that anti-EPS IgM is present in serum of RB50-infected mice. Further studies will revealed the fine chemical structure of *Bb* EPS and evaluated the contribution of anti-EPS immune response during clearance of *Bb* from respiratory tract.