Clostridium difficile Surface Polysaccharide-KLH Conjugate Vaccine Induced Th17-Featured Adaptive Immune Responses in Mice

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Abstract

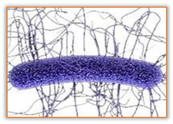
***Clostridium difficile* ("*C. difficile*")** is a Gram-positive, anaerobic, spore-forming, toxin-producing bacterium. It is the leading cause of antibiotics associated diarrhea. *C. difficile* infection related morbidity and mortality has been increasing in recent decades. As *C. difficile* is closely related to disturbed gut microbial flora caused by over usage of antibiotics, immunotherapy vaccines are being developed as an antibiotic-independent therapy. Toxin-based vaccine developed so far can reduce symptoms but cannot prevent recurrent infection, therefore, *C. difficile* surface layer proteins and various cell surface proteins are being targeted for blocking spore adhesion and vegetative bacteria colonization to prevent recurrent infection. As most of these proteins are heavily glycosylated, at Stellar Biotech, we explored the potential of *C. difficile* cell surface polysaccharides as potential immunotherapy vaccine candidate.

We challenged and vaccinated C57BL/6 mice with spores from non-virulent strain (ATCC 43255) and hyper virulent strains (ribotypes 106 and 027) as well as with vaccine prepared by conjugating 43255-derived polysaccharides to **Keyhole Limpet Hemocyanin (KLH)**, respectively. CD4+ T cells from the vaccinated mice were reacted *in vitro* with mitomycin-treated dendritic cells that were pretreated with 43255-derived polysaccharides, polysaccharide-BSA conjugate, and KLH. Cytokines released from the stimulated CD4+ T cells were profiled. All major Th1, Th2 and Th17 cytokines were studied. For the first time to our knowledge, we report here that (1) spores and vaccines prepared with polysaccharides from *C. difficile* induced strain-specific T cell-dependent immune responses; (2) Th17 responses were the dominant T cell responses; (3) polysaccharide-KLH conjugate, instead of polysaccharides alone, induced the generation of Th17 memory cells; (4) polysaccharides from different strains are different, as evidenced by the fact that the vaccines prepared with non-virulent strain 43255 could not protect infection caused by hyper virulent strains 106 and 027.

About Clostridium difficile

Clostridium difficile:

- Spore-forming, anaerobic, Gram-positive bacillus
- Causes diarrhea and colitis
- Responsible for antibiotics (e.g. clindamycin, penicillin and cephalosporin) overuse related diarrhea and colitis



C. difficile
Source: CDC

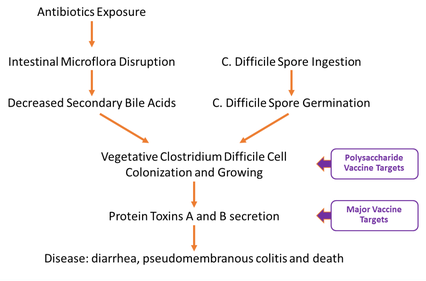
Clostridium difficile infection:

- Induced by disturbance of the normal flora of the colon
- Most common cause of acute infectious diarrhea in nursing homes and hospitals
- Diarrhea and related inflammation is believed to be mainly caused by two toxins (toxin A and B) secreted by vegetative bacteria
- Emergence of hypervirulent strains with reduced clinical responses and increased recurrence

Patient Outcomes (data from CDC 2011 study in USA):

- 500,000 infections per year
- 29,000 death within 30 days of initial diagnosis per year
- $4,800, 000, 000 in excess health care costs per year

Pathogenesis of *Clostridium difficile* Infection



Rationale, Methods and Results

Rationale

- Clostridium difficile spores and vegetative cells are heavily coated with polysaccharides
- Polysacchararides are utilized by vegetative C. difficile cells in colonization, which is essential for vegetative C. difficile cell population to expand
- Only vegetative C. difficile cells produce disease-causing toxins A and B

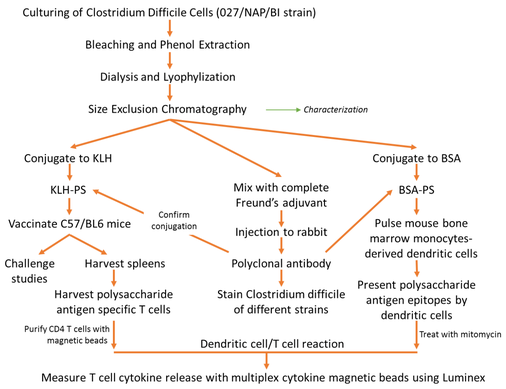
Goal

- Develop polysaccharide-targeting immunotherapy vaccine to block C. difficile spore adherence to intestine membrane and C. difficile vegetative cell colonization

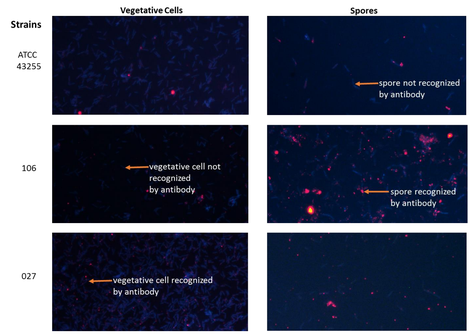
Twenty-five T Helper 17 (Th17) Cell-Related Cytokines Were Investigated

GM-CSF	Interleukin 13	Interleukin 27
Interleukin γ	Interleukin 15	Interleukin 28β
Interleukin 1β	Interleukin 17A	Interleukin 31
Interleukin 2	Interleukin 17E	Interleukin 33
Interleukin 4	Interleukin 17F	MIP-3$^{\alpha}$
Interleukin 5	Interleukin 21	TNFα
Interleukin 6	Interleukin 22	TNFβ
Interleukin 10	Interleukin 23	CD40 ligand
Interleukin 12p70		

C. Difficile Polysaccharide Vaccine Development & Functional Assay Characterization

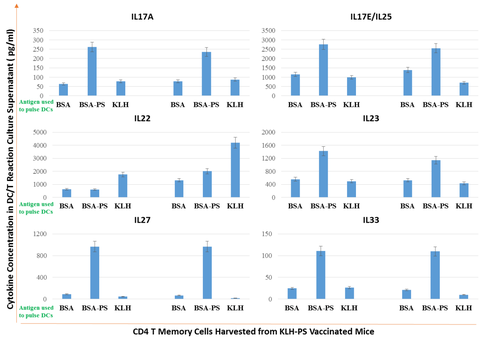


Polyclonal Antibody Generated with Polysaccharides from 027 Has Different Binding Affinity to Polysaccharides on C. Difficile Cells of Other Strains

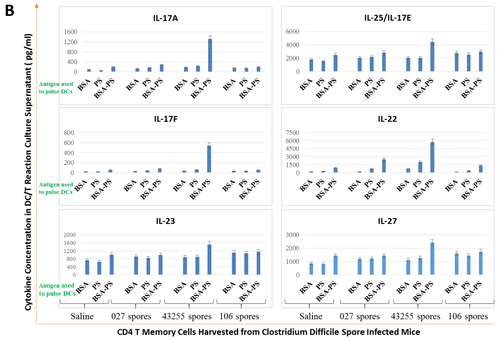


C. difficile vegetative cells and spores were stained with 1:1000 dilution of 4',6-diamidino-2-phenylindole (DAPI, blue) (1 mg/ml) for 1h. Vegetative cells and spores were blocked with 3% BSA for 1h, followed by staining with 1:500 dilution of rabbit anti-PSII for 2h, and with R-Phycoerythrin (PE) conjugated goat anti-rabbit IgG for 1h. Images were taken with Olympus FV1000 confocal microscopy, magnification 400X.

Conjugates Prepared with C. Difficile Cell Surface-derived Polysaccharide and KLH Induce Th17 Responses in Mice



Polysaccharide Antigens from Different C. Difficile Strains Have Strain-specificity in Inducing T Cell Dependent Immune Responses



Immature dendritic cells (DCs) were extracted from bone marrow of immune history free C57BL/6 mice, followed by pulsing with bovine serum albumin (BSA), C. difficile cell surface polysaccharide (PS)-BSA conjugate (BSA-PS), and KLH (**A**), or BSA, PS and BSA-PS conjugate (**B**)to generate matured DCs. Matured DCs were treated with mitomycin, then reacted with spleen-derived CD4 T cells from C57BL/6 mice that were injected with either KLH-PS (**A**) or challenged with spores of C. difficile of different strains (**B**). Cytokines released by activated CD4 T cells were measured with magnetic Th17 cytokine beads using Luminex. Cytokine concentration in DC/CD4 T cell reaction culture supernatant was measured three times and data are presented with standard deviation.

Summary

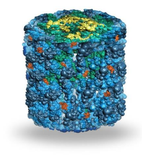
1. Clostridium difficile cell surface polysaccharide antigen, when conjugated to KLH, could induce T cell dependent adaptive immune responses in mice.
2. C. difficile spore infection could also induce T cell dependent adaptive immune responses in mice.
3. The T cell dependent adaptive immune responses induced by both C. difficile cell surface polysaccharide antigen-KLH conjugate and C. difficile spores were Th17-cell dominated responses.
4. In contrast to polysaccharide-BSA conjugate, polysaccharide alone induced much weaker activation of T cells harvested from mice that were infected with C. difficile spores.
5. Polysaccharides extracted from C. difficile cell surface showed significant strain-specific difference.
6. Interesting, the two new very infectious strains, 027 and 106, might have more similar polysaccharide structures, when compared to that from ATCC strain 43255.

Conclusions

1. Clostridium difficile cell surface polysaccharide-KLH conjugate is much more immunogenic than unconjugated polysaccharide.
2. Vaccines prepared with polysaccharide antigens extracted from one specific strain might not be able to protect infection induced by a different strain.
3. Newly emerged strains, 027 and 106, have significantly different polysaccharide 'fingerprints' than the old strains, e.g. ATCC 43255. This observation suggests vaccines prepared with antigens extracted from newer strains might be able to protect infection induced by older strains, but vaccines prepared with antigens extracted from older strains might not be able to protect infection induced by newer strains.
4. Vaccines prepared with antigens extracted from the two new strains, 027 and 106, might have some degree cross-strain protection to infection induced the other strain.

About KLH

Keyhole Limpet Hemocyanin (KLH) is a cylinder-shape didecamer (20-mer), which can dissociate into monomers (KLH subunits or suKLH). The subunit isoforms (approx. 360-400 kDa monomeric molecular weight) are each composed of 7 or 8 functional units. This complex molecular structure can be used to generate multiple product configurations.



Side View of KLH Molecule

Contacts & Resources

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