Clostridium difficile Surface Polysaccharide-KLH Conjugate Vaccine

Induced Th17-Featured Adaptive Immune Responses in Mice

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Abstract

Clostridium difficile: A Gram-positive, anaerobic, spore-forming, toxin-producing bacterium. It is the leading cause of antibiotics-associated diarrhea. C. difficile infection related mortality and mortality has been increasing in recent decades. As C. difficile is closely related to disturbed gut microbiota 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 candidates.

We challenged and vaccinated C57BL/6 mice with spores from non-virulent strain (ATCC 43255) and hyper virulent strains (strains 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 stained 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 in 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 43255 could not protect infection caused by hyper virulent strains 106 and 027.

Rationale

Clostridium difficile spores and vegetative cells are heavily coated with polysaccharides

Polysaccharides 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

Goals

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

C. difficile Polysaccharide Vaccine Development & Functional Assay Characterization

Cytokines Were Investigated

A

B

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

C. difficile vegetative cells and spores were stained with 1,000,000 dilution of F-6-amino-2-phenylindole (DAPI, blue) (1 mg/ml) for 1h. Vegetative cells and spores were blocked with 3% BSA for 1h. Immunostaining with 1,000,000 dilution of rabbit anti-PSI for 2h, and with RPhycoerythrin (RPh) conjugated goat anti-rabbit IgG for 3h. Images were taken with Olympus FV1000 confocal microscopy, magnification 400X.

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

For conjugate with polysaccharides from strain 43255, the conjugate showed clear staining (A) while for conjugate with polysaccharides from strain 027, the conjugate might not stain the target cells (B).

Rationale, Methods and Results

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

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

Summary

1. Clostridium difficile 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 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. Interestingly, 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 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 by the other strain.

About KLH

Keyhole Limpet Hemocyanin (KLH) is a cylinder-shaped blue protein (20-mer), which can incorporate into monomers (KLH subunits or subunit) and subunits (approx. 380-400 kDa monomer molecular weight) with each containing 7 or 8 functional units. This complex molecular structure can be used to generate multiple product configurations.

About 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
• 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

Pathogenesis of Clostridium difficile Infection

Antibiotics Exposure

• Immune-Mediated Disease

Decreased Resistance to Bacteria

C. difficile Spore Infection

• C. difficile Cell Lysate

Immune dendritic cells (DC) 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 (PS-BSA), and KLH (A), or BSA, PS, and BSA-PS conjugate (BSA) were generated mature DCs. Matured DCs were treated with mitomycin, then reacted with splenoid-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 Lumines. Cytokine concentration in DC/CD4 T cell reaction culture supernatant was measured three times and data are presented with standard deviation.

Contacts & Resources

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