

P026 Vaccination with *Clostridium difficile* PSII polysaccharide antigen adjuvanted with KLH induced broad-based enhancement of adaptive immune responses and protection in mice

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Abstract

Introduction

Clostridium difficile (CDI) infection has become a major source of antibiotic-associated diarrhea and placed a significant burden on global health care systems. Asymptomatic carriers of *C. difficile* strains have the potential to transmit spores or vegetative cells and contribute to the spread of CDI in long-term care facilities. The germination and colonization processes of bacteria can be considered as the earliest and essential steps for the development of primary and recurrent infection. Thus, disruption of germination, colonization, blockade of bacteria adhesion to gut or direct killing of cells using immunotherapies can be effective controls for both primary and relapse of CDI and be explored as an alternative means for protecting high-risk individuals. We assessed the immunogenicity and efficacy of a parenteral vaccine containing *C. difficile* PSII polysaccharide antigen conjugated to subunit KLH in a mouse CDI model.

Biochemistry and Immunogen

PSII is a hexaglycosyl polysaccharide with repeating blocks connected by a diester-phosphate. Polyclonal rabbit anti-PSII positively identified native PSII extracted from ten different epidemic strains of *C. difficile* suggesting that PSII is a conserved antigen. Similar positive immunoblot results were observed with PSII extracted from 027 strain using a hot-water-phenol extraction method. Rabbit anti-PSII antisera were able to bind to both spores (Day 5 culture) and vegetative cells (Day 3 culture) from three *C. difficile* strains (027, 106 and ATCC43255) using immunofluorescent method using pre-immunized serum as negative control. In the Western Blot analysis of PSII-KLH and PSII-BSA conjugates, rabbit serum immunized against native PSII had specificity to only PSII and not KLH or BSA.

Dendritic cell stimulation and Immunogenicity

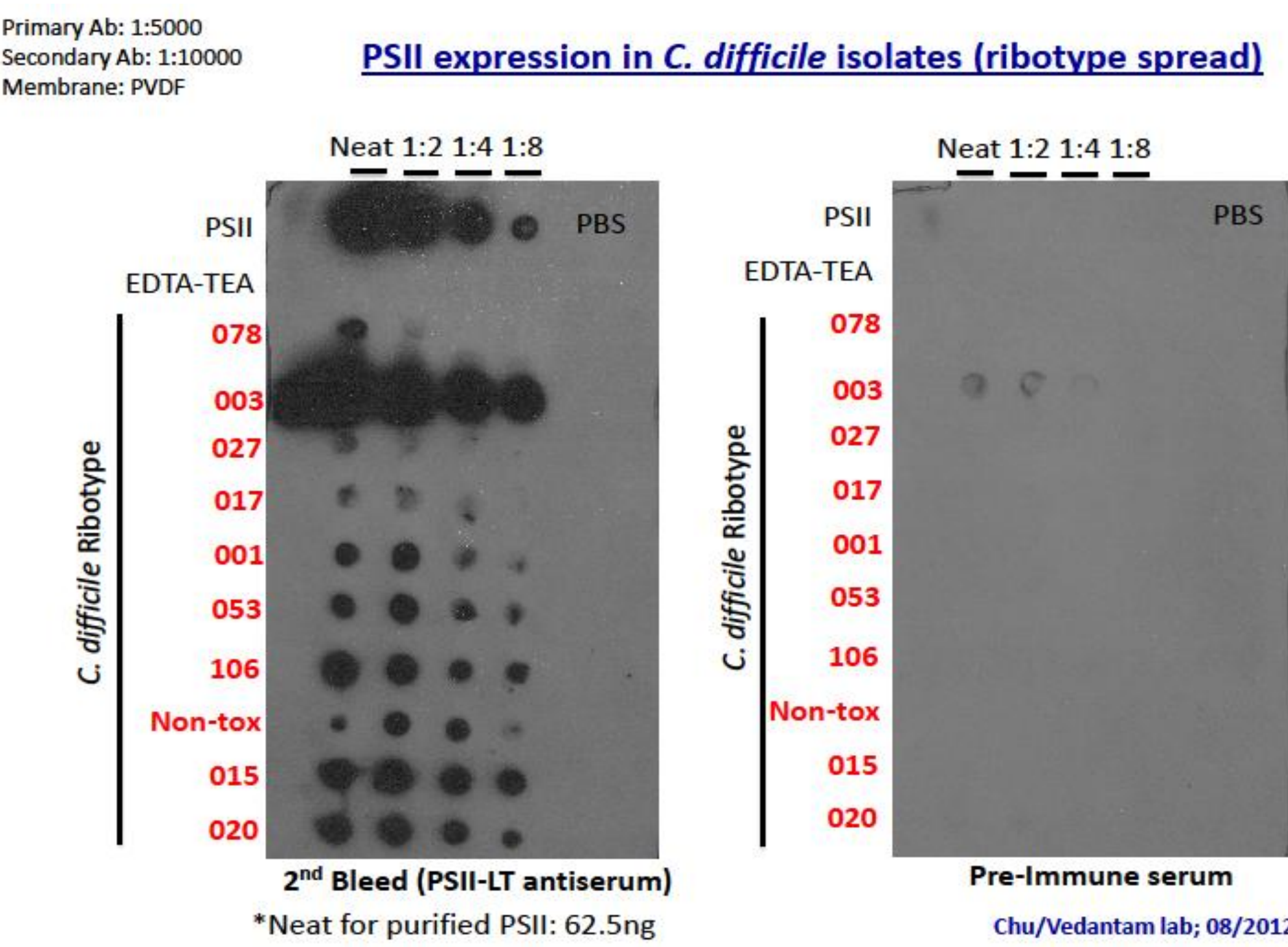
Recent study by Jafari et al. suggested that mucosal Th1/Th17 axis play a central role in establishing antimicrobial immunity to CDI (1). In the current study, we access the immunogenicity of the PSII-KLH conjugate on C57BL/6 dendritic cells and JAWII cell line. Bone marrow dendritic cells were pulsed with 30 ug/mL of PSII-KLH for 24 hours and monitored for cell surface maturation markers. PSII-KLH stimulated dendritic cells had increased expression of Class I MHC and CD86 costimulatory molecules. Ex vivo study of JAWII cell line also demonstrated activation of Th17>/Th1>Th2 cytokines including IL17, IL12 and IL4 (ranging between 1.6 to 1.9 fold increases (data not shown).

1. Jafari et al. *Clostridium difficile* Modulates Host Innate Immunity via Toxin-Independent and Dependent Mechanism(s). *PLoS One*. 2013 Jul 29;8(7):e69846. doi: 10.1371/journal.pone.0069846. Print 2013.

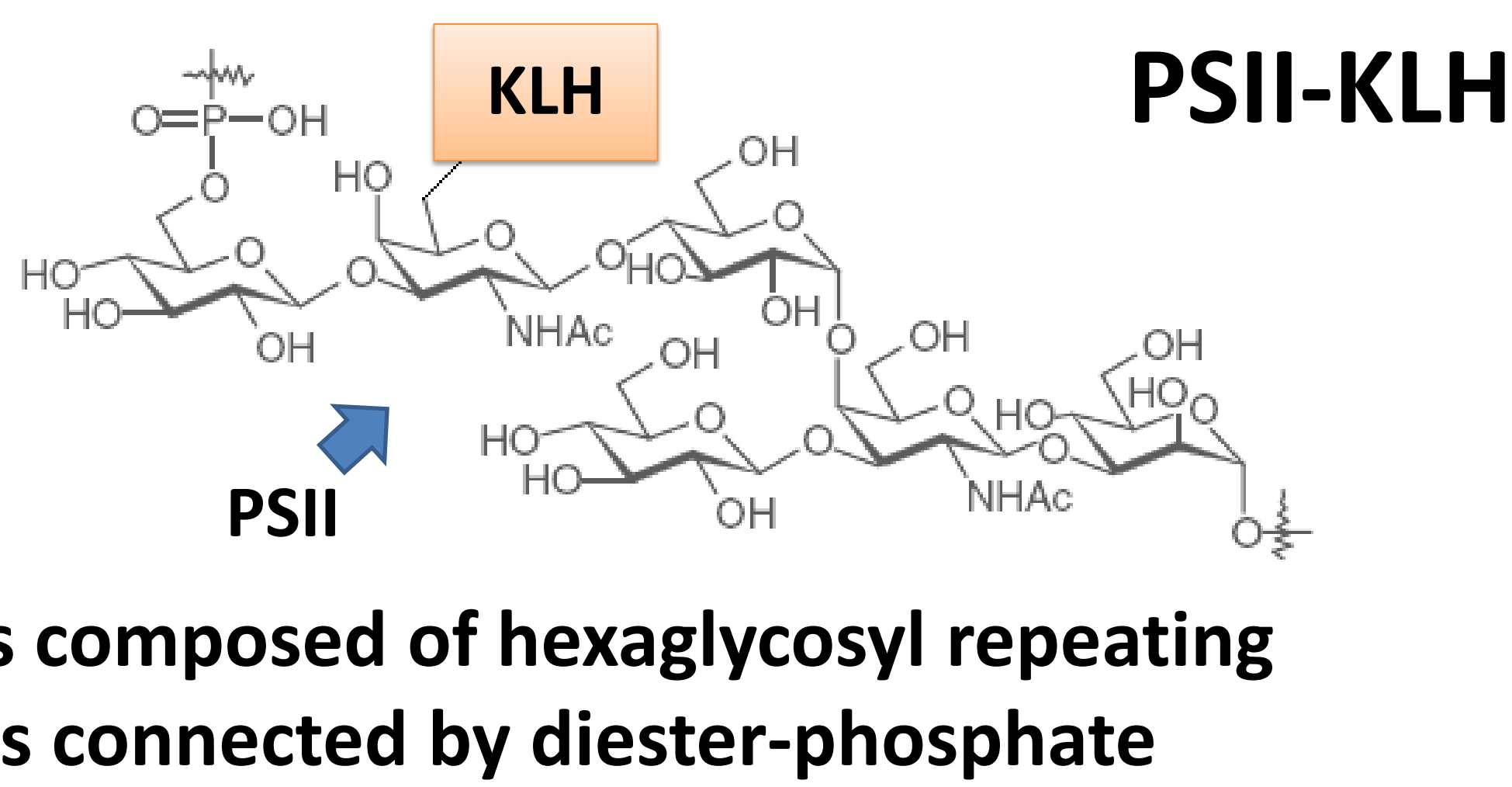
PSII-KLH Conjugate – Biochemistry and Immunogen

PSII is expressed on multiple C. difficile strains

The following *C. difficile* strains were tested positive for PSII –
•078, 003, 017, 001, 053, 106, 015, 020, 027
•027 subtypes include BI-1, BI-6, BI-8, BI-17, BI-23 (data not shown)

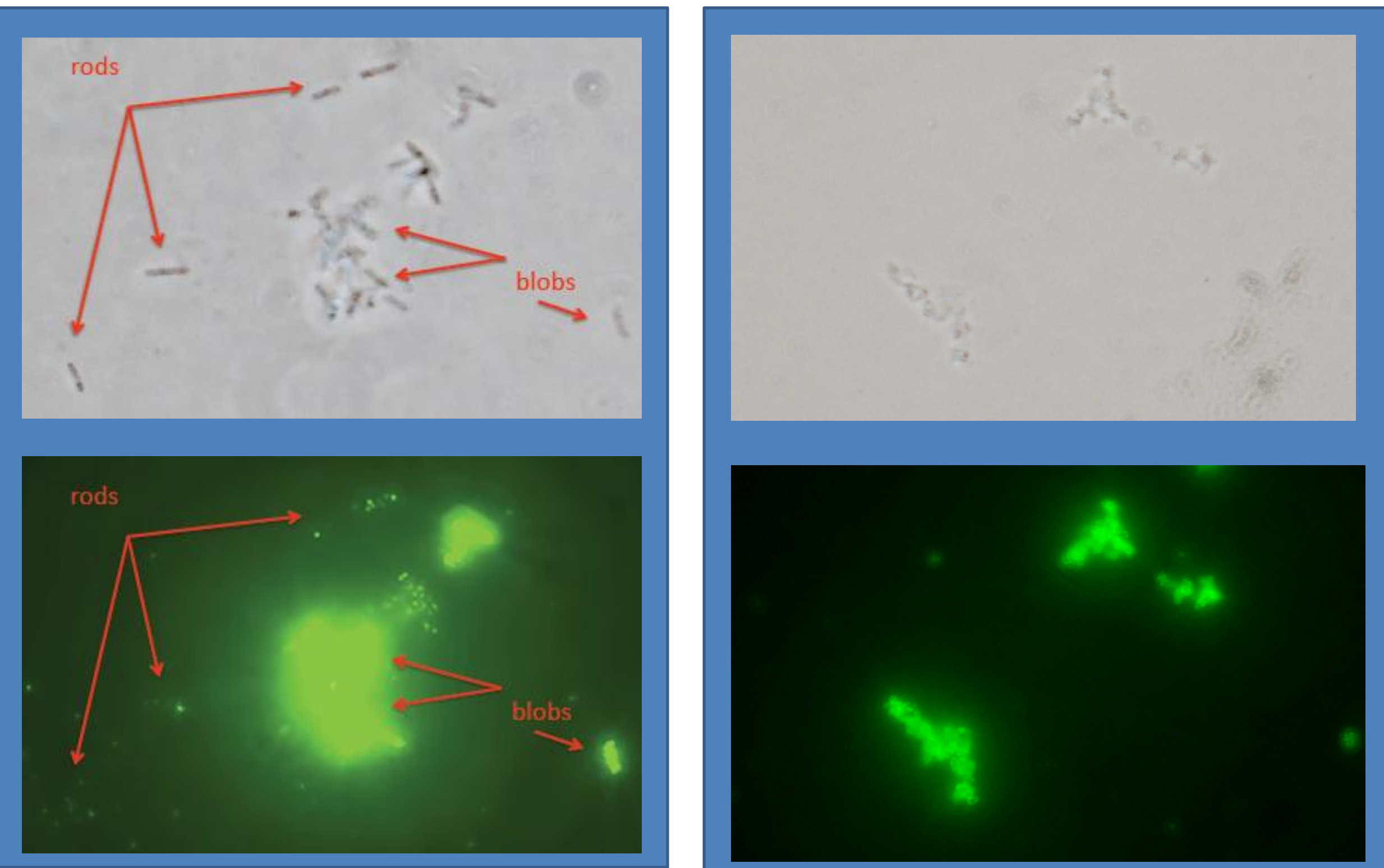


PSII –KLH Conjugate Clostridium difficile Vaccine
TEMPO oxidized PSII conjugated to subunit-KLH



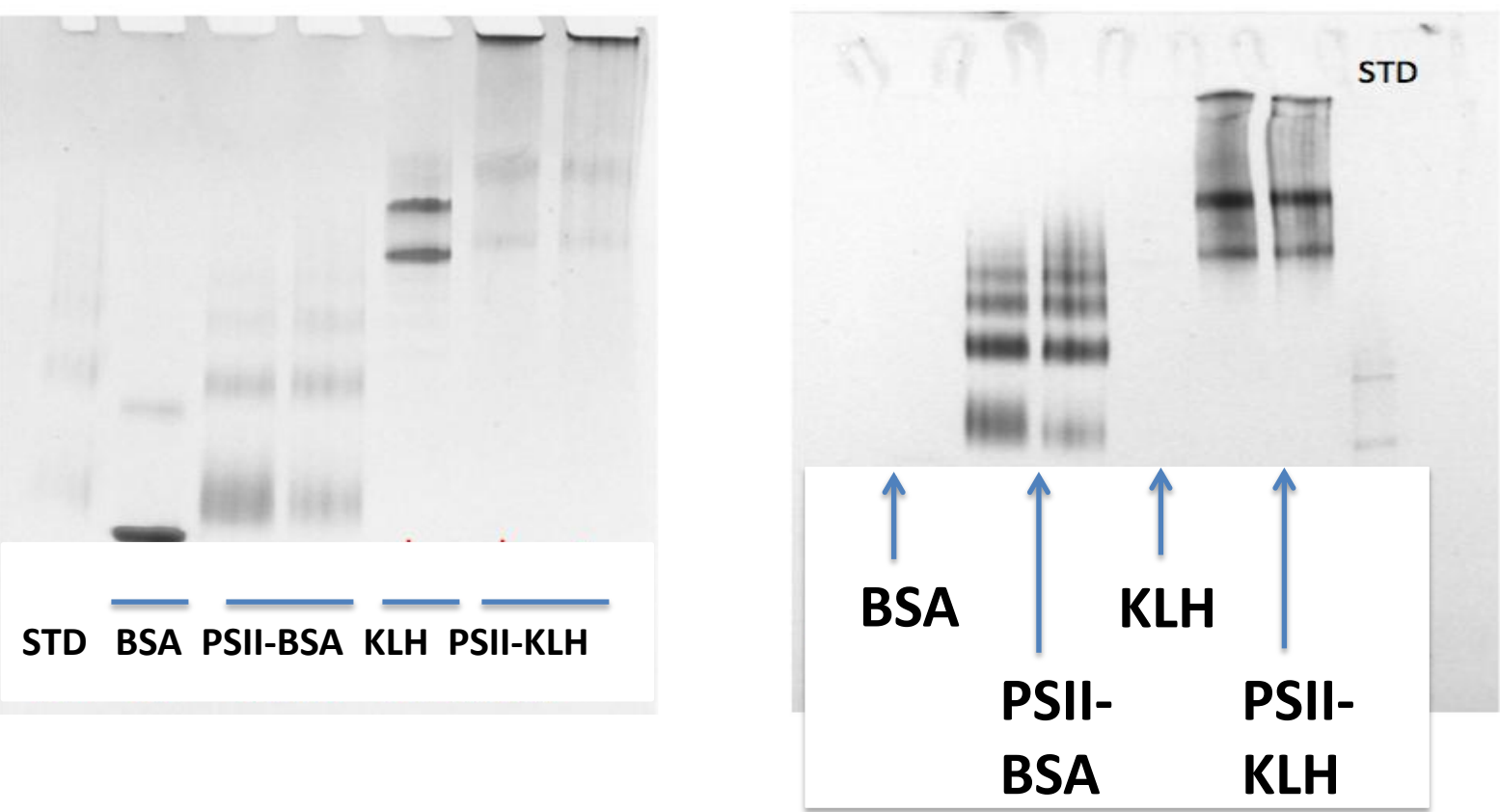
Polyclonal anti-PSII antisera recognize both spores and vegetative cells of C. difficile

Vegetative cells (Day 3 culture) **Spores(Day 5 culture)**



Polyclonal anti-PSII recognized only PSII and not the carrier proteins

Coomassie stained gel **Western Blot (Rabbit anti-PSII)**



PSII-KLH Vaccination and *C. difficile* challenge study

Mice were vaccinated with 3 intramuscular and subcutaneous injections of 100 ug PSII-KLH admixed in 100 ug of KLH on Day 1, 14 and 28. Sera samples collected on Day 35 showed high titers of anti-PSII IgG (data not shown). Following vaccination, C57BL6 mice were rendered susceptible to *C difficile* with multiple antibiotics and were then challenged with *C difficile* spores orally. Ninety percent of vaccinated mice (38 of 42) survived an otherwise an LD₅₀ dose of *C difficile* spore challenge. A total of 38 mice survived the first *C difficile* challenge were allowed to rest for 7 days, exposed to a 3 day course of antibiotic pretreatment, and challenged again with 10x LD₅₀ dose of *C difficile* spores. All vaccinated animals survived the second challenge whereas high mortality was observed (2 of 3) in the non-vaccinated control group. Studies are underway to correlate vaccination with adherent and non-adherent *C. difficile* burden and epithelial pathology.

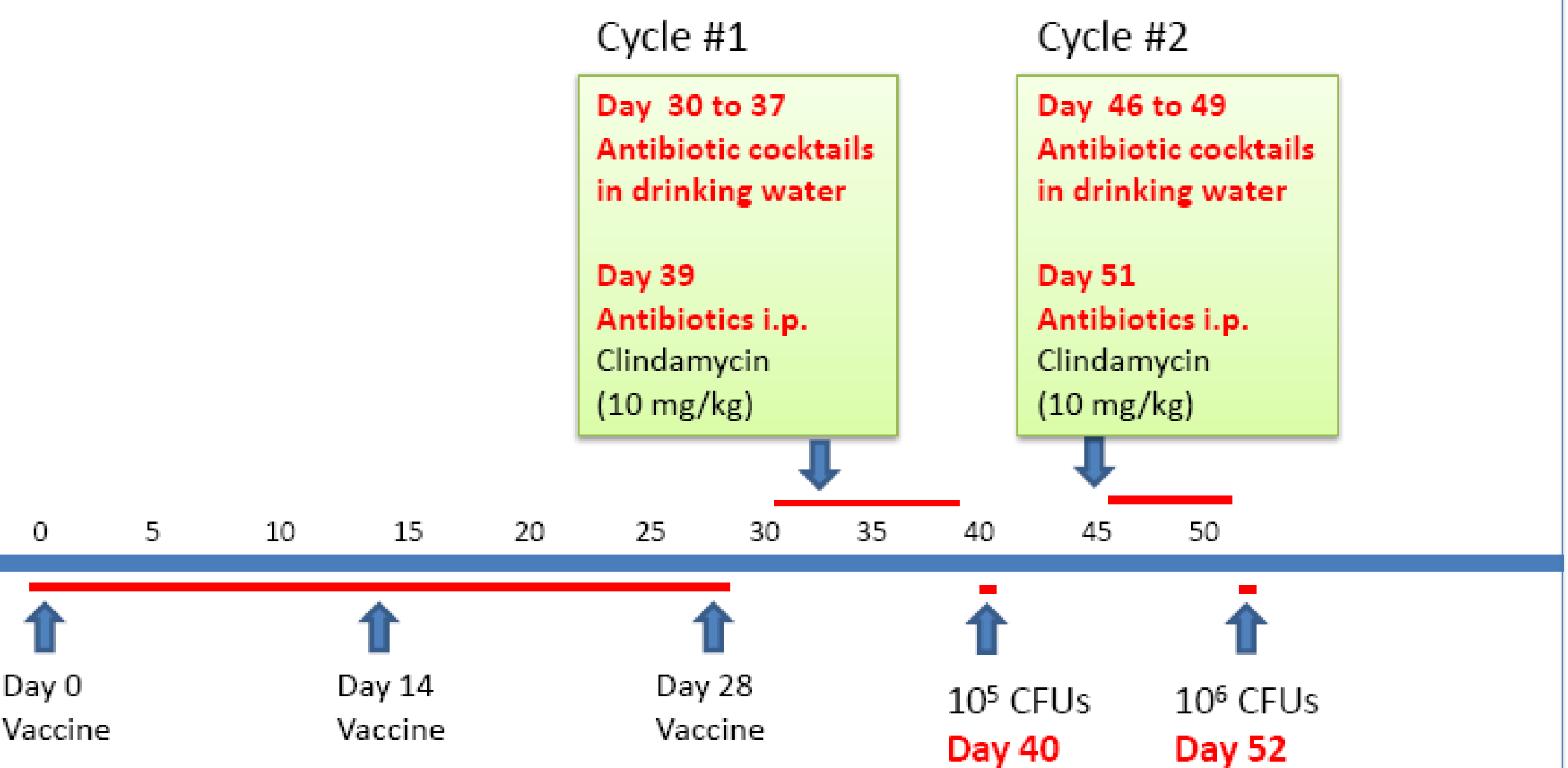
Development of PSII-KLH conjugate vaccine adjuvanted with subunit KLH is justified as a promising active immunotherapy approach.

Conclusion

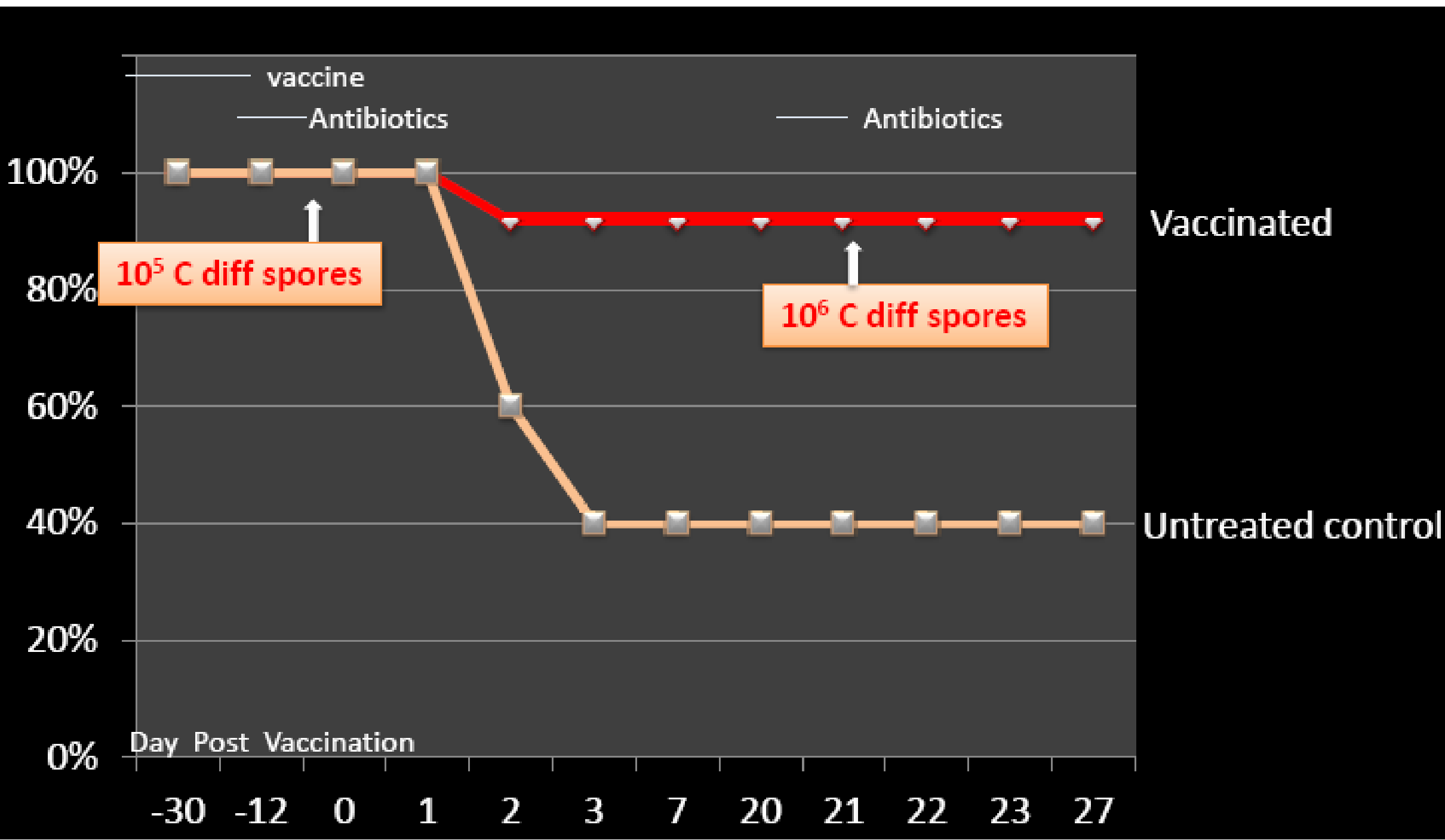
- PSII polysaccharide antigen is expressed on both *C. difficile* spores and vegetative cells
- PSII polysaccharide antigen seems to be conserve across many *C. difficile* strains
- PSII-KLH conjugate vaccine adjuvanted with KLH administered parentally is protective in mice challenged with *C. difficile* spores

C57BL6 C. difficile model

ATCC 43255 Spore oral gavage
100 ug KLH admixed with 100 ug PSII-KLH
Intramuscular & subcutaneous on day 1, 14 and 28



PSII-KLH conjugate vaccine adjuvanted with KLH protects 90% of mice challenged with C. difficile



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