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Phase 1 testing of detoxified LPS/group B meningococcal outer membrane protein vaccine with and without synthetic CPG 7909 adjuvant for the prevention and treatment of sepsis

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ABSTRACT

Background: Gram-negative bacteria (GNB) are a leading cause of nosocomial infection and sepsis. Increasing multi-antibiotic resistance has left clinicians with fewer therapeutic options. Antibodies to GNB lipopolysaccharide (LPS, or endotoxin) have reduced morbidity and mortality as a result of infection and are not subject to the resistance mechanisms deployed by bacteria against antibiotics. In this phase 1 study, we administered a vaccine that elicits antibodies against a highly conserved portion of LPS with and without a CpG oligodeoxynucleotide (ODN) TLR9 agonist as adjuvant.
Methods: A vaccine composed of the detoxified LPS (dLPS) from *E. coli* O111:B4 (J5 mutant) non-covalently complexed to group B meningococcal outer membrane protein (OMP). Twenty healthy adult subjects received three doses at 0, 29 and 59 days of antigen (10 μg dLPS) with or without CPG 7909 (250 or 500 μg). Subjects were evaluated for local and systemic adverse effects and laboratory findings. Anti-J5 LPS IgG and IgM antibody levels were measured by electrochemiluminesence. Due to premature study termination, not all subjects received all three doses.
Results: All vaccine formulations were well-tolerated with no local or systemic events of greater than moderate severity. The vaccine alone group achieved a ≥4-fold "responder" response in IgG and IgM antibody in only one of 6 subjects. In contrast, the vaccine plus CPG 7909 groups appeared to have earlier and more sustained (to 180 days) responses, greater mean-fold increases, and a higher proportion of "responders" achieving ≥4-fold increases over baseline.
Conclusions: Although the study was halted before all enrolled subjects received all three doses, the J5dLPS/OMP vaccine, with or without CpG adjuvant, was safe and well-tolerated. The inclusion of CpG increased the number of subjects with a ≥4-fold antibody response, evident even after the second of three planned doses. A vaccine comprising J5dLPS/OMP antigen with CpG adjuvant merits further investigation.
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1. Introduction

Gram-negative bacteria (GNB) are a leading cause of nosocomial infection, exceeded in one recent survey only by *C. difficile* infections [1]. Despite decades of intensive research, the morbidity and mortality from Gram-negative bacteremia and sepsis is unacceptably high [2]. The situation is further exacerbated by the dramatic increase in multi-antibiotic resistant (MDR) bacteria accompanied by a steady decline in the antibiotic pipeline [3]. Despite governmental attempts to encourage the development of new antibiotics by pharmaceutical companies, the development of antibiotic resistance is inevitable, making the useful life of a new

Abbreviations: TSH, thyroid stimulating hormone; ECL, electrochemiluminescence; GNB, Gram-negative bacteria; MDR, multidrug resistant; gMFI, geometric mean fold increase; OMP, outer membrane protein; dLPS, detoxified LPS; CpG, cytosine and guanosine triphosphate oligodeoxynucleotides joined by phosphorothioate.

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antibiotic uncertain [4,5]. Consequently, new approaches for the treatment of GNB are greatly needed.

Vaccines that elicit antibodies against bacterial pathogens have been successful in reducing the morbidity and mortality from infection, or in the case of *H. influenzae*, nearly eradicating lethal infections [6,7]. Antibodies against the lipopolysaccharide (LPS, or endotoxin) of GNB have been highly protective in experimental GNB infection, as well as in human infection [8–10]. While vaccines against nosocomial GNB pathogens have been developed and tested in human subjects, none have sought licensure in the United States, in part because of the effectiveness, until recently, of antibiotics [11–13].

Early investigation of LPS structure by several laboratories identified a highly conserved core region that joined the biologically active lipid A moiety to the highly variable carbohydrate region responsible for O antigen specificity. A whole killed vaccine was prepared from a mutant of *E. coli* O111:B4 (Rc chemotype, J5) that was unable to generate O antigens, thereby exposing conserved core LPS epitopes. Passive administration of post-immune antisera generated by the administration of this vaccine to healthy subjects demonstrated significant protection from shock and death in a large, multicenter randomized, control clinical trial [14]. We developed a new subunit formulation of the original J5 vaccine whereby purified J5 LPS was detoxified (J5dLPS) and non-covalently complexed with the outer membrane protein (OMP) of group B *N. meningitides* [15]. The resulting vaccine was highly immunogenic and protective in various preclinical models of sepsis caused by heterologous clinical isolates of GNB [15–20]. When tested in human subjects, this vaccine was well-tolerated with no systemic adverse effects and local reactions similar to those of licensed vaccines; however, this non-adjuvanted vaccine induced only a 2–4 fold increase in anti-J5 LPS antibodies over baseline levels [21].

Adjuvants are well established to increase antibody responses for kinetics, magnitude, breadth, and durability of antibody responses against co-administered antigens. Oligodeoxynucleotides (ODN) containing CpG motifs that activate immune cells via Toll-like receptor 9 (TLR9) have been shown to enhance antibody responses to a wide variety of antigens [22]. CPG 7909 is a 24-mer CpG ODN containing 3 CpG motifs that has been shown to significantly enhance antibody responses in several human clinical trials [23–27]. The subject of this report is a Phase 1 clinical study of J5dLPS vaccine administered alone or with CPG 7909 at two different doses.

2. Materials and methods

2.1. Vaccine and adjuvant

The J5dLPS/OMP vaccine was prepared at the Pilot Bioproduction Facility at the Walter Reed Army Institute of Research (WRAIR) in Silver Spring, MD under cGMP conditions as previously described [21]. *E. coli* O111:H4, J5 (Rc) mutant was originally obtained from Dr. Elizabeth Ziegler, San Diego, CA. The J5dLPS/OMP cGMP product (Lot 0376) was originally manufactured in 1996 and stored at $-20 \pm 5\,^{\circ}C$.

CPG 7909 is a synthetic CpG ODN of sequence TCGTCGTTTTGTCGTTTTGTCGTT, is manufactured with a nuclease-resistant phosphorothioate backbone. CPG 7909, generously provided by Pfizer (PF-3512676), was stored at 2–8 °C.

2.2. Study protocol

This single-center study intended to recruit 28–34 healthy subjects aged 18–50 years. The subjects were randomized to one of four study groups: (1) vaccine alone (10 μg, based on LPS content), (2) vaccine + CPG 7909 (500 μg), (3) vaccine + CPG 7909 (250 μg), or (4) placebo (normal saline). The primary objective of the study was to establish the safety and tolerability of the combination of vaccine and CPG 7909 when given together. The secondary objective was to determine if the combination of vaccine with CPG 7909 was more immunogenic than the vaccine alone and if the antibody response occurred earlier. This study was approved by the IRB of the University of Maryland, Baltimore.

Eligible subjects were to receive three immunizations in alternating deltoid muscle at Days 0, 29, and 59, based on the previous Phase 1 study performed with non-adjuvanted vaccine [21]. The subjects in the four study groups were to be immunized in three cohorts. Only the vaccinator, who did not monitor patient safety, was unblinded. For the first cohort, two subjects in each group received vaccine alone, vaccine + CPG 7909 (250 μg), or placebo. A second cohort received the vaccine alone ($n = 6$), vaccine + CPG 7909 (250 μg, $n = 6$), or vaccine + CPG 7909 (500 μg, $n = 2$). The third cohort was to be immunized with the vaccine + CPG 7909 (500 μg, $n = 6$) or placebo ($n = 2$). After each immunization, subjects were examined for local and systemic reactions at 24 and 48 h and reactogenicity and tolerability was recorded for 8 days. Blood samples for standard laboratory safety tests were obtained 7 days after each vaccination (i.e., days 7, 36, and 66) and for anti-core glycolipid antibody levels at days 14, 36, 66 as well as on days 120, 180, and 365. Baseline antibody levels were measured at day 0 before immunization. An immunology safety screen to monitor potential adverse effects of the adjuvant included ANA, anti-double-stranded DNA antibody, and TSH assays. EKGs were performed after each vaccination.

2.3. ELISA

A previously described ELISA assay for IgG and IgM antibody to J5 LPS was adapted to a proprietary platform that is a combination of electrochemiluminescence (ECL) detection and patterned arrays (Meso Scale Discovery [MSD]) according to the manufacturer's instructions. ECL detection uses secondary antibody labels that emit light when electrochemically stimulated that reduces background signals and improves sensitivity [28]. One microgram J5 LPS (List Biologics) in PBS was added to MSD MULTI-SPOT 96-well plates. Following addition of serum samples, incubation, and washing, SULFO-TAG® anti-human detection antibody was added to each well of the MSD plate and the wells were read in a SECTOR IMAGER 2400 reader. Data were analyzed using Microsoft Excel and MSD Discovery Workbench software (http://www.mesocale.com/CatalogSystemWeb/WebRoot/products/software.aspx).

Statistical analysis. IgG and IgM antibody levels to J5 LPS were analyzed by computing the geometric mean concentration (GMC), geometric mean-fold increase over baseline antibody levels (gMFI), and the proportion of responders (defined as >4-fold increase in antibody level over baseline) in each study group and at each time point. Confidence intervals for the geometric mean antibody levels were computed by transforming results to log scale, assuming normality assumptions were satisfied on this scale, and converting the computed interval back to the original scale. Exact 95% confidence intervals for the proportion of responders were also computed. An unplanned comparison was made between the proportion of responders in the vaccine alone and the combined vaccine + CPG 7909 groups. This analysis was performed with a Fisher Exact test and was consistent with the objective of determining if addition of CPG improved the level of antibody as well as the rapidity of response. Statistical analyses were performed using SAS software, version 9.3.

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