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Development of a synthetic Vi polysaccharide vaccine for typhoid fever

Yawei Ni [a,*], Michael J. Springer [a], Jianhua Guo [b], Isaac Finger-Baker [a], James P. Wilson [a], Ronald R. Cobb [a], Debra Turner [b], Ian Tizard [b]

[a] Research and Development, Nanotherapeutics, Inc., Alachua, FL, USA
[b] Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA

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ABSTRACT

Typhoid fever remains a serious public health problem with a high impact on toddlers and young children. Vaccines against the Vi capsular polysaccharide are efficacious against typhoid fever demonstrating that antibodies against Vi confer protection. The currently licensed Vi typhoid vaccines have however limited efficacy and are manufactured by a complex process from wild-type bacteria. Due to these inherent issues with the current vaccines, an alternative vaccine based on an O-acetylated high molecular weight (HMW) polygalacturonic acid (GelSite-OAc™) was generated. The HMW polygalacturonic acid shares the same backbone as the Vi polysaccharide of *Salmonella* Typhi. The GelSite-OAc™ has a high molecular weight (>1 × 10^6 Da) and a high degree of O-acetylation (DOAc) (>5 μmole/mg), both exceeding the potency specifications of the current Vi vaccine. Studies in Balb/c mice demonstrated that GelSite-OAc™ was highly immunogenic, inducing a strong antigen-specific antibody response in a DOAc- and dose-dependent manner which was comparable to or higher than those induced by the licensed Vi vaccine. Importantly, the GelSite-OAc™ was shown to be fully protective in mice against lethal challenge with *Salmonella* Typhi. Furthermore, the GelSite-OAc™ demonstrated a boosting effect or memory response, exhibiting a >2-fold increase in antibody levels upon the second immunization with either GelSite-OAc™ or the Vi vaccine. This novel boosting effect is unique among polysaccharide antigens and potentially makes GelSite-OAc™ effective in people under 2 years old. Together these results suggest that the GelSite-OAc™ could be a highly effective vaccine against *Salmonella* Typhi.

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1. Introduction

Typhoid fever is an acute and life-threatening febrile illness caused by *Salmonella enterica* serotype Typhi (*S.* Typhi). It is responsible for 16–33 million new typhoid fever cases and 500,000–600,000 deaths annually [1–3]. Contaminated food and water are the main sources of infection. The risk of infection is the highest in developing countries with poor sanitation including Asia, Africa, and Latin America. Several different antibiotics have been used to treat typhoid fever. However, multi-drug resistant strains of *S.* Typhi have emerged and rendered these costly treatments ineffective [4,5]. Thus, the widespread use of low-cost efficacious vaccines is still the most effective way to reduce the impact of typhoid fever.

Currently, there are two types of vaccines available for controlling typhoid fever, a Vi polysaccharide vaccine administered parenterally in a single dose and an oral live attenuated Ty21a vaccine [6,7]. The Vi vaccine is produced by costly and hazardous fermentation of *S.* Typhi wild type bacteria and elaborate purification processes. It is licensed for use in people ≥2 years and provides about 70% protection that lasts for two to three years [8]. An oral live vaccine in capsule form is currently licensed for persons over 5 years and provides a similar level of protection after four doses [8]. Thus, these vaccines provide limited protection and none are effective in people under 2 years.

Current Vi vaccines are based on the Vi capsular polysaccharide which is a linear alpha 1–4 linked polygalacturonic acid (PGA) that is N-acetylated at C2 and 60–90% O-acetylated at C3 of the galacturonic acid (Gal UA) residue. The O-acetylation at C3 and molecular weight are the two critical determinants of immunogenicity of the Vi polysaccharide and the potency indicators for the current Vi vaccines. Studies have shown that removal of the O-acetyl group at C3 reduces its immunogenicity [9–11]. Structural modeling of the Vi polysaccharide shows that the bulky nonpolar O-acetyl groups at C3 make up most of the surface of the polysaccharide molecule by protruding on both sides, whereas the carboxyl and

* Corresponding author at: KJ Biosciences LLC, 2151 Harvey Mitchell Parkway, Suite 306, College Station, TX 77840, USA.
E-mail address: yni@kjbiosciences.com (Y. Ni).

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N-acetyl (at C2) groups are mostly embedded or located close to the axis [12]. This is consistent with the O-acetyl group being the dominant immunogenicity determinant. Studies have also shown that the immunogenicity of the Vi polysaccharide decreases when its molecular weight is reduced [13–14]. The Vi vaccine, like other polysaccharide-based vaccines, acts as a T cell-independent antigen and does not elicit a booster response upon revaccination [15]. It is therefore not effective in infants or toddlers under 2 years. As a result, Vi polysaccharide-protein conjugate vaccines are being developed by covalent linking of Vi polysaccharide to a protein carrier [16–18].

Plant pectins share the same backbone with the Vi polysaccharide. They are alpha 1–4 linked polygalacturonic acid that is variably methylated. Those with a degree of methylation (DM) below 10% are considered as PGA [19,20]. The commercial low-methoxyl (LM) pectin or PGA has been O-acetylated and the resulting acetylated product was found to share the same antigenicity with native Vi polysaccharide [11,21]. However, it was not immunogenic in animals due to the low molecular weight ($\sim 4 \times 10^5$ Da) of the LM pectin or PGA used [18,21].

We have developed a synthetic Vi antigen (GelSite-OAc™) by O-acetylation of a novel high-molecular-weight polygalacturonic acid (GelSite®) from the Aloe vera plant. GelSite® is uniquely characterized by a high molecular weight ($>1 \times 10^6$ Da) and a very low degree of methylation (DM, <10%). These properties make it an ideal substrate for a synthetic Vi polysaccharide analog by O-acetylation (Supplementary Fig. S1). The GelSite® was successfully O-acetylated with a simple chemical reaction. We tested GelSite-OAc™ for its ability to immunize mice against *S.* Typhi. The results indicate that the GelSite-OAc™ can potentially serve as a typhoid Vi vaccine with distinct advantages over the current vaccines.

2. Materials and methods

2.1. GelSite polymer

All reagents used for GelSite-OAc™ manufacturing were obtained from Sigma Chemical Co (St. Louis, MO). High-molecular-weight polygalacturonic acid (HPGA or GelSite®) was isolated from *Aloe vera* L [22,23]. Its chemical and physical properties are summarized in Table 1. GelSite® has been manufactured as a lyophilized sodium salt with a high purity (>99%) under cGMP at a kilogram scale.

Table 1
Physical and chemical properties of GelSite®.

Molecular weight	$>1 \times 10^6$ Da (SEC; Dextran Standards)
Polydispersity (MW/Mn)	≤1.8
Anhydro-Gal UA content (w/w) %	80–90%
Neutral sugar content (w/w) %	<5%
Degree of methylation (% mole/mole)	<6%
Sodium content (w/w)	10–12%
Protein content (w/w)	<0.5%

2.2. O-acetylation of GelSite polymer

The Schweiger method [24] was adopted with modifications for O-acetylation of GelSite® after comparison with another method [25] as described in Supplemental Materials and is outlined in Fig. 1.

2.3. Determination of degree of O-acetylation

The method described by Hestrin [24] was used with modifications. The assay was conducted in 96-well plates. Samples were tested at ~0.2 mg/ml (w/v) in duplicate. Acetylcholine was used as the standard. DOAc was expressed as a molar ratio or percent of acetyl groups over Gal UA residues, or μM/mg – the unit used for DOAc specification for current Vi vaccine.

2.4. Determination of molecular weight

Molecular weight analysis was performed on a Waters 2695 HPLC with a Refractive Index detector and eight polysaccharide standards ranging from 5 kDa to 800 kDa (Shodex P-82 Pullulan Standards). Size exclusion separation was achieved using a mobile phase of 0.1 M ammonium acetate over a 90 min isocratic run at a flow rate of 0.4 mL/min.

All chromatograms were processed through Empower software. The observed retention time each standard was converted to elution volume.

2.5. Immunogenicity and protection

All animal studies were conducted with the approval of the Institutional Animal Use and Care Committee. The immunogenicity of GelSite-OAc™ was examined in 6–8 week old female Balb/c mice in comparison with a licensed Vi vaccine (Typhim Vi®; Sanofi-Pasteur). Groups of mice (n = 10) were immunized with GelSite-OAc™ or Typhim Vi vaccine at 2.5 μg/mouse or the indicated doses by intramuscular injection in the right hind leg in 50 μl PBS. Animals were immunized twice, four weeks apart. Serum samples were collected every two weeks. For cross-boosting experiments, groups of mice (n = 10) were immunized three times, four weeks apart with GelSite-OAc™ (with a DOAc of 155%) or Typhim Vi vaccine at 2.5 μg/mouse at the indicated times. Serum samples were collected every two weeks.

Specific antibodies were measured by ELISA in 96-well plates as described previously [27] with modifications. Briefly, the plates were coated with the polysaccharide antigens at 2 μg/ml in PBS at 4 °C overnight. They were blocked with 1% BSA in PBS at room temperature for 2 hrs followed by incubation of serial dilutions of serum samples in 1% BSA in PBS for 2 hrs at room temperature. After washing, the plates were incubated with 1:3000 dilution of anti-mouse IgG-alkaline phosphatase conjugate (Sigma Aldrich Co. St. Louis, MO) at RT for 1 hr before washing and addition of pNPP substrate. The OD was measured at 410 nm. The Vi polysaccharides obtained from International Vaccine Institute and

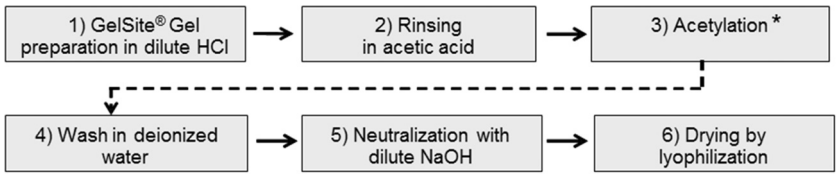

Fig. 1. The O-acetylation process for production of O-acetylated polygalacturonic acid (GelSite-OAc™). The asterisk indicates the use of a small amount of perchloric acid as the catalyst.

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