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Haloarchaeal gas vesicle nanoparticles displaying *Salmonella* antigens as a novel approach to vaccine development

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Abstract

A safe, effective, and inexpensive vaccine against typhoid and other *Salmonella* diseases is urgently needed. In order to address this need, we are developing a novel vaccine platform employing buoyant, self-adjuvanting gas vesicle nanoparticles (GVNPs) from the halophilic archaeon *Halobacterium* sp. NRC-1, bioengineered to display highly conserved *Salmonella enterica* antigens. As the initial antigen for testing, we selected SopB, a secreted inosine phosphate effector protein injected by pathogenic *S. enterica* bacteria during infection into the host cells. Two highly conserved *sopB* gene segments near the 3'- region, named *sop*B4 and *sop*B5, were each fused to the *gvpC* gene, and resulting SopB-GVNPs were purified by centrifugally accelerated flotation. Display of SopB4 and SopB5 antigenic epitopes on GVNPs was established by Western blotting analysis using antisera raised against short synthetic peptides of SopB. Immunostimulatory activities of the SopB4 and B5 nanoparticles were tested by intraperitoneal administration of SopB-GVNPs to BALB/c mice which had been immunized with *S. enterica* serovar Typhimurium 14028 Δ*pmrG-HM-D* (DV-STM-07), a live attenuated vaccine strain. Proinflammatory cytokines IFN-γ, IL-2, and IL-9 were significantly induced in mice boosted with SopB5-GVNPs, consistent with a robust Th1 response. After challenge with virulent *S. enterica* serovar Typhimurium 14028, bacterial burden was found to be diminished in spleen of mice boosted with SopB4-GVNPs and absent or significantly diminished in liver, mesenteric lymph node, and spleen of mice boosted with SopB5-GVNPs, indicating that the C-terminal portions of SopB displayed on GVNPs elicit a protective response to *Salmonella* infection in mice. SopB antigen-GVNPs were also found to be stable at elevated temperatures for extended periods without refrigeration. The results show that bioengineered GVNPs are likely to represent a valuable platform for antigen delivery and development of improved vaccines against *Salmonella* and other diseases.

Keywords: Salmonella; *Halobacterium*; vaccine; nanoparticle; gas vesicle

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1. Introduction

1.1. Salmonella *diseases*

Salmonella enterica, a Gram-negative intracellular pathogenic bacterium, infects humans and many warm blooded animals. Salmonellosis remains a serious problem in most developing countries and outbreaks are regularly seen in developed and industrialized countries[1-4]. Typhoid fever is caused by *S.* Typhi or *S.* Paratyphi, with global incidence of 21.7 million cases of Typhi and an additional 5.4 million Paratyphi cases resulting in 217,000 deaths per year[5]. Treatment is becoming more challenging due to increased prevalence of antibiotic resistance[6-8]. While two licensed vaccines for typhoid fever are commercially available, they have limited protection and/or may not be administered to immunocompromised people or children and infants. As a result, there is a critical need to develop more effective *Salmonella* vaccine candidates, which would also be safe and easily scalable, and inexpensive to produce and deliver. A versatile, particulate antigen delivery system, gas vesicle nanoparticles (GVNPs), which can be bioengineered to express multiple *Salmonella* antigens is likely to offer significant advantages over currently available vaccines, due to reduced risk and improved effectiveness.

1.2. Salmonella *pathogenesis and vaccine status*

Salmonella enterica includes 2,500 serovars infecting humans, and several are of public health importance, including *S.* Typhi and *S.* Paratyphi, the causative agents of typhoid and paratyphoid fever[2,9,10]. Transmission occurs through the fecal-oral route, upon ingestion of contaminated water and food. Occurrence of the disease may be confirmed by isolation of the pathogen, detection of antibodies against *Salmonella* specific O (somatic) and H (flagellar) antigens in the serum, or most sensitively, PCR based methods which utilize specific primers designed against unique regions of their genomes[8]. Treatment however is becoming more challenging, due to increased prevalence of multiple drug resistant (MDR) strains[6,7]. The need for improved vaccines against typhoid fever has been amply underscored by recent WHO reports[3,4].

Development of effective vaccines against *Salmonella* diseases is challenging due to its complex lifecycle[11]. The facultative intracellular pathogen enters the host by crossing intestinal epithelial cells via Peyer's patches and infecting monocytes, macrophages, and dendritic cells. The bacteria can then travel to the mesenteric lymph nodes (MLN), spleen, and liver via circulating phagocytes. The host defense mechanisms for clearing *Salmonella* involve stimulation of both the adaptive and innate immune systems[12]. The importance of Th1 cells was shown by depletion of IFN-γ[13,14] and $CD4^+$ T cells have been shown to play a significant role in immunity induced by *Salmonella* flagellin[15,16] and live attenuated *Salmonella*[17]. B cell depletion studies and immunization studies in Ig-deficient mice also indicate the importance of B cells for immunity against *Salmonella*[18,19]. *Salmonella* during its intracellular life in macrophages also induces a modification of lipopolysaccharides (LPS) recognized by TLR-4 and triggers a downstream signaling cascade to evoke host immune response. As a result of these complexities, it is clear that potent antigens as well as adjuvants are important in potentiating the immune response and modulating its quality.

Early efforts to develop an effective vaccine against *Salmonella* began with whole, killed cells, which were shown to be moderately effective in field trials in the 1960s, but most countries have eliminated its use due to undesired side effects. Currently, two licensed commercial vaccines for typhoid fever, a subunit (Vi polysaccharide or Vi PS) and a live attenuated *S.* Typhi strain (Ty21a), are commercially available. However, the use of these vaccines is limited because of the short period of protection and lack of effectiveness in small children. The Vi PS vaccine given in a single dose provides protection for only 3 years, while the live oral vaccine Ty21a requires 3-4 doses for ~7 years of protection. For Ty21a, the limitations include requirement of large numbers (10^9) of bacteria, its inactivation by stomach acidity, and limited period of protection. The Ty21a vaccine cannot be used by children under the age of 6 or immunocompromised individuals. The Vi PS does not induce a switched antibody response, requires frequent boosts, and also cannot be used to immunize infants under the age of 2. Additionally, there are no licensed vaccines against *S.* Paratyphi A or B.

In order to address these limitations, we are developing an improved vaccine utilizing an innovative new adjuvant and antigen delivery system, gas vesicle nanoparticles (GVNPs)[20,21]. To select antigens for display, we conducted