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Haloarchaeal gas vesicle nanoparticles displaying *Salmonella* SopB antigen reduce bacterial burden when administered with live attenuated bacteria

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Abstract

Innovative vaccines against typhoid and other *Salmonella* diseases that are safe, effective, and inexpensive are urgently needed. In order to address this need, buoyant, self-adjuvating gas vesicle nanoparticles (GVNPs) from the halophilic archaeon *Halobacterium* sp. NRC-1 were bioengineered to display the highly conserved *Salmonella enterica* antigen SopB, a secreted inosine phosphate effector protein injected by pathogenic bacteria during infection into the host cell. Two highly conserved *sopB* gene segments near the 3'-coding region, named *sopB4* and *B5*, were each fused to the C-terminal coding region of the *gvpC* gene, and resulting GVNPs were purified by centrifugally accelerated flotation. Display of SopB4 and B5 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 recombinant GVNPs to BALB/c mice which had been immunized with *S. enterica* serovar Typhimurium 14028 *pmrG*-HM-D (DV-STM-07), a live attenuated vaccine strain. Proinflammatory cytokines IFN- γ , IL-2, and IL-9 were significantly

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Contributions

All authors must have materially participated in the research and/or article preparation. PD: participated in research design and execution and manuscript preparation, VDN and AB: conducted the animal research and assisted with manuscript preparation, RK, SB, and FE: conducted molecular and microbial research, DC and SD: were responsible for research design, supervision and manuscript preparation. All authors have approved the final article.

Conflict of Interest

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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 GVNP elicit a protective response to *Salmonella* infection in mice. SopB antigen-GVNPs were found to be stable at elevated temperatures for extended periods without refrigeration in *Halobacterium* cells. The results all together show that bioengineered GVNP are likely to represent a valuable platform for the development of improved vaccines against *Salmonella* diseases.

Keywords

antigen display; nanoparticle; *Salmonella*; Haloarchaea; enteric disease

1. Introduction

Salmonella enterica are Gram-negative pathogenic bacteria which cause enteric diseases that are a serious problem in most developing countries, and are also responsible for occasional deadly outbreaks in developed and industrialized countries [1-4]. *S. enterica* serovar Typhi and Paratyphi, causative agents of typhoid fever, are responsible for global incidence of over 21.7 million cases and 217,000 deaths per year [5], with treatment becoming more challenging due to increased prevalence of antibiotic resistance [6,7]. As a result, improvements leading to vaccines against typhoid fever that are safe, effective, easily available, and inexpensive are critical. Also critical are formulations that permit distribution to regions of the world where cold chains are frequently unavailable.

Inactivated whole-cell vaccine for typhoid fever has been replaced with subunit (Vi polysaccharide or Vi PS) and live attenuated *S. enterica* serovar Typhi (Ty21a) vaccines [2,8]. However, their value is limited because of the short period of protection, need for multiple boosts, as well as the lack of effectiveness in small children [9]. The Vi PS vaccine given in a single injectable subunit dose provides 70% protection for only 3 years, while the live, oral attenuated Ty21a vaccine requires 3-4 doses of liquid vaccine, or 4 doses of capsules for ages 5 and up, resulting in 53-78% protection for ~7 years [2]. Ty21a requires a large dose (10^9 cells) of bacteria, is unstable at unrefrigerated temperatures, and cannot be used by children under the age of 6 or by immunocompromised individuals. Vi PS requires boosts every 2-3 years, and cannot be used to immunize infants under the age of 2, and the emergence of Vi negative *Salmonella* Typhi strains makes it unsuitable for future use [10-11]. Additionally, neither Vi PS nor Ty21a confers protection to *S. Paratyphi* A.

Haloarchaea, such as *Halobacterium* sp. NRC-1, constitute novel cell factories for antigen expression and vaccine delivery with several advantages over conventional hosts, including stability and scalability. They are known to be safe and non-toxic to humans and display many stress tolerant properties, including survival of desiccation, heat, cold, UV, ionizing radiation, and high salinity (~3 - 5 M NaCl) [12,13]. They are genetically engineered through a facile DNA transformation system and well-developed, regulated expression

vectors [14-16]. *Halobacterium* sp. NRC-1 cells are lysed by simple hypotonic conditions, providing an easy method for releasing their cytoplasmic components, including expressed antigenic proteins from pathogenic microbes.

Halobacterium sp. NRC-1 cells contain intracellular buoyancy organelles called gas vesicles, which are novel self-adjuvating and bioengineerable nanoparticles that are being developed as antigen delivery systems (Fig. 1) [17-23]. The nanoparticles are lemon-shaped structures, about 350-450 nm long and 150-250 nm in diameter, and have a thin (20 Å) lipid-free, rigid membrane composed solely of proteins surrounding a gas-filled space. The large number of protein molecules exposed on the surface of the gas vesicle nanoparticles (GVNPs) provides a unique scaffold for the display of antigenic proteins in an ordered array. Moreover, the nanoparticles are easily purified by cell lysis using hypotonic solutions and centrifugally accelerated flotation.

We developed *Halobacterium* sp. NRC-1 GVNPs for antigen delivery via fusion of antigenic sequences to the *gvpC* gene [17,24-27]. The GvpC protein functions by binding to the GVNP surface and contributing to growth and stability of the nanoparticles. GvpC tolerates modification by genetic truncations or fusions to foreign proteins without abrogating gas vesicle formation. Replacement of the C-terminal region of GvpC with foreign proteins results in display of their antigenic sequences on the surface of the nanoparticles. Extensive immunostimulatory effects of GVNPs displaying antigens from SIV and Chlamydia have been studied [26-30].

In order to identify new targets for developing *Salmonella* vaccines, SopB was selected as an antigenic protein candidate coded for in the SPI-1 pathogenicity island of *S. enterica* serovar Typhimurium [31]. SopB is a secreted inositol phosphate phosphatase, which is an effector protein that is essential for host cell invasion via the SPI needle, during the intestinal stage, and causes generation of a favorable vacuole environment where the bacteria can grow and avoid immune defenses [32]. The SopB protein was identified through bioinformatic analysis of MHC-I and MHC-II binding epitopes and tested by administration of live attenuated *Salmonella* followed by SopB DNA vaccines [31,33]. Challenge with a virulent strain of *Salmonella* in a murine model showed that the C-terminal regions of SopB (named B4 and B5) were most effective in reducing the bacterial burden in organs.

In the current study, we have fused highly conserved C-terminal portions of SopB to haloarchaeal gas vesicle nanoparticles and tested their efficacy in antigen delivery and immunostimulation. The results indicate that the nanoparticle platform is highly effective in eliciting a strong immune response and reducing the organ bacterial burden in a murine model of typhoid fever.

2. Materials and methods

2.1. Bioinformatic analysis

For bioinformatic analysis, 100 SopB protein sequences were downloaded from NCBI and after exclusion of 34 partial sequences, the remaining 66 complete sequences were aligned

using Clustal W version 1.83 [34]. Final alignments used SopB sequences from representative *S. enterica* serovars: Typhimurium LT2 (accession number NP_4600640), [35]; Typhi CT18 (accession number NP_455588), [36]; Paratyphi A ATCC 9150 (accession number YP_1509890, [37]; and Paratyphi B SPB7 (accession number YP_001588678).

2.2. Gene design and synthesis

The *sopB* gene from the *S. enterica* serovar Typhimurium LT2 chromosome (NCBI accession number NC_003197.1, nucleotides 1,178,231-1,179,916) was codon-optimized by employing the codon usage table for predicted genes in the fully sequenced *Halobacterium* sp. NRC-1 genome [38]. Sequences for the SopB4 (303 bp) and B5 (501 bp) were synthesized commercially, incorporating flanking *AfeI* sites for cloning (Life Technologies, Carlsbad, CA, USA).

2.3. Cloning, expression, purification, and Western blotting of haloarchaeal nanoparticles

Synthetic SopB4 and B5 gene fragments were cloned into a unique *AfeI* site in the expression plasmid pSD104 in an *E. coli* DH5 α host strain. Constructs were verified by sequencing using external and internal primers (see Supplementary Table 1) to identify presence, orientation, and sequence of regions coding SopB4 and B5. *Halobacterium* sp. SD109 deleted for the *gvp* gene cluster [39] was transformed with pSD104*sopB4* and pSD104*sopB5* using the PEG-EDTA method. Transformants were selected on CM⁺ medium containing 20 μ g/ml mevinolin, DNA was extracted using the method previously described [14], followed by PCR amplification using primers to *gvpC* and *sopB4* and *sopB5* regions. Lawns of the two constructed strains along with *Halobacterium* sp. NRC-1 were plated and grown at 37 °C for 10 days. Nanoparticles were prepared as previously described [21]. Prior to inoculation, aliquots of GVNPs were spun down in microfuge tubes in an Eppendorf 5417C centrifuge (Eppendorf AG, Hauppauge, NY, USA) for 10 minutes, and resuspended in a total volume of 100 μ l PBS (to a final concentration of 1 μ g/ μ l). Western blotting was carried out as previously described using antisera against peptides of SopB4 and SopB5 (EALHQLLGNDLRPEARPGGW and RVGDENIWQSVKGISSLITS, respectively) (Pierce Biotechnology, Inc., Rockford, IL, USA) [40].

2.4. Immunization and organ and sera testing

Cohorts of five 4-6-week-old male and female BALB/c mice were immunized via intraperitoneal (i.p.) route with 0.5 ml PBS containing 10³ cfu attenuated vaccine strain *S. enterica* serovar Typhimurium 14028 *pmrG*-HM-D (DV-STM-07) [33,41]. In one week intervals, mice were injected i.p. with 0.5 ml PBS containing 100 μ g wild-type NRC-1 GVNPs, SopB4-GVNPs, or SopB5-GVNPs in two boosts. Blood was collected by tail-bleeds, prevaccination and seven days post second boost, and sera prepared. Analysis of IFN- γ , TNF- α , IL-2, and IL-9 responses was performed on mouse sera on a Luminex 100 analyzer (Luminex, Austin, TX, USA).

Seven days after the last boost, mice were orally challenged with 10⁸ cfu of virulent *S. enterica* serovar Typhimurium strain 14028 [42]. Mice were sacrificed after 7 days and bacterial burden was determined in liver, mesenteric lymph node (MLN), and spleen by

homogenization of the tissue by bead beater and plating of serial dilutions on *Salmonella-Shigella*-Agar plates (HiMedia, Mumbai, India).

Spleenocytes were isolated by homogenization and treated with red blood cell (RBC) lysis buffer. Cells were counted and 10^6 cells were used for flow cytometric analysis, using FITS and PE conjugated CD4⁺ and CD8⁺ T cell-specific monoclonal antibodies (BD Biosciences, San Jose, CA, USA), respectively. Data was analyzed using WinMDI software as previously described [31].

3. Results

3.1. Conservation of SopB protein

The *Salmonella* SopB protein was previously identified as a potentially useful antigen for display and vaccination [31]. Best protection was observed with the C-terminal regions, named B4 and B5 (amino acids 300-400 and 395-561, respectively) (Fig. 2). When the B4 and B5 regions from diverse *S. enterica* serovars (Typhimurium, Typhi, and Paratyphi A and B) were compared, ~99% conservation was observed. Out of a total of 11 variant positions in the 561 amino acid-long SopB protein, a single variant was found in the B4 region and 2 variants were found in the B5 region. For B4, a serine residue at position 377 was changed to arginine only in *S. Paratyphi A*. In B5, an isoleucine at position 478 in *S. Typhimurium* was changed to leucine in *S. Typhi* and histidine in both *S. Paratyphi A* and B, and a leucine at position 481 was changed to phenylalanine in *S. Typhi*. These results show an extremely high degree of conservation for the SopB protein, especially in the B4 and B5 regions, making these regions attractive for a broadly effective vaccine against *Salmonella* diseases.

3.2. Display of *Salmonella* SopB antigenic polypeptides on haloarchaeal nanoparticles

In order to display the SopB4 and B5 regions on the surface of GVNP in *Halobacterium*, we synthesized codon-optimized gene fragments and cloned them into pSD104 the expression plasmid, in the *gvpC* gene [21,26]. The resulting plasmids, pSD104sopB4 and pSD104sopB5, contained the respective SopB coding regions inserted in-frame. These were transformed into *Halobacterium* sp. SD109, a gas vesicle-deficient (Vac⁻) mutant of the wild-type NRC-1 strain, with a deletion of the entire *gvp* gene cluster [39]. Plasmid-containing strains were grown under mevinolin selection on agar plates, and colonies were examined for a Vac⁺ (pink, turbid, producing GVNP) phenotype [43]. Vac⁺ transformants containing plasmid pSD104sopB4 or pSD104sopB5 were lysed, and GVNP were isolated by centrifugally accelerated flotation [21]. The SopB4 and B5 displaying nanoparticles were electrophoresed on 12 % SDS-polyacrylamide gels and antigenic proteins were identified by Western blotting analysis using rabbit polyclonal antisera prepared against synthetic peptides corresponding to the B4 and B5 regions [31,40]. Proteins of expected sizes were observed by Western analysis in the purified GVNP from the expression strains, *Halobacterium* sp. SD109 (pSD104sopB4) and *Halobacterium* sp. SD109 (pSD104sopB5), but not from the GVNP from the wild-type negative control strain (*Halobacterium* sp. NRC-1) (Fig. 3). These findings confirmed that GVNP displaying SopB4 and B5 were successfully produced and purified.

3.3. Immunization and cytokine response

SopB4 and B5 fragments from the C-terminal regions of SopB were found to be effective antigens in eliciting a cellular immune response and limiting bacterial burden in mice when DNA vaccine vectors were administered i.p. after immunization with the attenuated strain in past work [31]. In order to determine the effect of *Halobacterium* GVNPs displaying SopB4 and B5 antigens, GVNPs were tested in a similar manner, with initial immunization with the attenuated strain followed by boosting with SopB4 and B5-GVNPs. *S. Typhimurium pmrG*-HM-D attenuated strain was administered via the i.p. route with 10^3 cfu followed by boosting twice with 100 µg each of SopB4, B5, or wild-type NRC-1 nanoparticles respectively, 1 and 2 weeks post immunization.

To determine the cytokine response, mice immunized with the attenuated strain and boosted with wild-type, SopB4, or SopB5 GVNPs were bled and sera tested by Luminex analyzer. Among the panel of cytokines tested, the most notable changes were for mice immunized with SopB5-GVNP. Serum levels of IFN- γ , IL-2, and IL-9, were found to be significantly increased in sera from SopB5-GVNP immunized mice compared to pre-immunized sera or wild-type NRC-1 GVNP immunized mice, indicating immunostimulatory effects of the nanoparticles resulting in induction of proinflammatory cytokines involved in response to intracellular bacterial pathogens (Fig. 4). However, no induction for TNF- α was observed (data not shown), consistent with previous observations [31].

3.4. Bacterial burden and T cell response

One week following immunizations and boosting, mice were challenged orally with 10^8 cfu of the virulent *S. Typhimurium* strain STM 14028. The mice were sacrificed one week post-challenge and their liver, spleen, and MLN were isolated. Bacterial burdens were determined for each organ by plating dilutions of tissue homogenate and counting cfu's, normalized to organ weight (cfu per 10 mg). In each of the three organs, bacterial burden was lower by at least two orders of magnitude for mice immunized with SopB5-GVNP (Fig. 5). A similar result of lower bacterial burden was found with SopB4-GVNP immunized mice for spleen. CD4⁺ T-cells were also found to be elevated 2-4 fold in the spleen of mice immunized with SopB5-GVNP compared to wild-type (Fig. 6). These results are consistent with significant immunostimulatory effects of nanoparticles, resulting in reduced bacterial burden in organs, with greater effectiveness observed for SopB5 over SopB4-GVNPs.

4. Discussion

As a new approach toward the goal of a long-lived, effective, and stable vaccine against *Salmonella* [1,2], we employed buoyant gas vesicle nanoparticles from the halophilic archaeon *Halobacterium* sp. NRC-1. We successfully displayed highly conserved *Salmonella* SopB antigens on GVNPs and observed powerful immunostimulatory and protective effects. Proinflammatory cytokines (IFN- γ , IL-2, and IL-9) were significantly induced in sera, CD4⁺ T cells were induced in spleen, and bacterial burden was significantly reduced in liver, MLN, and spleen of mice. All together, our results showed that bioengineered GVNPs displaying *Salmonella* antigens may be valuable for development of new and innovative vaccines.

A most remarkable aspect of the GVNP platform is that it represents a versatile antigen display system from *Halobacterium* sp. NRC-1, a nonpathogenic model microorganism that is a member of the 3rd Domain of life, the Archaea [12]. GVNP display unusual and useful properties, including extreme stability, high immunogenicity, and ease of genetic engineerability, as well as abundance and relative ease of purification. Their size (~150-450 nm), shape (lemon or spindle), and composition (proteinaceous and lipid-free) all suggest that they may be useful for antigen delivery. Our findings with GVNP displaying fragments of diverse antigenic proteins, fused to GvpC, including *Salmonella* SopB provided in this report, confirms that the nanoparticles are indeed valuable for antigen presentation [26,27]. The yields of SopB nanoparticles were also found to be impressive and sufficient quantities for testing resulted from ordinary laboratory sized cultures. The use of codon-optimized gene fragments likely contributed to improved yields not seen in past work. The SopB nanoparticles were also found to be stable over extended periods of time and had very long shelf lives, persisting in the haloarchaeal host cells for months at room temperature and weeks at elevated temperatures (45 °C) in a purified form (our unpublished results). These findings suggest that the GVNP are likely to be candidates for distribution in the absence of a cold chain, and therefore should be valuable for addressing the need for shelf-stable vaccines in tropical endemic areas of the world.

The immunogenicity of the SopB5-GVNP was found to be highly effective in producing desired immunostimulatory responses necessary for combating enteropathogens like *S. Typhi* that exhibit both intracellular and extracellular phases in their lifestyles. Past work has shown that GVNP are taken up by macrophages and slowly processed (over the course of 3-5 days) for presentation [29]. Specific induction of IgG1 was found, as was induction of IL-8, a proinflammatory cytokine [44], IL-10 an anti-inflammatory and immunosuppressive Th2 cytokine [45], and IL-18, a cytokine known to induce both Th1 and 2. The current work with GVNP extends these findings, with additional significant induction of proinflammatory cytokines observed, including IFN- γ and IL-9, which correlate with induction of the Th1 pathway, and IL-2, which has broader implications, including priming and maintaining both Th1 and Th2 differentiation [46,47].

S. Typhimurium is known to produce systemic disease in mice, similar to typhoid, resulting from *S. Typhi* in humans. *S. Typhimurium* administered orally spreads from the intestines via the MLN to the liver and spleen [48,49]. Remarkably, recovery of virulent *S. Typhimurium* from these key organs was found to be significantly reduced or abrogated when mice, immunized with attenuated *S. Typhimurium*, were boosted with SopB5-GVNP. These findings strongly suggest that the SopB5 nanoparticles elicit important immune responses that have the potential to prevent systemic disease in mice. Previous studies have shown that oral delivery of a single dose of an attenuated *Salmonella* strain resulted in *Salmonella*-specific IgM, IgG2c, and intestinal IgA production, and that further boosts did not alter the levels. However, when delivered intravenously, IgM, IgG2c antibodies were induced and increased further with boosting, indicating systemic B-cell response, leading to protection [50]. Furthermore, in TLR11⁻ mice, which are susceptible to *S. Typhi* infection, IP administration of heat-killed wild type *S. Typhi* resulted in protection and elevated levels of IgG and IgA, and CD4⁺ T cells [51]. Our findings are consistent with CD4⁺ T cell

induction in the spleen and robust Th1 and humoral responses after SopB5-GVNP boosts, resulting in a protective immune response effectively targeting and limiting *Salmonella* burden in key organs.

The present study provides the foundation for the development of a new, stable, innovative vaccine platform for protection against *Salmonella* diseases, capitalizing on the immunostimulatory activities of buoyant gas vesicle nanoparticles from *Halobacterium* sp. NRC-1. The advantages of this GVNP antigen delivery platform for eliciting protection and addressing stability in endemic areas lacking a cold chain appear to be potentially extremely valuable. The protective properties of recombinant GVNPs may be combined with other existing approaches, such as live attenuated vaccine strains as demonstrated here, and offer significant improvements over current vaccines. Our recent ability to bioengineer GVNPs with multiple proteins offers the possibility to develop multivalent GVNPs [17], which potentially allow us to extend the capabilities of the system by incorporating additional T cell epitopes, resulting in costimulatory signals and improved protection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. WHO. Typhoid vaccines. Wkly. Epidemiol. Rec. 2000; 75:257–264. [PubMed: 10974947]
2. WHO. Typhoid vaccines. Wkly. Epidemiol. Rec. 2008; 83:49–58. [PubMed: 18260212]
3. Hohmann EL. Nontyphoidal salmonellosis. Clin. Infect. Dis. 2001; 32:263–269. [PubMed: 11170916]
4. Mastroeni, P.; Maskell, D. *Salmonella* infections: clinical, immunological, and molecular aspects. Cambridge University Press; Cambridge, UK: 2006.
5. Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. Bull. World Health Organ. 2004; 82:346–353. [PubMed: 15298225]
6. Helms M, Ethelberg S, Molbak K. International *Salmonella* Typhimurium DT104 infections, 1992–2001. Emerg. Infect. Dis. 2005; 11:859–867. [PubMed: 15963280]
7. Poppe C, Smart N, Khakhria R, Johnson W, Spika J, Prescott J. *Salmonella* Typhimurium DT104: a virulent and drug-resistant pathogen. Can. Vet. J. 1998; 39:559–565. [PubMed: 9752592]
8. Fraser A, Paul M, Goldberg E, Acosta CJ, Leibovici L. Typhoid fever vaccines: systematic review and meta-analysis of randomised controlled trials. Vaccine. 2007; 25:7848–7857. [PubMed: 17928109]
9. McGregor AC, Waddington CS, Pollard AJ. Prospects for prevention of *Salmonella* infection in children through vaccination. Curr. Opin. Infect. Dis. 2013; 26:254–262. [PubMed: 23591641]

10. Pulickal AS, Callaghan MJ, Kelly DF, Maskey M, Mahat S, Hamaluba M, Dongol S, Adhikari N, Thorson S, Basynat B, Murdoch DR, Farrar JJ, Pollard AJ. Prevalence and genetic analysis of phenotypically Vi- negative *Salmonella* typhi isolates in children from Kathmandu. Nepal. J. Trop. Pediatr. 2013; 59:317–320. [PubMed: 23598894]
11. Martin LB. Vaccines for typhoid fever and other salmonellos. Curr. Opin. Infect. Dis. 2012; 25:489–499. [PubMed: 22825288]
12. DasSarma, S.; DasSarma, P. Encyclopedia of Life Sciences. Wiley; London: 2012. Halophiles.. DOI: 10.1002/9780470015902.a0000394.pub3
13. DasSarma S, Berquist BR, Coker JA, DasSarma P, Müller JA. Post-genomics of the model haloarchaeon *Halobacterium* sp. NRC-1. Saline Syst. 2006; 2:3. [PubMed: 16542428]
14. Berquist, BR.; Müller, JA.; DasSarma, S. Genetic systems for halophilic archaea.. In: Oren, A.; Rainey, F., editors. Methods in microbiology. Vol. 35. Elsevier/Academic; London: 2006. p. 649-680.
15. Karan R, Capes MD, DasSarma P, DasSarma S. Cloning, overexpression, purification, and characterization of a polyextremophilic β -galactosidase from the Antarctic haloarchaeon *Halorubrum lacusprofundi*. BMC Biotechnol. 2013a; 13:3. [PubMed: 23320757]
16. Karan R, DasSarma P, Balcer-Kubiczek E, Weng RR, Liao CC, Goodlett DR, Ng WV, DasSarma S. Bioengineering radioresistance by overproduction of RPA, a mammalian-type single-stranded DNA-binding protein, in a halophilic archaeon. Appl Microbiol Biotechnol. 2013b [Epub ahead of print] PubMed PMID:24292079.
17. DasSarma S, Karan R, DasSarma P, Barnes S, Ekulona F, Smith B. An improved genetic system for bioengineering buoyant gas vesicle nanoparticles from Haloarchaea. BMC Biotech. 2013; 13:112.
18. DasSarma S, Arora P. Genetic analysis of the gas vesicle gene cluster in haloarchaea. FEMS Microbiol. Lett. 1997; 153:1–10.
19. Shively, JM.; Cannon, GC.; Heinhorst, S.; Bryant, DA.; DasSarma, S.; Bazylnski, D.; Preiss, J.; Steinbüchel, A.; Docampo, R.; Dahl, C. Encyclopedia of Life Sciences. Wiley; London: 2011. Bacterial and Archaeal Inclusions.. DOI: 10.1002/9780470015902.a0000302.pub3
20. Cai L, Zhao DH, Hou J, Wu JH, Cai SF, DasSarma P, Xiang H. Cellular and organellar membrane-associated proteins in haloarchaea: Perspectives on the physiological significance and biotechnological applications. Science China Life Sci. 2012; 55:404–414.
21. Halladay JT, Ng W-L, DasSarma S. Genetic transformation of a halophilic archaebacterium with a gas vesicle gene cluster restores its ability to float. Gene. 1992; 119:131–136. [PubMed: 1398080]
22. Belenky M, Meyers R, Herzfeld J. Subunit structure of gas vesicles: a MALDI-TOF mass spectrometry study. Biophys. J. 2004; 86:499–505. [PubMed: 14695294]
23. Chu LJ, Chen MC, Setter J, Tsai YS, Yang H, Fang X, Ting YS, Shaffer SA, Taylor GK, von Haller PD, Goodlett DR, Ng WV. New structural proteins of *Halobacterium salinarum* gas vesicle revealed by comparative proteomics analysis. J. Proteome. Res. 2011; 10:1170–1178. [PubMed: 21158390]
24. DasSarma S, Arora P, Lin F, Molinari E, Yin LR. Wild-type gas vesicle formation requires at least ten genes in the gvp gene cluster of *Halobacterium halobium* plasmid pNRC100. J. Bacteriol. 1994; 176:7646–7652. [PubMed: 8002589]
25. Halladay JT, Jones JG, Lin F, MacDonald AB, DasSarma S. The rightward gas vesicle operon in *Halobacterium halobium* plasmid pNRC-100: identification of the gvpA and gvpC gene products by use of antibody probes and genetic analysis of the region downstream of gvpC. J. Bacteriol. 1993; 175:684–692. [PubMed: 8423144]
26. Stuart ES, Morshed F, Sremac M, DasSarma S. Antigen presentation using novel particulate organelles from halophilic archaea. J. Biotechnol. 2001; 88:119–128. [PubMed: 11403846]
27. Stuart ES, Morshed F, Sremac M, DasSarma S. Cassette-based presentation of SIV epitopes with recombinant gas vesicles from halophilic archaea. J. Biotech. 2004; 114:225–237.
28. Sremac M, Stuart ES. Recombinant gas vesicles from *Halobacterium* sp. displaying SIV peptides demonstrate biotechnology potential as a pathogen peptide delivery vehicle. BMC Biotech. 2008; 8:9.

29. Sremac M, Stuart ES. SIVsm Tat, Rev, and Nef1: functional characteristics of r-GV internalization on isotypes, cytokines, and intracellular degradation. *BMC Biotech.* 2010; 10:54.
30. Childs TS, Webley WC. In vitro assessment of halobacterial gas vesicles as a *Chlamydia* vaccine display and delivery system. *Vaccine.* 2012; 30:5942–5948. [PubMed: 22846397]
31. Nagarajan AG, Balasundaram SV, Janice J, Karnam G, Eswarappa SM, Chakravorty D. sopB of *Salmonella enterica* serovar Typhimurium is a potential DNA vaccine candidate in conjugation with live attenuated bacteria. *Vaccine.* 2009; 27:2804–2811. [PubMed: 19428891]
32. Agbor TA, McCormick BA. Salmonella effectors: important players modulating host cell function during infection. *Cell Microbiol.* 2011; 13:1858–1869. [PubMed: 21902796]
33. Negi VD, Singhamahapatra S, Chakravorty D. *Salmonella enterica* serovar Typhimurium strain lacking pmrG-HM-D provides excellent protection against salmonellosis in murine typhoid model. *Vaccine.* 2007; 25:5315–5323. [PubMed: 17574312]
34. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucl. Acids Res.* 1994; 22:4673–4680. [PubMed: 7984417]
35. McClelland M, Sanderson KE, Spieth J, Clifton SW, Latreille P, Courtney L, Porwollik S, Ali J, Dante M, Du F, Hou S, Layman D, Leonard S, Nguyen C, Scott K, Holmes A, Grewal N, Mulvaney E, Ryan E, Sun H, Florea L, Miller W, Stoneking T, Nhan M, Waterston R, Wilson RK. Complete genome sequence of *Salmonella enterica* serovar Typhimurium LT2. *Nature.* 2001; 413:852–856. [PubMed: 11677609]
36. Parkhill J, Dougan G, James KD, Thomson NR, Pickard D, Wain J, Churcher C, Mungall KL, Bentley SD, Holden MT, Sebaihia M, Baker S, Basham D, Brooks K, Chillingworth T, Connerton P, Cronin A, Davis P, Davies RM, Dowd L, White N, Farrar J, Feltwell T, Hamlin N, Haque A, Hien TT, Holroyd S, Jagels K, Krogh A, Larsen TS, Leather S, Moule S, O'Gaora P, Parry C, Quail M, Rutherford K, Simmonds M, Skelton J, Stevens K, Whitehead S, Barrell BG. Complete genome sequence of a multiple drug resistant *Salmonella enterica* serovar Typhi CT18. *Nature.* 2001; 413:848–852. [PubMed: 11677608]
37. McClelland M, Sanderson KE, Clifton SW, Latreille P, Porwollik S, Sabo A, Meyer R, Bieri T, Ozersky P, McLellan M, Harkins CR, Wang C, Nguyen C, Berghoff A, Elliott G, Kohlberg S, Strong C, Du F, Carter J, Kremizki C, Layman D, Leonard S, Sun H, Fulton L, Nash W, Miner T, Minx P, Delehaunty K, Fronick C, Magrini V, Nhan M, Warren W, Florea L, Spieth J, Wilson RK. Comparison of genome degradation in Paratyphi A and Typhi, human-restricted serovars of *Salmonella enterica* that cause typhoid. *Nat. Genet.* 2004; 36:1268–1274. [PubMed: 15531882]
38. Kennedy SP, Ng WV, Salzberg SL, Hood L, DasSarma S. Understanding the Adaptation of *Halobacterium* Species NRC-1 to its extreme environment through computational analysis of its genome sequence. *Genome Res.* 2001; 11:1641–1650. [PubMed: 11591641]
39. Ng W-L, Arora P, DasSarma S. Large deletions in Class III Gas Vesicle-Deficient Mutants of *Halobacterium halobium*. *Syst. Appl. Microbiol.* 1994; 16:560–568.
40. Shukla HD, DasSarma S. Complexity of gas vesicle biogenesis in *Halobacterium* sp. strain NRC-1: Identification of five new proteins. *J. Bacteriol.* 2004; 186:3182–3186. [PubMed: 15126480]
41. Negi VD, Nagarajan AG, Chakravorty D. A safe vaccine (DV-STM-07) against *Salmonella* infection prevents abortion and confers protective immunity to the pregnant and new born mice. *PLoS One.* 2010; 5(2):e9139. [PubMed: 20161765]
42. Jarvik T, Smillie C, Groisman EA, Ochman H. Short-term signatures of evolutionary change in the *Salmonella enterica* serovar Typhimurium 14028 Genome. *J. Bacteriol.* 2010; 192:560–567. [PubMed: 19897643]
43. DasSarma S, Halladay JT, Jones JG, Donovan JW, Giannasca PJ, Tandeau de Marsac N. High-frequency mutations in a plasmid-encoded gas vesicle gene in *Halobacterium halobium*. *Proc. Natl. Acad. Sci. USA.* 1988; 85:6861–6865. [PubMed: 16593983]
44. D'Elia RV, Harrison K, Oyston PC, Lukaszewski RA, Clark GC. Targeting the “cytokine storm” for therapeutic benefit. *Clin. Vaccine Immunol.* 2013; 20:319–327. [PubMed: 23283640]
45. Dinarello CA. Historical insights into cytokines. *Eur. J. Immunol.* 2007; 37:S34–45. [PubMed: 17972343]

46. Cope A, Le Friec G, Cardone J, Kemper C. The Th1 life cycle: molecular control of IFN- γ to IL-10 switching. *Trends Immunol.* 2011; 32:278–286. [PubMed: 21531623]
47. Liao W, Lin JX, Leonard WJ. IL-2 family cytokines: new insights into the complex roles of IL-2 as a broad regulator of T helper cell differentiation. *Curr. Opin. Immunol.* 2011; 23:598–604. [PubMed: 21889323]
48. Voedisch S, Koenecke C, David S, Herbrand H, Förster R, Rhen M, Pabst O. Mesenteric lymph nodes confine dendritic cell-mediated dissemination of *Salmonella enterica* serovar Typhimurium and limit systemic disease in mice. *Infect. Immun.* 2009; 77:3170–3180. [PubMed: 19506012]
49. Griffin AJ, Li LX, Voedisch S, Pabst O, McSorley SJ. Dissemination of persistent intestinal bacteria via the mesenteric lymph nodes causes typhoid relapse. *Infect. Immun.* 2011; 79:1479–1488. [PubMed: 21263018]
50. Lee S-L, Liang L, Juarez S, Nanton MR, Gondwe EN, Msefula CL, Kayala MA, Necchi F, Heath JN, Hart P, Tsolis RM, Heyderman RS, MacLennan CA, Felgner PL, Davies DH, McSorley SJ. Identification of a common immune signature in murine and human systemic Salmonellosis. *Proc. Nat. Acad. Sci. USA.* 2012; 109:4998–500. [PubMed: 22331879]
51. Mathur R, Oh H, Zhang D, Park S-G, Seo J, Koblansky A, Hayden MS, Ghosh S. A mouse model of *Salmonella* Typhi infection. *Cell.* 2012; 151:590–602. [PubMed: 23101627]

Highlights

Made a significant advancement toward an improved *Salmonella* vaccine

Bioengineered a buoyant, stable, and safe nanoparticle from a salt-loving microbe

Displayed highly conserved *Salmonella* SopB antigenic proteins on the nanoparticles

Mice immunized with SopB-nanoparticles showed negligible bacterial burden in organs

Robust immune response including CD4+T cells and proinflammatory cytokines observed

Fig. 1. *Halobacterium* sp. NRC-1 gas vesicle nanoparticles (GVNPs). A. Phase contrast micrograph of purified GVNPs, which appear as phase bright dots (bar is 112.5 μ m). B. Transmission electron micrograph of negatively stained GVNPs (bar is 500 nm) [17].

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300      310      320      330      340      350      360      370      380
S. Typhimurium str. LT2  SVGLLTASNIFGKECTMVEDQMRWQSLTQPKMIHLKIRNKDGLQTVKIKPDVAAFNVGVNELALKLGFGLKASDSYNAREALHQLLGN
S. Typhi str. CT18      .....R.....
S. Paratyphi A str. 9150 .....
S. Paratyphi B str. SPB7 .....

390      400      410      420      430      440      450      460      470
S. Typhimurium str. LT2  DLRPFARGGGRVGEVLAQYDDNYEVVNTLARIKDIWNQNHKDKGGEVYLAQRLAMLAHEIDAVPANNCKSGKDKTGMDSREIKREII
S. Typhi str. CT18      .....L.....
S. Paratyphi A str. 9150 .....H.....
S. Paratyphi B str. SPB7 .....H.....

480      490      500      510      520      530      540      550      560
S. Typhimurium str. LT2  SLHQTTHMLSAFGSLPDSGGKIFQKVLNSGNLEIQKNTGGAGNKVAKNLSPEVLNLSYQKRVGDEINWQSVKGISLLITS
S. Typhi str. CT18      .....F.....
S. Paratyphi A str. 9150 .....
S. Paratyphi B str. SPB7 .....

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Fig. 2.

Aligned partial sequences of SopB proteins from *Salmonella enterica* serovar Typhimurium, Typhi and Paratyphi. The neighbor-joining alignment is shown for the B4 (underlined) and B5 (broken underlined) regions from *S. enterica* serovar Typhimurium LT2 (S. Typhimurium LT2), *S. enterica* serovar Typhi CT18 (S. Typhi CT18), *S. enterica* serovar Paratyphi A ATCC 9150 (S. Paratyphi A), and *S. enterica* serovar Paratyphi B SPB7 (S. Paratyphi B). A six amino acid region of overlap is indicated by the heavy underline.

Fig. 3.

Western blotting analysis of *Halobacterium* GVNPs. A. Blot probed with SopB4 antiserum is shown between molecular weight markers 70 KDa and 100 KDa (lane labeled MW) for *Halobacterium* wild-type (lane labeled NRC-1) and *Halobacterium* SD109 (pSD104dsopB4) (lane labeled B4) GVNPs. B. Blot probed with SopB5 antiserum is shown between molecular weight markers 70 KDa and 100 KDa (lane labeled MW) for *Halobacterium* wild type (lane labeled NRC-1) and *Halobacterium* SD109 (pSD104dsopB5) (lane labeled B5) GVNPs.

Fig. 4.

ELISAs for proinflammatory cytokines, IFN- γ , IL-2 and IL-9 in GVNP immunized mouse sera. Concentrations of cytokines (pg/ml) are indicated on the vertical axes and the types of GVNP used for immunizations indicated in the horizontal axes. Dark gray bars denote preimmune sera and light gray bars, sera postimmunization. NRC: Wild type *Halobacterium* sp. NRC-1 GVNP; B4: *Halobacterium* SD109 (pSD104dsopB4) GVNP; B5: *Halobacterium* SD109 (pSD104dsopB5) GVNP. Standard deviations are indicated by error bars.

Fig. 5.

Bacterial burden in the liver, MLN and spleen of immunized and challenged mice. The cfu numbers are plotted on the vertical axes. GVNP identities are plotted on the horizontal axes. Cohorts of 5 mice were used in each case, with average values for surviving mice indicated by “X” and with standard deviations indicated by error bars. All mice were initially immunized with attenuated *S. enterica* serovar Typhimurium 14028 *pmrG*-HM-D and challenged with virulent *S. enterica* serovar Typhimurium 14028 orally.

Fig. 6. CD4⁺ T cell assay for immunized mice. Flow cytometric analysis of spleenocytes are shown with CD4⁺ T cell-specific monoclonal antibodies of animals immunized with wild-type (WT), SopB4-GVNP (B4), and SopB5-GVNP (B5) (on the horizontal axis), with percent cell population plotted (on the vertical axis). Standard deviations are indicated by error bars.