Argomenti per il primo gruppo di tesi
Vaccini per il cancro 1.
PD-1-siRNA delivered by attenuated *Salmonella* enhances the antimelanoma effect of pimozone

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Abstract
Melanoma is one of the most aggressive skin cancers worldwide. Although there has been much effort toward improving treatment options over the past few years, there remains an urgent need for effective therapy. Immunotherapy combined with chemotherapy has shown great promise in clinical trials. Here, we studied the cooperative effects of the small molecule drug pimozone, which has a therapeutic effect in melanoma, and RNA interference (RNAi) targeting PD-1, an important immune checkpoint molecule involved in tumor immune escape. PD-1 siRNA was delivered by attenuated *Salmonella* to melanoma-bearing mice in combination with pimozone. Our results demonstrated that the combination therapy had the optimal therapeutic effect on melanoma. The mechanisms underlying the effect involved the induction of apoptosis and an enhanced immune response. This study suggests that immunotherapy based on PD-1 inhibition combined with anticancer drugs could be a promising clinical strategy for the treatment of melanoma.

Introduction
Metastatic melanoma is one of the most aggressive skin cancers worldwide, and there is no effective treatment currently1. Surgical resection remains the cornerstone of curative treatment at the early stages of the disease but offers only a small chance for curing metastatic melanoma. The addition of radiotherapy and chemotherapy is not effective2. As a result, the prognosis of metastatic melanoma is poor, with an average survival time of less than 1 year3. Therefore, more effective treatment strategies for melanoma are urgently required. Pimozone, a Food and Drug Administration (FDA)-approved psychiatric drug and effective dopamine antagonist, was first administered to patients with metastatic melanoma as early as 19794. Previous studies by us and other researchers have shown that pimozone has certain therapeutic effects on melanoma5,6. Although favorable responses have been documented, the therapeutic effect must be further improved. Recent studies revealed a promising strategy of combining immunotherapy with chemotherapy, which may further improve cancer treatment.

Immunotherapy has been successfully applied to the treatment of several human cancers7. The blockade of immune checkpoints, a newly emerging idea in antitumor immunotherapy, has exhibited curative effects and thus has potential as a new way to cure cancer8,9. Programmed death 1 (PD-1) is an important immune checkpoint molecule that can enable tumor cells to escape the host immune response through the suppression of effector T-cell function and the induction of T-cell exhaustion10,11. In addition, multiple basic research and clinical studies have
Cancer Immunotherapy: Priming the Host Immune Response with Live Attenuated Salmonella enterica

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In recent years, cancer immunotherapy has undergone great advances because of our understanding of the immune response and the mechanisms through which tumor cells evade it. A century after the first immunotherapy attempt based on bacterial products described by William Coley, the use of live attenuated bacterial vectors has become a promising alternative in the fight against cancer. This review describes the role of live attenuated Salmonella enterica as an oncolytic and immunotherapeutic agent, due to its high affinity for tumor tissue and its ability to activate innate and adaptive antitumor immune response. Furthermore, its potential use as delivery system of tumor antigens and immunomodulatory molecules that induce tumor regression is also reviewed.

1. Introduction

Cancer is among the first causes of death in millions of individuals throughout the world [1]. The development of adverse effects and resistance to chemotherapy and radiotherapy, as well as the difficulty inherent to the elimination of metastatic cells, are some of the elements that underscore the need to search for better treatment alternatives with greater selectivity and effectiveness against tumor cells. Recent studies have documented the crucial role of the immune response in the elimination of tumors [2]; this fact has allowed to propose immunotherapy as an encouraging alternative in cancer treatment [3], by potentiating the host immune response activation or by acting in synergy with conventional treatments. In this context, the concept of using bacteria as agents against cancer described over a century ago [4] recently has generated great interest, as a result of the development of live attenuated bacterial vectors safe for human use, such as Salmonella enterica. This bacterium has proven usefulness in antitumoral therapy, by inducing innate and adaptive immune response in preclinical and clinical assays, which has led the tumor elimination without secondary effects [5], making Salmonella enterica a great candidate to cancer immunotherapy.

1.1. Bacteria in Antitumor Immunotherapy. The association of bacteria and antitumor activity was described in 1813, with observations of Vautier on tumor regression in patients with gangrene after Clostridium perfringens infection [6]. Subsequent studies by Coley, documented since 1890, demonstrated that “Coley’s toxin,” constituted by Streptococcus pyogenes and Serratia marcescens, could immunotherapeutically treat patients with sarcomas, lymphomas, myelomas, and melanomas [4, 7]. Research initiated by Holmgren in 1935 [8], on the antitumor activity of the attenuated strain of Mycobacterium bovis, the Calmette-Guérin Bacillus (BCG), culminated in this approval of this strain in 1976, for intravesical application in patients with bladder superficial transitional cell carcinoma [9], a treatment modality that is currently still in use.

To date, the immunotherapeutic antitumor effect of bacteria has been proven in the genus Bifidobacterium, Clostridium, Listeria, Escherichia, and Salmonella [10, 11].
Review

Listeria monocytogenes as a Vector for Cancer Immunotherapy: Current Understanding and Progress

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Abstract: Listeria monocytogenes, a Gram-positive facultative anaerobic bacterium, is becoming a popular vector for cancer immunotherapy. Indeed, multiple vaccines have been developed utilizing modified Listeria as a tool for generating immune responses against a variety of cancers. Moreover, over a dozen clinical trials testing Listeria cancer vaccines are currently underway, which will help to understand the utility of Listeria vaccines in cancer immunotherapy. This review aims to summarize current views on how Listeria-based vaccines induce potent antitumor immunity and the current state of Listeria-based cancer vaccines in clinical trials.

Keywords: Listeria; cancer; vaccine; immunotherapy; bacteria

1. Introduction

The proficiency of the immune system to recognize and eliminate cancer cells, a process summarily called immunosurveillance, is well documented [1]. Failure of immunosurveillance enables the clinical development of cancer and has motivated the search for strategies to rearm and restore effective immune responses to malignant cells. One such strategy includes vaccine development against cancer. In general, cancer vaccines are composed of antigens found in tumor cells, often referred to as tumor-associated antigens (TAA), paired with adjuvants designed to induce an immune response. Antigen-specific T-cell responses induced by cancer vaccines have the potential to produce more targeted elimination of cancer cells than conventional chemotherapy, as well as lead to durable memory responses capable of challenging cancer recurrence. This review aims to summarize one particular approach to cancer vaccination, employing Listeria monocytogenes vectors, and its current progress in terms of preclinical development and clinical trials.

Listeria monocytogenes (Lm) is a Gram-positive bacteria most widely known for its ability to infect humans and produce a variety of symptoms, including gastroenteritis, meningitis, and encephalitis [2]. In general, the human immune system mounts potent innate and adaptive immune responses capable of controlling Lm infections. As a result, serious infection by Lm is rare and typically limited to elderly, pregnant, or immunocompromised patients [2]. Decades worth of research on the properties that make Lm immunogenic and how these properties can be exploited have led to its advancement as a vaccine platform for cancer immunotherapy in clinical trials.

Lm has numerous features that make it an attractive vector for cancer immunotherapy. While other vectors may be inhibited through neutralizing antibodies, Lm infection triggers only modest humoral responses which fail to block reinfection [3,4]. This permits repeated administration of Lm-based
Fattori che influenzano i vaccini e generici effetti di un vaccino. 2
Factors That Influence the Immune Response to Vaccination

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SUMMARY ................................................................................................................................. 2
INTRODUCTION ...................................................................................................................... 2
FACTORs INFLUENCING VACCINE RESPONSES .................................................................. 2
Intrinsic Host Factors ............................................................................................................. 2
  Age ................................................................................................................................. 2
  Sex ................................................................................................................................. 5
  Genetics .......................................................................................................................... 5
  Comorbidities .................................................................................................................. 5
Perinatal Host Factors .......................................................................................................... 10
  Gestational age ............................................................................................................. 10
  Birth weight .................................................................................................................. 12
  Breastfeeding ............................................................................................................... 12
  Maternal antibodies ...................................................................................................... 12
  Maternal infections during pregnancy .......................................................................... 13
  Other maternal factors ................................................................................................. 14
Extrinsic Factors .................................................................................................................. 14
  Infections ...................................................................................................................... 14
  Parasites ....................................................................................................................... 16
  Antibiotics, probiotics, and prebiotics ......................................................................... 16
  Microbiota ..................................................................................................................... 20
  Preexisting immunity ................................................................................................. 21
Behavioral Factors .............................................................................................................. 21
  Smoking ....................................................................................................................... 21
  Alcohol consumption ................................................................................................. 21
  Exercise ....................................................................................................................... 21
  Acute psychological stress ......................................................................................... 21
  Chronic psychological stress ...................................................................................... 24
  Sleep ............................................................................................................................. 24
Nutritional Factors .............................................................................................................. 24
  Body mass index ........................................................................................................ 24
  Nutritional status ......................................................................................................... 25
  Micronutrients (vitamins A, D, and E and zinc) .......................................................... 25
  Enteropathy ................................................................................................................ 25
Environmental Factors ...................................................................................................... 25
  Rural versus urban environment ................................................................................ 25
  Geographic location ................................................................................................... 27
  Season .......................................................................................................................... 27
  Family size .................................................................................................................. 27
  Toxins .......................................................................................................................... 27
Vaccine Factors ................................................................................................................... 27
  Vaccine type, product, and strain ............................................................................... 27
  Adjuvants ..................................................................................................................... 27
  Vaccine dose ............................................................................................................... 29
  Administration Factors .............................................................................................. 29
  Vaccination schedule ................................................................................................. 29
  Vaccination site .......................................................................................................... 29
  Vaccination route ....................................................................................................... 29
  Needle size .................................................................................................................. 30
  Time of day ................................................................................................................ 30
  Coadministered vaccines ......................................................................................... 30
  Coadministered drugs ............................................................................................... 31
(continued)
Nonspecific effects of oral vaccination with live-attenuated *Salmonella Typhi* strain Ty21a

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Epidemiological and immunological evidence suggests that some vaccines can reduce all-cause mortality through nonspecific changes made to innate immune cells. Here, we present the first data to describe the nonspecific immunomodulatory impact of oral vaccination with live-attenuated *Salmonella Typhi* strain Ty21a. We vaccinated healthy adults with Ty21a and assessed aspects of innate and adaptive immunity over the course of 6 months. Changes to monocyte phenotype/function were observed for at least 3 months. Changes to innate and adaptive immune cell cytokine production in response to stimulation with vaccine and unrelated nonvaccine antigens were observed over the 6-month study period. The changes that we have observed could influence susceptibility to infection through altered immune responses mounted to subsequently encountered pathogens. These changes could influence all-cause mortality.

**INTRODUCTION**

Epidemiological evidence has demonstrated that live-attenuated vaccines can reduce all-cause mortality (1, 2). The strongest evidence has been collected in resource-poor settings after vaccination with oral polio vaccine (OPV), Bacille Calmette-Guérin (BCG), and measles-containing vaccines (3–7). Recently, the World Health Organization Special Advisory Group of Experts has recommended that further research should be undertaken to better understand the nonspecific impact of vaccination on all-cause mortality (8, 9).

The effects of vaccination are believed to be the result of the generation of innate immune memory through epigenetic modification (10–12). These changes may manifest themselves in the form of phenotypic variation among circulating innate cell populations, as well as altered cytokine production in response to in vitro cell stimulation (13).

Toll-like receptor 5 (TLR-5) engagement at the mucosal surface of the gastrointestinal tract is known to enhance immune responses to influenza vaccination (14). It has been demonstrated that the live-attenuated oral *Salmonella* vaccine, Ty21a, has the capacity to aid in the regression of bladder cancer, which is believed to be the result of TLR engagement (15). We have also observed enhanced cellular responses to influenza virus at the duodenal mucosa 18 days after vaccination with Ty21a (16). We therefore hypothesized that oral vaccination with Ty21a may have the capacity to generate innate immune memory and alter immune responses to unrelated pathogens.

A number of *Salmonella*-based vectors are in development (17, 18) and an increased understanding of the off-target impacts of live-attenuated strains of *Salmonella* could further inform their development. The identification of bacteria that generate innate immune memory may lead to the development of therapeutics, vectors, and adjuvants capable of reducing all-cause mortality and/or enhancing the efficacy of vaccines targeting a wide array of unrelated pathogens.

We have previously shown that vaccination with Ty21a generates long-lived vaccine-specific peripheral immune responses (19). Here, we have assessed the wider, off-target impact of vaccination with Ty21a on human immunity at 14 days, 3 months, and 6 months after vaccination. We assessed innate immune cell surface marker expression as well as interferon-γ (IFN-γ) [T helper 1 (Th1)], interleukin-4 (IL-4) (Th2), IL-17A (Th17), transforming growth factor-β (TGF-β) (regulatory), and tumor necrosis factor-α (TNF-α) (Th1) production among B cells, CD4+ T cells, CD8+ T cells, monocytes, mucosal-associated invariant T (MAIT) cells, and γδ T cells after in vitro stimulation with a range of antigens.

**RESULTS**

**Volunteer recruitment**

Volunteers aged between 18 and 60 years were invited to participate in this study. Volunteers were excluded from participation if they had previously been immunized with Ty21a or if they had previously visited a typhoid-endemic region (Asia or sub-Saharan Africa). Volunteers were also excluded if they were pregnant, if they were taking any immunosuppressive medications, or if they had a chronic illness. There were no substantial differences between the groups with regard to age or gender; however, it should be noted that considerably more females than males were recruited to both the vaccinated group and the control group (Table 1).

**Monocyte phenotype**

We measured the relative expression intensity of CD11b (integrin αM), CD11c (integrin αD), CD14, CD16 (FcεRII), CD18 (integrin β2), CD64 (FcγRI), CD123 (IL-3RA), CD206 (mannose receptor), CD303 (BDCA-2), HLA-DR (human leukocyte antigen - DR isotype), TLR-4, and TLR-5 on the surface of nonstimulated CD14+ monocytes from vaccinated and unvaccinated volunteers by flow cytometry. A sequential gating strategy was used to identify populations of interest (Fig. 1). We compared the phenotypic properties of cells isolated.
Vaccini e nanoparticelle. 3 (x2)
Nanovaccine: A novel approach in immunization

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Abstract
Despite great advances in the field of vaccination, there are still needs for novel and effective vaccines because still no effective vaccines have been produced for some diseases such as malaria, acquired immune deficiency syndrome (AIDS), and tuberculosis. Furthermore, many of the existing vaccines have disadvantages such as failure to stimulate completely the immune system, in vivo instability, high toxicity, the need for cold chain, and multiple administrations. Nanotechnology has been raised as a powerful tool for solving these problems in this regard. Generally, nanovaccines are a new generation of vaccines using nanoparticles (NPs) as carriers and/or adjuvants. Due to the similar scale (size) between the NPs and pathogens, the immune system can be stimulated well, resulting in triggered cellular and humoral immunity responses. Other benefits of the nanovaccines include their better stability in blood flow to increase the shelf life in blood, enhanced immune system stimulation, no need for booster doses, no need to maintain the cold chain, and ability to create active targeting. In addition, nanovaccines have raised the hope to treat diseases such as rheumatoid arthritis, AIDS, malaria, and chronic autoimmune, and so forth.

KEYWORDS
adjuvant, nanoparticle, nanovaccine, vaccine delivery

1 | INTRODUCTION

Since Edward Jenner developed the first vaccine in 1796, vaccination has been playing an important role in the prevention of many diseases and health promotion (Riedel, 2005). Currently, about 70 different types of vaccines against more than 30 infectious agents are licensed to produce and distribute around the world. However, many scientists have attempted to construct improved vaccines over the years, and also some progress have been achieved in this regard but there are still many problems remaining in this process (Kaufmann, McElrath, Lewis, & Del Giudice, 2014). No effective vaccine has been made yet for many diseases, such as malaria (Crompton, Pierce, & Miller, 2010), tuberculosis (Brennan, 2005; Ravilione & Sills, 2016), human immunodeficiency virus (Barouch, 2008; Feinberg & Moore, 2002; Nabel, 2003), and so forth (Gasone, 2008; Glass et al., 2006; Hayat, Gargari, & Nazarian, 2016; Smiley, 2008). Live attenuated vaccines have no necessary safety (Maldonado, 2002), and other vaccines (inactivated, toxoid, and recombinant) do not adequately stimulate the immune system and require booster doses or adjuvant (Siegrist, 2007; Wilson-Welder et al., 2009; Figure 1). On the other hand, there are always needs for vaccines that do not require cold chain (Chen & Kristensen, 2009; Kummer et al., 2014).

Nowadays, nanotechnology has been introduced in many fields of medicine and could solve many medical problems (Kim & Ersu, 2015; Nikajie, 2015; Nobile, Nobile, D’Amore, Adorno, & Grassia, 2016; Singla & Singla, 2014). In the field of vaccination, the nanotechnology can also help to improve the problems of traditional vaccines (Kim et al., 2014). Nanotechnology refers to the science related to nanoparticles (NPs). NPs are known as particles with a size of 10−100 nm and in some cases up to 1,000 nm. Properties of materials at the nanoscale could be strengthened or weaken, or even it is possible that materials at nanoscale find new properties; many of these characteristics have been identified and many are still unknown. NPs include liposomes, dendrimers, micelles, buckminsterfullerene, carbon nanotube and metallic NPs (Bainbridge, 2001; Bhushan, 2010; Mohrnan & Chen, 2006; Figure 2).
Induction of Potent Neutralizing Antibody Responses by a Designed Protein Nanoparticle Vaccine for Respiratory Syncytial Virus

Graphical Abstract

Authors
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In Brief
A computationally designed self-assembling nanoparticle that displays 20 copies of a trimeric viral protein induces potent neutralizing antibody responses.

Highlights
- Design of a self-assembling protein immunogen displaying 20 copies of profusion RSV F
- In vitro assembly yields highly ordered immunogens with tunable antigen density
- The nanoparticle immunogens induce potent neutralizing antibody responses
- Fusion of DS-Cav1 to the trimeric nanoparticle subunit stabilizes the antigen

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Vaccini per *Pseudomonas*.4
Genome-Based Approach Delivers Vaccine Candidates Against Pseudomonas aeruginosa

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High incidence, severity and increasing antibiotic resistance characterize Pseudomonas aeruginosa infections, highlighting the need for new therapeutic options. Vaccination strategies to prevent or limit P. aeruginosa infections represent a rational approach to positively impact the clinical outcome of risk patients; nevertheless this bacterium remains a challenging vaccine target. To identify novel vaccine candidates, we started from the genome sequence analysis of the P. aeruginosa reference strain PAO1 exploring the reverse vaccinology approach integrated with additional bioinformatic tools. The bioinformatic approaches resulted in the selection of 52 potential antigens. These vaccine candidates were conserved in P. aeruginosa genomes from different origin and among strains isolated longitudinally from cystic fibrosis patients. To assess the immune-protection of single or antigens combination against P. aeruginosa infection, a vaccination protocol was established in murine model of acute respiratory infection. Combinations of selected candidates, rather than single antigens, effectively controlled P. aeruginosa infection in the in vivo model of murine pneumonia. Five combinations were capable of significantly increase survival rate among challenged mice and all included PAS340, a hypothetical protein exclusively present in P. aeruginosa. PAS340 combined with PA3526-MoY gave the maximum protection. Both proteins were surface exposed by immunofluorescence and triggered a specific immune response. Combination of these two protein antigens could represent a potential vaccine to prevent P. aeruginosa infection.

Keywords: Pseudomonas aeruginosa, reverse vaccinology, vaccine, respiratory infection, mouse model

INTRODUCTION

P. aeruginosa infections are among the most severe public health issues. This opportunistic bacterium belongs to the multi-drug resistant (MDR) ESKAPE pathogens, along with Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa. According to data from Centers for Disease Control, P. aeruginosa is responsible for millions of infections each year in the community, 10–12% of all healthcare-associated infections, with more than 300,000 cases annually in the EU, USA and Japan (1). Patients hospitalized in intensive care units (ICU) ran a high risk of acquiring P. aeruginosa as they
Construction of a Protective Vaccine Against Lipopolysaccharide-Heterologous Pseudomonas aeruginosa Strains Based on Expression Profiling of Outer Membrane Proteins During Infection

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Pseudomonas aeruginosa is a ubiquitous opportunistic pathogen, which causes infectious disease in patients with cystic fibrosis and compromised immunity. P. aeruginosa is difficult to eradicate because of its intrinsic resistance to most traditional antibiotics as well as acquired resistance mechanisms after decades of antibiotic usage. A full understanding of the P. aeruginosa pathogenesis mechanisms is necessary for the development of novel prevention and treatment strategies. To identify novel vaccine candidates, here we comprehensively examined the expression levels of all the known outer membrane proteins in two P. aeruginosa strains in a murine acute pneumonia model. OprH was one of the most highly expressed proteins during infection. In addition, OprH is known to be highly immunogenic and accessible by host proteins. Thus, it was chosen as a vaccine candidate. To further identify vaccine candidates, 34 genes highly expressed during infection were evaluated for their contributions in virulence by testing individual transposon insertion mutants. Among them, fpwA, hasFl, and foxA were found essential for bacterial virulence and therefore included in vaccine construction. Immunization with a mixture of FpwA, HasFl, and FoxA rendered no protection, however, while immunization by OprH refolded in liposomes elicited specific opsonic antibodies and conferred protection against two lipopolysaccharide-heterologous P. aeruginosa strains (PA14 and PA103). Overall, by studying the expression profile of the P. aeruginosa outer membrane proteins during infection, we identified OprH as a potential vaccine candidate for the prevention of lung infection by P. aeruginosa.

Keywords: Pseudomonas aeruginosa, vaccine, OprH, outer membrane proteins, immunization

INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen which can cause various human infections, especially in immunocompromised and cystic fibrosis patients (1, 2). P. aeruginosa is intrinsically highly resistant to a variety of antibiotics, and biofilm formation can further increase resistance by 1,000-fold (3). It is often difficult to eradicate P. aeruginosa despite intense antibiotic treatment (4).
Killed but metabolically active *Pseudomonas aeruginosa*-based vaccine induces protective humoral- and cell-mediated immunity against *Pseudomonas aeruginosa* pulmonary infections

Elodie Meynet a, David Laurin a, b, Jean Luc Lenormand a, Boubou Camara a, Bertrand Toussaint a, Audrey Le Gouëllec a, b

https://doi.org/10.1016/j.vaccine.2018.02.040

Abstract

*Pseudomonas aeruginosa* (Pa) is a significant cause of morbidity and mortality, especially in cystic fibrosis patients. Its eradication is difficult due to a wide phenotypic adaptability and an increase of its resistance to antibiotics. After the failure of non-vaccine approaches, which mainly targeted humoral immunity, the development of a vaccine against *Pa* is essential to control the infection.
VACCINI PER LA TUBERCOLOSI
Research and development of new tuberculosis vaccines: a review [version 2; referees: 3 approved, 1 approved with reservations]

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Abstract
Tuberculosis kills more people worldwide than any other single infectious disease agent, a threat made more dire by the spread of drug-resistant strains of Mycobacterium tuberculosis (MtB). Development of new vaccines capable of preventing TB disease and new MtB infection is an essential component of the strategy to combat the TB epidemic. Accordingly, the WHO considers the development of new TB vaccines a major public health priority. In October 2017, the WHO convened a consultation with global leaders in the TB vaccine development field to emphasize the WHO commitment to this effort and to facilitate creative approaches to the discovery and development of TB vaccine candidates. This review summarizes the presentations at this consultation, updated with scientific literature references, and includes discussions of the public health need for a TB vaccine, the status of efforts to develop vaccines to replace or potentiate BCG in infants and develop new TB vaccines for adolescents and adults; strategies being employed to diversify vaccine platforms; and new animal models being developed to facilitate TB vaccine development. A perspective on the status of these efforts from the major funders and organizational contributors also is included. This presentation highlights the extraordinary progress being made to develop new TB vaccines and provides a clear picture of the exciting development pathways that are being explored.

Keywords
Tuberculosis, Mycobacterium tuberculosis, vaccines, immunization

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Any reports and responses or comments on the article can be found at the end of the article.

This article is included in the World TB Day collection.
Review

Novel vaccine candidates against Mycobacterium tuberculosis

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ABSTRACT

Tuberculosis (TB) is now among the top ten causes of mortality worldwide being resulted in 1.7 million deaths including 0.4 million among people with HIV in 2016. The Bacille Calmette-Guerin (BCG) is the only available TB vaccine which fails to provide consistent protection against pulmonary TB in adults and adolescents despite being efficacious in protecting infants and young children. From the most severe, often deadly forms of TB disease, to achieving elimination by 2050, we will need new interventions including more improved vaccines that are effective in adult individuals who have not been infected with Mycobacterium tuberculosis as well as latent infected or immunocompromised subjects. In recent decades, multiple new vaccine candidates including whole cell vaccines, adjuvanted proteins, and vectored subunit vaccines have entered into clinical trials. These novel TB vaccines are hoped to provide encouraging safety and immunogenicity under various conditions including prevention of TB disease in adolescents and adults, as BCG replacement boosters, or as therapeutic vaccines to reduce the duration of TB therapy. In this review, we will discuss the status of novel TB vaccine candidates currently under development in preclinical or clinical phases.

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Contents

1. Introduction ........................................ 181
2. Prime-boost vaccines ................................ 182
3. TB vaccine candidates in preclinical phase .................. 182
3.1. H54 + CAM0 ................................. 182
3.2. rRECA/M [vm1] ................................ 182
4. TB vaccine candidates in phase I ....................... 183
4.1. MVA/M ................................. 183
4.2. MBvac ...................................... 183
4.3. ChAdOx1/LSA ................................ 184
4.4. Ad5 Ag85A .................................. 184
5. TB vaccine candidates in phase Ia .................. 184
5.1. TB/RU-04L ................................... 184
5.2. RUB ..................................... 185
5.3. H/MB6E/IC 1 ................................ 185
5.4. Hsc KCH .................................. 185
5.5. ID33 + GIA GE .................................. 185
6. TB vaccine candidates in phase II .................. 185
6.1. VPM 1002 (rRECA/m3) .......................... 185
6.2. M7 + AS01F .................................. 185
6.3. DAB-001 ................................... 186
7. TB vaccine candidates in phase III .................. 186

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Review Article

Current trends in tuberculosis vaccine

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ABSTRACT

Despite the global efforts made to control tuberculosis (TB) and the large number of available new anti-TB drugs, TB still affects one-third of the world population. The conventional vaccine bacille Calmette–Guérin (BCG) shows varying efficacy in different populations, and there are safety issues in immunocompromised patients. Hence, there is an urgent requirement for a new and better TB vaccine candidate than BCG. There are several alternate vaccines available for TB such as DNA, subunit, adjuvant, and live-attenuated vaccines. Use of auxotrophic vaccine is an emerging technology. Newer vaccine technologies include vaccine delivery methods such as adenovirus- and cytomegalovirus (CMV)-based vector delivery, chimeric monoclonal antibody, single-chain fragment variable, RNA-lipoplexes, and nanoparticle-based technology. Based on its application, TB vaccines are classified as conventional, prophylactic, booster, therapeutic, and reinfection preventive vaccines. Currently, there are 12 vaccine candidates in clinical trials. In this review, we have briefly discussed about each of these vaccines in different phases of clinical trials. These vaccines should be analyzed further for developing a safe and more efficacious vaccine for TB.

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“Structural vaccines”. 6
Structure-Based Vaccine Antigen Design

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respiratory syncytial virus, coronavirus, influenza, nanoparticle display, vaccine development, platform technology, immunization, X-ray crystallography, electron microscopy

Abstract
Enabled by new approaches for rapid identification and selection of human monoclonal antibodies, atomic-level structural information for viral surface proteins, and capacity for precision engineering of protein immunogens and self-assembling nanoparticles, a new era of antigen design and display options has evolved. While HIV-1 vaccine development has been a driving force behind these technologies and concepts, clinical proof-of-concept for structure-based vaccine design may first be achieved for respiratory syncytial virus (RSV), where conformation-dependent access to neutralization-sensitive epitopes on the fusion glycoprotein determines the capacity to induce potent neutralizing activity. Success with RSV has motivated structure-based stabilization of other class I viral fusion proteins for use as immunogens and demonstrated the importance of structural information for developing vaccines against other viral pathogens, particularly difficult targets that have resisted prior vaccine development efforts. Solving viral surface protein structures also supports rapid vaccine antigen design and application of platform manufacturing approaches for emerging pathogens.
Laser micro-structured Si scaffold-implantable vaccines against 
Salmonella Typhimurium

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ABSTRACT

Salmonella Typhi is responsible for typhoid fever in humans. Despite the efforts, the development of long-lasting vaccines has failed and the available vaccines display only moderate activity, being considered as “international travelers’s” vaccines. Taking advantage of the previously described implantable vaccine technology consisting on 3D laser-microstructured Si scaffolds loaded with antigen-seeded macrophages, the present study aimed to apply an antigenic stimulus of whole extracts of S. Typhimurium, which is the mouse analogue of the human Salmonella Typhi, and examine its ability to mount specific antibody response. After defining the experimental conditions for specific anti-S. Typhimurium IgG production in vitro, antigen-seeded macrophages loaded onto the 3D Si-scaffolds were implanted to mice, while parallel experiments used conventional Freund-complete-adjuvant vaccination protocols. The results showed that only the implantable vaccine protocol could mount a specific antibody response 14 days after implantation. The cytokine profile showed increase of IL-10 and IFN-γ in the case of implantable and conventional vaccination respectively, 7 days after implantation. Morphological studies on the excised scaffolds 14 days after implantation, showed the development of a well-structured adherent monolayer, establishing multiple contacts with lymphocytes in favor to immune response development. Based on the hypothesis that both stimulatory and suppressive components in the vaccination preparation, could affect the overall activity, peptidoglycan was applied as an antigen to the vaccination protocols. Surprisingly, peptidoglycan was shown to induce a mitogenic rather than specific immunogenetic response. In this case, histological analysis of the excised scaffolds showed a restricted layer of adherent cells with cytoplasmatic extensions, but hard to distinguish cell contacts with lymphocytes. Finally, the presented results showed a differential behavior of antigen presenting cells in accordance to the antigenic stimulus and consequently the activation state of the cells. Tailoring the micro/sub-micron 3D structures and chemistry of Si scaffolds, could control cell behavior according to the user’s needs.

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1. Introduction

Salmonella belongs in the family of Enterobacteriaceae and is a prototype Gram-negative intracellular bacterial pathogen, which lives as saprophyte or parasite within eukaryotic cells. Typically, Salmonella infects individuals through food consumption or drinking water and transmission uses the fecal-oral root, where it invades intestinal epithelial cells in the distal ileum. It infects many different animal hosts where the majority of diseases in animals and humans are caused by Salmonella enterica subspecies and range from local gastroenteritis to death [1].

Since typhoid fever, which is caused by serovar Typhi and Paratyphi is manifested in >20 million cases and accounts for more than 200,000 deaths per year [2], it has been a great interest in vaccine development. Attenuated whole cell vaccines have been replaced by an orally administered attenuated strain (Ty21a) and parenteral Vi capsular polysaccharide antigen which are mostly considered as traveler’s vaccines due to their moderate efficacy and duration [3]. The need of in-depth study of host-pathogen interactions during Typhi infection turned scientists in defining animal experimental models. Although serovar Typhi only infects humans, Salmonella typhimurium-infected mice display many

**Abbreviations:** FCA, freund complete adjuvant; SEM, scanning electron microscopy; PC, peptidoglycan; IL-2, interleukin-2; IL-10, interleukin-10; IFN-γ, interferon-gamma; TNF-α, tumor necrosis factor-alpha; HSA, human serum albumin.

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0264-410X/© 2019 Elsevier Ltd. All rights reserved.
Implantable vaccine development using in vitro antigen-pulsed macrophages absorbed on laser micro-structured Si scaffolds

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ABSTRACT

To overcome the limiting antigenic repertoire of protein sub-units and the side effects of adjuvants applied in second generation vaccines, the present work combined in vitro and in vivo manipulations to develop biomaterials allowing natural antigen-loading and presentation in vitro and further activation of the immune response in vivo. 3-dimensional laser micro-textured implantable Si-scaffolds supported mouse macrophage adherence, allowed natural seeding with human serum albumin (antigens) and specific antibody and inflammatory cytokine production in vitro. Implantation of Si-scaffolds loaded with antigen-activated macrophages induced an inflammatory reaction along with antigen-specific antibody production in vivo, which could be detected even 30 days post implantation. Analysis of implant histology using scanning electron microscopy showed that Si-scaffolds could be stable for a 6-month period. Such technology leads to personalized implantable vaccines, opening novel areas of research and treatment.

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1. Introduction

In order to avoid virulence and infectivity of vaccines, novel technologies have replaced bacterial or viral inactivation with sub-unit vaccines. Highly purified antigenic peptides or their corresponding DNA are usually emulsified into adjuvants and used for vaccination. Although such approaches eliminate infectivity of the pathogen itself, non-specific side effects of the adjuvants cannot be safely monitored yet. On the other hand, small antigenic peptides cannot be immunogenic by themselves and therefore the use of adjuvants is obligatory for the initiation of an inflammatory reaction that could increase chances for antigen presentation. Furthermore, small antigenic peptides have limited affinity for complexation with MHC class II antigens for presentation. Because of the extended polymorphism of MHC in the human race, only a limited number of MHC polymorphisms will display the right affinity for the provided antigenic epitopes to proceed to specific immune stimulation.

The vision towards an antigen-specific immune stimulation in vivo would be to find a way to trigger T-helper cells without the need of adjuvants. During a natural infection, because of the size and the complexity of the antigen (immunogenic), the organism develops a polyclonal immune response without the need of external manipulation. The idea developed in the present study was to allow antigen presentation to occur in vitro on implantable substrates, which upon implantation in vivo could stimulate specific immune response. The advantages of the in vitro antigen presentation process in case of a pathogen would be first, to allow natural epitope selection for loading onto self-MHC and second to avoid infectivity of the pathogen, since whole attenuated or dead organisms could be provided to the culture for a short period of time, necessary for antigen presentation and thereafter cleared out from the culture. Furthermore, the advantage of using an implantable biomaterial would be first, to provide a focal nest of antigen-loaded APCs capable to trigger a specific immune response and second, the material itself could cause a systemic inflammatory response [1] attracting immune cells to the implantation site.

The implantable scaffolds used herein consisted of 3-dimensional (3D) micropatterned silicon (Si)-based substrates with tunable morphology and chemistry, which have been successfully used in vitro for fibroblast and nerve cell growth [2−4] and are preferred versus 2D surfaces because they provide more usable area and adhesion points for cell growth [5,6]. The choice of a so far
Vaccini per Salmonella: vari approcci.
Overview of the Typhoid Conjugate Vaccine Pipeline: Current Status and Future Plans

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Typhoid fever remains a common and serious disease in populations that live in low- and middle-income countries. Treatment usually consists of antibiotics, but problems with drug-resistant strains have been increasing in endemic countries, making treatment prolonged and costly. Improved sanitation and food hygiene have been effective in controlling the disease in industrialized world, but these steps are associated with socioeconomic progress that has been slow in most of the affected areas. Therefore, vaccination is an effective way to prevent the disease for the short to medium term. Oral typhoid vaccine and Vi polysaccharide typhoid vaccine (Vi polysaccharide) have been available for many years, yet a large population, in particular infants and children aged <2 years, remains at higher risk. Recently, with the availability of Vi polysaccharide–based conjugate vaccines and funding to support vaccination from the Gavi alliance, there is great momentum for typhoid prevention efforts. Supply of the vaccine will be critical, and there are multiple efforts to make new typhoid vaccines accessible and available to populations that desperately need them.

Keywords. Salmonella Typhi, typhoid conjugate vaccines, immunogenicity, safety.

DISEASE AND PATHOGEN

Typhoid (enteric) fever is an important cause of morbidity and mortality. It is caused by infection with Salmonella enterica serovar Typhi (S. Typhi), a gram-negative bacterium that invades the body via the small intestines and colonizes macrophages in the reticuloendothelial system, where it is shed into the bloodstream [1, 2]. Symptoms of the resulting disease typically include prolonged fever, frontal headache, malaise, and marked loss of appetite, sometimes accompanied by abdominal pain, nausea, and, in severe cases, intestinal perforation and neurological complications [3]. Symptoms typically subside in 7–21 days, but mortality is estimated at 1%–5% of hospitalized patients [4–6]. In a small percentage of cases, the bacteria may also colonize the gallbladder, leading to a chronic carrier state [3].

Between 11.9 and 26.9 million cases of typhoid fever occur each year in low- and middle-income countries [7]. Most cases can be treated effectively with antibiotics. However, antibiotic resistance is a challenge for effective treatment of typhoid, and treatment is likely to become increasingly problematic with the spread of multidrug-resistant strains [8]. Vaccination against typhoid has proven to be an effective preventive intervention, especially when coupled with hand-washing, treatment of household water, and provision of adequate sanitation and other preventive measures [9].

In 2008, the World Health Organization (WHO) recommended vaccination of all children in areas where the disease is common and of those at high risk [10]. At a meeting held on 17–19 October 2017 in Geneva, Switzerland, the Strategic Advisory Group of Experts on Immunization recommended the introduction of typhoid conjugate vaccine (TCV) for infants and children aged >6 months as a single dose in typhoid-endemic countries [11]. Typhoid vaccines are on the WHO's list of essential medicines, which are the most effective and safe medicines needed within a health system [12].

VACCINE CANDIDATE PIPELINE AND STATUS

Whole Cell S. Typhi Vaccine

Almroth Edward Wright, Richard Pfeiffer, and Wilhelm Kolle developed the first typhoid vaccine in 1896 [13]. It was a heat-killed, phenol-preserved, and acetone-killed lyophilizable injectable whole-cell S. Typhi vaccine that was used in England and Germany. The efficacy of this vaccine was assessed in a trial in 1960 in Yugoslavia, Russia, Poland, and Guyana. Although licensed in few countries, this vaccine is no longer used due to its side effects.

The Live Attenuated Ty21a

Due to limitations of the killed whole-cell vaccine, there was a need to develop a more competent vaccine candidate. With the knowledge that a live attenuated strain elicits more immune response, attenuated Salmonella strains were considered for vaccine development. Ty21a, the first live oral attenuated Salmonella vaccine (sold as Vivovax by Berna Biotech, now Crucell and now PaxVax), was developed in Switzerland by chemical mutagenesis of wild-type S. Typhi strain Ty2 [14, 15].
Comparative immunogenicity and efficacy of equivalent outer membrane vesicle and glycoconjugate vaccines against nontyphoidal *Salmonella*

Francesca Miccoli, Simona Rondini, Renzo Alfinito, Luisa Lanzilao, Francesca Necchi, Aurel Negrea, Omar Rossi, Cornelia Brandt, Simon Clare, Pietro Mastromenfi, Rino Rappuoli, Allan Saul, and Galman A. MacLennan

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Contributed by Rino Rappuoli, August 9, 2018 (sent for review May 4, 2018; reviewed by S. Abigail and Brian M. Greenwood)

Nontyphoidal *Salmonella* cause a devastating burden of invasive disease in sub-Saharan Africa with high levels of antimicrobial resistance. Vaccination has potential for a major global health impact, but no licensed vaccine is available. The lack of commercial incentive makes simple, affordable technologies the preferred route for vaccine development. Here we compare equivalent Generalized Modules for Membrane Antigens (GMMMA) outer membrane vesicles and O-antigen CRM197 glycoconjugates to deliver lipopolysaccharide O-antigens in bivalent *Salmonella* Typhimurium and Enteritidis vaccines. Salmonella strains were chosen and tolI deleted to induce GMMMA production. O-antigens were extracted from wild-type bacteria and conjugated to CRM197. Purified GMMMA and glycoconjugates were characterized and tested in mice for immunogenicity and ability to reduce Salmonella infection. GMMMA and glycoconjugate O-antigens had similar structural characteristics, O-acetylation, and glucosylation levels. Immunization with GMMMA induced higher anti-O-antigen IgG than glycoconjugate administered without Adjuvax. With Adjuvax, antibody levels were similar. GMMMA induced a diverse antibody isotype profile with greater serum bactericidal activity than glycoconjugate, which induced almost exclusively IgG1. Immunization reduced bacterial colonization of mice subsequently infected with *Salmonella*. The Typhimurium numbers were reduced for each GMMMA strain vaccinated with GMMMA compared with glycoconjugate. Enteritidis burden in the tissues was similar in mice immunized with either vaccine. With favorable immunogenicity, low cost, and ability to induce functional antibodies and reduce bacterial burden, GMMMA offer a promising strategy for the development of a nontyphoidal *Salmonella* vaccine compared with established glycoconjugates. GMMMA technology is potentially attractive for development of vaccines against other bacteria of global health significance.

nontyphoidal; *Salmonella*; vaccines; GMMMA; vesicles

Invasive nontyphoidal *Salmonella* (INTS) disease is a leading cause of death and morbidity in developing countries (1–3). Nontyphoidal *Salmonella* are responsible for up to 35% of community-acquired bloodstream infections in sub-Saharan Africa with an average case fatality rate of 19% (4). The effectiveness of antibiotic treatment is hampered by the difficulty in making a diagnosis, the sudden onset of the disease, and the growing frequency of multidrug resistance (1, 2, 5). Higher incidence and increased severity of INTS disease have been observed in young children below 72 mo of age, in patients with malaria, anemia, malnutrition, HIV, sickle cell disease, and hemolysis (6–9). Moreover, the Global Burden of Disease Study 2015 estimated that NTS is the third commonest cause of diarrheal deaths at 90,300 (95% uncertainty interval, 34,100–185,100) (10).

*Salmonella* enterica serovars Typhimurium and Enteritidis are responsible for 91% of the cases of INTS disease reported in Africa (4) and a similar proportion of NTS diarrheal disease. A bivalent vaccine against these two serovars could represent a valuable public health intervention. Several groups have been working on the development of glycoconjugate, protein-based, vaccine-based, and live attenuated vaccines against NTS (11), but none has entered clinical trials over the last 16 y. Hence, a licensed vaccine is still a long way off. This lack of progress relates primarily to the absence of a commercial incentive to develop such a vaccine. Hence, a technology that could produce large quantities of an effective vaccine simply and at low cost would be enormously valuable for advancing a vaccine against this devastating disease.

The serovar-specific O-antigen (OAg) moiety of *Salmonella* lipopolysaccharide (LPS) is the principal target of protective immunity (12–14). LPS molecules are composed of lipid A (endotoxin) attached to the 3-deoxy-d-manno-octulosonic acid (KDO) terminus of the conserved core region, which is linked to

**Significance**

Bacteria, such as nontyphoidal *Salmonella*, are responsible for a large global burden of disease. Due to limited need in developed countries and consequent lack of commercial incentive, vaccines are unavailable against many bacteria. Glycoconjugates constitute the standard bacterial vaccine approach, but can be costly, particularly where multivalent preparations are required. This report compares a low-cost vaccine-based technology, known as Generalized Modules for Membrane Antigens (GMMMA), with glycoconjugate in bivalent vaccines against nontyphoidal *Salmonella*. In head-to-head immunogenicity and infection studies in mice, GMMMA performed at least as well as equivalent glycoconjugate vaccine, indicating good potential of this approach. Given that many bacteria are amenable to genetic engineering for GMMMA production, the GMMMA strategy could provide a breakthrough for a range of needed bacterial vaccines.


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Conflict of interest statement: F.M., S.R., L.L., F.N., O.R., D.R., and A.S. are employees of the GSK group of companies. M.N. and C.A.M. were employees of OVG (now GSK) during part of the study.

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This work was initiated at the Novartis Vaccine Institute for Global Health. In March 2015 the Novartis nontyphoidal vaccines business was acquired by the GSK group of companies. Therefore the company became GlaxoSmithKline Biologicals SA.

This article contains supporting information online at www.pnas.org/cgi/content/full/10.1073/pnas.1807655115/DCSupplemental.

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IgG Responses to Porins and Lipopolysaccharide within an Outer Membrane-Based Vaccine against Nontyphoidal Salmonella Develop at Discordant Rates

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ABSTRACT Antibodies acquired after vaccination or natural infection with Gram-negative bacteria, such as invasive Salmonella enterica serovar Typhimurium, can protect against disease. Immunization with naturally shed outer membrane vesicles from Gram-negative bacteria is being studied for its potential to protect against many infections, since antigens within vesicles maintain their natural conformation and orientation. Shedding can be enhanced through genetic modification, and the resulting particles, generalized modules for membrane antigens (GMMA), not only offer potential as vaccines but also can facilitate the study of B-cell responses to bacterial antigens. Here we show that the response to immunization with GMMA from S. Typhimurium (STMGMMA) provides B-cell-dependent protection and induces antibodies to two immuno-dominant antigens, lipopolysaccharide (LPS) and porins. Antibodies to LPS O antigen (O-Ag) markedly enhance protection in the spleen, but this effect is less marked in the liver. Strikingly, IgG responses to LPS and porins develop with distinct kinetics. In the first week after immunization, there is a dramatic T-cell-independent B1b-cell-associated induction of all IgG isotypes, except IgG1, to porins but not to LPS. In contrast, production of IgG1 to either antigen was delayed and T-cell dependent. Nevertheless, after 1 month, cells in the bone marrow secreting IgG against porins or LPS were present at a similar frequency. Unexpectedly, immunization with O-Ag-deficient STMGMMA did not substantially enhance the anti-porin response. Therefore, IgG switching to all antigens does not develop synchronously within the same complex and so the rate of IgG switching to a single component does not necessarily reflect its frequency within the antigenic complex.

IMPORTANCE Vaccines save millions of lives, yet for some infections there are none. This includes some types of Salmonella infections, killing hundreds of thousands of people annually. We show how a new type of vaccine, called GMMA, that is made from blebs shed from the Salmonella cell wall, works to protect against infection in mice by inducing host proteins (antibodies) specifically recognizing bacterial components (antigens). The rate of development of IgG antibody to antigens within GMMA occurs with different kinetics. However, the antibody response to GMMA persists and is likely to provide prolonged protection for those who need it. These results help show how antibody responses to bacterial antigens develop and how vaccines like GMMA can work and help prevent infection.
Vaccini contro Stafilococco.8 (x 2 studenti)
PanRV: Pangeneome-reverse vaccinology approach for identifications of potential vaccine candidates in microbial pangeneome

Kanwal Naz, Anam Naz, Shifa Tariq Ashraf, Muhammad Rizwan, Jamil Ahmad, Jan Baumbach and Amjad Ali

Abstract
Background: A revolutionary diversion from classical vaccinology to reverse vaccinology approach has been observed in the last decade. The ever-increasing genomic and proteomic data has greatly facilitated the vaccine designing and development process. Reverse vaccinology is considered as a cost-effective and proficient approach to screen the entire pathogen genome. To look for broad-spectrum immunogenic targets and analysis of closely-related bacterial species, the assimilation of pangeneome concept into reverse vaccinology approach is essential. The categories of species pangeneome such as core, accessory, and unique genes sets can be analyzed for the identification of vaccine candidates through reverse vaccinology.

Results: We have designed an integrative computational pipeline termed as PanRV that employs both the pangeneome and reverse vaccinology approaches. PanRV comprises of four functional modules including i) Pangeneome Estimation Module (PEM) ii) Reverse Vaccinology Module (RVM) iii) Functional Annotation Module (FAM) and iv) Antibiotic Resistance Association Module (ARM). The pipeline is tested by using genomic data from 301 genomes of Staphylococcus aureus and the results are verified by experimentally known antigenic data.

Conclusion: The proposed pipeline has proved to be the first comprehensive automated pipeline that can precisely identify putative vaccine candidates exploiting the microbial pangeneome. PanRV is a Linux based package developed in JAVA language. An executable installer is provided for ease of installation along with a user manual at https://sourceforge.net/projects/panrv2/.

Keywords: PanRV, Pangeneome, Core genome, Reverse vaccinology, Microbial species, Vaccine targets, And therapeutic targets

Background
Microbial species are rapidly evolving and acquiring multi-drug resistance, making existing therapies ineffective [1]. Hence, there is a need to identify broad-spectrum therapeutic targets, which will be effective against a range of closely related microbial pathogens. Advancements in genome sequencing technologies and high-throughput bioinformatics analyses have assisted the basic in-vivo vaccine design via in-silico practices [2]. The genomes of thousands of pathogenic microbes have been sequenced so far, and are available for scientific exploration such as antibiotic resistance determination and finding alternative therapeutic targets [3]. Due to genomic diversity in bacterial species, a large number of variable genes accumulate in species gene pool ultimately resulting in the species pangeneome expansion [4]. Therefore, considering a single representative (genome) from such a species is not sufficient to estimate the exact pangeneome and is unfavorable to be targeted for broad-spectrum therapeutics. On the other hand, closely related bacterial species share a large
Protection against *Staphylococcus aureus* Colonization and Infection by B- and T-Cell-Mediated Mechanisms

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**ABSTRACT** *Staphylococcus aureus* is a major cause of morbidity and mortality worldwide. *S. aureus* colonizes 20 to 80% of humans at any one time and causes a variety of illnesses. Strains that are resistant to common antibiotics further complicate management. *S. aureus* vaccine development has been unsuccessful so far, largely due to the incomplete understanding of the mechanisms of protection against this pathogen. Here, we studied the role of different aspects of adaptive immunity induced by an *S. aureus* vaccine in protection against *S. aureus* bacteremia, dermonecrosis, skin abscess, and gastrointestinal (GI) colonization. We show that, depending on the challenge model, the contributions of vaccine-induced *S. aureus*-specific antibody and Th1 and Th17 responses to protection are different: antibodies play a major role in reducing mortality during *S. aureus* bacteremia, whereas Th1 or Th17 responses are essential for prevention of *S. aureus* skin abscesses and the clearance of bacteria from the GI tract. Both antibody- and T-cell-mediated mechanisms contribute to prevention of *S. aureus* dermonecrosis. Engagement of all three immune pathways results in the most robust protection under each pathological condition. Therefore, our results suggest that eliciting multipronged humoral and cellular responses to *S. aureus* antigens may be critical to achieve effective and comprehensive immune defense against this pathogen.

**IMPORTANCE** *S. aureus* is a leading cause of healthcare- and community-associated bacterial infections. *S. aureus* causes various illnesses, including bacteremia, meningitis, endocarditis, pneumonia, osteomyelitis, sepsis, and skin and soft tissue infections. *S. aureus* colonizes between 20 and 80% of humans; carriers are at increased risk for infection and transmission to others. The spread of multidrug-resistant strains limits antibiotic treatment options. Vaccine development against *S. aureus* has been unsuccessful to date, likely due to an inadequate understanding about the mechanisms of immune defense against this pathogen. The significance of our work is in illustrating the necessity of generating multipronged B-cell, Th1-, and Th17-mediated responses to *S. aureus* antigens in conferring enhanced and broad protection against *S. aureus* invasive infection, skin and soft tissue infection, and mucosal colonization. Our work thus, provides important insights for future vaccine development against this pathogen.

**KEYWORDS** B-cell responses, *Staphylococcus aureus*, T-cell immunity, adaptive immunity, vaccines

*S. aureus* is a leading cause of community- and healthcare-associated bacterial infections and postsurgical wound infections (1–4). Skin and soft tissue infections (SSTIs) are a common type of community-acquired *S. aureus* infection, which can be recurrent in many individuals (5, 6). *S. aureus* also causes severe invasive disease, such as bacteremia, meningitis, endocarditis, osteomyelitis, pneumonia, sepsis, and...
Vaccini contro *Brucella*. 9
Research paper

Attenuated *Salmonella* secreting *Brucella* protective antigens confer dual-faceted protection against brucellosis and salmonellosis in a mouse model

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**ARTICLE INFO**

**ABSTRACT**

We demonstrated the use of attenuated *Salmonella* strains secreting *Brucella* antigens SodA, Omp19, BLS, and PrpA as live vaccine candidates against *Brucella abortus* infection and presented their cross-protection against *Salmonella* infections using a BALB/c mouse model. Here, a single immunization with each individual strain was capable of establishing significantly high (p < 0.05) *Brucella*-specific systemic immunoglobulin (IgG) and secretory IgA (sIgA) responses compared to control mice. Upon stimulation of the splenocytes harvested from immunized mice with the respective antigens SodA, Omp19, BLS, and PrpA, significant increases in splenocyte proliferative responses against all four antigens versus PBS and vector controls were observed (p < 0.05). Additionally, interferon-γ and interleukin-4 secretion clearly demonstrated an upsurge of these cytokines in all four strains upon immunization compared to the control groups. However, a significantly high response was noted in the mice groups immunized with *Salmonella* secreting SodA and Omp19 only. Upon virulent *Brucella abortus* 544 challenge, all four antigens presented a significantly high protection index (PI) in the spleen, as follows: 0.85 for SodA, 0.96 for Omp19, 0.65 for BLS, and 0.66 for PrpA. In contrast, in the liver, the same antigens resulted in PI values of 1.37, 1.14, 1.12, and 1.81, respectively. Immunological profiling of immunized mice against *Salmonella*-specific immune responses also showed significant elicitation of both humoral and cell-mediated immune responses as measured by IgG, sIgA, splenocyte proliferation, and cytokine induction. In addition, full protection against virulent *Salmonella* challenge was shown with no mortality in immunized mice, whereas 100% (8/8) mortality was observed in control mice over a two-week post-*Salmonella* challenge. In conclusion, we show that the live attenuated *Salmonella* delivering *Brucella* protective antigens may efficiently confer dual protection against both brucellosis and salmonellosis in immunized mice.

1. Introduction

Brucellosis is a disease caused by a group of intracellular Gram-negative bacteria of the genus *Brucella*. It has previously been recognized as a class B bioterror agent by the United States Centers for Disease Control and Prevention (Dabrak et al., 2014). It infects both humans and animals by way of ingestion; inhalation; or contact exposure, where already-infected organisms can act as a reservoir of infection (Yang et al., 2013). The disease rarely results in death in humans; however, its outcomes remain severely debilitating. It also poses a significant economic loss in domesticated animals due to the loss of progeny, a reduction in milk yield, and infertility (Xavier et al., 2009). The lack of a safe vaccine for both animal and human brucellosis remains a significant hurdle to overcome. As a result, many scientific research investigations have been launched in search of alternative vaccine strategies for the conventional live forms of *Brucella* vaccines such as *Brucella abortus* S19 and *Brucella melitensis* Rev-1, which preserve considerable residual virulence (Yang et al., 2013). Recent studies have also demonstrated the ability of attenuated *Salmonella* strains to deliver various heterologous antigens in order to induce protective immune responses against various infections (Kim et al., 2018; Lakiathara and Lee, 2017; Osorio et al., 2009). Considering the *Salmonella* pathogenesis, an anti-*Brucella* vaccine could be developed using recombinant *Salmonella* strains, which express and secrete *Brucella* protective antigens in order to deliver protection against *Brucella abortus* infection. As an intracellular pathogen, *Salmonella* can be employed to deliver protective *Brucella* antigens directly into macrophages for efficient antigen presentation (Goo et al., 2012). Additional advantages such as an intrinsic adjuvant effect (Chaudhuri and Lee, 2013) and sufficiency of a single inoculation for a lasting immunity (Lakiathara and Lee, 2017), ability to multiply and present an amplitude of antigens, and the active penetration of natural barriers that protect subunit

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Brucellosis vaccines based on the open reading frames from genomic island 3 of *Brucella abortus*

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**ABSTRACT**

*Brucella abortus* is the etiological agent of brucellosis, a zoonotic disease affecting cattle and humans. This disease has been partially controlled in cattle by immunization with live attenuated *B. abortus* S19 and RB51 strains. However, use of these vaccine strains has been associated with safety issues in animals and humans. New vaccines have since emerged in the prevention of brucellosis, particularly DNA vaccines, which have shown effectiveness and a good safety profile. Their protective efficacy in mice is associated with the induction of Th1 type and cytotoxic T cell mediated immune response against structural antigens and virulence factors expressed during *B. abortus* infection. Some antigenic candidate for vaccine design against brucellosis (mainly DNA vaccines) have been obtained from genomic island 3 (GI-3) of *B. abortus*, which encodes several open reading frames (ORFs) involved in the intracellular survival and virulence of this pathogen. The immunogenicity and protection conferred by these DNA vaccines in a murine model is reviewed in this article, suggesting that some of them could be safe and effective vaccine candidates against to prevent *B. abortus* infection.

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**Contents**

1. Introduction ................................................................. 2028
2. Bovine brucellosis vaccines ............................................. 2029
3. Vaccines against Brucella based on genomic island 3 (GI-3) open reading frames .............................................. 2031
   3.1. Subunit of flagellar protein FliG vaccine encoded by BAB1_0260 ORF ........................................... 2032
   3.2. Hypothetical protein encoded by BAB1_0263 ORF .......................................................... 2032
   3.3. Hypothetical protein with an Sre homology 3-like domain encoded by BAB1_0267 ORF .................. 2033
   3.4. ImmA/mrE metallo-endopeptidase family encoded by BAB1_0270 ORF .................................... 2033
   3.5. Hypothetical protein GCTA encoded by BAB1_0278 ORF ...................................................... 2033
   3.6. Multi-epitope and multivalent DNA vaccines based on GI-3 ORFs ........................................ 2034
4. Conclusions and future perspectives .................................. 2035

Acknowledgements .......................................................... 2035
Conflict of interest ......................................................... 2035
Authors contributions .................................................... 2035
References ........................................................................ 2035

1. Introduction

*Brucella abortus* is a facultative intracellular pathogen that causes brucellosis, an endemic zoonosis affecting bovines and humans in several regions of the world. It is a Gram-negative bacterium characterized as a small, microaerophilic, non-spore-forming, slow growing, coccobacillus [1]. This pathogen is one of the most virulent species of *Brucella* genus infecting humans [2]; infection usually occurs through ingestion of contaminated food or through direct contact with infected animals [3]. After initial contact with the host, *Brucella* adheres to and penetrates the
Intranasally administered anti-Brucella subunit vaccine formulation induces protective immune responses against nasal Brucella challenge

Amal Senevirathne, Chamith Hewawaduge, Irshad A. Hajam, Jonathan Lalsiamthara, John Hwa Lee

Article Info

Abstract

The present study was aimed to develop a safe and effective anti-Brucella subunit vaccine for mucosal protection against the respiratory exposure of Brucella infection. A chitosan-based Brucella nasal vaccine (BNV) was formulated using well-known Brucella immunogens, sodC, omp19, BLS and PrpA and tested against nasal Brucella challenge in BALB/c mice. The mice were intra-nasally vaccinated with sterile phosphate buffer saline (PBS), BNV or BNV plus Brucella LPS, and human (systemic IgG and mucosal IgA) and cell-mediated immune responses were analyzed. Results showed that mice vaccinated with either BNV or BNV plus LPS elicited significantly (p < 0.05) high IgG and IgA responses compared to the PBS control. The IgG responses were significantly (p < 0.05) higher than IgA levels, which showed almost comparable levels observed in either intestines or in lungs. Furthermore, the IgG and IgA responses against each individual component of the BNV formulation indicated that omp19 induced highest levels of both IgG and IgA levels than the other constituents of BNV formulation. Upon re-stimulation of the splenocytes with Brucella whole cell lysate, significantly (p < 0.05) high IFN-γ levels, lymphocyte proliferation, and CD4+ T cell responses were observed in mice vaccinated with BNV or BNV plus LPS. Upon sub-lethal nasal challenge with wild-type Brucella abortus, vaccinated mice showed significant reduction of Brucella recovery in lungs and spleen compared to the PBS control. This study indicates that BNV formulation with or without Brucella LPS efficiently induced humoral and cell-mediated immune responses and conferred significant protection against the sub-lethal Brucella challenge.

1. Introduction

Brucella infection remains as a significant zoonotic threat throughout the world. It is caused by a group of Gram-negative bacteria of the genus Brucella (Yang et al., 2013). The bacterium can be easily disseminated by ingestion, inhalation or contact exposure (Surendran et al., 2013) and is considered as a category B bioterrorism agent by the Center for Disease Control in the United States (“Emergency Preparedness and Response,” 2018). Lack of human vaccines demonstrates the vulnerability of human population and, therefore, warrants intensive research investigations into development of an effective and safe vaccines against human brucellosis. Currently available live attenuated Brucella abortus S19, B. abortus RB51, and B. melitensis Rev-1 vaccine strains are licensed to control livestock brucellosis (Lalsiamthara and Lee, 2017). However, residual virulence associated with these vaccine strains have not been completely eliminated (Godfroid et al., 2011). Due to the virulence of livestock Brucella vaccines on humans, the professionals dealing with animal vaccination and veterinary care are particularly vulnerable for the disease. These facts emphasize the need for alternative safe vaccines that can be equally potent on animals and particularly for humans for the control of brucellosis. It is a fact that Brucella species can infect epithelial cells allowing the entry via mucosal surfaces (Bhattacharjee et al., 2006; Foester et al., 2013; Clapp et al., 2016), moreover, Brucella species are highly infection through aerosol route (Dogany and Dogany, 2013). Therefore, a vaccine which could elicit mucosal immunity especially in the nasopharyngeal region would be possibly the ideal way to contain Brucella infections in place of acquisition. To accomplish this strategy, in the present study, we have chosen nasal route of administration to deliver vaccine antigens. Further to enhance the safety of the vaccine more towards human use, we used Brucella subunit proteins which could be substantially safer than currently available live forms of Brucella vaccines. In order to formulate an anti-Brucella nasal vaccine (BNV), four highly conserved Brucella antigens, namely Cu-Zn superoxide dismutase (sodC), outer membrane protein 19 (omp19), lumazine synthase (BLS) and proline racemase subunit A (PrpA) were selected as