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Current Opinion in
Immunology

Recombinant bacterial vaccines

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Vaccines are currently available for many infectious diseases caused by several microbes and the prevention of disease and death by vaccination has profoundly improved public health globally. However, vaccines are not yet licensed for use against many other infectious diseases and new or improved vaccines are needed to replace suboptimal vaccines, and against newly emerging pathogens. Most of the vaccines currently licensed for human use include live attenuated and inactivated or killed microorganisms. Only a small subset is based on purified components and even fewer are recombinantly produced. Novel approaches in recombinant DNA technology, genomics and structural biology have revolutionized the way vaccine candidates are developed and will make a significant impact in the generation of safer and more effective vaccines.

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Current Opinion in Immunology 2012, 23:1–6

This review comes from a themed issue on
Vaccines
Edited by Alan Aderem and Cecil Czerkinsky

0952-7915/\$ – see front matter

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<http://dx.doi.org/10.1016/j.coi.2012.03.013>

Introduction

Vaccines for infectious diseases have come a long way, starting from the Jenner's cowpox vaccines two centuries ago to the present day vaccines designed using recent technologies like reverse and structural vaccinology. The field of vaccinology, primarily founded on Pasteur's principles has yielded several effective vaccines. In fact, today, the vast majority of licensed vaccines are live attenuated or killed/inactivated microorganisms [1]. However, given that there are still several deadly diseases that lack an efficient vaccine, it is becoming pertinent to apply newer technologies to quicken the search for better and safer vaccines [2]. The advent of genetic engineering has given scientists the ability to rationally design both individual microbial components as well as whole organisms. Furthermore, the application of functional genomics and structural biology has allowed the identification of many promising new antigens. Although at present there are few licensed vaccines that are based on recombinant technology, it is quite clear that novel genetic

technologies have a crucial role to fulfill in the years to come. The aim of this review is to illustrate the use of recombinant DNA technology in bacterial vaccine development using examples of existing and future vaccines.

Recombinant subunit vaccines

The main drivers for recombinant protein-based vaccine development are developing less reactogenic, more potent, safer, better characterized vaccines, in addition to developing vaccines that provide broader protection against multiple serotypes/serogroups of a bacterium. The first of these vaccines was the highly pure surface antigen of the hepatitis B virus expressed in yeast [3]. The second, a *Bordetella pertussis* subunit vaccine (acellular vaccine) containing three highly pure proteins provided a safe and efficient alternative to chemical inactivation and pioneered the application of structure–function studies to produce a genetically modified pertussis toxin [4]. These novel approaches were based on studying the microorganism in terms of pathogenicity and immunogenicity, to identify factors involved in virulence suitable as vaccine candidates and have been the basis of development of some new vaccines currently available or in clinical development.

The licensed anthrax vaccine (AVA) is a cell-free filtrate from an attenuated strain of *Bacillus anthracis*. Although it is US FDA approved, concerns regarding its undefined composition, extensive dosing regimen and local reactogenicity have highlighted the need to develop new anthrax vaccines. The recombinant protective antigen (PA)-based vaccines currently in clinical trials have been produced in bacterial cell culture from *B. anthracis* [5] and *E. coli* [6] and are potentially superior to the current anthrax vaccine. Protein-based approaches are being evaluated for several important human pathogens including *Staphylococcus aureus*, *Streptococcus pneumoniae*, Group A and Group B streptococci (GBS).

Many of the vaccine antigens identified during the past few decades was through conventional subunit vaccines approaches. These require information about virulence and disease pathogenesis, which is lacking for difficult pathogens. In addition, it involves a labor-intensive process of pathogen culturing, purification of individual components and testing for their ability to induce protective immunity.

The advent of whole-genome sequencing led to a new strategy termed reverse vaccinology (RV) that enables the selection of potential vaccine candidates starting from the genomic information of a bacterial strain [7].

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The application of RV to the human pathogen *Neisseria meningitidis* serogroup B (MenB) allowed the identification of new surface-exposed protein antigens able to induce protective immunity in mice [8[•]]. The main protective antigens (NHBA, fHbp and NadA) were combined in a multicomponent MenB vaccine [9], which after successful preclinical studies has been through the long path of vaccine development including testing safety and immunogenicity in humans [10–13] and a large scale phase III clinical trial in infants that was the basis for a European license application. Clinical trials showed that the vaccine is safe and able to induce strong bactericidal antibodies responses. The pioneering work on MenB shows that protein-based vaccine can be developed against encapsulated bacteria that are usually targeted by polysaccharide conjugate vaccines.

As the genomic era progresses, RV has evolved with the application of pan-genomics. Maione *et al.* have applied the pan-genome concept to GBS and identified subunit protein antigens protective against GBS infection in mice [14[•]]. Characterization of the newly identified vaccine antigens revealed that three of them are able to form pilus-like structures extending from the bacterial surface [15]. A recent modification of RV is subtractive reverse vaccinology, which involves multi-strain genome analysis in design of universal vaccines. With the aim of developing a vaccine that will not interfere with the natural commensal gut flora, Gomes Moriel *et al.* applied a subtractive reverse vaccinology approach performing a comparative genome analysis between pathogenic extraintestinal *E. coli* (ExPEC) and commensal *E. coli* strains to identify genes specific for pathogenic strains. Nine antigens were found to be protective in a mouse model of sepsis and furthermore conserved among several pathogenic *E. coli* strains [16].

Recent genomic and genome-based approaches have allowed the identification of a significant number of new vaccine antigens against diseases that are still an unmet medical need [17]. In order to strengthen the protective activity of the single antigens, to increase strain coverage and to avoid escape mutants, most new subunit-based vaccines will be constituted by combinations of multiple antigens. One potential strategy to simplify the manufacturing process of such multicomponent vaccines is expression of proteins as polyprotein fusions. This approach was used for the recombinant proteins of MenB [9] and has been also recently reported for *S. pneumoniae*. Harfouche *et al.* engineered a fusion protein containing the three known pilus RrgB variants (RrgB321) that protected against the infection caused by all pilus-positive *S. pneumoniae* strains [18].

An important advantage of multicomponent vaccines is that antibodies directed to the antigens will have multiple modes of action. Antigens may directly activate the

classical complement pathway and induce opsonophagocytosis or indirectly interfere with colonization of invasive strains and/or prevent binding of complement regulators on the bacterial surface, as recently shown in the case of *N. meningitidis* fHbp [19].

The move toward fully recombinant vaccines produced in recombinant strains of *E. coli* or other cell culture methods has resulted in products with more demanding manufacturing and downstream processing. These challenges perhaps contribute to the lack of introduction of many new recombinant vaccines. However, the extra manufacturing and processing steps will be justified if the resulting vaccines provide the necessary safety and efficacy benefits needed.

Recombinant bacterial strains

Whole cell vaccines are a common vaccine strategy for many diseases, especially when the immune responses involved are complex. Historically, many attenuated vaccine strains like Bacille Calmette Guerin (BCG) and *Salmonella typhi* Ty21a were generated by continuous passage or chemical mutagenesis [20]. With recombinant technology, pathogens can now be manipulated to generate non virulent, but immunogenic strains. Targeted attenuated mutants of various human pathogens have shown promise in animal models of infection [21,22]. Further, attenuated bacteria can also be engineered to overexpress antigens of interest. A recent example of successful use of recombinant strains in humans is live attenuated enterotoxigenic *E. coli* expressing colonization factors and toxin subunits (ACE527) [23].

In the last decade, recombinant whole cell vaccines have been the focus of groups working toward better tuberculosis (TB) vaccines. BCG, an attenuated strain of *Mycobacterium tuberculosis* (Mtb) from the 1900s is the only available licensed vaccine against TB despite data demonstrating its ineffectiveness against pulmonary disease in adults [24,25]. Given that a strong T cell response is considered key in combating Mtb, there is much emphasis on creating BCG strains that evoke better cellular immune responses. A few different recombinant BCG strains are currently in various phases of clinical trials. rBCG30, a recombinant BCG strain overexpressing an immunodominant antigen (Ag85B) was more efficient than BCG in guinea pig models of infection [26] and has successfully completed a phase 1 clinical trial [27]. Grode *et al.* developed a novel approach based on improving access of mycobacterial antigens to the MHC I pathway by listeriolysin-mediated induction of the escape of Mtb from the phagolysosome into the cytoplasm, which results in better CD8 T cell stimulation [28[•]]. The rBCGΔ*ureC::hly* (VPM 1002) vaccine showed improved efficacy in preclinical models, and furthermore successfully completed safety studies in humans [29]. Finally, Aeras-422 encodes for perfringolysin and multiple TB antigens,

combining approaches in VPM 1002 and rBCG30 [22]. In addition, highly attenuated auxotrophic mutants of *Mtb* also show promise as potential vaccine candidates [30].

Bacterial carriers

Lactic acid bacteria (LAB), mainly lactococci and lactobacilli have been used as vehicles for the effective delivery wide array of molecules including vaccine candidates, anti-infectives, and human cytokines to mucosal tissues [31[•]]. These bacteria are considered safe, are amenable to genetic manipulation and have been shown to stimulate both systemic and mucosal immune responses [32]. *L. lactis* expressing the tetanus toxin fragment C protected mice from lethal systemic challenge with tetanus toxin when administered both orally and intranasally [33]. Recently it was reported that lactococci expressing hybrids of type 1 and type 2 GBS pili proteins can assemble within lactococci and confer protection against lethal challenge with GBS [34]. Potential superiority of LAB strains over purified proteins was demonstrated in a study with pneumococcal surface protein A (PspA) expressing *L. lactis* in respiratory challenge with virulent pneumococci [32]. Although there are many successful protection studies in animal models, there is a lack of data demonstrating their effectiveness in humans.

The *Salmonella* spp. are an important group of bacterial vaccine carrier strains widely exploited across various disciplines of medicine. Two major *Salmonella* serovars, *S. typhimurium* in mice and *S. typhi* in humans are used for vaccine carrier studies. Important rationale for *Salmonella*-based vaccine development include a *S. typhimurium* mouse model for preclinical analysis and a licensed live attenuated human vaccine Ty21a. *Salmonella* can also deliver directly into the antigen presenting cells and induce a strong innate immune response. *Salmonella* strains are bacterial carriers for heterologous bacterial proteins, DNA, immunomodulators and tumor antigens [35]. Attenuated *Salmonella* mutant strains in bacterial biosynthetic and regulatory pathways (*purD*, *aroA*, *phoP/phoQ*) were demonstrated to be safe for use in humans [36]. Mutant *Salmonella* strains expressing heterologous antigens have been shown to induce protection against various pathogens including *Bacillus anthracis* and *Helicobacter pylori* [37,38[•]].

Salmonella strains have been used extensively as a carrier for DNA vaccines. Protection of mice against a lethal challenge with *Listeria monocytogenes* was observed toward *S. typhimurium* DNA encoding for *L. monocytogenes* (LLO and ActA) virulence factors [39]. A novel genetic approach employing the type III secretion pathway effectors was used to allow translocation of the cancer antigen survivin to the host cytosol, allowing efficient T cell responses and inhibition of tumor growth murine glioblastoma [40]. In addition to *Salmonella*, a series of recombinant *L. monocytogenes*-strains expressing various tumor antigens have

been developed for cancer antigen delivery, some of which are currently in clinical trials [41].

OMV-based vaccines

The most successful outer membrane vesicle (OMV)-based vaccine is perhaps the one against MenB. The main limitation of OMV vaccines is its strain-specificity. In MenB, PorA, the major protective antigen of the OMV-based vaccine is highly variable across different isolates of *N. meningitidis*. Multivalent OMV vaccines have been derived by genetically engineering meningococci to increase vesicle production, reduce LPS toxicity and increase expression of protective antigens (Opc, fHbp and NadA) [42–44]. Interest in OMV-based vaccines has expanded to other bacterial pathogens, including *S. typhimurium*, *B. burgdorferi*, *B. pertussis*, and *P. aeruginosa* [45]. Heterologous proteins can also be incorporated into nascent OMVs by fusion with periplasmic or outer membrane proteins. For example, Schroeder and Aebischer reported *Salmonella* OMVs carrying *Leishmania* antigens fused to an *E. coli* autotransporter that spontaneously integrates into the OM [46].

New approaches

Vaccines based on protein structure

Structural vaccinology, a recent approach based on the use of information derived from protein structure and epitope mapping can be used in the rational design of new recombinant vaccines, particularly against antigens characterized by a complex antigenic variability. This approach has been applied to design fully synthetic proteins to induce multivalent protection. Cell surface pili, protective antigens of GBS, exist as six immunogenically different but structurally similar variants. Based on 3-D structure information, a synthetic protein comprising protective domains from all six variants was constructed and shown to protect mice against challenge with all type 2a pilus-carrying strains [47].

The *N. meningitidis* protective antigen fHbp has more than 300 amino acid variations that are classified into three distinct antigenic variant groups. Immunogenic regions from all the three variants, defined by extensive structural and immunologic analysis, were incorporated into a single molecule. This fHbp, containing a conserved backbone and an engineered surface with multiple immunogenic regions, was capable of inducing broad cross-protection against MenB strains harboring all three variant groups [48^{••}]. The structure-based design of multiple immunodominant antigenic surfaces on a single protein scaffold represents an effective way to create broadly protective vaccines.

E. coli engineered for glyconjugate production

A major issue with the use of polysaccharides conjugated to proteins as vaccines is the cost involved in production and their efficiency. Using recombinant technology,

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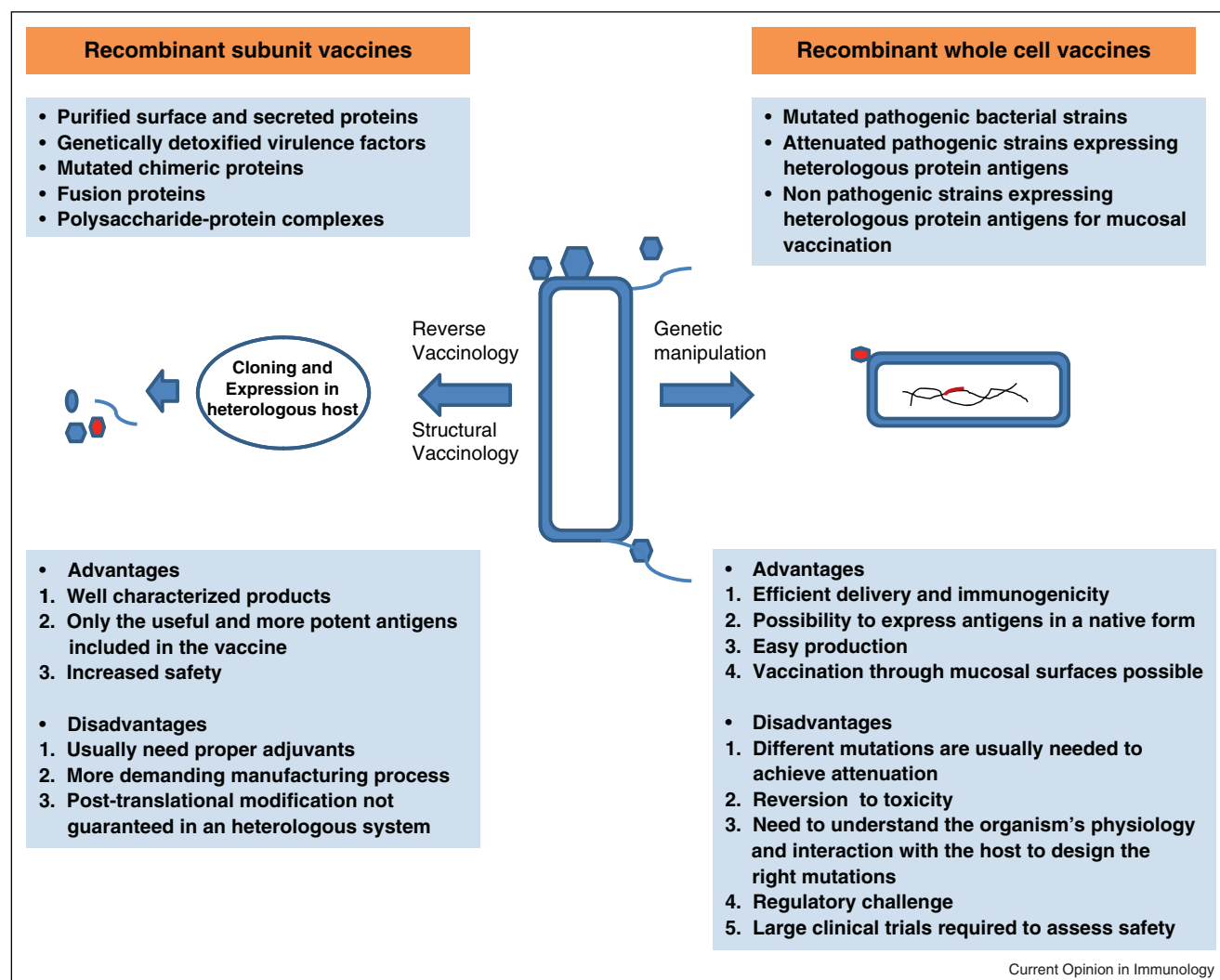
E. coli have been engineered to produce desired protein–polysaccharide complexes [49,50]. Glyconjugates can be synthesized *in vivo* in the bacterium by cloning the entire gene loci required for polysaccharides, the required protein carrier and the conjugating enzyme PglB into *E. coli*. Purification of glycoconjugates from *E. coli* is easier, cheaper, avoids culturing pathogenic bacteria and moreover *in vivo* conjugation preserves the natural conformation of epitopes, thereby resulting in better immune responses [51].

Conclusions

Recombinant DNA technology has indeed changed the face of vaccinology by enabling rational design

vaccines against complex human diseases (Figure 1). Despite excellent evidence of success of recombinant vaccines in pre-clinical and clinical studies, few recombinant vaccines are licensed for human use. This can however be attributed to the intense regulatory processes revolving around recombinant vaccine production. Improved adjuvant technologies, new approaches like systems biology and advanced immunological studies on vaccine mechanisms will all play a role in shaping the next generation of vaccines. Furthermore, with the advent of the first synthetic bacterium, applications of synthetic biology in vaccine design will be the most rational approach in the years to come.

Figure 1



Recombinant Bacterial Vaccines: A comparison of recombinant subunit and recombinant whole cell vaccines. The two main categories of recombinant bacterial vaccines, subunit and whole cell, their major constituents, how they are made, and the advantages and disadvantages of each are illustrated.

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