First steps towards a vaccine against *Acinetobacter baumannii*

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Abstract

Acinetobacter baumannii has become an important cause of human infections, most notably in the hospital setting. In addition, the global dissemination of multidrug resistant strains has complicated effective antibiotic therapy of infections produced by this pathogen, necessitating the development of novel treatment and prevention strategies. Active and passive immunization approaches have begun to be explored in experimental animal models as potential alternative therapies for *A. baumannii*. In the present review, we discuss the advantages and disadvantages of each therapeutic strategy with respect to *A. baumannii* infections, and summarize the recent studies that have explored these approaches. The single antigen candidates that have been tested include, the outer membrane protein OmpA, the membrane transporter Ata, the biofilm-associated protein Bap, the K1 capsular polysaccharide and the membrane associated polysaccharide poly-*N*-acetyl-β -(1-6)-glucosamine. Strategies employing multicomponent antigens include inactivated whole cells, outer membrane complexes and outer membrane vesicles. The strengths and limitations of each approach are discussed and the challenges that remain to be addressed for successful *A. baumannii* vaccine development are highlighted.

Keywords: *Acinetobacter baumannii*, Vaccine, Passive immunization.

INTRODUCTION

Acinetobacter baumannii is a Gram-negative coccobacillus that has been associated with a number of human infections including pneumonias, bloodstream infections, meningitis, urinary tract infections, and skin and soft tissue infections. The majority of infections produced by *A. baumannii* are hospital acquired, although community acquired infections have been reported [1]. Within the hospital setting,
A. baumannii has the ability to persist on surfaces within the patient environment for long periods of time, and is thought to be transmitted from patient to patient by contaminated equipment and contact with hospital personnel [2]. Infectionscaused by this organism have been especially problematic in critically-ill patients in intensive care units, and are associated with increased patient morbidity and mortality [1]. The prevalence of infections caused by *A. baumannii* can vary widely between institutions and geographic locations. However, a recent report from the European Centre for Disease Control and Prevention which included data from over 900 hospitals from 29 countries indicated that *A. baumannii* accounted for 3.6% of all hospital acquired infections, 8.7% of all pneumonias/lower respiratory tract infections, and 4.1% of all bloodstream infections [3].

The clinical management of infections caused by
A. baumannii has become increasingly difficult due to the high prevalence of infections caused by multidrug resistant strains. In many cases, these strains are resistant to the majority of clinically-available antibiotics, leaving clinicians with fewoptions that still retain adequate antimicrobial activity against the infecting organism. As an example, the carbapenem class of antibiotics has traditionally been a mainstay of treatment for infections caused by *A. baumannii*. However, the global emergence of strains with resistance to carbapenems over the proceeding two decades has severely compromised the efficacy of this antibiotic class for the treatmentof *A. baumannii* infections. This is evidenced by a recent European point prevalence study that included data from 29 countries in which 81.2% of isolates for which susceptibility data were available demonstrated non-susceptibility to carbapenems [3]. This worrisome trend has prompted the increasing use of novel therapeutic approaches, one of which isthe reintroduction of colistin for the treatment of infections caused by multidrug resistant gram negative infections. Colistin is an “old” peptide antibiotic that was introduced half a century ago, although its use has not been widespread in recent decades due to concerns regarding nephrotoxicity [4]. More recently, however, colistin has been used increasingly in the treatment of infections for which local epidemiology indicates that traditional therapies, such as carbapenems, will not have adequate activity.

Unfortunately, strains that acquire resistance in response to treatment with colistin have been described [5-7], making the emergence of pandrug resistant strains, with resistance to all clinically-used antibiotics, a reality that has already been reported in sporadic cases [8, 9].

In this current context of antibiotic resistant *A. baumannii*, the development of novel treatment and prevention strategies is of interest. While antibiotic stewardship practices and hospital hygiene-based approaches will undoubtedly play a role in combating the appearance of resistant strains, new therapeutics with the ability to prevent and/or treat infections caused by highly resistant strains of *A. baumannii* could provide an important alternative to existing treatments, which are increasingly ineffective [10]. Vaccination represents a therapeutic approach that has the potential to reduce patient morbidity and mortality, and at the same time help to prevent the emergence of resistance by decreasing clinicians' dependence on antibiotics for treating infections caused by *A. baumannii*. A handful of preclinical studies have begun to characterize different vaccine candidates in animal models of infection. In general, these vaccines can be divided into two broad categories, vaccines based on single purified bacterial antigens, and vaccines that contain multiple antigens. In this review, we summarize the results that have been obtained from these initial studies, and comment on the remaining challenges that still must be addressed for successful development of a vaccine against *A. baumannii*.

ACTIVE IMMUNIZATION *VS.* PASSIVE IMMUNIZATION/ANTIBODY-BASED THERAPY

Active immunization, which involves the administration of an antigen that stimulates a protective immune response, and passive immunization/antibody-based therapy, which relies on the administration of antibodies with antibacterial activity, both represent potential novel therapeutic approaches which could be effective for prevention and/or treatment of infections caused by *A. baumannii*. Advantages of the active immunization approach are that it is a cost- effective method with a proven track record regarding safety and efficacy. The major drawback of active immunization for preventing infections caused by *A. baumannii* is that patients at risk for infection must be identified with sufficient lead time to allow for the host to mount a protective immune response after immunization. Due to the fact that *A. baumannii* produces predominately nosocomial infections, it may not be possible to vaccinate acute patients admitted urgently with sufficient lead time for achieving protective immunity. In contrast, antibody-based passive immunization has the potential to provide instantaneous protective immunity, thus avoiding the problem of lead time that could occur with active immunization. In addition, antibodies with antibacterial activity could potentially be used for treating established *A. baumannii* infections, either alone or in combination with traditional antibiotic therapy. The drawbacks associated with antibody-based approaches are that they are largely unproven in the clinical setting for bacterial infections, and that they are typically significantly more costly than active immunization approaches. Regardless of the immunization approach that is employed, active vaccination and passive immunization, in many cases, both rely on the identification of an antigen(s) that can induce protective immunity. In the sections below, the antigens that have been tested in preclinical models are presented and their advantages and disadvantages discussed.

SINGLE ANTIGEN VACCINES

Vaccines based on a single bacterial antigen are attractive because high levels of antigen purity can be obtained, thus avoiding the presence of contaminating bacterial components such as lipopolysaccharide (LPS). In addition, manufacturing and regulatory issues may be less cumbersome for vaccines based on a single, well-defined bacterial component (Table **1**). A potential disadvantage of a vaccine based on a single antigen includes the possibility that not all circulating strains express the chosen antigen, likely resulting in the ineffectiveness of the vaccine against such a strain. It is also possible that bacteria may

adapt to the selective pressure produced by an immune response generated by immunization through downregulation of the antigen targeted by the vaccine. In this scenario, the downregulation of a single antigen may be more readily achieved than the downregulation of several targeted antigens. Similarly, the targeting of a single antigen may result in the selection and expansion of a variant that was a minority member of the bacterial population and does not possess the epitope to which the vaccine-induced antibodies are directed. The single antigen vaccines that have been studied for *A. baumannii* include three outer membrane proteins, outer membrane protein A (OmpA), the biofilm associated protein (Bap) and a surface autotransporter (Ata), and the surface polysaccharide poly-*N*-acetyl-β -(1-6)- glucosamine (PNAG) and the capsular polysaccharide.

Acinetobacter baumannii OmpA is a transmembrane protein that shares sequence and structural homology with the OmpA superfamily of proteins, which are highly conserved among Gram-negative bacteria [11]. OmpA has been implicated in a number of virulence traits expressed by *A. baumannii* including induction of host cell apoptosis, adherence, biofilm formation and surface motility [12-14]. OmpA may also play a role in bacterial survival and/or dissemination during infection given data showing that blood bacterial loads in mice with *A. baumannii* pneumonia were lower in mice infected with an *ompA* mutant compared to the wild type strain [13]. Lou *et al*. identified OmpA as a target of the humoral immune response produced in mice during sublethal intravenous infection with *A. baumannii* [15]. Based on these findings, purified recombinant OmpA was combined with an aluminium hydroxide adjuvant and used for immunization. Vaccination stimulated high levels of OmpA-specific antibodies and provided partial protection from intravenous infection in a diabetic mouse model. Vaccination also resulted in a 10-fold reduction in tissue bacterial loads compared to control mice. Importantly, anti-OmpA antibodies were shown to enhance opsonophagocytic killing of *A. baumannii in vitro*, and were able to mediate passive protection in mice. OmpA is known to be a predominant component of the bacterial outer membrane, and sequence analysis has shown that it is highly conserved between *A. baumannii* strains [15], supporting the use of this antigen for vaccine development.

Bap is an 854 kDa surface exposed protein that is involved in biofilm formation [16]. Fattahian *et al*. evaluated a 371 amino acid region of Bap that had previously been predicted to be a conserved functional motif in the native protein as a vaccine antigen [17]. Three administrations of the Bap subunit in combination with Freund's complete and incomplete adjuvants produced antigen-specific antibodies that were able to recognize intact bacterial cells in a whole cell ELISA. Immunized mice had lower bacterial loads in spleen and liver 18 hours after intraperitoneal infection, and showed increased survival compared to unimmunized controls. An important consideration for a vaccine based on Bap is the expression of this protein in circulating clinical strains of *A. baumannii,* since it has been shown that not all strains produce biofilm [18], and it is unknown if strains express Bap independent of whether or not biofilm is produced.

The *A. baumannii* Ata protein is a surface-exposed, trimeric autotransporter that has been shown to participate in biofilm formation and the adhesion of *A. baumannii* cells to host extracellular and basement membrane proteins such as collagen type IV [19]. Ata also appears to play a role in pathogenesis as mice infected with wild type *A. baumannii* showed decreased survival compared to mice infected with an isogenic mutant strain lacking Ata expression in an intraperitoneal model of infection. Rabbit antisera to Ata were able to block the binding of *A. baumannii* to immobilized collagen type IV and promote the opsonophagocytic killing and complement-dependent bactericidal killing of *A. baumannii* strains *in vitro*, including multidrug resistant clinical isolates [20]. Passive immunization of mice with Ata antisera resulted in reduced lung bacterial loads after intranasal infection compared to control mice receiving non-immune serum, demonstrating that antibodies against Ata have antibacterial activity *in vivo*. It remains to be seen if the antibacterial activity mediated by Ata antibodies is sufficient for reducing post-infection mortality in animal models that evaluate survival. As with other antigens, the presence and expression of Ata in circulating strains of *A. baumannii* is a critical issue. Bentancor *et al*. used PCR to demonstrate that the *ata* gene was present in 44/75 (58.6%) of isolates from different geographic locations, and that the levels of surface expression of Ata in PCR-

positive isolates showed considerable variation, indicating that the presence and expression of Ata varies between clinical strains.

The surface-associated polysaccharide PNAG is produced by a variety of Gram-positive and Gram-negative bacterial pathogens and consists of linked subunits of N-acetyl- D-glucosamine [21, 22]. In *A. baumannii*, PNAG is synthesized by the products of the *pgaABCD* genes, as a mutant lacking *pgaABC* genes did not produce PNAG [23]. PNAG has been shown to play a role in biofilm formation in *A. baumannii*. Bentancor *et al*. raised rabbit antisera against a synthetic nonameric oligonucleotide to evaluate the ability ofPNAG-specific antibodies to mediate bacterial killing and protection from infection [24]. The antisera were able topromote the opsonophagocytic killing of a PNAG-producing strain, but not its isogenic mutant lacking the *pgaABC* genes. In addition, the antisera were able to promote opsonophagocytolysis of four multidrug resistant clinical isolates. In animal models of pneumonia and bacteremia, passive immunization with the anti-PNAG sera resulted in lower bacterial loads in lung and blood, respectively. Protection studies evaluating survival after administration of PNAG antiserawere not reported. The presence of the *pgaABCD* locus in a collection of 30 multidrug resistant clinical isolates was evaluated by PCR, and showed that all 30 produced amplification products corresponding to the predicted size [23]. An immunoblot assay demonstrated that of these 30 strains, 14 showed high levels of PNAG expression, 14 showed low levels of expression, and 2 demonstrated no PNAG expression. The use of PNAG as a vaccine antigen is supported by studies with other PNAG-expressing bacterial species that have shown encouraging results in preclinical models [25, 26].

The *A. baumannii* capsular polysaccharide has been shown to play a role in bacterial growth and survival in human ascites fluid, human serum and a rat soft tissue model ofinfection [27]. Based on these findings, and the fact that the capsular polysaccharide is surface exposed and that bacterial capsules have formed the basis for vaccines against a numberof pathogens [28-30], Russo *et al.* characterized a monoclonal antibody directed against the K1 capsular polysaccharide of *A. baumannii* in passive immunization studies [31]. Their results showed that the monoclonal antibody could promote neutrophil-mediated bactericidal activity *in vitro* and reduce post-infection bacterial loads in a rat soft tissue model of infection. Using an immunoassay, the monoclonal antibody reacted with 13 of 100 (13%) strains of *A. baumannii* from different geographic locations and isolated fromdifferent body sites or environmental sources. Clearly in the case of the capsular polysaccharide more work is necessaryin order to characterize the different capsular serotypes in circulating strains in order to facilitate broad coverage of a potential vaccine or antibody-based therapy.

VACCINES CONTAINING MULTIPLE BACTERIALANTIGENS

Vaccines containing multiple bacterial components, such as whole cells or membrane complexes, have the advantage of potentially producing an immune response against multiple antigens. This may result in increased vaccine coverageof strains within a bacterial species compared to vaccines based on single antigens given that antigen expression can vary widely between strains. Potential disadvantages of thesetypes of vaccines include difficulties associated with achieving consistent levels of all vaccine components between production lots, and the presence of bacterial components that could produce unwanted side effects such as LPS. Multi-antigen vaccines against *A. baumannii* that have been reported to date include a vaccine based on outer membrane complexes, an inactivated whole cell vaccine and a vaccine consisting of outer membrane vesicles (OMVs).

Outer membrane complexes are prepared by isolating whole bacterial membranes and then solubilizing the inner membrane component using a detergent before subsequent removal. Proteomic analysis of outer membrane complexes prepared from the *A. baumannii* ATCC 19606 strain grownin laboratory media identified 61 protein components, 41 of which were predicted to be located on the cell surface [32]. Immunization of mice with the outer membrane complexes induced antibodies against multiple bacterial outer membrane proteins that were able to recognize surface proteins from multiple clinical

isolates. Immunized mice infected with *A. baumannii* using an intraperitoneal sepsis model demonstrated dramatically reduced post-infection tissue bacterial loads and lower serum levels of the pro-inflammatory cytokines IL-6, IL-1β and TNF-a compared to unimmunized controls. Vaccinated mice also showed increased survival after infection, including after infection with a pandrug resistant clinical isolate. Importantly, treatment of previously infected mice with antisera raised against outer membrane complexes 1 hour after infection was able to therapeutically rescue mice from infection. These results indicate that a mixture of outer membrane components can induce a potent immune response. However, the use of outer membrane complexes as a vaccine is limited by the difficult nature of standardizing the levels of the multiple components that are present in the vaccine preparation.

Vaccines based on whole bacterial cells have the advantage of potentially inducing a response against multiple surface antigens in their native conformation. An inactivated whole cell vaccine prepared by formalin inactivation of the ATCC 19606 strain was highly immunogenic and produced antibodies against multiple bacterial outer membrane proteins [33]. Immunized mice showed reduced bacterial loads and serum cytokine levels compared to control mice, and increased survival after infection with the ATCC 19606 strain and two clinical isolates. While these results are promising, concerns about using whole cells due to the high levelsof LPS present a crucial limitation.

OMVs are vesicles produced from the bacterial outer membrane that are actively produced by numerous Gram- negative bacteria [34]. They are typically 20-200 nm in size and consist of multiple bacterial components including periplasmic and outer membrane proteins, as well as LPS. *A.baumannii* OMVs have been shown to contain a number of potential virulence factors and immune modulating proteins [35]. In vaccination studies, *A. baumannii* OMVs were highly immunogenic, producing antibodies against multiple bacterial outer membrane proteins [36]. Mice vaccinated with OMVs had reduced post-infection tissue bacterial loads and increased survival after intraperitoneal infection compared to control mice. Similar to whole cell vaccines, vaccines based on OMVs have the advantage of presenting multiple antigens in their native form. Unfortunately, OMVs, like whole cells, have the limitation of containing high levelsof LPS. OMVs that have been detergent extracted to remove LPS, or OMVs isolated from strains genetically modified to lack LPS have been employed as vaccines for other bacterial species [37], raising the possibility that this approach may hold promise for *A. baumannii* OMVs as well.

REMAINING CHALLENGES

The studies summarized above demonstrate the proof of concept that protective immunity can be achieved against *A. baumannii* through both active and passive immunization. However there is clearly a great deal of further work that must be performed before these therapies can begin to be considered as viable alternative therapies for treating and preventing infections caused by this pathogen. An ideal antigen would be present on the cell surface, highly conserved between strains within the species, and be highly expressed during infection. Although some of the candidates described above hold promise, the continued identification of antigens that meet such criteria is warranted. An additional aspect that must be clarified is the definition of the correlates of protective immunity for *A. baumannii*. While passive immunization experiments indicate that antibodies alone are sufficient for providing protection against infection, the role of thecell-mediated immune response has not been characterized. The findings that the cell-mediated immune response may play a role in controlling infections caused by *Pseudomonas aeruginosa*, a bacterium that is phylogenetically related to *A. baumannii* and produces similar types of infections, supports the idea that this aspect of the immune response may have importance [38, 39]. Finally, although a number of animal models of infection by *A. baumannii* have been developed [1], in some cases these models are not ideal for characterizing the protective capacity of vaccines. Due to the low virulence of *A. baumannii* in mice, some models employ neutropenic mice or virulence enhancing agents (e.g. porcine mucin) in order to achieve mortality, although a recent study has identified a strain the produces lethal infection without manipulation [40]. These limitations demonstrate the importance of the further development of additional models that more accurately reflect human infection with *A. baumannii* (i.e. models that permit bacterial growth and do not

provokea cytokine “storm” in order to produce mortality). Although further studies are required, the continued development of active and passive immunization strategies for *A. baumannii* is of interest given their potential for reducing the morbidity and mortality produced by this drug resistant pathogen.

CONFLICT OF INTEREST

MJM owns stock in and act as scientific advisors for Vaxdyn, S.L., a biotechnology company developing vaccinesfor multidrug resistant bacteria, including *A. baumannii*. The other authors declare no potential conflicts of interest.

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LIST OF ABBREVIATIONS

Ata	=	*Acinetobacter* trimeric autotransporter
Bap	=	Biofilm-associated protein
ELISA	=	Enzyme-linked immunosorbant assay
LPS	=	Lipopolysaccharide
OmpA	=	Outer membrane protein A
OMV	=	Outer membrane vesicles
PNAG	=	poly-*N*-acetyl-β-(1-6)-glucosamine

Table 1. Advantages and disadvantage of single and multiple antigen vaccine strategies.

Vaccine Type	Advantages	Disadvantages
Single antigen vaccines	Well-defined composition, low levels of reactogenic impurities, existence of standardized methods for industrial production	Concerns regarding expression of the antigen in all strains, adaptation to immune pressure via antigen down-regulation more feasible, purification process can alter native antigen conformation
Multicomponent vaccines	Higher stain coverage due to targeting of multiple antigens, reduced risk of adaptation due to immune pressure, antigens can be maintained in their native conformation	Difficult to standardize all vaccine components between production lots, presence of impurities that could produce side effects (e.g. LPS)

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