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Antibodies can be used for the prevention and treatment of bacterial infections in animal models of disease. Current antibody technology allows the generation of high affinity human/humanized antibodies that can be optimized for antibacterial activity and *in vivo* biodistribution and pharmacokinetics. Such antibodies have exquisite selectivity for their bacterial target antigen and promise efficacy and safety. Why are there no monoclonal antibody products approved for the treatment or prevention of bacterial infections? Can antibodies succeed where antibiotics are failing? Some antibody therapies are currently being evaluated in clinical trials but several have failed despite positive data in animal disease models. This review will discuss the pros and cons of antibody therapeutics targeted at bacterial infections.

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Introduction

An increasing number of serious bacterial infections are caused by organisms resistant to antibiotics, including vancomycin-resistant *Enterococcus* (a significant problem in immunocompromised patients), multiply resistant *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, common causative agents in hospital-acquired pneumonia and other infections. Chronic *P. aeruginosa* infection is also a major factor in the progressive decline in lung function in cystic fibrosis (CF) patients. Antibodies that kill drug-resistant bacteria typically exploit mechanisms distinct from those of antibiotics and are therefore unlikely to select for cross-resistance. Such antibodies thus provide attractive new therapeutic options for some of the most intractable bacterial infections. Two alternative approaches to antibacterial antibody development are discussed here: exploiting antibodies directly targeting the bacterial surface; or antibodies that act indirectly, protecting normal host immunity by neutralizing bacterial toxins or other virulence factors essential for pathogenicity and maintenance of infection.

Direct targeting to bacterial cell surfaces

Antigens on the bacterial cell surface are appealing targets for antibody-based intervention because they provide the potential for blocking antibodies to interfere with bacterial colonization as well as marking the bacteria for destruction by immune effector responses (opsonization). In some cases, antibodies with direct bactericidal activity have been identified, such as a single-chain antibody to the spirochete *Borellia* [1]. Similarly, anti-idiotype antibodies which mimic a yeast killer toxin have been reported to have direct cell-killing activity against a broad spectrum of Gram-positive bacteria in addition to yeasts, fungi, and mycobacteria [2]. These antibodies are yet to be tested in the clinic but they may overcome the need for a competent host immune response for efficacy. In most cases, however, antibodies to bacterial surface components rely on the recruitment of an array of antibody-directed immune effector functions. Antibodies with appropriate constant region isotypes (chiefly human IgG1 and IgG3) can recruit cells of the immune system with multiple activities: stimulating phagocytosis of bacteria by macrophages; inducing antibody-directed cellular cytotoxicity (ADCC) by macrophages or NK cells; activating the complement cascade; and generating the oxidative burst from neutrophils. (IgM antibodies also potently induce complement and ADCC activities). Furthermore, all antibody variable regions are capable of catalyzing redox reactions from singlet oxygen provided by activated neutrophils, leading to the generation of several highly potent oxidizing agents directly harmful to bacteria [3], including ozone, a potent antibacterial agent which also stimulates inflammatory responses [4]. Indeed, the inflammation induced by complement activation and ozone generation has the potential to recruit additional elements of the immune system to further boost immunity.

Direct cell-targeting antibodies in development

Antibodies targeting *Staphylococcus*

Two hyperimmune human polyclonal antibody preparations (Altastaph and Veronate) have recently been evaluated for the prevention of *S. aureus* infection in very low birth weight (VLBW) infants but both have failed to show sufficient efficacy [5]. Inadequate immune effector cell responses in premature infants may have contributed to the lack of efficacy in these trials. It is also possible that

Table 1

Antibodies to bacterial targets in clinical studies.

Pathogen	Antibody	Target	Antibody form	Company	Ref	Clinical studies
Staphylococcus	Veronate	*S. aureus* surface components	Human polyclonal	Inhibitex	[5]	Failed Phase 3
Staphylococcus	Altastaph	*S. aureus* vaccine	Polyclonal purified from vaccinees	Nabi	[5]	Failed Phase 2
Staphylococcus	Pagibaximab	Lipoteichoic acid (surface antigen)	Chimeric IgG1k	Medimmune	[6]	Phase 2 completed
Staphylococcus	Aurexis (tefibazumab)	Clumping Factor A (surface antigen)	Humanized IgG1k	Inhibitex	[7,8]	Phase 2 completed
Staphylococcus	Aurograb	GrfA (surface efflux pump)	Recombinant human scFv	Novartis	[9]	Failed Phase 2
B. anthracis	ABthrax (PAmAb; raxibacumab)	Protective antigen (toxin)	Recombinant human IgG1λ	HGS	[36,37]	Surrogate efficacy and Phase 1 completed[a]
B. anthracis	Anthim	Protective antigen (toxin)	Deimmunized Mab	Elusys	[38]	Surrogate efficacy and Phase 1 completed[s]
B. anthracis	Valortim (MDX-1303)	Protective antigen (toxin)	Human IgG1k	Medarex/ Pharmathene	[39]	Surrogate efficacy and Phase 1 completed[s]
C. difficile	MDX-066 MDX-1388	Toxins A and B	Human IgG1	Medarex and MBL	[41]	Phase 2 ongoing
E. coli (Shiga toxin producing)	Shigamabs	Shiga toxins Stx1 and Stx2	*NA*	Thallion	[40]	Phase 1 completed
P. aeruginosa	Anti-*Pseudomonas* IgY	*P. aeruginosa*	Chicken polyclonal	Immunsystem AB	[10]	Phase 1/2 ongoing
P. aeruginosa serotype 011	KBPA101	LPS	Human monoclonal IgM	Kenta Biotech	Kenta website	Phase 2 ongoing
P. aeruginosa	KB001	PcrV (toxin delivery by the TTSS)	PEGylated Humaneered™ Fab′	KaloBios	KaloBios website	Phase 2 ongoing

NA: not available.

[a] Therapies for inhalation anthrax are evaluated under the FDA 'animal rule'; safety and pharmacokinetics are tested in human volunteers.

naturally occurring antibodies may have low activity because bacteria have evolved to be able to evade the human immune system. Monoclonal antibodies specifically targeting surface components with important functions in bacterial colonization or infection may be more effective. There are three monoclonal antibodies against *S. aureus* currently in clinical development, Pagibaximab, Aurexis, and Aurograb (see Table 1), but none have yet demonstrated significant efficacy.

Pagibaximab is a chimeric IgG1 antibody recognizing the surface component lipoteichoic acid of *S. aureus* and *S. epidermidis*. A Phase 2 clinical study in which VLBW infants were given up to three doses at 60 or 90 mg/kg showed evidence of prophylactic activity but only at the highest dose of antibody [6], again perhaps reflecting the comparatively poor immune response in premature infants.

Aurexis, a humanized IgG1 antibody to *S. aureus* Clumping Factor A (ClfA), a protein implicated in mediating adherence to damaged endothelia and inhibiting phagocytosis, has activity in prophylactic and therapeutic models in rabbits [7]. Results of a Phase 2 clinical study in which a single 20 mg/kg dose of Aurexis was administered in adult patients with *S. aureus* bacteremia showed no statistically significant responses, although fewer deaths occurred and no worsening of sepsis occurred in the antibody-treated group [8].

Aurograb is a single-chain antibody variable fragment (scFv) that binds to the *S. aureus* surface protein GrfA [9]. GrfA is a drug-efflux pump and Aurograb increases the sensitivity of *S. aureus* to vancomycin by the inhibition of the pump. Because Aurograb does not have an Fc-region, it is expected to have a half-life of only a few minutes *in vivo*, requiring frequent dosing. A Phase 2 study of Aurograb (dosed twice-daily at 1 mg/kg) in combination with vancomycin for deep-seated *S. aureus* infections failed to show efficacy (Novartis new release URL: http://www.novartis.-com/newsroom/media-releases/en/2008/1247203.shtml).

Antibodies to *P. aeruginosa* surface antigens in development

A polyclonal antibody preparation (IgY; Table 1) from the eggs of hens immunized with *P. aeruginosa* has been evaluated in an open-label study in CF children whose first *P. aeruginosa* colonization was eradicated with antibiotics. Recently published data from patients treated for up to 12 years provide evidence of a trend to efficacy with only 2/17 patients who gargled daily with the chicken

antibody showing recolonization with *P. aeruginosa* compared with 7/23 in a control group [10].

For the development of monoclonal antibodies, there is a paucity of surface antigens conserved across different strains of *P. aeruginosa* to which useful antibodies can be generated. Several groups have raised antibodies against the *O*-linked polysaccharide side chains of lipopolysaccharide (LPS) of *P. aeruginosa* but the LPS side chains are highly variable and contribute at least 20 serotypes among clinical isolates. Multiple human antibodies raised against a total of 10 serotypes in mice transgenic for human immunoglobulin genes have shown activity in animals [11,12], raising the prospect of an oligoclonal antibody preparation to treat at least the majority of serotypes. A human IgM antibody specific for serotype 011 (KBPA101) developed by Kenta Biotech, is currently in Phase 2 clinical trials, with other antibodies against other serotypes in preclinical development (Kenta Biotech website; URL: http://www.kentabiotech.com). IgM antibodies, however, have shorter *in vivo* half-lives than IgG antibodies, are sensitive to the many secreted bacterial proteases (see below), and may be costly to deliver. Furthermore, expression of *O*-linked LPS side chains is reduced in mucoid strains that are prevalent in the lungs of CF patients [13].

The bacterial flagellum is a comparatively conserved structure and two monoclonal antibodies against two common flagellum variants have shown activity in a mouse burn model of *P. aeruginosa* infection [14]. The antibodies collectively recognize up to 98% of clinical isolates but the need to combine two antibodies and the possibility of selection of flagellum-negative variants may have been factors in the lack of further development.

Drawbacks of antibodies to bacterial surface components

Many bacterial infections are opportunistic and the existence of persistent infection indicates effective evasion of host immune response. In almost all CF patients having chronic lung infections with *P. aeruginosa*, high titers of opsonizing antibodies can be measured in the systemic circulation yet the infectious pathology still progresses. Indeed, antibodies from CF patients have been demonstrated to be ineffective at mediating phagocytic killing [15].

One of the major concerns in targeting the bacterium directly is the selection of escape variants, which may arise through a wide variety of mechanisms in addition to the selection of variants that do not retain the antibody-binding site. Production of a bacterial capsule, for example, can provide effective protection against opsonization. Capsules are typically permeable to antibodies but mask antibodies bound to the bacterial surface, preventing contact with receptor-bearing effector cells [16].

Bacteria typically have multiple mechanisms to evade immune responses and block antibody effector functions. *S. aureus* produces two proteins, Protein A and Sbi, that bind directly to antibody Fc regions and inhibit complement activation and Fc-receptor binding [17]. Protein A also binds variable regions of some antibodies and so can inhibit the activity of some Fab and scFv molecules as well [17]. Similar antibody-binding proteins are found in other bacteria, such as the Protein G and Protein H of *Streptococcus pyogenes*.

The importance of neutrophils in the antibody response makes these cells a major target for bacterial evasion. The chemotaxis inhibitory protein of *S. aureus* (CHIPS) binds both the formyl peptide receptor and complement C5a receptor to inhibit neutrophil recruitment to sites of infection [18•]. Similarly, complement activation is a frequent target for bacterial immune evasion mechanisms. *S. aureus*, for example, has at least five complement-inhibitory proteins [18•]. Both CHIPS and the staphylococcal complement inhibitor (SCIN) show strict specificity for human complement [19] which may be a significant factor in determining the differences in the responses to some antibodies in animal models compared with human disease.

Many bacteria secrete proteases that inactivate antibody effector functions by cleaving at the hinge region of IgG and IgM, separating the Fc-region from the antigen-binding site. The resulting Fab or $F(ab')_2$ fragments compete for binding to the infecting bacteria further preventing effective immune clearance [20]. *P. aeruginosa* secretes at least four distinct proteases that are pathogenic determinants abundant in the sputum of CF patients [21,22,23]. Interestingly, in the human airway a number of antiproteinases, for example α_1 protease inhibitor (α_1-PI) are secreted as a protective mechanism for the epithelial surface, but *P. aeruginosa* secretes a potent inhibitor of α_1-PI, the phenazine derivative, pyocyanin [24]. *S. aureus* also produces four major proteases that directly or indirectly degrade antibodies [25,26]. Thus, passive immunotherapy that relies on antibody effector functions is likely to be negatively impacted by secreted proteases capable of inactivating IgG. Antibody therapies that do not rely on Fc effector function may have a greater chance of success.

Lastly, the access of antibodies may be limited by biofilm formation. Many pathogenic bacteria switch morphology between a free-swimming planktonic form and a sessile mucoid form that forms the biofilm, creating a reservoir of organisms comparatively inaccessible to antibiotics. Biofilm formation is triggered by a variety of environmental conditions and can be induced by the immune response [27]. In CF, the lungs tend to be colonized by mucoid forms of *P. aeruginosa*. Although these are less pathogenic they can seed pathogenic planktonic bacteria throughout the lungs leading to progressive lung damage. The switch

to biofilm growth involves a profound antigenic shift with the downregulation of *O*-linked LPS side chains and upregulation of alginate production making the identification of antibodies to *P. aeruginosa* surfaces that will detect all clinical strains particularly challenging [13].

Antibodies to an exopolysaccharide of *S. epidermidis*, poly *N*-acetyl glucosamine (PNAG) have been shown to be ineffective in opsonic killing of bacteria in a biofilm because of high levels of non-cell-associated soluble antigen [28]. These authors showed by immunofluorescence analysis that the biofilm did not present a significant diffusion barrier and suggest that the PNAG shed from cells in the biofilm simply acts as a decoy to reduce the level of antibodies capable of reaching the bacterial cell surface. This has important implications because it suggests that appropriate choice of antibodies to epitopes not shed from the cell surface may permit better access to bacteria growing in biofilms. Thus, for example, Alopexx is in preclinical development with an antibody, F598, specific for a deacetylated form of *S. aureus* PNAG preferentially retained on the bacterial surface, in contrast to the acetylated form abundant in biofilms. The antibody has potent activity in animal models and deacetylated PNAG is also found in *S. epidermidis* and even *E. coli*, implying that such antibodies may have a broad spectrum of activity [29]. The conserved alginate molecule of *P. aeruginosa* is another target for which choice of the precise epitope may be crucial. One such antibody, F429, against a carboxylic-acid component of alginate mediates phagocyte-dependent killing and is protective against both mucoid and nonmucoid strains in a pulmonary infection model, because of rapid induction of alginate expression *in vivo* [30]. The antibody is currently in preclinical development at Aridis, designated Aerucin. However, it remains to be seen if improved access of antibodies to bacteria in biofilms will be sufficient for clinical efficacy; such antibodies are also subject to the other bacterial evasion strategies described above.

Indirect neutralization of pathogenic bacteria

Bacteria produce a number of diffusible virulence factors such as pigments, proteases and toxins as well as 'signaling' molecules. Neutralizing virulence factors represent an 'indirect' targeting approach to bacterial infections, its success relying upon the ability of the host immune system to clear the infection. Several antibodies to toxins and signaling molecules are currently being evaluated.

It is now recognized that bacteria synthesize factors that regulate the metabolic activity of other bacteria within their vicinity. This 'communication' can be either intra-species or inter-species and is termed quorum sensing.

Gram-negative bacteria communicate through a number of diffusible autoinducers (AIs) including *N*-acyl homocysteine lactones and *N*-acyl homoserine lactones (C-12 inducers) [31]. Expression of the AIs regulates the expression of virulence factors that directly contribute to colonization and dissemination of the pathogen. The C-12 AIs have also been shown to interfere with host cell signaling in cells of the immune system and can induce apoptosis in macrophages [32]. In animal studies, mice with a high titer of antibodies to *N*-acyl homoserine lactone following immunization were shown to be more resistant to *P. aeruginosa* challenge [33] and an anti-C-12 antibody protects macrophages from C-12-induced cytotoxicity [34]. Gram-positive bacterial signaling is mainly through small peptides. A monoclonal antibody to *S. aureus* autoinducing peptide 4 (AIP-4) suppressed pathogenicity in murine disease models of infection [35]. The results in *in vitro* and *in vivo* models of bacterial infection suggest that neutralizing quorum sensing molecules may provide a new and effective treatment modality although the importance of quorum sensing in pathogenicity in humans is unknown. The concentrations of AIs in infected tissues are relatively high (μM) and it may be difficult to deliver sufficient antibody to provide effective neutralization during an ongoing infection. Success of the approach may depend on efficient antibody delivery and rapid clearance of immune complexes.

Many pathogenic bacteria secrete protein toxins that are key virulence factors. Neutralization of these toxins should render the bacteria less pathogenic and allow clearance of bacteria by the immune system. Currently, several antibody approaches targeting such toxins are in clinical trials (Table 1).

ABthrax is a recombinant human antibody with high affinity for *Bacillus anthracis* protective antigen, the receptor-binding component of anthrax toxin. The antibody provides complete protection against a lethal bacterial challenge in animal models [36]. A Phase 1 clinical trial has been completed with this antibody [37] and further development is in progress. Two other antibodies to protective antigen are also in clinical development having shown activity in animal models of inhalation anthrax; a deimmunized antibody [38] and a human antibody, Valortim [39].

Hemolytic-uremic syndrome (HUS) is a serious complication of infection by Shiga toxin-producing *E. coli* (S-TEC). A chimeric antibody that neutralizes the toxin Stx2 is protective in animal models and has been tested in Phase 1 safety study in humans [40]. Further studies are planned with a pair of antibodies recognizing both Stx2 and Stx1 (Thallion website; URL: http://www.thallion.com).

The pathogenic role of *C. difficile* in intestinal disease is well established. This organism can cause mild self-limiting diarrhea or fulminating pseudomembranous colitis. The pathogenicity of this microbe is in part dependent

upon two immunologically distinct toxins: A and B. Human antibodies that neutralize either toxin A or B have been evaluated in a hamster and mouse model of infection. Combination of an anti-A toxin antibody and anti-B antibody demonstrated the best *in vivo* efficacy [41]. Clinical trials with this pair of antibodies are ongoing (Medarex website; URL: http://www.medarex.com/Development/Pipeline.html).

Many Gram-negative bacteria are able to inject toxins directly into adjacent cells using a protein syringe-like structure called the type III secretion system (TTSS), providing a mechanism for blocking the activity of multiple toxins with a single therapeutic agent [42•]. Indeed, acquisition of an active TTSS can be causally related to pathogenicity, as in STEC described above [40,42•]. In the case of *P. aeruginosa*, an important pathogen in immunocompromised people and those on a ventilator, almost all environmental isolates possess the TTSS-encoding genes but different strains vary in the exotoxins secreted. Pathogenicity correlates with the expression of the TTSS and the exotoxins secreted [43,44]. A monoclonal antibody, Mab166, directed against the PcrV needle-tip protein of the *P. aeruginosa* TTSS, inhibits TTSS function and is therapeutically active in animal models of acute *P. aeruginosa* infection [45,46]. An engineered human Fab′ fragment has also been generated against the PcrV protein, derived from Mab166 by a process of antibody humaneering^{TM}. This antibody fragment, conjugated to polyethylene-glycol (PEG), is currently in clinical development for the treatment of *P. aeruginosa* lung infections in CF and in mechanically ventilated patients (KB001; KaloBios website; URL: http://www.kalobios.com/kb_pipeline_001.php). This drug candidate has novel properties that address potential problems with antibody-based antibacterial therapeutics. The activity of the Fab fragment is important in reducing sensitivity to *Pseudomonas* proteases that degrade IgG antibodies and PEGylation both extends *in vivo* half-life and further reduces susceptibility to proteolytic inactivation. The ability of the Fab fragment to inactivate the TTSS results in prevention of toxin injection into cells and also prevents the direct killing of macrophages by the TTSS that occurs even in the absence of toxins [47]. KB001 is thus an exciting new approach to the treatment of *P. aeruginosa* infections that is not dependent on antibody effector function.

The neutralization and clearance of bacterial toxins provides a logical antibacterial therapeutic approach. A human immune serum that neutralizes botulinum toxin is approved for the treatment of infants with suspected botulism [48] and a horse serum is approved for the treatment of adults. The success of antitoxin approaches depends upon early diagnosis of infection and in some cases, the ability to neutralize multiple unrelated toxins. For the latter, a monoclonal antibody is likely to be less effective than a polyclonal approach. The direct comparison of single toxin neutralization and double toxin neutralization with *C. difficile* toxin monoclonal antibodies in the clinic will be instructive. The inhibition of the *P. aeruginosa* TTSS represents a monoclonal antibody approach that effectively neutralizes several protein toxins that correlate with pathogenicity in humans and should test the utility of antivirulence approaches in human bacterial infections [49].

Conclusions and future prospects

There are currently no FDA-approved antibacterial monoclonal antibody products. Those evaluated to date may not have had optimal activity. Some, for example, have had short *in vivo* half-lives (scFv and IgM) requiring frequent dosing. Others may not have had appropriate specificity. Centoxin, the first monoclonal antibody to be developed against a bacterial cell-wall component, was ultimately shown to have inadequate specificity [50•]. In other cases, antibodies requiring immune effector functions have been tested in patients with weakened or immature immune systems. Species-specificity in bacterial immune evasion mechanisms may also have contributed to differences between the results in animal models and human clinical trials.

There is now a greater understanding of immune evasion mechanisms, and the need to match antibody target epitopes and mechanism of action, as well as optimizing antibody delivery to the site of infection.

One novel approach to addressing the problem of bacterial complement evasion is being pursued by Elusys. The major route of clearance of complement-opsonized immune complexes in humans is via the complement receptor 1 (CR1) expressed on erythrocytes. Erythrocytes coated with bacteria are rapidly cleared via the liver. Elusys has exploited this system to target immune complexes directly to CR1 using an anti-CR1 antibody. In mouse models, an anti-CR1 antibody cross-linked to an antibody to *S. aureus* Protein A to generate a heteropolymer (designated ETI-211) has shown rapid clearance of *S. aureus* from the blood stream (within 30 min) and demonstrated survival benefits in prophylactic and therapeutic models [51]. This approach may be attractive for enhancing bacterial clearance for the treatment of bacteremia although it may be less effective for tissue infections in which access to erythrocytes is limited.

Alternative methods for reducing virulence may provide the opportunity to interfere with bacterial defenses and allow more effective clearance by natural host mechanisms. Bacterial toxins in particular represent a class of highly conserved targets which play an essential role in pathogenicity. Neutralizing either the toxins or toxin delivery systems such as the TTSS of Gram-negative bacteria provides the potential to intervene in the

pathology of the disease without selecting escape variants and may provide effective means of prevention or treatment of some of the most intractable bacterial diseases.

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