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REVIEW ARTICLE

ANTIBODY-BASED ANTIBACTERIAL AGENTS: AN EMERGING OPTION

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SUMMARY

The use of antibodies as antibacterials precedes the antibiotic era and historically provided the first therapeutic tool to help control the burden of infectious diseases. Multiple advances in the production of therapeutic antibodies, primarily through the application of monoclonal methodology, as well as refinements in the identification of key bacterial components that could be targeted to avert pathogenesis, have resulted in a renewed interest in the development of antibodies to control bacterial infection. Several bacteria known for their virulence and resulting high morbidity and mortality have been the focus of intense investigation in this regard. This review presents a comprehensive assessment of the multiple agents currently under development. Although the rationale and preclinical work that preceded the development of many of these agents has been robust, this has not translated into an agent with demonstrated effects in clinical trials convincing enough to warrant approval for human use. The potential reasons for this apparent discrepancy are also discussed. Hopefully, candidate drugs with sufficient beneficial effects will be identified as further advances are made in both the methodologies applied for the development of these potential therapeutics and, as importantly, in our understanding of key pathophysiological mechanisms amenable to intervention.

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INTRODUCTION

Infectious diseases have played a significant role in human history, being a major contributor to both serious morbidity and mortality. Up until the late 19th century, infections were a predominant cause of death and with little hope for control of the most serious conditions. Following the work by Roux and Yersin in 1888 (1), Emil von Behring and his colleague Shibasaburo Kitasato demonstrated in 1890 that an immune serum produced in horses inoculated with diphtheria could be administered to diseased individuals and effect a cure (2). This was the birth of passive immunotherapy as a treatment option and opened a new era in the fight against infectious diseases (3). Consequently, the first Nobel Prize in Medicine was awarded in 1901 to von Behring for his work on serum therapy and its application against diphtheria. The responses observed to this therapeutic paradigm were so dramatic that there was hope for the control of human disease mediated by infectious organisms through the application of serum therapy (4). However, very early on it was already noted that this approach had some serious limitations, in particular due to recipient reactions (5), but also in great part due to variability and lack of purity in the preparations available. The dawn of the antibiotic era in the 1940s, through its effectiveness in controlling bacterial infection, virtually displaced the use of antibodies as a therapeutic option in the treatment of infections. Nevertheless, the use of serum therapy for the treatment of serious viral infections remained, although its role was recognized mainly as an adjuvant. Most of the preparations were based on the pooling of high-titer immune human sera, which posed some challenges as to the activity and safety of the preparations available. No major development in the field occurred until Milstein and Köhler reported in 1975 on their development of a technique for the production of monoclonal antibodies based on the creation of fused cells they defined as hybridomas (6), and they shared the Nobel prize in 1984 for this work. This discovery ushered a new era in the development of antibody-based therapies, given the specificity and purity of the preparations obtained. Eventually, this led to the development of the first monoclonal antibody (MAb) for human therapeutic use. In 1986, an MAb directed at T lymphocytes, muromonab-CD3 (Orthoclone OKT3; Janssen-Cilag), was approved for the prevention of kidney transplant rejection (7, 8). Since then, a total of 24 antibodies for either therapeutic or diagnostic indications have received approval in the U.S. (9).

Of note, only one of these is directed at an infectious organism: palivizumab against respiratory syncytial virus (RSV) (10, 11). Given that most viral infections are not amenable to direct antibiotic therapy, it is not surprising to see that for many years most of the efforts for the development of antibody therapies as anti-infectious agents have been directed primarily at viruses. Despite this, besides RSV, no other monoclonal antibody directed at a viral entity has been developed and approved. However, the field is rapidly evolving, with continuously evolving sophistication in the techniques and approaches being utilized (12-14).

The last decade has seen a rising problem with bacterial resistance to antibiotics, as well as an increased prevalence of infections with more virulent organisms (15). This has been compounded by the threat of bioterrorism with the use of highly virulent toxin-producing organisms (16). Most alarming is the rising prevalence of antibiotic resistance among bacteria previously held as widely sensitive to antibiotics, as well as the emergence of bacteria intrinsically highly resistant to antibiotics as the etiology of common infections both in the hospital setting as well as in the community (17, 18). Perhaps of greatest concern is a lag in the development of more potent agents within the known classes of antibiotics, and more so for the development of new classes of antibiotics that could potentially be less amenable to bacterial resistance (19). In parallel to this, significant improvements in the development and manufacturing of monoclonal antibodies, including the development of humanized antibodies and refinements in the use of cell lines for synthesis, have greatly enhanced the feasibility of producing reliable therapeutic products for human use. All of these facts have led to a renewed interest in the development of antibodies for the treatment of infectious diseases. As a consequence, a number of programs targeted at specific infectious agents have come along (Table I) and will be the focus of this review. However, although some of these hold great promise, no agent has yet proven convincingly enough to be efficacious to merit approval as a therapeutic agent.

STAPHYLOCOCCI

Staphylococcus aureus is a Gram-positive organism that has high carriage prevalence in the population and is a common member of

Table I. *Monoclonal antibodies in clinical development for indications associated with bacterial infection or fungal infections (list is not exhaustive).*

Microorganism	Antibody	Targeted epitope	Stage of development
S. aureus	Tefibazumab	Clumping factor A	Lack of efficacy Program terminated
S. aureus	Aurograb®	EMRSA-15	Lack of efficacy Program terminated
S. aureus	Pagibaximab	Lipotechoic acid	Phase III study to be completed 2011
S. mutans	Carorx®	Adhesin SA I/II	Lack of efficacy Program terminated
P. aeruginosa	Panobacumab	LPS-O polysaccharide	Awaiting confirmatory trials
P. aeruginosa	KB-001	TTSS PcrV	Phase I/II studies completed
B. anthracis	Raxibacumab	Protective antigen (PA)	Added to strategic stockpile Awaiting FDA approval
B. anthracis	Anthim®	Protective antigen (PA)	Phase I study
B. anthracis	Thraxiva®	Protective antigen (PA)	Phase I study
B. anthracis	Valortim®	Protective antigen (PA)	Phase I study
C. botulinum	XOMA 3AB	Toxin A	Phase I study
C. difficile	MK-3415A	Toxin A/toxin B	Phase III study
E. coli	Urtoxazumab	Stx2	Phase I completed
E. coli	CαStx1/ CαStx2	Stx1/Stx2	Phase I completed
E. coli	Edobacomab	Endotoxin	Lack of efficacy Program terminated
E. coli	Nebacumab	Endotoxin	Lack of efficacy Program terminated
C. albicans	Efungumab	Hsp90	Lack of efficacy Program terminated
C. neoformans	MAb18b7	Glucuronoxylomannan	Phase I completed No further development

the normal flora in humans. In healthy carriers it can be found on nasal passages, skin and mucous membranes. As a pathogen, it is an important cause of morbidity and mortality, causing a wide range of suppurative infections, as well as food poisoning and toxic shock syndrome. A complex process that involves increased susceptibility in the host, breakdown of natural barriers and the production of virulence factors by the organism leads to both the development and severity of the infectious process (20). *S. aureus* is armed with a number of toxins that provide it with great virulence and damaging effects on the host tissues, as well as the ability to trigger a cascade of systemic effects. Interest in controlling this bacterium with drugs other than antibiotics has increased in the recent past given the widespread emergence of antibiotic-resistant strains both in the hospital setting and in the community. Several antibodies targeting toxins and virulence factors have been evaluated for their ability to block the pathogenicity of *S. aureus*. This has led to the development of concentrated immunoglobulin preparations targeting surface components such as the capsular polysaccharide antigens (Altastaph®; obtained from subjects immunized with staphylococcal polysaccharides 5 and 8) or the microbial surface components recognizing adhesive matrix molecules ("MSCRAMMs") clumping factor A (Veronate®). However, none of these proved successful in clinical trials on populations at risk (21-24). This has in part been perceived as related to the relative immune impairment of the subjects, as well as potentially perhaps from lack of sufficient affinity of the antibodies generated (25). In spite of these disappointing results, further research on the development of antibodies directed at these targets led to the identification of monoclonal antibodies as a strategy to enhance the affinity and activity of the antibodies with hopes of producing the intended clinical benefit. Encouraged by the results of preclinical studies, potential therapeutic candidates are at this point in time in different stages of clinical development. These include tefibazumab (targeting clumping factor A), Aurograb® (targeting an ABC transporter) and pagibaximab (targeting lipoteichoic acid [LTA]).

Tefibazumab is a humanized MAb version of a murine antibody directed at *S. aureus* clumping factor A (*clfA*) (26). The MSCRAMM clumping factor A is a staphylococcal surface protein involved in binding to fibrinogen (27). It promotes attachment to various host tissues and cell types, as well as biomaterials. In a rabbit endocarditis model, pooled human serum from donors with high titers against clumping factor A given in combination with vancomycin resulted in fewer animals with bacteremia and a smaller bacterial load than animals given vancomycin alone (28). Further studies with intravenous immunoglobulin showed some positive trends in a phase II clinical trial and this was taken as evidence for a potential beneficial role of targeting clumping factor A (21). However, a subsequent study with this preparation in a neonatal population failed to show a beneficial effect in sepsis due to *S. aureus* and *Staphylococcus epidermidis* (24).

Further work on this target led to the development of tefibazumab, with an intended application as adjunctive therapy to treat serious staphylococcal infections. However, a phase II clinical study in patients with *S. aureus* bacteremia given tefibazumab in addition to standard therapy failed to achieve statistically significant differences in a composite clinical endpoint (29). The manufacturer of the antibody, Inhibitex, then halted further clinical development of the drug.

Aurograb® is a human-derived single-chain antibody fragment lacking the immunoglobulin Fc domain and targeted at EMRSA-15, a 61-kDa ABC transporter expressed by epidemic strains of methicillin-resistant *S. aureus* (MRSA) (30). EMRSA-15 was identified as an important immunodominant target through the analysis of serum from patients affected by serious MRSA infections (31). This ABC transporter has high homology to YkpA from *Bacillus subtilis* and is putatively involved in cell wall biosynthesis (21). Antibodies raised against this target have intrinsic antistaphylococcal activity and bind to the bacterial cell surface. Aurograb® was then developed as an adjuvant for the treatment of deep-seated MRSA infections. Evidence for its activity against antibiotic-resistant *S. aureus* was obtained through in vitro testing, demonstrating a synergistic effect in combination with the antibiotic vancomycin, with reductions in the MIC for vancomycin by as much as 40-fold (32). However, a subsequent clinical trial (ClinicalTrials.gov Identifier NCT00217841) to determine the overall response (clinical and bacterial) to Aurograb® plus vancomycin in adult hospitalized patients with severe staphylococcal infections failed to reach endpoints, and the drug development program was eventually dropped (33).

Pagibaximab is a monoclonal antibody for the prevention of staphylococcal sepsis that is being primarily investigated as a prophylactic agent in infants with low birth weight, a high-risk population for staphylococcal infections. The antibody consists of a humanized mouse chimeric antibody against LTA, producing opsonic activity against staphylococci. The antibody has been shown in preclinical studies to confer protection against both *S. aureus* and *S. epidermidis* (34). Subsequent normal volunteer studies proved the antibody to be safe and well tolerated, with a long half-life and induction of dose-related serum anti-LTA and opsonophagocytic activity (35). Favorable safety and pharmacokinetics were demonstrated in early clinical trials with very-low-birth weight infants (36). In a subsequent clinical trial with two dose levels of the antibody (60 and 90 mg/kg) against placebo, a trend for those treated with the higher dose of fewer positive blood cultures due to staphylococci was seen (0% in the 90 mg/kg group, 10% in the 60 mg/kg group and 13% in the placebo group) (37). Furthermore, an antibody level > 500 μg/mL was identified as a target for protection given that cases of sepsis occurred mostly among individuals with antibody levels below this cut off. Subsequent pharmacokinetic modeling studies have been performed to define dosing regimens that achieve and maintain serum levels of ≥ 500 μg/mL (38). Following these encouraging early results, a larger clinical trial has been implemented with plans to enroll a large number of infants at risk (ClinicalTrials.gov Identifier: NCT00646399). Subjects will be monitored for the occurrence of neonatal sepsis, as determined by the presence of clinical signs and symptoms and positive blood cultures. The study is expected to be completed in 2011. In addition, the potential for pagibaximab to act as an adjuvant to antibiotic therapy for serious MRSA infections has been investigated in an animal model with promising results (39).

STREPTOCCOCUS MUTANS

S. mutans is a common member of the flora of the oral cavity that is involved in the development of dental caries and tooth decay. As such, it has been for many years the focus of investigation for the prevention of tooth decay. A key event in the pathophysiology of

dental caries is the adhesion of the microorganism to the salivary pellicle of the teeth surface mediated through bacterial surface adhesins. The adhesin SA I/II is present in all *S. mutans* strains capable of promoting tooth decay. Treatment with an antibody directed at SA I/II completely prevented caries in an animal model, while caries developed in control animals (40). The mechanism proposed for this effect is that following decontamination of the oral cavity, the local bacterial population is regenerated by the proliferation of several species that compete for the oral niche. By blocking the ability of *S. mutans* to adhere to teeth, favorable conditions are then created for more benign populations to out-compete *S. mutans* for space and become the predominant flora (41, 42). A murine IgG_1 MAb (Guy's 13) directed at the SA I/II protein has been proven in both animal and human studies to block the ability of *S. mutans* to adhere to teeth and proliferate (43). This finding led to the development of a hybrid fusion murine monoclonal secretory antibody (sIgA/G) having constant regions of IgG and IgA. As an additional innovation, DNA encoding the combining regions of the Guy's 13 antibody was cloned and used to create a transgenic tobacco plant that produces the antibody (namely, a "plantibody") (44). In preliminary small human studies, the topical application of the antibody to teeth was shown to prevent re-colonization with *S. mutans* for a prolonged period of time and protect teeth (45). However, a subsequent randomized clinical trial failed to show any protective effect on the recolonization with *S. mutans* after oral decontamination (46). A possible reason advanced to explain this discrepant result was that the concentration of antibody used in the second trial was 35-40% lower and subjects may have received a dose below the required threshold for effectiveness. Further development of this antibody has not occurred.

PSEUDOMONAS AERUGINOSA

P. aeruginosa is a ubiquitous environmental organism that is a major opportunistic pathogen in certain diseases where host defense mechanisms are impaired, such as cystic fibrosis (CF), extensive burns, cancer and immunodeficiencies, as well as in hospital-acquired infections. It is particularly noted as a major reason for disease progression and mortality among patients with CF (47). In the hospital setting, its role as a significant cause of morbidity and mortality is clearly recognized in the setting of ventilator-associated pneumonia (VAP) (48). The pathogenicity of *P. aeruginosa* is in great part related to its large toxin repertoire. As with other Gram-negative bacteria, different secretion systems allow *P. aeruginosa* to utilize an array of bacterial products by either releasing them into the surrounding environment or directly injecting them into host cells through the use of secretion systems. Of note is the expression in strains of *P. aeruginosa* of the type III secretion system (TTSS). Although most toxins produced by bacteria are secreted into the surrounding extracellular environment, the TTSS allows bacteria to inject potent toxins directly into the cell, as well as create pores in the cell (49). The TTSS consists of three protein complexes that are expressed and assembled in a highly coordinated fashion: a secretion apparatus, a translocation apparatus and an array of specific toxins and chaperones (50, 51). *P. aeruginosa* secretes via the type III secretion system four potent toxins (ExoS, ExoT, ExoU and ExoY) that effect cell death and are recognized as risk factors for death in nosocomial pneumonia (52). It has also been demonstrated that phagocytic cells in the lung are injected with ExoU and this effectively eliminates one important mechanism of defense, thus facilitating the pathogenesis of pneumonia caused by *P. aeruginosa* (53). In addition, because of its multiple mechanisms of antibiotic resistance, there is a rising incidence of multidrug resistance challenging the ability to treat infections due to *P. aeruginosa* (54). This has led to decades of intense investigation into the development of protective strategies for populations at risk through either vaccines or passive immunization (55). Although an array of key immunogens, including virulence factors, have been characterized, very little progress has been made towards the identification and approval of an effective candidate. Currently, two candidate MAbs have shown promise in early clinical trials: panobacumab and KB-001.

Panobacumab (KBPA-101) is a fully human pentameric IgM MAb which is directed against the lipopolysaccharide (LPS) *O*-polysaccharide moiety of *P. aeruginosa* serotype IATS O11. This specific serotype accounts for 20% of clinical *P. aeruginosa* isolates. Early work with the use of an octovalent *P. aeruginosa O*-polysaccharide-toxin A conjugate vaccine in CF patients at risk for infection showed some promising results, with induction of strong antibody responses (56, 57). Larger studies with this vaccine failed to show a long-term protective effect from chronic infection, and at present there is no vaccine approved for this use (58). However, it had been noted in earlier studies that immunized patients who maintained a high-affinity anti-LPS antibody response had a significant reduction in the rate of infection (57). This was taken as an indication for the importance of achieving high titers of an antibody with high affinity to effect protection (59). Following on this lead, the development of panobacumab also introduced the concept of utilizing an IgM antibody to elicit complement activation and effect bacterial killing. The antibody was identified by first obtaining B-cell-enriched peripheral blood lymphocytes from a volunteer immunized with a *P. aeruginosa O*-polysaccharide-toxin A conjugate vaccine to then generate human hybridoma cell lines producing MAbs specific for individual LPS serotypes (60). Subsequent preclinical studies in mouse models of pulmonary infection demonstrated that the antibody administered systemically reaches the lung space and effects protection from mortality and enhances clearance of infection (61). The antibody has already been shown in healthy volunteer studies to be safe and non-immunogenic, with linear pharmacokinetics (62). In a subsequent small open-label study with patients affected by nosocomial pneumonia due to *P. aeruginosa*, the pharmacokinetics and safety of panobacumab were investigated (63). Patients received three infusions of 1.2 mg/kg of panobacumab 3 days apart, at the same time that they continued antibiotic therapy and other cares appropriate for their condition. Of note is that two of three of the patients enrolled had polymicrobial infection in association with *P. aeruginosa*. This study demonstrated the drug to be safe, well tolerated, free from immunogenicity and to have favorable kinetics. In addition, higher clinical cure and survival rates than those expected given the condition of these patients were observed, and this was more notable among those patients who received all the intended doses, being taken as suggestive of efficacy (64). This awaits more detailed and larger studies for confirmation.

KB-001 is a humanized murine IgG pegylated Fab antibody directed at PcrV, a component of the TTSS of *P. aeruginosa*. PcrV is located at the tip of the injector apparatus and is crucial for the toxin translo-

cation process to proceed (65). In animal models of infection with *Pseudomonas*, blockade of PcrV with antibody or immunization effectively enhances survival and response to infection (65, 66). Of note is that in a model of chronic infection with PcrV expressing *P. aeruginosa*, blockade of PcrV with antibody led to significant improvements in inflammatory changes in the lung, and this without effecting bacterial clearance (67). This suggests that by blocking one key virulent mechanism the inflammatory response is modulated so that the infection is not as damaging to the lungs.

This observation is of high relevance to the infection seen in patients with CF. In the lungs of CF patients, abnormalities in the airways surface milieu lead to an environment prone to chronic infection with *P. aeruginosa*, among other organisms (68). The damage to the lungs is a consequence of an exuberant inflammatory response to the presence of the bacteria and not solely due to the bacterial activity itself. Thus, modulating the inflammatory response can have significant beneficial effects. KB-001 has been tested in two different patient populations: patients with acute VAP due to *P. aeruginosa* and patients with CF chronically infected with *P. aeruginosa*. After favorable normal volunteer studies, only small early clinical trials have been completed to date in both patient populations. For VAP patients, a randomized, double-blind, placebo-controlled study was conducted in 10 centers in France to evaluate safety, tolerability, pharmacokinetics and immunogenicity (69). To participate in this study, patients had to be colonized with *P. aeruginosa* at enrollment but be free from any signs of infection. Subjects were randomized to a single i.v. infusion of placebo or two dose levels of KB-001 (3 or 10 mg/kg) and then followed clinically for 28 days. No safety concerns were noted during the study and analysis of bronchoalveolar lavage fluid (BALF) revealed a dose-dependent penetration of KB-001 into the airspace. Of interest, trends towards improved clinical outcomes were observed in the subjects randomized to active treatment, with more subjects alive at day 28 without *Pseudomonas* infection (40% active groups combined vs. 20% placebo group). For CF patients with *P. aeruginosa*, a randomized safety and tolerability study was completed of a single dose of KB-001 at two dose levels of 3 and 10 mg/kg (70). Participants in this study were assessed for microbiological, inflammatory and pulmonary function parameters at baseline and 4 weeks following infusion. Patients had a large bacterial burden in their respiratory secretions, reflected by a mean *P. aeruginosa* density of 7.7 log CFU/g of sputum at enrollment. Overall, a single infusion of KB-001 had a good safety profile and no patient developed anti-KB-001 antibodies. As in the VAP study, despite the relatively small sample size, important positive trends were noted in the active treatment groups in inflammatory parameters when compared to the placebo group (71). The encouraging findings from these studies await confirmation in larger efficacy studies.

BACILLUS ANTHRACIS

B. anthracis is the etiological agent of anthrax, an infection that can affect the skin, lungs or gastrointestinal tract. Of special note is inhalational anthrax, a highly lethal pulmonary infection with rapid progression to respiratory failure and death. This has resulted in its long-standing recognition as a potential agent for application as a biological weapon (72). *B. anthracis* spores, when inhaled, are deposited in the alveolar spaces of the lungs, being ingested by resident macrophages. Macrophages will mobilize to local lymph nodes, where spores will germinate over the course of a few days to weeks (73). The pathogenicity of anthrax is primarily due to the action of two potent toxins that effect cell death: lethal factor (LF) and edema factor (EF). These toxins inhibit normal immune system functioning, interfere with signal transduction pathways and ultimately cause cell death. Metabolically active *B. anthracis* releases protective antigen (PA), which forms heptamers that bind to the anthrax toxin receptors (*ANTXR*), of which two related cell-surface proteins have been recognized as such: anthrax toxin receptor 1 (tumor endothelial marker 8; *ANTXR1*) and anthraxin toxin receptor 2 (capillary morphogenesis gene 2 protein; *ANTRX2*) (74). The heptamers on the cell surface then bind LF and EF, resulting in a protein–receptor complex that is internalized into the cell, allowing LF and EF to effect cell death (75). This identified PA as a factor to target to block a key step in the pathogenicity of anthrax. Thus, multiple efforts have been directed at producing effective means of both active and passive immunization against PA as a way of preventing and controlling inhalational anthrax. Four monoclonal antibodies have been developed against PA, one of which is awaiting FDA approval for human use and three others are currently at different stages of clinical development.

Raxibacumab is a recombinant human IgG_1 MAb directed at PA that inhibits the binding of PA to the cell surface receptors. Preclinical studies with this antibody demonstrated protection from death in a rat model of anthrax infection (76). These studies were followed by animal efficacy studies and human volunteer safety and pharmacokinetic studies (77). This innovative strategy followed the 2002 animal rule regulations that allow the FDA to approve drugs that demonstrate efficacy only in animal models, provided the drug would have a reasonable health benefit in humans and the drug is deemed safe for human use (78). The animal rule can be applied in the case of infections that usually have a low incidence, such as anthrax, and where human studies will be deemed impractical and/or unethical. In the clinical studies with raxibacumab, a total of 483 subjects received 1 or 2 intravenous doses of the antibody. The half-life of the antibody was in the range of 20–22 days, and serum levels were maintained for 28 days at a concentration greater than or equal to the highest concentration of PA in the serum of monkeys exposed to lethal doses of *B. anthracis* (77). Animal studies in rabbits and monkeys proved the efficacy of the antibody, with protection from death in both prophylactic use as well as post-exposure. As a result of this favorable evidence, raxibacumab was deemed safe and effective for human use and added to the Strategic National Stockpile as an agent for the control of inhalational anthrax (79). However, due to questions on the human pharmacokinetics, this antibody is still awaiting FDA approval.

Three other MAbs targeting anthrax are also in different phases of clinical development. ETI-204 (Anthim®; Elusys Therapeutics, Inc.) was developed by engineering a panel of PA-neutralizing human antibodies and selecting them for high affinity (80). When administered to rabbits as a single intravenous injection prior to exposure to a lethal aerosolized anthrax spore challenge, significant protection from death was noted (81). Studies of administration 24 and 36 hours after exposure to the spore challenge also demonstrated significant protection from death, as well as freedom from bacteriemia, among the surviving animals. Based on these results, ongoing clinical trials are assessing the safety and pharmacokinetics of ET-204 in humans (ClinicalTrials.gov Identifier: NCT00138411).

Two additional MAbs with encouraging preclinical profiles also in clinical trials are AVP-21D9 and MDX-1303. AVP-21D9 (Thravixa®; Emergent Biosolutions, Inc.) is a human IgG_1 MAb specific for PA that inhibits the heptamer assembly and renders it ineffective (82). The antibody has been shown in rabbits to protect from lethal spore exposure (83) and is currently undergoing phase I studies to evaluate pharmacokinetics and immunogenicity (ClinicalTrials.gov Identifier: NCT01202695). MDX-1303 (Valortim®; PharmAthene, Inc.) is directed at blocking PA and is currently in phase I trials to assess the safety and pharmacokinetics of a single-dose infusion (ClinicalTrials.gov Identifier: NCT01265745).

CLOSTRIDIUM

Clostridium are Gram-positive bacteria which are obligate anaerobes and capable of producing dormant spores and potent toxins. Although fairly sensitive to antibiotic therapy, it is the effects of their toxins that make these bacteria lead to significant morbidity and mortality. Three species are of note –*Clostridium botulinum, Clostridium diffficile* and *Clostridium tetani*– with active efforts to develop MAbs for the first two.

C. botulinum is the agent responsible for botulism. The disease is mediated by the effects of a potent neurotoxin that irreversibly binds to the neuromuscular junction, inducing paralysis (84). Seven antigenic variants of the toxin exist, with human disease being primarily due to toxins A, B and E. Most of the disease in the U.S. is related to toxins A and B. Infants are particularly at risk for significant morbidity and mortality from botulism. Given the potency of the toxin and its lethality when untreated, it has also been recognized as a potential threat as a bioterrorism agent (85). The lethal effects of the toxin can be effectively blocked by immunoglobulin, and horse serum had been used for many decades for this purpose. Human globulin obtained from volunteers with high titers of antitoxin induced by immunization has been available since 2006, and a large study demonstrated the beneficial effects of this preparation in the infant population (86). Following on this observation, MAbs directed at blocking the effects of botulinum toxin have been investigated. A combination of three human MAbs (XOMA 3AB) with high affinity for toxin A has been shown to effectively block the toxin activity and protect animals from lethality (87). This antibody in currently undergoing evaluation in a phase I safety and pharmacokinetic study (ClinicalTrials.gov Identifier: NCT01357213).

C. diffficile is the etiological agent of pseudomembranous colitis, as well as a good proportion of cases of antibiotic-associated diarrhea. Pseudomembranous colitis is the result of colonization of the lower bowel with toxin-producing strains of *C. difficile*. Selection of *C. difficile* to the gut bacterial flora after treatment with antibiotics is a well-recognized risk factor, and particularly among debilitated individuals. Once toxigenic *C. difficile* proliferates, toxins A and B are secreted. These toxins are large proteins with cytotoxic effects to the intestinal mucosa, leading to mucosal shedding, ulceration and focal exuberant inflammation. The disease is of particular severity among certain high-risk groups, such as the elderly and the immunosuppressed. Although effective antibiotic therapy directed against *C. difficile* is available, there is a recognized risk of disease recurrence and failure to eradicate infection (88). Multiple studies have identified a lack of antibody response to the toxins as a risk factor for recurrence and human pooled immunoglobulin contains IgG antitoxin with neutralizing activity (89). Small non-controlled studies suggested that intravenous immunoglobulin administration has beneficial effects on individuals affected by *C. difficile* colitis (90, 91). However, subsequent larger non-controlled studies failed to confirm a beneficial effect (92, 93). More specific therapy with the use of antibodies has been pursued with human MAbs directed at both toxin A and B (MK-3415A; Merck & Co., Inc.), since animal models suggest that it is important to target both toxins to achieve the strongest effects (94). In a randomized, controlled trial, a significant decrease in the rate of recurrent infections was noted, but this was not paralleled by any significant improvements in outcomes related to the acute incident infection (95). These findings await confirmation through larger clinical trials comparing combination antibody versus either antibody alone and placebo (ClinicalTrials.gov Identifier: NCT01241552).

ESCHERICHIA COLI

E. coli is a ubiquitous bacterium that is a prevalent colonizer of the human gut. It can have pathogenic potential in immunosuppressed hosts or when barrier disruption occurs, such as in intestinal perforations. However, its role as a pathogen more commonly occurs with toxin-producing strains of *E. coli* leading to significant morbidity and a significant risk for mortality. Shiga toxin-producing *E. coli* (STEC) is recognized as an emerging foodborne pathogen that can produce bloody diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS), especially in developed countries. STEC produces two toxin types, Stx1 and Stx2, with Stx2 being more commonly secreted by *E. coli* serotype O157. As a specific therapy for the toxin effects is not available, current medical therapy including antibiotics is inadequate to prevent life-threatening complications (96). Thus, there is an ongoing interest in developing therapeutics that will block the activity of the toxins (97). A protective role for antibodies to Stx2 has been demonstrated among household non-affected contacts of individuals affected by HUS (98, 99). Urtoxazumab is a humanized MAb developed against Stx2 (100), and its efficacy has been demonstrated in animal models even when administered after infection had been established (101). Following normal volunteer safety and pharmacokinetic studies, a pharmacokinetic study in children infected with *E. coli* O157, a population at risk for HUS, also showed a favorable safety profile (102). Independently, a second set of MAbs, CαStx1 and CαStx2, have been developed in tandem for the treatment of HUS. CαStx1 and CαStx2 are chimeric mouse–human antibodies targeting Stx1 and Stx2, respectively. Their safety and pharmacokinetic profiles have already been demonstrated in healthy volunteers alone and in combination (103, 104). Further randomized trials to demonstrate the efficacy of these antibodies are pending.

Many Gram-negative bacteria, including *E. coli,* produce endotoxins that effect significant deleterious effects in the host, including the development of sepsis and shock. There are shared characteristics of endotoxin among Gram-negative bacteria that make it an attractive target for antibody development, since the pathogenicity of different species could potentially be attenuated with one drug. Immunotherapy with human polyclonal antiserum obtained by immunizing healthy volunteers against the endotoxin core of the J5 mutant of *E. coli* 0111:B4 was shown to reduce mortality in patients with Gram-negative bacteremia (105). Following this observation,

endotoxin from this same J5 *E. coli* strain was utilized to develop edobacomab, a murine IgM MAb intended for the treatment of sepsis (106). Animal model studies demonstrated a beneficial effect with protection from death (107, 108). Subsequent early clinical trials demonstrated a favorable safety profile and suggested a protective effect with positive trends in sepsis-related outcomes (109-111). However, a larger confirmatory trial failed to show a beneficial effect and the program was terminated (112). A similar experience was observed with nebacumab, a human MAb developed by producing hybridoma cells with splenocytes from humans immunized with the J5 *E. coli* endotoxin. Although earlier studies suggested efficacy (113), confirmatory trials failed to demonstrate a clear-cut beneficial effect (114). No further developments have occurred with this potential application.

CANDIDA ALBICANS

C. albicans is a common yeast fungus that frequently colonizes human skin and the gastrointestinal and genitourinary tracts. Systemic infection with *Candida* is a significant reason for morbidity and mortality among immunocompromised and hospitalized debilitated individuals. Efungumab (Mycograb®; NeuTec Pharma) was selected to target the immunodominant epitope of *Candida* hsp90. In studies of animal models of *Candida* infection, significant protective effects were noted from the combination of the antibody with antifungal therapy (115, 116). Efungumab was intended for use in combination with antifungals for the treatment of serious systemic *Candida* infections, primarily cases of invasive infections in adults. A randomized trial demonstrated beneficial effects in combination with the antifungal drug amphotericin (117). However, following concerns over the quality and safety of the antibody, as well as what was considered as insufficient evidence for efficacy, the drug was denied approval (118). Subsequent to this, the antibody was modified by substituting a cysteine felt to be contributing to aggregation of the antibody. This modification did not lead to an improvement in the antibody activity and it performed poorly in vitro (119). This program has since been discontinued.

CRYPTOCOCCUS NEOFORMANS

C. neoformans is an encapsulated yeast that is typically found in soil contaminated with bird excrement. It is a cause of serious opportunistic infections, particularly of the nervous system, in immunocompromised individuals, including those with HIV infection and post-transplant. One of the characteristic features of *C. neoformans* is its prominent polysaccharide capsule, which confers it protection from phagocytes. Components of the capsule can elicit antibody responses and high antibody titers can be found in healthy individuals. However, the ability of these natural antibodies to promote opsonization and killing is limited. Selection of MAbs directed at specific epitopes led to the identification of antibodies that can mediate a protective effect through complement activation (120, 121). One such antibody, MAb18b7, developed against capsular glucuronoxylomannan, was shown in animal models to enhance clearance of infection (122). MAb18b7 was then developed as a mouse MAb for clinical application. A dose-escalation safety and pharmacokinetic study was conducted in HIV-infected individuals recovering from *C. neoformans* meningitis (123). This study revealed favorable kinetics and a tolerable side effect profile. In addition, a decrease in the levels of serum *C. neoformans* antigen was noted, which waned over time. However, the development of human anti-mouse antibody titers was noted. No further development of this product has been reported.

DISCUSSION

The use of antibodies as a strategy to control bacterial infection has a long history and remains an attractive therapeutic option given its potential to circumvent the current pressing issues of emerging bacterial resistance to antibiotics. In addition, antibodies have the ability to control bacterial infection by making use of mechanisms inherent to the host, and thus are devoid of complicating issues with toxicities, drug interactions or induction of resistance or super-infection with selected virulent bacterial strains. The rapidly evolving methodology in the development of human antibodies with high affinity has renewed the interest in this therapeutic option and led to multiple candidates at different stages of testing. Nevertheless, other than palivizumab for RSV, no antibody has received approval for human use and several failures have occurred, questioning the viability of this approach. Several issues need to be taken into consideration to reconcile this apparent lack of translation from the in vitro and animal study observations. First, it must be kept in mind that for the most part antibodies do not have a direct bactericidal activity, but rather the killing of the microorganisms is primarily effected by the action of phagocytes and the activation of the complement system. Then, the presence of impaired immune responses, as is likely the case in many of the patient populations that have been subjected to clinical trials, could limit the strength of the antibacterial effect. Bacteria themselves have evolved a number of mechanisms to evade the immune system to be able to produce both acute and chronic infection. These will effectively block the downstream effects of antibodies that have bound to their intended epitopes, rendering the antibody ineffective despite its effectiveness in binding to the target site. Among the mechanisms reported are blocking the Fc regions of the bound antibody by specific secreted products, producing antibody-binding proteins to the variable region and blocking the activation of complement (124-126).

Secondly, the antibody is developed based on its specificity for the pathogen being targeted, which, although a strength, does have an inherent weakness. Although the antibodies are selected on the basis of targeting an epitope that is common in the strains of interest, there could always be limitations due to the presence of variants that do not present the antibody binding site or are able to mask the antibody by their bacterial capsule (127), effectively blocking the ability of the antibody to elicit further immune responses in the host.

Thirdly, issues related to antibody dosing and pharmacokinetics may need further evaluation. Some of the studies reviewed suggested that protection is contingent on achieving a given level of circulating antibody (38, 57), which will not necessarily become apparent from either animal models or healthy volunteer studies, as not only kinetics of the sick patient population may be different, but also issues such as bacterial burden and activity at the specific site of infection may play a role.

Despite these shortcomings, it is likely that refinements in methodology, as well as in dissecting more clearly key mechanisms of dis-

ease pathogenesis, including those evasion mechanisms present in bacteria, will eventually lead to the identification of effective drugs. Crucial to this will also be improving the design of the clinical studies to demonstrate efficacy, and particularly the definition of clear endpoints. However, it must be kept in mind that, for the most part, these potential approved drugs may only play an adjuvant role in the treatment, since antibiotics could still be required to effect eradication of the offending organism in the presence of impaired immune defense mechanisms.

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