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Magic bullets for the 21st century: the reemergence of immunotherapy for multi- and pan-resistant microbes

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In our current world, antibiotic resistance among pathogenic microbes keeps getting worse with few new antibiotics being pursued by pharmaceutical companies. Modern-day immunotherapies, reminiscent of the serotherapy approaches used in the early days of antimicrobial treatments, are a potential counter-measure, but are usually limited by the narrow spectrum against target antigens. Surprisingly, many multidrug-resistant (MDR) bacteria share a common surface polysaccharide, poly-β-1,6-*N*-acetylglucosamine (PNAG). Natural antibodies to PNAG are present in normal human sera, but are not protective. However, human monoclonal antibodies (MAbs) or polyclonal antisera raised to a deacetylated glycoform of PNAG mediate opsonic killing and protect mice against infections due to all PNAG-positive MDR pathogens tested. An MAb is currently in Phase II clinical trials. These discoveries could lead to utilization of antibodies to PNAG for either therapeutic use in patients infected by PNAG-producing MDR bacteria or prophylactic use in patients at risk of developing MDR infections.

Keywords: poly-*N*-acetylglucosamine, PNAG, broad-spectrum vaccines, antibiotic resistance

The fate of the antibiotic miracle

At the beginning of the 20th century Dr Paul Ehrlich was searching for magic bullets—substances that could be injected into the blood to fight disease. He is credited with developing one of the first chemotherapeutic treatments for an infectious agent, namely salvarsan (followed later by neosalvarsan) for syphilis.[1] While quite controversial at the time and certainly not without serious toxicity, these arsenical compounds nonetheless proved we could actually cure infectious diseases with small molecules. Now, over 100 years later we are confronted with one of the largest problems in healthcare—an inability to treat many serious infections as pathogens become highly resistant to the numerous magic bullets developed in the past century. Compounding this problem is the absence from the drug pipeline of new antibiotics, particularly those with novel mechanisms of action.[2]

Broad-spectrum antibodies against antibiotic-resistant pathogens

This situation warrants new types of treatments and/or approaches to either prevent or treat infections due to multidrug-resistant (MDR) bacteria. Such developments are urgently required, now more than ever, with resistance to carbapenems described in all major Enterobacteriaceae,[3] and with more and more settings where there are no antibiotics left to treat patients infected with pan-resistant bacteria. Active vaccination and passive immunotherapy are leading candidates. Among the most common, and certainly the most successful, bacterial antigens targeted by protective antibodies are the surface polysaccharides. Excitingly, one of these, a poly-β-1,6-*N*-acetylglucosamine (PNAG) antigen, has recently been found as a surface polysaccharide on many serious MDR bacteria, including methicillin-resistant *Staphylococcus aureus* and extended-spectrum β-lactamase (ESBL)-producing and carbapenemase-producing Enterobacteriaceae, as well as less common pan-resistant bacteria such as *Acinetobacter baumannii* and members of the *Burkholderia cepacia* complex (BCC). Encouragingly, a fully human monoclonal antibody (MAb F598) to PNAG[4] has successfully completed a Phase I safety and pharmacokinetic dose-escalation trial in healthy adults,[5] and is currently undergoing a Phase II trial, raising a provocative question: can MAb F598, by targeting many of the MDR and even pan-resistant bacteria, become the first known broad-spectrum therapeutic antibody?

Pre-clinical studies of antibodies to PNAG

The protective value of antibodies to bacterial surface polysaccharides has been strongly validated by successful use of this strategy to produce vaccines effective against several bacterial pathogens,[6] including *Streptococcus pneumoniae*,[7] *Neisseria meningitidis*,[8] *Haemophilus influenzae* type b[9] and *Salmonella enterica* serovar Typhi.[10] However, with this approach, only the specific bacterial capsule serotypes in the vaccines are targeted

and none of the licensed vaccines are useful for any of the most frequent MDR bacteria. In contrast, the antibodies raised to PNAG isolated from *S. aureus* were not only able to mediate opsonic killing and provide protective immunity against this pathogen in murine blood and skin infections,[11] but were also opsonic and protective against *Escherichia coli*[12] and BCC[13] in a murine model of peritonitis and against pneumonia caused by *A. baumannii* (Table 1).[14]

Additionally, PNAG is often a major component of biofilms of MDR organisms, another property contributing to virulence. In staphylococci, synthesis of PNAG occurs via proteins encoded by genes in the staphylococcal *icaADBC* locus (*ica* for intercellular adhesin).[15] PNAG is produced by *E. coli* using proteins encoded in the related, but somewhat differently organized, *pgaABCD* locus (*pga* for polyglucosamine).[16] Strikingly, there is increasing evidence that many of the MDR bacterial species involved in both community- and hospital-acquired infections carry the genes involved in PNAG synthesis (*ica* or *pga* loci) (Table 2).

If antibodies to PNAG were to be extensively used, a legitimate concern would arise regarding the consequences of a decrease or loss of expression of PNAG during infection. Fortunately, selection of strains deficient in PNAG production during or after immunotherapy would probably be associated with a loss of virulence and would lead to strains potentially unable to produce a strong biofilm. Furthermore, *S. aureus* and *E. coli* strains with mutations in the *ica* or *pga* loci had diminished virulence in several different murine infection models.[12,17] While further studies are needed in this area, the conserved synthesis of PNAG by genetically divergent Gram-positive and Gram-negative pathogens implies this molecule has an important role in microbial biology that has led to a positive selection for maintenance of PNAG synthesis by diverse microorganisms.

Logically, as PNAG is produced by major commensal bacteria of the gastrointestinal tract (*E. coli*) or the skin (*Staphylococcus epidermidis*), we might expect antibodies to PNAG to be present in most human sera. Indeed, all human sera tested to date in various studies have natural antibodies to PNAG.[18,19] However, the antibodies found in about 95% of these normal sera bind predominantly to the highly acetylated glycoform of PNAG produced by commensal microbes. These natural antibodies do not appear to provide vigorous opsonic killing or immune protection *in vitro* or *in vivo*.[18] However, studies over the past several years have validated that antibodies induced by conjugate vaccines composed of carrier proteins bound to either chemically deacetylated PNAG or a synthetic oligosaccharide of non-acetylated glucosamines have opsonic and protective effects comparable to those that mediate immunity to other encapsulated bacteria.[20] Similarly, the opsonic and protective MAb F598 was identified initially by its ability to bind to both native and deacetylated PNAG,[4] and the immunological property distinguishing the opsonic/protective from non-opsonic/non-protective antibodies was determined to be the ability of the functional MAbs to deposit complement onto the target bacterial surfaces.[4]

Table 1. Antibodies to PNAG: *in vivo* activity against bacteria associated with MDR infections

Bacterial species	Method of immunization	Challenge route	Animal model
Staphylococcus aureus	intravenous[a]	intravenous	kidney infection[11]
	intravenous[b]	intravenous	bacteraemia[20]
	intraperitoneal[b]	intraperitoneal	lethality[20]
	intraperitoneal[b]	subcutaneous	skin abscesses[21]
Escherichia coli	intraperitoneal[b]	intraperitoneal	lethality[12]
Acinetobacter baumannii	intranasal[b]	intranasal	pneumonia[14]
	intravenous[b]	intravenous	bacteraemia[14]
Burkholderia cepacia complex	intraperitoneal[b]	intraperitoneal	lethality[13]

[a]Passive and active immunization.
[b]Passive immunization.

Table 2. PNAG production by bacteria associated with MDR infections

Bacterial species	Genetic locus	Phenotype	Publication
Staphylococcus aureus	*icaADBC*	biofilm formation	15
Escherichia coli	*pgaABCD*	biofilm formation	16
Acinetobacter baumannii	*pgaABCD*	biofilm formation	22
Klebsiella pneumoniae	*pgaABCD*	under investigation	13
Enterobacter cloacae	*pgaABCD*	under investigation	13
Burkholderia cepacia complex	*pgaABCD*	biofilm formation	23
Stenotrophomonas maltophilia	*pgaABCD*	under investigation	13

Antibodies to PNAG: clinical studies

A potential use of antibodies to PNAG would be via therapeutic intervention in the early stages of an infection to prevent the development of a more serious sepsis, facilitating the immune system's ability to eliminate pathogens. In the case of MDR bacteria, PNAG-targeted immunotherapy might also compensate for suboptimal antibiotic therapy. Another strategy for use of these antibodies would be to give a preventive dose for patients with a high risk of developing infections, such as critically ill individuals in the intensive care unit (ICU). Addressing this approach, there is an ongoing Phase II randomized, double-blind, placebo-controlled trial to assess the pharmacokinetics, pharmacodynamics and safety of MAb F598 in mechanically ventilated patients in the ICU (http://clinicaltrials.gov/ct2/show/NCT01389700?term=SAR279356&rank=1).

Defining the proper population of patients to target is a major challenge in the clinical development of PNAG-specific immunotherapies, as predicting which individuals might develop an infection due to an MDR pathogen is quite difficult. But if clinical trials are successful in finding an effective means to use MAb or polyclonal immunotherapy against the known and to be discovered PNAG-producing pathogens, immunotherapy targeting PNAG may have a broad spectrum of activity. Thus we might

realistically ask: will MAbs or antibodies to PNAG be a 21st century version of Dr Ehrlich's magic bullet?

Transparency declarations

G. B. P. is an inventor of Intellectual Property (IP) (PNAG Vaccine and human monoclonal antibody to PNAG) that is licensed by Brigham and Women's Hospital (BWH) to Alopexx Vaccines LLC and Alopexx Pharmaceuticals LLC, companies in which G. B. P. owns equity. As an inventor of the IP, he also has the right to receive a share of licensing-related income (royalties, fees) through BWH from Alopexx Pharmaceuticals and Alopexx Vaccines. G. B. P.'s interests were reviewed and are managed by the BWH and Partners healthcare in accordance with their conflict of interest policies. D. R. and D. S.: none to declare.

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