Engineering 38 (2024) 69–76

Contents lists available at ScienceDirect

Engineering

journal homepage: www.elsevier.com/locate/eng

Research
Natural Medicine—Review

Antibodies Targeting a Conserved Surface Polysaccharide Are Protective Against a Wide Range of Microbial Pathogens Producing β-1–6-Linked Poly-*N*-Acetylglucosamine (PNAG)

Xi Lu [a,#], Guoqing Li [a,#], Jing Pang [a,#], Xinyi Yang [a], Colette Cywes-Bentley [b], Xuefu You [a], Gerald B. Pier [b,*]

[a] Beijing Key Laboratory of Antimicrobial Agents, Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China
[b] Division of Infectious Diseases, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA

ARTICLE INFO

Article history:
Received 23 June 2023
Revised 18 August 2023
Accepted 11 September 2023
Available online 18 October 2023

Keywords:
Poly-*N*-acetylglucosamine
Conjugate vaccine
Monoclonal antibody

ABSTRACT

The β-1–6-linked poly-*N*-acetylglucosamine (PNAG) polymer is a conserved surface polysaccharide produced by many bacteria, fungi, and protozoan (and even filarial) parasites. This wide-ranging expression makes PNAG an attractive target for vaccine development, as it potentially encompasses a broad range of microorganisms. Significant progress has been made in discovering important properties of the biology of PNAG expression in recent years. The molecular characterization and regulation of operons for the production of PNAG biosynthetic proteins and enzymes have been studied in many bacteria. In addition, the physiological function of PNAG has been further elucidated. PNAG-based vaccines and PNAG-targeting antibodies have shown great efficacy in preclinical research. Furthermore, clinical tests for both vaccines and antibodies have been carried out in humans and economically important animals, and the results are promising. Although it is not destined to be a smooth road, we are optimistic about new vaccines and immunotherapeutics targeting PNAG becoming validated and eventually licensed for clinical use against multiple infectious agents.

© 2023 THE AUTHORS. Published by Elsevier LTD on behalf of Chinese Academy of Engineering and Higher Education Press Limited Company. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

1.1. From exopolysaccharides (EPSs) to β-1–6-linked poly-N-acetylglucosamine (PNAG)

EPSs are complex structures synthesized by microorganisms such as bacteria, fungi, algae, and protozoan parasites [1–3]. They are long chains of repeating sugar units that are produced in response to environmental stress, nutrient limitation, or other stimuli. Microorganisms can use EPSs as a protective layer or as a means of attaching themselves to surfaces. In addition, EPSs have industrial and biomedical applications, including uses in food, pharmaceuticals, and cosmetics [4]. For example, the EPSs produced by some bacteria can be used as thickeners, stabilizers, and emulsifiers in food products, while the EPSs made by fungi have been studied for their potential as probiotics [5], which can help promote the growth of beneficial gastrointestinal (GI) tract bacteria. EPSs can be either homopolysaccharides, which consist of a single type of monomer repeated in a linear or branched chain, such as cellulose, chitin, and the conserved microbial surface PNAG, or as heteropolysaccharides, which consist of multiple types of monomers in varying ratios and sequences, including xanthan gum, hyaluronic acid, and alginate [6]. The structure and composition of EPSs can vary greatly between bacterial strains and even within a single strain under different growth conditions, making EPSs a diverse and complex class of molecules [7].

PNAG is a linear homopolymer composed of *N*-acetylglucosamine (GlcNAc) monosaccharide units linked together by β-1–6 glycosidic bonds, as shown in Fig. 1. To date, PNAG has been detected on the surface of over 75 microbial organisms (see Fig. 2 for examples), including but not limited to Gram-positive bacteria (*Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*), *Enterococcus faecalis* (*E. faecalis*), and *Bacillus subtilis* (*B. subtilis*)), Gram-negative bacteria (*Escherichia coli* (*E. coli*), *Yersinia pestis* (*Y. pestis*), and *Acinetobacter baumannii* (*A. baumannii*)), fungi (*Streptomyces* and *Candida albicans* (*C. albicans*)), and protozoa (*Plasmodium* spp.) [8], as listed in

* Corresponding author.
E-mail address: gpier@bwh.harvard.edu (G.B. Pier).
# These authors contributed equally to this work.

https://doi.org/10.1016/j.eng.2023.09.012
2095-8099/© 2023 THE AUTHORS. Published by Elsevier LTD on behalf of Chinese Academy of Engineering and Higher Education Press Limited Company.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

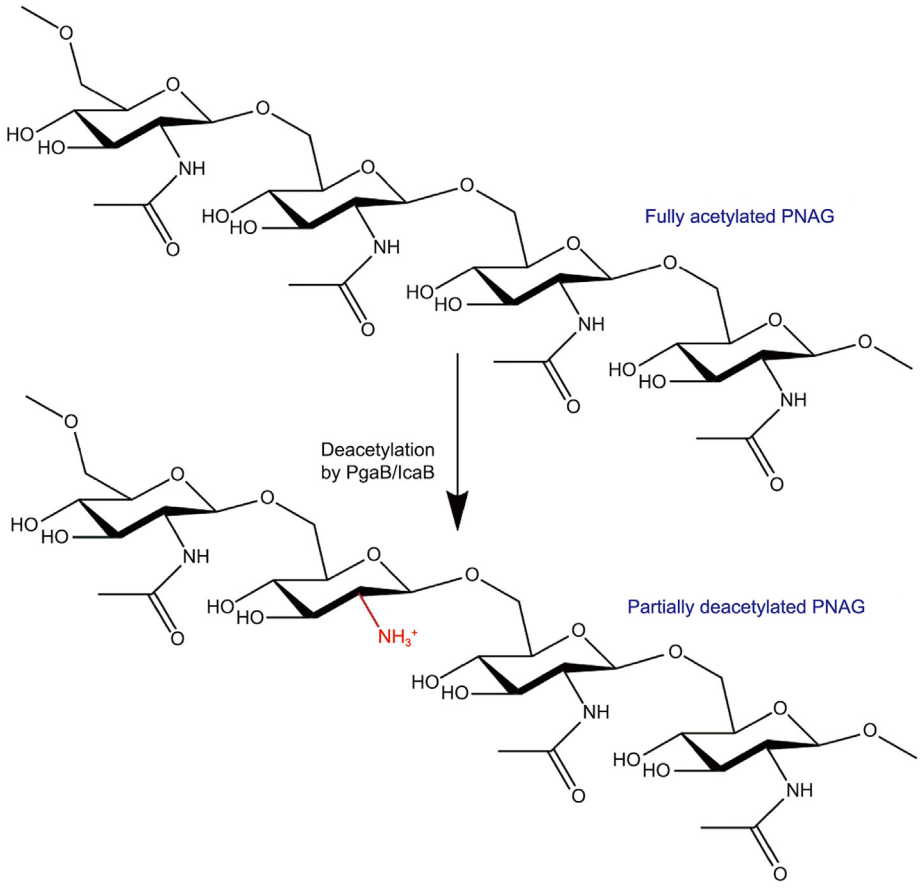

Fig. 1. Structure of fully acetylated and partially deacetylated PNAG. Pga: polyglucosamine; Ica: intercellular adhesin.

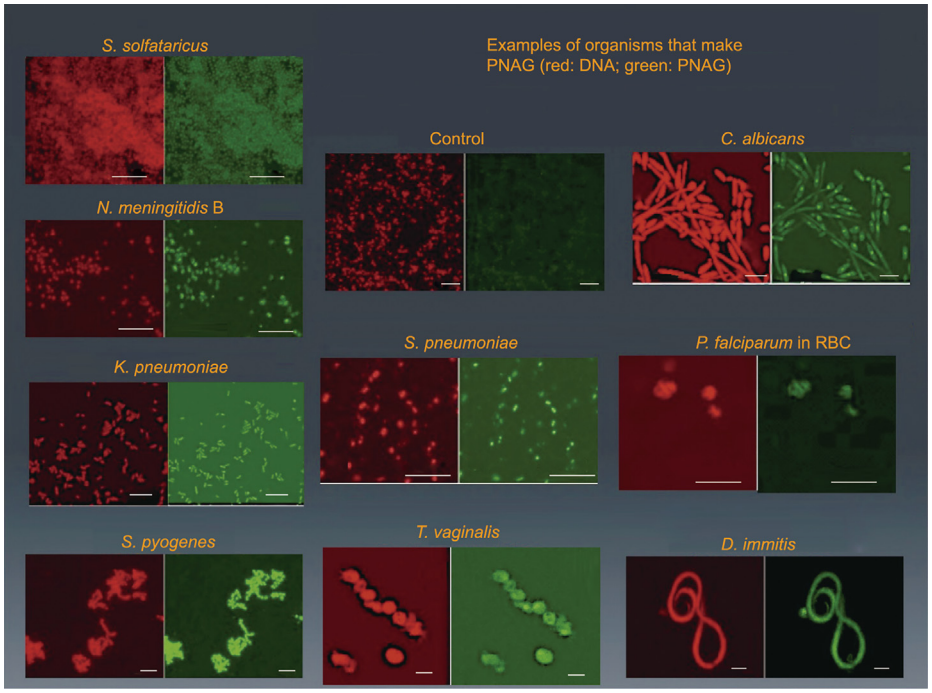

Fig. 2. Examples of organisms making PNAG. All controls were negative for reaction with a control monoclonal antibody (mAb) as represented by the single panel labeled control. The control mAb has constant regions identical to those of the mAb to PNAG used for detection. The detection of PNAG used a directly labeled human immunoglobulin G1 (IgG1) mAb. Scale bars: 10 μm. RBC: red blood cell; *S. solfataricus*: *Sulfolobus solfataricus*; *N. meningitidis*: *Neisseria meningitidis*; *K. pneumoniae*: *Klebsiella pneumoniae*; *S. pyogenes*: *Streptococcus pyogenes*; *S. pneumoniae*: *Streptococcus pneumoniae*; *T. vaginalis*: *Trichomonas vaginalis*; *P. falciparum*: *Plasmodium falciparum*; *D. immitis*: *Dirofilaria immitis*.

Table 1
Microbes documented to produce PNAG [1].

Microbe	Microbe	Microbe
E. coli	*Bordetella bronchiseptica*	*Streptococcus equi* subspecies *equi* [11]
A. baumannii	*Listeria monocytogenes*	*Streptococcus equi* subspecies *zooepidemicus*
Shigella spp.	*Bordetella parapertussis*	*Actinobacillus pleuropneumoniae*
Bordetella pertussis	*Mycobacterium tuberculosis*	*A. actinomycetemcomitans* [12]
Y. pestis [9]	*C. albicans*	*Babesia bovis* [13]
K. pneumoniae	*S. aureus*	*Babesia bigemina*
Yersinia enterocolitica	*Neisseria gonorrhoeae*	*Babesia divergens*
Enterobacter cloacae	*S. epidermidis*	*Babesia microti*
Yersinia pseudotuberculosis	*Neisseria meningitides*	*E. faecalis* [14]
B. subtilis	*S. pneumoniae*	*Streptomyces lividans* [15]
T. vaginalis	*Vibrio parahemolyticus*	*Francisella tularensis* [16]
Plasmodium spp.	*S. pyogenes*	*Aspergillus fumigatus* [17]
Hemophilus ducreyi	*Burkholderia cepacia* complex [10]	*Aspergillus flavus* [17]
Bacteroides fragilis	*Streptococcus agalactiae*	*B. subtilis* [18]
Hemophilus influenzae	*Stenotrophomonas maltophilia*	*Fusarium solani* [17]

Table 1 [1,9–18]. Recently, PNAG has been detected on the filarial worm *Dirofilaria immitis* (*D. immitis*), the cause of heartworm in canines (Fig. 2). In all the studies involving the immunohistochemical detection of PNAG on microbial surfaces, confirmatory experiments have been carried out in which the cells are treated with either the PNAG-degrading enzyme dispersin B or a control enzyme chitinase, which does not degrade PNAG due to the differences in the linkages of the GlcNAcs in PNAG (β-1–6) and chitin (β-1–4) [1].

PNAG has also been referred to as the polysaccharide intercellular adhesin (PIA) in *Staphylococci*, polyglucosamine (Pga) in *E. coli* and *Enterococci*, and the *Bordetella* polysaccharide (BPS) in *Bordetella* species. It is synthesized by the proteins encoded by the genes in the hemin storage locus (Hms+) of *Y. pestis*.

1.2. Biosynthesis of PNAG

The biosynthesis pathways of PNAG have been studied for several decades but have only been fully elucidated in a limited number of bacterial strains. In Gram-negative bacteria, such as *E. coli* [19,20], PNAG is produced via a synthase-dependent pathway that consists of an inner membrane-spanning synthase protein, PgaC, a small inner membrane protein with two N-terminal transmembrane helices, PgaD, and an outer membrane-spanning β-barrel porin, PgaA, that functions as an outer-membrane-associated tetratricopeptide repeat (TPR)-containing protein. The synthase PgaC has a glycosyltransferase domain on its cytosolic face, which can use uridine diphosphate (UDP)–GlcNAc to synthesize PNAG precursors; the precursors are then translocated across the inner membrane into the periplasm. PgaD can combine with PgaC to form the PgaCD complex, which is a novel type of cyclic dimeric guanosine monophosphate (c-di-GMP) receptor. If it cannot form a complex or bind to c-di-GMP, PgaD is rapidly degraded [21]. PgaB sits in the periplasm and has an N-terminal deacetylase domain and a C-terminal PNAG binding domain with glycoside hydrolase activity. It is a low-efficiency deacetylase that partially de-*N*-acetylates the precursor PNAG (3%–5% in *E. coli*, 10% in *A. actinomycetemcomitans*, and 15%–20% in *A. baumannii* [12]); PgaB also plays an important role in disrupting PNAG-dependent biofilms [22]. The mature PNAG is exported by PgaA located in the outer membrane and subsequently anchored onto the cell surface by currently unknown mechanisms. All four genes in the *pga* operon are essential for the production of PNAG. The second messenger, c-di-GMP, can bind to PgaCD to stabilize the interaction of the complex and allosterically activate the glycosyltransferase activity of PgaC, which controls the initial step of PNAG synthesis [21].

In *Staphylococci*, the *icaABCD* locus is a homologue of *pgaABCD* and is responsible for the production of PNAG [23]. The plasma-membrane protein intercellular adhesin A (IcaA) is a low efficacy *N*-acetylglucosaminyltransferase that initiates biosynthesis using UDP–GlcNAc and binds to IcaD to form a complex to promote the synthesis of mature PNAG. The limited-length PNAG (less than 20 residues) synthesized by the IcaAD complex is elongated by IcaC, which may also modify PNAG with O-succinylate groups (6%–10% of GlcNAc residues in *S. epidermidis* and *S. aureus*; no O-succinylation reported in *E. coli* [7]). Afterward, the metal-dependent IcaB introduces net charges to PNAG via deacetylation (5%–40%). IcaB can be deleted from the *S. epidermidis* genome, which results in the production of fully acetylated PNAG; however, this structure is poorly retained on the cell surface. The regulator IcaR that is encoded upstream of *icaABCD* is a suppressor that binds to the promoter region upstream of *icaA*. Antibiotics and environmental conditions affect the binding of IcaR to DNA, which regulates the transcription of the *icaABCD* operon. In addition, the 3′ untranslated region (UTR) of *icaR* has been demonstrated to control *icaR* messenger RNA (mRNA) translation [24]. The proteins that are encoded by the *icaABCD* genes and involved in the biosynthesis of PNAG are shown in Fig. 3.

Partial deacetylation is important in order for PNAG to obtain the net charge that facilitates the binding of PNAG to microbial surfaces. In *Bordetella bronchiseptica*, deacetylation of PNAG is required for the formation of a robust three-dimensional biofilm. As mentioned above, the fully acetylated PNAG in *S. epidermidis* was unable to bind to the cellular surface and was mostly present in the culture media.

Aside from the two mentioned operons, *icaABCD* and *pgaABCD*, *hmsHFRS* in *Y. pestis*, *epsHIJK* in *B. subtilis*, *matAB* in *Streptomyces*, *epxA* in *E. faecalis*, and *sezMV* in *Streptococcus equi* (*S. equi*) have all been shown to either encode PNAG biosynthetic proteins or are needed for PNAG synthesis [8,15]. As these organisms—which lack a clear 3–4 gene operon to produce PNAG biosynthetic proteins—have not been studied for the molecular basis underlying PNAG synthesis, the current state of the art for understanding the variation in synthesis pathways for PNAG remains limited.

Although numerous microorganisms have been shown or predicted to produce PNAG, production can also be species or even strain-specific. While *Pseudomonas aeruginosa* (*P. aeruginosa*) does not carry genes for PNAG synthesis, it was able to produce this structure once the *pga* operon was introduced into a *P. aeruginosa* strain that was also unable to produce the native alginate polysaccharide [18]. In *Salmonella,* which is closely related to *E. coli*, no *pga* operon has been identified, and it has been proposed that the loss of PNAG is necessary during *Salmonella* speciation to permit

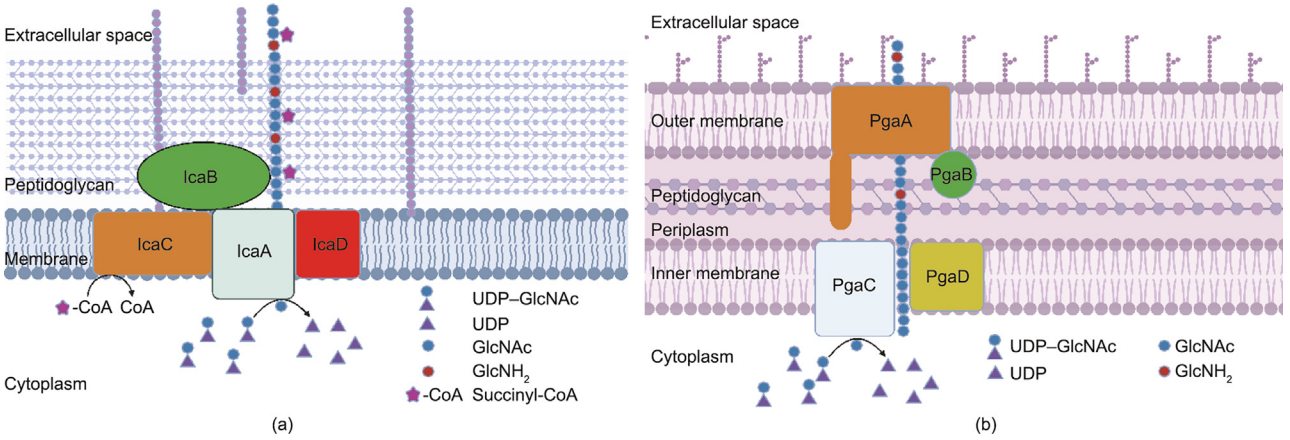

Fig. 3. Proteins involved in the biosynthesis of PNAG. (a) The *ica* locus of Gram-positive bacteria such as *S. aureus*; (b) the *pga* locus of Gram-negative bacteria such as *E. coli*. $GlcNH_2$: glucosamine; CoA: coenzyme A.

survival inside the host [25]. In the *S. epidermidis* A/B cluster, the occurrence rate of *ica* genes is about 37%, compared with 4% in the B cluster, while most strains of *S. aureus* carry the *ica* operon [26].

1.3. Functions of PNAG

Due to the importance of PNAG in biofilm formation and the relationship between biofilms and antibiotic resistance, PNAG production affects bacterial susceptibility to antibiotics. It has been reported in multiple studies that, compared with *ica*-negative staphylococcal strains, the resistance of *ica*-positive *S. epidermidis* and *S. aureus* strains to a variety of antibiotics, including β-lactams, quinolones, aminoglycosides, macrolides, and glycopeptides, is increased when PNAG is expressed [26]. In *Bordetella pertussis*, the presence of BPS on the bacterial cell surface was shown to enhance resistance to antimicrobial peptides [27].

PNAG can act as an adhesin, allowing bacteria to attach to abiotic surfaces or to other cells; this makes PNAG an important factor in microbial adherence to the surfaces of polymeric substrates and biomaterials, and in bacterial inter-cellular adhesion. PNAG was found to be more often produced by strains of *S. epidermidis* that cause biofilm-dependent catheter-related infections when compared with skin-colonizing strains, revealing an important role of PNAG in biofilm formation under high-shear flow conditions [28]. The accumulation of PNAG on the surface of *Streptomyces* was found to mediate the attachment of the bacteria to hydrophilic surfaces [15]. In a *clsA* null mutant of *Streptomyces lividans* with inhibited pellet formation in liquid-grown cultures, overexpression of PNAG restored the pellet formation phenomenon [15]. In *E. faecalis*, EPSs including PNAG facilitate bacterial penetration into semisolid surfaces and translocation through human epithelial cell monolayers [14].

2. Conjugate vaccines targeting PNAG

2.1. PNAG-based vaccines against various pathogens

The development of vaccines against PNAG has been a focus of numerous research efforts, as such a vaccine holds the potential to be a preventive measure against a wide range of infections and pathogens, since PNAG is produced by numerous microbial pathogens. An effective vaccine directed toward this antigen could greatly decrease the burden of community-acquired and hospital-acquired diseases caused by PNAG-producing microorganisms. Studies have shown that protective antibodies to PNAG were elicited when a deacetylated glycoform of PNAG, termed dPNAG (with < 30% acetate substituents on the glucosamine amides), was used in conjugate vaccines, whereas highly acetylated PNAG does not induce functional antibodies [29]. Vaccines and antibodies related to PNAG are listed in Table 2 [1,10,11,13,14,16,17,30–47].

A review published in 2016 [8] summarized the progress of PNAG-based vaccines up to that year; thus, the present work mainly focuses on updating vaccine development targeting PNAG since that time.

(1) **Update on PNAG-based vaccines for bacteria.** ① *E. coli*. In a recent study, Pons et al. [30] used a transposon-sequencing (TnSeq) library to investigate the genes required for systemic dissemination and brain infection by *E. coli* K1. Their findings showed that PNAG served as a crucial virulence factor for bacterial crossing of the blood–brain barrier and, due to its localization on the outer surface, was an ideal target for protective antibodies. Through their research, they established that antibodies elicited by a PNAG-based conjugate vaccine protected mice against neonatal meningitis caused by *E. coli* K1. Importantly, the vaccination of pregnant dams resulted in the transfer of antibodies to PNAG to the pups, which were then protected against *E. coli* K1 infection. In addition, this study showed that another major neonatal pathogen, *Streptococcus agalactiae* or Group B *streptococcus*, also expressed PNAG, and that the antibodies to PNAG were similarly protective against neonatal meningitis in mouse pups. ② *Streptococcus pneumoniae* (*S. pneumoniae*) and *S. aureus*. Zaidi et al. [35] showed that antibodies to PNAG exhibited therapeutic efficacy in mouse models of *S. pneumoniae* and *S. aureus* conjunctivitis, validating the protective capacity of antibodies to PNAG in this ocular tissue. ③ *Rhodococcus equi* (*R. equi*). *R. equi* is a primary cause of severe granulomatous pneumonia in foals; however, there is no licensed vaccine available for prevention. Like *Mycobacterium tuberculosis*, *R. equi* is an intracellular pathogen. In a study conducted by Cywes-Bentley et al. [11], pregnant horse mares were given a PNAG-based vaccine six and three weeks prior to their predicted date of parturition. In horses, there is no placental transfer of antibodies from pregnant mares to foals; rather, the foals must consume the colostrum produced during the first 48 h of life, which is rich in serum proteins, including immunoglobulins. The foals absorb these through their GI tracts during this short period, and the antibodies enter their circulation. A study conducted over two foaling seasons reported 91% protection against an intratracheal challenge with virulent *R. equi* compared with foals whose mothers received the control vaccine. In a follow-up study, foals

Table 2
PNAG-related vaccines and antibodies studied in various infection models.

Pathogen	Type of pathogen	Infection model	Vaccine or antibody used	Refs.
Group B *streptococcus*	Gram-positive bacteria	Neonatal meningitis	9GlcNH$_2$-TT, mAb F598	[30]
S. aureus	Gram-positive bacteria	Skin abscess	9GlcNH$_2$-Hla, 5GlcNH$_2$-TT	[31–33]
		Corneal keratitis	9GlcNH$_2$-TT, mAb F598	[34]
		Pneumonia	9GlcNH$_2$-Hla	[31]
		Nasal colonization	9GlcNH$_2$-Hla	[31]
		Bacterial conjunctivitis	mAb F598	[35]
		Bacteremia	dPNAG conjugated to DT or clumping factor A (ClfA), PNAG-containing glycosylated outer membrane vesicles (glycOMVs)	[16,32,36]
		Prosthetic osteomyelitis	9GlcNH$_2$-TT	[37]
S. pyogenes	Gram-positive bacteria	Skin infection causing lethal systemic infection	9GlcNH$_2$-TT	[1]
Listeria monocytogenes	Gram-positive bacteria	Lethal sepsis	9GlcNH$_2$-TT	[1]
S. pneumoniae	Gram-positive bacteria	Systemic infection	9GlcNH$_2$-TT	[1]
		Bacterial conjunctivitis	mAb F598	[35]
E. faecalis	Gram-positive bacteria	Surface penetration of agar	mAb F598	[1,14]
Rhodococcus equi	Gram-positive bacteria	Pneumonia	5GlcNH$_2$-TT	[11,38–41]
E. coli	Gram-negative bacteria, carbapenemase-producing *E. coli*	Lethal peritonitis	9GlcNH$_2$-TT, anti-dPNAG-DT	[42,43]
		Peritonitis	9GlcNH$_2$-TT	[44]
	Shiga toxin-producing *E.coli*	STEC infection	9GlcNH$_2$-TT, 9GlcNH2-Stx1B	[45]
	CTX-M-producing *E. coli*	Neonatal meningitis	9GlcNH$_2$-TT, mAb F598	[30]
Burkholderia cepacia complex	Gram-negative bacteria	Lethal peritonitis	9GlcNH$_2$-TT	[10]
N. meningitidis	Gram-negative bacteria	Neonatal meningitis	9GlcNH$_2$-TT	[1]
Enterobacter cloacae	Gram-negative bacteria, carbapenemase NDM-1-producing Enterobacteriaceae	Lethal peritonitis	9GlcNH$_2$-TT	[42]
K. pneumoniae	Gram-negative bacteria, carbapenemase NDM-1-producing Enterobacteriaceae	Lethal peritonitis, bacteremia	9GlcNH$_2$-TT	[42]
P. aeruginosa PA14 Tn::*algD* (pUCP18::*pga*)	Gram-negative bacteria	Lethal peritonitis	9GlcNH$_2$-TT	[42]
A. baumannii	Gram-negative bacteria	Pneumonia	9GlcNH$_2$-TT	[46]
		Bacteremia	9GlcNH$_2$-TT	[46]
Francisella tularensis	Gram-negative bacteria	Systemic infection	5GlcNH$_2$-TT, PNAG-containing outer membrane vesicles	[16]
Microbiota-derived cells or fragments after allogeneic hematopoietic cell transplantation	Microbiota-derived cells and fragments translocating to GI submucosa	Acute graft-versus-host disease	5GlcNH$_2$-TT	[47]
C. albicans	Fungal keratitis	Fungal keratitis	9GlcNH$_2$-TT	[1]
Aspergillus flavus	Fungal keratitis	Fungal keratitis	9GlcNH$_2$-TT, mAb F598	[17]
A. fumigatus	Fungal keratitis	Fungal keratitis	9GlcNH$_2$-TT, mAb F598	[17]
Fusarium solani	Fungal keratitis	Fungal keratitis	9GlcNH$_2$-TT, mAb F598	[17]
Plasmodium berghei ANKA	Parasite	Malaria	9GlcNH$_2$-TT	[1]
Babesia bovis	Tick-borne apicomplexan parasites	Acute babesiosis (no protection)	5GlcNH$_2$-TT	[13]

P. aeruginosa does not produce PNAG, the indicated strain was genetically engineered to inactivate its ability to produce the usual surface polysaccharide alginate and the genes for PNAG production then introduced to achieve PNAG expression in this recombinant laboratory strain. Tn::*algD (pUCP18*::*pga)*: *P. aeruginosa* PA14 with a transposon (Tn) insertion in the *algD* gene to inactivate alginate production (PA14 Tn::*algD*) with a cloned *pga* locus (PA14 Tn::*algD* (pUCP18::*pga*)) was negative for alginate, but positive for PNAG production; CTX-M: cefotaximase-Munich; NDM: New Delhi metallo-β-lactamase; STEC: Shiga toxin-producing *E. coli*; 9GlcNH$_2$-TT: nonameric β-1–6-linked glucosamine oligosaccharides conjugated to a tetanus toxoid; Hla: alpha-hemolysin; 5GlcNH$_2$-TT: pentameric β-1–6-linked glucosamine oligosaccharides conjugated to a tetanus toxoid; DT: diphtheria toxin; Stx1B: Shiga toxin 1B.

were given either normal horse plasma or a horse plasma derived from donors immunized with the PNAG-targeting vaccine to produce a hyperimmune plasma (HIP). This preparation protected 100% of the recipients from any signs of infection, compared with all of the recipients of the control plasma getting a diagnosis of *R. equi* pneumonia. Notably, the antibodies to PNAG protected against a lung infection, whereas most of the currently used anti-bacterial vaccines for human infections primarily protect against disseminated bloodstream infections.

In the study showing protection against the intracellular pathogen *R. equi*, it was notable that the research group further investigated the mechanism of protection mediated by the antibody against an intracellular organism. The researchers showed that a range of pathogens that survive inside human monocyte-derived macrophages could be killed in the presence of the antibody to PNAG, complement, and human neutrophils, as the PNAG antigen was strongly expressed on the surface of the infected macrophage, where it could serve as a target for the antibody. Thus, the antibody to PNAG, along with complement and phagocytes, lysed the infected cells and released the intracellular bacteria, which were then killed by these components in a typical opsonic-killing setting [11].

Another study by Kahn et al. [38] revealed that HIP produced against PNAG was more effective in the opsonophagocytic killing

of *R. equi* than an HIP produced against *R. equi* whole cells and the vacuolar-associated protein (VAP) virulence factor. Moreover, the researchers found that the quantity and activity of antibodies against PNAG in HIP were positively correlated with protection against pneumonia in foals caused by *R. equi*. In another study, the same group assessed the relative efficacy of HIP to PNAG and *R. equi* to safeguard foals against *R. equi* pneumonia. They discovered that, under field conditions, PNAG HIP did not offer better protection to foals against *R. equi* pneumonia than a commercially available *R. equi* HIP product [39], although both were likely more effective than non-HIPs.

(2) **Update on the efficacy of PNAG-targeting antibody against fungal pathogens.** A study by Zhao et al. [17] showed the efficacy of the antibody to PNAG against clinically important fungal pathogens including *Aspergillus flavus, Aspergillus fumigatus*, and *Fusarium solani* in a murine model of corneal keratitis. The researchers demonstrated that the antibody to PNAG was protective in conjunction with T cells producing interleukin (IL)-17 and IL-22, providing a potential treatment option against infections from PNAG-producing fungal pathogens. These findings augmented an earlier report on the protective efficacy of the antibody to PNAG against *C. albicans* keratitis [1].

(3) **Update on PNAG-based vaccines for parasites.** Although many microbial organisms produce PNAG, not all are susceptible to control in an experimental-infection model. Taus et al. [13] discovered that PNAG was shared among tick-borne pathogens such as *Babesia bovis* (*B. bovis*), *Babesia bigemina*, *Babesia divergens*, *Babesia microti*, and *Babesia* WA1. In their study, calves that were immunized with synthetic pentameric β-1–6-linked glucosamine oligosaccharides conjugated to a tetanus toxoid ($5GlcNH_2$-TT) produced antibodies that displayed *in vitro* opsonophagocytic activity against *S. aureus*, and their sera reacted to *B. bovis*. Despite these findings, the anti-$5GlcNH_2$-TT vaccine was unable to protect the calves from experimental *B. bovis* infection.

2.2. New settings for the application of PNAG-based vaccines

The activation of neutrophil granulocytes by bacteria following allogeneic hematopoietic cell transplantation has been shown to promote acute graft-versus-host disease (GVHD). Interestingly, active and passive immunization against PNAG resulted in the elimination of microbiota-derived inflammatory factors, which could have been either complete cells or cellular fragments such as extracellular vesicles; this reduced the uncontrolled activation of neutrophils and prevented death from GVHD. This finding offers a possible new strategy to interfere with acute GVHD without impacting the commensal intestinal microbiota as antibiotics do [47].

2.3. New techniques applied in PNAG-based vaccine development

Although traditional conjugate vaccines are effective, they have several limitations, such as the complicated and time-consuming processes required for polysaccharide isolation and purification, and the conjugation or synthesis of oligosaccharides for conjugation. To overcome these issues, Stevenson et al. at Cornell University [16] tested a method based on the production of outer-membrane vesicles (OMVs) from a strain of *E. coli* with a reduced lipopolysaccharide (LPS) toxicity. OMVs are naturally occurring nanosized spherical structures that are produced by all Gram-negative bacteria; they consist of proteins, lipids, and glycans derived from the bacterial outer membrane and periplasm. OMVs have potential as a vaccine delivery platform, and OMVs isolated from *Neisseria meningitides* have already been incorporated into commercial vaccine formulations for human use [48,49].

The researchers at Cornell University proposed a different approach to develop vaccine candidates that can target PNAG. They showed that producing PNAG from proteins encoded by *pgaABCD* genes on a recombinant plasmid, along with overexpression of the *icaB*-encoded deacetylase protein from *S. aureus* to enhance the reduction of acetate substituents, allowed this construct to be incorporated into OMVs in laboratory strains of *E. coli*, leading to a highly deacetylated glycoconjugated OMV vaccine [16]. The resultant OMV glycoconjugate vaccine was efficacious at inducing a functional antibody to PNAG-producing microbes and protected mice against *S. aureus* and *Francisella tularensis* infections.

3. A PNAG-targeted vaccine and a mAb in clinical trials

3.1. AV0328

AV0328 is the designation for the conjugate vaccine targeting PNAG; it is a clinical product tested in humans and produced under good manufacturing processes. In preclinical trials in animals with a comparable construct, AV0328 induced protection against a wide range of microbial pathogens associated with infections by eliciting a protective antibody response. The results from *in vitro* experiments indicated that the animal antibodies elicited by the AV0328 vaccine demonstrated efficacy in killing every PNAG-producing pathogen tested, including over ten different microbial species [50].

The safety and efficacy of AV0328 were evaluated in 16 human subjects in a phase I clinical trial (NCT02853617). The subjects were divided into four groups and given escalating doses of 15, 30, 75, or 150 μg twice, 28 days apart. The vaccine was well tolerated, with no serious adverse events reported. Minor and temporary injection site reactions were observed in all dose groups. At the two highest doses administered (75 and 150 μg twice), the vaccine induced a notable increase in antibody titers against the PNAG antigen. Moreover, there was a positive indication of protective immunity, as evidenced by complement activation and binding to the PNAG antigen. The vaccine also demonstrated robust bactericidal activity against antibiotic-resistant strains of *Neisseria gonorrhea* and *Neisseria meningitidis* (serogroups A, B, C, W, and Y), as well as the opsonic killing of multi-drug resistant *S. pneumoniae* strains, multi-drug-resistant *Klebsiella pneumonia* strains, colistin, and multi-drug-resistant *E. coli* and *S. aureus* strains, including methicillin-resistant *S. aureus* (MRSA) clinical isolates [51].

PNAG has been found to be expressed by 100% of 36 pneumococcal strains tested so far, including the serotypes contained in Pneumovax and the recently approved Prevnar 20 vaccine. With this knowledge, the AV0328 vaccine has the potential to be a single, non-serotype-specific vaccine that could potentially provide coverage for most—if not all—pneumococcal serotypes. Although further research is needed, AV0328 could serve as a complementary vaccine that expands coverage to many of the remaining 100+ *S. pneumoniae* serotypes not currently covered by existing vaccines. Therefore, AV0328 could be an important step toward developing a more comprehensive vaccine against pneumococcal infections.

3.2. F598—The mAbs to PNAG

Human or humanized mAbs are being explored as potential treatments for bacterial, viral, and fungal infections, as well as for a variety of inflammatory diseases, due to their low immunogenicity and few side effects. In the case of PNAG, mAbs specific to different epitopes, based on the acetylation level of the PNAG

antigen used for the initial screening of promising hybridomas, were developed using *in vitro* assays that measured complement deposition and opsonophagocytic killing. These mAbs were subsequently tested for their ability to protect mice *in vivo*.

Three fully human mAbs (i.e., all genetic elements to produce the mAbs were of human origin) were identified and tested based on differential abilities to bind to both dPNAG and fully acetylated PNAG. Compared with two of the mAbs (mAbs F628 and F630) that had little or no binding to dPNAG, a fully human mAb called mAb F598 exhibited better binding to both dPNAG and native PNAG (where the glycoform is expressed on microbial cell surfaces). F598 exhibited superior activity in opsonophagocytic killing assays *in vitro* and increased protection *in vivo*. Furthermore, switching the mAb F598 from the human immunoglobulin G2 (IgG2) to the IgG1 subclass resulted in a modest improvement in complement component 3 (C3) deposition, along with a 25%–30% increase in opsonic killing activity. In a mouse study, a dose of 10 $mg \cdot kg^{-1}$ body weight of mAb F598 was found to provide significant protection against *S. aureus* experimental infections, which is comparable to the dose of the humanized mAb Palivizumab given to infants for prophylaxis against respiratory syncytial virus infection.

Human IgG1 mAb F598 is undergoing clinical assessments for the prevention and treatment of serious bacterial, fungal, and protozoal infections. A phase I trial has been completed, and F598 was found to be well-tolerated and safe with a 20–30 d half-life following a single administration. Therefore, F598 successfully passed phase I and is being pursued in phase II clinical trials in passive protection studies. The first trial is likely to be administrated to individuals upon admission to intensive care units, in order to assess F598's impact on infectious outcomes.

3.3. Crystal structure of mAb F598 to PNAG

To gain a better understanding of how antigens and antibodies interact, researchers conducted a structural analysis of the fragment antigen binding (Fab) of mAb F598 and the oligosaccharides of PNAG [52]. The Fab portion of mAb F598 was isolated and co-crystallized with monomeric GlcNAc and a nonameric PNAG oligosaccharide in order to gain insight into the molecular determinants of the binding of the antibody to the PNAG antigen. The findings showed that F598 recognizes PNAG through a large groove-shaped binding site that spans both the light and heavy chains of the antibody and can accommodate at least five GlcNAc residues. The Fab–GlcNAc complex was found to have a deep binding pocket in which the monosaccharide and a core GlcNAc of the oligosaccharide were positioned almost identically. These results suggest that F598 uses an anchored binding mechanism to recognize PNAG.

4. A future outlook on PNAG-based vaccines

The data generated in preclinical studies and the completed clinical trials supports the pursuit of other indications of PNAG-based vaccines as well. It should be noted that there is growing evidence to suggest that PNAG-positive microbes may be critical factors in a wide range of diseases not usually associated with infections. Ineffective removal of PNAG-expressing microbes or their fragments is being investigated for its contribution to the development of diabetes, Alzheimer's disease, and other chronic debilitating conditions. There are an increasing number of conditions for which an infection has been identified as the initial trigger for inflammation and associated tissue destruction. Although they are not the initial focus of scientists working on PNAG-based vaccines, such areas have great future potential.

5. Conclusions

In conclusion, PNAG is a complex carbohydrate structure found on the surface of various microorganisms, including bacteria, fungi, and protozoa. It plays a crucial role in microbial biofilm formation, adhesion to surfaces, and intercellular interactions. The biosynthesis of PNAG involves a series of enzymatic reactions and is regulated by specific genes and environmental conditions. Notably, the broad distribution of PNAG among organisms that use a diverse set of genes encoding biosynthetic enzymes to produce this conserved molecule indicates that a strong degree of convergent evolution has occurred, which would be consistent with PNAG playing a critical role in microbial biology.

The diverse functions and extensive production of PNAG make it an attractive target for the development of a vaccine and antibodies that could be protective against a wide range of infectious diseases caused by PNAG-producing pathogens. Several PNAG-based vaccines and antibodies have been studied in various infection models, demonstrating promising results in preventing infections and reducing the burden of diseases. However, further research is needed to fully understand the biosynthesis pathways and regulatory mechanisms of PNAG and to optimize the development of PNAG-based vaccines. In summary, PNAG is a versatile and important molecule involved in microbial physiology and pathogenesis. Harnessing its potential through targeted vaccines and therapies could contribute to the prevention and treatment of infections caused by PNAG-producing microorganisms, ultimately improving public health. Continued exploration of PNAG and its applications will undoubtedly provide valuable insights into the field of microbiology and pave the way for innovative strategies to combat infectious diseases.

Acknowledgments

The work was supported by the National Natural Science Foundation of China (32141003 and 81703399) and the Chinese Academy of Medical Sciences Innovation Fund for Medical Sciences (CIFMS; 2021-I2M-1-026).

Compliance with ethics guidelines

Gerald B. Pier has a financial interest in Alopexx, Inc., a company developing broad-spectrum immune therapeutics, which targets the polysaccharide, PNAG, for the prevention, treatment, and mitigation of bacterial, fungal, and parasitic infections. Dr. Pier's interests were reviewed and are managed by Brigham and Women's Hospital (BWH) and Mass General Brigham in accordance with their conflict of interest policies. Colette Cywes-Bentley is one of the inventors of intellectual properties (use of human mAb to PNAG and use of PNAG vaccines) that are licensed by BWH to Alopexx Inc. As inventors of intellectual properties, they also have the right to receive a share of licensing related income (royalties, fees) through BWH from Alopexx Inc. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

Xi Lu, Guoqing Li, Jing Pang, Xinyi Yang, and Xuefu You declare that they have no conflict of interest or financial conflicts to disclose.

References

[1] Cywes-Bentley C, Skurnik D, Zaidi T, Roux D, Deoliveira RB, Garrett WS, et al. Antibody to a conserved antigenic target is protective against diverse prokaryotic and eukaryotic pathogens. Proc Natl Acad Sci USA 2013;110(24):E2209–18.

[2] Kocer AT, Inan B, Usul SK, Özçimen D, Yilmaz MT, Işıldak I. EPSs from microalgae: production, characterization, optimization and techno-economic assessment. Braz J Microbiol 2021;52(4):1779–90.
[3] Abdalla AK, Ayyash MM, Olaimat AN, Osaili TM, Al-Nabulsi AA, Shah NP, et al. EPSs as antimicrobial agents: mechanism and spectrum of activity. Front Microbiol 2021;12:664395.
[4] Tounsi L, Hentati F, Hlima HB, Barkallah M, Smaoui S, Fendri I, et al. Microalgae as feedstock for bioactive polysaccharides. Int J Biol Macromol 2022;221:1238–50.
[5] Jawad I, Tawseen HB, Irfan M, Ahmad W, Hassan M, Sattar F, et al. Dietary supplementation of microbial dextran and inulin exerts hypocholesterolemic effects and modulates gut microbiota in BALB/c mice models. Int J Mol Sci 2023;24(6):5314.
[6] Nadzir MM, Nurhayati RW, Idris FN, Nguyen MH. Biomedical applications of bacterial EPSs: a review. Polymers 2021;13(4):530.
[7] Whitfield GB, Marmont LS, Howell PL. Enzymatic modifications of EPSs enhance bacterial persistence. Front Microbiol 2015;6:471.
[8] Skurnik D, Cywes-Bentley C, Pier GB. The exceptionally broad-based potential of active and passive vaccination targeting the conserved microbial surface polysaccharide PNAG. Expert Rev Vaccines 2016;15(8):1041–53.
[9] Yoong P, Cywes-Bentley C, Pier GB. Poly-*N*-acetylglucosamine expression by wild-type *Yersinia pestis* is maximal at mammalian, not flea, temperatures. mBio 2012;3(4):e00217–12.
[10] Skurnik D, Davis MR Jr, Benedetti D, Moravec KL, Cywes-Bentley C, Roux D, et al. Targeting pan-resistant bacteria with antibodies to a broadly conserved surface polysaccharide expressed during infection. J Infect Dis 2012;205 (11):1709–18.
[11] Cywes-Bentley C, Rocha JN, Bordin AI, Vinacur M, Rehman S, Zaidi TS, et al. Antibody to poly-*N*-acetyl glucosamine provides protection against intracellular pathogens: mechanism of action and validation in horse foals challenged with *Rhodococcus equi*. PLoS Pathog 2018;14(7):e1007160.
[12] Shanmugam M, Oyeniyi AO, Parthiban C, Gujjarlapudi SK, Pier GB, Ramasubbu N. Role of de-*N*-acetylase PgaB from *Aggregatibacter actinomycetemcomitans* in exopolysaccharide export in biofilm mode of growth. Mol Oral Microbiol 2017;32(6):500–10.
[13] Taus NS, Cywes-Bentley C, Johnson WC, Pier GB, Fry LM, Mousel MR, et al. Immunization against a conserved surface polysaccharide stimulates bovine antibodies with opsonic killing activity but does not protect against *Babesia bovis* challenge. Pathogens 2021;10(12):1598.
[14] Ramos Y, Rocha J, Hael AL, van Gestel J, Vlamakis H, Cywes-Bentley C, et al. PolyGlcNAc-containing exopolymers enable surface penetration by non-motile *Enterococcus faecalis*. PLoS Pathog 2019;15(2):e1007571.
[15] Van Dissel D, Willemse J, Zacchetti B, Claessen D, Pier GB, van Wezel GP. Production of poly-β-1,6-*N*-acetylglucosamine by MatAB is required for hyphal aggregation and hydrophilic surface adhesion by *Streptomyces*. Microb Cell 2018;5(6):269–79.
[16] Stevenson TC, Cywes-Bentley C, Moeller TD, Weyant KB, Putnam D, Chang YF, et al. Immunization with outer membrane vesicles displaying conserved surface polysaccharide antigen elicits broadly antimicrobial antibodies. Proc Natl Acad Sci USA 2018;115(14):E3106–15.
[17] Zhao G, Zaidi TS, Bozkurt-Guzel C, Zaidi TH, Lederer JA, Priebe GP, et al. Efficacy of antibody to PNAG against keratitis caused by fungal pathogens. Invest Ophthalmol Visual Sci 2016;57(15):6797–804.
[18] Roux D, Cywes-Bentley C, Zhang YF, Pons S, Konkol M, Kearns DB, et al. Identification of poly-*N*-acetylglucosamine as a major polysaccharide component of the *Bacillus subtilis* biofilm matrix. J Biol Chem 2015;290 (31):19261–72.
[19] Kwan DH, Withers SG. Periplasmic de-acylase helps bacteria don their biofilm coat. Proc Natl Acad Sci USA 2014;111(30):10904–5.
[20] Little DJ, Li G, Ing C, DiFrancesco BR, Bamford NC, Robinson H, et al. Modification and periplasmic translocation of the biofilm exopolysaccharide poly-β-1,6-*N*-acetyl-*D*-glucosamine. Proc Natl Acad Sci USA 2014;111 (30):11013–8.
[21] Steiner S, Lori C, Boehm A, Jenal U. Allosteric activation of exopolysaccharide synthesis through cyclic di-GMP-stimulated protein–protein interaction. EMBO J 2013;32(3):354–68.
[22] Little DJ, Pfoh R, Le Mauff F, Bamford NC, Notte C, Baker P, et al. PgaB orthologues contain a glycoside hydrolase domain that cleaves deacetylated poly-β(1,6)-*N*-acetylglucosamine and can disrupt bacterial biofilms. PLoS Pathog 2018;14(4):e1006998.
[23] Balducci E, Papi F, Capialbi E, Del Bino L. Polysaccharides' structures and functions in biofilm architecture of antimicrobial-resistant (AMR) pathogens. Int J Mol Sci 2023;24(4):4030.
[24] De los Mozos IR, Vergara-Irigaray M, Segura V, Villanueva M, Bitarte N, Saramago M, et al. Base pairing interaction between 5′- and 3′-UTRs controls *icaR* mRNA translation in *Staphylococcus aureus*. PLoS Genet 2013;9(12):e1004001.
[25] Echeverz M, Garcia B, Sabalza A, Valle J, Gabaldon T, Solano C, et al. Lack of the PGA exopolysaccharide in *Salmonella* as an adaptive trait for survival in the host. PLoS Genet 2017;13(5):e1006816.
[26] Nguyen HTT, Nguyen TH, Otto M. The staphylococcal exopolysaccharide PIA—biosynthesis and role in biofilm formation, colonization, and infection. Comput Struct Biotechnol J 2020;18:3324–34.
[27] Fullen AR, Gutierrez-Ferman JL, Yount KS, Love CF, Choi HG, Vargas MA, et al. BPS polysaccharide of *Bordetella pertussis* resists antimicrobial peptides by functioning as a dual surface shield and decoy and converts *Escherichia coli* into a respiratory pathogen. PLoS Pathog 2022;18(8):e1010764.
[28] Arciola CR, Baldassarri L, Montanaro L. In catheter infections by *Staphylococcus epidermidis* the intercellular adhesion (*ica*) locus is a molecular marker of the virulent slime-producing strains. J Biomed Mater Res 2002;59(3):557–62.
[29] Maira-Litrán T, Kropec A, Goldmann DA, Pier GB. Comparative opsonic and protective activities of *Staphylococcus aureus* conjugate vaccines containing native or deacetylated staphylococcal poly-*N*-acetyl-β-(1–6)-glucosamine. Infect Immun 2005;73(10):6752–62.
[30] Pons S, Frapy E, Sereme Y, Gaultier C, Lebreton F, Kropec A, et al. A high-throughput sequencing approach identifies immunotherapeutic targets for bacterial meningitis in neonates. EBioMedicine 2023;88:104439.
[31] Pozzi C, Wilk K, Lee JC, Gening M, Nifantiev N, Pier GB. Opsonic and protective properties of antibodies raised to conjugate vaccines targeting six *Staphylococcus aureus* antigens. PLoS One 2012;7(10):e46648.
[32] Skurnik D, Merighi M, Grout M, Gadjeva M, Maira-Litran T, Ericsson M, et al. Animal and human antibodies to distinct *Staphylococcus aureus* antigens mutually neutralize opsonic killing and protection in mice. J Clin Invest 2010;120(9):3220–33.
[33] Gening ML, Maira-Litrán T, Kropec A, Skurnik D, Grout M, Tsvetkov YE, et al. Synthetic β-(1→6)-linked *N*-acetylated and nonacetylated oligoglucosamines used to produce conjugate vaccines for bacterial pathogens. Infect Immun 2010;78(2):764–72.
[34] Zaidi T, Zaidi T, Yoong P, Pier GB. *Staphylococcus aureus* corneal infections: effect of the Panton-Valentine leukocidin (PVL) and antibody to PVL on virulence and pathology. Invest Ophthalmol Visual Sci 2013;54 (7):4430–8.
[35] Zaidi TS, Zaidi T, Pier GB. Antibodies to conserved surface polysaccharides protect mice against bacterial conjunctivitis. Invest Ophthalmol Visual Sci 2018;59(6):2512–9.
[36] Maira-Litrán T, Bentancor LV, Bozkurt-Guzel C, O'Malley JM, Cywes-Bentley C, Pier GB. Synthesis and evaluation of a conjugate vaccine composed of *Staphylococcus aureus* poly-*N*-acetyl-glucosamine and clumping factor A. PLoS One 2012;7(9):e43813.
[37] Søe NH, Jensen NV, Jensen AL, Koch J, Poulsen SS, Pier GB, et al. Active and passive immunization against *Staphylococcus aureus* periprosthetic osteomyelitis in rats. In Vivo 2017;31(1):45–50.
[38] Kahn SK, Cywes-Bentley C, Blodgett GP, Canaday NM, Turner-Garcia CE, Vinacur M, et al. Antibody activities in hyperimmune plasma against the *Rhodococcus equi* virulence-associated protein A or poly-*N*-acetyl glucosamine are associated with protection of foals against rhodococcal pneumonia. PLoS One 2021;16(8):e0250133.
[39] Kahn SK, Cywes-Bentley C, Blodgett GP, Canaday NM, Turner-Garcia CE, Flores-Ahlschwede P, et al. Randomized, controlled trial comparing *Rhodococcus equi* and poly-*N*-acetyl glucosamine hyperimmune plasma to prevent *R equi* pneumonia in foals. J Vet Intern Med 2021;35(6):2912–9.
[40] Cohen ND, Kahn SK, Cywes-Bentley C, Ramirez-Cortez S, Schuckert AE, Vinacur M, et al. Serum antibody activity against poly-*N*-acetyl glucosamine (PNAG), but not PNAG vaccination status, is associated with protecting newborn foals against intrabronchial infection with *Rhodococcus equi*. Microbiol Spectrum 2021;9(1):e00638-21.
[41] Rocha JN, Dangott LJ, Mwangi W, Alaniz RC, Bordin AI, Cywes-Bentley C, et al. PNAG-specific equine IgG1 mediates significantly greater opsonization and killing of *Prescottella equi* (formerly *Rhodococcus equi*) than does IgG4/7. Vaccine 2019;37(9):1142–50.
[42] Skurnik D, Roux D, Pons S, Guillard T, Lu X, Cywes-Bentley C, et al. Extended-spectrum antibodies protective against carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother 2016;71(4):927–35.
[43] Cerca N, Maira-Litrán T, Jefferson KK, Grout M, Goldmann DA, Pier GB. Protection against *Escherichia coli* infection by antibody to the *Staphylococcus aureus* poly-*N*-acetylglucosamine surface polysaccharide. Proc Natl Acad Sci USA 2007;104(18):7528–33.
[44] Mellata M, Mitchell NM, Schödel F, Curtiss R 3rd, Pier GB. Novel vaccine antigen combinations elicit protective immune responses against *Escherichia coli* sepsis. Vaccine 2016;34(5):656–62.
[45] Lu X, Skurnik D, Pozzi C, Roux D, Cywes-Bentley C, Ritchie JM, et al. A poly-*N*-acetylglucosamine–Shiga toxin broad-spectrum conjugate vaccine for Shiga toxin-producing *Escherichia coli*. mBio 2014:5(2):e00974-14.
[46] Bentancor LV, O'Malley JM, Bozkurt-Guzel C, Pier GB, Maira-Litrán T. Poly-*N*-acetyl-β-(1–6)-glucosamine is a target for protective immunity against *Acinetobacter baumannii* infections. Infect Immun 2012;80(2):651–66.
[47] Hülsdünker J, Thomas OS, Haring E, Unger S, Núñez NG, Tugues S, et al. Immunization against poly-*N*-acetylglucosamine reduces neutrophil activation and GVHD while sparing microbial diversity. Proc Natl Acad Sci USA 2019;116(41):20700–6.
[48] Kulp A, Kuehn MJ. Biological functions and biogenesis of secreted bacterial outer membrane vesicles. Annu Rev Microbiol 2010;64:163–84.
[49] Gorringe AR, Pajón R. Bexsero: a multicomponent vaccine for prevention of meningococcal disease. Hum Vaccines Immunother 2012;8(2):174–83.
[50] alopexx.com [Internet]. Cambridge: Alopexx incorporation; [cited 2023 Oct 10]. Available from: https://www.alopexx.com/pipeline/vaccine-av0328.
[51] ClinicalTrials.gov [Internet]. Bethesda: National Library of Medicine; [cited 2023 Oct 10]. Available from: https://clinicaltrials.gov/study/NCT02853617.
[52] Soliman C, Walduck AK, Yuriev E, Richards JS, Cywes-Bentley C, Pier GB, et al. Structural basis for antibody targeting of the broadly expressed microbial polysaccharide poly-*N*-acetylglucosamine. J Biol Chem 2018;293 (14):5079–89.