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Diverse Mechanisms of Protective Anti-Pneumococcal Antibodies

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The gram-positive bacterium *Streptococcus pneumoniae* is a leading cause of pneumonia, otitis media, septicemia, and meningitis in children and adults. Current prevention and treatment efforts are primarily pneumococcal conjugate vaccines that target the bacterial capsule polysaccharide, as well as antibiotics for pathogen clearance. While these methods have been enormously effective at disease prevention and treatment, there has been an emergence of non-vaccine serotypes, termed serotype replacement, and increasing antibiotic resistance among these serotypes. To combat *S. pneumoniae*, the immune system must deploy an arsenal of antimicrobial functions. However, *S. pneumoniae* has evolved a repertoire of evasion techniques and is able to modulate the host immune system. Antibodies are a key component of pneumococcal immunity, targeting both the capsule polysaccharide and protein antigens on the surface of the bacterium. These antibodies have been shown to play a variety of roles including increasing opsonophagocytic activity, enzymatic and toxin neutralization, reducing bacterial adherence, and altering bacterial gene expression. In this review, we describe targets of anti-pneumococcal antibodies and describe antibody functions and effectiveness against *S. pneumoniae*.

Keywords: *Streptococcus pneumoniae*, monoclonal antibody, opsonophagocytic, immune evasion, pneumococcal vaccination

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INTRODUCTION

Streptococcus pneumoniae is a gram-positive opportunistic bacterial pathogen that colonizes the upper respiratory tract, and is a leading cause of bacterial infections worldwide (Weiser et al., 2018), (Denny and Loda, 1986). Bacterial colonization is a precursor to pneumococcal disease, which can manifest as otitis media, pneumonia, septicemia and meningitis. *S. pneumoniae* is found in up to 27-65% of children and <10% of adults in a commensal carriage state (Yahiaoui et al., 2016). In 2000, it was estimated that *S. pneumoniae* was responsible for over 800,000 deaths in children annually (O'Brien et al., 2009). Due to the continued high burden of disease despite an effective vaccine, *S. pneumoniae* was designated a priority pathogen by the World Health Organization in 2017 (WHO, 2017). Multiple colonization events in the lifetime of an individual result in serum antibody responses to the capsular polysaccharide (CPS) (Weinberger et al., 2008) and protein antigens (McCool et al., 2002; Zhang et al., 2006; Prevaes et al., 2012; Turner et al., 2013). The CPS is a critical virulence factor for *S. pneumoniae*

survival during invasive disease, and the CPS can inhibit the innate immune response *via* inhibition of phagocytosis, preventing recognition of the bacteria by host receptors, and evasion of neutrophil extracellular traps (Hyams et al., 2010). Each pneumococcal serotype, of which 100 serotypes have been identified, has a distinct CPS that varies in its biochemical and antigenic structure (Ganaie et al., 2020). The most effective preventative measure against pneumococcal infection is vaccination with either a 23-valent pneumococcal polysaccharide vaccine (PPSV23) or a multivalent pneumococcal conjugate vaccine (PCV7, PCV10, PCV13, PCV15, or PCV20). While the CPS is the primary antigen in both vaccines, the CPS antigens in PCV-based vaccines are linked to a carrier protein to induce T-dependent antibody responses leveraging the hapten-carrier effect. However, due to the limited serotypes included in PCVs, protection is incomplete, and this has caused an increase of non-vaccine serotypes in carriage and disease (Klugman, 2009; von Gottberg et al., 2014). Nonencapsulated strains of *S. pneumoniae* are also able to colonize the nasopharyngeal tract and are not affected by current vaccines (Keller et al., 2016). These strains have unique surface proteins that allow for colonization and virulence in the absence of the CPS (Keller et al., 2016).

In light of limited serotype coverage, increasing prevalence of non-vaccine serotypes, and increasing antibiotic resistance among non-vaccine serotypes, conserved protein antigens and other molecules have also been studied as potential vaccine candidates, as these would presumably induce broadly reactive antibodies that would be effective against a greater number of serotypes than CPS-based vaccines (Daniels et al., 2016). Multiple antigens have been tested in animal models and clinical trials, including the toxin pneumolysin (Ply) (Kamtchoua et al., 2013; Odutola et al., 2017; Hammitt et al., 2019), pneumococcal surface protein A (PspA) (Briles et al., 2000b; Nabors et al., 2000; Frey et al., 2013), pneumococcal surface adhesin A (PsaA) (Wang et al., 2010; Moffitt and Malley, 2016), pneumococcal choline binding protein A (PcpA) (Glover et al., 2008; Verhoeven et al., 2014; Xu et al., 2017), pneumococcal histidine triad protein (PhtD) (Denoël et al., 2011b; Kaur et al., 2014; André et al., 2021), Phosphorycholine (PC) (Trolle et al., 2000; Tanaka et al., 2007), Neuraminidase (Nan) (Janesch et al., 2018) and choline binding proteins (CbpA, CbpG) (Mann et al., 2006; Hernani et al., 2011; Ricci et al., 2011). Antibodies are a key component of pneumococcal immunity, and their mechanisms of action are important to understand for vaccine design efforts (**Figure 1** and **Table 1**). Here we review anti-pneumococcal antibodies, including antibody targets and known mechanisms of action.

Antibodies to the Pneumococcal Capsular Polysaccharide

One of the most important virulence factors of *S. pneumoniae* is the CPS, which encases the bacterium and is the defining antigen for serotype identification. Currently, 100 distinct capsule serotype structures have been identified, with at least 30 serotypes being responsible for invasive pneumococcal disease (Luck et al., 2020). The CPS is typically negatively charged, which allows the bacteria to avoid mucous entrapment and enables colonization of the nasopharynx (Magee and Yother, 2001; Nelson et al., 2007; Li et al., 2013). As the CPS encases the bacterium, it protects deeper antigenic structures, and inhibits the binding of immunoglobulins, complement components, and C-reactive protein (Weiser et al., 2018). The CPS also reduces opsonization with C3b/iC3b, thereby impairing the receptors of phagocytic cells from interacting with antibody Fc regions (Abeyta et al., 2003; Hyams et al., 2010). It has been hypothesized that the CPS also protects bacteria from entrapment by neutrophil extracellular traps (Wartha et al., 2007). Due to the vital importance of the CPS to bacterial survival and virulence, it has long been a therapeutic target. PPSV23 and PCV vaccines have been approved for use in humans and have been widely effective. However, there has been a rise in the incidence of nonvaccine serotypes, and serotypes 3 and 19A have also persisted despite vaccination efforts (Wantuch and Avci, 2018; Linley et al., 2019).

A key function of CPS targeting vaccines is to elicit antibodies that have opsonophagocytic activity, which has been well defined to be a predictor of protection (Poehling et al., 2006; Ekström et al., 2007; Goldblatt et al., 2010; Prymula et al., 2011; Dagan et al., 2013; Lee et al., 2014) (**Figure 2**). Indeed, *in vitro* opsonophagocytic assays are the gold standard for measuring antibody-mediated immunity (Romero-Steiner et al., 1997; Song et al., 2013; Toh et al., 2021). In addition, antibody agglutination activity is a correlate of protection (Bull, 1915), as the establishment of colonization by *S. pneumoniae* is a critical first step in disease pathogenesis, and antibody agglutinating activity inhibits colonization (Bogaert et al., 2004; Simell et al., 2012) (**Figure 2**). For example, CPS-specific antibodies from nasal washes of vaccinated individuals are important for protection against colonization (Mitsi et al., 2017), and human mAbs targeting the CPS can have both opsonophagocytic and agglutination activity *in vitro* (Babb et al., 2021).

While a majority of described antibodies targeting the CPS rely on increasing opsonophagocytic and agglutinating activity to offer protection, nonopsonic antibodies to the pneumococcal CPS can also offer protection. The first study to discover this phenomenon found that a mouse IgG_1, mAb 1E2, targeting the serotype 3 CPS was unable to opsonize and kill bacteria *in vitro* yet was protective *in vivo* (Tian et al., 2009). In several follow up studies, mAb 1E2 was shown to alter the expression of over 50 genes (Doyle et al., 2021), which included genes involved in quorum sensing and increased fratricide (Yano et al., 2011). Upon interaction with mAb 1E2, iron uptake (*piuB* gene) was increased, sensitivity to oxidative stress (*dpr* gene) was amplified, and rapid capsule shedding was observed (Doyle et al., 2021) (**Figure 2**). mAb 1E2 also reduces colonization in mice (Doyle and Pirofski, 2016). A mechanistic study on mAb 1E2 revealed that neutrophils were not needed for its protective efficacy (Tian et al., 2009), but macrophages were required (Sarah et al., 2012). These studies demonstrate that anti-CPS antibody functions beyond opsonophagocytic and agglutination activity can protect against pneumococcal disease, and such functions may be important to consider in current and future vaccines.

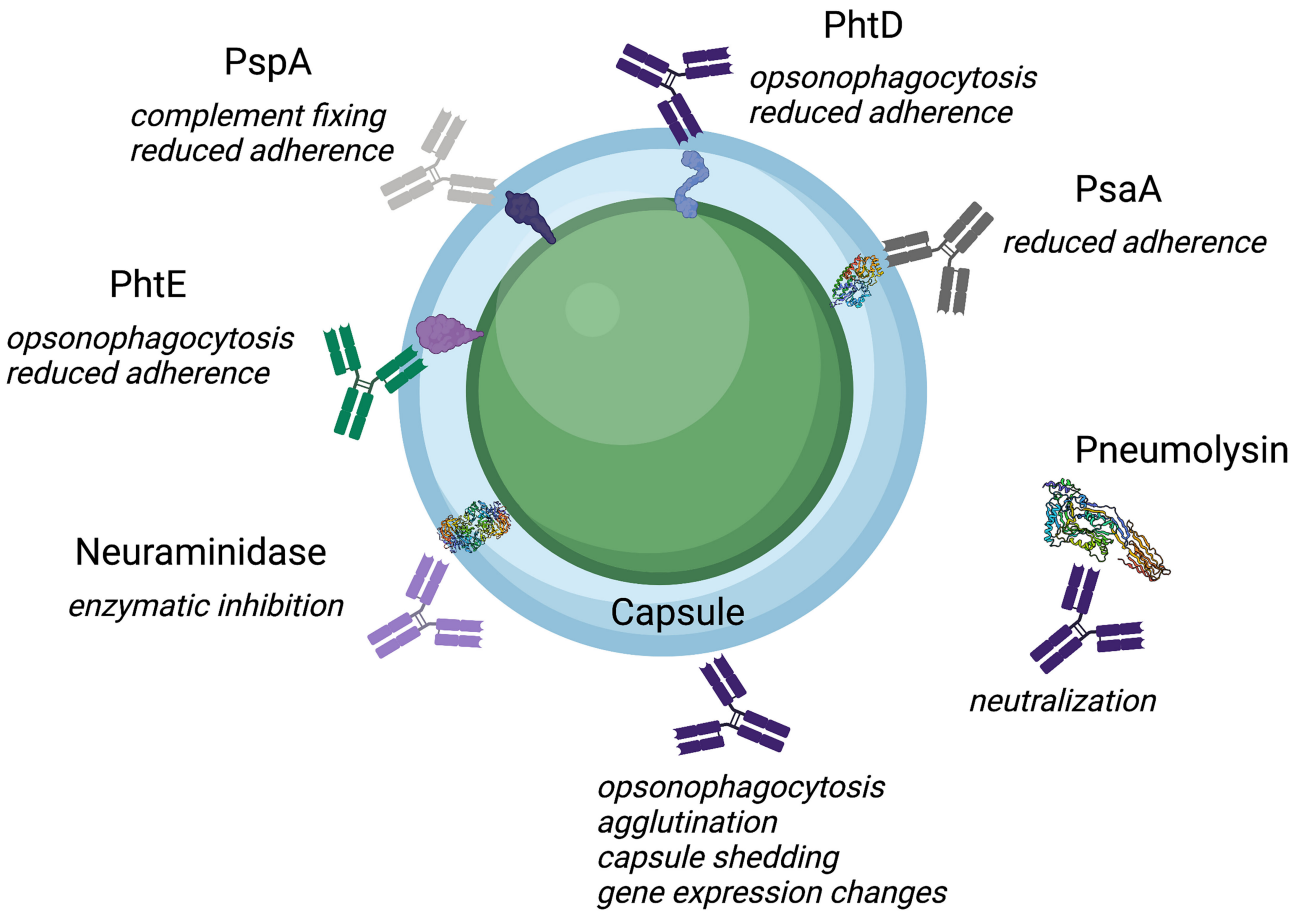

FIGURE 1 | Illustration of the diverse mechanisms and targets of anti-pneumococcal antibodies. Protein antigens are displayed within the capsule as cartoons or as protein models when available from the protein data bank.

Serotype specific IgG targeting the CPS is sufficient to protect against the homologous serotype, and additional antibody isotypes beyond IgG can offer protection. Serotype 8 specific mAbs NAD (an IgA) and D11 (an IgM) have been examined *in vitro*, and NAD was shown to increase complement deposition (**Figure 2**) whereas D11 was not, and neither NAD nor D11 promoted significant neutrophil mediated killing in an opsonophagocytic assay (Burns et al., 2003). In the presence of bacteria, complement, and D11 or NAD, a decrease in IL-8 secretion by neutrophils was observed (Burns et al., 2003). Both the D11 and NAD antibodies were found to be protective against infection in the mouse model (Burns et al., 2003). Additional studies with D11 revealed treated mice had significantly less IFN-γ, MIP-2, IL-12, MCP-1/JE, and TNF-α compared to control mice (Burns et al., 2005). mAb A7, a serotype 3-specific IgM, induced a decrease in keratinocyte-derived chemokine, IL-6 and macrophage inflammatory protein-2 in mAb treated mice, similar to levels seen in penicillin treated mice (Fabrizio et al., 2007). Together these studies demonstrate that the connection between antibody-mediated protection and immunomodulation plays a key role in protection *in vivo* and should be further explored.

Antibodies to Pneumococcal Proteins

Pneumococcal Surface Protein A

While vaccine-induced CPS-specific antibodies can protect against colonization with vaccine-included serotypes, natural colonization leads the induction of both CPS-specific antibodies and anti-protein antibodies (Wilson et al., 2017). Pneumococcal surface protein A (PspA) is one of the most prevalent antigens on the surface of *S. pneumoniae* and plays a major role in protective immunity (Khan and Jan, 2017). PspA aids the bacteria in evading the bactericidal activity of neutrophil extracellular traps (Martinez et al., 2019), inhibits complement (Tu et al., 1999; Ren et al., 2004; Ren et al., 2012) by reducing the amount of C3 that is deposited on the bacteria (Mukerji et al., 2012), and binds lactoferrin, which likely blocks the active site of apolactoferrin responsible for bacterial killing (Shaper et al., 2004; Bitsaktsis et al., 2012; Mirza et al., 2016). PspA is classified into 3 families with 6 clades based on sequence similarities in the variable N-terminal α-helical region (Hollingshead et al., 2000). Numerous studies have demonstrated the protective effects of PspA-based vaccines, which lead to increased survival and decreased bacterial

TABLE 1 | Antigens evaluated in clinical trials and animal models.

Antigen	Antigen function	Antibody function	Tested as Vaccine?	Species tested	Vaccine status	Protection	Antibody Therapy	Protection	
Capsular polysaccharide	Inhibition of phagocytosis Blocking host receptors Evasion of NETs	Opsonophagocytosis Agglutination Capsule shedding Gene expression	Yes	Murine Human	FDA approved	Yes	Yes	Yes	Burns et al., 2003; Burns et al., 2005; Fabrizio et al., 2007; Tian et al., 2009; Yano et al., 2011; Sarah et al., 2012; Doyle and Pirofski, 2016; Keller et al., 2016; Mitsi et al., 2017; Babb et al., 2021; Doyle et al., 2021
Phosphorylcholine	Bacterial adherence	Reduced adherence	Yes	Murine	Murine model	Yes	Yes	Yes	Wallick et al., 1983; Briles et al., 1992; Fischer et al., 1995; Trolle et al., 2000; Tanaka et al., 2007
PspA	Inhibition of complement deposition Binding lactoferrin Evasion of NETs	Increase C3 deposition Inhibition of lactoferrin binding Enhancing NETs	Yes	Murine Human	Phase 1	Protection not yet assessed in humans. Yes, in animal models	Yes	Yes	Wu et al., 1997; Briles et al., 2000b; Briles et al., 2000c; Nabors et al., 2000; Ferreira et al., 2006b; Daniels et al., 2010; Bitsaktsis et al., 2012; Frey et al., 2013; Piao et al., 2014; Nagano et al., 2018; Nakahashi-Ouchida et al., 2021
PsaA	ABC transporter of Zn2+ and Mn2+ Attachment to epithelial cells Protection against oxidative stress	Reduced adherence	Yes	Murine Human	Phase 1	Protection not yet assessed in humans. Partial protection in animal models	ND	ND	Talkington et al., 1996; Briles et al., 2000a; Miyaji et al., 2001; Romero-Steiner et al., 2003; Oliveira et al., 2006; Pimenta et al., 2006; Romero-Steiner et al., 2006; Wang et al., 2010
PhtD	Inhibition of complement deposition Zn^{2+} acquisition Adherance to epithelial cells	Increase C3 deposition Reduced adherence Opsonophagocytosis	Yes	Murine Macaque Human	Phase1/2	Yes, in animal models. Did not reduce colonization or otitis media in humans	Yes	Yes	Wizemann et al., 2001; Denoël et al., 2011a; Denoël et al., 2011b; Godfroid et al., 2011; Bologa et al., 2012; Khan and Pichichero, 2012; Seiberling et al., 2012; Plumptre et al., 2013b; Ravinder et al., 2014; Berglund et al., 2014; Leroux-Roels et al., 2014; Odutola et al., 2017; Papastamatiou et al., 2018; Visan et al., 2018; Hammitt et al., 2019; André et al., 2020; Huang et al., 2021
Neuraminidase	Sugar acquisition Inhibition of complement deposition Adherence to endothelial cells Promotes biofilm formation	Enzymatic inhibition	No	ND	ND	ND	Yes	No	Janesch et al., 2018
Pneumolysin	Cytotoxic activity Inhibition of complement deposition Biofilm formation	Neutralization	Yes	Murine Macaque Human	Phase1/2	Yes in animal models. Did not reduce colonization or otitis	Yes	Yes	García-Suárez et al., 2004; Ferreira et al., 2006a; Sanders et al., 2010; Lu et al., 2014; Mann et al., 2014; Hermand et al., 2017; Odutola et al., 2017; Hammitt et al., 2019; Petukhova

(Continued)

TABLE 1 | Continued

Antigen	Antigen function	Antibody function	Tested as Vaccine?	Species tested	Vaccine status	Protection	Antibody Therapy	Protection	
						media in humans			et al., 2020; Thanawastien et al., 2021
CbpG	Cleavage of casein and fibronectin	Opsonophagocytosis	Yes	Murine	Murine model	Yes	Yes	Yes	Mann et al., 2006; Kazemian et al., 2018
PcpA	Adherence to epithelial cells	Increase C3 deposition Reduced adherence Opsonophagocytosis	Yes	Murine Human	Phase 1	Protection not yet assessed in humans. Yes, in animal models	Yes	Yes	Glover et al., 2008; Verhoeven et al., 2014; Brooks et al., 2015; Ochs et al., 2016; Xu et al., 2017; Visan et al., 2018
PspC/CbpA	Adherence to epithelial cells Binding complement factor H, C4BP, and vitronectin	Increase C3 deposition Block fH binding Opsonophagocytosis	Yes	Murine	Murine model	Protective against certain serotypes	Yes	Protective when used with other antibodies	Cao et al., 2007; Lu et al., 2008; Ferreira et al., 2009; Hernani et al., 2011; Ricci et al., 2011; Mann et al., 2014; Chen et al., 2015

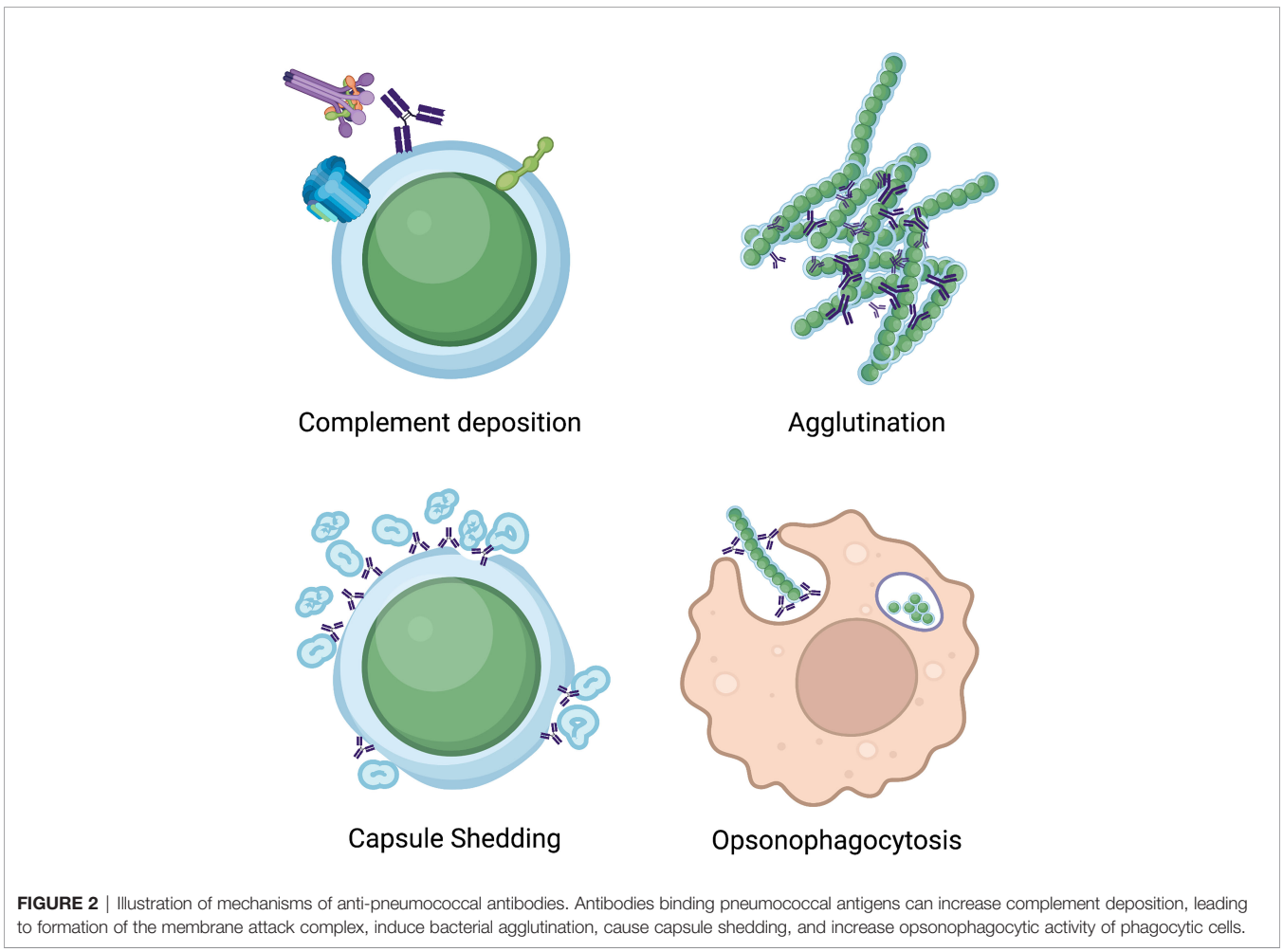

FIGURE 2 | Illustration of mechanisms of anti-pneumococcal antibodies. Antibodies binding pneumococcal antigens can increase complement deposition, leading to formation of the membrane attack complex, induce bacterial agglutination, cause capsule shedding, and increase opsonophagocytic activity of phagocytic cells.

burden in animal models (Wu et al., 1997; Briles et al., 2000c; Ferreira et al., 2006b; Daniels et al., 2010; Piao et al., 2014; Nagano et al., 2018; Nakahashi-Ouchida et al., 2021). It has also been demonstrated that antibodies targeting PspA can have their function enhanced *via* targeting activating FcγRI with fusion proteins consisting of PspA and IgG2a Fc, which then is able to enhance PspA-specific immune responses (Wiedinger et al., 2020). Another study demonstrated that by fusing a humanized single-chain antibody component (in which the variable domain binds to FcγRI) to PspA, protection could be achieved in the absence of adjuvant (Bitsaktsis et al., 2012). This vaccine could be enhanced further by adding an additional FcγRI binding moiety to the vaccine mentioned above (Kumar et al., 2020). PspA vaccines have advanced to human clinical trials and demonstrated safety and robust antibody responses (Briles et al., 2000b; Nabors et al., 2000; Frey et al., 2013), however, concerns exist about using full length PspA due to its sequence homology with human cardiac myosin (Ginsburg et al., 2012).

Vaccine derived antibodies targeting PspA mainly function by increasing complement C3 deposition leading to increased phagocytosis. Passive immunization with an anti-PspA mAb XiR278 protected mice from infection at 10x the LD_{50} when given before or shortly after pneumococcal infection (Swiatlo et al., 2003). Another study generated broadly reactive anti-PspA mouse mAbs, one of which, mAb 140H1, bound to 98% of the 48 strains tested, which encompasses the clinically relevant PspA clades 1-5 (Kristian et al., 2016). mAb 140H1 was shown to be protective in *in vivo* mouse sepsis and pneumonia models, including 24 hrs after intranasal infection (Kristian et al., 2016). This study also demonstrated that mAb 140H1 and others were able to increase C3 deposition on the surface of bacteria and increase bacterial killing in opsonophagocytic killing assays. This suggests that the complement deposition induced by these anti-PspA mAbs facilitates phagocytic uptake and killing by neutrophils (Kristian et al., 2016). In support of this observation, increased C3 deposition on the surface of bacteria *via* anti-PspA mAb binding has been demonstrated in numerous studies (Ren et al., 2004; Ren et al., 2012; Bitsaktsis et al., 2012; Goulart et al., 2013; Khan et al., 2015; Genschmer et al., 2019; Wiedinger et al., 2020). Additional functions for anti-PspA mAbs include inhibition of lactoferrin by anti-PspA mAbs (Bitsaktsis et al., 2012), and enhanced trapping and killing of *S. pneumoniae* by neutrophil extracellular traps (Martinez et al., 2019).

Pneumococcal Surface Adhesin A

The pneumococcal surface adhesin A (PsaA) is a multifunctional surface exposed lipoprotein that is found in all known serotypes (Sampson et al., 1997). PsaA is an ABC transporter binding protein that is capable of transporting Zn^{2+} and Mn^{2+} (Dintilhac et al., 1997; Lawrence et al., 1998; Jakubovics and Jenkinson, 2001; W et al., 2004). PsaA can also facilitate attachment to host cells (Sampson et al., 1994; Novak et al., 2000; Romero-Steiner et al., 2003; McAllister et al., 2004; Romero-Steiner et al., 2006) and protect against oxidative stress (Tseng et al., 2002). The binding receptor for PsaA is E-cadherin, the cell-cell junction protein found in respiratory epithelial cells (Anderton et al., 2007). PsaA employs multiple effects on host cells including cytoplasmic foaming, excessive vacuolation, and nuclear structure changes leading to colonization and internalization of the bacteria (Anderton et al., 2007). It was recently discovered that PsaA interacts with human Annexin A2 (ANXA2) (Hu et al., 2021). Immunization studies with recombinant PsaA showed a significant reduction in recovery time against pneumococcal carriage (Briles et al., 2000a; Miyaji et al., 2001; Oliveira et al., 2006; Pimenta et al., 2006; Wang et al., 2010). However when used in a systemic challenge model those protective effects were either minimal (Talkington et al., 1996) or not significant (Wang et al., 2010). Anti-PsaA antibodies can reduce the ability of *Streptococcus pneumoniae* to bind to Detroit 562 cells (pharyngeal epithelial cells) *in vitro* (Romero-Steiner et al., 2003; Romero-Steiner et al., 2006).

Pneumococcal Histidine Triad Proteins

The pneumococcal histidine triad proteins are a group of four proteins (PhtA, PhtB, PhtD, PhtE) that are present on the surface of *S. pneumoniae* (Adamou et al., 2001). PhtD is the most conserved of the group with 91-98% identity across strains isolated from cases of invasive disease (Yun et al., 2015). While the function of PhtD is not fully understood, it is important for attachment to respiratory epithelial cells *in vitro* (Khan and Pichichero, 2012; Plumptre et al., 2013a; Kallio et al., 2014). Additionally, PhtD has been shown *in vitro* to aid in Zn^{2+} acquisition and ultimately bacterial homeostasis as Zn^{2+} is a vital nutrient (Ogunniyi et al., 2009; Eijkelkamp et al., 2016). PhtD can also reduce complement deposition *via* Factor H (FH) (Ogunniyi et al., 2009). Several studies have demonstrated that immunization with PhtD or other Pht antigens elicits antibodies that protect against sepsis, pneumonia, and reduced colonization in animal models (Wizemann et al., 2001; Denoël et al., 2011a; Denoël et al., 2011b; Godfroid et al., 2011; Plumptre et al., 2013b; Ravinder et al., 2014; Papastamatiou et al., 2018; André et al., 2020). In a non-human primate model, vaccination with a combined PhtD/pneumolysin formulation was protective against lethal challenge (Denoël et al., 2011b). PhtD-based vaccine candidates have advanced to clinical trials, and several trials have demonstrated that immunization with PhtD was well tolerated and immunogenic (Bologa et al., 2012; Khan and Pichichero, 2012; Seiberling et al., 2012; Berglund et al., 2014; Leroux-Roels et al., 2014; Hammitt et al., 2019). However in a clinical trial in 6-12 month old infants, vaccination with PhtD/dPly/PCV13 did not show an increase in vaccine efficacy against acute otitis media compared to the standard PCV13 vaccine (Hammitt et al., 2019). In an additional study, a PHiD-CV/dPly/PhtD vaccine containing a 10-valent polysaccharide conjugate (10VT) combined with pneumolysin toxoid and pneumococcal histidine triad protein D was immunogenic but no improvement in pneumococcal carriage in infants was seen regardless of dose or schedule (Odutola et al., 2017). Vaccine induced PhtD antibodies were shown to increase complement C3b deposition on the bacterial surface and increase phagocytosis.

Antibodies targeting PhtD have demonstrated protection in preclinical animal models. Anti-PhtD polyclonal antibodies are

able to reduce colonization (Ravinder et al., 2014) and protect against lethal sepsis (Brookes et al., 2015) in a passive transfer mouse model. The use of anti-PhtD mAbs has also been explored. Human anti-PhtD mAbs are protective against both sepsis and pneumonia models of infection in mice when given prior to infection (Huang et al., 2021). Additionally, administration of an anti-PhtD mAb (PhtD3) 24 hours post infection was able to rescue mice from infection (Huang et al., 2021). An *in vivo* mechanism for anti-PhtD antibodies has not been fully elucidated, however, a recent study demonstrated that when depleting complement *via* cobra venom factor the protective effect of anti-PhtD antibodies was lost (Visan et al., 2018). Furthermore, depletion of macrophages but not neutrophils also resulted in loss of protection (Visan et al., 2018). The mechanism of anti-PhtD antibodies has been tested *in vitro* where it was observed that antibodies are able to inhibit bacterial attachment to epithelial cells (Kallio et al., 2014; Kaur et al., 2014), promote complement deposition (André et al., 2021) (Visan et al., 2018), and increase bacterial phagocytosis (Visan et al., 2018; Malekan et al., 2020; André et al., 2021; Huang et al., 2021).

Neuraminidase

S. pneumoniae utilizes neuraminidases for sugar acquisition, and these neuraminidases are a key virulence factor of which three have been described: NanA, NanB and NanC (Cámara et al., 1994; Berry et al., 1996; Xu et al., 2011; Hammond et al., 2021). These neuraminidases cleave terminal sialic acids providing essential nutrients (King et al., 2006; Burnaugh et al., 2008), interfere with C3 deposition (Dalia et al., 2010), promote biofilm formation (Parker et al., 2009), and are required for adherence and invasion of brain endothelial cells (Uchiyama et al., 2009). Due to the multitude of essential functions for *S. pneumoniae* these neuraminidases provide an attractive target for therapeutic antibodies. Utilizing an *in vitro* assay with differentiated airway epithelial cells, it was shown that anti-neuraminidase mAbs can preserve the terminal epithelial sugar composition during *S. pneumoniae* infection leading to a 10-20 fold decrease in bacterial growth compared to control mAbs (Janesch et al., 2018). Similar effects were seen in an *in vivo* mouse model, where anti-neuraminidase mAbs led to a reduction in desialylation of the airways (Janesch et al., 2018). However, in an acute murine pneumonia treatment with the anti-neuraminidase mAbs there was no effect on survival, lung burden, or host inflammatory responses (Janesch et al., 2018). While anti-neuraminidase mAbs exert an inhibitory effect on the neuraminidases, this does not lead to a positive effect on mortality or bacterial burden in a murine model of acute pneumonia (Janesch et al., 2018).

Pneumolysin

The virulence factor pneumolysin (Ply) is a cholesterol-dependent cytolysin that plays an essential role in pneumococcal disease. Pneumolysin lacks a secretory signal and is localized in the cytoplasm or the cell wall, and is released during autolysis and bacterial growth (García-Suárez et al., 2007; Price and Camilli, 2009). Pneumolysin exhibits a wide range of effects, including cytotoxic activity (Steinfort et al., 1989; Rubins et al., 1992; Rayner et al., 1995) to host cells, increasing bacterial penetration (Ring et al., 1998; Zysk et al., 2001), increasing inflammation (Feldman et al., 1991; Cockeran et al., 2001; Yoo et al., 2010; Subramanian et al., 2019), blocking complement (Paton et al., 1984; Mitchell et al., 1991; Rubins et al., 1995; Alcantara et al., 2001), and being a vital factor in biofilm formation and pneumococcal transmission (Shak et al., 2013; Marks et al., 2014; Zafar et al., 2017). Due to its conserved nature between strains and serotypes (Han and Zhang, 2019), (Kanclerski and Möllby, 1987) pneumolysin has been well studied as a therapeutic target. Numerous studies investigating antibodies targeting Ply have demonstrated their protective efficacy. Ply renders platelets nonfunctional and inhibits platelet-thrombus formation in whole blood, and an *in vitro* study demonstrated that anti-Ply antibodies are able to neutralize Ply and rescue platelet function (Jahn et al., 2020). Murine derived anti-pneumolysin mAbs were able to neutralize its cytolytic activity and inhibit binding of pneumolysin to cholesterol (Kucinskaite-Kodze et al., 2020). In a recent study, anti-pneumolysin human polyclonal antibodies did not decrease adherence of TIGR4 to A549 cells *in vitro*, however, in an *in vivo* murine model, passive transfer of anti-pneumolysin antibodies did show a significant decrease in colonization of TIGR4 (Kaur et al., 2014). Biofilm formation plays a key role in pneumococcal colonization and Ply is important for biofilm formation, independent of its cytolytic activity (Shak et al., 2013). High serum levels of anti-Ply antibodies have been correlated to delayed colonization in infants (Holmlund et al., 2006; Francis et al., 2009), and low anti-Ply antibodies may be a predisposing factor in developing pneumococcal pneumonia (Huo et al., 2004). Inactive Ply mutants are able to induce antibodies that neutralize Ply *via* an *in vitro* anti-hemolytic assay (Kamtchoua et al., 2013). Several studies have demonstrated that immunization with Ply generates anti-pneumolysin antibodies that are protective (Sanders et al., 2010; Lu et al., 2014; Mann et al., 2014; Hermand et al., 2017; Petukhova et al., 2020; Thanawastien et al., 2021). The direct use of anti-pneumolysin mAbs was protective when administered intravenously in a lethal intranasal model of infection in mice (García-Suárez et al., 2004). However, a recent study showed that using a DNA vaccine vector, low levels of anti-pneumolysin antibodies were generated that were not protective in a septic challenge model in mice (Ferreira et al., 2006a). In human clinical trials, anti-pneumolysin antibodies derived from vaccination are able to neutralize pneumolysin (Kamtchoua et al., 2013) but no differences were seen in colonization, bacterial load, or clearance due to the vaccine compared to controls (Odutola et al., 2017; Hammitt et al., 2019). While antibodies targeting Ply have been shown to function through the neutralization of its cytolytic activity, antibody inhibition of other Ply functions such as adherence, complement blocking, and biofilm formation have not been demonstrated.

Choline Binding Proteins

The virulence factor CbpG is an adhesin with putative serine protease activity in both colonization and sepsis (Galán-Bartual

et al., 2015). The protease portion of CbpG is able to cleave casein and fibronectin, and enzymatic activity is able to remain intact regardless of being surface bound or secreted (Mann et al., 2006). Another closely related antigen, CbpM, was demonstrated to bind to fibronectin facilitating *S. pneumoniae* attachment to epithelial cells (Afshar et al., 2016). Vaccination with recombinant CbpG induces antibodies that are able to confer protection against colonization to a limited degree and provide robust protection against systemic infection (Mann et al., 2006). In another study, mice immunized with CpbG or CbpM and challenged *via* intraperitoneal injection had a significant increase in protection and clearance of bacteria as early as 48 hours post infection (Kazemian et al., 2018). Serum containing anti-CbpG and anti-CbpM antibodies from the aforementioned study were also found to increase neutrophil mediated opsonophagocytosis (Kazemian et al., 2018). Finally, a passive transfer model using anti-CbpG and anti-CbpM antibodies in mice demonstrated a protective effect against *S. pneumoniae* challenge although not as robust as that seen in immunization studies with the antigens (Kazemian et al., 2018). Currently, antibodies targeting CbpG and CbpM have been shown to increase neutrophil mediated opsonphagocytosis, but other functions of these antibodies need to be further elucidated.

PcpA is another member of the choline binding protein family that is under the control of the manganese-dependent regulator *psaR*, and high concentrations of Mn suppress expression (Johnston et al., 2006). Immunization with PcpA elicits antibody responses that provide protection against lung and systemic infections (Glover et al., 2008; Verhoeven et al., 2014; Xu et al., 2017), but do not protect against colonization in the nasopharynx (Sánchez-Beato et al., 1998). In phase 1 human trials PcpA was used in a trivalent recombinant vaccine containing PhtD, PlyD1 and PcpA, the vaccine demonstrated immunogenicity with an increase in antibody concentration, however protection was not assessed (Brooks et al., 2015). Passive transfer of human anti-PcpA polyclonal antibodies in a murine challenge model were able to mediate protection (Ochs et al., 2016). The adherence effect of PcpA on the surface of bacteria was successfully blocked by utilizing Fab fragments targeting PcpA, which blocked adherence of *S. pneumoniae* to human epithelial cells *in vitro* (Khan et al., 2012). Macrophages but not neutrophils were required for anti-PcpA antibody protective efficacy in a passive transfer model (Visan et al., 2018). Additionally, anti-PcpA antibodies are able to enhance compliment C3b deposition (Visan et al., 2018), increase phagocytosis and block adherence of the bacteria.

CbpA/PspC is involved in the adhesion and colonization of the nasopharynx and in the binding of pIgR (Kerr et al., 2006; Orihuela et al., 2009). PspC is important in the recruitment and binding of complement factor H (Dave et al., 2001; Hammerschmidt et al., 2007; Ricci et al., 2011), evading complement by binding of C4BP (Dieudonné-Vatran et al., 2009; Haleem et al., 2019), and prevention of terminal complement complex mediated lysis by binding vitronectin (Voss et al., 2013; Kohler et al., 2015). Due to its role in virulence on multiple fronts, it is a key immunogenic target, however, while it is present in all clinically relevant serotypes, it is quite variable making it a difficult target for vaccination efforts (Brooks-Walter et al., 1999; Iannelli et al., 2002; Georgieva et al., 2018). This high degree of variability has led to mixed results when PspC has been used for immunization. Studies have demonstrated that immunization with PspC alone (Hernani et al., 2011; Ricci et al., 2011) or a multivalent approach with other pneumococcal antigens PspA and/or Ply are protective (Cao et al., 2007; Mann et al., 2014; Chen et al., 2015). However, other studies have shown that immunization with PspC is not protective (Lu et al., 2008; Ferreira et al., 2009; Ricci et al., 2011). These different results are likely due to the high variability of PspC between different serotypes. Passive immunization with anti-PspC antibodies has demonstrated protection (Mann et al., 2014), however, once again mixed results have been seen where passive immunization with anti-PspC antibodies alone were not protective but showed a protective and synergistic effect once administered with anti-PspA and anti-ClbP antibodies (Cao et al., 2007). PspC antibodies can increase complement deposition (Ricci et al., 2011), interfere with fH binding (Ricci et al., 2011; Glennie et al., 2016) and promote opsonophagocytic killing of *S. pneumoniae* (Ricci et al., 2011; Georgieva et al., 2018) *in vitro*.

Other Antigens

Phosphorylcholine

S. pneumoniae contains phosphorylcholine (PC) also known as ChoP, a structural component that is linked to bacterial adherence *via* the platelet activating factor receptor (PAF-R) (Cundell et al., 1995; Iuchi et al., 2019). Several key virulence proteins such as the CBPs and PspA are attached to the cell wall *via* PC (Rosenow et al., 1997). PC-dependent binding to the epithelial receptor asialo-GM1 has also been demonstrated (Sundberg-Kövamees et al., 1996). PC expression is essential to the bacteria as mutant bacteria that do not express PC are unable to colonize the upper respiratory tract in mice and are less virulent in murine sepsis models (Kharat and Tomasz, 2006). The C-reactive protein (CRP) recognizes PC and initiates the classical complement pathway increasing phagocytosis of *S. pneumoniae* (Mold et al., 1982). Due to PCs important role in adherence and association with key proteins, it offers a promising therapeutic target. Early on in the field it was demonstrated that anti-PC antibodies in normal mouse serum provide protection against intravenous pneumococcal challenge (Briles et al., 1981). Anti-PC mAbs were also found to be protective against several different serotypes in a murine infection model (Briles et al., 1992). One study demonstrated that pretreating *S. pneumoniae* with an anti-PC mAb reduced the adherence of *S. pneumoniae* with high levels of PC but not low levels of PC in both *in vitro* and *in vivo* models (Iuchi et al., 2019). Several studies have concluded that immunization with PC elicits anti-PC antibodies that are able to enhance clearance of *S. pneumoniae* and provide protection against pneumococcal infection (Wallick et al., 1983; Fischer et al., 1995; Trolle et al., 2000; Tanaka et al., 2007).

CONCLUSION

This review has discussed the important role of anti-pneumococcal antibodies in protection against pneumococcal infections. While currently approved vaccines only target the pneumococcal capsule of the bacteria *via* immunization, more recent studies have demonstrated the potential of targeting conserved antigens that help bacteria evade the immune system. The antimicrobial effects of anti-pneumococcal antibodies are wide ranging and impressive, and include an increase in complement deposition, enhanced opsonophagocytic activity, amplified NET and lactoferrin mediated killing, interference with attachment and penetration of host cells, neutralization of cytotoxic proteins, and modulation of the inflammatory response. A greater understanding of how anti-pneumococcal antibodies function is crucial. Studies to date have demonstrated that optimal antibody responses are unlikely to target one antigen but multiple antigens with different functions essential to *S. pneumoniae* fitness and/or survival. This synergistic approach may be our most successful path against an ever-evolving pathogen. The emergence of non-vaccine serotype and associated antibiotic resistance of pneumococcal isolates illustrates the need for vaccines that are capable of eliciting antibodies with greater serotype coverage and/or mAb treatments targeting conserved surface exposed antigens.

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Conflict of Interest: AG and JM have applied for a provisional patent application covering human monoclonal antibody sequences for prevention and treatment of pneumococcal infection.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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