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Outsmarting Pathogens with Antibody Engineering

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Abstract

There is growing interest in identifying antibodies that protect against infectious diseases, especially for high-risk individuals and pathogens for which no vaccine is yet available. However, pathogens that manifest as opportunistic or latent infections express complex arrays of virulence-associated proteins and are adept at avoiding immune responses. Some pathogens have developed strategies to selectively destroy antibodies, whereas others create decoy epitopes that trick the host immune system into generating antibodies that are at best nonprotective and at worst enhance pathogenesis. Antibody engineering strategies can thwart these efforts by accessing conserved neutralizing epitopes, generating Fc domains that resist capture or degradation and even accessing pathogens hidden inside cells. Design of pathogen-resistant antibodies can enhance protection and guide development of vaccine immunogens against these complex pathogens. Here, we discuss general strategies for design of antibodies resistant to specific pathogen defense mechanisms.

1. INTRODUCTION

The immune system provides critical defenses against invading pathogens that are increasingly important in the face of rising resistance against small-molecule drugs and a shrinking pipeline of new antimicrobials. Protective antibodies are key components of any immune response because they can neutralize secreted toxins, block interactions with host cells, and recruit immune system defenses. These antibodies can be elicited by natural infection or vaccination or be administered passively as purified proteins. Indeed, monoclonal antibodies are now available to treat five infectious diseases: respiratory syncytial virus (RSV), anthrax, recurrent *Clostridium difficile*, SARS-CoV-2, and Ebola virus (1).

The earliest efforts used the entire pathogen with its complex set of antigens in attenuated or inactivated vaccines while subsequent efforts aimed to identify a pathogen's Achilles' heel: a single target that cripples the pathogen when bound by an antibody. This latter approach supported design of successful vaccines against tetanus, diphtheria, and other diseases and antibodies against RSV, anthrax, and *C. difficile*. However, it has proven challenging to develop effective vaccines and antibody therapeutics against more complex pathogens for which natural immunity is often insufficient to prevent reinfection. These pathogens encode diverse proteins whose expression is orchestrated by complex regulatory pathways, with few antigens expressed across different disease states. Expression of functionally redundant virulence factors and strain variation can further complicate target selection. In addition, many pathogens have evolved diverse immune evasion strategies and, in some cases, can avoid elimination after phagocytosis. Even pathogens with relatively few genes, such as the influenza and SARS-CoV-2 viruses, have managed to rapidly evade immune responses against the few proteins targeted, emphasizing the need for more sophisticated antibodies.

This review focuses on general strategies bacterial and viral pathogens use to evade capture by antibodies and protein engineering efforts to overcome them. This includes targeting pathogens that shield vulnerable epitopes by identifying antibodies binding functionally or otherwise conserved epitopes that are less susceptible to antigenic drift. Engineering can render antibodies resistant to pathogen efforts to degrade, capture, or block antibody functions. Finally, antibodies can disrupt pathogen schemes to evade host immune responses by suppressing complement activation or sheltering inside cells. These approaches are expected to contribute to development of successful therapeutics for otherwise challenging pathogens.

2. TARGETING PATHOGENS THAT SHIELD VULNERABLE EPITOPES

A key requirement for antibody targeting is facile recognition of pathogen-associated molecules. The best targets are expressed by most if not all strains in different tissues and during multiple stages of infection, are readily accessible, and either perform functions critical for disease progression or recruit opsonins that mediate pathogen destruction. Unsurprisingly, this provides selective pressures for pathogens to conceal these vulnerable epitopes. Structure-function studies of viral glycoproteins in complex with neutralizing and non-neutralizing antibodies have revealed common mechanisms of antibody escape, including antigenic drift, epitope shielding, and immune redirection to dominant but nonprotective epitopes. These insights, in conjunction with new epitope-specific and target-agnostic antibody discovery tools (2, 3), support design of more resilient antibody therapeutics.

2.1. Antibodies Binding Conserved Epitopes that Resist Antigenic Drift

Considerable effort has been invested in identifying antibodies that target conserved epitopes on enveloped viruses, including RSV, influenza, HIV, and coronaviruses, to develop broadly reactive antibodies and vaccines that protect against many strains. These viruses employ a fusogenic glycoprotein to invade host cells that first attaches to a host cell receptor and then undergoes dramatic conformational changes to bring the viral and host membranes together and mediate membrane fusion. Efforts to identify neutralizing antibodies have focused on the receptor-binding domains to block this initial interaction. Indeed, these regions are highly immunogenic, and antibodies binding key epitopes can be potently neutralizing. Seminal work with the RSV F fusion protein demonstrated that the prefusion conformation contains many more neutralizing epitopes than the postfusion conformation and that these can be lost during conformational change (4). Antibody D25 binds the prefusion site Ø to block receptor binding and neutralize RSV ∼50-fold more potently than the antibody in current clinical use that recognizes a postfusion epitope (Palivizumab) (5, 6). Because of these promising results, D25 is progressing through clinical trials as Nirsevimab with an extended half-life Fc to allow for single-dose administration (7).

Identifying such potent antibodies has been challenging for other viruses, due in part to high sequence variation in receptor-binding domains, which often limits antibody neutralization to a few strains. In the case of SARS-CoV-2, multiple antibodies blocking interactions between the fusogenic spike protein and ACE2 receptor (**Figure 1**) received emergency use authorization only to be rapidly rendered ineffective due to mutations in newer variants (8, 9). Current efforts focus on identifying neutralizing antibodies that engage conserved residues, often in cryptic epitopes, that are less susceptible to antigenic drift. These antibodies can be rare, but examples have been reported that neutralize across sarbecovirus clades or bind receptor binding motifs presented by multiple SARS-CoV-2 variants (10, 11). Nanobodies are particularly useful in this situation, because their small size and long CDR3 loops can access epitopes that are not available to IgG antibodies and are therefore less susceptible to antigenic drift resulting from human immune system pressures. One effort with immunized camels identified hundreds of CDR3 nanobody families targeting five new neutralizing epitopes on spike (12, 13). Among these were ultrapotent nanobodies that bind with picomolar affinities and neutralize multiple SARS-CoV-1 and -2 strains in animal models, while their high stabilities support nasal administration to achieve therapeutic concentrations at the infection site. To elicit antibodies binding the conserved neutralizing class 4 and 1/4 epitopes, immunization with nanoparticles decorated with mosaics of receptor binding domains from eight strains appears promising (14, 15). The intense efforts in this area highlight the challenges and promise of finding cross-reactive antibodies that neutralize the variable receptor binding domain.

An alternative approach to identifying antibodies binding rare, conserved neutralizing epitopes is to identify those binding functionally constrained epitopes. On fusogenic glycoproteins, this includes the highly conserved region that undergoes conformational change, alternately called the stem, stalk, or S2 domain. In influenza, serum antibodies binding this region correlated with protection in an infection study (16), and these antibodies appear less susceptible to viral evasion (17). Whereas most neutralizing antibodies bind strain-specific epitopes in the receptor-binding head domain of hemagglutinin, those binding the fusogenic stalk can neutralize multiple virus groups by inhibiting the conformational rearrangements necessary for membrane fusion in low-pH endosomes and by recruiting Fc effector functions (18) (**Figure 1**). Guthmiller et al. (19) identified a highly conserved hemagglutinin anchor epitope that is common in the human B memory repertoire. Antibodies binding this epitope stabilize hemagglutinin near the viral membrane to neutralize a broad range of H1 strains as well as potential pandemic H2 and H5 strains and the common A388V stalk escape variant. Similarly, both the 3A3 antibody (20) and 7A3 nanobody (21) target the highly conserved betacoronavirus hinge to bind a wide range of strains, and 7A3 has been shown to protect human ACE2-expressing mice from delta SARS-CoV-2

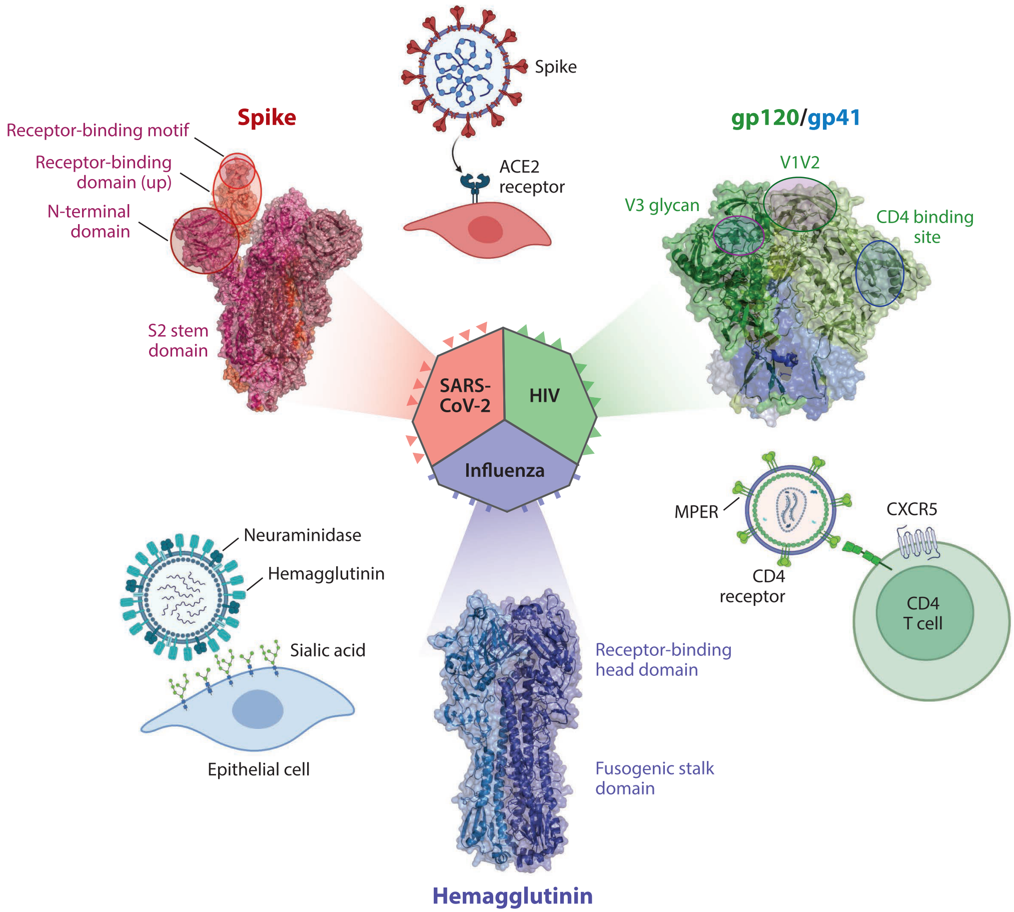

Figure 1

Key domains and antibody-targeted vulnerable sites in viral fusogen proteins from SARS-CoV-2, HIV-1, and influenza. For each virus, the overall interactions with the respective cellular receptor are shown along with the structure of each corresponding fusogen, indicating key domains and epitopes. Structures for influenza hemagglutinin H3 are from PDB 4FNK, for the HIV-1 envelope from PDB 5CJX, and for the SARS-CoV-2 spike from PDB 7SXT. Abbreviation: MPER, membrane-proximal external region. Adapted from images created with BioRender.

infection. Antibodies binding conserved stem epitopes can recognize a broad range of strains, but their contributions to protection are not yet fully understood.

These successes have stimulated interest in identifying highly conserved epitopes on other target proteins, including those expressed by complex bacterial pathogens. Protective antibodies binding the bacterial surface can directly mediate phagocytosis via Fc receptors or activate complement, leading to C3b opsonization followed by phagocytosis or direct lysis. A key constraint is that the antigen surface density must be sufficiently high to support the antibody and receptor clustering necessary to trigger these events; in the case of *Neisseria meningitidis*, >757 factor H proteins per cell appear to be required (22). The target protein should also be essential, because bactericidal antibodies can drive antigen loss, as was observed for the protein pertactin, which is included in many *Bordetella pertussis* vaccines (23). To this end, antibodies binding the conserved and protective *Streptococcus pneumonia* antigen pneumococcal histidine triad protein PhtD were discovered by screening human donor cells (24). These bind antigen on encapsulated bacteria from several serotypes and protected mice against primary and secondary challenges (25), offering a potential alternative to targeting the >100 distinct capsule serotypes individually. Similarly, conserved regions of the Lyme disease pathogen *Borrelia burgdorferi* outer surface protein VlsE (variable major protein-like sequence, expressed) have been identified, although their therapeutic potential remains to be determined (26). These reports are exciting because they suggest that antibodies binding bacterial surface proteins can be used to complement those binding bacterial carbohydrates.

2.2. Antibodies Accessing Concealed Epitopes

Solvent-exposed epitopes are accessible to antibodies, which exerts pressure for antigenic drift to evade antibody binding. Accordingly, pathogens can shade conserved and functionally important epitopes. One strategy is presenting highly immunogenic but nonprotective decoy epitopes to limit antibody recognition of adjacent vulnerable epitopes. For example, antibody IgG22 binds an epitope conserved on MERS-CoV and SARS-CoV-1 and -2 stem domains and prophylactically protected mice from MERS-CoV and SARS-CoV-2 lethal challenges (27). Antibodies binding this highly conserved epitope are rare because it is adjacent to an immunodominant and hypervariable loop. Deleting this loop from the immunogen elicited high levels of IgG22-like antibodies that protected mice against different β-human coronaviruses. Similarly, the human cytomegalovirus gB fusogen contains a highly conserved site 1, spanning residues 69–78 in antigenic domain 2, that elicits potently protective antibodies. This site is effectively masked by antibodies binding the adjacent site 2, spanning residues 50–54, and the immunodominant antigenic domain 1, both of which elicit primarily non-neutralizing antibodies (28). Extrapolating from the IgG22 report, future immunogen design efforts may be able to focus immune responses on gB site 1 and subdominant but protective epitopes in other antigens.

A second strategy is the use of glycosylation sites to shield vulnerable epitopes. The HIV-1 envelope is decorated with >25 *N*-linked glycosylation sites that shield broadly neutralizing epitopes, including the V1V2 site, comprising the first and second variable regions on gp120 (**Figure 1**). V1V2-binding antibodies correlated with protection in the phase III RV144 Thai vaccine trial by mediating Fc effector functions (antibody-dependent cellular phagocytosis and complement activation), rather than broad neutralization (29). This region elicits cross-reactive antibodies in many donors but requires extensive somatic hypermutation and long CDRH3 loops to access the epitope (30). The CAP256-VR26 antibody lineage binds the V1V2 region and elicits potent Fc-mediated protection (31) while tolerating the loss of spike glycans N160 and N156, unlike the prior V1V2-binding antibody PG9 (30). Immunization with cocktails of V1V2-scaffold immunogens that varied in glycosylation and multimerization states elicited modestly neutralizing but functionally active Fc responses in macaques (32). Unlike other broadly HIV-neutralizing antibodies, which require years of maturation and extensive somatic hypermutation, CAP256-VRC26.08 contained far fewer mutations, which could simplify strategies to elicit similar antibodies by vaccination.

A third strategy is structural blockade of vulnerable epitopes. For example, receptor-binding epitopes may be transiently exposed only when needed to engage a receptor. This was reported for the SARS-CoV-1/-2 cross-reactive antibody CR3022, which binds a cryptic epitope that is concealed when the receptor-binding domain is in the down state but exposed when this domain converts to the up state to engage the ACE2 receptor (33). Similarly, the broadly neutralizing anti-HIV antibodies 3BNC117 and 10-1074 bind only when CD4 engages the HIV-1 envelope to transiently expose the vulnerable CD4 binding site (34, 35). Coadministration of soluble CD4 or CD4 mimics exposes the epitope for antibody binding; accordingly, nonhuman primates vaccinated with HIV-1 gp120 variants showed improved antibody-dependent cellular cytotoxicity (ADCC) against HIV-1-infected T cells only in the presence of a small-molecule CD4 mimic

(36). The membrane-proximal external region of the gp41 envelope protein elicits the broadest neutralizing monoclonal antibodies (e.g., 4E10, LN01, VRC42) (37), but these are rare because this epitope is only transiently exposed during the pre- to post-fusion transition (38, 39). These reports suggest that coadministration of two antibodies may be required to access some epitopes: one to stabilize the target protein in an open state (e.g., a receptor mimic) and a second to bind the sensitive epitope.

2.3. Antibodies Targeting Variable Antigens

The above examples highlight the challenges of targeting vulnerable epitopes on viruses that require just a few proteins to infect cells. Applying these strategies to bacterial pathogens expressing many more surface antigens presents additional challenges because, in many cases, which key molecule to target is not readily apparent. For example, influenza has only 8 gene segments, whereas the opportunistic pathogen *Pseudomonas aeruginosa* has 321 core essential genes shared by all strains, with a pan-genome of 5,316 genes distributed across different isolates (40). Further complicating matters, many of the surface proteins are shielded from immune access by a capsule or expressed at low levels incompatible with triggering Fc functions.

Given their abundance, accessibility, and immunogenicity, bacterial carbohydrates (lipopolysaccharides and capsular polysaccharides) have been primary targets. Indeed, vaccination with glycoconjugates of the *N. meningitidis* serogroup A capsule nearly eliminated clinical cases of this serogroup. Unfortunately, these molecules are also highly variable: Although *P. aeruginosa* lipopolysaccharide vaccines elicit protective immunity in animal models (41), there are >20 O-antigen serotypes, many with multiple subtypes (42), which would necessitate development of serotype-specific therapeutics. To explore this option, antibody KBPA101 was developed to target the O-11 serotype common in clinical isolates. Despite a modest 600 nM functional affinity, KBPA101 potently promotes opsonophagocytic killing due to its formulation as a pentavalent IgM isotype and was evaluated in clinical trials (43). As a more general approach, antibody F598 was developed to target the surface polysaccharide poly-*N*-acetyl-D-glucosamine expressed by a range of pathogenic bacteria. This antibody induces complement-dependent opsonic and bactericidal effects against multiple organisms, including *Listeria monocytogenes*, *Streptococcus pyogenes*, and *Burkholderia* species (44). Structural analyses of antibody–polysaccharide complexes have revealed the importance of acetyl groups in recognition that foreshadows development of additional potent yet specific anti-polysaccharide antibodies (45–48).

Given the diversity of bacterial carbohydrates and the challenges in generating antibodies against these flexible molecules, conserved surface proteins may be better suited as antibody targets. An antibody binding the essential *Escherichia coli* outer membrane export protein BamA was identified that binds a single extracellular loop to disrupt BamA function. Antibody binding induced bacterial stress responses, disrupted outer membrane integrity, and killed bacteria at subnanomolar concentrations (49). However, because outer membrane proteins are buried beneath the surface carbohydrates, this antibody was effective only against strains with a minimal lipopolysaccharide structure. The same group identified antibodies against a different *E. coli* outer membrane protein, LptD, only to find that no antibody-accessible loop impacts protein function (50). These reports highlight the challenges of selecting appropriate bacterial targets a priori, although successful antigens, such as the *N. meningitidis* factor H (22) and *S. pneumonia* PhtD proteins (24) discussed in Section 2.1, have been identified. To instead isolate antibodies using a target-agnostic approach, DiGiandomenico et al. (51) identified antibodies from infected and healthy humans that bound intact *P. aeruginosa* cells and screened them for opsonophagocytic killing. A panel of single-gene knockout *P. aeruginosa* isolates revealed that all selected antibodies bound the surface-exposed polysaccharide Psl, which is conserved across multiple strains and expressed in multiple disease states. The lead anti-Psl antibody Cam-003 binds 85% of 173 *P. aeruginosa* clinical isolates and, despite an affinity of just 144 nM, provided strong protection in a mouse acute lethal pneumonia model. It was evaluated in clinical trials as part of a bispecific antibody, discussed in Section 4.4 below (52).

Pathogen adaptability necessitates creativity in antibody targeting approaches. This can include antibody discovery strategies using antigenic baits from multiple strains to identify cross-reactive antibodies against a known target, an approach that has identified a range of antibodies binding different viral epitopes (20). To expand these approaches to more complex bacterial pathogens, target-agnostic approaches followed by extensive screening for the desired function have shown promise. Although not discussed here, prediction of immune escape pathways via machine learning, computation (53), and in vivo (54) and in vitro pathogen passaging (55–57) is also expected to support development of resilient antibody therapeutics. These reports highlight the promise of identifying therapeutic antibodies for challenging targets such as *Mycobacterium tuberculosis* (58) and malaria (59).

3. TARGETING PATHOGENS THAT UNDERMINE ANTIBODY FC FUNCTIONS

Although many efforts have focused on developing neutralizing or blocking antibodies, the contributions of Fc effector functions to protection are increasingly recognized. These can be mediated by human IgG1 and IgG3 isotypes, which bind host Fc receptors to mediate ADCC by natural killer (NK) cells, antibody-dependent cellular phagocytosis by neutrophils and macrophages, and complement-dependent cytotoxicity. These responses rely on Fc interactions with the complement component C1q and the classical activating Fc receptors CD16A and CD32A, which are highly dependent on antigen density and epitope accessibility on the target cell surface (reviewed in 60). Although Fc interactions can lead to antibody-dependent enhanced disease, most notably in dengue infection (61, 62), they typically represent a powerful arm of the adaptive immune response that can complement neutralizing antibodies. Unfortunately, this has also provided selective pressures for pathogens to evade these functions by degrading or sequestering antibodies. Antibodies engineered to resist cleavage, block protease activities, or resist antibody sequestration present opportunities to defuse these immune evasion strategies.

3.1. Antibodies that Resist Pathogenic Protease Activities

Enzymes such as papain (from the tropical fruit papaya, *Carica papaya*) and pepsin (an enzyme in the mammalian digestive tract) are commonly used to generate Fab and $F(ab')_2$ antibody fragments by cleaving sequences in the upper and lower hinge regions, respectively (63). Several human matrix metalloproteinases (MMPs) associated with tumor invasion and inflammation, such as MMP-3 and MMP-7, can also cleave immunoglobulins (64), suggesting these features are common across phylogenies. As part of an immune evasion repertoire, bacteria can secrete enzymes with homologous functions. For example, the streptococcal pyrogenic exotoxin B (SpeB), immunoglobulin G–degrading enzyme (IdeS), and endoglycosidase (EndoS) are secreted by *S. pyogenes* and cleave antibodies. Whereas SpeB has broad specificity for immunoglobulins (65), IdeS is specific for the IgG hinge (66), and EndoS removes the sugar attached to residue N297 required for Fc receptor binding. Similarly, *Staphylococcus aureus* produces glutamyl endopeptidase V8 (GluV8), which cleaves immunoglobulins in the upper hinge (67), whereas *P. aeruginosa* secretes abundant quantities of elastase B (LasB), which also cleaves immunoglobulins, possibly in the hinge region (68).

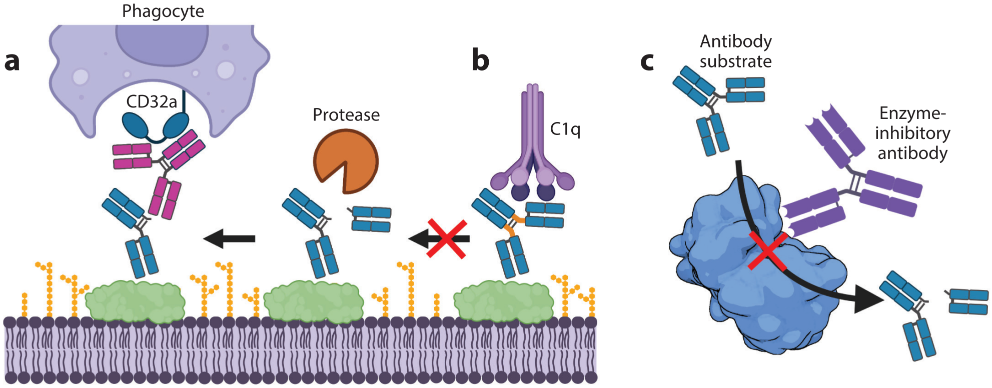

Figure 2

Antibodies that resist pathogenic protease activities to sustain recognition by host immune proteins, such as the C1q component of complement and CD32a Fc receptor found on phagocytes. (*a*) Antibodies recognizing hinge epitopes exposed after cleavage restore Fc functions to cleaved antibodies. (*b*) Antibodies with engineered hinge regions no longer serve as suitable substrates for pathogenic proteases. (*c*) Antibodies that block the activity of pathogenic proteases by directly blocking access to the active site (*shown*) or noncompetitive allosteric mechanisms (*not shown*) can protect antibody functions. Shown is the structure of LasB (PDB 3DBK). Adapted from images created with BioRender.

Although useful for biotechnology applications, antibody cleavage can be detrimental to a protective immune response. Indeed, adding protease inhibitors decreased antibody cleavage while increasing C3b complement deposition and neutrophil phagocytosis of *S. aureus* bacteria (69). Most IgG-specific proteases employ a two-step process: One heavy chain hinge is cleaved, generating an intermediate, singly cut product (64, 67), followed by slower cleavage of the second hinge to generate Fc and Fab or $F(ab')_2$ fragments. Singly cleaved IgG retains antigen-binding activity but can no longer promote effector functions (70). Auto-antibodies binding cleaved IgG1 are observed in many individuals, showing that this intermediate product is immunogenic and physiologically relevant (71, 72). This accumulation of cleaved antibodies on the bacterial surface inhibits access of intact antibodies to antigens, thereby reducing recruitment of Fc effector functions. Hence, even incomplete antibody cleavage can effectively evade immune responses (73).

To restore Fc effector functions lost by antigen-bound and cleaved antibodies, the use of antibodies that recognize the new epitopes created by cleavage has been explored (**Figure 2*a***). These intact antibodies can then reconstitute the severed link between the target cell and Fc receptors on phagocytic cells. Jordan et al. (71) evaluated this approach by using antibodies binding the *S. aureus* surface that are rapidly cleaved by GluV8 in a rabbit model of infection. The authors showed that immunization with peptides similar to antibody hinge sequences elicited a strong antibody response that specifically recognized GluV8-cleaved antibodies. Rabbits immunized with hinge-like peptides had reduced *S. aureus* colonization levels, and sera from these animals recovered complement-dependent cytotoxicity in an in vitro assay, compared with an unimmunized group.

Engineering cleavage-resistant antibodies is another strategy to counter proteolytic degradation. Although IgG1 is the most abundant isotype in the human body and the most common isotype for therapeutic antibodies, IgG2 is generally more resistant to proteolytic cleavage (74). However, IgG2 binds more weakly to CD16a and C1q than IgG1 and accordingly mediates reduced Fc-dependent killing and complement deposition (75). Efforts to combine the resilience of IgG2 with the function of IgG1 resulted in chimeras in which the IgG2 lower hinge and C_H2 sequences replaced those of IgG1. Unfortunately, antibodies also bind classical Fc receptors and C1q via interactions on the conserved lower hinge–C_H2 interface, particularly residues

E233/L234/L235/G236/G237/P238 (EU numbering) (76), an accessible region that serves as a substrate for many proteases. Accordingly, mutations that increased antibody resistance to cleavage also negatively impacted Fc receptor binding and recruitment of immune effector functions.

To address the need for antibody protease resistance while preserving effector functions an IgG1/IgG2 hybrid antibody was generated. Kinder et al. (77) first introduced the IgG2 lower hinge into an IgG1 by introducing the substitutions E233P/L234V/L235A and deleting Gly236. As expected, this construct lost its ability to promote complement killing and phagocytosis in in vitro assays. Mutations previously established to selectively activate either complement killing or opsonophagocytosis were then introduced into this chimera. Variant 2h-DE (S239D/I332E) restored Fc receptor binding, while variant 2h-AA (K326A/E333A) restored complement killing. Combining these mutations to produce 2h-DAA (239D/K326A/E333A) and 2h-AEA (K326A/I332E/E333A) restored both complement killing and opsonophagocytic activities while retaining resistance to cleavage by multiple proteases (*S. pyogenes* IdeS, *S. aureus* GluV8, MMP-3, and MMP-12) (**Figure 2*b***). Incorporation of protease-resistant Fc domains may support development of antibody therapeutics to treat bacterial infections whose armory includes antibody-degrading proteases.

Directly targeting proteases with neutralizing antibodies is an alternative approach to block proteolytic activity and has the advantage of blocking cleavage of all substrates. This approach was explored initially for tumor-associated MMPs, resulting in identification of antibodies that act as competitive inhibitors with K_i values ∼5 nM (78). It was extended to the *P. aeruginosa* pseudolysin (LasB) protease, which supports early infection by cleaving elastin, collagen, IgA, IgG, and complement proteins (for a review, see 79). Small-molecule inhibitors generally suffer from poor specificity or activity, although recent molecules were reported to have K_i values of ∼120–160 nM (80). As an alternative approach, Santajit et al. (81) identified antibodies blocking LasB activity from the lymphocytes of healthy volunteers. Two clones were shown to bind LasB with modest EC_{50} values of ∼100 nM and demonstrated concentration-dependent enzyme inhibition (**Figure 2*c***). Given that this protease is conserved and abundantly expressed by *P. aeruginosa*, LasB neutralization may improve the efficacy of vaccine-elicited or therapeutic antibodies against this pathogen in the early stages of lung infection (82).

Antibody therapeutics that are resistant to proteolytic cleavage may improve their protective capacity and can be achieved by identifying Fc substitutions that resist degradation. An attractive feature of Fc engineering is that once a suitably engineered Fc is developed, it can be combined with Fab arms binding any antigen. Alternatively, an enzyme-inhibiting antibody could be developed for each protease of interest. If supported by studies with monoclonal antibodies, appropriately engineered protease immunogens could enhance protection conferred by naturally and vaccine-elicited polyclonal antibodies.

3.2. Antibodies that Block Bacterial Fc-Binding Proteins

Various bacterial pathogens express virulence factors that bind conserved antibody sequences. These captured antibodies can then shield the pathogen, blocking immune access to other surface antigens, antagonizing Fc effector functions, and even altering immune cell signaling. Although multiple organisms secrete antibody-binding proteins, including the *S. aureus* second immunoglobulin-binding protein (Sbi), *S. pyogenes* proteins G and M, and *Peptostreptococcus magnus* protein L (83), *S. aureus* protein A is the most studied. Protein A is expressed by all colonizing isolates and is composed of five homologous domains, each able to bind the Fc of all human IgG isotypes except IgG3 and all mouse IgG isotypes except IgG1 with high affinity (K_D values of 2.6–14 nM) (84, 85). When attached to the bacterial cell wall via a C-terminal anchor,

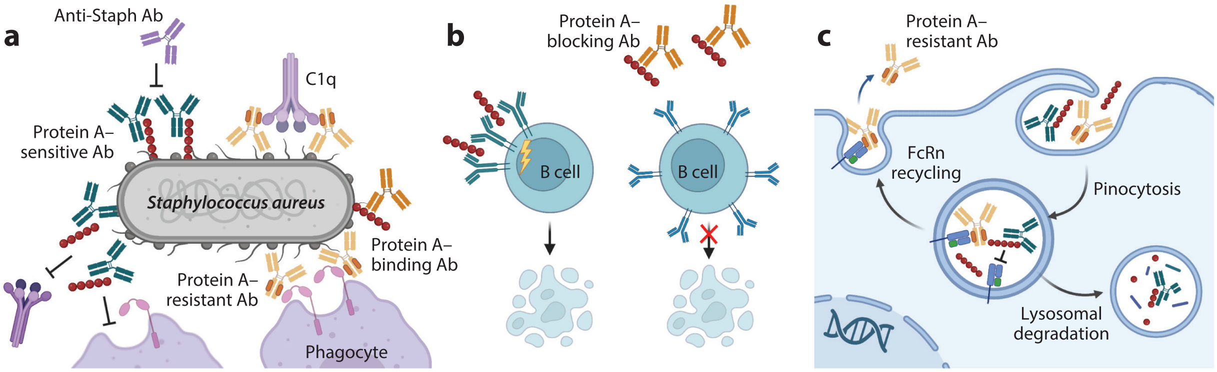

Figure 3

Antibodies that resist capture by Fc-binding protein A. *Staphylococcus aureus* protein A disrupts antibody responses in multiple ways but can be restored by Fc domains with reduced protein A affinity (most IgG3 allotypes or engineered IgG1 domains) or antibodies that bind protein A to block Fc capture. (*a*) Membrane-bound protein A can block antibody Fc binding to the low-affinity Fc receptors CD16b and CD32a, block the Fc hexamerization required for efficient recruitment of C1q to activate complement, and shield the bacterial surface from recognition by antibody Fab domains. Secreted protein A can (*b*) crosslink V_H3 domains to trigger B cell receptor activation and apoptotic collapse and (*c*) block antibody recycling by Fc/FcRn binding to reduce antibody half-life. Unmodified antibodies are shown in green, Fc engineered antibodies are shown in yellow, and antiprotein A antibodies are shown in orange. Abbreviations: Ab, antibody; FcRn, neonatal Fc receptor. Adapted from images created with BioRender.

protein A may block access of anti-*Staphylococcus* antibodies to surface antigens. It also prevents antibody-dependent complement activation by interfering with IgG hexamer formation (86) and opsonophagocytic killing by sterically blocking Fc interactions with the low-affinity Fc receptors (87) (**Figure 5**). Additionally, some protein A is released from the bacterial surface and can bind the framework of V_H3-type B-cell receptors or antibody Fc domains. Cross-linking of B-cell receptors activates B cells, leading to a clonal expansion and apoptotic collapse that limits anti-*Staphylococcus* responses (88) (**Figure 3*b***). Binding to the Fc domains of free antibodies can block interactions with the neonatal Fc receptor (FcRn) during cellular recycling to target the antibody for lysosomal degradation (89) (**Figure 3*c***).

Direct targeting of protein A with the antigen-binding paratopes of monoclonal antibodies has been explored to block its Fc-binding activities and support bacterial opsonophagocytosis. Antibody 514G3 binds a protein A epitope that is accessible on the bacterial surface in the presence of serum antibodies. When produced as a human IgG3, the only human isotype not captured by protein A due to an H435R substitution present in most allotypes (90), 514G3 mediated enhanced *S. aureus* killing versus an isotype control in an opsonophagocytic assay (91). In a mouse model, it demonstrated protection when administered prior to lethal challenge with methicillin-resistant *S. aureus*, whereas a lower antibody dose exhibited synergy with vancomycin antibiotic treatment. Antibody 514G3 was subsequently evaluated in a phase I/II study in patients hospitalized with *S. aureus* bacteremia (NCT02357966), although results have yet to be reported. Antibody 3F6 binds protein A and the related protein Sbi to block Fc capture and B cell cross-linking effects (**Figure 3*b***). Passive immunization with 3F6 as a mouse IgG2a protected neonatal mice against bloodstream infection and allowed for higher serum IgG titers against other *S. aureus* antigens (92, 93).

Design of antigens that elicit strong 512G3- and 3F6-like protein A responses has been pursued, with the goal of reducing protein A affinity for the Fc. An initial detoxified variant with lysine substitutions in residues 9 and 10 and alanine at residues 36 and 37 in each protein A domain named SpA_{KKAA} exhibited negligible Fc and 100-fold reduced V_H3 affinity. Unlike wild-type protein A, immunization with SpA_{KKAA} elicited high-titer antibodies that promoted opsonophagocytic killing and protected mice against bloodstream *S. aureus* infection. However, SpA_{KKAA} continued to cause animal distress, suggesting it retains some B cell superantigen activities. Accordingly, a second rational design effort combined the Q9E/Q10E changes with an S33E or T substitution to generate variants SpA_{QQE} and SpA_{QQT} that further reduce V_H3 cross-linking capacity (94). These variants exhibit negligible affinity for Fc and Fab while showing reduced mast cell degranulation and retaining the immunogenic and protective qualities of SpA_{KKAA}. Immunogens like SpA_{KKAA} better elicit antibodies disrupting protein A functions because the key epitope remains exposed even in the presence of Fc. This approach shows promise for design of immunogens for other Fc-binding proteins.

3.3. Antibodies Engineered to Resist Capture by Fc-Binding Proteins

To support development of monoclonal antibodies for therapeutic use, the antibody Fc domain can be engineered to reduce affinity for pathogenic Fc-binding proteins. Prior work demonstrated that antibodies with higher affinity for the activating CD16a and CD32a host receptors due to afucosylation or amino acid sequence changes have enhanced effector functions that are relevant for cancer (95–97). However, the potential impacts of these and related Fc changes on infectious disease responses are less well understood.

To determine whether antibodies that resist protein A capture mediate more potent *S. aureus* phagocytosis, an antibody binding the abundant surface glycopolymer wall teichoic acid was selected. Two residue changes that reduce protein A binding affinity (H435R and Y436F) (98) were introduced into the human IgG1 Fc domain. The resulting antibody mediated equally efficient phagocytosis of bacteria regardless of whether they express protein A (87). Subsequent rational Fc engineering identified four amino acid changes (H435R, T307Q, Q311V, A387V) that reduced protein A but not FcRn binding. When combined with Fab arms from the protein A–binding antibody 3F6, the resulting 3F6-hIgG1$^{R\text{-}QVV}$ antibody reduced kidney colonization and abscess formation in infected mice, with protection appearing to correlate with enhanced activation of C1q at the bacterial surface (99). Complementary approaches to enhance antibody activity against *S. aureus* involve using the IgG3 isotype, which has advantages in addition to protein A resistance (100), or HexaBodies, whose enhanced Fc–Fc interactions may limit protein A effects.

Analogous Fc-binding proteins are expressed by herpesviruses, including the gE/gI of alphaherpesviruses and the gp68 and RL11 family of the betaherpesvirus cytomegalovirus. These proteins bind all human IgG isotypes, with gE/gI and gp68 binding near the C_H2/C_H3 elbow to competitively inhibit FcRn binding (101, 102), whereas gp34 binds near the hinge to antagonize binding to classical Fc receptors such as CD16a. When these viral Fc receptors are expressed on an infected cell surface, they can capture the Fc domain of antibodies binding adjacent viral glycoproteins. This antagonism inhibits Fc binding to and signaling through host Fc receptors on an immune cell (103, 104) and can result in antibody internalization (103, 105) for lysosomal degradation or repackaging to cloak the virion with antibodies (106, 107). These proteins appear to limit antibody efficacy; for instance, the herpes simplex virus gE/gI complex inhibits antibody-mediated elimination of infected cells in vitro and in vivo (108). Similarly, antibodies binding cytomegalovirus proteins bound human Fc receptors and induced ADCC more potently when virus strains lacking the viral Fc receptors were used to infect fibroblasts (109).

This suggests that Fc domains could be engineered to enhance the potency of non-neutralizing anti-herpesvirus responses such as ADCC. An initial report modified the antibody Fc to include the S239D and I332E residues, which increase Fc-CD16A affinity by ~100-fold (95). When combined with Fab arms binding the cytomegalovirus glycoprotein gB, which is expressed on the infected cell surface, a ~3-fold increase in NK cell degranulation was observed for modified versus wild-type IgG1 when multiple antibodies binding the same antigen were pooled (110). Reasoning that Fc modifications to instead reduce viral Fc receptor binding while retaining affinity for host Fc receptors would provide a wider therapeutic window, we used directed evolution and yeast display to engineer the human IgG1 Fc domain for 70-fold reduced gp68 affinity and 45-fold reduced gp34 affinity but unaltered binding to FcRn and CD16A. When combined with Fab arms binding the major viral glycoprotein gB, modified Fc G2 eliminated antibody internalization while restoring CD16a signaling and ADCC activities when incubated with cytomegalovirus-infected fibroblasts and human NK cells (A.N. Qerqez, K. Hoffman, A.G. Lee, A.K. Mishra, G. Delidakis, A. Chowdhury, et al., manuscript submitted).

Preventing pathogen sequestration of antibodies can allow the host immune system to respond effectively to infection. In the *S. aureus* example, antibody 514G3 blocks protein A binding to any antibody Fc, whereas use of the IgG3 Fc prevents protein A disruption of therapeutic antibody activities. These reports provide a proof of concept for antibodies targeting protein A that may be extended to other Fc-binding proteins. Combined with advances in Fc engineering technologies (60), these reports highlight the opportunities to leverage knowledge of antibody-escape pathways to develop designer Fc domains that mediate potent effector functions against specific pathogens.

4. TARGETING PATHOGENS THAT UNDERMINE HOST IMMUNITY

Antibodies binding conserved and accessible targets often require host immune responses to prevent or resolve an infection. In these situations, opsonized pathogens can be destroyed by a variety of mechanisms, including complement lysis, phagocytosis, or NK-mediated killing of infected cells. For example, simply tagging a pathogen with an antibody to mediate phagocytosis via Fc receptors instead of the natural invasion pathway can deliver the pathogen to the lysosome for destruction (111). In response, bacterial and viral pathogens have evolved strategies to evade these protective immune functions, including proteins that disrupt the complement cascade or impair the ability of host immune cells to capture and destroy internalized pathogens. Antibodies blocking immune-evasive proteins can restore immune function, while engineered antibodies can target bacteria in secluded cellular niches.

4.1. Antibodies that Preserve Complement Activities

The classical complement pathway is activated by abundant surface antigens that allow for binding and hexamerization of IgG antibodies or, more effectively, binding by the naturally pentameric or hexameric IgM molecule (**Figure 4**). These antibodies can then engage the six globular heads of the C1q protein to activate a proteolytic cascade (112). The resulting C3b deposition on the pathogen surface mediates phagocytosis by engaging complement receptors on leukocytes, particularly complement receptor 3. If C3b densities are high, the lytic membrane attack complex can form to lyse gram-negative bacteria and virions. Because not all antigens are spatially arranged to support efficient formation of IgG hexamers, residue changes (E430G and E345K or -R) were identified that independently increase the Fc–Fc interactions supporting hexamer formation, leading to increased C1q binding and 5–7-fold enhanced complement activation (113). When combined with an antibody targeting a conserved *Neisseria gonorrhoeae* lipooligosaccharide epitope, these engineered HexaBody Fc domains mediated enhanced bacterial clearance from mice as compared to an unmodified Fc using mechanisms that required complement activation only (114).

Complement evasion strategies are common in bacterial pathogens, including the production of capsules to shield antibody access to antigens, surface antigens that recruit complement inhibitors, and proteases that cleave complement proteins (**Figure 4**). Antibodies targeting these

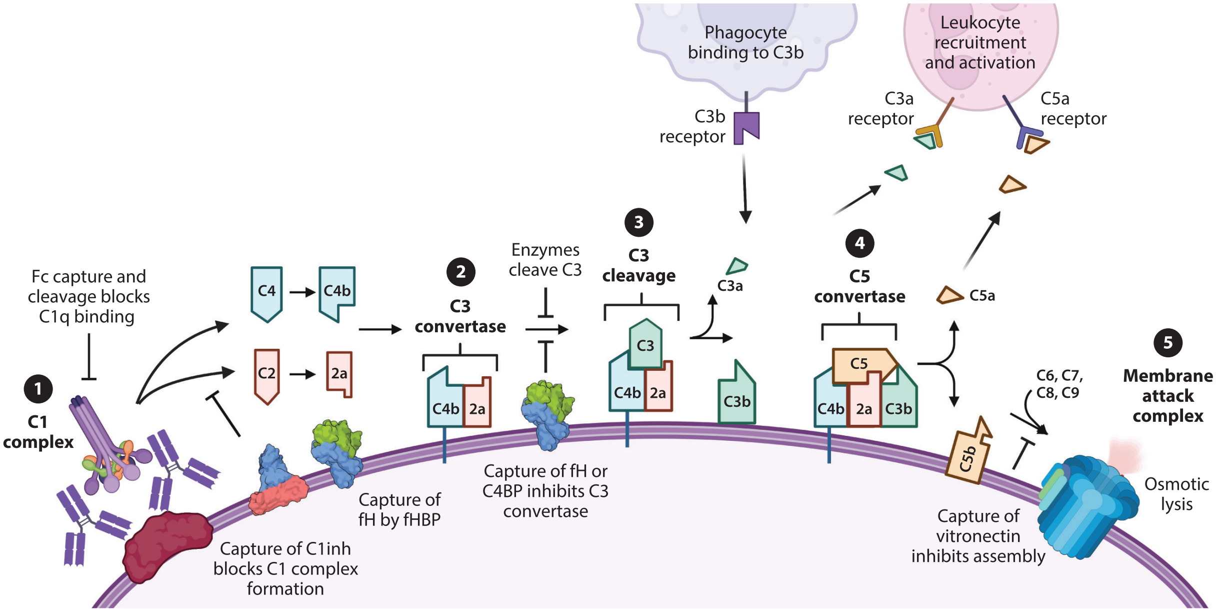

Figure 4

Microbial disruption of the classical complement cascade by recruiting inhibitors or degrading complement proteins. Key steps of the classical pathway of antibody activation are shown: ① The complement proteins C1q and then C1r and C1s bind hexamerized IgG or IgM on the pathogen surface to form the C1 complex. ② Complement components C2 and C4 are cleaved to produce membrane-bound C4b and C2a, which form the C3 convertase. ③ This cleaves C3 to release C3a and deposit C3b covalently on the membrane. ④ When C3b levels are high, it joins the C3 convertase to form the C5 convertase, which cleaves C5 to release C5a and deposit C5b on the membrane. ⑤ Components C6–C9 join C5b to form the membrane attack complex and osmotically lyse the target membrane. Released C3a and C5a are chemoattractants for leukocytes, while membrane-bound C3b can engage complement receptors such as CR3 to trigger phagocytosis. The lectin pathway follows a similar cascade but is initiated by the mannose-binding lectin complex, which recruits C1q; the alternate pathway results from spontaneous C3 cleavage and C3b deposition to enter at the C3 convertase step using an alternate C3b/Bb complex. Many of these steps can be inhibited by pathogen components, including proteins that bind or cleave Fc to inhibit C1q recruitment (e.g., protein A and staphylokinase), proteins that recruit host complement regulators (e.g., the *Neisseria* fHBP, which recruits fH, and *Bordetella pertussis* Vag8, which recruits C1 inhibitor), and enzymes that degrade complement components (e.g., staphylokinase depletes C3). Engineering efforts to overcome these strategies include the use of HexaBodies, which favor C1q binding; antibodies resistant to capture by Fc-binding proteins or cleavage by bacterial proteases; and antibodies that block recruitment of complement inhibitors such as factor H. Abbreviations: fH, factor H; fHBP, factor H binding protein. Adapted from images created with BioRender.

antigens could simultaneously block inhibitor binding and recruit C1q to the pathogen surface, thereby contributing to bacterial killing (115). This has been demonstrated most convincingly with antibodies binding *Neisseria* bacteria, an important cause of bacterial meningitis and sepsis for which the classical complement pathway dominates bactericidal killing (114). When three antibodies binding different epitopes on the *N. meningitidis* factor H binding protein (fHBP) were compared, only the antibody blocking recruitment of the factor H (fH) complement inhibitor showed human complement–mediated bacterial lysis (116). The high antibody/fH affinity was likely critical for the antibody to successfully inhibit the ∼1,000-fold weaker affinity fH/fHBP interaction. fHBP is included in two licensed meningococcal serogroup B vaccines and induces high titers of bactericidal antibodies (117). However, fHBP is speculated to form complexes with fH in serum that would limit development of protective antibodies that competitively inhibit the fH/fHBP interaction. To address this, an engineered fH immunogen was developed that includes two amino acid changes (R41S and H248L) that reduce fH affinity by >250-fold (118). After immunization of rhesus macaques, this modified fHBP elicited 3-fold higher serum IgG titers and 150-fold higher serum bactericidal titers than the native fHBP, which correlated with increased deposition of the complement component C4b on live bacteria.

These data suggest that targeting complement-evading antigens expressed by other organisms may support vaccine development efforts. For example, a goal for future *B. pertussis* vaccines is to better limit human colonization, yet the organism is quite resistant to complement-mediated lysis. Accordingly, its four proteins involved in complement inhibition (the C4b-binding protein Fha, the C1 esterase inhibitor binding Vag8, and BapC and the *Bordetella* resistant to killing A) are attracting interest as future vaccine antigens and may benefit from engineering, as fHBP did (119).

4.2. Antibodies that Protect Leukocyte Functions

Many bacteria produce leukotoxins, which impair immune cells' ability to capture and destroy pathogens. *S. aureus* produces a suite of pore-forming leukocidins with redundant and complementary functions, including α-hemolysin (Hla) and the five bicomponent cytotoxins HlgAB, HlgCB, LukSF, LukED, and LukGH (120). Antibodies binding just Hla have been evaluated in the clinic, with suvratoxumab appearing promising but not meeting end points in phase II clinical trials (121), whereas tosatoxumab (122) is currently in phase III clinical trials to evaluate prevention of ventilator-associated pneumonia caused by *S. aureus* (NCT03816956). To simultaneously neutralize multiple toxins and provide superior protection, a monoclonal antibody was engineered to bind a single epitope conserved among Hla and four additional leukocidins (123). A high-affinity anti-Hla antibody was initially isolated from a human yeast display library, followed by randomization and selection to select for antibodies binding a highly conserved microdomain involved in pore formation. This effort yielded a single antibody that binds Hla, HlgB, LukF, and LukD with K_D values <2.2 nM for each antigen and protected mice in lethal pneumonia and bacteremia models (124). This was combined with a second antibody specifically targeting the leukocidin LukGH (125). A cocktail of these two antibodies that neutralized six different *S. aureus* toxins met phase I end points (126) and is being evaluated in phase II clinical trials (NCT02940626) under the name ASN100.

Once opsonizing antibodies bind conserved epitopes on the pathogen surface, secretion of leukotoxins may limit bacterial killing before or after phagocytosis. In this situation, antibodies blocking leukotoxin activities are expected to synergize with opsonizing antibodies. This is the case for *Bordetella* species, including *B. pertussis*, which secrete the 177-kDa adenylate cyclase toxin. This protein binds leukocytes via the $\alpha_M\beta_2$ integrin receptor, after which the N-terminal catalytic domain is translocated to the cytosol, where it binds calmodulin and rapidly converts available ATP to cAMP (127). This activity dysregulates cell signaling and reduces opsonophagocytosis and bacterial clearance during the early stages of infection (128, 129). We showed that neutralizing antibodies competitively inhibit receptor binding (130, 131) and synergize with opsonizing anti-pertactin antibodies in a mouse pneumonia model (132). Tkaczyk et al. (133) illustrated the same concept, showing that an opsonizing antibody-binding ClfA synergized with an antibody-neutralizing Hla in lethal pneumonia and bacteriemia models of *S. aureus* infection.

4.3. Antibody–Antibiotic Conjugates Targeting Internalized Bacteria

Although intracellular bacteria are traditionally viewed as having obligate or facultative intracellular lifestyles, there is an increasing appreciation that phagocytosed bacteria can serve as a reservoir of viable organisms to seed infection (134). For example, *S. aureus* is readily phagocytosed by macrophages and neutrophils (134). Although most internalized bacteria are killed, a fraction resist killing and use the phagocytes to mediate dissemination to other sites. For *S. aureus* specifically,

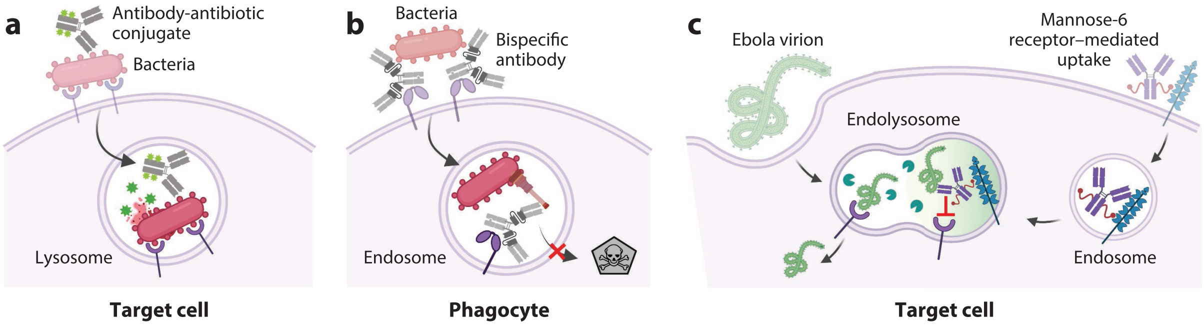

Figure 5

Antibodies that target intracellular pathogens. (*a*) Antibody–antibiotic conjugates bind bacterial surface antigens and are internalized with the bacteria by natural or phagocytic mechanisms. Once internalized, the antibiotic is released by resident enzymes to kill co-localized bacteria. (*b*) Bispecific antibody MEDI3902 uses one binding site to bind the Psl surface antigen on *Pseudomonas aeruginosa* and mediate phagocytosis. After translocation to the endosome, the second antibody-binding site blocks type III secretion to support endosome acidification and bacterial killing. (*c*) The mannose-6-phosphate receptor can mediate antibody transfer to an endolysosome that may already contain Ebola virions. Once co-localized, the antibody can block Ebola–receptor interactions to prevent viral escape into the cellular cytosol. Adapted from images created with BioRender.

intracellular survival helps the bacteria evade innate immune defenses and destroy neutrophils by programmed necrosis (135), with leukocidins mediating bacterial survival/escape through induction of programmed necrosis (136). Moreover, many viruses perform membrane fusion events required for entry into host cells in the low-pH endosome (e.g., influenza and Ebola). In both cases, the intracellular pathogen is largely inaccessible to the immune system, antibodies, and antibiotics. Clever engineering strategies can deliver multifunctional antibodies to these intracellular organisms to support pathogen destruction by natural immune cell functions or co-delivered antibiotics.

Opsonizing antibodies have been engineered to co-localize antibiotics or other anti-virulence molecules with intracellular pathogens. Multifunctional antibody–antibiotic conjugates rely on the antibody component binding an abundant bacterial surface antigen to tag it for phagocytosis into an endosome, which may harbor previously internalized bacteria (**Figure 5*a***). Local proteases then release the antibiotic to kill all bacteria within that vesicle. Lehar et al. (137) developed this strategy to target *S. aureus* using an antibody binding the conserved β-*O*-linked *N*-acetylglucosamine sugar on cell wall teichoic acid. This was expressed as a THIOMAB with six unpaired cysteines to allow conjugation to a peptide linker and antibiotic. In the neutrophil phagosome, cathepsin B cleaved the linker to release the antibiotic, a rifamycin derivative that retains potent antibacterial activity in low-pH environments (137, 138). In vitro, the antibody–antibiotic conjugate killed bacteria internalized by macrophages and endothelial and epithelial cells. In mice, prophylactic administration prevented kidney colonization better than vancomycin, although neither the antibody alone nor pooled sera could prevent infection spread. The promise of this strategy is supported by favorable phase I clinical trial results of the staph-targeting DSTA4637S (NCT03162250) (139).

This strategy depletes poorly accessible bacterial reservoirs that would otherwise present a source of recurrent infection. Although bactericidal activity is a key requirement for new antibiotics, these molecules often also possess undesirable host toxicities. Conjugation to an antibody offers the benefits of reducing the therapeutic dose while extending the circulating half-life and localizing the antibiotic to the infection site before release. This strategy could diminish systemic toxicities while achieving high local antibiotic concentrations within the phagosome necessary to kill the bacteria. Accordingly, it has been extended to *P. aeruginosa*, using an antibody binding the abundant lipopolysaccharide O antigen to enhance antibiotic potency ∼100-fold (from single-digit micromolar minimum inhibitory concentrations), indicating that antibody–antibiotic conjugates could revitalize antibiotics with modest potencies (140). It may also be appropriate for obligate pathogens such as *Burkholderia* species (141).

4.4. Bispecific Antibodies to Access Internalized Pathogens

Bispecific antibodies use an analogous approach in which one binding site tags the pathogen for phagocytosis while the other performs a complementary function, such as blockade of virulence factors that render the intracellular site hospitable to the pathogen. The most advanced therapeutic in this class is MEDI3902, which recently completed phase II clinical trials (NCT02696902) (142). One arm of MEDI3902 uses the Cam-003 binding site to bind the abundant Psl exopolysaccharide on the *P. aeruginosa* surface, activate complement, and mediate neutrophil phagocytosis (**Figure 5*b***). The other arm binds PcrV to block type III secretion of toxins such as ExoS into the host cell cytosol. This blockade supports phagosome acidification and enhanced bacterial killing (143, 144). A similar approach was used to neutralize filoviruses including Ebola, the cryptic glycoprotein receptor-binding site of which is exposed by proteases present in late endosomes, a site generally inaccessible to antibodies. Bispecific antibodies were generated with one arm blocking the receptor-binding site at endosomal pH while the other arm mediated phagocytosis and endosomal delivery by binding a conserved non-neutralizing epitope on the virion (145). This concept could be extended to block the function of other proteins that support pathogen escape or maintain the endosome as a hospitable environment for the pathogen, such as the *S. aureus* leukotoxins (136).

The above strategies require the antibody and pathogen to engage prior to internalization, which may present challenges for treating an established infection or eliminating all intracellular pathogens. As an alternative, antibodies can be delivered separately to join pathogens already present in an endosome or lysosome. This can occur by pinocytosis and FcRn-mediated antibody transport across epithelial cells; during this process, antibody-containing endosomes can fuse with those containing influenza virions to neutralize hemagglutinin and prevent fusion of the influenza virus and host cell membranes (146). If any antibody-bound virions escape the endosome as part of the natural infective cycle, the cytosolic E3 ubiquitin ligase and Fc receptor TRIM21 can redirect the virion for proteasomal destruction. This appears to be the mechanism by which VP6-specific antibodies protect against rotavirus (147). Alternatively, antibodies can be directed to the lysosome by receptor-mediated endocytosis when designed to target the mannose-6-phophate receptor used to deliver enzyme replacement therapies (148). This approach was used to develop a second-generation bispecific Ebola endosomal neutralizing antibody that is resistant to viral escape (149) (**Figure 5*c***). Finally, there is evidence that adding a cell-penetrating peptide to an IgG will mediate transport into the cellular cytoplasm (150). This can allow for antibodies to disrupt the hepatitis B virus life cycle (150) or limit infection by the intracellular pathogen *Ehrlichia chaffeensis* (151).

Three complementary strategies are described here to target pathogens hiding within cells. Antibodies can block the activities of cytotoxins that prevent host cell killing of internalized bacteria, or they can mediate targeted delivery of an antibiotic and the bacterium into the phagosome for intracellular bacterial killing. In both cases, identification of a bacterial surface antigen as an antibody target and binding of the antibody to the pathogen before phagocytosis are essential for success. Newer approaches target delivery of a protective antibody to intracellular compartments that already contain a pathogen and may have advantages for treating established infections.

5. CONCLUSIONS

Rising population density, travel frequency, and numbers of immunocompromised individuals have increased opportunities for pathogens to cause disease. Antibody engineering tools can help define mechanisms of pathogen immune evasion and counter them by identifying functionally relevant antibody targets, engineering antibodies that render antibody evasion tactics useless, and hunting pathogens hidden inside host cells. These efforts are performed with the knowledge that any new therapeutic strategy may exert selective pressures that drive emergence of organisms with altered traits. However, the persistence of many pathogens, coupled with the emergence of new pathogens, underscores the need for innovative approaches to prevent and treat infections.

SUMMARY POINTS

1. Many pathogens excel at concealing critical epitopes from antibody detection. Effective antibody therapeutics can access these vulnerable sites using strategies such as ultra-long CDR3 loops or nanobodies to access recessed sites or by targeting functionally constrained or highly conserved, often cryptic, epitopes.
2. While bacterial surface carbohydrates are abundant, accessible, and immunogenic, they are also highly variable among strains and often poor targets for antibodies. Antibodies binding conserved epitopes on surface proteins may allow for targeting of a broader range of strains with a single antibody.
3. Although non-neutralizing antibodies that trigger complement and Fc receptor activation are increasingly recognized as key components of successful immune responses, their contributions to protection are not understood fully and merit additional study.
4. Multiple bacterial and viral pathogens evade antibody effector functions by binding or cleaving antibody Fc domains. Antibodies engineered to resist capture or cleavage may confer enhanced protection.
5. Many pathogens recruit complement regulatory proteins to their surface to avoid triggering this arm of the immune system. Antibodies binding these surface proteins to block inhibitor recruitment may support complement activation while also serving as opsonins.
6. Many pathogens enter the host cell cytosol from the protected space of an endosome or phagolysosome. Antibodies engineered to access these intracellular spaces and block pathogens at the site of entry may help eliminate protected pathogen reservoirs.

DISCLOSURE STATEMENT

A.N.Q., R.P.S., and J.A.M. are inventors on pending or published patents related to novel antibodies binding pertussis antigens and SARS-CoV-2 S2 domain, engineered SARS-CoV2 spike antigens, and Fc domains engineered for increased efficacy against cytomegalovirus. J.A.M. is on the Scientific Advisory Boards of Janux and Releviate. She previously received funding from Synthetic Biologics. A.N.Q. is currently employed by Denali; R.P.S. is currently employed by Abbvie.

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