Functions of Antibodies

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ABSTRACT Antibodies can impact pathogens in the presence or in the absence of effector cells or effector molecules such as complement, and experiments can often sort out with precision the mechanisms by which an antibody inhibits a pathogen in vitro. In addition, in vivo models, particularly those engineered to knock in or knock out effector cells or effector molecules, are excellent tools for understanding antibody functions. However, it is highly likely that multiple antibody functions occur simultaneously or sequentially in the presence of an infecting organism in vivo.

The most critical incentive for measuring antibody functions is to provide a basis for vaccine development and for the development of therapeutic antibodies. In this respect, some functions, such as virus neutralization, serve to inhibit the acquisition of a pathogen or limit its pathogenesis. However, an antibody can also enhance replication or contribute to pathogenesis. This review will emphasize those functions of the antibody that are potentially beneficial to the host; a separate article is devoted to a discussion of antibody-dependent enhancement of infection. In addition, this article will focus on the effects of antibodies on the organisms themselves, rather than on the toxins the organisms may produce. Finally, the role of antibody in modulating T-cell immunity is not discussed in detail.

INTRODUCTION
In the setting of infectious diseases, antibody function refers to the biological effect that an antibody has on a pathogen or its toxin. Thus, assays that measure antibody function are differentiated from those that strictly measure the ability of an antibody to bind to its cognate antigen. Examples of antibody functions include neutralization of infectivity, phagocytosis, antibody-dependent cellular cytotoxicity (ADCC), and complement-mediated lysis of pathogens or of infected cells.

Antibodies can impact pathogens in the presence or in the absence of effector cells or effector molecules such as complement, and experiments can often sort out with precision the mechanisms by which an antibody inhibits a pathogen in vitro. In addition, in vivo models, particularly those engineered to knock in or knock out effector cells or effector molecules are excellent tools for understanding antibody functions. However, it is highly likely that multiple antibody functions occur simultaneously or sequentially in the presence of an infecting organism in vivo.

ANTIBODY FUNCTIONS INDEPENDENT OF EFFECTOR CELLS OR EFFECTOR MOLECULES
Antibodies are capable of having an impact on organisms in the absence of effector cells or effector molecules such as complement. For the most part, the impact of antibodies by themselves can be measured in vitro as
neutralization of organism infectivity. Neutralization is herein referred to as the ability of the antibody by itself to inhibit the infection of susceptible cells or, in the case of some extracellular organisms, to inhibit an initial pathogenic step. Importantly, as described below, neutralization involves many potential mechanisms. Furthermore, it should be emphasized that other antibody functions in addition to neutralization may ultimately be involved in the prevention or clearance of infection, even by antibodies that neutralize the relevant organism in vitro (1).

Neutralization of Infectivity

In vitro, antibodies are capable of blocking the infectivity or pathogenesis of viruses, bacteria, parasites, and fungi. Neutralization generally occurs as a result of interfering with an organism’s attachment to host tissues. However, it is now clear that several mechanisms account for neutralization and that a single antibody or antibodies with different specificities can neutralize a given organism, at least in vitro, through multiple mechanisms.

Preattachment neutralization

Some antibodies have been shown to inhibit infectivity by binding to organisms and causing them to aggregate. Aggregation or agglutination by immunoglobulin A (IgA) may allow more efficient entrapment of bacteria in mucus and subsequent clearance by peristalsis (2, 3). Although aggregation is more likely to occur with polymeric IgA and IgM, some neutralizing IgG antibodies can aggregate poliovirus; the aggregation results in less infectivity, probably by reducing the number of encounters between virus and host cells (4, 5).

Antibodies have also been shown to immobilize or “paralyze” organisms, such as the channel catfish pathogen Ichthyophthirius multifiliis (6). The IgA monoclonal antibody (mAb) Sal4 can render Salmonella enterica serovar Typhimurium immobile, independently of agglutination, although Sal4 also specifically interferes with uptake into epithelial cells. Antibodies directed against Pseudomonas aeruginosa flagella inhibit motility of that organism (7). Polyclonal antibodies, induced by immunizing mice with Vibrio cholerae outer membrane vesicles, protect suckling mice from oral V. cholerae challenge, likely by inhibiting the motility of the organism (8). Antibody may slow the random movement of human immunodeficiency virus type 1 (HIV-1) in vaginal mucus, presumably reducing the number of times the virus can make contact with the epithelial surface; this antibody function appears to rely in part on Fc interactions with components of the mucus (9).

Some antibodies appear to destabilize organisms, rendering them noninfectious. For example, the antifoot-and-mouth-disease virus mAb 4C9 disrupts virion capsids, possibly by mimicking the virus’ cell receptor (10). A neutralizing antibody against the E1 glycoprotein of Sindbis virus also induces conformational changes (11). Binding of HIV-1 gp120 can result in the shedding of gp120, leaving the transmembrane glycoprotein on the surface. However, the overall effect of such shedding on neutralization sensitivity is unclear (12). mAbs binding to a surface protein of Borrelia can kill the organism by inducing pores in the outer membrane (13). A mAb directed against fungal heat shock protein 90, a component of yeast cell walls, directly inhibits the growth of Candida (14, 15) and works in synergy with antifungal drugs to inhibit Cryptococcus neoformans (16). IgG1 and IgM mAbs that bind to the C. neoformans capsule affect gene expression, lipid biosynthesis, cellular metabolism, and protein phosphorylation or susceptibility to amphotericin B (17). Other mechanisms by which antibody inhibits bacterial and fungal infections directly and prior to attachment have been described (18, 19, 20).

Interference with pathogen attachment

Antibodies that bind to pathogen ligands essential for attachment of the pathogen to its host receptor have been described for many pathogens. In the case of viruses, such antibodies generally inhibit infectivity without altering their cognate antigen, thus strictly inhibiting by virtue of steric interference. This mechanism of virus inhibition has been described for many enveloped and nonenveloped antibodies. Well-studied example are antibodies against HIV-1 gp120 that interfere with binding of gp120 to CD4 (21). In addition, antibodies that neutralize, among others, flaviviruses (22), Newcastle disease virus (23), papillomavirus (24), and rotavirus (25) may do so by interfering with attachment. Some antibodies that block virus attachment do not bind directly to the site on the virus involved with attachment. For example, an antibody against human rhinovirus type 14 binds to surrounding viral structures but nonetheless sterically hinders interactions between the virus and its intercellular adhesion molecule-1 host receptor (26).

The stoichiometry of antibody-antigen interactions required for neutralization has been studied for many viruses, and evidence supports a “multiple hit” phenomenon in which neutralization requires the engagement of more than one antibody on the virion (27). Both antibody affinity and the accessibility of epitopes on the organism are the critical factors in determining whether antibody
binding will exceed the threshold required for neutralization. Thus, for example, one cannot necessarily predict neutralizing potency by measuring antibody affinity alone or on the basis of epitope specificity. Antibody Fab or F(ab')2 fragments are often capable of providing sufficient blockade of attachment to inhibit neutralization. These and other details regarding virus neutralization, including kinetics and requirements for steric hindrance can be found elsewhere \(^{12, 28, 29}\).

Adhesion of bacteria to the surface of host cells or tissue allows targeting of the organism to a specific cell type and allows the bacteria to resist physical removal by hydrodynamic shear forces \(^{30}\). Thus, adhesion is a first step in bacterial pathogenesis. The molecules responsible for bacterial adhesion are known as adhesins and are generally incorporated into pili or fimbriae \(^{30, 31}\). These adhesins are targets for antibodies that, in a manner somewhat analogous to virus neutralization, can inhibit attachment \(^{32, 33, 34}\). Thus, vaccines have been developed in order to elicit antibodies directed against adhesions. In most cases, this strategy has failed because of sequence variation in the structural proteins of fimbriae. Nonetheless, vaccination with FimH was able to reduce bladder infection of mice and monkeys with uropathogenic Escherichia coli \(^{35, 36, 37}\). The use of this vaccination strategy in humans is made difficult by a shared epitope between FimH and human LAMP-2 and thus a fear of autoimmunity \(^{38}\). Another example of adherence inhibition was described by Manjarrez-Hernandez et al., who found that secretory IgA (sIgA) in breast milk was able to inhibit the adherence of enteropathogenic E. coli to cells \(^{39}\). An mAb against lipoarabinomannan, a surface lipoglycan of Mycobacterium tuberculosis, is able to prevent adherence of M. tuberculosis to human monocyte-derived macrophages \(^{40}\). Antibodies can also inhibit the attachment of bacteria to abiotic surfaces \(^{41}\).

Antibodies to merozoite surface protein 1 (MSP1) on Plasmodium spp. can protect rodents against infection \(^{42}\). One mechanism that might account for this is inhibition of the attachment of the parasite to red blood cells \(^{43, 44}\). Plasmodium-infected red blood cells express Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1), which mediates binding to host endothelia and placenta. Antibodies have been elicited that can inhibit the interaction between infected erythrocytes and chondroitin sulfate proteoglycan, their ligand on placenta \(^{45}\). Inhibition of binding in this manner would not impact infection per se, but might influence pathogenesis.

As with other organisms, binding of fungi to host-cell surfaces is a first step in infection. mAb 2G8, directed against β1,3-glucan, can inhibit binding of Candida albicans to human epithelial cells \(^{49}\). It should be noted that mAb 2G8 also directly inhibits fungal growth and facilitates antifungal activity of human polymorphonuclear neutrophils \(^{49, 50}\). Other antibodies can also inhibit adhesion of C. albicans to HEC cells \(^{51}\) and of C. neoformans to a human lung epithelial cell line \(^{52}\). As with bacteria, antibodies can inhibit C. albicans adherence to abiotic surfaces \(^{53}\).

In the case of several parasites that infect the gastrointestinal tract, the mechanisms by which Ig, and, in particular, IgA, may function are unclear, but it is likely that the inhibition of attachment plays a role \(^{46}\). For example, mucosal anti-Giardia IgA antibodies may prevent infection by inhibiting attachment of the organism to the intestinal epithelium \(^{47}\). Intestinal IgE antibodies might contribute to the elimination of Trichinella spiralis in rats, possibly by blockade of the attachment to intestinal epithelium \(^{48}\), and immune serum can block the attachment of Cryptosporidium parvum to epithelial cells \(^{49}\).

Finally, antibodies generated against the host receptors themselves can also block infection of a number of different organisms \(^{50, 51, 52, 53, 54, 55, 56, 57}\).

Postattachment neutralization

Inhibition of fusion/entry

Intracellular pathogens can be neutralized by antibodies at postattachment steps in their life cycle. In the case of viruses, several studies have identified antibodies that inhibit fusion of viral and host membranes or entry into susceptible target cells. For enveloped viruses, antibodies can block an interaction between a viral protein necessary for fusion and its cellular receptor \(^{58}\). mAb 2F5, an HIV-1-neutralizing antibody, may block fusion of HIV-1 by obstructing the juxtaposition of viral and cellular membranes \(^{29, 59}\). 4E10, another HIV-1-neutralizing mAb, may interfere with the formation of fusion-competent complexes of gp41 \(^{60}\). In the case of West Nile virus, a neutralizing monoclonal antibody likely sterically constrains low-pH-mediated rearrangements of E proteins \(^{61, 62}\). Similarly, anti-influenza virus hemagglutinin (HA) antibodies can hinder the low pH-induced structural changes necessary for fusion of viral and endosomal membranes \(^{63, 64}\). It is possible that anti-influenza HA antibodies can inhibit both attachment and postattachment steps \(^{65}\).

An interesting twist on fusion inhibition was described for an mAb against influenza virus HA. The mAb becomes internalized at acid pH through the Fc neonatal...
receptor (FcRn) and reduces viral replication following apical exposure of Madin-Darby canine kidney cells to influenza virus. As virus, mAb, and FcRn colocalize within endosomes, it is possible that inhibition of infectivity occurs by interfering with fusion of viral envelope and endosomal membranes (66).

Nonenveloped viruses generally enter cells by endocytosis, and escape from the endocytic vesicle is mediated by capsid protein. Antibodies against polio virus may stabilize the capsid and prevent the structural rearrangements necessary for vesicle escape (29, 67, 68).

**Inhibition of other steps in organism life cycles**

A number of studies have revealed the ability of antibodies to inhibit organisms once they have successfully entered cells. In order for intracellular neutralization to be accomplished, antibodies must be internalized by host cells. Internalization of antibodies can occur as a result of coating of the organism, in which case, the coated organism must be capable of cell entry, or through Fc receptors. In addition, cells have been engineered to express intracellular antibodies (intrabodies) for potential therapeutic purposes (69). Intracellular neutralization can potentially interrupt an organism’s life cycle by interfering with the release, replication, or expression of genomic material. As an example, adenovirus type 5 antihexon mAb (9C12) allows viral attachment, cell entry, and intracellular transport of the virus to the nuclear periphery (70). Nonetheless, 9C12 neutralizes virus infectivity, likely by interfering with capsid uncoating and the release of viral genome (70). A rabbit anti-HPV16 L2 serum was able to neutralize HPV16 pseudoviruses through a mechanism that appeared to involve, at least in part, blocking the transport of viral genome to the nucleus (71).

IgA directed against surface proteins or glycoproteins can mediate neutralization of Sendai virus, influenza virus, and measles virus within susceptible target cells (72, 73, 74). In addition, IgA directed against measles virus M and N proteins, which are internal to the membrane, can inhibit measles virus replication within Vero cells (73, 75). Polymeric IgA or sIgM can intracellularly block the transcytosis of HIV-1 through epithelial cells. Although the epithelial cells are not thought to be a target of HIV-1 infection and replication, such blocking of transcytosis could block access in vivo to subepithelial CD4+ cells (76). Similarly, IgA inhibits transcytosis of rotavirus through polarized Caco-2 cells (77). IgA can also introduce a conformational change in the rotavirus VP6 trimer, which is exposed after internalization of virus. The structural change results in transcriptionally incompetent particles (78, 79).

A novel mechanism of intracellular virus inhibition was described by Mallery et al., wherein antibody bound to adenovirus interacted with cytosolic TRIM21; this interaction resulted in the antibody-bound virus being targeted to the proteasome for degradation (80). This mechanism of inhibition would not be expected to work with enveloped virus, since the antibody would be shed along with the envelope prior to internalization within the cytoplasm.

An interesting example of intracellular antibody function in a bacterial infection was described by Wang et al., who showed that a mAb against *Anaplasma phagocytophilum* inhibits morulae formation within HL-60 cells (a human promyelocytic leukemia cell line) (81). A mAb against listeriolysin O, the pore-forming toxin of *Listeria monocytogenes*, blocks *L. monocytogenes* infection within macrophages. Inhibition likely occurs as a result of intracellular neutralization of a secreted *Listeria* virulence factor (82).

With respect to parasites, IgA is reported to inhibit the replication of *Toxoplasma gondii* in enterocytes (83). In addition, a mouse monoclonal IgG2b antibody, which enters host fibroblasts upon invasion of the antibody-treated organism, inhibits the intracellular growth of *T. gondii* (84).

**INHIBITION OF LATER STEPS**

Antibodies are capable of binding to nascent viruses and inhibiting their liberation from infected cells. This function has been described for antibodies directed against the neuraminidase of influenza A virus (85). It has also been suggested that the antibody directed against influenza A virus M2 protein influences the efficiency of virus budding (86). An mAb against rubella virus E1 glycoprotein was reported to delay the release of virus, perhaps by affecting virion assembly (87).

**ANTIBODY FUNCTIONS DEPENDENT ON COMPLEMENT**

Activation of the complement cascade by antibody can result in the lysis of organisms or of infected cells (88). In addition, organisms bound by complement can be internalized by phagocytic cells, with resultant clearance of the organism. Internalization through complement receptors on antigen-presenting cells can also result in the processing of antigen for presentation to T lymphocytes. The details of complement activation have been reviewed elsewhere (88). It is important to note that antibodies that bind and activate complement may also
directly inhibit pathogens in the absence of complement. Complement activation may also have an indirect effect on pathogens by recruiting and activating leukocytes to sites of infection (89, 20). Similarly, complement-activating antibodies may engage Fc receptors (see below). The Ig subtype and IgG subclass of antibody are major determinants of complement activation (91). For the most part, in this review, we limit the discussion to antibodies that affect pathogens in the presence of complement but that, in the absence of complement, either have no or reduced antimicrobial activity.

A role for IgM and complement in limiting West Nile virus infection in mice has been suggested (92). More recently, Vogt et al. determined that a nonneutralizing mouse IgG1 mAb decreased West Nile virus load in mice in a manner that required C1q as well as phagocytic cells and FcγRIII (93). C1q, as well as FcγRs, contributed to the enhancement of CD4+ T-cell responses mediated by nonneutralizing anti-respiratory syncytial virus (RSV) antibody during RSV infection (94).

Antibodies that both neutralize and mediate complement-dependent lysis of influenza virus-infected cells may provide broader strain cross-reactivity than antibodies that only neutralize (95). Furthermore, the addition of complement has long been known to increase the infectivity-inhibiting activity of neutralizing antibodies against several viruses, including influenza viruses (96), Newcastle disease virus (97), herpes simplex virus (98), and Japanese encephalitis virus (99). Paramyxoviruses represent an interesting case in terms of the role of complement, since one study has shown that antibody can neutralize human parainfluenza virus type 2 with little contribution by complement, whereas neutralization of mumps virus and simian virus 5 was dependent on complement (100).

In a mouse model of respiratory syncytial virus, passive immunization of a nonneutralizing mAb was shown to protect animals from intranasal challenge. The mAb lost protective activity as a Fab, and decomplementation of mice reduced the degree of protection (101). Similarly, protective mAbs against Semliki Forest virus lose some effect in complement-depleted mice (102).

HIV-1 Env-specific antibodies are capable of lysing HIV-1-infected cells or virus in the presence of complement. However, such complement-mediated effects are inhibited by the presence of regulators of complement activation found on infected cells or on the virus itself (103, 104). Recently, complement-mediated phagocytosis of apoptotic, HIV-1-infected T cells by polyreactive antibodies has been reported (105). Another study has found that antibody from human immunodeficiency virus type 2 (HIV-2)-infected subjects is more potent than that from HIV-1-infected subjects in complement-mediated inactivation of the respective virus. Given the multiple potential consequences of complement, either directly or indirectly, its role in human immunodeficiency virus (HIV) infection in vivo remains unsettled (106, 107, 108).

Natural antibodies, generally of the IgM subtype, activate complement and can neutralize influenza virus (109). Moreover, natural IgM recognizing influenza virus or a surface protein of *Leishmania* may be involved in regulating CD4+ or CD8+ T cells through complement (110, 111).

A unique function of antibody is to initiate the clearance of pathogens via complement activation and binding to erythrocyte complement receptor 1 (CR1); the result of such binding sequesters the pathogen from invading susceptible tissue and may facilitate the destruction of the organisms by tissue macrophages (112). This phenomenon was first noted for bacteria by Nelson in 1953 (113).

Bacterial pathogens have developed strategies to evade the effects of complement. However, in the presence of specific antibody, effective activation of complement can result in the death or clearance of organisms such as *Neisseria meningitidis*, *Neisseria gonorrhoeae*, and *Haemophilus influenzae* (114). Individuals with complement deficiencies are at higher risk of infection with these organisms, and, in the case of *N. meningitidis* and *H. influenzae*, vaccine-induced antibody may protect through complement-mediated bacterial killing (115, 116). However, even with late complement component deficiencies, C3b deposition allows antibodies to kill the organisms by complement-mediated phagocytosis (115, 116, 117, 118, 119).

Antibody-mediated complement lysis of *Legionella pneumophila* is ineffective; however, organisms opsonized with both antibody and complement are phagocytosed by polymorphonuclear leukocytes (PMNs), although killing of ingested bacteria is limited (120). Vaccination of humans with an oral typhoid vaccine, M01ZH09, results in antibodies that are bactericidal to *S. enterica* serovar Typhi in the presence of complement; the antibodies also promote phagocytosis of *S. enterica* serovar Typhi by macrophages in a complement-independent manner (121).

Antibody and complement augment proinflammatory cytokine production of human peripheral blood mononuclear cells stimulated with *C. albicans*, which could be a factor in host defense against *C. albicans* infection (122). Han et al. found that protective IgM or IgG3 mAbs more efficiently bind C3 to the yeast cell than does a nonprotective mAb and that protection is likely
associated with enhanced phagocytosis and killing (123). In *C. neoformans*, immune serum or an IgG1 mAb localize C3 at the edge of the organism’s capsule, allowing phagocytosis through complement receptors (124). IgM also promotes complement deposition and PMN phagocytosis of *C. neoformans* (125). Interestingly, IgM, IgA, and IgG1 can promote the phagocytosis of *C. neoformans* through complement receptors in the absence of component; this occurs because of an antibody-mediated change in the organism’s capsule that allows an interaction with complement receptors (126).

Non-specific autologous antibodies can opsonize *P. falciparum*-parasitized erythrocytes, activate complement, and clear the infected cells through phagocytosis (127). Interestingly, antibody and complement deposition and phagocytosis are increased with erythrocytes from individuals with glucose-6-phosphate dehydrogenase deficiency, sickle trait, and β-thalassemia; it has been proposed that this antibody-mediated phenomenon underlies the protection against falciparum malaria in individuals with certain genetic disorders of red blood cells (127, 128, 129, 130). Antibody and complement can promote the killing of *P. falciparum* blood forms by THP-1 cells (a myelomonocytic cell line) and neutrophils (131, 132). In addition, antibody-dependent complement-mediated lysis of schizonts results in growth inhibition of *P. falciparum* (133). Antibodies to *P. falciparum* gametes can abolish infectivity of the gametes to mosquitoes; the gametes are lysed in the presence of complement and antibody, and antibody that binds to gametes but does not lyse them does not abolish mosquito infectivity (134, 135, 136, 137). Antibody has also been shown to clear experimental *Trypanosoma brucei* infection in a manner dependent on C3 and associated with uptake of organisms in the liver (138). Moreover, clearance of African trypanosomes by IgM, which is a major factor in controlling parasitemia, is mediated by complement and CR3 (139). Complement may also be involved in the pathogenesis of severe malaria (140, 141).

Finally, it is important to note that antibodies that neutralize in vitro only in the presence of complement may protect in vivo through other means (142).

**ANTIBODY FUNCTIONS DEPENDENT ON Fc-Fc RECEPTOR INTERACTIONS**

Much of the biological activity of antibody is mediated through interactions between Fc and Fc receptors found on a number of cells important for host defense. The engagement of Fc receptors by immune complexes (ICs) results in several downstream effects, depending on the Fc receptor-bearing cell, the form of the IC, the cytokine milieu, and the presence of complement. Fc receptor-mediated antibody activity can impact virus, bacteria, fungi, and parasites and can have beneficial or adverse consequences to the host.

Fc receptors have been identified for IgG (FcγRI), IgE (FcεRI), and IgA (FcαR), and for both IgA and IgM (FcαR/μ). Five FcRs have been identified in humans: FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa, and FcγRIIIb that differ from one another in their cellular distribution, function, and binding to IgG Fc (143, 144). There are two known forms of FcR and one expressed form each of FcRs and FcRα/μ (144). FcγRIIa, FcγRIIb, and FcγRIIIb are each encoded by polymorphic genes that result in phenotypically different receptors with respect to binding to different IgG subclasses (145, 146, 147, 148, 149). As a rule, IgG1 and IgG3 bind best to FcγRs, whereas IgG2 and IgG4 bind less well (150). Despite the similar magnitude of IgG1 and IgG3 binding to FcγRs, it has been reported that IgG3 mAbs are less able to mediate phagocytosis of antibody-coated red blood cells than are IgG1 mAbs, whereas ADCC activity of IgG3 is greater than that of IgG1 (151). In addition, glycosylation of the Fc segment of antibody can impact binding to FcRs (152, 153, 154, 155).

Interactions between Fc and FcRs can result in the death of pathogens or of cells infected with pathogens by a process known as antibody-dependent cellular cytotoxicity (156, 157, 158). Fc-FcR interactions are also important for phagocytosis of pathogens or of infected cells, although phagocytosis can occur in the absence of antibody or in the presence of antibody through other receptors (including complement receptors) (144). Engagement of FcRs can also inhibit intracellular pathogens without apparently killing the host cell (159). Modulation of inflammation is another FcR-mediated antibody function that can impact several pathogens (160, 161, 162, 163, 164). Finally, studies have documented the impact of FcR engagement on assays used to measure the neutralizing activity of antibodies (165, 166, 167, 168).

**Antibody-dependent cellular cytotoxicity**

ADCC occurs when antibody forms a bridge between an infected target cell (or directly with some pathogens) and an Fc-bearing effector cell. The result of this three-way interaction is the death of the target cell, either by lysis or apoptosis. ADCC is likely to play an important role in the clinical effects of antitumor mAbs, such as rituximab and trastuzumab, but its role in infections is less clear and complicated by the multiple functions of antibody (169, 170, 171).
ADCC, first described against virus-infected cells by Shore et al. for HSV-1 (172), becomes most interesting with regard to antibodies that protect animals but that poorly neutralize the pathogen in vitro. Nonneutralizing mAbs directed against HSV-2 glycoproteins can protect mice after a footpad injection of a lethal dose of HSV-2. The mAbs are equally efficient in protection in complement-sufficient and complement-deficient mice (173). More recently, Gorander et al. found that vaccination of mice with glycoprotein G of HSV-2 plus CpG could protect animals from vaginal challenge with HSV-2. The protective vaccine was associated with CD4+ T-cell interferon-γ responses. In addition, the vaccine resulted in nonneutralizing antibodies that mediated ADCC and might have been involved in protection (174). Chu et al. found that passive infusion of IgG antibodies decreased symptoms and mortality and decreased vaginal viral quantity in normal mice infected with HSV-2; although the antibody had neutralizing activity, protection was significantly diminished in mice lacking FcγR expression (175). Influenza A virus M2 vaccination results in partial protection of mice from influenza A infection that is mediated by nonneutralizing antibodies; ADCC is likely involved, since protection is not dependent on complement, whereas NK cells depletion reduces the protective effect (176).

The role of ADCC in HIV-1 and other lentivirus infections has been reviewed recently (156, 177). A great deal of correlative evidence in monkeys, as well as in humans, suggests a role for ADCC or other FcR-mediated antibody activities in preventing or modulating lentivirus infections. A more definitive study has demonstrated that mutations in the Fc segment of antibody that abrogate FcγR binding render a neutralizing mAb (IgG1b12) less protective in vivo against vaginal SHIV challenge than the unmutated mAb (1). However, whether the decreased protection is due to a lack of ADCC or to some other FcγR-mediated activity remains unknown.

There is scant literature on the role of ADCC in bacterial infections. However, complement-independent killing of bacteria in vitro in the presence of “killer” lymphocytes has been described for N. meningitidis and Shigella flexneri (178, 179). IgA, as well as IgG, in combination with lymphocytes from murine gut-associated lymphoid tissue, is reported to mediate ADCC against S. flexneri and Salmonella spp. (180, 181). Similarly, the same group found antibacterial activity against Streptococcus pneumoniae by mouse lung lymphocytes in conjunction with IgA (182). ADCC has also been described for Ehrlichia risticii and Coxiella burnetii-infected cells and for Brucella abortus, although, as with other bacteria, the role, if any, of ADCC in these infections in vivo is unknown (183, 184, 185). In the case of C. burnetii, passive antibody treatment can protect mice from C. burnetii infection in common γ-chain knockout mice, suggesting that Fc-FcγR interactions were not required for protection (186).

ADCC has been documented in vitro for a number of parasites. IgG opsonized T. spiralis larvae are susceptible to ADCC by eosinophils, neutrophils, and monocytes (187, 188). Schistosomula are killed by eosinophils, macrophages, or platelets in the presence of specific antibody, including IgE and IgG (189, 190, 191, 192). In vivo protection of rats from Schistosoma mansoni infection is likely the result of such IgE-mediated ADCC (193). Antibodies that bind poorly to FcRs, such as IgM, IgG2, and IgG4, can inhibit ADCC against schistosomula and have been epidemiologically linked to increased susceptibility of infection in humans (194, 195, 196). ADCC mediated by IgG or IgE and by macrophages, eosinophils, or neutrophil effector cells has also been shown in vitro to kill larval or adult filaria (197, 198, 199, 200, 201, 202). In vivo, clearance of Brugia malayi microfilaria is very likely mediated through ADCC (203). ADCC activity against trypanosomes and other parasites has also been documented (204, 205, 206, 207, 208, 209, 210).

It is important to note that many of the effector cells mediating ADCC against parasites are capable of antibody-mediated phagocytosis as well. In both ADCC and phagocytosis, organisms are killed and radioisotopes or dyes are released, and distinguishing between these two antibody functions requires careful consideration (204, 205).

Finally, antibodies may also inhibit infections in a manner that requires the components of ADCC (i.e., infected target cells, antibody, and FcR-bearing effector cells) but does not necessarily rely on target cell lysis. Thus, antibody-dependent cell-mediated inhibition of P. falciparum has been described, where the development of intracellular parasites is blocked in a manner dependent on blood monocytes and antibody; triggering of both FcγRIla and FcγRIIIa may be required, but erythrocyte target cells do not appear to be killed (159, 211, 212). Forthal et al. have described antibody-dependent cell-mediated virus inhibition (ADCVI) with measles virus HIV, simian immunodeficiency virus (SIV) and simian-human immunodeficiency virus (SHIV) (1, 160, 213, 214). ADCVI is a measure of virus inhibition occurring as a result of antibody-FcR interactions and is likely dependent on combinations of ADCC, phagocytosis, and chemokine/cytokine production.
Phagocytosis

The internalization and degradation of antibody-coated pathogens by phagocytes via FcRs has been well described for a number of organisms and is likely a critical antibody function for clearance of pathogens in vivo. Since phagocytosis and ADCC often require the same components (antibody and effector cells), it may be difficult to definitively and specifically demonstrate a role for phagocytosis in preventing or modulating infections in animals or humans.

With respect to viruses, passive infusion of antibodies results in the rapid elimination of cell-free organisms from the blood of animals (215, 216). This is consistent with information indicating that the rate of clearance of antigens by the reticuloendothelial system is greatly increased in the presence of specific antibodies (217, 218).

A recent example comes close to demonstrating a key role for phagocytosis in preventing a viral infection (93). In that study, a poorly neutralizing antibody against the West Nile virus envelope could reduce viremia in mice via an FcγRIII- and C1q-dependent mechanism that required phagocytic cells. Since NK cells did not seem to be involved, it is less likely that ADCC played a significant role in protection. However, it remains possible that the lysis of infected cells mediated by the phagocytes, in addition to or instead of phagocytosis of antibody-coated virus, was involved in protection. A possible role for phagocytosis in clearing influenza virus from the lungs of mice was suggested by Fujisawa (219). In that study, both PMNs and the passive infusion of neutralizing antibody were required for maximum viral clearance and survival. A particularly interesting point about this study was the need for PMNs despite the high neutralizing titer of the infused immune serum; this finding is consistent with that of Hessell et al. where maximal protection against SHIV was afforded by a neutralizing antibody that engaged Fc receptors (1). Huber et al., using passive antibody transfer in FcRγ-/-mice, also concluded that phagocytosis is important in the clearance of influenza virus (220). In all of these studies, however, it is not possible to precisely define the antibody function responsible for protection, because phagocytosis, ADCC, or soluble factors could have contributed.

An interesting phenomenon related to phagocytosis was described by Chan et al. (221) who showed that inhibition of dengue virus phagocytosis, by aggregating virus and cross-linking of FcγRIIb, resulted in neutralization of virus infectivity.

Phagocytosis of antibody-coated infected cells, in addition to phagocytosis of immune complexed cell-free virus, could be a contributor to protection, although virus-infected cells can be phagocytosed in the absence of antibody (222, 223). Surprisingly, two studies indicated that neither human monocytes nor human neutrophils were able to phagocytose IgA or IgG immune complexes formed with influenza virus in vitro (224, 225).

FcγR-mediated phagocytosis and clearance of Borrelia pertussis has been demonstrated in vitro and in a mouse model (226). Similarly, natural and vaccine-induced antibodies mediate phagocytosis of S. pneumoniae, and such FcγR-mediated phagocytosis may play a role in protection (227). In FcγRIIb-deficient mice, phagocytosis and survival after S. pneumoniae challenge are both improved relative to control mice, although the survival advantage is reversed after immunization followed by challenge with a high dose of bacteria (227). In the later case, it is likely that inflammatory cytokines triggered by interactions between antipneumococcal antibody and activating FcγRs—in the absence of the inhibitory FcγRIIb—were responsible for the higher mortality. Other studies in mice have found that antibody-mediated protection from S. pneumoniae does not depend on FcγRs (228). In humans, IgG2, a relatively inefficient activator of complement, is thought to be important in protection against S. pneumoniae. Although IgG2 is also relatively poor at engaging FcγRs, it binds best to the H isoform of FcγRIIa and to the V isoform of FcγRIIIa (150). Consistent with a role for FcγR-mediated phagocytosis, there appears to be an association between homozygosity for the R isoform of FcγRIIa and severe or invasive pneumococcal disease (229, 230). Moreover, PMNs from FcγRIIa HH homozygous donors have higher phagocytic activity against antibody-opsonized S. pneumoniae (231). In the case of N. meningitidis, complement-mediated clearance or bactericidal activity appears to be more important than FcγR-mediated phagocytosis (see above). However, FcγR-mediated phagocytosis can be demonstrated in vitro (232, 233). Furthermore, some studies, but not all, have found associations between FcγRIIa genotypes and susceptibility to or severity of meningococcal infection (233, 234, 235, 236, 237, 238). Finally, γδT cells capable of phagocytosing antibody-opsonized E. coli via FcγRIIa have been described (239).

Opsonization and phagocytosis by IgG subclass-switched mouse mAbs has been described for C. neoformans (240). Passive infusion of the mAbs had some effect on clearing yeast from mice, but the phagocytic activity in vitro did not correlate well with clearing of organisms in vivo (241). A study of C. neoformans phagocytosis has suggested a specific receptor for IgG3
in mice different from the known FcγRs (242). A recent study using X-linked immunodeficient mice indicated that IgM promotes containment of C. neoformans in the lungs by augmenting phagocytosis (243).

In many cases, parasites may be too large for phagocytosis: lysosomal and parasitic membranes fuse after Fc-FcR (γ, α, or ε) interactions, resulting in the lysis of parasites extracellularly (244). However, IgG from individuals living in malaria-endemic areas can mediate the phagocytosis of P. falciparum-infected erythrocytes by monocytes (245). In addition, the PfEMP1 is the major target of antibodies that mediate phagocytosis, and anti-PfEMP1 antibodies are associated with a reduced risk of developing symptomatic malaria (246).

OTHER ANTIBODY FUNCTIONS

Apart from specific effects on organisms, antibodies may modulate inflammation and thereby indirectly affect pathogenesis. Such immune modulation is well described for FcγR triggering by immune complexes, which result in the generation, secretion, or repression of various pro- or anti-inflammatory substances (247, 248, 249, 250, 251, 252, 253, 254, 255, 256). In addition, modulation of cytokines by FcRs themselves, internalization of immune complexes via FcRs can result in the engagement of toll-like receptors, adding a further layer of complexity and control over inflammation (255, 257, 258, 259, 260).

An example of the role of FcγRs in inflammation was the demonstration that soluble FcγRII, by competing with IC binding to cellular FcγRs, can limit the inflammation due to the IC (i.e., the Arthus reaction) (261). Soon thereafter, it was established that the Arthus reaction was markedly attenuated in FcγRIIb IgG transgenic mice (262). These types of studies have important implications for autoimmune diseases (263).

Engagement of FcγRIIb by IgG immune complexes serve to regulate B-cell activity and survival and may serve as a means of maintaining peripheral tolerance for B cells (264, 265, 266). Immune complex binding and internalization via FcγRs also result in dendritic cell maturation and in efficient major histocompatibility complex class I-restricted presentation of the exogenous peptides making up the immune-complexed antigen (267).

Another important mechanism of immune modulation by antibodies is through the activation of complement components, which can then serve as chemotactic agents (268). Moreover, CSa anaphylatoxin is involved in immune complex-mediated injury in part because it results in a shifting of the balance between activating and inhibitory FcγRs toward a more inflammatory phenotype (269). Activation of C3bi on immune complexes can result in blunting of the inflammatory response by diverting interactions of the IC away from FcγRs and toward CR3 (270).

CONCLUSIONS

The inhibitory effects of antibodies on pathogenic organisms have been documented since the late 1800s (271). Since that time, much has been learned regarding the mechanisms that underlie the antimicrobial activity of antibodies. However, antibodies often have multiple functions in vitro and in vivo, either directly or through interactions with FcRs or complement. Modern tools, such as knockout mice or antibodies engineered to abrogate or enhance functions have proven useful for more precise explorations of antibody function. Nonetheless, major questions regarding the way in which an antibody functions in vivo remain, and multiple activities are likely to contribute to the antimicrobial effect.

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Functions of Antibodies


