SPECIAL FEATURE REVIEW

The protective potential of Fc-mediated antibody functions against influenza virus and other viral pathogens

Hillary A Vanderven[1,2] & Stephen J Kent[2,3,4]

1 Discipline of Biomedicine, College of Public Health, Medical and Veterinary Sciences, James Cook University, Douglas, QLD, Australia

2 Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, University of Melbourne, Parkville, VIC, Australia

3 Australian Research Council Centre of Excellence in Convergent Bio-Nano Science and Technology, University of Melbourne, Parkville, VIC, Australia

4 Melbourne Sexual Health Centre, Department of Infectious Diseases, Alfred Health, Central Clinical School, Monash University, Clayton, VIC, Australia

Abstract

In recent years, there has been a renewed interest in utilizing antibody fragment crystallizable (Fc) functions to prevent and control viral infections. The protective and therapeutic potential of Fc-mediated antibody functions have been assessed for some clinically important human viruses, including HIV, hemorrhagic fever viruses and influenza virus. There is mounting evidence that influenza-specific antibodies with Fc-mediated functions, such as antibody-dependent cellular cytotoxicity and antibody-dependent phagocytosis, can aid in the clearance of influenza virus infection. Recent influenza challenge studies and intravenous immunoglobulin G therapy studies in humans suggest a protective role for Fc effector functions *in vivo*. Broadly reactive influenza antibodies with Fc-mediated functions are prevalent in the human population and could inform the development of a universally protective influenza vaccine or therapy. In this review, we explore the utility of antibodies with Fc-mediated effector functions against viral infections with a focus on influenza virus.

INTRODUCTION

Structural features of antibodies allow them to mediate a diverse array of antiviral effector functions. Antibodies are composed of the fragment antigen-binding region and the fragment crystallizable (Fc) region. The antibody fragment antigen-binding domains recognize antigen, whereas the Fc region contains binding sites for Fc receptors (FcRs) and complement. The Fc domain is historically considered to be the invariant region of the antibody, but it displays significant heterogeneity owing to variation in the antibody isotypes [immunoglobulin G (IgG), IgA, IgM, IgE and IgD], subclasses (IgG1, IgG2, IgG3 and IgG4), allotypes and modifications (e.g. glycosylation).[1,2] Each antibody isotype and subclass has the capacity to selectively engage specific FcRs with distinct effector functions at different sites in the body. While IgG is the major isotype in the blood, IgA is dominant in mucosal tissues. Human IgG1 and IgG3 are the most functional subclasses because of their greater affinity for activating FcRs.[3–5] IgG contains two N-glycosylation sites at asparagine 297, one on each heavy chain. The core Fc glycan is a biantennary heptasaccharide to which fucose, galactose and sialic acid can be added at variable levels.[1] Differential glycosylation alters the flexibility and structure of IgG Fc, changing FcR binding affinity and functionality.[6,7]

Fc gamma receptors (FcγRs) are classified into two types based on their ability to engage the dominant conformational states of the IgG Fc domain. Type I FcγRs belong to the Ig receptor superfamily and bind to unsialylated Fc domains in an open confirmation.[8] Type I

FcγRs include classical activating (FcγRI, FcγRIIa, FcγRIIc, FcγRIIIa and FcγRIIIb) and inhibitory FcγRs (FcγRIIb). Type II FcγRs are members of the C-type lectin family, such as DC-SIGN and CD23, that bind to sialylated Fc domains in a closed confirmation.[8] The different FcγR subfamilies have unique cellular expression patterns, isotype/subclass binding and affinities for modified Fc domains. The diversity in FcγRs adds another level of complexity to the Fc-FcγR effector system by driving different immunomodulatory pathways depending on the context of viral infection and antigen.

Antibodies are a key component of protective immunity against viruses. Neutralization of extracellular virus can prevent infection by inhibiting viral attachment, entry and fusion with host cells. It has, however, become evident that neutralization alone does not predict the protective capacity of antibodies targeting viral pathogens. Viruses can evade recognition by neutralizing antibodies (NAbs) as a result of their intracellular localization and high mutation rates. Fc-functional antibodies can recognize more conserved epitopes on viral antigens compared with NAbs.[9] As such, viruses represent a major target of Fc-mediated effector functions. Despite their relatively small genomes, multiple viruses have evolved and maintained anti-Fc/FcR mechanisms, highlighting the importance of Fc functions in the antiviral immune response. Herein, we examine the contributions of Fc-mediated antibody functions to antiviral immunity with a focus on influenza virus.

THE NEED FOR BETTER PROTECTION AGAINST INFLUENZA VIRUS

Seasonal influenza vaccines are reformulated and administered yearly, but influenza epidemics still cause 290 000–650 000 deaths annually.[10] The protection afforded by influenza vaccination is limited by several factors including antigenic drift, antigenic shift and suboptimal responsiveness in high-risk groups. Avian-origin influenza viruses (H5N1 and H7N9) also pose a significant threat to global health, particularly if they acquire the ability to transmit efficiently from human-to-human.

Influenza vaccination and infection primarily generate antibodies targeting the major envelope glycoprotein hemagglutinin (HA), which is required for influenza virus attachment and entry into cells. The classical hemagglutination inhibition (HAI) assay is frequently used to assess protection following influenza vaccination.[11] HAI assays detect a subset of NAbs that block influenza virus attachment by binding to epitopes surrounding the receptor-binding site in the HA head domain. A serum HAI titer of 40 correlates with a ≈50% reduction in the rate of influenza virus infection.[12] However, HAI antibodies that bind in the hypervariable HA head are typically strain-specific and are not protective against drifted or emerging influenza viruses with pandemic potential.

The development of a universally protective influenza vaccine is a global priority. Influenza-specific antibodies that protect through Fc-mediated functions preferentially target conserved epitopes on the HA molecule, including the highly conserved HA stem domain.[9,13] Antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent phagocytosis (ADP) and antibody-dependent complement activation are increasingly recognized as mediators of influenza immunity and may inform the development of more universal influenza vaccines and immunotherapies.

FC-MEDIATED EFFECTOR FUNCTIONS OF ANTIBODIES AND FCRS

ADCC

ADCC is induced by human FcγRIIIa on natural killer (NK) cells and monocytes/macrophages binding to the IgG Fc, primarily of IgG1 and IgG3.[3] Human neutrophils express FcγRIIIb and may also mediate ADCC in some form.[14] ADCC is initiated when FcγRs on immune cells are engaged by the Fc of IgG bound to viral antigens on the surface of infected cells.[15] Multimeric engagement of FcγRs leads to immune cell activation, which triggers the release of preformed cytolytic granules (containing granzymes and perforin) and causes apoptosis of the infected cell.[16] Immune cell activation can also lead to the secretion of antiviral cytokines and chemokines.[17,18] The clearance of virus-infected cells, as well as the release of cytokines, contributes to antiviral immunity by reducing virus replication and spread.

Passive transfer of monoclonal antibodies (mAbs) into mice is frequently used to assess the protective role of Fc-mediated antibody functions during virus challenge, but it is worth noting two important caveats associated with this strategy. First, mAbs often do not have the same post-translational modifications (e.g. glycosylation) as their *in vivo* equivalent because mAbs are expressed from recombinant plasmids in cell culture systems. Altered glycosylation of the Fc domain can greatly impact the functionality of mAbs *in vivo*. Second, mice express a novel activating receptor, FcγRIV, with functional similarity to human FcγRIIIa. However, murine FcγRIV differs from human FcγRIIIa in cellular distribution and isotype binding.[3] Murine FcγRIV engages the Fc of

IgG2a, IgG2b and IgE, but not murine IgG1 or IgG3. FcγRIV is also expressed on murine monocytes/ macrophages and neutrophils, but not on NK cells.[3] While some mechanistic conservation between NK cell- and monocyte-mediated ADCC has been demonstrated,[19,20] the mechanism underlying neutrophil-mediated ADCC is only currently being investigated. Disparities between human and mouse FcRs represent a limitation of murine studies, but FcγR-humanized mice have been developed to partially overcome this limitation.[21]

ADP

Uptake of antibody-opsonized virions or apoptotic infected cells is referred to as ADP. Human FcαRI, FcγRI and FcγRIIa on phagocytes are engaged by the Fc region of IgA or IgG bound to viral antigen,[3,22] leading to phagocytosis of immune complexes (ICs) or infected cells. Following ADP, engulfed virions or infected cells are degraded in the phagolysosome. Phagocytes, such as monocytes/macrophages and dendritic cells, can also present viral peptides on major histocompatibility complex molecules to T cells and release proinflammatory cytokines. Phagocytosis occurs independently of antibody during viral infection but is enhanced in the presence of virus-specific IgG.[23,24]

Antibody-dependent complement activation

The complement system comprises the classical, lectin and alternative pathways. The classical pathway is activated when C1q interacts with antibodies, typically IgM and IgG, bound to antigens on the surface of virus or virally infected cells.[25] Agalactosylated IgG and polymeric IgA can also activate the lectin pathway through interactions with mannan-binding lectin.[26,27] Antibody-dependent complement activation can lead to direct complement-dependent lysis of enveloped virus or virally infected target cells through the insertion of a membrane attack complex.[28] Antibody-dependent complement activation can also cause the deposition of opsonins (e.g. C3b) on the surface of virus or virally infected cells. Opsonization may lead to neutralization of virus, complement-dependent phagocytosis of virions or complement-dependent cellular cytotoxicity of infected cells.[25,28]

Many viral pathogens have evolved mechanisms to evade the complement system, indicating that complement plays an important role in the outcome of virus infection and disease. Viruses pirate soluble and membrane-bound complement regulatory proteins, express viral complement regulators and exploit complement receptors for cellular entry. Influenza virus subverts complement by blocking the interaction of C1q with antibodies (via M1 protein) and acquiring a membrane attack complex-inhibitory protein (CD59) in its envelope during budding.[28] Further, human C3b is not efficiently deposited onto the surface of H1N1 2009 pandemic A/California/07/2009 [A(H1N1)pdm09] influenza virions *in vitro*.[29] Thus, the A(H1N1)pdm09 virus is only susceptible to classical and alternative complement pathways when bound by influenza-specific antibodies.[29] Pre-existing antibodies with complement-dependent lysis activity correlate with protection against clinical A(H1N1)pdm09 influenza in children, independently of ADCC.[30]

Additional Fc-mediated functions via nonclassical FcRs

Nonclassical FcRs also contribute to the immune response against viruses. The neonatal FcR regulates the persistence of IgG in serum and transports IgG across cellular barriers including the placenta, the vascular and mucosal epithelia.[31] Furthermore, neonatal FcR has been shown to facilitate antibody-dependent intracellular neutralization of influenza virus. Unusual HA head-specific IgG, that only binds at an acidic pH, can be taken up by neonatal FcR-mediated transcytosis.[32] When transcytotic vesicles containing IgG fuse with endosomes containing influenza virions, the intracellular virus can be neutralized following endosomal acidification. This neonatal FcR-mediated antibody-dependent intracellular neutralization prevents fusion of viral and endosomal membranes, thereby trapping influenza virions in the lysosome.[32]

ICs can engage a variety of different FcRs on immune cells and trigger divergent Fc functions depending on their composition. Type II FcγRs engage ICs containing sialylated IgG Fc domains, which can result in anti-inflammatory activity [via DC-SIGN during intravenous immunoglobulin (IVIG) therapy] or high-affinity antibody production (via B-cell CD23 engagement).[33] Increased sialylation correlates with greater antibody affinity following human influenza vaccination.[34–36] It has been proposed that influenza vaccination results in the production of sialylated anti-HA IgG antibodies by plasmablasts, which form ICs with vaccine antigen.[33,36] The sialylated ICs are delivered to lymph node germinal centers by subcapsular macrophages and noncognate B cells through interactions with CD23.[34,37] Sialylated ICs may associate with follicular dendritic cells prolonging contact between B cells and influenza antigen, driving affinity maturation of antibodies in the germinal center (GC).[33,36] Furthermore, sialylated ICs increase B-cell expression of inhibitory FcγRIIb through engagement of

CD23.[33,36] Influenza antigens in ICs can engage the B-cell receptor and FcγRIIb on GC B cells simultaneously, which increases the threshold of B-cell activation and drives the selection of higher-affinity B cells.[33,36] A similar mechanism of antigen deposition in B-cell follicles by sialylated IC–complement interactions has recently been described for HIV.[38]

IgA is a first line of defense against viruses in the mucosa. Dimeric IgA can be transported across the mucosal epithelium by an FcR called polymeric Ig receptor, allowing access to mucosal sites.[39] Owing to its presence in the respiratory tract and its polyvalency, IgA has significant protective potential against influenza virus.[40] Indeed, high levels of nasal IgA correlated with protection in a human influenza challenge study.[41] The Fc domain of mucosal IgG also interacts weakly with mucins. The avidity of IgG Fc–mucin interactions is enhanced when multiple IgGs bind to the same virion causing mucosal trapping, which has been demonstrated for HIV and herpesvirus.[42]

During infections with nonenveloped viruses, antibodies bound to the viral capsid are transported into the cytosol and are detected by TRIM21, an intracellular FcR. TRIM21 recognizes the Fc domains of multiple antibody isotypes and mediates antibody-dependent intracellular neutralization of nonenveloped viruses like adenovirus.[43]

FC EFFECTOR FUNCTIONS CONTRIBUTE TO ANTIBODY-MEDIATED INFLUENZA PROTECTION *IN VIVO*

An early study by Corti *et al.* showed that passive transfer of an HA stem-specific broadly NAb (bNAb), FI6, fully protected mice and ferrets from lethal influenza virus challenge.[44] A mutant FI6 deficient in FcγR engagement (FI6-LALA) only protected 40% of mice from a lethal dose of A/Puerto Rico/8/1934, indicating that the antiviral activity of FI6 is predominantly Fc-mediated.[44] Infusion of FcγRIV knockout mice with an HA stem bNAb (6F12) resulted in increased weight loss and a 50% decrease in survival compared with wild-type mice, confirming the importance of ADCC for *in vivo* protection.[9] Since these initial findings, it has been widely reported that HA stem bNAbs provide Fc-dependent protection in mice and have ADCC activity *in vitro*.[9,13,45–47] Conversely, strain-specific HA head mAbs confer Fc-independent protection.[9,13] DiLillo *et al.* showed that bNAbs targeting conserved epitopes in the HA head and in the neuraminidase glycoprotein also require FcγR engagement to protect mice from lethal influenza virus challenge.[13] A newly isolated bNAb, H3v-47, binds to a novel epitope spanning the receptor-binding site and vestigial esterase subdomains in the HA head.[48] H3v-47 protected mice by blocking influenza virus egress from cells, but also engaged FcγRs and activated NK cells *in vitro*.[48] Broadly reactive mAbs capable of binding across both lineages of influenza B virus were recently isolated and shown to confer Fc-dependent protection in mice.[49] Collectively, these studies suggest that an antibody's ability to engage FcγRs and confer Fc-mediated protection is largely epitope driven *in vivo*.

The impact of epitope localization on ADCC is complex. A study suggested that for efficient induction of ADCC two points of contact are required between the immune cell and the influenza-infected cell. The proposed points of contact are (1) FcγR and IgG Fc and (2) cell surface sialic acid and influenza HA.[50] Stem-specific bNAbs mediate potent ADCC as they do not block sialic acid-binding sites in the HA head. Conversely, strain-specific HAI+ mAbs block sialic acid-binding sites and cannot efficiently induce ADCC.[50] HAI+ mAbs can also inhibit ADCC by stem bNAbs or polyclonal IgG through competitive binding to HA (Figure 1b, last panel).[51] Infusion of a single Fc-functional mAb can be protective in influenza-infected mice, but the polyclonal nature of the humoral response adds an extra level of complexity. A polyclonal antibody pool contains a mixture of ADCC-mediating (broadly reactive HAI–) and ADCC-inhibiting (strain-specific HAI+) antibodies. As such, the contribution of ADCC to influenza immunity may vary depending on the ratio of ADCC-mediating to ADCC-inhibiting antibodies in an individual. The significance of this "second point of contact" has not been investigated for other viruses or Fc functions.

ADP may also play a key role in clearing influenza virions and restricting viral replication. An early study showed that FcγR-deficient (FcγR$^{-/-}$) mice had increased morbidity and mortality following influenza virus infection. Murine macrophages were capable of phagocytosing antibody-opsonized influenza virions, but NK cells were not required for antibody-dependent clearance of virus.[52] ADP was also proposed as the main mechanism of Fc-mediated protection in H7N9-infected mice.[47] HA stem-specific IgG and IgA can induce reactive oxygen species production by neutrophils, but this process requires FcR engagement and ADP of influenza virions.[53] A recent study showed that depletion of murine alveolar macrophages, but not NK cells, decreased protection by HA stem bNAbs.[46] Because murine macrophages express an array of FcγRs (including FcγRI, FcγRIII and FcγRIV), depletion models cannot distinguish between different macrophage-mediated Fc functions (e.g. ADCC *versus* ADP). As such, the exact mechanism of Fc-mediated protection *in vivo* is not clear and warrants further investigation in single FcγR knockout mice.
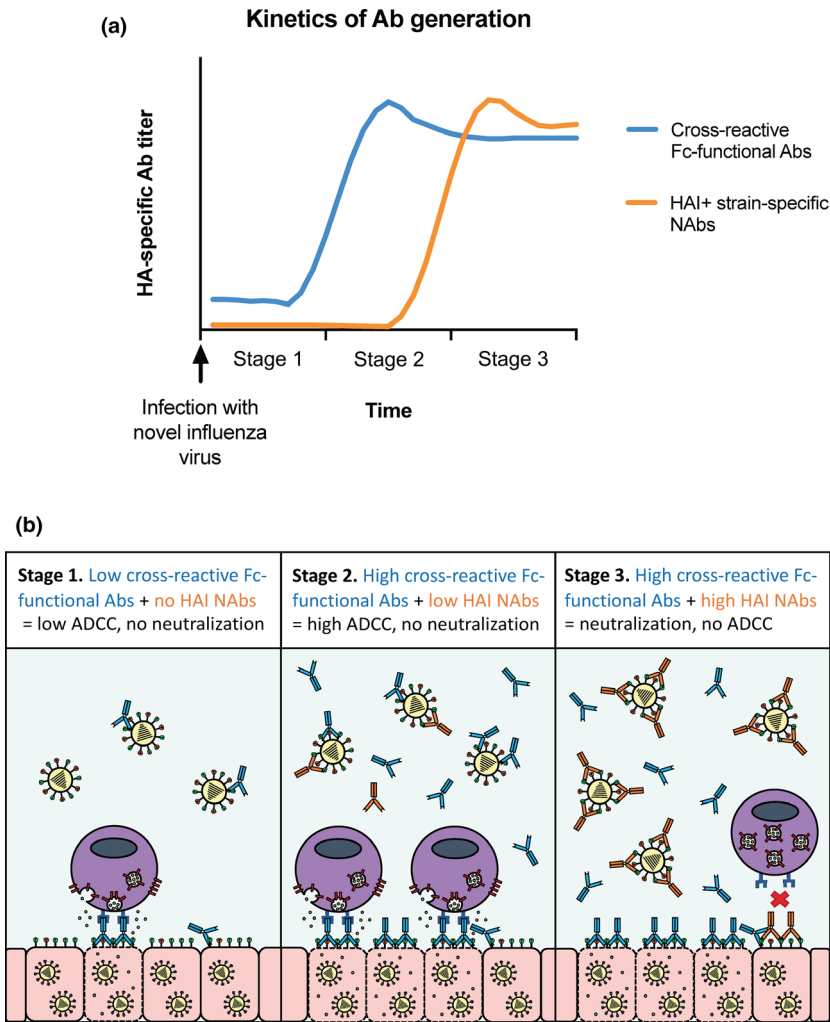
Figure 1. Proposed kinetics of cross-reactive fragment crystallizable (Fc)-functional and hemagglutination inhibition (HAI)+ strain-specific hemagglutinin (HA) antibodies following infection with a novel influenza virus. **(a)** Approximately <10 days postinfection (stage 1) with a novel influenza virus, preinfection or baseline levels of cross-reactive Fc-functional HA antibodies (Abs; blue line) are present as a result of prior influenza virus exposures and no HAI+ strain-specific neutralizing antibodies (orange line) have been generated. Approximately <15 days postinfection (stage 2) with a novel influenza virus (timing appears to vary depending on the infecting strain[47] and may reflect antigenic distance from prior influenza virus exposures as well as antibody affinity), cross-reactive Fc-functional HA antibodies are boosted and reach peak levels, while low levels of HAI+ strain-specific neutralizing antibodies (NAbs) are starting to be produced. Approximately >15 days postinfection (stage 3) high levels of cross-reactive Fc-functional antibodies are maintained and HAI+ strain-specific NAbs are boosted and reach peak levels. **(b)** In stage 1, very early postinfection with a novel influenza virus, no strain-specific HAI+ NAbs are present and low baseline levels of antibody-dependent cellular cytotoxicity (ADCC) activity may be detectable. In stage 2, high levels of ADCC activity are detected because of the rise in cross-reactive Fc-functional antibodies and the production of low, subneutralizing levels of HAI+ strain-specific NAbs has begun. In stage 3, high levels of HAI+ strain-specific NAbs are neutralizing free influenza virions and inhibiting ADCC.

FC-MEDIATED ANTIBODY FUNCTIONS IN HUMAN INFLUENZA VACCINATION AND INFECTION STUDIES

Influenza-specific ADCC was first described in human samples in 1977.[54] Human peripheral blood leukocytes with low concentrations of cell-associated HA antibody were capable of killing influenza-infected cells *in vitro*.[54]

In the early 1980s, ADCC activity was detected in sera collected from influenza-infected or vaccinated children. Serum antibodies with ADCC activity were broadly reactive, generated earlier than HAI antibodies and capable of targeting both HA and neuraminidase glycoproteins.[55] Purified populations of human NK cells, monocytes and neutrophils all mediated detectable levels of ADCC against influenza-infected cells *in vitro*. Human

NK cells rapidly (within 2–6 h) mediated potent ADCC of influenza-infected cells at low antibody concentrations (1:45 000 serum dilution).[55] Thus, NK cells are frequently used in surrogate ADCC assays *in vitro*. Other immune cell types likely contribute to influenza-specific ADCC *in vivo*, but their involvement is not well understood yet.

Humans are repeatedly exposed to different strains of influenza virus from early childhood, leading to the generation of cross-reactive Fc-functional antibodies. Young adults have detectable serum ADCC activity against an H3N2 influenza virus that circulated in 1968, to which they lack previous exposure and have undetectable HAI titers.[56] Healthy adults also have ADP activity against an array of influenza HA proteins.[23] ADCC and ADP activities against H5N1 avian influenza viruses have been reported in humans,[23,57–59] despite the fact that these avian-origin viruses do not circulate in the human population. Similarly, intravenous IVIG preparations pooled from thousands of human donors have detectable ADCC and ADP activities against HA proteins from noncirculating subtypes of influenza virus, such as H2N2, H4N6 and H5N1.[23,58] Broadly cross-reactive antibodies with ADCC activity may increase with age. Terajima *et al.* found that children and adults, but not infants, had high titers of ADCC-mediating antibodies against avian H5N1- and H7N9-infected cells. A strong positive correlation was observed between age and serum ADCC titer against H7N9-infected cells.[60] Jegaskanda *et al.* showed that adults aged ≥45 years had higher pre-2009 pandemic levels of serum ADCC activity against the A(H1N1)pdm09 than younger adults or children,[57] which may have contributed to their increased protection during the 2009 pandemic. Together, these studies indicate that serum antibodies with ADCC activity are common in humans, accumulate with age and may contribute to protective immunity against avian-origin influenza viruses with pandemic potential.

Seasonal influenza vaccination with trivalent or quadrivalent influenza vaccines is routine in developed countries, especially for high-risk groups. Immunization with inactivated seasonal influenza vaccines has been shown to boost serum ADCC activity against vaccine viruses in children, older adults (aged ≥ 65 years), health-care workers, pregnant women and HIV+ adults.[55,59,61–63] Vaccination with a trivalent influenza vaccine also increased ADP activity against HA proteins from the vaccine strains in adults.[64] Because older adults are more susceptible to influenza morbidity and mortality, there is a growing interest in developing more protective vaccines for this group. Serum ADCC activity was increased in older adults who seroconverted following trivalent influenza vaccination, but the ADCC response was modest and variable.[59] Many countries have

approved administration of adjuvanted and high-dose seasonal influenza vaccines to older adults, with the aim of improving response to vaccination. Whether adjuvanted and high-dose influenza vaccines are more effective at inducing Fc-functional antibodies in older adults than standard trivalent or quadrivalent influenza vaccines is not known. Influenza vaccination can also boost Fc-functional antibodies targeting divergent strains of influenza virus. Healthy adults immunized with a quadrivalent influenza vaccine demonstrated increased serum ADCC activity against HA proteins from the H3N2 vaccine virus as well as an antigenically drifted H3N2 virus.[65] Similarly, a monovalent inactivated subunit vaccine against the A(H1N1)pdm09 virus increased titers of ADCC-mediating antibodies against heterologous group 1 influenza viruses.[66] Vaccines against avian-origin H5N1 and H7N9 influenza viruses have been studied in preparation for a possible pandemic. Two doses of an adjuvanted H7N9 influenza vaccine elicited a robust, cross-reactive ADCC response capable of targeting antigenically drifted H7N9 viruses, including a highly pathogenic human isolate.[67] Similar findings were also reported for adjuvanted H5N1 vaccines.[65] Influenza vaccination can induce ADCC-mediating antibodies, but different vaccine types and formulations may vary in their ability to elicit an Fc-functional antibody response in humans.

Studying naturally acquired human influenza infection is challenging because of the absence of preinfection samples, limited information regarding previous influenza exposures and variable timing and severity of disease presentation. Humans with naturally acquired influenza infections have greater serum ADCC activity than uninfected controls, but the timing of symptom presentation (or hospital admission) is typically too late to observe a rise in ADCC activity over time.[55,66,68] We showed that humans who succumbed to H7N9 infection had reduced Fc-functional antibodies, in magnitude and breadth, compared with those who survived infection.[68] Humans who recovered from severe influenza infections (H7N9 and seasonal) had higher levels of FcγR-binding antibodies against the homologous infecting strain and heterosubtypic influenza A viruses.[68] Cross-reactive ADCC-mediating antibodies may be generated more rapidly than NAbs following natural influenza infection.[68,69] As influenza is an acute infection, the early production of cross-reactive Fc-functional antibodies could be critically important in controlling infection prior to the generation of strain-specific NAbs (Figure 1).

Unlike most natural infection cohorts, experimentally infected humans have preinfection samples to define baseline levels of ADCC and are usually infected with influenza viruses that cause relatively mild disease.

Humans experimentally infected with the A/Wisconsin/67/2005(H3N2) influenza virus showed a significant increase in serum ADCC activity against the HA protein of the infecting virus and infected cells.[66] Individuals with more clinical symptoms during experimental influenza infection also exhibited a greater rise in serum ADCC activity to the HA protein of the infecting virus,[66,70] possibly because of higher viral replication and antigen availability. Following experimental influenza infection, a small number of humans ($n = 3$) with high preinfection ADCC antibody titers (>320) showed reduced disease severity and less detectable virus compared with those with lower ADCC titers.[66] These preliminary findings suggest that high baseline levels of serum ADCC activity may reduce viral load and influenza disease severity. Large experimental and natural infection cohorts are needed to draw specific conclusions about the protective role of Fc-functional antibodies during human influenza.

Antibody-based therapies represent a promising treatment option for patients hospitalized with severe influenza. A small pilot study ($n = 24$) showed that infusion of an influenza-enriched IVIG (Flu-IVIG) increased serum ADCC activity in patients with severe influenza infections.[69] A larger clinical trial ($n = 308$) carried out by the same INSIGHT group revealed that high titers of HAI antibodies in Flu-IVIG-infused patients were, overall, not associated with improved clinical outcomes.[71] A robust rise in serum HAI titers postinfusion with Flu-IVIG did not result in any clinical benefit for patients with severe influenza A. Despite the absence of a rise in HAI titer, the clinical benefits of Flu-IVIG infusion were evident in the subgroup of patients with severe influenza B infection, suggesting a possible role for antibodies with non-neutralizing Fc functions.[71]

OTHER VIRAL TARGETS OF FC-MEDIATED ANTIBODY FUNCTION

Because influenza HA is prone to antigenic drift, there has been a renewed interest in Fc-functional antibodies targeting more conserved influenza antigens such as nucleoprotein (NP) and the ectodomain of matrix 2 protein (M2e). NP is expressed on the surface of influenza-infected cells *in vitro* and may provide a conserved target for antibodies with Fc functions.[72] Infusion of NP antibodies protects mice from lethal heterosubtypic influenza virus infection in an FcγR- and CD8 T-cell-dependent manner.[73] The precise mechanism of NP antibody-dependent protection in mice is unclear, but it has been suggested that FcγR-mediated uptake of NP antibody ICs by dendritic cells may increase viral antigen presentation to CD8 T cells. However, the addition of ICs consisting of human anti-NP mAbs and

inactivated influenza virus did not increase antigen presentation by dendritic cells *in vitro*.[74] Healthy and influenza-infected humans have NP-specific antibodies capable of cross-linking FcγRIIIa and activating NK cells.[70] Terajima *et al.* reported that sera from children and adults mediated ADCC of H7N9-infected cells.[60] Additional analyses of these samples showed high titers of ADCC-mediating antibodies against H7N9 NP, with undetectable or low ADCC titers against H7N9 HA and neuraminidase glycoproteins.[75] These studies suggest that antibodies targeting NP may be able to mediate ADCC against a diverse spectrum of influenza viruses, but future experiments with human NP mAbs are needed to characterize their protective mechanism and therapeutic potential.

The M2e is found on the surface of influenza-infected cells and may be accessible to antibodies with Fc-mediated functions. Infusion of anti-M2e immune serum protects wild-type mice, but not FcγR$^{-/-}$ mice, from lethal A/Puerto Rico/8/1934 infection.[76] Prophylactic and therapeutic treatment of mice with a human M2e-specific mAb (Z3G1) reduced viral load in the lungs and protected mice from lethal influenza virus challenge through FcγR- and complement-dependent mechanisms.[77] Another human M2e mAb (Ab1-10) activated NK cells and killed influenza-infected as well as matrix 2-expressing cells *in vitro*.[78] More conserved influenza antigens may be important targets for Fc-functional antibodies and could inform the development of universal influenza vaccines and immunotherapies.

THE DELICATE BALANCE: IMMUNITY *VERSUS* IMMUNOPATHOLOGY

Many studies highlight the therapeutic and protective potential of Fc-functional antibodies during viral infection, but relatively few examine their capacity to enhance viral disease. Extracellular viral antigens (released from dead or dying cells) can bind to uninfected bystander cells causing tissue damage. Two murine studies have described enhanced lung pathology following vaccination with a known influenza ADCC epitope or infusion with non-neutralizing mAbs after H3N2 virus challenge.[79,80] No difference in adverse effects was reported for influenza-infected humans infused with Flu-IVIG compared with placebo in a recent clinical trial.[71] Nevertheless, it is essential that antibodies with Fc-mediated functions are assessed as rigorously for immunopathology and tissue damage as they are for antiviral effects, particularly in the context of antibody-based therapies and next-generation vaccines.

PROTECTIVE POTENTIAL OF FC-MEDIATED ANTIBODY FUNCTIONS DURING OTHER VIRAL INFECTIONS

A vast body of literature has examined the protective effects of Fc-mediated antibody functions against viral pathogens including HIV, herpes simplex virus (HSV), Ebola virus (EBOV), Marburg virus and Dengue virus (DENV). The RV144 HIV vaccine trial identified ADCC as an important correlate of protection against HIV infection in vaccine recipients.[81] Further, HIV+ controllers, who control HIV replication without antiretroviral therapy, demonstrate increased breadth of HIV-binding antibodies and higher ADCC activity compared with individuals with progressive HIV infection.[82]

Fc-mediated protection of neonatal mice from HSV was first described in the early 1980s. Adoptive transfer of a combination of human leukocytes and subneutralizing levels of antibody were highly protective against lethal HSV infection in neonatal mice,[83] but it was later discovered that HSV expresses a viral FcγR that binds to the Fc region of human (not murine) IgG. The HSV FcγR blocks Fc-mediated functions of IgG including ADCC and complement activation.[84] Expression of viral FcγRs is an immune evasion strategy that can limit the protective potential of Fc-functional antibodies against some clinically important members of the Herpesviridae family including HSV, varicella-zoster virus and cytomegalovirus.

Several recent studies have suggested that ADCC may be critical for protection and treatment of hemorrhagic fever viruses, including EBOV and Marburg virus. Treatment efficacy of EBOV-specific mAbs in mice correlated with their ADCC activity in vitro. Further, NK cell-deficient mice treated with these EBOV-specific mAbs showed reduced viral clearance compared with wild-type mice, suggesting that NK cell-mediated ADCC is a major component of the anti-EBOV immune response.[85] Gunn et al. found that non-neutralizing and poorly neutralizing EBOV-specific mAbs with Fc effector functions (including NK cell activation and ADP) provided protection in vivo.[86] A promising rabies-vectored Marburg virus vaccine (FILORAB3) achieved >90% protection against a murine-adapted Marburg virus challenge, despite the absence of NAbs. Sera from FILORAB3-vaccinated mice had high levels of ADCC activity in vitro and $FcγR^{-/-}$ mice were not protected by FILORAB3 vaccination.[87]

While Fc-dependent protection against viruses has been widely studied, Fc-mediated functions have also been linked to antibody-dependent enhancement of certain viral infections. Antibody-dependent enhancement has been widely reported for DENV infection. A small fraction (<15%) of human DENV infections that occur in the presence of reactive, non-neutralizing IgG progress into dengue shock syndrome or dengue hemorrhagic fever.[88] Wang et al. recently showed that dengue shock syndrome/dengue hemorrhagic fever patients respond to DENV infection by producing IgG1 with afucosylated Fc domains, which enhances IgG affinity for FcγRIIIa and triggers a decrease in platelets possibly by ADCC. This suggests that Fc-mediated antibody functions not only lead to antibody-dependent enhancement of DENV infection, but also enhance disease severity in the presence of certain Fc modifications.[88]

CONCLUSIONS

The ability of antibodies to engage FcRs and mediate antiviral effector functions has been described for many important viral pathogens, including influenza virus. Recent studies have illustrated that Fc-mediated effector functions, in addition to direct neutralization, are required for protection against influenza virus infection. Despite the recent interest in influenza-specific ADCC and ADP, several important questions remain unanswered. Additional studies are needed to determine the precise mechanism(s) of Fc-mediated protection in vivo and to identify the influenza antigen(s) capable of eliciting the most universally protective humoral response. The antibody Fc region has substantial potential for use in the development of novel immunotherapies and vaccines targeting clinically relevant viruses.

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CONFLICT OF INTEREST

All authors report no conflicts of interest.

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