

# The role of Fc–Fc $\gamma$ R interactions in IgG-mediated microbial neutralization

Stylianos Bournazos, David J. DiLillo, and Jeffrey V. Ravetch

The Laboratory of Molecular Genetics and Immunology, The Rockefeller University, New York, NY 10065

**Antibodies are bifunctional molecules, containing a variable Fab domain that mediates binding specificity and a constant Fc domain that bridges antibody-coated targets with Fc $\gamma$ R-expressing cells that mediate effector functions. Although traditional mechanisms of antibody-mediated neutralization of microbes have been largely thought to result from Fab–antigen interactions, recent studies suggest that recruitment of Fc $\gamma$ R-expressing effector cells by antibodies is a major *in vivo* mechanism of antibody-mediated protection from infection. In this article, we review Fc $\gamma$ R biology, compare mammalian Fc $\gamma$ R families, and summarize recent evidence demonstrating the crucial role that Fc–Fc $\gamma$ R interactions play during *in vivo* protection from infection.**

## CORRESPONDENCE

Jeffrey V. Ravetch:  
ravetch@rockefeller.edu

## Abbreviations used:

A/I, activating/inhibitory;  
HA, hemagglutinin; ITAM,  
immunoreceptor tyrosine-  
based activation motif;  
ITIM, immunoreceptor  
tyrosine-based inhibitory motif;  
RSV, respiratory syncytial virus.

Seminal studies by Emil von Behring on the activity of diphtheria antiserum during the late 19th century provided physicians with the first effective therapeutic intervention against infectious diseases (Behring, 1890; Behring and Kitasato, 1890). For over five decades, antibody-based therapy in the form of serum therapy had been effectively used for the prevention and treatment of bacterial and viral infections, including—but not limited to—diphtheria, pneumococcal pneumonia, streptococcal, and meningococcal diseases. The widespread use of this successful approach is historically reflected by the establishment of specialized institutes (e.g., the Institut für Serumforschung und Serumprüfung in Berlin and the Statens Serum Institut in Copenhagen) entirely devoted to the development and validation of serum-based therapeutics. However, after the discovery of broad-spectrum antimicrobial agents, such as antibiotics and virus inhibitors, antibody therapy against infectious diseases was largely abandoned and reserved only to confer protection after exposure to venoms and tetanus toxoids and for prevention of respiratory syncytial virus (RSV) in high-risk infants. Indeed, antimicrobial drugs are currently the first choice for the treatment of several pathogenic infectious diseases. However, the emergence of new pathogenic microorganisms, the wide dissemination of multidrug-resistant strains, and the long-term toxicity and poor compliance associated

with chronic infections, as well as their relative inefficacy of antimicrobial drugs in immunocompromised individuals clearly highlight the need for novel therapeutic strategies for the prevention and treatment of infectious diseases.

Substantial advances in antibody technologies over the past decades revolutionized our approaches to generating highly specific, well-tolerated mAbs with exceptional *in vivo* activity. With over 40 FDA-approved mAbs currently in clinical use, antibody-based therapeutics are a first-line therapy for several neoplastic and autoimmune disorders, demonstrating unsurpassed efficacy and safety compared with conventional therapeutic interventions (Chan and Carter, 2010; Page et al., 2014). The success of antibody-based therapy has motivated the development of mAbs against infectious diseases, with two antibodies already licensed: palivizumab and raxibacumab for the prevention and treatment of RSV and anthrax infection, respectively. Many more are currently in clinical trials, including antibodies against rabies, influenza, HIV-1, and *Clostridium difficile* (Migone et al., 2009; Lowy et al., 2010; Gogtay et al., 2012; Yoshihara et al., 2013; Caskey et al., 2015). Similarly, an oligoclonal antibody cocktail (ZMapp) has been used as post-exposure therapy during the recent 2014 Ebola pandemic (Qiu et al., 2013).

S. Bournazos and D.J. DiLillo contributed equally to this paper and share first authorship.

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Despite the successful experiences from serum therapy against infectious diseases in the past, the reintroduction of antibodies as therapeutic modalities against infections has previously faced skepticism, mainly because of the highly specific nature of antibodies that normally target restricted epitopes, thereby presenting limited breadth against other microbial subspecies. However, progress in recombinant antibody strategies and in-depth study of human responses to infectious diseases have recently led to the identification, isolation, and characterization of mAbs with broad and potent neutralizing activity, especially for antigenically variable microbes like influenza and HIV-1 (Burton et al., 2012; Klein et al., 2013; Laursen and Wilson, 2013). Recent results from preclinical evaluation studies strongly suggest the use of these broadly neutralizing antibodies as promising and effective therapeutic molecules against infectious diseases, offering significant advantages over conventional antimicrobial agents (Corti et al., 2011; Klein et al., 2012; Tan et al., 2012; Barouch et al., 2013; Laursen and Wilson, 2013; Shingai et al., 2013; DiLillo et al., 2014; Caskey et al., 2015). Indeed, antibodies present remarkably little toxicity and are not subject to multidrug resistance mechanisms, and their highly specific antigenic reactivity ensures that nontargeted microbes, such as those comprising the normal microbial flora, remain unaffected. Additionally, with a serum half-life of up to 3 wk, antibodies provide sustained protection for a vastly extended timeframe compared with many antimicrobial drugs.

More importantly, the capacity of antibodies to initiate and regulate effector functions through their Fc domain is a key component of their *in vivo* protective activity. Although the neutralizing activity of antibodies has been previously considered to be solely the outcome of Fab-antigen interactions, it has become apparent that their *in vivo* activity is highly dependent on interactions of the IgG Fc domain with its cognate receptors, Fc $\gamma$  receptors (Fc $\gamma$ R), expressed on the surface of effector leukocytes (Pincetic et al., 2014). In this review, we will focus on Fc $\gamma$ R expression and function, compare Fc $\gamma$ R biology between humans and other experimental mammalian species, and evaluate the contributions of Fc $\gamma$ R family members during *in vivo* antibody-mediated protection against infectious diseases. We will highlight the recent observations that demonstrate the importance of Fc effector functions for optimal activity of broadly neutralizing mAbs against various infectious agents (Bournazos et al., 2014c; DiLillo et al., 2014) and suggest approaches for using this information for the development of second generation, Fc-optimized neutralizing mAbs.

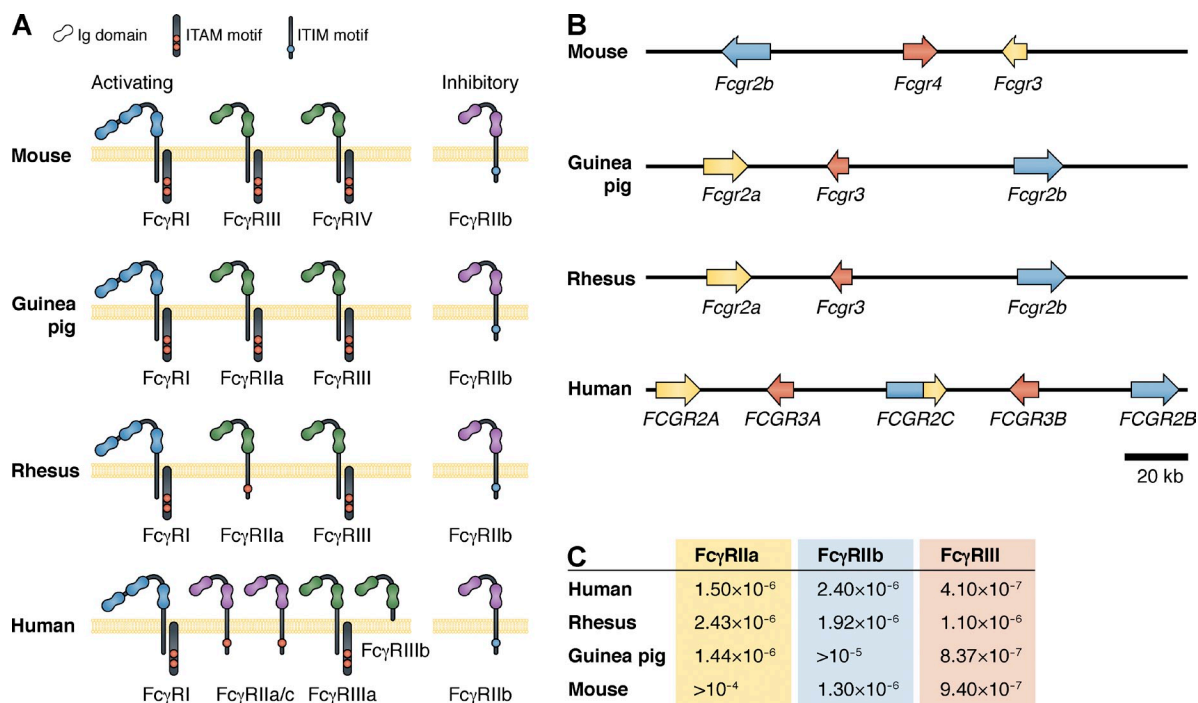
### Fc $\gamma$ R function and activities

IgG antibodies engaged in immune complexes or antibodies coating the surface of opsonized cells or microbes mediate downstream effector functions by binding to either type I or type II Fc $\gamma$ R (Pincetic et al., 2014). Type I Fc $\gamma$ R include the canonical Fc $\gamma$ R for IgG and are members of the immunoglobulin superfamily. Type II Fc $\gamma$ R include CD209 (DC-SIGN in humans and SIGN-R1 in mice) and CD23 and fall into the C-type lectin receptor family. The conformational state of

the IgG Fc domain, physiologically regulated by the precise composition of the complex biantennary N-linked glycan attached to Asn297, determines whether type I or type II Fc $\gamma$ R are engaged by an IgG Fc. IgG Fc domains with a terminal sialic acid attached to the core heptasaccharide conjugated to the amino acid backbone of the IgG Fc domain at Asn297 adopt a flexible, “closed” conformational state, thereby permitting binding to type II Fc $\gamma$ R while engagement of type I Fc $\gamma$ R is inhibited. In contrast, nonsialylated IgG Fcs adopt an “open” conformation that suppresses engagement of type II Fc $\gamma$ R and promotes interactions with type I Fc $\gamma$ R members (Sondermann et al., 2013; Ahmed et al., 2014; Pincetic et al., 2014).

Several previous studies clearly demonstrated that type II Fc $\gamma$ R engagement by sialylated IgG Fcs results in active suppression of antibody-mediated and T cell-mediated inflammation (Anthony and Ravetch, 2010; Anthony et al., 2012; Schwab and Nimmerjahn, 2013; Fiebiger et al., 2015). In contrast, engagement of type I Fc $\gamma$ R results in an array of pleiotropic proinflammatory and immunomodulatory consequences. Fc $\gamma$ R cross-linking triggers antibody-dependent cellular cytotoxicity and/or phagocytosis (ADCC/ADCP), in which IgG bridges target cells or microbes and Fc $\gamma$ R-expressing effector cells to mediate cytotoxicity or phagocytosis (Nimmerjahn and Ravetch, 2008). Other Fc $\gamma$ R-mediated effector functions include maturation and activation of APCs, including DCs, enhanced antigen uptake and presentation by APCs, cellular activation and release of cytokines and chemokines by innate effector cells, regulation of affinity maturation of B cells in the germinal center by setting thresholds for B cell activation, and plasma cell survival and regulation of antibody production (see Fig. 2; Pincetic et al., 2014).

The type I Fc $\gamma$ R family is a group of structurally and functionally related receptors that belong to the immunoglobulin superfamily and share highly conserved intracellular signaling components (Fig. 1 A). Fc $\gamma$ R are broadly classified into either activating or inhibitory Fc $\gamma$ R based on the presence of intracellular immunoreceptor tyrosine-based activation motifs (ITAMs) or immunoreceptor tyrosine-based inhibitory motifs (ITIMs), respectively, that have the capacity to transduce immunostimulatory or immunosuppressive signals after receptor cross-linking by IgG immune complexes (Nimmerjahn and Ravetch, 2008). For humans, activating Fc $\gamma$ R include Fc $\gamma$ RI, Fc $\gamma$ RIIa, Fc $\gamma$ RIIc, and Fc $\gamma$ RIIIa, whereas Fc $\gamma$ RIIb is the sole inhibitory Fc $\gamma$ R. Fc $\gamma$ RIIIb, a GPI-linked receptor exclusively expressed at high levels on neutrophils, functions in concert with the activation receptor Fc $\gamma$ RIIa to mediate cellular responses. Both activating and inhibitory Fc $\gamma$ R are expressed by most cells of the innate immune system. Notable exceptions include NK cells, which express only activating Fc $\gamma$ R (Fc $\gamma$ RIIIa in humans), and B cells, which express only the inhibitory Fc $\gamma$ RIIb. With the exception of Fc $\gamma$ RI, the Fc $\gamma$ R are of low affinity for IgG and thus do not engage monomeric IgG at physiological conditions. These receptors must engage multimeric immune complexes or IgG-coated targets to trigger receptor cross-linking and subsequent cellular responses. The cellular outcome



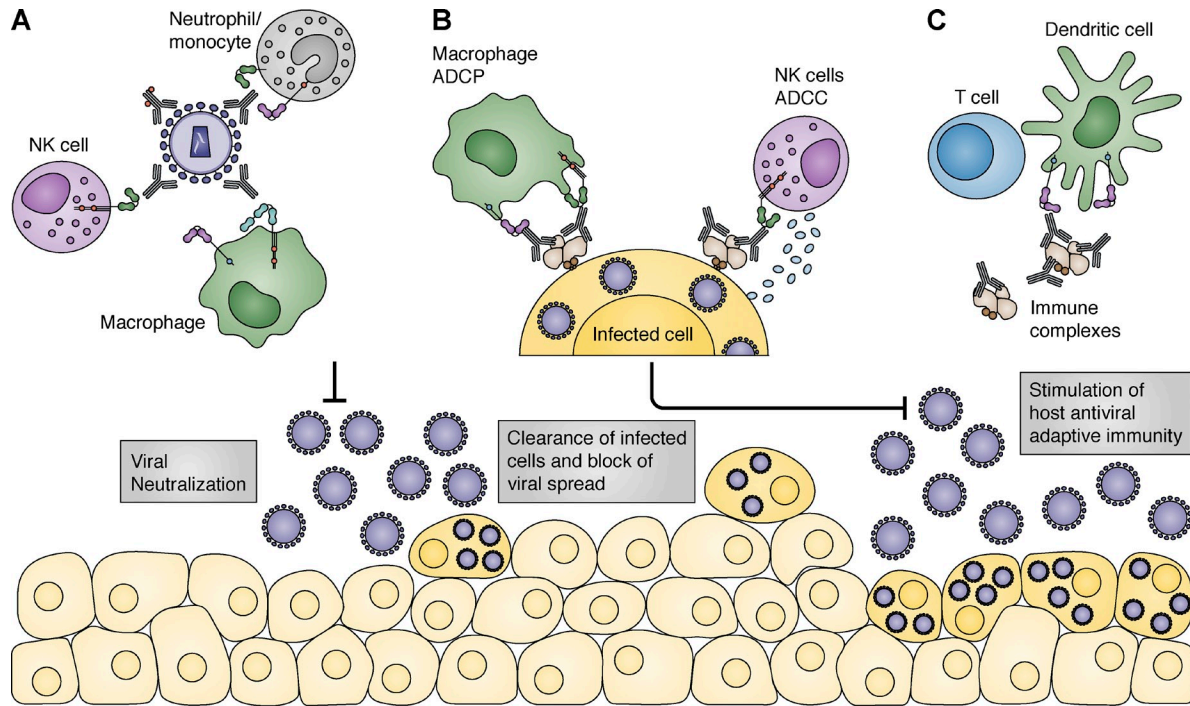
**Figure 1. Overview of the Fc $\gamma$ R family.** (A) Schematic representation of the different Fc $\gamma$ R classes. Fc $\gamma$ Rs are broadly categorized as activating or inhibitory, based on the presence of an intracellular ITAM (red) or ITIM (blue) that transduces activating or inhibitory signals upon receptor cross-linking by IgG complexes. The extracellular domain of Fc $\gamma$ Rs consists of two (three for Fc $\gamma$ RI) Ig domains that mediate IgG binding. (B) Genomic organization of the Fc $\gamma$ R locus in the indicated species. With the exception of Fc $\gamma$ RI, all Fc $\gamma$ R genes are mapped at a common, highly conserved locus. The unique organization of the human Fc $\gamma$ R locus is the result of nonhomologous recombination that gave rise to additional Fc $\gamma$ R genes (*FCGR2C* and *FCGR3B*). (C) Human IgG1 binding affinities ( $K_d$  [M]) to the low-affinity Fc $\gamma$ Rs of human, rhesus, guinea pig, and mouse. Fc $\gamma$ Rs among different species are grouped based on sequence homology and named after the corresponding human orthologue.

of IgG interactions with Fc $\gamma$ Rs is governed by the affinity of the Fc for the specific Fc $\gamma$ R and the expression pattern of those receptors on the effector cells. Therefore, the outcome of IgG-mediated inflammation and immunity is largely determined by balancing activating or inhibitory signals transduced by activating or inhibitory Fc $\gamma$ Rs, respectively (Nimmerjahn and Ravetch, 2006).

Because most effector leukocytes express both activating and inhibitory Fc $\gamma$ Rs, the relative ratio of the binding affinities of these receptors for a specific IgG Fc determines the outcome of the IgG–Fc $\gamma$ R interaction. These binding affinities are regulated by the amino acid sequences of the different IgG Fc subclasses and the IgG Fc's N-linked glycan structure. Thus, the IgG Fc composition can dramatically influence the in vivo outcome of Fc $\gamma$ R engagement by immune complexes or opsonized cells by directing the effector cell into either a pro- or antiinflammatory state. For example, the mouse (m)IgG2a subclass engages the activating mFc $\gamma$ RIV (orthologue of human Fc $\gamma$ RIIIa) with 100-fold greater affinity compared with the inhibitory mFc $\gamma$ RIIb receptor, whereas mIgG1 preferentially engages the inhibitory mFc $\gamma$ RIIb receptor (Nimmerjahn and Ravetch, 2005). Thus, mIgG2a antibodies have an activating/inhibitory (A/I) ratio (ratio of the Fc's affinity for the relevant activating Fc $\gamma$ R versus the affinity for the inhibitory Fc $\gamma$ R) of  $\sim 70$ . In contrast, mIgG1 antibodies

have an A/I ratio of 0.1. The in vivo activities of mIgG2a and mIgG1 antibodies correspond to their A/I ratios, and cytotoxic antibodies of the mIgG2a subclass potently kill target cells in vivo, whereas mIgG1 antibodies have minimal activity (Nimmerjahn and Ravetch, 2005). Similarly in humans, human (h)IgG2 and hIgG4 poorly interact with human Fc $\gamma$ Rs, whereas hIgG1 and hIgG3 interact more strongly (Nimmerjahn and Ravetch, 2007).

Apart from the IgG subclass and glycan composition, additional determinants for the regulation of IgG–Fc $\gamma$ R interactions exist, including IgG immune complex size and the localization of Fc $\gamma$ Rs within membrane microdomains that favor interactions with intracellular signaling proteins (Floto et al., 2005; Bournazos et al., 2009a; Lux et al., 2013). Furthermore, allelic and copy number variants of the human Fc $\gamma$ R genes affect binding affinities to human IgG, as well as receptor activity and expression (Bournazos et al., 2009b). These variants modulate in vivo antibody effector function, as cancer patients carrying the high-affinity allelic variants of Fc $\gamma$ RIIIa and Fc $\gamma$ RIIIa (V158 and H131, respectively) demonstrate greater responses to antitumor mAb therapies (Cartron et al., 2002; Weng and Levy, 2003; Musolino et al., 2008). Indeed, selective engagement of certain Fc $\gamma$ R classes by IgG has been shown to determine the outcome of passive antibody treatment in vivo, suggesting that Fc-mediated pathways play a key



**Figure 2. Fc $\gamma$ R-mediated effector pathways during viral infection.** Interactions of the IgG Fc domain have pleiotropic effects that contribute to the *in vivo* protective activity of antibodies during infection. (A) IgG-opsonized viral particles are cleared by Fc $\gamma$ R-expressing effector leukocytes, like neutrophils, macrophages, and NK cells. (B) Additionally, IgG binding to infected cells expressing viral proteins on their surface recruits effector leukocytes, such as macrophages and NK cells, through Fc–Fc $\gamma$ R interactions. Infected cells are thereby cleared by Fc $\gamma$ R-expressing leukocytes, limiting the viral reservoir and preventing further viral spreading. (C) Lastly, IgG–antigen immune complexes generated during these steps have the capacity to stimulate host immune responses through Fc $\gamma$ R engagement on DCs, inducing cellular maturation and enhancing antigen presentation to T cells.

role in modulating the effector activities of an antibody. For example, enhanced engagement of activating Fc $\gamma$ Rs by anti-tumor mAbs greatly improves their cytotoxic activity (Clynes et al., 2000; Smith et al., 2012; Goede et al., 2014), whereas selective binding to the inhibitory Fc $\gamma$ RIIb is necessary for optimal activity of agonistic anti-CD40 mAbs (Li and Ravetch, 2011). In the context of infectious diseases, Fc–Fc $\gamma$ R interactions readily amplify the *in vivo* protective activity of neutralizing antibodies through opsonization and clearance of microbes and their toxins, as well as by cytotoxic killing of infected cells (Fig. 2; Bournazos et al., 2014a,c; DiLillo et al., 2014). Additionally, Fc $\gamma$ R engagement by immune complexes may induce pleiotropic proinflammatory and immunomodulatory functions with the potential to initiate sustained antimicrobial immune responses (Fig. 2).

### Fc $\gamma$ R interspecies conservation

With the exception of the high-affinity Fc $\gamma$ RI, all members of the Fc $\gamma$ R family are mapped at a common Fc $\gamma$ R locus (located at 1q23 for *Homo sapiens*) that is highly conserved among species. Indeed, the ancestral Fc $\gamma$ R locus that encompasses the genes coding for the low-affinity Fc $\gamma$ Rs (Fc $\gamma$ RIIa/b/c and Fc $\gamma$ RIIIa/b) shares a common genomic organization that can be traced back early in evolutionary history and presents substantial similarities among different mammalian species

(Fig. 1 B). Furthermore, the capacity of other mammalian Fc $\gamma$ Rs to interact with human IgG molecules is indicative of the high degree of Fc $\gamma$ R gene similarity (Fig. 1 C).

Despite such similarities in the Fc $\gamma$ R structure and function, there are several fundamental differences among mammalian species stemming mainly from the unique characteristics of human Fc $\gamma$ Rs. For example, the human Fc $\gamma$ R locus exhibits a unique organization pattern that is the result of a nonhomologous recombination event of the ancestral Fc $\gamma$ R locus that gave rise to human *FCGR2C* and *FCGR3B* genes encoding for Fc $\gamma$ RIIc and Fc $\gamma$ RIIIb, respectively (Qiu et al., 1990). Likewise, Fc $\gamma$ R expression patterns on different leukocyte types differ substantially between different species. For example, nonhuman primate neutrophils express Fc $\gamma$ RI, Fc $\gamma$ RIIa, and Fc $\gamma$ RIIb, whereas in humans, neutrophils express Fc $\gamma$ RIIa, Fc $\gamma$ RIIb, and Fc $\gamma$ RIIIb (unpublished data). Likewise, mouse monocyte-derived DCs express Fc $\gamma$ RI, Fc $\gamma$ RIIb, Fc $\gamma$ RIII, and Fc $\gamma$ RIV, contrary to their human counterparts that express only Fc $\gamma$ RIIa and Fc $\gamma$ RIIb. Similar interspecies differences have been observed in other cell types, including NK cells, macrophages, and other effector leukocytes (Smith et al., 2012).

Such differences in the Fc $\gamma$ R structure and expression pattern among different mammalian species commonly used as infectious disease models do not allow for the precise evaluation

of the *in vivo* Fc effector activities of neutralizing antibodies. Careful characterization of the Fc $\gamma$ R expression pattern and their capacity to interact with human IgG molecules is therefore required before the assessment of the *in vivo* activity of antibodies in other experimental species. Additionally, recently developed mouse strains humanized for Fc $\gamma$ R genes could be used to accurately assess the contribution of Fc effector pathways in the *in vivo* protective activity of neutralizing antibodies (Smith et al., 2012; Bournazos et al., 2014b). Such strains encompass specific deletions of all mouse Fc $\gamma$ R genes, with human Fc $\gamma$ Rs introduced as BAC transgenes with expression under the control of their endogenous promoters and regulatory elements (Smith et al., 2012). This approach ensures proper human Fc $\gamma$ R expression patterns and cell type specificity. Indeed, Fc $\gamma$ R-humanized mice faithfully recapitulate the unique characteristics of human Fc $\gamma$ R functional and structural diversity, with their Fc $\gamma$ R expression profiles among the various leukocyte types mirroring precisely those observed in humans (Smith et al., 2012). Fc $\gamma$ R-humanized mice have been successfully used to assess the contribution of Fc effector function of passively administered antibodies to their *in vivo* protective activity in models of influenza, HIV-1, and anthrax infection (Bournazos et al., 2014a,c; DiLillo et al., 2014). Additionally, the Fc $\gamma$ R-humanized mouse model has become a useful preclinical evaluation platform for comparing the *in vivo* activity of Fc domain-engineered variants of antibodies with enhanced capacity to engage particular human Fc $\gamma$ R classes (Smith et al., 2012; Bournazos et al., 2014a; DiLillo and Ravetch, 2015).

### Fc effector mechanisms during infection

The traditional notion that antibody-mediated neutralization of a microbe or toxin is the result of Fab-antigen interactions has relied on *in vitro* assays, which fail to reproduce the diversity of receptors, effector cells, and microenvironments that contribute to protection during *in vivo* conditions. Such *in vitro* assays provide limited information on the precise Fc effector mechanisms that participate *in vivo* to mediate host protection against microbial or toxin challenge. By focusing on *in vivo* studies in the following sections, using examples from influenza, HIV-1, and other infectious diseases, we will review recent data demonstrating that Fc-Fc $\gamma$ R interactions are crucial for mediating optimal *in vivo* protection by neutralizing mAbs. In view of this evidence, it is now clear that ideal antibody-based therapeutics need to combine potent and broad neutralization capabilities with optimal Fc effector function to mediate maximal *in vivo* protective effects.

**Influenza.** Antibodies that neutralize influenza virus traditionally have been thought to function in one of three ways, based on *in vitro* neutralization experiments. First, anti-hemagglutinin (HA) head antibodies disrupt virus attachment to sialic acids on the surface of target cells to block viral entry (Wiley et al., 1981; Knossow et al., 2002). Second, some anti-HA head and anti-neuraminidase (NA) antibodies may function to prevent viral budding and the release of progeny. Finally, anti-HA stalk

antibodies can block viral fusion with the target cell (Ekiert et al., 2009). However, recent studies have more carefully clarified the *in vivo* mechanisms of viral neutralization and revealed that Fc-Fc $\gamma$ R interactions are required for effective anti-influenza mAb-mediated protection from lethal influenza virus infection *in vivo*. For example, vaccines targeting the ectodomain of matrix protein 2 (M2e) of influenza A virus induce anti-M2e antibodies. M2e is not expressed on the viral particle, and anti-M2e antibodies do not neutralize virus *in vitro*. However, anti-M2e antibodies are protective against influenza infection in mice; this protection is mediated by alveolar macrophages expressing activating Fc $\gamma$ Rs (El Bakkouri et al., 2011; Lee et al., 2014), which likely mediate cytotoxicity or phagocytosis of antibody-coated infected cells. Furthermore, anti-M2e antibodies of the mIgG2a subclass, which preferentially engage mouse-activating Fc $\gamma$ Rs, mediate *in vivo* protection, with no activity observed for the mIgG1 subclass, which engages the inhibitory Fc $\gamma$ RIIb (Schmitz et al., 2012). Furthermore, Fc $\gamma$ R expression by macrophages was required for protection mediated by passive serum transfers from mice immunized with H1N1 virus (Huber et al., 2001). Collectively, these results suggest that infection of cells with influenza virus leads to cell surface expression of viral proteins, such as M2e, that serve as targets for antibodies that trigger Fc $\gamma$ R-mediated clearance of infected cells.

HA is the most abundant glycoprotein expressed on the influenza viral envelope, as well as on influenza-infected cells. Influenza HA is composed of two major domains: the antigenically variable globular head and the relatively conserved stalk. Because of its immunodominance and high level of antigenic variation, the majority of the immune response is directed at the HA head domain, and most antibodies reactive with the HA head domain only neutralize a single influenza strain. In contrast, antibodies targeting the conserved HA stalk domain have been found to be broadly neutralizing, reactive with a wide range of influenza subtypes. Recent studies have mechanistically assessed the requirements for Fc-Fc $\gamma$ R interactions during *in vivo* protection mediated by either anti-HA stalk broadly neutralizing antibodies or strain-specific anti-HA head antibodies (Corti et al., 2011; DiLillo et al., 2014). Although unnecessary for *in vitro* neutralization, interactions with activating Fc $\gamma$ Rs were required for *in vivo* protection mediated by a panel of broadly neutralizing anti-HA stalk antibodies after lethal influenza infection (DiLillo et al., 2014). This Fc $\gamma$ R-mediated protection required interactions with the IgG Fc domain after viral entry into target cells, suggesting that HA-expressing, antibody-opsonized infected cells were targeted for clearance by ADCC/ADCP mechanisms, consistent with an earlier study (Corti et al., 2011). Interestingly, strain-specific anti-HA head mAbs protected mice from lethal infection independently of Fc-Fc $\gamma$ R interactions, a property of these mAbs that correlates with their inability to interact with Fc $\gamma$ Rs and trigger ADCC during *in vitro* assays (DiLillo et al., 2014). In contrast, anti-HA stalk broadly neutralizing mAbs potently interact with Fc $\gamma$ Rs and trigger ADCC *in vitro*. Thus, at least two classes of neutralizing antibodies are co-selected in response to viral challenge to provide

multiple pathways for antiviral protection: those that require the Fc $\gamma$ R effector system and induce clearance of infected cells and those that do not. Recent results have shown that all antibodies with a wide breadth of reactivity (broadly neutralizing antibodies) analyzed thus far demonstrate a dependence on Fc–Fc $\gamma$ R interactions to mediate protection in vivo (unpublished data), suggesting that Fc $\gamma$ R engagement is a common mechanism of action for all anti-HA broadly neutralizing antibodies.

Because of their dependence on Fc–Fc $\gamma$ R interactions to mediate protection in vivo, a recent study has addressed whether a broadly neutralizing hIgG1 antibody could be Fc engineered for augmented protection in vivo in the context of the human Fc $\gamma$ R system in Fc $\gamma$ R–humanized mice (DiLillo et al., 2014). By introducing point mutations in the hIgG1 Fc domain of an anti-HA stalk broadly neutralizing antibody to selectively enhance interactions with activating human Fc $\gamma$ Rs, survival was enhanced at least twofold, and weight loss was decreased after lethal influenza challenge compared with anti-HA stalk mAb with a wild-type human IgG1 Fc domain. Thus, optimizing interactions between neutralizing antibodies and the Fc $\gamma$ R system is an attractive approach to enhancing the efficacy of passively administered anti-influenza antibody therapeutics.

**HIV.** For years, the development and use of potent neutralizing antibodies against HIV have been a challenge for efforts to control HIV-1 infection. Indeed, HIV-1 presents several unique structural and functional determinants that conventional antibody strategies must overcome to block viral entry to target cells. For example, the envelope glycoprotein of HIV-1 (env) is present at remarkably low density on the virus surface, thereby precluding high-avidity concurrent interactions of both IgG Fab arms (Klein and Bjorkman, 2010). In addition, its highly glycosylated structure forms a glycan shield that masks sites of potential vulnerability, further contributing to the limited immunogenicity of the HIV-1 env (Burton et al., 2012; Moore et al., 2012). HIV-1 env also exhibits substantial diversity (up to 35% amino acid sequence divergence), attributed to the high virus mutation rate and its capacity to remain latent for several years, even in chronically treated patients, as well as the chronicity of infection, during which antibody responses exert selection pressure on the virus (Korber et al., 2001; Gaschen et al., 2002). Although these immune evasion mechanisms greatly compromise the host's capacity to mount potent broadly neutralizing antibody responses, early clinical studies have identified a small fraction of infected individuals that develop affinity-matured antibodies with broad activity against diverse, cross-clade virus isolates (Simek et al., 2009). These individuals, commonly referred to as “elite neutralizers,” have inspired the development and use of broadly neutralizing antibodies as a therapeutic modality to prevent and control HIV-1 infection. Thanks to recent advancements in B cell cloning techniques, the systematic isolation and characterization of broadly neutralizing antibodies from elite neutralizers has become a reality, leading to the development over the past 5 yr of several dozen anti-HIV-1

env mAbs with potent and broad activity (reviewed in Klein et al. [2013]). Passive administration of these broadly neutralizing mAbs has been shown to confer sterilizing immunity against SHIV challenge in macaques and HIV-1 infection in humanized mouse models (Mascola et al., 2000; Hessel et al., 2007; Balazs et al., 2012). More importantly, effective control of virus replication in HIV-1–infected humanized mice and in SHIV-infected nonhuman primates by these broadly neutralizing mAbs clearly suggests their potential clinical use to control HIV-1 infection in humans (Klein et al., 2012; Barouch et al., 2013; Horwitz et al., 2013; Shingai et al., 2013). Indeed, administration of the broadly neutralizing anti-CD4bs mAb, 3BNC117, in chronically infected HIV-1 patients successfully suppressed viremia for several days after mAb infusion (Caskey et al., 2015).

Previous studies have suggested a role for Fc $\gamma$ Rs during HIV-1 infection, as indicated by the association of genetic variants of Fc $\gamma$ RIIa with the clinical progression of AIDS (Forthal et al., 2007). Given broadly neutralizing anti-HIV env mAbs were isolated and characterized only recently, the precise Fc effector mechanisms that contribute to their in vivo activity have also only recently been elucidated. Despite the lack of an in vivo HIV-1 infection model that faithfully recapitulates the complete virus infection cycle, human effector cells, and Fc $\gamma$ R diversity, the role of Fc–Fc $\gamma$ R interactions during in vivo mAb activity has been assessed using complementary in vivo models (Klein et al., 2012; Pietzsch et al., 2012; Smith et al., 2012). In particular, using a mouse model for HIV-1 entry, comparison of the in vivo protective activity of a panel of broadly neutralizing mAbs targeting different HIV-1 env epitopes revealed increased activity for the mIgG2a subclass compared with mIgG1 (Bournazos et al., 2014c). This finding is consistent with the capacity of the IgG2a subclass to interact preferentially with activating mFc $\gamma$ Rs, thereby inducing clearance of mAb–opsonized viral particles through activating Fc $\gamma$ R engagement (Nimmerjahn and Ravetch, 2005). Similar effects have been observed in studies using HIV-1–infected humanized mice in models of mAb-mediated post-exposure prophylaxis and therapy (Bournazos et al., 2014c; Halper-Stromberg et al., 2014). Assessment of the in vivo protective activity of Fc variants of anti-HIV-1 mAbs with differential Fc $\gamma$ R binding capacities revealed that administration of mAb variants optimized for enhanced affinity to activating Fc $\gamma$ Rs resulted in substantially improved and durable suppression of viremia in humanized mice with established HIV-1 infection (Bournazos et al., 2014c). Likewise, in a model of post-exposure prophylaxis using HIV-1–infected humanized mice, mAb variants with diminished Fc $\gamma$ R binding capacity failed to suppress viremia, contrary to wild-type hIgG1 mAbs (Halper-Stromberg et al., 2014). Similar effects have been previously observed in a nonhuman primate pre-exposure prophylaxis model, in which Fc domain variants with minimal capacity for Fc $\gamma$ R engagement provided no protection against SHIV challenge (Hessel et al., 2007). These findings clearly suggest a key role for Fc $\gamma$ R pathways during the in vivo protective activity of anti-HIV-1 mAbs and indicate that

selective engagement of activating Fc $\gamma$ R3 greatly enhances their *in vivo* activity, guiding the development of a new generation of broadly neutralizing mAbs optimized for Fc effector function.

**Other infectious diseases.** Similar to the influenza and HIV-1 infection systems, the contribution of Fc $\gamma$ R-mediated interactions to the *in vivo* protective activity of mAbs extends to other viral, fungal, and bacterial diseases. For example, the generation and evaluation of a nonfucosylated glycovariant of the FDA-approved anti-RSV IgG, palivizumab, resulted in significantly improved *in vivo* protective activity compared with the parental mAb (Hiatt et al., 2014). Furthermore, mAbs against the pneumococcal capsular polysaccharide have been shown to require Fc–Fc $\gamma$ R interactions to protect mice from pneumococcal pneumonia after challenge with *Streptococcus pneumoniae* (Weber et al., 2012). Common to divergent infectious disease models, several studies have noted differential *in vivo* activity of mouse IgG subclass variants of protective mAbs, thereby providing evidence for a crucial role for Fc $\gamma$ R effector pathways. Despite having comparable activities *in vitro*, the *in vivo* protective activity of these mAbs correlated precisely with their capacity to engage activating Fc $\gamma$ R3; mIgG2a subclass variants always exhibited improved efficacy *in vivo* compared with mIgG1. This phenomenon has been observed for mAbs protective against bacterial (*Staphylococcus aureus*) and fungal (*Cryptococcus neoformans*) infections, as well as the anthrax toxin (Sanford et al., 1990; Schlageter and Kozel, 1990; Abboud et al., 2010; Varshney et al., 2014). In all cases, an increase in the capacity of a mAb to interact with activating Fc $\gamma$ R3 (increased A/I ratio) was accompanied by improved *in vivo* activity without any impact on their Fab-mediated functions. Based on these findings, efforts to generate mAbs with enhanced *in vivo* activity led to the development of Fc domain-engineered variants with selective binding to activating Fc $\gamma$ R3 (Bournazos et al., 2014a). For example, Fc optimization of an anti-anthrax lethal toxin mAb to selectively engage activating human Fc $\gamma$ R3 substantially improved its protective activity when tested in Fc $\gamma$ R-humanized mice challenged with *Bacillus anthracis* (Bournazos et al., 2014a). Collectively, these findings provide substantial evidence for the contribution of Fc effector function to the *in vivo* activities of protective mAbs in several infectious disease model systems.

### Concluding remarks

Antibodies have become major therapeutic tools with potential treatment applications in a variety of disease settings, especially infectious diseases. Ideal therapeutic antibodies to treat infection should be selected based not only on their antigen-binding characteristics (i.e., specificity, affinity, and broad binding capability) and neutralization ability (based on *in vitro* assays), but also for optimal engagement of the appropriate Fc $\gamma$ R family members and recruitment of effector cells. Such Fc optimization can be achieved by altering the Fc glycan or introducing Fc point mutations that augment the Fc domain's affinity for the appropriate Fc $\gamma$ R, as is currently being tested in cancer therapeutics (Natsume et al., 2009). Thus, combining

superior Fc-independent neutralization and Fc engineering for optimal Fc-dependent effector function will produce the most effective therapeutic antimicrobial antibodies to treat infectious disease in patients.

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