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Beyond binding: antibody effector functions in infectious diseases

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Abstract

Antibodies play an essential role in host defence against pathogens by recognizing microorganisms or infected cells. Although preventing pathogen entry is one potential mechanism of protection, antibodies can control and eradicate infections through a variety of other mechanisms. In addition to binding and directly neutralizing pathogens, antibodies drive the clearance of bacteria, viruses, fungi and parasites via their interaction with the innate and adaptive immune systems, leveraging a remarkable diversity of antimicrobial processes locked within our immune system. Specifically, antibodies collaboratively form immune complexes that drive sequestration and uptake of pathogens, clear toxins, eliminate infected cells, increase antigen presentation and regulate inflammation. The diverse effector functions that are deployed by antibodies are dynamically regulated via differential modification of the antibody constant domain, which provides specific instructions to the immune system. Here, we review mechanisms by which antibody effector functions contribute to the balance between microbial clearance and pathology and discuss tractable lessons that may guide rational vaccine and therapeutic design to target gaps in our infectious disease armamentarium.

More than a century ago, the crucial importance of the humoral immune response in immunity against infection was shown[1]. Specifically, the passive transfer of serum from animals with tetanus or diphtheria provided protection to non-immune animals, which demonstrated the presence of a substance — antibodies — that could confer protection[1]. These observations gave rise to passive serum therapy, which was used for decades to treat infections such as diphtheria, tetanus, scarlet fever, pneumococcal pneumonia and meningitis[2]. Today, serum therapy has been largely replaced by vaccines and antibiotics.

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However, following the development of the theory of antibody formation[3], the discovery of phagocytosis by macrophages[4] and further definition of the immunological origin of antibody diversity[5,6], the groundwork for the development of hybridoma technologies emerged, giving rise to the monoclonal antibody era.

Though studies in infectious diseases led to the discovery of antibodies, monoclonal therapeutics have not been exploited as aggressively against pathogens as they have against other targets. More than 45 monoclonal anti bodies have been licensed for the treatment of autoimmune or oncological diseases[7], but only four mono clonal antibodies have been licensed for infectious disease targets: palivizumab for the prevention of respiratory syncytial virus (RSV) infection in high-risk infants[8], raxibacumab and obiltoxaximab for the prevention and treatment of inhaled anthrax[9] and bezlotoxumab as adjunctive therapy for recurrent *Clostridium difficile* infections[10]. This may be due to the fact that though the mechanism of action of anticancer antibodies is clearly related to the specific destruction of tumour cells or enhancement of tumour-specific T cell immunity[11], the mode of action required for the elimination of pathogens is less certain. Importantly, unlike tumours, some antibodies have been implicated in both protection against and enhancement of infectious diseases (for example, in dengue fever)[12]. Thus, complexities related to the potential pathological consequences of imperfect therapeutic design have potentially slowed the development of the monoclonal therapeutic industry for the treatment of infections. More recently, however, there has been a renaissance of activity in this field[13,14] as a greater understanding is gained of the breadth of antibody functions that may be exploited to eliminate pathogens.

Although technological advances in genomic sequencing have revolutionized the depth and pace of B cell repertoire analysis[15], leading to monoclonal antibody discovery for infectious agents, there is an increasing amount of data suggesting that antigen specificity is insufficient to guarantee protective immunity. Instead, understanding the mechanisms of antibody-mediated protection beyond simple antigen recognition may provide crucial clues for the generation of effective therapeutic antibodies or vaccines. Although antibody production represents the primary correlate of protection induced by nearly all clinically approved vaccines, vaccine-specific titres alone do not always predict efficacy[16,17]. For example, antibody titres fail to predict parasiteaemia following sporozoite challenge after vaccination with candidate malaria vaccines that use the same antigen but follow different regimens. Both a strategy using an adenovirus vaccine vector primed with a protein boost and a regimen consisting of a protein boost alone provided 50% protection from infection. However, protection was observed at significantly different antibody titres[18], with protective circumsporozoite-specific antibody titres in the viral vector-primed arm falling within the range of the non-protected, protein-boosted group; these results suggest that qualitative antibody profiles, beyond antibody quantities, determine protection against malaria. Furthermore, immune-correlate analyses in the trial of the first moderately protective HIV vaccine, RV144 (REFS 19–21), suggested that total HIV-envelope-specific antibody levels were not correlated with protection. Instead, epitope-specific IgG antibodies, levels of specific vaccine-targeting isotypes and/or subclasses (IgA and IgG3) and antibody-dependent cellular cytotoxicity (ADCC) were associated with prevention from infection with HIV. In addition, for several clinically approved vaccines, including those that prevent

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pneumococcal, meningococcal and influenza disease, measures of antibody functionality, including opsonophagocytosis, bactericidal activity and haemagglutination, provide a more robust measure of protective immunity[16] compared with titres alone. Thus, defining the unique qualitative features of antibodies that distinguish individuals who develop exacerbated disease or increased protection represents one rational path towards the improved design of protective vaccines or monoclonal therapeutics.

In this Review, we summarize the structure and functions of different isotypes and subclasses of antibodies that are found in humans. We then cover the various receptors and signalling pathways that are modulated by antibody binding on different cells. Finally, we discuss the various antibody effector functions that exist and contemplate how an increased knowledge of these functions could aid the design of future vaccines and therapeutics.

Antibody features that impact function

All antibodies possess two functional domains — one that confers antigen specificity, known as the antigen-binding fragment (Fab), and another that drives antibody function, known as the crystallizable fragment (Fc). Each antibody has two Fab domains and one Fc domain, generating a 'Y'-shaped or 'T'-shaped molecule that can either exist as a monomer or form multimers (the latter in the case of IgM and IgA). Fab domains are essential to the adaptive nature of the humoral response and evolve during an immune response to improve affinity to a foreign antigen[22,23]. The Fc domain, although referred to as the constant domain, also changes rapidly during an immune response to elicit distinct innate immune effector functions. Specifically, the Fc domain variants include five isotypes (IgM, IgD, IgG, IgA and IgE), each with unique structural features that impact antibody function (BOX 1; FIG. 1). For example, the pentameric form of IgM has both enhanced avidity to antigens with multi-site binding[24] and an ability to bind to complement[25], enabling this isotype to drive the destruction or phagocytic clearance of organisms early in infection when the affinity of the humoral immune response is still developing. By contrast, IgD has increased hinge flexibility and forms a 'T' shape[26] due to heavy glycosylation[27], which allows for greater epitope binding and synergy with IgM early in infection, particularly within mucosal tissues where it is localized[28–30]. Notably, IgG has been the principal isotype utilized in monoclonal therapeutics[27,31] because of its high serum abundance, long half-life, critical role in antipathogen control and destruction, extensive available structural and functional data and amenability for protein engineering and production. Importantly, IgA provides protection both in the blood as a monomer and in mucosal tissues as secretory IgA; an IgA dimer that is complexed to a heavily glycosylated polypeptide chain called the secretory component[32]. In addition, IgE is involved in the response to helminths, where substantial structural rigidity must be overcome to enable binding to its cognate Fc receptor (FcR) and drive persistent antibody effector function for weeks to months against parasites and allergens[33].

The IgG and IgA isotypes can be further divided into six subclasses (IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) that also have unique structural differences, primarily in the hinge region, though additional variation exists[34]. Differential hinge lengths and disulfide bonds influence the effector functions of antibodies, with longer and more flexible structures being associated with an enhanced ability to bind to antigen, complement and/or FcRs[35]. As such,

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IgG3 has the greatest functional potency, followed by IgG1. IgG2 and IgG4 have the least functional potency of the subclasses[36]. In addition, the hinge region is vulnerable to proteolytic enzymes[37–39], and the hinge can play a role in immune complex formation[40]. Thus, despite the functional potency of IgG3, it has the shortest half-life among all the subclasses, which is related to its susceptibility to hinge proteolysis and antibody recycling by the neonatal FcR (FcRn)[41]. Similarly, IgA1 has a unique, 16-amino-acid sequence in the hinge that renders it more susceptible to proteolytic cleavage by pathogens[42]. By contrast, IgA2 has a shorter hinge that confers more resistance to proteolytic lysis, which probably accounts for its increased abundance at mucosal membranes. Thus, even within each isotype, further Fc domain variation by subclasses provides crucial differences in function, persistence and distribution.

Fc domains can be additionally tuned via post-translation modification of the glycans that are attached at specific sites on each isotype; this modification has been shown to profoundly impact antibody stability, half-life and function for IgG[43] (BOX 2). For example, IgG1 glycosylation occurs at a single conserved residue (asparagine 297) in the heavy chain (CH2 domain) of the Fc domain, where as many as 36 different glycan structures may be attached, with some of the resulting glycoforms extensively interrogated in their ability to drive unique effector functions[27] (FIG. 1). The monoclonal therapeutics community has shown that the removal of fucose from the IgG1 glycan can substantially improve the ability of antibodies to engage and deploy the cytolytic function of natural killer (NK) cells[44,45]. However, some antibody hinges may even potentiate the neutralizing activity of the Fab domain[46], which highlights the dynamic interaction between the Fab and Fc domains of IgG. Glycosylation on other isotypes is complex, as they harbour many more glycosylation sites, which may differentially modulate antigen binding, antibody function, localization, stability and degradation (FIG. 1). Thus, extensive variation on the Fc domain in combination with the enormous diversity in potential Fab domains enables the immune system to rapidly evolve the targeting and functional potency of the humoral response during infection.

Antibody sensors

The specific effector functions that are triggered by antibodies are determined by the receptors to which the antibody Fc domain binds and the specific innate immune cells on which these FcRs are expressed[47]. These sensors include both classical FcRs and non-classical C-type lectin receptors (CLRs), which are differentially expressed across distinct innate immune cell subsets and tissues, thus enabling the deployment of targeted effector functions (TABLE 1). The mammalian FcRs belong to a conserved family of glycoproteins of the immunoglobulin superfamily[48], with distinct FcRs that interact with each isotype[49–53]. In addition, beyond the initiator of the complement cascade, C1q, dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN), FcεRII (also known as CD23) and mannose-binding lectin (MBL) are among the CLRs that bind to the Fc domain of antibodies[49,54,55]. Furthermore, the intracellular non-classical FcR TRIM21, which is ubiquitously expressed in the cytosol of many non-immune and immune cells[56], may interact with and clear antibody-opsonized targets that have breached the cellular or endocytic membrane. Last, FcRn[57] and the polymeric immunoglobin receptor (pIgR)[58] are involved in the transfer of antibodies across the placenta and mucosal membranes,

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respectively. Thus, a diverse network of sensors (complement components, FcRs, CLRs and TRIM21) as well as receptors (FcRn and pIgR) exist within the mammalian system to respond rapidly to antibody-bound pathogens and to maintain immunity across tissues and compartments.

Variation in FcRs, including both nucleotide polymorphisms and gene copy number variants, can impact the interaction of FcRs with human isotypes and thereby skew antibody effector function. For example, multiple gene duplication and recombination events followed by functional mutations (changes in gene sequence that alter the function of the gene product) have resulted in the generation of three distinct genes within the FcγRI family (*FCGR1A*, *FCGR1B* and *FCGR1CP*), three genes within the FcγRII family (*FCGR2A*, *FCGR2B* and *FCGR2C*) and two genes within the FcγRIII family (*FCGR3A* and *FCGR3B*). As an example of how genetic variation can affect FcR function, the single nucleotide poly morphism (SNP) identified by the unique accession number rs1801274 significantly alters FcγRIIa (also known as CD32a) affinity for IgG2 and IgG3: the cytosine allele encoding an arginine at position 131 has low affinity, while the thymine allele encoding a histidine has high affinity[59] for IgG2 and IgG3. These polymorphisms have clearly illustrated both the immunoprotective and pathological functions of antibodies in several infectious and oncological diseases. For example, the low affinity R/R allele is correlated with progression-free survival in patients with neuroblastoma who have been treated with an anti-GD2 mouse IgG3 antibody targeted against disialoganglioside (which is expressed on tumours of neuroectodermal origin)[60]. However, the same low affinity R/R allele is associated with decreased progression-free survival in patients with metastatic colorectal cancer treated with the chimeric IgG1 antibody cetuximab, which is targeted against epidermal growth factor[61]. Similarly, in the setting of many infections such as malaria[62,63], HIV[64] and pneumococcal pneumonia[65,66], both improved host protection and exacerbated disease progression are independently observed in patients with lower affinity alleles of *FCGR2A*, which encodes FcγRIIa, suggesting that host-protective Fc effector functions during infection may in some cases be detri mental. In fact, for other infections such as dengue virus, in which antibody-dependent enhancement (ADE) of disease is mediated by FcRs, SNP studies show a protective role for lower affinity *FCGR2A* variants, which can reduce viral entry into cells[67]. For *FCGR3A*, which encodes FcγRIIIa (also known as CD16a), the SNP rs396991 alters the receptor binding affinity for IgG: a valine at position 158 is associated with a higher affinity for IgG1 and IgG3 and increased NK cell activity compared with the phenylalanine variant[68], which exhibits lower affinity for the same IgG subclasses. Similar to *FCGR2A* SNPs, higher affinity *FCGR3A* alleles have been associated with both decreases and increases in the risk of infection. For example, high-affinity *FCGR3A* alleles are associated with a decreased risk of contracting acute poliomyelitis[69] but have also been associated with harm in the context of increased HIV acquisition in low-risk populations[70] and aggravated disease progression in HIV-positive individuals[71]. Thus, for SNPs in both *FCGR2A* and *FCGR3A*, which are the best characterized, both immuno protective and pathological effects are associated with variation in receptor affinity for antibodies. Although additional polymorphisms exist in *FCGR2A* and *FCGR3A* that can impact *in vitro* Fc effector activities, their physiological significance in infectious risks remains to be clarified.

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Given their broad expression profiles, antibody sensors such as FcRs can be recruited in different combinations on distinct innate immune cells, providing an additional level of functional tuning. Other than the high-affinity FcRs (FcγRI (also known as CD64), FcεRI and Fcα/μR (also known as CD351)), the majority of FcRs and CLRs possess a low affinity for antibodies. Thus, individual antibodies that interact with FcRs bind weakly and therefore signal poorly[72] (FIG. 2). Consequently, innate immune activation only occurs upon antibody–antigen multimerization and complex formation, which enhances the avidity of the inter actions of FcRs or CLRs on the cell surface with antibody Fc domains[73,74]. This ensures that innate immune effector function is only deployed in the presence of the pathogen (which is bound by several antibodies simultaneously) and enables innate immune cells to integrate information across multiple and sometimes heterogeneous FcRs or CLRs that aggregate on the surface of effector cells. In humans, only FcγRIIb and in some instances FcαRI (also known as CD89) are inhibitory[49]. Thus, the combinatorial engagement of activating and inhibitory FcRs and/or CLRs provides the ability to fine-tune effector function both spatially and temporally in response to infection[75].

Antibody-mediated effector functions

The Fab and Fc domains act together to drive antibody effector function and pathogen clearance. The Fc domain can structurally augment or inhibit affinity and avidity between the Fab domain and antigen[46,76–78]. In addition, the Fc domain can recruit complement and innate immune cells to engage in effector functions such as opsonophagocytosis and cytotoxicity as directed by the Fab domain. Finally, the stoichiometry of Fab domain binding to the antigen determines the quality of immune complex formation, which is crucial in signalling through receptors that bind to the Fc domain to initiate effector functions[79,80]. Moreover, antibody function is potentiated with increasing immune complex size, in such a way that antibody subclasses thought to have negligible affinity for FcRs are able to bind and activate innate immune effector function when present in large (but not small) immune complexes[79]. Additionally, antibody:antigen ratios can impact effector activity[80,81], where antibody excess or shortage can disrupt the optimal configuration of immune complexes required to drive the activation of effector functions (FIG. 2). This is probably related to the need for optimal antibody-mediated FcR cross linking, which may be too sparse with low antibody titres or lead to poor FcR clustering when antibody levels are too high due to the generation of small immune complexes[81]. Thus, an ideal stoichiometry likely exists for protective humoral immune responses for any pathogen, dictated by the mechanism of antibody action, the landscape of immune cells at the site of infection and the life cycle of the pathogen of interest.

Antibody-mediated neutralization

The simplest mechanism by which antibodies can prevent disease is via direct neutralization of a pathogen or toxin. The Fab domain may bind to specific pathogen targets, thus preventing microbial interactions with host cell receptors and thereby blocking infection or disease (FIG. 3). Although antibody-mediated neutralization is classically associated with the inhibition of bacterial toxins and pathogen entry into cells, other types of virulence factors can be targeted (for example, factors involved in biofilm development). Moreover,

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whereas Fab domain-mediated direct biophysical neutralization has been thought to explain the protective efficacy of passive antibody transfer in toxin-mediated diseases, there is growing evidence that supports the contribution of Fc domain functions in the protective efficacy of these therapeutics.

Neutralization of toxins.—Many pathogenic bacteria (*Corynebacterium diphtheriae*, *Bordetella pertussis*, *Vibrio cholerae*, *Bacillus anthracis*, *Clostridium botulinum*, *Clostridium tetani* and enterohaemorrhagic *Escherichia coli*) can cause disease via the release of toxins. The administration of therapeutic antibodies that neutralize these secreted toxins remains an integral part of the treatment of these diseases (FIG. 3a). For example, in anthrax (associated with *B. anthracis*), the disease is caused by anthrax toxin, which is composed of a binding protein known as protective antigen and two enzymes, oedema factor and lethal factor. Together, these factors can inhibit immune responses and kill host cells. Three treatments are used clinically, two IgG1 monoclonal antibodies (raxibacumab and obiltoxaximab)[9] and a polyclonal pool of immunoglobulins from humans who have been immunized with a subunit anthrax vaccine, Anthrasil (Cangene, USA)[82]. *In vitro* binding assays show that the monoclonal antibody inhibits protective antigen interaction with cellular receptors, which prevents toxin-mediated killing and confers protection from lethal challenge *in vivo*[83,84]. Furthermore, passive transfer studies of the Fab domain[85] in mice deficient in T cells, B cells, complement or FcRs have suggested that Fab domain binding to protective antigens alone is sufficient to confer protection, without activation of downstream Fc domain effector functions[86]. However, the Fc domain does still have a significant impact. Specifically, in cell lines that express high levels of FcRs, the neutralization of anthrax toxin by polyclonal serum is significantly impaired in the presence of FcR-blocking antibodies[87]. In addition, altering the Fc domain by subclass switching[76] or site-directed mutagenesis to modify FcR engagement[88] significantly impacts the *in vivo* protective efficacy of the toxin-specific antibodies. In this case, mutations in the Fc domain that enhance interactions with activating FcRs resulted in increased *in vitro* neutralization and *in vivo* protection. Thus, the Fc domain can substantially impact polyclonal Fab domain-mediated neutralization of anthrax toxin and may also have an effect on specific neutralizing monoclonal antibodies. In addition, the Fc domain has been implicated in neutralization by toxin-specific antibodies against *C. difficile*[89] and *E. coli*[90], which provides (previously underappreciated) opportunities to enhance the design of monoclonal therapeutics to these and additional pathogens.

Neutralization of pathogen entry and replication.—Antibodies can also bind directly to pathogens and prevent their entry into cells, thus restricting replication, dissemination and disease progression. A fraction of individuals infected with HIV are able to mount broadly neutralizing antibody responses against highly diverse strains of HIV[91,92] that provide complete protection from infection in non-human primates (NHPs)[93,94]. Similarly, antibodies specific for hepatitis C virus[95,96], Zika virus[97], West Nile virus[98], dengue virus[99] and *Plasmodium* spp.[100,101] have been identified that can block the entry of the pathogen into a cell. In addition, other neutralizing antibodies can limit infection via the inhibition of crucial intracellular processes such as adenoviral uncoating[102], nuclear translocation of human papilloma virus (HPV) DNA[103], rotavirus transcription[104], measles

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virus assembly[105] and vacuolar replication of intra cellular pathogens such as *Listeria monocytogenes*[106] and *Anaplasma phagocytophilum*[107].

As in the case of antibodies that neutralize toxins, the neutralization of pathogen entry and replication may also be enhanced by the antibody Fc domain. For example, the passive administration of an Fc domain variant of the broadly neutralizing HIV-specific IgG1 b12, with a decreased affinity for FcRs, compromised neutralizing immunity to HIV in NHPs[108]. Moreover, the protective efficacy of other broadly neutralizing antibodies against HIV were also compromised when engineered with an Fc domain variant that had decreased FcR binding, providing further evidence that some neutralizing antibodies require Fc domain effector function to contain the virus[46]. Similarly, certain neutralizing antibodies against influenza virus fail to provide protection in FcR-deficient mice[77,109], which is potentially linked to *in vitro* ADCC, antibody-dependent neutrophil-mediated phagocytosis and nitric oxide production.

Neutralization of microbial virulence factors.—Beyond toxin or pathogen neutralization, antibodies may modify disease progression via the inhibition of microbial virulence factors that mediate invasion and pathogenesis. These antibodies target various mechanisms by which pathogens can attach to host cells, control quorum sensing and regulate biofilm formation. For commensal organisms, attachment to mucosal surfaces is one of the first crucial steps in colonization of a host. Human polyclonal IgG antibodies specific for *Streptococcus pneumoniae* cell surface proteins block adherence of the bacteria to airway epithelial cells via the Fab domain, and passive transfer of Fab domains decreases nasopharyngeal colonization in mice[110]. For *Staphylococcus aureus* and *Staphylococcus epidermidis*, biofilms not only serve as a nidus for infection but also support resistance to antimicrobials by preventing antimicrobials from reaching their target and downregulating the targets themselves in the altered bacterial physiology within a biofilm. Pathogen-specific antibodies can directly, as well as through neutrophil opsonophagocytosis, inhibit biofilm formation *in vitro*[111] (FIG. 3b). These examples highlight additional aspects of the infectious process where antibodies, either directly or via Fc domain effector mechanisms, contribute to pathogen growth inhibition.

More invasive pathogenic strategies of pathogens involve penetration beyond physical mucosal barriers in which mucins play a critical role. In this context, *in vitro* studies show that antibodies may interfere with viral infection by trapping pathogens in the dense coat of mucins that decorate all mucosal membranes[112], providing a means to sequester pathogens and prevent further infiltration[112] (FIG. 3c). The ability of antibodies to trap pathogens in mucins is Fc-dependent[113] and is tuned by Fc domain glycosylation[112]. Along these lines, the Fc domain has also been implicated in Fab domain-mediated neutralization of pathogen virulence factors in *Cryptococcus* infection. Although the polysaccharides in the capsule of this pathogen are able to inhibit phagocytosis and antigen presentation[114,115], *Cryptococcus*-specific antibodies are able block the activities of these fungal polysaccharides. Intriguingly, Fab domain recognition of *Cryptococcus* glucuronoxylomannan, a critical component of these capsular virulence factors, is significantly influenced by the antibody isotype. Unique *Cryptococcus* capsule staining patterns are observed when distinct isotypes are used, which highlights the critical influence of the Fc domain on potentially altering Fab domain angle

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and flexibility[116]. Thus, collaboration at both ends of the antibody is key to antibody-mediated recognition and function.

Antibody-mediated complement activation

Both pentameric IgM and clusters of particularly glycosylated IgG antibodies that form stable hexameric complexes[117] are able to recruit complement, which is found ubiquitously in the blood and tissues of mammals[118,119] (FIG. 3d). Complement may be activated via three distinct pathways that diverge in the molecules that initiate the cascade: first, the classical pathway involving the initial recruitment of C1q to the immune complex; second, the lectin pathway involving the recruitment of MBL to antibody-opsonized material; or third, the alternative pathway that is independent of either C1q or MBL[118,119]. Ultimately, the complement cascade leads to first, the production of peptide mediators of inflammation, which recruit phagocytes and activate the endothelium by increasing surface thrombogenicity (thus increasing the tendency of blood cells to clot) and up-regulating adhesion molecules; second the opsonization of immune complexes through complement receptors found on innate immune cells; and third, the assembly of the membrane attack complex to directly destroy the target[118,119].

The crucial role of complement for the clearance of immune-complexed microorganisms is demonstrated in individuals who have complement deficiencies and those who lack a spleen, who exhibit an increased susceptibility to infections, particularly by capsular bacteria (for example, *S. pneumoniae*, *Neisseria meningitides* and *Haemophilus influenzae* type B)[120]. More specifically, human genetic complement deficiencies can lead to recurrent disseminated *Neisseria* infections[120]. Moreover, iatrogenic deficiencies that are caused by treatment with eculizumab, a monoclonal antibody that targets the final stages of complement activation, are associated with an elevated risk of disseminated bacterial infection[121]. Finally, meningococcal disease incidence is inversely proportional to the prevalence of bactericidal antibodies that depend on complement fixation to kill bacteria. Complement-fixing antibodies also represent the correlate of protection for the 4CMenB (meningococcal Type B) vaccine, which highlights the crucial role of complement in the control of meningococcal disease[122–124]. In addition, antibody-mediated complement activation has been implicated in protective immunity against influenza virus[125], West Nile virus[126], vaccinia virus[127], *Plasmodium*[128] and even *Cryptococcus* infections[129,130]. Although it is clear that antibody-mediated recruitment of complement is key to predicting protective immunity in these infectious diseases, the precise mechanism or mechanisms by which complement activation (cytotoxic, inflammatory, phagocytic, etc.) may contribute to the control and/or clearance of many of these pathogens continue to be incompletely understood.

Antibody-mediated complement activation also has a key role in driving the adaptive immune response[131,132] (FIG. 3i). Specifically, real-time, two-photon microscopy studies show that complement-opsonized immune complexes captured by complement receptors on naive non-cognate B cells are transferred to follicular dendritic cells (FDCs) that are located within germinal centres (GCs)[133]. Antigen-specific B cells within the GCs, with sufficient antigen-specific affinity, are able to capture processed antigens from the surface of FDCs to

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present to follicular CD4$^+$ T cells, which then provide survival signals to the B cells that are required for the evolution of the humoral response[132]. Additionally, the binding of complement-coated immune complexes on antigen-specific B cells is able to increase B cell receptor signalling, thus lowering the activation threshold required for B cell survival. Interestingly, although complement-deficient mice can mount weak humoral immune responses, mice with B cell-specific deficiencies in complement receptors exhibit both compromised induction of humoral immunity and compromised maintenance of antibody responses[134]. This finding highlights the key role of antibody–complement interactions, not only in innate antipathogen activity but also in the promotion of long-lived adaptive immunity.

Antibody-dependent cellular cytotoxicity

The ability of antibodies to direct the cytotoxic destruction of cells, via a mechanism termed ADCC, has been exploited extensively by monoclonal therapeutics that target tumours[11]. Classical ADCC results from the crosslinking of FcγRIIIa on NK cells, which results in the release of perforin and/or granzyme that drives cell death in the target tumour cell (FIG. 3g). To harness this activity further, the monoclonal therapeutic field has explored Fc domain modifications, such as point mutations that enhance NK cell-mediated cytotoxicity[31]. More naturally, alterations of the antibody Fc domain glycan have also been exploited to improve ADCC. For example, removal of fucose from the IgG glycan can increase ADCC by enhancing antibody affinity for FcγRIIIa[45,135]. The addition of a bisecting *N*-acetylglucosamine (GlcNAc) prevents the addition of fucose and similarly enhances ADCC[136]. Although monoclonal developers have focused largely on optimizing NK cell effector functions, other innate immune cells express FcγRIIIa (macrophages and DCs)[137–139] and FcγRIIIb (also known as CD16b) (neutrophils) (TABLE 1) and may also contribute to the tumour clearance that is driven by these potentiated monoclonal therapeutics[140,141].

ADCC has also been implicated in the control and clearance of many pathogens (FIG. 3g). Circulating antibodies that have an elevated capacity to drive ADCC correlate with the spontaneous control of HIV[142] and are associated with protection from HIV infection following vaccination[143,144]. Antibodies with increased capacity to activate ADCC also correlate with influenza-specific antibody-mediated protection[125,145], the control and killing of malaria para sites[146,147] and the killing of *Chlamydia trachomatis*[148] and more recently have been associated with control of *Mycobacterium tuberculosis*[149]. Data also suggest that IgA-directed and IgE-directed ADCC may protect against extracellular organisms, including helminths such as *Schistosoma mansoni*[150,151], through the non-classical induction of degranulation by eosinophils and platelets that promotes pathogen clearance (FIG. 3j).

In HIV infection, the role of ADCC has gained tremendous traction both in the context of preventing infection and in the context of monoclonal antibody-mediated approaches to ‘cure’ the infection or drive viral control and long-term viral remission[152–154]. The goal is to induce the host immune system to target latently infected cells for killing by ADCC. Specifically, viral replication can be suppressed with passive administration of potent HIV-specific neutralizing monoclonal antibodies in the absence of antiretroviral therapy in

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patients[155–158] and in NHPs[159–161]. However, after the antibody is cleared from the system, the virus always rebounds, which suggests that there is limited ADCC-mediated elimination of the virally infected cellular reservoir. Thus, aggressive efforts are underway to engineer antibodies to potentiate cellular reservoir killing, particularly within tissues where the viral reservoir may hide, such as lymph nodes and the central nervous system[152]. Importantly, defining the principles to effectively augment monoclonal-mediated 'cure' efficacy against HIV may pave the way for the rational development of curative therapeutics that target a much broader range of chronic viral and bacterial infections.

Antibody-dependent cellular phagocytosis

Opsonophagocytosis, or the clearance of pathogens marked by immune complexes from the circulation, is mediated by mononuclear phagocytes (monocytes, macrophages and DCs) and granulocytes (neutrophils, eosinophils, basophils and mast cells) following the ligation of complement receptors and/or classical and non-classical FcRs[162] (FIG. 3d–f). Beyond pathogen clearance, opsonophagocytosis may be accompanied by the secretion of antimicrobial peptides, release of metalloproteinases, secretion of cytokines, production of lipid mediators and presentation of antigens. Thus, antibody-mediated phagocytosis not only clears antibody-opsonized targets but also shapes the extra cellular milieu and ensuing immunological memory to the pathogen.

Phagocytosis of antibody-coated pathogens involves the formation of endocytic vesicles that mature through fusion with different endosomal compartments. The crosslinking of different FcRs results in signalling via immunoreceptor tyrosine-based activation motifs (ITAMs) and in some instances immuno receptor tyrosine-based inhibitory motifs (ITIMs), which both impact the re-organization of microtubules to enable phagosome formation[162]. At the initiation of phagocytic clearance of immune complex-opsonized pathogens, differential FcR signalling determines the fate of the endocytosed complex. Thus, ITAM recruitment leads to the rapid trafficking of pathogens to lysosomes for their degradation and directed antigen processing for presentation to T cells[163]. By contrast, ITIM signalling results in the retention of whole patho gen antigens for subsequent transfer to B cells for the induction of humoral immunity[164]. Phagocytic cells may integrate additional information via cooperative signals between FcRs and other pattern recognition receptors, such as CLRs and Toll-like receptors, which are found on the surface of the effector cell or within endocytic compartments[165–167]. This collaborative signalling may lead to additional effector functions, which includes the release of proteases, defensins, cytokines, reactive oxygen species (ROS) and reactive nitrogen species that together recruit and arm additional innate effector cells[167,168].

Interestingly, although some pathogens take advantage of antibody-independent phagocytosis to take up residence in innate immune cells, antibody-dependent, Fc domain-mediated phagocytosis can preferentially drive the activation of microbial destructive pathways. For example, *Legionella pneumophila* uses a special ized secretion system to enhance its own uptake and establish infection within replication-permissive vacuoles inside the host cell. By contrast, *in vitro* and *in vivo* mouse studies show that antibody-opsonized *L. pneumophila* are readily targeted to lysosomal compartments where they are cleared, substantially impacting bacterial burden[169]. Similar findings have been described for

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Salmonella, where rerouting into the lyso some in an FcγRIII-dependent manner in bone marrow-derived DCs enhances antigen presentation and T cell activation[170]. Thus, the presence of antibodies and FcR signalling redirects trafficking of the bacteria into degradative pathways that are directly antimicrobial and promotes protective host immune responses.

Antibody-dependent phagocytosis may activate distinct immune effector functions, which depend on the cell type that has been recruited to clear the pathogen. For example, in macrophages, differential engagement of FcR-mediated phagocytosis can regulate the production of inflammatory cytokines, oxidative bursts, the clearance of immune complexes and polarization towards the M1 (classic, inflammatory) or M2 (alternative, growth and repair) macrophage state[139]. In myeloid DCs, FcR-induced opsonophagocytosis drives maturation, differentiation, upregulation of the MHC gene products, increased expression of co-stimulatory molecules and increased antigen presentation for T helper cell activation[171] (FIG. 3h). By contrast, in neutrophils, FcR-mediated phagocytosis initiates a strong NADPH-dependent oxidative burst within the phagosome that generates highly toxic ROS, degranulation to deliver antimicrobial enzymes such as myeloperoxidase and neutrophil serine proteases and the formation of neutrophil extra cellular traps (NETs) that kill extracellular bac teria such as *S. aureus*[172]. Thus, depending on the presence of particular innate immune cells within a particular tissue compartment or at a specific time point following infection, distinct innate immune effector functions (FIG. 3) may be deployed by immune-complex-opsonized pathogens, which results in a tightly regulated sequence of innate immune effector functions.

Antibody-dependent enhancement of disease

Importantly, some pathogens have evolved mechanisms to exploit the complement and FcR pathways, and in some cases antibody-mediated responses are not protective and can enhance disease[12,173]. The classical example of this is in the case of viral infections, where primary infection and/or immunization may result in incomplete protective humoral immunity that enhances the subsequent infection of host cells with partially antibody-opsonized viruses, which can cause a severe infection. As mentioned above, ADE has been clearly observed in dengue virus infection. Dengue virus has four different serotypes, and prior infection with a specific viral serotype provides long-lived protection against the same serotype but only partial protection against one of the other three serotypes, thus facilitating access of the other viral serotypes to immune cells and thereby enhancing infection and disease. Specifically, cross-reactive antibodies raised following the first infection form immune complexes with the second viral serotype that stabilize the virus[174]; this stabilization promotes viral uptake within macrophages[175], leading to dengue haemorrhagic fever and shock syndrome[176,177]. This phenomenon is not limited to dengue virus serotypes but crosses to other flaviviruses such as Zika virus, where both plasma from patients recovering from dengue virus infection and dengue virus envelope-specific monoclonal antibodies have been shown *in vitro* to enhance Zika virus infection[178]. Similarly, ADE activity has been observed for Murray Valley encephalitis virus[12], West Nile virus[80] and RSV. In the case of RSV, the vaccination of a paediatric cohort in the 1960s resulted in enhanced infections, which are thought to have been driven by vaccine-induced

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antibodies[179,180]. Specifically, the formalin-fixed RSV vaccine virus may have induced antibodies to poorly protective targets, which were probably enhancing rather than protective, driving exacerbated immune activation and disease. Thus, the experiences with RSV and dengue virus clearly illustrate that antibody titres alone are not sufficient to predict protective versus pathological outcomes. However, ADE is not only associated with viral infection; it has also been implicated in *Leishmania* infection. Here, IgG-bound *Leishmania* amastigotes are taken up by macrophages through the engagement of FcRs, resulting in IL-10 production, which downregulates nitric oxide synthase and inhibits T helper 1 cell and IFNγ responses[181–183]. Thus, defining the specific antibody effector functions that drive protection, rather than disease, in viral, bacterial and potentially even fungal infections is absolutely crucial, as not all functional humoral immune responses provide protection.

The promise

An emerging appreciation of the broad effector mechanisms of action that are induced by antibodies, beyond binding, is providing a framework for the rational design of effective monoclonal therapeutics or next-generation vaccines, which are based on defined correlates of protective immunity. Importantly, the combination of monoclonal antibodies that target non-overlapping epitopes is more efficacious than a single antibody[184]. This strategy has been particularly resonant in the recent Ebola virus outbreak, where cocktails as opposed to single monoclonal antibodies were used to treat and reverse disease[185–187]. Thus, combinations of antibodies may not only provide a higher evolutionary barrier for pathogens to overcome but also form more effective immune complexes that together may drive more effective immune control. Although titres and neutralization provide a reductionist framework to measure the overall levels and one potential function of the humoral immune response, alternative emerging tools that probe antibody specificity, affinity, function and glycosylation and the role of other isotypes may provide a more granular and objective approach for the identification of previously underappreciated protective humoral responses[144,149,188–191] (FIG. 4). Moreover, antibody Fc profiles may also influence the transfer of immune activity across the placenta[57] and into mucosal surfaces[192] and the central nervous system[193] and may drive increased adaptive immunity via antigen delivery to DCs[194,195], which highlights additional opportunities to improve immunity. However, it is crucial to note that although correlates of disease are not necessarily directly related to mechanisms of protection, they can provide a path to improved vaccine and/or therapeutic design via the iterative optimization of vaccines that selectively promote the target immune profile, or they may raise hypotheses related to the mechanism or mechanisms of antibody action against the pathogen.

Although antibiotics and vaccines have been revolutionary in treating a large number of infectious diseases, gaps remain in our ability to treat many deadly pathogens and drug-resistant organisms. These gaps may be addressed by our emerging appreciation for the complex mechanisms of action of antibodies, which can target multiple stages and processes within the life cycle of a pathogen in physiologically relevant tissues via the recruitment and orchestration of the innate and adaptive immune system. Thus, design efforts may begin to extend beyond improving neutralization, binding specificity and affinity to now gain access to the large array of innate antimicrobial effector functions that are able to target incoming

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pathogens and to direct their function in an antigen-specific manner against current and emerging pathogens. Though the promise of harnessing antibody effector functions in next-generation vaccine design and monoclonal therapeutics appears distant, the strides made since the days of early antibody research provide compelling evidence that it is possible to realize the promise.

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Glossary

Monoclonal therapeutics

Treatments utilizing immunoglobulins that are engineered with a single antigenic specificity. Current monoclonal therapeutics approved by the US Food and Drug Administration (FDA) involve a range of immune targets, which are important in cancer and autoimmune diseases, as well as three infectious disease targets.

Affinity

The strength of the interaction between an antigen and antibody. K_a, the affinity constant, is influenced by pH, temperature and buffer and ranges from below 10^5 mol^{-1} to above 10^{12} mol^{-1}. Affinity and K_d, the equilibrium dissociation constant, are inversely related.

Avidity

The overall strength of the antibody–antigen complex. Itis dependent on affinity, valency of the antibody and antigen, and structural arrangements of the interacting parts.

Complement

A system that consists of a large number of plasma proteins that follow a cascade of reactions, which induce antimicrobial and inflammatory responses.

Immune complex

An aggregate complex formed from the binding of several antibodies to an antigen that can exist as a solitary unit and/or further multimerize to induce antibody effector function.

Glycoforms

Isoforms of glycans that can exist on proteins in a set of specific states. For example, 36 distinct glycoforms can theoretically be attached at a single conserved residue (asparagine 297) on the crystallizable fragment (Fc) domain of an IgG1 antibody.

Antibody-dependent enhancement

A phenomenon where pre-existing cross-reactive antibodies bind to cells and enhance host cell entry of a pathogen, its replication and the host inflammatory response to infection, thus exacerbating pathogenesis and disease.

Direct neutralization

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Inhibition of a pathogen or microbial component by direct binding of antibody to the antigen in the absence of a target host cell. By contrast, non-neutralizing antibody functions involve additional host immune factors to generate antimicrobial functions.

Biofilms

Collections of microorganisms that adhere to each other and produce an extracellular matrix on living or non-living surfaces. Biofilms can be found in the natural and humanized environment, with uniquely resilient growth phenotypes not observed in single cells.

Membrane attack complex

A complex formed by terminal complement components that create transmembrane channels directly on the surface of bacteria or an infected host cell, which disrupt the cell membrane, leading to membrane destabilization and death.

Metalloproteinases

Protease enzymes that contain a catalytic metal ion in their active site and that cleave and inactivate proteins. Matrix metalloproteinases can degrade extracellular matrix proteins and act on pro-inflammatory cytokines, chemokines and other proteins to modulate inflammation and immunity.

Neutrophil extracellular traps

(NETs). Extracellular chromatin studded with granular and selected cytoplasmic proteins that bind to pathogens. NETs are produced through a process called NETosis in neutrophils, which is induced in response to microbial components, antibodies and reactive oxygen species.

***Leishmania* amastigotes**

Leishmaniasis is a vector-borne disease caused by an obligate intracellular protozoa of the genus *Leishmania*. Sandfly-to-human transmission occurs at the promastigote stage; the promastigotes then transform into amastigotes that replicate in human cells, to be taken up by the sandfly and complete the life cycle.

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Box 1 |

The structures and functions of different antibody isotypes and subclasses

IgM: As a monomer, IgM associates with transmembrane invariant accessory chains, forming the B cell receptor. IgM is secreted as a pentamer, which is linked by disulfide bonds and a single J chain. Multimerization enhances avidity via multi-site binding[24,74] and complement binding via a central crystallizable fragment (Fc) bulge[25]. IgM possesses five *N*-linked glycoslation sites.

IgD: Like IgM, IgD is a membrane receptor on naive mature B cells, but it can also be secreted. The long IgD hinge length increases its flexibility, resulting in a 'T'-shaped structure[26] and thus enabling binding to low surface density epitopes. In the respiratory mucosa[28,30], IgD binds to basophils and mast cells[29], thus inducing the production of antimicrobial peptides and inflammatory cytokines. IgD possesses three *N*-linked and four *O*-linked glycan sites.

IgG: IgG accounts for 10–20% of the protein found in serum. The four subclasses in humans, named IgG1–IgG4 in order of their abundance, each contain unique hinges[34] that are vulnerable to pathogen[39] and host[37,38] proteolytic enzymes. Longer and more flexible hinges (IgG1 and IgG3 hinges are longer than those of IgG2 and IgG4) enhance binding to antigen and complement Fc receptors (FcRs)[35,36] and thus effector function. IgGs possess a single *N*-glycan site at asparagine 297, which is known to tune antibody function. For IgG3, one additional *N*-linked glycan site has been described at asparagine 392 (REF. 196) as well as three *O*-linked sites in the hinge[197].

IgA: IgA1 has a flexible, heavily *O*-glycosylated hinge that induces a 'T' shape[198], which is vulnerable to pathogen cleavage[42], whereas IgA2 has a more rigid 'Y'-shaped hinge. Monomeric IgA is present in the serum. By contrast, dimeric IgA, which is linked by disulfide bridges to a J chain[32] and complexed to a glycosylated polypeptide chain (secretory component) that is derived from the polymeric immunoglobulin receptor, is secreted into the mucosa. IgA1 possesses two *N*-linked and four *O*-linked glycan sites, and IgA2 possesses five *N*-linked glycan sites.

IgE: IgE is present in the serum at low concentrations with the shortest half-life of all the isotypes. IgE is potent and once bound can remain fixed to the high-affinity FcεRI on mast cells for weeks to months[199]. IgE possesses seven *N*-linked glycan sites, including asparagine 394, which is critical for binding to FcεRI[200].

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Box 2 |

Antibody glycosylation impacts effector function

The impact of glycosylation on IgG1 secretion, stability, function and immunogenicity has been thoroughly investigated in antibody engineering[31]. This is due to the clear impact of glycan changes on antibody structure, flexibility and affinity for crystallizable fragment (Fc) receptors (FcRs) or C-type lectin receptors (CLRs), antibody subcellular transport, secretion, clearance, solubility and conformation. IgG1 *N*-linked glycosylation improves or inhibits FcR and/or CLR binding and induces conformational changes in the Fc domain[166,201,202]. For example, *N*-linked glycosylation at asparagine 297 in the Fc domain of IgG1 helps maintain its molecular quaternary structure and stability, which is essential in modulating binding affinity to different receptors and thus function. Furthermore, the removal of fucose from the core biantennary structure of the IgG1 glycan enhances FcγRIIIa binding and is linked to antibody-dependent cellular cytotoxicity[44,45,135]. Moreover, the addition of sialic acid is thought to improve Fc domain binding to non-classical FcRs[202,203]. In addition, the removal of galactose residues improves IgG1 interaction with mucins[112]. For IgD, the maintenance of the Fc domain structure by glycans is necessary for secretion[204]. For IgA, *N*-linked glycosylation of the J chain is required for dimerization, binding to the polymeric immunoglobulin receptor (pIgR) and transport across the mucosal epithelium[58]. The IgA secretory component, which is derived from the proteolytic cleavage of pIgR, has seven *N*-linked glycosylation sites that are occupied by a large diversity of carbohydrate structures that are involved in anchoring the secreted IgA to the mucosal lining[205]. Conversely, *N*-linked glycosylation of serum IgA provides galactose terminating glycans for the asialoglycoprotein receptor, which mediates IgA immune complex clearance from the blood[206]. In addition, a specific *N*-linked glycan site at asparagine 384 in IgE is responsible for driving anaphylactic shock[200]. Thus, Fc domain glycosylation plays a central role in antibody localization and function, and in some cases, modulation of antibody glycosylation is exploited to shape antibody effector function and regulatory mechanisms.

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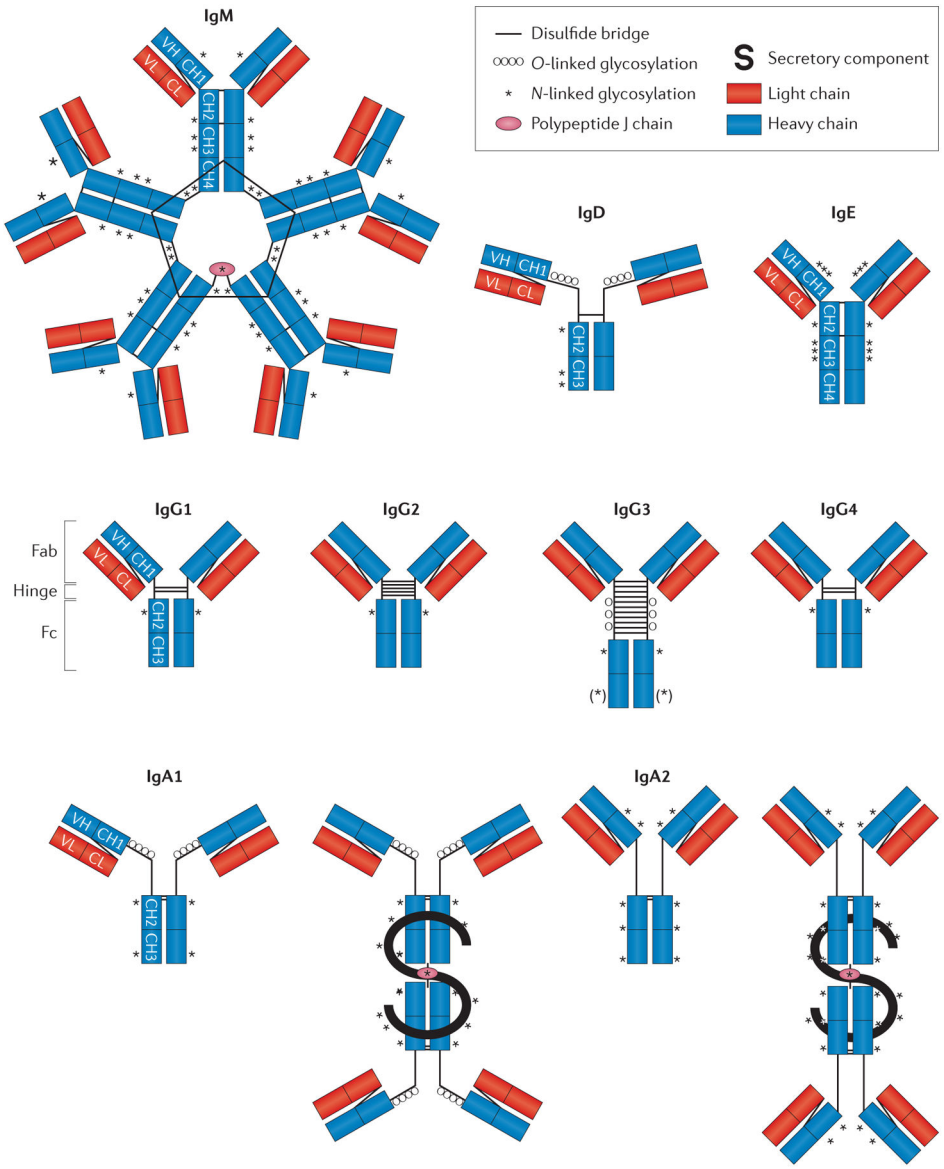

Figure 1 |. Antibody isotypes and subclasses.

The basic structure of a human antibody consists of two functional domains linked by a hinge region. The domains include an antigen-binding fragment (Fab) domain that binds to antigens and a crystallizable fragment (Fc) domain that binds to host sensors that deploy effector functions. Each antibody molecule is composed of four chains with two identical heavy chains (blue) and two identical light chains (red). These are further divided into variable (VH or VL) domains and constant (CH or CL) domains, which form the Fab and the Fc domains. Fc domain diversity is generated during an immune response via the selection of different antibody isotypes, subclasses and post-translational glycosylation profiles. Five isotypes (IgM, IgD, IgG, IgA and IgE) and six subclasses (IgG1–4, IgA1 and IgA2) exist in humans. Each isotype or subclass exists as monomers and/or multimers, linked by disulfide bridges, polypeptide J chains or the secretory component, which is a proteolytic cleavage product of the polymeric immunoglobulin that remains associated with

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secreted dimeric IgA. Three structural features influence their flexibility and/or conformation and hence impact antibody–receptor and even antigen binding; these features include hinge length and flexibility, the number and location of disulfide bridges and *O*-linked or *N*-linked glycosylation. Together, changes in isotype or subclass and glycosylation influence the capacity of any antibody to interact with innate immune receptors and thereby deploy distinct innate immune effector functions.

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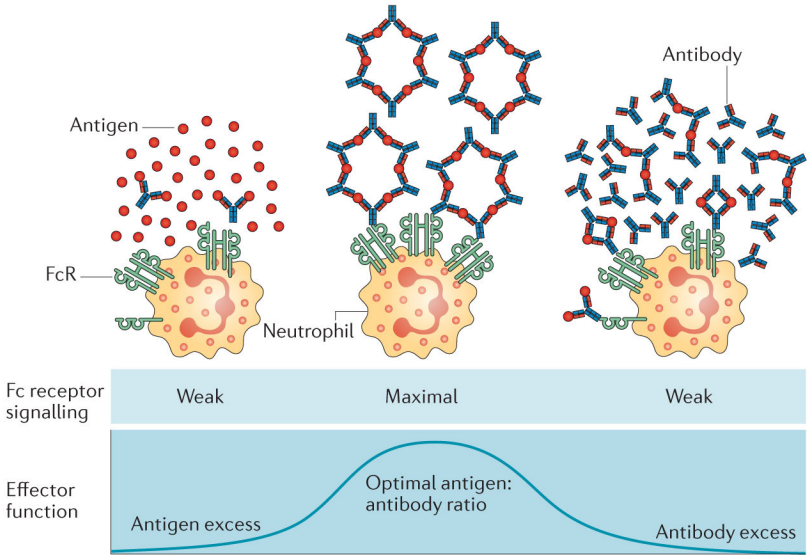

Figure 2 |. Antigen, antibody and Fc receptor stoichiometry in effector function.
Antibodies collaboratively generate immune complexes that drive innate immune effector function. Antigen–antibody complex quality is influenced by both the crystallizable fragment (Fc domain) characteristics of the antibody (how strongly it will bind to Fc receptors) and the ratio of antigen to antibody, which will greatly affect the size and shape of immune complexes. The size and shape of the immune complex likely influences both the number and conformation of Fc domain sensors that may be engaged on the surface of innate effector cells. Immune complex stability is driven by multiple bonds that enhance the binding between Fc domains and Fc domain sensors. In a state of antigen excess (and antibody scarcity, left side) or antibody excess (and antigen scarcity, right side), immune complex quality shifts towards small complexes that cluster fewer Fc domain sensors on the surface of innate immune cells. Conversely, at an optimal antibody:antigen ratio (centre), larger, more stable immune complexes are generated that are able to cluster a larger number of Fc domain sensors, thereby driving optimal innate immune effector activation. FcR, Fc receptor.

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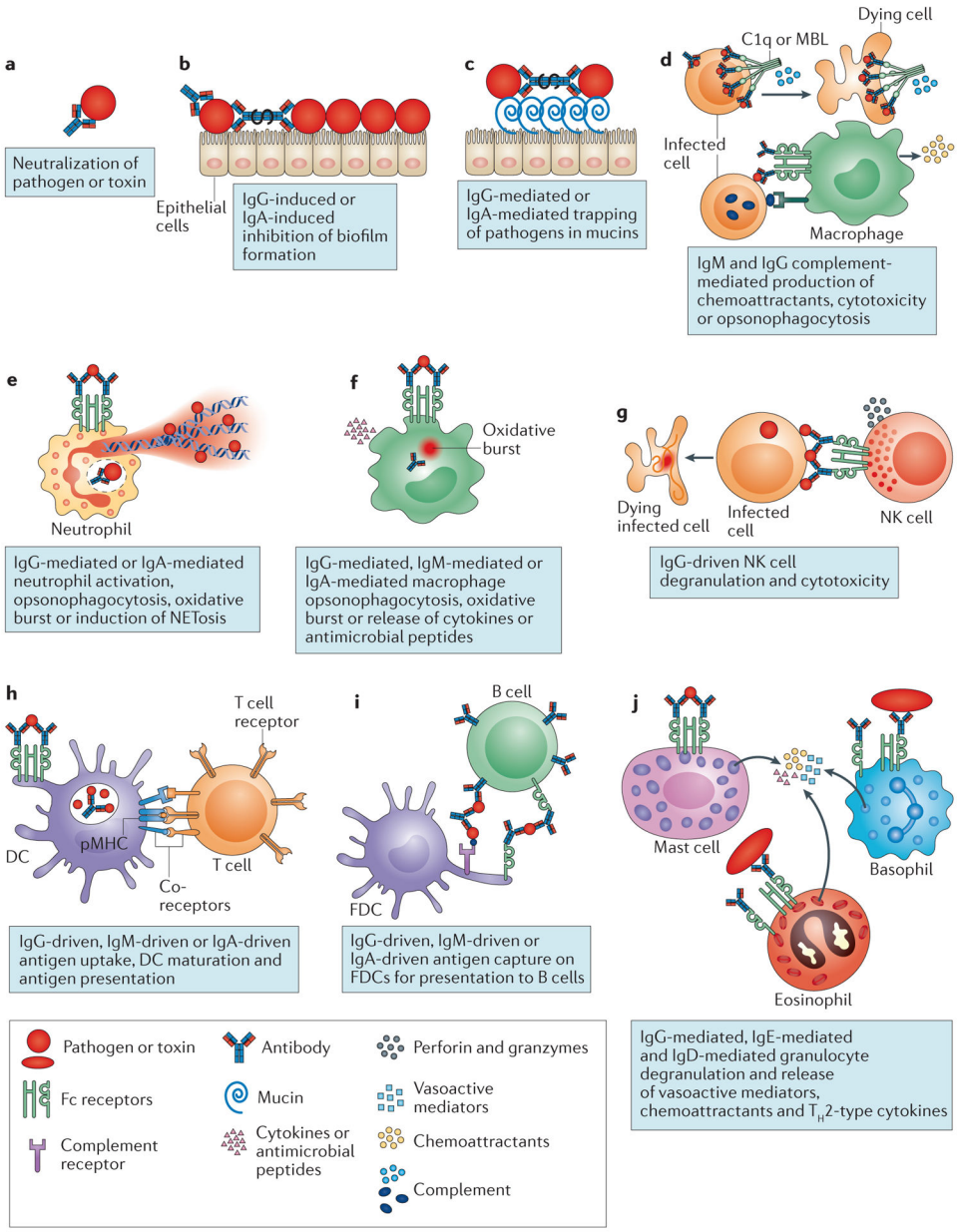

Figure 3 |. Antibody effector functions.

Antibodies are able to deploy a plethora of effector functions over the course of an infection. These include but are not limited to the following: **a** | The direct neutralization of toxins or microorganisms. **b** | The neutralization of microbial virulence factors, such as those involved in quorum sensing and biofilm formation. **c** | The trapping of pathogens in mucins. **d** | The activation of complement to drive phagocytic clearance or destruction, generate chemoattractants or anaphylatoxins such as C3a and C5a or complement fragment opsonins such as C3b or induce lysis through the membrane attack complex. **e** | The activation of neutrophil opsonophagocytosis, oxidative bursts, production of lytic enzymes and chemoattractants, or the formation of neutrophil extracellular traps (NETs) of chromatin and antimicrobial proteins. **f** | The induction of macrophage opsonophagocytosis, oxidative

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bursts or antimicrobial peptide release. **g** | The activation of natural killer (NK) cell degranulation to kill infected cells. **h** | The enhancement of antigen uptake, processing and presentation by dendritic cells (DCs) to T cells. **i** | The presentation of antigens by follicular dendritic cells (FDCs) to B cells. **j** | The degranulation of mast cells, basophils and eosinophils to release vasoactive substances, chemoattractants and T helper 2 (T_H2)-type cytokines in the setting of allergens or parasitic infections. Fc, crystallizable fragment; MBL, mannose-binding lectin; pMHC, peptide–MHC complex.

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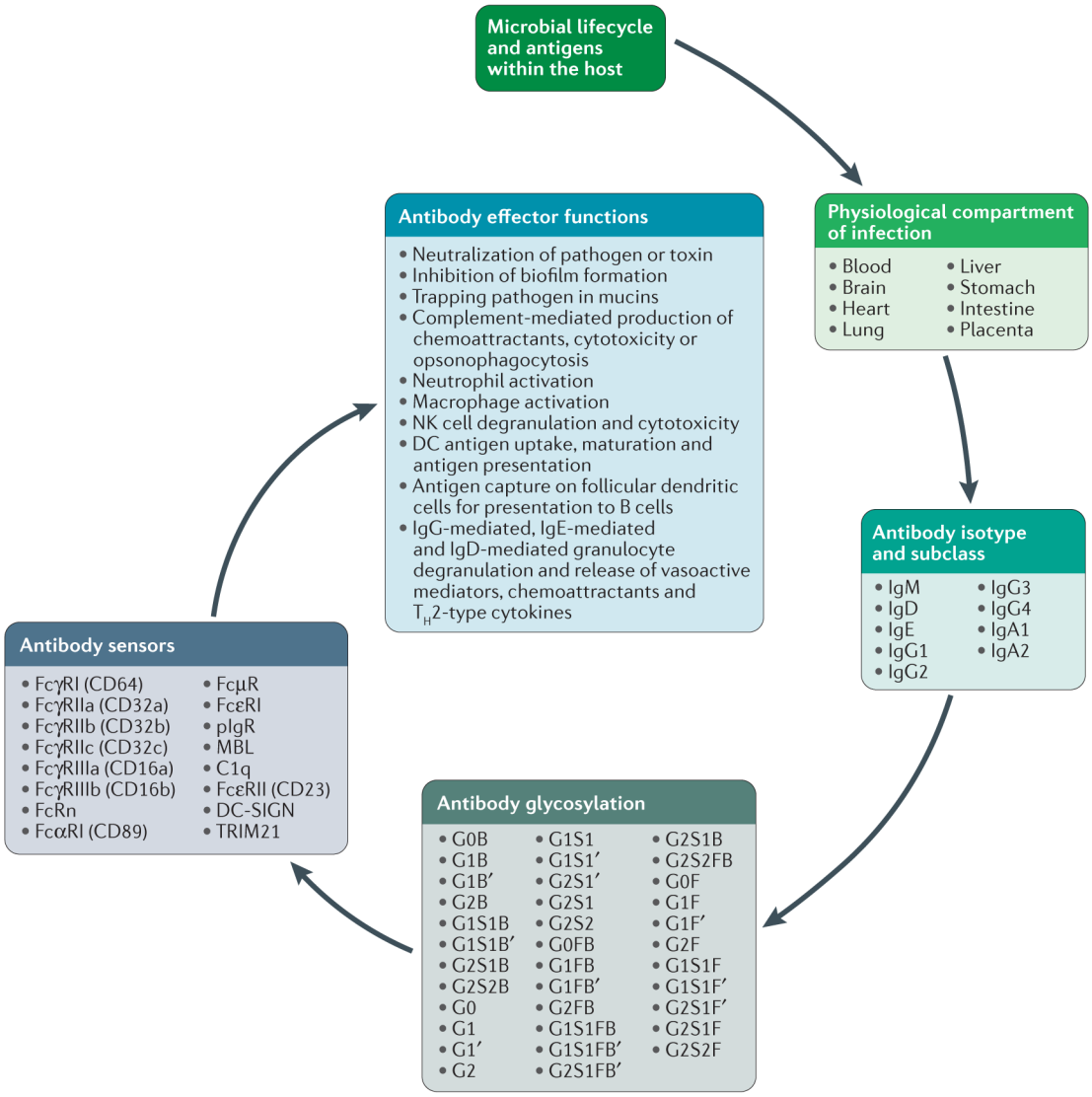

Figure 4 |. Factors influencing humoral activity in response to infection.

Microbial life cycles, including tissue tropism and disease pathogenesis, can dynamically impact humoral immunity over the course of an infection. Factors that include the spectrum of antigens recognized by the host over the course of infection, the inflammatory profiles driven by the pathogen and the physiological compartment or compartments where infection occurs may alter the landscape of humoral immune responses that may provide protection. These infection profile features in turn influence the quality of the humoral immune response, such that antibody specificity and antibody function are rapidly customized to effectively target the pathogen. Thus, based on the inflammatory profile and tissue compartment, the humoral immune response rapidly explores the combinatorial diversity of different isotypes, subclasses and crystallizable fragment (Fc domain) glycovariants to selectively recruit the Fc domain sensors available on innate immune cells at the site of infection. In the context of antibody glycosylation in the figure, G refers to galactose, S refers to sialic acid, F refers to fucose and B refers to bisecting *N*-acetylglucosamine. DC,

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dendritic cell; DC-SIGN, dendritic cell-specific ICAM3-grabbing non-integrin; GPI, glycosyl phosphatidylinositol; MBL, mannose-binding lectin; NK, natural killer; pIgR, polymeric immunoglobulin receptor; T_H2, T helper 2.

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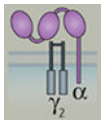
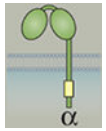
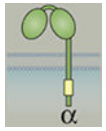
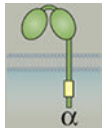
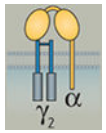
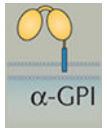
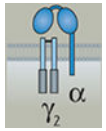
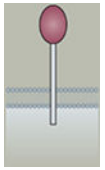
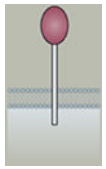
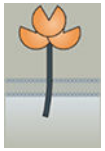
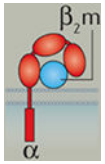
Table 1 |

Expression of Fc domain sensors

Cell type	FcγRI (CD64)	FcγRIIa (CD32a)	FcγRIIb (CD32b)	FcγRIIc (CD32c)	FcγRIIIa (CD16a)	FcγRIIIb (CD16b)	FcαRI (CD89)	Fcα/μR (CD351)	FcμR	FcεRI	FcεRII (CD23)	DC-SIGN	FcRn
													
Adaptive immunity													
B cell	−	−	+	+	−	−	−	+	+		+	+/−	+
CD4+ T cell	−	(+/−)			(+)			(+)	+	(+)	+		−
CD8+ T cell	−				(+)				+				−
Innate immunity													
DC	(+)	+	+	+	+	+	+/−		+	+		+	+
NK cell	−	−	−	+	+	−	−			+	+		−
Neutrophil	(+)	+	+	+	−	+	+				(+)		+
Monocyte	+	+	+	+	+	+	+			+	(+)	+	+
Macrophage	+	+	+	+	+	+	+/−			+	(+)	+	+
Microglia	(+)	(+)	(+)		(+)								
Eosinophil	(+)	+	+			(+)	+			+	(+)		
Basophil	(+)	+	+		−	+			−	+	(+)		
Mast cell	(+)	+	+		−				−	+			
Non-immune cells													
Platelet		+					+			+		+	
Epithelial cell													+
Placental cell													+
Endothelial cell			+										+

+, receptor is present on the cell; −, receptor is not present on the cell; +/−, receptor is present on a subset of cells; (+), receptor is inducible; (+/−), receptor is inducible on a subset of cells; empty cell, it is unknown whether receptor is present. β_2m, β_2-microglobulin; DC, dendritic cell; DC-SIGN, dendritic cell-specific ICAM3-grabbing non-integrin; Fc, crystallizable fragment; FcRn, neonatal Fc receptor; GPI, glycosyl phosphatidylinositol; ITIM, immunoreceptor tyrosine-based inhibitory motif; NK cell, natural killer cell. Images in the table have been adapted with permission from REF. 49, Macmillan Publishers Limited; REF. 53, Frontiers; REF. 72, Springer and REF. 207 © 1999 BioScientifica Limited.