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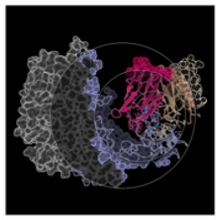

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REVIEW

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With great power, comes great responsibility: the importance of broadly measuring Fc-mediated effector function early in the antibody development process

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ABSTRACT

The field of antibody therapeutics is rapidly growing, with over 210 antibodies currently approved or in regulatory review and ~ 1,250 antibodies in clinical development. Antibodies are highly versatile molecules that, with strategic design of their antigen-binding domain (Fab) and the domain responsible for mediating effector functions (Fc), can be used in a wide range of therapeutic indications. Building on many years of progress, the biopharmaceutical industry is now advancing innovative research and development by exploring new targets and new formats and using antibody engineering to fine-tune functions tailored to specific disease requirements. In addition to considering the target and the disease context, however, the unique features of each therapeutic antibody trigger a diverse set of Fc-mediated effector functions. To avoid unexpected results on safety and efficacy outcomes during the later stages of the development process, it is crucial to measure the impact of antibody design on Fc-mediated effector function early in the antibody development process. Given the breadth of effector functions antibodies can deploy and the close interplay between the antibody Fab and Fc functional domains, it is important to conduct a comprehensive evaluation of Fc-mediated functions using an array of antigen-specific biophysical and cell-mediated functional assays. Here, we review antibody and Fc receptor properties that influence Fc effector functions and discuss their implications on development of safe and efficacious antibody therapeutics.

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Introduction

The field of antibody therapeutics is rapidly growing, with over 210 antibodies currently approved or in regulatory review globally (www.antibodysociety.org/antibody-therapeutics-product-data), and, as of October 2023, ~1250 antibodies in clinical development.[1] Antibodies are very versatile molecules, and with strategic design of their two functional domains, the antigen-binding domain (Fab) and the Fc, which mediates effector functions, can be used in a wide range of therapeutic indications, including cancer, autoimmune diseases, and infections, as well as cardiovascular, neurological, ophthalmic, and musculoskeletal disorders.[2]

Building on past knowledge and experience, the biopharmaceutical industry is now exploring new targets and new formats and using antibody engineering to fine tune antibodies for greater efficacy and safety.[2] Among therapeutic antibodies, defined here as recombinant protein-based molecules with at least one antigen-binding site derived from an antibody gene and evaluated for therapeutic use, only 5% of those currently marketed or under regulatory review, and 5% of those in late-stage clinical studies, lack an Fc domain (Figure 1a, b). Of the therapeutic antibodies with an Fc currently marketed or in regulatory review and of those in late-stage clinical studies, at least 46% and 45%, respectively, are Fc engineered (Figure 1c, d).[3,4]

Due to advances in antibody engineering techniques, coupled with a deeper understanding of the mechanisms involved in antibody effector functions and disease pathogenesis, antibodies are now powerful tools that can be customized to drive specific functions tailored to individual diseases. However, with this great power comes great responsibility, and thus several factors must be considered to improve both efficacy and safety of next-generation antibodies. The effects of new formats and antibody engineering modifications are dependent on the target and the disease context.[5,6] To avoid unexpected results on safety and efficacy outcomes during the later and more expensive stages of the development process and consequently reduce risk, the impact of antibody design on Fc-mediated effector function must be systematically measured at the outset of the process.

Antibodies and Fc receptors

Canonical antibody therapeutics comprise two identical Fab domains and one Fc domain. The Fc domain mediates effector function by engaging Fc receptors (FcRs) expressed on a variety of immune cells, and complement component C1q, the recognition molecule of the classical complement pathway. The Fc domain of IgGs also regulates the antibody serum half-life by interacting with the neonatal Fc receptor (FcRn) on endothelial cells.

Of the five major classes of human Ig, IgG, IgA, IgM and IgE have been considered for therapeutic purposes. These isotypes share a similar structure, but differ in valency, size,

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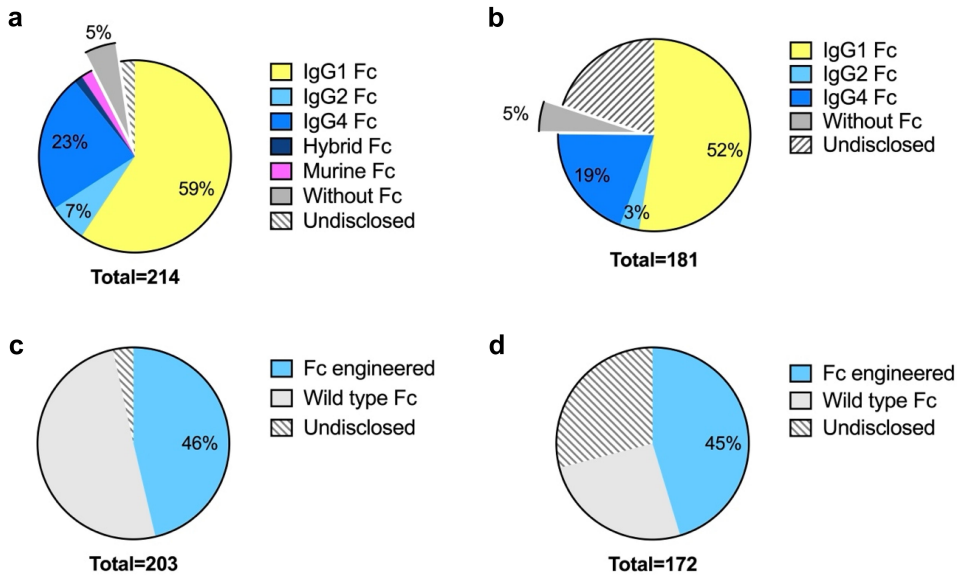

Figure 1. Fc characteristics of antibody therapeutics: pie charts representing the distribution of the different types of Fc and the proportion of molecules without an Fc in antibodies therapeutics that are currently approved or in regulatory review (a) and in antibodies in late-stage clinical studies (b). Pie charts representing the proportion of antibodies with a protein- or glycoengineered Fc that are currently approved or in regulatory review (c) and in antibodies in late-stage clinical studies (d); cohorts analyzed in C and D are Fc containing antibody therapeutics. Data refers to commercially developed antibody therapeutics defined as recombinant protein-based molecules containing at least one antigen binding site derived from an antibody gene.[3,4].

amino acid sequence, hinge presence and flexibility, glycosylation, and charge, which translate into different biodistribution, ability to engage Fc receptors and complement, and consequently effector function.[7,8]

For each of these isotypes, there are specific FcRs expressed on a variety of immune cells. FcRs can be categorized into two classes based on the conformation the antibody Fc region can adopt: type I receptors, belonging to the Ig superfamily and binding antibody Fc in an open conformation; and type II receptors, belonging to the C-type lectin family and binding the antibody Fc in a closed conformation. Open and closed conformations are determined by the sialylation level of the antibody. Sialic acid conjugation results in a closed conformation of the Fc that masks the binding sites for type I receptors and reveals those for type II receptors.[9]

Type I receptors include FcγRs for IgG, FcαRI/CD89 for IgA, FcμR for IgM, Fcα/μR for IgA/IgM and FcεRI for IgE. Type II receptors include DC-SIGN/CD209 and FcεRII/CD23.[10]

IgG can also bind the neonatal FcR (FcRn) in a pH-dependent manner. FcRn is expressed on the intestinal epithelium, placenta and vascular endothelium and is involved in IgG transport across epithelial barriers and IgG biodistribution. Serum antibodies are continuously internalized by vascular endothelial cells. FcRn prolongs IgG serum half-life by binding IgG at acidic pH in the endosome and protecting it from degradation. IgG is then recycled back to the plasma membrane where, at physiological pH, it is released.

As shown in Figure 1a, all the currently approved antibody therapeutics and the majority of those in clinical study belong to the IgG isotype, and specifically to the IgG1, IgG2, and IgG4 subclasses,[3] although single assets representing IgG3[11] and IgE[12] have also entered the clinic. IgG cognate receptors, FcγRs, can be classified as FcγRI/CD64, FcγRIIa/CD32a, FcγRIIb/CD32b, FcγRIIIa/CD16a, FcγRIIIb/CD16b. FcγRI is the only high-affinity receptor for IgG. The remaining receptors primarily interact with IgG that is part of an immune complex with antigen. Activating FcRs exert their function through the intracellular immunoreceptor tyrosine-based activation motif (ITAM) domain and, depending on the strength of their signaling and the cellular context, they can drive different effector functions. FcγRIIIb is attached to the cell membrane via a glycophosphadylinositol (GPI) anchor and does not have an intracellular ITAM domain. This receptor is not considered a canonical activating receptor but has been reported to mediate effector functions in neutrophils via FcγRIIa or integrins.[13] FcγRIIb is the only inhibitory receptor, exerting its function through immunoreceptor tyrosine-based inhibitory motifs (ITIM), able to dampen the signaling induced by activating FcRs.

Antibodies can engage with a variety of immune cells expressing different levels of activating and inhibitory FcRs. It is important to highlight that the balance of activating and inhibitory FcRs and the antibody affinity for these receptors are crucial in regulating antibody-mediated effector function. A comprehensive analysis of the binding affinities of antibody/antigen immune complexes for a broad set of FcRs and complement is therefore of paramount importance to delineate potential antibody-mediated effector functions and thereby to facilitating a more rational approach to therapeutic antibody development strategy.

Fc-mediated effector functions

Thanks to the variety of immune cells and molecules the antibody Fc can engage, antibody therapeutics can drive several types of direct and indirect effector functions (Figure 2). Direct Fc-mediated effector functions mainly comprise complement-

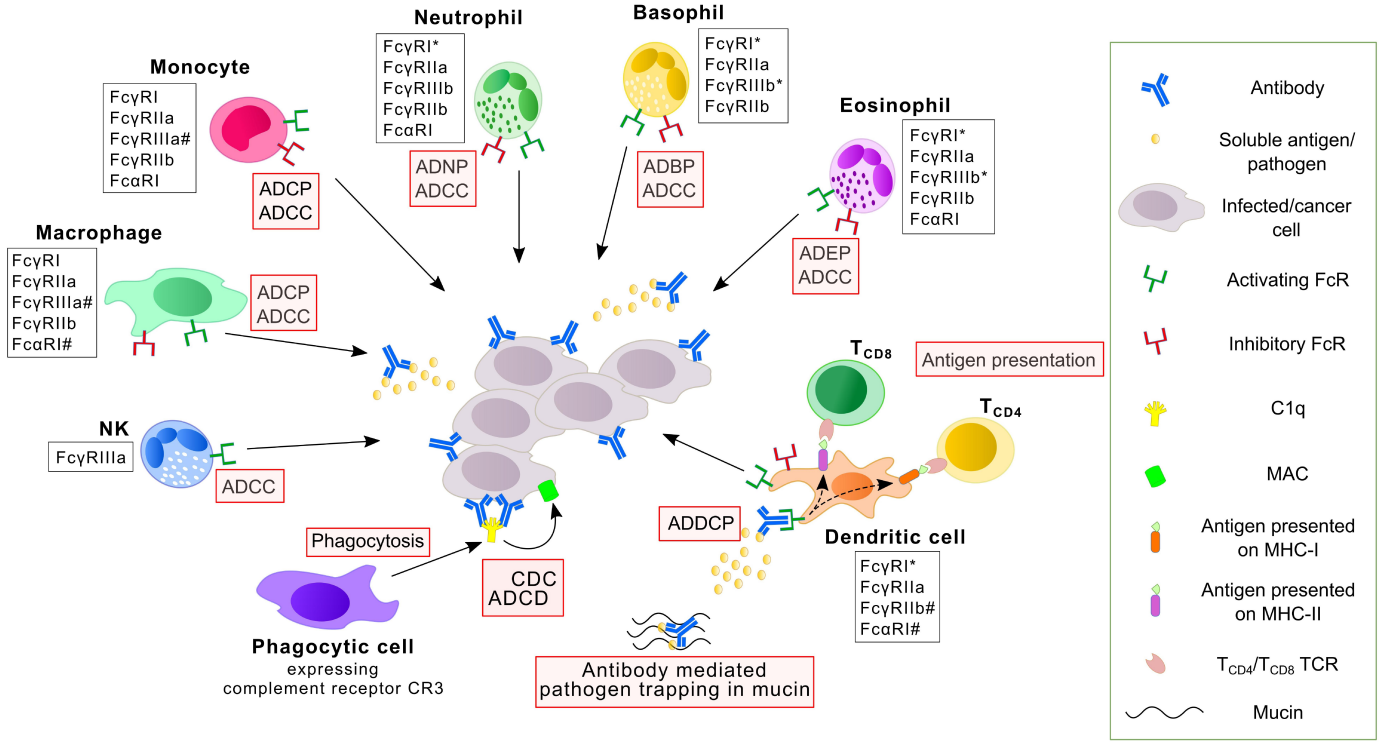

Figure 2. Fc-mediated effector functions: the schematic depicts the variety of cells expressing Fc receptors (FcRs) and the different types of Fc-mediated effector functions. Depending on the affinity for each FcR, antibodies can engage different type of cells and mediate different effector functions. Direct effector functions include: complement-dependent cytotoxicity (CDC), antibody-dependent complement deposition (ADCD), antibody-dependent cell cytotoxicity (ADCC), antibody-dependent cell phagocytosis (ADCP) and, for pathogen-targeting antibodies, antibody-dependent mucin binding (ADMB). Antibodies can also mediate indirect effector functions such as antigen presentation with priming of both CD4+ and CD8+ T cells. ADCD can also facilitate phagocytosis by cells expressing complement receptor CR3. Engagement of FcRs on immune cells can also result in cytokine release, or antibody-dependent enhancement (ADE) of infection or disease (not depicted in this schematic). Excessive cytokine release and ADE of infection or disease are Fc-mediated functions that pose safety issues. The boxes underneath each cell name show the expressed FcRs, * is for inducible expression, # is for expression in a subset of the cells.[8,9,14] The red boxes show the types of Fc-mediated effector function. Unlabelled molecules and cells are described in the key box on the bottom.

dependent cytotoxicity (CDC), antibody-dependent cell cytotoxicity (ADCC), and antibody-dependent cell phagocytosis (ADCP), and, for pathogen-specific antibodies, antibody-mediated pathogen trapping in mucin. Indirect Fc-mediated effector functions mainly comprise antibody-dependent cytokine release and antigen presentation.[15,16]

ADCC is mainly mediated by natural killer (NK) cells, granulocytes, and myeloid cells. During ADCC, antibodies bound to the target cell via their Fab region engage activating FcRs on effector cells via their Fc region, resulting in the release of the contents of cytotoxic granules that kill the target cell.[8,16]

ADCP is mainly mediated by phagocytic cells, such as macrophages, monocytes, and neutrophils, however, other cells such as dendritic cells, eosinophils, and basophils can mediate ADCP.[17] During ADCP, antibodies opsonizing a target cell engage, via their Fc region, activating FcRs on effector cells, resulting in the internalization and degradation of the target cell.

CDC is initiated by the binding of the Fc domain of target-bound antibodies and the six-headed globular protein C1q, which triggers the activation of the classical complement cascade, ultimately resulting in the formation of a membrane attack complex (MAC) that mediates the lysis of target cells.[7] Only IgM and IgG classes can fix complement. Antibody-dependent complement deposition (ADCD) can also facilitate phagocytosis through complement receptor CR3 on phagocytic cells.[18]

An indirect effector function mediated by the antibody Fc is antigen presentation. Antigen- presenting cells (APC), such as dendritic cells, can engage and internalize antigen–antibody immune complexes in an FcR-dependent fashion. Immune complex internalization can lead to dendritic cell maturation, upregulation of co-stimulatory molecules, and antigen presentation on both MHC class I and MHC class II, priming both CD4+ and CD8+ T cell-mediated immune responses.[10,19–21]

Antibody therapeutics can also promote the release of pro-inflammatory cytokines from multiple cell types in an Fc-dependent manner, a phenomenon that can indirectly support antibody-mediated killing but can also pose safety risks,[22] as discussed in more detail below.

Other immune effector functions, such as trogocytosis, have been described. Trogocytosis can be mediated by several immune cells, such as T cells, B cells, NK cells, dendritic cells, monocytes/macrophages, neutrophils, endothelial cells, fibroblasts, eosinophils, and basophils, and consists in the transfer of membrane fragments with functional integrity between cells. Trogocytosis does not always result in cell death and is therefore not discussed further in this review.[23]

Given the multitude of Fc-mediated effector functions, and the variety of cells involved, it is evident that, for a comprehensive

overview of antibody-mediated effector functions, an array of assays performed with the different cell types is required. ADCC and ADCP assays are usually performed with effector and target cell co-cultures, where the target cells can be tumor cells or virus-infected cells, but they can also be performed using beads instead of target cells.[6,24–26] Effector cells can be primary cells or established cell lines. ADCC is usually evaluated using NK cells because this is the cell type most involved in this type of mechanism; however, other cells such as neutrophils can trigger ADCC.[27,28] ADCP is usually evaluated using monocytes or macrophages, but other cells, such as neutrophils, dendritic cells, eosinophils, and basophils, have recently been reported to mediate phagocytosis.[17] It is therefore clear that a preclinical evaluation of Fc-mediated effector functions based only on CDC, NK-mediated ADCC, and monocyte/macrophage-mediated ADCP is far from exhaustive, and a use of a broader set of assays for a comprehensive analysis is imperative.

We and others have developed several platforms for high-throughput functional assays such as CDC, ADCC, and ADCP that can be used to broadly measure Fc-mediated functions in a large set of candidates during the early stage of antibody therapeutic development.[6,24–26,29] These assays, alongside biophysical assays for the evaluation of antibody glycosylation profile and antigen-specific binding affinities for FcRs, would facilitate the selection of optimal leads in terms of functionality, biodistribution, and safety.

Considerations for antibody therapeutic design

Fc-mediated effector functions are influenced by many factors involving not only the Fc domain but also the Fab domain of the antibody. Antigen binding, antibody structure, and flexibility are dependent on amino acid sequence, glycosylation, and charge, and affect the antibody affinity for FcRs and complement, consequently influencing antibody effector function, biodistribution, and safety.[7,30]

Critical considerations for Fab and Fc design depending on therapeutic target and purpose (Figure 3) are discussed below.

Fc design

Isotype

The currently marketed therapeutic antibodies belong to the IgG class, but IgA, IgE, and IgM isotypes are also currently being evaluated as therapeutics. The four subclasses of IgG (IgG1, IgG2, IgG3, and IgG4) differ in their affinity for FcγRs and consequently in their ability to mediate effector functions.[31] IgG1 and IgG3 have greater capacity to fix complement and higher affinity for activating FcγRs compared to IgG2 and IgG4. IgG1 is the isotype of choice when the antibody is designed to mediate effector function such as ADCC, ADCP, and CDC. IgG3 is the most potent subclass in mediating Fc effector functions due to its superior ability to fix complement and engage activating FcγR. Although the clinical development of IgG3 antibodies has been limited by their short half-life (typically ~7 days compared to ~21 days for the other IgG subclasses), possible immunogenicity, and manufacturing concerns,[7,32] efforts are ongoing to leverage IgG3 as a therapeutic modality.[33,34] IgG2 and IgG4 have low or no ability to fix complement and low affinity for activating FcγRs compared to IgG1. These two subclasses are therefore often used for the design of antibodies that do not require or aim to silence Fc effector function.[7,8,35] IgG2 and IgG4 antibodies

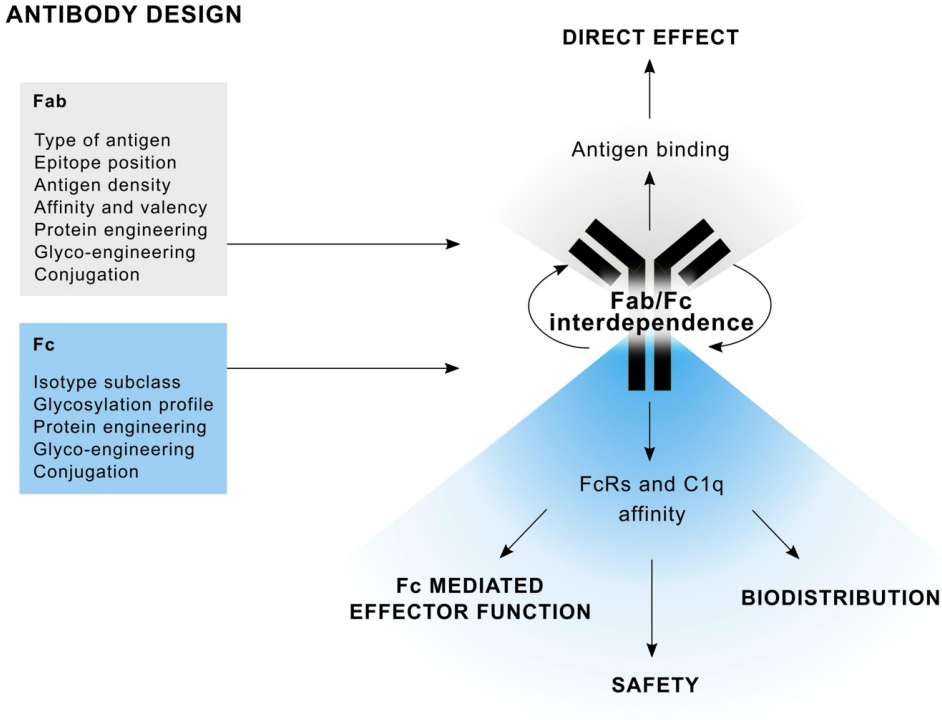

Figure 3. Antibody design: schematic depicting the variables to consider during antibody design (boxes on the left) and the effect of ab and c design on antibody function due to ab/c interdependence (schematic on the right).

have been approved for a variety of cancer and non-cancer indications, accounting for 7% and 25% of the currently approved therapeutic antibodies, respectively (Figure 1a). Furthermore, IgG2 and IgG4 is the isotype of choice of 6% and 19%, respectively, of the current late-stage clinical pipeline (Figure 1b).

IgA antibodies can form monomers and dimers and are the most abundant class at mucosal surfaces. There is a growing interest in this isotype for the design of cell-depleting therapeutic antibodies, due to the ability of IgA to engage FcαR on neutrophils and elicit a more potent ADCC compared to IgG.[36] However, there are currently no IgA antibodies in the clinic, likely due to their short half-life, heterogeneous glycosylation profile and challenging manufacturing characteristics, which pose severe technical limitations.[37] Alternative approaches to take advantage of IgA's potency, by engineering IgG1 Fc with IgA domains or by engineering IgA antibodies for better biodistribution, are currently under investigation.[37–40]

IgE antibodies have high affinity for FcεR expressed on mast cells, basophils, macrophages, and monocytes and have no known inhibitory receptors, resulting in the ability of IgE to trigger strong pro-inflammatory effector functions, such as ADCC, ADCP, as well as cytokine release and possible antigen presentation. These characteristics, make IgE a valid alternative to IgG1 in cancer immunotherapy. There is currently one IgE antibody in clinical study.[12]

IgM antibodies can form hexamers and pentamers and therefore present a stronger binding avidity to the target. For this reason, the IgM isotype can be a rational choice for low expression or difficult targets, such as lipopolysaccharides, glycolipids, and glycans. IgM avidity can also enhance antibody neutralization properties (for example, the anti-SARS-CoV-2 IgM in the clinic),[41] and has the ability to cluster receptors and increase agonism (for example, the anti-DR5 IgM in the clinic).[42] Moreover, IgM can potently activate complement and mediate CDC.

Fc protein engineering

Besides the choice of the isotype, protein engineering of the Fc domain can be used to further fine tune the antibody effector function, as well as to improve safety and biodistribution.

Introducing point mutations targeting the Fc-binding domains for FcRs, C1q, and FcRn is the most widely used technique for modulating Fc-mediated effector function. There are currently over 100 combinations of point mutations that can be employed (extensively reviewed in Damelang et al.[7]), each one with a different effect on binding to FcRs, C1q, and FcRn and consequently different effector function. Besides point mutations, protein engineering can be used to create antibody isotype hybrids, for modulating FcR binding, generating bispecifics, or to promote hexamerization.

Protein engineering can be used to increase affinity for FcRs. For antibodies with cell depleting function, point mutations can be introduced to increase affinity for FcRs, ideally skewing the antibody binding toward activating receptors, thus enhancing cell-mediated effector functions. Conversely, agonist antibodies or those designed to dampen B cell activation, can be Fc engineered to skew binding affinity toward the inhibitory receptor FcγRIIb. Agonist antibodies have been demonstrated to better cluster the target receptor and induce signaling, when binding to FcγRIIb via their Fc in a process called scaffolding. Antibody binding to FcγRIIb has been demonstrated to act as a scaffold to help cluster the target receptors and promote signaling for anti-OX40 and anti-CD137 antibodies,[43] and for an anti-DR5 antibody,[44] resulting in enhanced T cell activation and apoptosis, respectively. However, point mutations to increase affinity to FcγRIIb could also increase affinity for FcγRIIa and lead to unexpected side effects,[45] which highlights the need for a comprehensive screening for FcγRs binding and antibody effector functions when using these mutations.

Another approach to enhance antibody effector function, currently investigated in preclinical settings, combines IgG1 serum stability and effector function properties with IgA's ability to trigger a more potent ADCC compared to IgG1, by generating IgG1/IgA2 hybrids, or IgG1 grafted with IgA domains.[37]

For some antibodies, such as blocking antibodies (including checkpoint inhibitors, antibodies blocking ligand/receptor interaction, and certain neutralizing antibodies) or antibody-drug conjugates (ADCs), Fc effector function is not essential and, in some cases, it has been demonstrated that removing effector function could improve efficacy and safety. To achieve this, antibodies are engineered to decrease or abrogate antibody binding to FcRs, either by introducing point mutations or by creating antibody isotype hybrids, replacing the region containing the binding site for FcRs and C1q on IgG1 with that of IgG2 or IgG4.[7,46,47]

Monomeric IgG has been shown to have low affinity for the single globular heads of C1q, and therefore hexamerization is required to properly bind C1q and initiate the complement cascade.[7] Indeed IgG subtypes inherently differ in their ability to recruit C1q via multivalent interactions governed by their oligomerization status.[48] Several Fc engineering approaches have been developed to enhance the ability of IgG to fix complement. These include the introduction of point mutations increasing IgG affinity for C1q,[5] and the introduction of domains, such as IgM tail-piece,[49] or point mutations[50] able to promote IgG hexamerization. It should be noted that, given the proximity of the domains involved in the binding to C1q and FcγRs, mutations designed to affect complement binding are also likely to affect binding to FcγRs and vice versa.

IgG antibodies can also be engineered to increase their serum half-life. This is achieved by introducing mutations affecting the IgG affinity for FcRn. Different combinations of mutations have been used to increase or reduce IgG affinity for FcRn at low pH and/or neutral pH, depending on the therapeutic purpose. Some mutations have demonstrated improved IgG serum half-life from ~21 days up to ~3 months, increasing efficacy and reducing dosing frequency.[51–53] It is important to highlight that, mutations increasing serum half-life might also influence effector function, which can affect safety and efficacy and should be carefully considered and evaluated during the antibody discovery process.

Depending on the combination of mutations used, the antibody can preferentially bind certain activating receptors over

others, and preferentially engage certain type of immune cells and not others. This should be considered when designing the antibody, depending on the disease settings and therapeutic purpose, and systematically evaluated during the antibody discovery process. Furthermore, the complex outcomes of these mutations and their combinations must be empirically determined by conducting cellular effector function assays that have been developed with disease and immune system biology in mind.

Fc glycosylation

Besides antibody amino acid sequence, another feature that can influence Fc receptors' affinity, and consequently effector function, is antibody glycosylation. IgG lacking or with reduced fucose have increased affinity for FcγRIIIa and are able to trigger more potent ADCC compared to fucosylated IgG.[54–56] Conversely, terminal sialic acid confers to IgG antibodies anti-inflammatory properties, while the pro- or anti-inflammatory role of terminal galactose is more controversial.[57] For these reasons, a thorough analysis of glycosylation during the antibody discovery process could be informative on the type of effector function the antibody mediates.

Fc glycoengineering

Antibodies are glycoproteins, and, as mentioned above, glycans influence the affinity for Fc receptors and Fc-mediated effector functions. Glycoengineering can be used to remove or enhance IgG effector function.

Impairment of the effector function of IgG1 antibodies can be achieved by introducing a point mutation to replace the asparagine at position 297 (N297) with a variety of substitutions,[47,58,59] thus removing the glycosylation site on wild type IgG1 antibodies. There are currently four aglycosylated therapeutic antibodies approved and one in regulatory review.[3]

Enhanced effector function can be obtained by removing fucose from the glycan structure of IgG, which results in increased binding to FcγRIIIa and does not affect FcγRIIb, skewing the antibody affinity toward activating receptors. A total of four afucosylated and three low-fucose IgG therapeutics have been approved so far.[3]

Fab design and Fab/Fc interdependence

The Fab domain of the antibody is responsible for antibody specificity. Canonical antibody therapeutics present two identical Fab fragments, resulting in monospecific bivalent monomers (IgA, IgE, IgG, IgM), tetravalent dimers (IgA), decavalent pentamers and dodecavalent hexamers (IgM). Over the past decade, advances in protein engineering techniques to modify avidity and specificity resulted in the development of a series of novel antibody formats, with mono or multivalent bispecific or multispecific properties.

The Fab region and Fc region exert different functions, but are closely connected as one can influence the function of the other.[60] The Fc region can influence the Fab region's apparent affinity for the target and, by binding to specific cognate FcRs, improve the efficacy of certain neutralizing[16] and agonistic antibodies.[61–63]

Fc-mediated effector functions can be in turn influenced by antibody affinity, avidity, ability to form immune complexes and the size of the immune complexes.[64,65] For example, Mazor and colleagues, in a study with a series of affinity-modulated antibodies targeting CD4, EGFR, or HER2, demonstrated that antibodies with intermediate affinity were able to trigger stronger effector functions compared to high-affinity antibody variants.[66] The researchers suggested that the slower off-rates of high-affinity antibodies promote greater bivalent binding, which reduces the density of Fc domains on the target cell surface. This lower Fc density may, in turn, result in diminished functional responses. In contrast, antibodies with faster dissociation rates allow for higher levels of monovalent binding at saturating concentrations, leading to increased cell-surface opsonization. This higher Fc domain density enhances effector functions.

The nature of the antigen, its distribution on the cell surface, and the position of the epitope are also important for Fc-mediated effector functions. In a study using two anti-CD52 and anti-CD20 therapeutic antibodies and a panel of fusion proteins presenting a CD20 or CD52 epitope at different distances from the plasma membrane, ADCC and CDC have been shown to be more active for epitopes positioned in proximity with the cell membrane, while ADCP requires a minimum distance of the epitope from the plasma membrane.[67] A different study evaluated a threshold for the antigen distance from the plasma membrane for efficient elicitation of macrophage-mediated ADCP.[68] Harnessing epitope location for improving antibody effector function is now a critical step for antibody therapeutic design and has been demonstrated as a valid strategy also for the design of bispecific antibodies.[69]

IgG-mediated CDC is another example of Fc-mediated function influenced by the Fab domain. Monomeric IgG has low affinity for the single globular heads of C1q, and therefore, antigen-driven IgG hexamerization is required to properly bind C1q and initiate the complement cascade. Antigen driven hexamerization is highly dependent on antibody flexibility, antigen size, density, mobility, and epitope position.[7]

The characteristics of the Fab region described above are carefully designed and optimized during the early stages of the antibody discovery process. Given the now recognized ability of Fc and Fab region to influence each other's function, it is therefore critical that optimal leads should be evaluated also in the context of Fc-mediated effector functions for a comprehensive immune profile of development candidates.

For decades, Fc and Fab regions have been considered to act independently, and therefore during early stages of antibody therapeutic development, emphasis has been predominantly on the optimization of the Fab region for binding to the target antigen. A better understanding of the close Fab-Fc interplay and the role of Fc-mediated functions in antibody safety and efficacy, highlights the importance of measuring Fc-mediated functions in the early stages of the antibody therapeutic development pipeline. This can facilitate the selection of optimal Fab-Fc combinations in terms of efficacy, safety and biodistribution (Figure 3). These assays should provide biophysical methods for screening antigen-specific affinities for FcRs,

C1q and FcRn and the antibody glycosylation profile, as well as complement and cell-based functional assays to measure antigen-specific effector functions. It is important to emphasize that, because of the close relationship of Fab and Fc regions, both binding and functional assays should be performed in the presence of the antigen to have a full overview on the antibody functionality. In other words, the field of therapeutic antibody development needs to progress from assessing the bipartite immune complexes (FcR-antibody or antibody-antigen interactions) to tripartite immune complexes (FcR-antibody-antigen) for the elucidation of physiologically relevant interactions and hence clinically relevant outcomes.

Considerations on safety: when "silencing" is not truly silent

The Fc domain is also involved in several potential therapeutic antibody side effects. Depending on the therapeutic purpose, some antibodies do not require Fc-mediated effector function, and, for some, the Fc's ability to engage FcRs could pose safety risks.

ADCs, a rapidly growing format in the antibody clinical pipeline, do not necessarily require mediating Fc effector functions; on the contrary the engagement of FcRs can lead to ADC internalization by immune cells, resulting in off-target effects. An example is trastuzumab emtansine (TDM-1), which has been reported to be internalized by megakaryocytes in a FcγRIIa-dependent manner, leading to thrombocytopenia.[70] For this reason, several strategies to eliminate FcR binding have been implemented for the development of ADCs. However, the actual abrogation of FcR affinity and off-target cell toxicity should be thoroughly evaluated during the discovery process for each novel ADC.[35]

Engagement of FcRs can also lead to antibody-dependent enhancement (ADE) of infection or disease whereby antibodies can facilitate the cellular entry of viruses even when the cell lacks the expression of the viral receptor. Fc engineering to abrogate FcRs affinity can prevent ADE, a strategy that has been recently used for anti-SARS-CoV-2 antibodies.[51] To date, one approved anti-SARS-CoV-2 antibody has been designed with reduced effector function.[3]

One of the most dangerous side effects of antibody therapeutics is cytokine release syndrome (CRS), which occurs when excessive release of cytokines leads to endothelial damage and multi organ failure.[71] Antibodies that have provoked CRS in humans are the anti-CD3 muromonab,[72] the anti-CD52 alemtuzumab,[73] the anti-CD28 TGN1412,[74] and, in rare cases, the anti-CD20 rituximab.[75] For these antibodies, CRS has been demonstrated to be Fc mediated, either by engaging FcγRIIIa on NK cells (alemtuzumab)[73] or by binding FcγRIIb and using it as a scaffold to better cluster the target receptor (TGN1412).[76,77] The mechanism responsible for TGN1412-induced CRS has been widely debated. The isotype of choice for the design of the antibody was an IgG4, chosen because of its low capacity to trigger effector functions. However, IgG4 can mediate effector function under certain conditions and has an affinity for FcγRIIb comparable to IgG1. An initial evaluation excluded an Fc-mediated role, mainly because when introducing the Fc-silencing L235E mutation, the antibody retained the ability to trigger pro-inflammatory cytokine release.[78] Follow-up studies have instead demonstrated that TGN1412's superagonistic activity is Fc-mediated and involves FcγRIIb. Hussein and colleagues have shown that T cells in high density peripheral blood mononuclear cell culture respond to TGN1412, and their response is dependent on FcγRIIb expression on monocytes.[77] Chenoweth and colleagues, in a thorough evaluation of the effect of Fc engineering mutations on TGN1412 binding to FcRs, have demonstrated that introducing the L235E mutation in the TGN1412 antibody abrogated the affinity for activating FcγRs, but not for FcγRIIb. This could explain the ability of a TGN1412 L235E mutant to trigger CRS and suggests a scaffolding Fc-mediated mechanism.[76] The TGN1412 story is a clear example of how crucial it is to broadly evaluate the impact of antibody design on FcR binding and on wanted and unwanted Fc-mediated effector functions.

As discussed above, the implications of Fc modifications are dependent on the antibody target and therapeutic context, and the impact of Fc design on FcRs binding and antibody effector function needs to be systematically screened during the early phase of antibody therapeutic development to avoid unexpected and sometimes very serious results in the clinic. In particular, when the antibody is designed to not have effector function, the silencing of Fc-mediated functions should be thoroughly evaluated using not only the canonical three assays (NK-mediated ADCC, monocyte-mediated ADCP and CDC), but also an array of assays using a variety of immune cell types. Functional screening should be also complemented by a set of biophysical assays, such as glycosylation profile and antigen-specific FcRand C1q binding.

Conclusion

Advances in antibody engineering techniques and in the understanding of the mechanisms involved in antibody effector functions, together with a deeper knowledge of disease biology, have resulted in the biopharmaceutical industry engaging in new antibody targets, new formats, and using antibody engineering to fine tune antibody functions. These new advances in antibody discovery could potentially enable ad hoc tailoring of antibodies depending on the disease. However, to make the best of such a powerful tool, several factors need to be considered. In the past decade, there has been increased acceptance of the close relationship between Fab and Fc, and the implications of antibody design on Fc-mediated effector function, biodistribution, and safety, which need to be considered and implemented during the discovery process of next-generation therapeutic antibodies. The burst of innovative antibody discovery together with the variety of possible antibody effector functions and their safety implications, highlight the need for a broad and systematic approach for Fc effector function profiling in the early stages of the antibody therapeutic development pipeline.

The regulatory guidance for evaluating Fc-mediated effector function is mainly limited to three assays: NK-mediated ADCC, monocyte-mediated ADCP, and CDC. However, we expect that improved preclinical evaluation of Fc-mediated effector functions will result from a broader array of high

throughput biophysical and cell-based assays performed in the presence of the target antigen i.e., tripartite immune complexes (Figure 4). Such a broader evaluation will both reduce risk in the more expensive later development steps and provide the basis to address additional questions from regulators. Ideally, ADCC and ADCP assays should not be reporter assays, but measure effective cytotoxicity and phagocytosis. Furthermore, given the variety of immune cells antibodies can engage, cell-based assays should be performed using a comprehensive set of both established cell lines and primary cells of human origin. To further inform on safety, supernatants from each assay should be then analyzed for cytokine release. Cell-based assays should be complemented by biophysical assays evaluating binding affinities for FcRs and glycosylation profiling. An integrated analysis of biophysical and cell-based assays should then be able to inform the possible effector functions the antibody can mediate in vivo, antibody serum half-life, and safety, based on empirical data. The impact of antibody design on Fc function should be considered early and tested empirically in the antibody development process to select optimal leads in terms of maximal function and safety. Early identification of failures and elimination of uncertainties in the early stages of the antibody discovery process is a risk-mitigation strategy that can facilitate product development and enhance patient safety. Finally, these assays should ideally be performed under a quality management system for supporting regulatory filing, to ensure consistency, reliability, and Good Clinical Laboratory Practices compliance, in order to generate reliable and reproducible high-quality data adhering to regulatory standards.

The complex and interconnected factors that influence antibody effector functions present substantial challenges in designing Fc domains of antibody therapeutics to achieve desired functional outcomes. A major hurdle is the current lack of understanding of the rules governing the interdependence of these factors, making it difficult to accurately predict effector functions during Fc domain design. Broad profiling of Fc-dependent functions, as described here, through systematic studies of Fc domain modifications that probe the variables influencing antibody effector functions, could generate valuable datasets. These datasets could be used to train predictive algorithms, enabling accurate effector function predictions. Recent studies have begun advancing the field toward large-scale Fc domain screening,[6,47,79] a critical step in developing accurate predictive models. Such models may eventually enable a "plug-and-play" approach to Fc domain design, streamlining the development of antibody therapeutics with tailored functional profiles.

Antibodies have been, and will remain for the foreseeable future, among the most powerful modalities for therapeutic intervention. Like any cutting-edge field, therapeutic antibodies face their own specific challenges. Every challenge we face is an opportunity for growth. It would behoove us, the field at large, to acknowledge that with great power, comes great responsibility, and great opportunity.

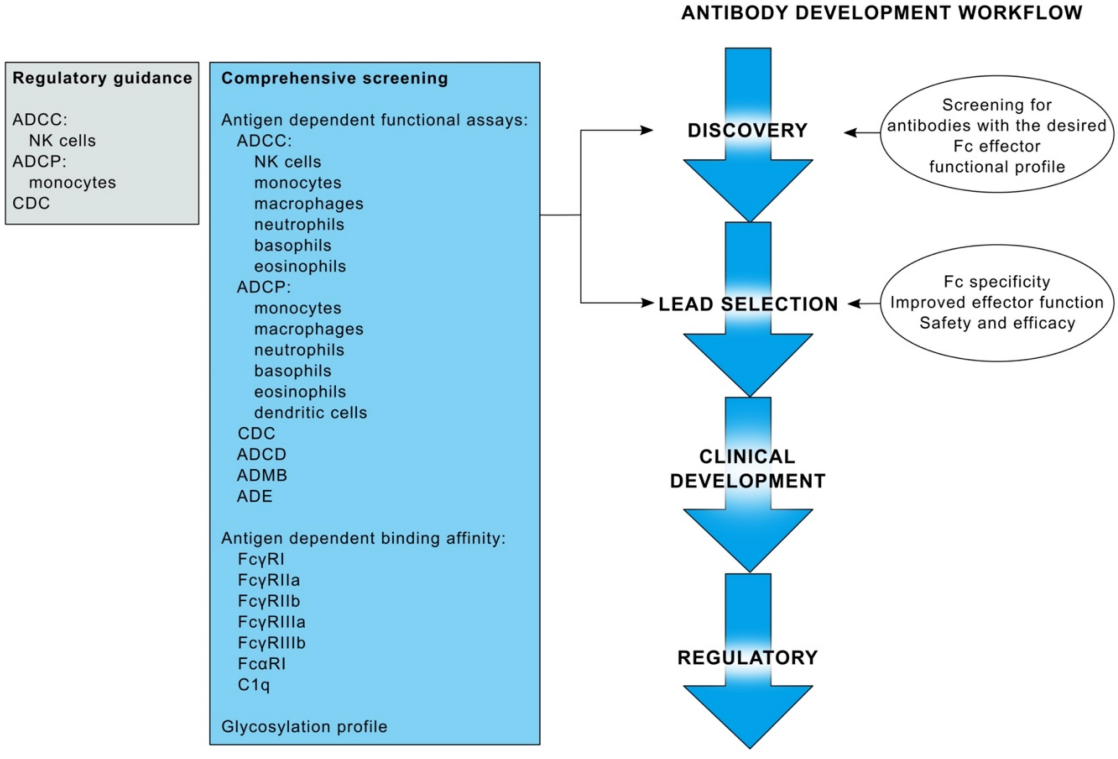

Figure 4. Early and broad screening of Fc-mediated function for optimal antibody development: schematic depicting the antibody development workflow and where a comprehensive screening of Fc-mediated functions would be beneficial. The boxes on the left illustrate the assays complying with the current regulatory guidance (in grey) and the broad variety of assays to be used for a comprehensive screening of Fc-mediated functions (in blue).

Abbreviations

ADC	Antibody-drug conjugate
ADCC	Antibody-dependent cell cytotoxicity
ADCD	Antibody-dependent complement deposition
ADCP	Antibody-dependent cell phagocytosis
ADE	Antibody-dependent enhancement
ADMB	Antibody-dependent mucin binding
APC	Antigen presenting cell
C1q	Complement component 1q
CD	Cluster of differentiation
CDC	Complement dependent cytotoxicity
CRS	Cytokine release syndrome
Fab	Fragment antigen-binding
Fc	Fragment crystallizable
FcR	Fc receptor
FcRn	Neonatal Fc receptor
FcαR	Fc receptor alpha
FcγR	Fc receptor gamma
FcμR	Fc receptor mu
FcεR	Fc receptor epsilon
GPI	glycophosphadylinositol
Ig	Immunoglobulin
MAC	Membrane attack complex
MHC	Major histocompatibility complex
NK	Natural killer
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
TDM-1	Trastuzumab emtansine

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References

1. Crescioli S, Kaplon H, Chenoweth A, Wang L, Visweswaraiah J, Reichert JM. Antibodies to watch in 2024. MAbs. 2024;16 (1):2297450. doi:https://doi.org/10.1080/19420862.2023.2297450.
2. Kaplon H, Crescioli S, Chenoweth A, Visweswaraiah J, Reichert JM. Antibodies to watch in 2023. MAbs. 2023;15 (1):2153410. doi:https://doi.org/10.1080/19420862.2022.2153410.
3. The Antibody Society. Therapeutic monoclonal antibodies approved or in regulatory review. [Accessed 8562 Dec 10]. www.antibodysociety.org/antibody-therapeutics-product-data.
4. The Antibody Society, inc. Antibody therapeutics in late-stage clinical studies. [Accessed 2024 Dec 10]. www.antibodysociety.org/antibodies-in-late-stage-clinical-studies/.
5. Moore GL, Chen H, Karki S, Lazar GA. Engineered Fc variant antibodies with enhanced ability to recruit complement and mediate effector functions. MAbs. 2010;2(2):181–189. doi:https://doi.org/10.4161/mabs.2.2.11158.
6. Gunn BM, Lu R, Slein MD, Ilinykh PA, Huang K, Atyeo C, Schendel SL, Kim J, Cain C, Roy V, et al. A Fc engineering approach to define functional humoral correlates of immunity against Ebola virus. Immunity. 2021;54(4):815–828.e815. doi:https://doi.org/10.1016/j.immuni.2021.03.009.
7. Damelang T, Brinkhaus M, van Osch TLJ, Schuurman J, Labrijn AF, Rispens T, Vidarsson G. Impact of structural modifications of IgG antibodies on effector functions. Front Immunol. 2023;14:1304365. doi:https://doi.org/10.3389/fimmu.2023.1304365.
8. Galvez-Cancino F, Simpson AP, Costoya C, Matos I, Qian D, Peggs KS, Litchfield K, Quezada SA. Fcγ receptors and immunomodulatory antibodies in cancer. Nat Rev Cancer. 2024;24 (1):51–71. doi:https://doi.org/10.1038/s41568-023-00637-8.
9. Pincetic A, Bournazos S, DiLillo DJ, Maamary J, Wang TT, Dahan R, Fiebiger B-M, Ravetch JV. Type I and type II Fc receptors regulate innate and adaptive immunity. Nat Immunol. 2014;15(8):707–716. doi:https://doi.org/10.1038/ni.2939.
10. DiLillo DJ, Ravetch JV. Fc-receptor interactions regulate both cytotoxic and immunomodulatory therapeutic antibody effector functions. Cancer Immunol Res. 2015;3(7):704–713. doi:https://doi.org/10.1158/2326-6066.Cir-15-0120.
11. Clarke JM, Stinchcombe T, Gu L, Mamdani H, Antonia SJ, Simon GR, Sonpavde GP, Ready NE, Crawford J, Campa M, et al. Results from a first-in-human phase 1B study of a complement factor H inhibitor (GT103) in patients with non-small cell lung cancer (NSCLC). J Clin Oncol. 2023;41(16_suppl):9128–9128. doi:https://doi.org/10.1200/JCO.2023.41.16_suppl.9128.
12. Spicer J, Basu B, Montes A, Banerji U, Kristeleit R, Miller R, Veal GJ, Corrigan CJ, Till SJ, Figini M, et al. Safety and anti-tumour activity of the IgE antibody MOv18 in patients with advanced solid tumours expressing folate receptor-alpha: a phase I trial. Nat Commun. 2023;14(1):4180. doi:https://doi.org/10.1038/s41467-023-39679-9.
13. Brandsma AM, Jacobino SR, Meyer S, ten Broeke T, Leusen JH. Fc receptor inside-out signaling and possible impact on antibody therapy. Immunol Rev. 2015;268(1):74–87. doi:https://doi.org/10.1111/imr.12332.
14. Bournazos S, Gupta A, Ravetch JV. The role of IgG Fc receptors in antibody-dependent enhancement. Nat Rev Immunol. 2020;20 (10):633–643. doi:https://doi.org/10.1038/s41577-020-00410-0.
15. Delidakis G, Kim JE, George K, Georgiou G. Improving antibody therapeutics by manipulating the Fc domain: immunological and structural considerations. Annu Rev Biomed Eng. 2022;24 (1):249–274. doi:https://doi.org/10.1146/annurev-bioeng-082721-024500.
16. Lu LL, Suscovich TJ, Fortune SM, Alter G. Beyond binding: antibody effector functions in infectious diseases. Nat Rev Immunol. 2018;18(1):46–61. doi:https://doi.org/10.1038/nri.2017.106.
17. Gunn BM, Bai S. Building a better antibody through the Fc: advances and challenges in harnessing antibody Fc effector functions for antiviral protection. Hum Vaccin Immunother. 2021;17 (11):4328–4344. doi:https://doi.org/10.1080/21645515.2021.1976580.
18. Huang ZY, Hunter S, Chien P, Kim M-K, Han-Kim T-H, Indik ZK, Schreiber AD. Interaction of two phagocytic host defense systems: Fcγ receptors and complement receptor 3. J Biol Chem. 2011;286(1):160–168. doi:10.1074/jbc.M110.163030.
19. Boruchov AM, Heller G, Veri MC, Bonvini E, Ravetch JV, Young JW. Activating and inhibitory IgG fc receptors on human DCs mediate opposing functions. J Clin Invest. 2005;115 (10):2914–2923. doi:10.1172/jci24772.
20. Dhodapkar KM, Kaufman JL, Ehlers M, Banerjee DK, Bonvini E, Koenig S, Steinman RM, Ravetch JV, Dhodapkar MV. Selective blockade of inhibitory Fcγ receptor enables human dendritic cell maturation with IL-12p70 production and immunity to antibody-coated tumor cells. Proc Natl Acad Sci USA. 2005;102 (8):2910–2915. doi:10.1073/pnas.0500014102.
21. Dhodapkar KM, Krasovsky J, Williamson B, Dhodapkar MV. Antitumor monoclonal antibodies enhance cross-presentation of cellular antigens and the generation of myeloma-specific killer T cells by dendritic cells. J Exp Med. 2002;195(1):125–133. doi:10.1084/jem.20011097.
22. Kinder M, Greenplate AR, Strohl WR, Jordan RE, Brezski RJ. An Fc engineering approach that modulates antibody-dependent cytokine release without altering cell-killing functions. MAbs. 2015;7(3):494–504. doi:10.1080/19420862.2015.1022692.
23. Zhao S, Zhang L, Xiang S, Hu Y, Wu Z, Shen J. Gnawing between cells and cells in the immune system: friend or foe? A review of

trogocytosis. Front Immunol. 2022;13:791006. doi:10.3389/fimmu.2022.791006.
24. Ackerman ME, Moldt B, Wyatt RT, Dugast A-S, McAndrew E, Tsoukas S, Jost S, Berger CT, Sciaranghella G, Liu Q, et al. A robust, high-throughput assay to determine the phagocytic activity of clinical antibody samples. J Immunol Methods. 2011;366(1–2):8–19. doi:10.1016/j.jim.2010.12.016.
25. Fischinger S, Fallon JK, Michell AR, Broge T, Suscovich TJ, Streeck H, Alter G. A high-throughput, bead-based, antigen-specific assay to assess the ability of antibodies to induce complement activation. J Immunol Methods. 2019;473:112630. doi:10.1016/j.jim.2019.07.002.
26. Karsten CB, Mehta N, Shin SA, Diefenbach TJ, Slein MD, Karpinski W, Irvine EB, Broge T, Suscovich TJ, Alter G, et al. A versatile high-throughput assay to characterize antibody-mediated neutrophil phagocytosis. J Immunol Methods. 2019;471:46–56. doi:10.1016/j.jim.2019.05.006.
27. Furumaya C, Martinez-Sanz P, Bouti P, Kuijpers TW, Matlung HL. Plasticity in pro- and anti-tumor activity of neutrophils: shifting the balance. Front Immunol. 2020;11:2100. doi:10.3389/fimmu.2020.02100.
28. Heemskerk N, van Egmond M. Monoclonal antibody-mediated killing of tumour cells by neutrophils. Eur J Clin Invest. 2018;48(2):e12962. doi:10.1111/eci.12962.
29. Camacho-Sandoval R, Jiménez-Uribe A, Tenorio-Calvo AV, López-Morales CA, Muñoz-García L, Montes-Luna A, García-Xolalpa HL, Velasco-Velázquez M, Pavón L, Pérez-Tapia SM, et al. Taking advantage of a high-throughput flow cytometer for the implementation of an ADCC assay for regulatory compliance. Biotechnol Rep (Amst). 2020;26:e00456. doi:10.1016/j.btre.2020.e00456.
30. Nimmerjahn F, Ravetch JV. Fcγ receptors as regulators of immune responses. Nat Rev Immunol. 2008;8(1):34–47. doi:10.1038/nri2206.
31. Bruhns P, Iannascoli B, England P, Mancardi DA, Fernandez N, Jorieux S, Daëron M. Specificity and affinity of human Fcγ receptors and their polymorphic variants for human IgG subclasses. Blood. 2009;113(16):3716–3725. doi:10.1182/blood-2008-09-179754.
32. Chu TH, Patz EF Jr., Ackerman ME. Coming together at the hinges: therapeutic prospects of IgG3. MAbs. 2021;13(1):1882028. doi:10.1080/19420862.2021.1882028.
33. Armstrong GB, Lewis A, Shah V, Taylor P, Jamieson CJ, Burley GA, Lewis W, Rattray Z. A first insight into the developability of an immunoglobulin G3: a combined computational and experimental approach. ACS Pharmacol & Transl Sci. 2024;7(8):2439–2451. doi:10.1021/acsptsci.4c00271.
34. Saito S, Namisaki H, Hiraishi K, Takahashi N, Iida S. A stable engineered human IgG3 antibody with decreased aggregation during antibody expression and low pH stress. Protein Sci. 2019;28(5):900–909. doi:10.1002/pro.3598.
35. Hoffmann RM, Coumbe BGT, Josephs DH, Mele S, Ilieva KM, Cheung A, Tutt AN, Spicer JF, Thurston DE, Crescioli S, et al. Antibody structure and engineering considerations for the design and function of antibody drug conjugates (ADCs). Oncoimmunology. 2018;7(3):e1395127. doi:10.1080/2162402x.2017.1395127.
36. Brandsma AM, Bondza S, Evers M, Koutstaal R, Nederend M, Jansen JHM, Rösner T, Valerius T, Leusen JHW, Ten Broeke T, et al. Potent Fc receptor signaling by IgA leads to Superior killing of cancer cells by neutrophils compared to IgG. Front Immunol. 2019;10:704. doi:10.3389/fimmu.2019.00704.
37. van Tetering G, Evers M, Chan C, Stip M, Leusen J. Fc engineering strategies to advance IgA antibodies as therapeutic agents. Antibodies (Basel). 2020;9(4):70. doi:10.3390/antib9040070.
38. Borrok MJ, Luheshi NM, Beyaz N, Davies GC, Legg JW, Wu H, Dall'Acqua WF, Tsui P. Enhancement of antibody-dependent cell-mediated cytotoxicity by endowing IgG with FcαRI (CD89) binding. MAbs. 2015;7(4):743–751. doi:10.1080/19420862.2015.1047570.
39. Heinkel F, Verstraete MM, Cao S, Li J, Farber P, Stangle E, Silva-Moreno B, Peng F, Dixit S, Boulanger MJ, et al. Engineering a pure and stable heterodimeric IgA for the development of multispecific therapeutics. MAbs. 2022;14(1):2141637. doi:10.1080/19420862.2022.2141637.
40. Kelton W, Mehta N, Charab W, Lee J, Lee C-H, Kojima T, Kang T, Georgiou G. IgGA: a "cross-isotype" engineered human fc antibody domain that displays both IgG-like and IgA-like effector functions. Chem Biol. 2014;21(12):1603–1609. doi:10.1016/j.chembiol.2014.10.017.
41. Strohl WR, Ku Z, An Z, Carroll SF, Keyt BA, Strohl LM. Passive immunotherapy against SARS-CoV-2: from plasma-based therapy to single potent antibodies in the race to stay ahead of the variants. BioDrugs. 2022;36(3):231–323. doi:10.1007/s40259-022-00529-7.
42. Wang BT, Kothambawala T, Wang L, Matthew TJ, Calhoun SE, Saini AK, Kotturi MF, Hernandez G, Humke EW, Peterson MS, et al. Multimeric anti-DR5 IgM agonist antibody IGM-8444 is a potent inducer of cancer cell Apoptosis and synergizes with chemotherapy and BCL-2 inhibitor ABT-199. Mol Cancer Ther. 2021;20(12):2483–2494. doi:10.1158/1535-7163.Mct-20-1132.
43. Zhang D, Goldberg MV, Chiu ML. Fc engineering approaches to enhance the agonism and effector functions of an anti-OX40 antibody. J Biol Chem. 2016;291(53):27134–27146. doi:10.1074/jbc.M116.757773.
44. Li F, Ravetch JV. Apoptotic and antitumor activity of death receptor antibodies require inhibitory Fcγ receptor engagement. Proc Natl Acad Sci USA. 2012;109(27):10966–10971. doi:10.1073/pnas.1208698109.
45. Mimoto F, Katada H, Kadono S, Igawa T, Kuramochi T, Muraoka M, Wada Y, Haraya K, Miyazaki T, Hattori K, et al. Engineered antibody Fc variant with selectively enhanced Fc RIIb binding over both Fc RIIaR131 and Fc RIIaH131. Protein Eng Des Sel. 2013;26(10):589–598. doi:10.1093/protein/gzt022.
46. An Z, Forrest G, Moore R, Cukan M, Haytko P, Huang L, Vitelli S, Zhao JZ, Lu P, Hua J, et al. IgG2m4, an engineered antibody isotype with reduced fc function. MAbs. 2009;1(6):572–579. doi:10.4161/mabs.1.6.10185.
47. Hale G, De Vos J, Davy AD, Sandra K, Wilkinson I. Systematic analysis of Fc mutations designed to reduce binding to Fc-gamma receptors. mAbs. 2024;16(1):2402701. doi:10.1080/19420862.2024.2402701.
48. Frischauf N, Strasser J, Borg EGF, Labrijn AF, Beurskens FJ, Preiner J. Complement activation by IgG subclasses is governed by their ability to oligomerize upon antigen binding. Proceedings of the National Academy of Sciences; Vol. 121. 2024. p. e2406192121. doi:10.1073/pnas.2406192121.
49. Sopp JM, Peters SJ, Rowley TF, Oldham RJ, James S, Mockridge I, French RR, Turner A, Beers SA, Humphreys DP, et al. On-target IgG hexamerisation driven by a C-terminal IgM tail-piece fusion variant confers augmented complement activation. Commun Biol. 2021;4(1):1031. doi:10.1038/s42003-021-02513-3.
50. de Jong RN, Beurskens FJ, Verploegen S, Strumane K, van Kampen MD, Voorhorst M, Horstman W, Engelberts PJ, Oostindie SC, Wang G, et al. A novel platform for the potentiation of therapeutic antibodies based on antigen-dependent formation of IgG hexamers at the cell surface. PLOS Biol. 2016;14(1):e1002344. doi:10.1371/journal.pbio.1002344.
51. House RV, Broge TA, Suscovich TJ, Snow DM, Tomic MT, Nonet G, Bajwa K, Zhu G, Martinez Z, Hackett K, et al. Evaluation of strategies to modify anti-SARS-CoV-2 monoclonal antibodies for optimal functionality as therapeutics. PLOS ONE. 2022;17(6):e0267796. doi:10.1371/journal.pone.0267796.
52. Rondeau E, Scully M, Ariceta G, Barbour T, Cataland S, Heyne N, Miyakawa Y, Ortiz S, Swenson E, Vallee M, et al. The long-acting C5 inhibitor, Ravulizumab, is effective and safe in adult patients with atypical hemolytic uremic syndrome naïve to complement inhibitor treatment. Kidney Int. 2020;97(6):1287–1296. doi:10.1016/j.kint.2020.01.035.
53. Yu XQ, Robbie GJ, Wu Y, Esser MT, Jensen K, Schwartz HI, Bellamy T, Hernandez-Illas M, Jafri HS. Safety, tolerability, and

pharmacokinetics of MEDI4893, an investigational, extended-half-life, Anti-Staphylococcus aureus alpha-toxin human monoclonal antibody, in healthy adults. Antimicrob Agents Chemother. 2017;61(1). doi:10.1128/aac.01020-16.
54. Forthal DN, Gach JS, Landucci G, Jez J, Strasser R, Kunert R, Steinkellner H. Fc-glycosylation influences Fcγ receptor binding and cell-mediated anti-hiv activity of monoclonal antibody 2G12. J Immunol. 2010;185(11):6876–6882. doi:10.4049/jimmunol.1002600.
55. Shields RL, Lai J, Keck R, O'Connell LY, Hong K, Meng YG, Weikert SHA, Presta LG. Lack of fucose on human IgG1 N-Linked oligosaccharide improves binding to human FcγRIII and Antibody-dependent cellular toxicity. J Biol Chem. 2002;277(30):26733–26740. doi:10.1074/jbc.M202069200.
56. Shinkawa T, Nakamura K, Yamane N, Shoji-Hosaka E, Kanda Y, Sakurada M, Uchida K, Anazawa H, Satoh M, Yamasaki M, et al. The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. J Biol Chem. 2003;278(5):3466–3473. doi:10.1074/jbc.M210665200.
57. Shade K-TC, Anthony RM. Antibody glycosylation and inflammation. Antibodies. 2013;2(3):392–414. doi:10.3390/antib2030392.
58. Tao MH, Morrison SL. Studies of aglycosylated chimeric mouse-human IgG. Role of carbohydrate in the structure and effector functions mediated by the human IgG constant region. J Immunol. 1989;143(8):2595–2601. doi:10.4049/jimmunol.143.8.2595.
59. Wilkinson I, Hale G. Systematic analysis of the varied designs of 819 therapeutic antibodies and Fc fusion proteins assigned international nonproprietary names. MAbs. 2022;14(1):2123299. doi:10.1080/19420862.2022.2123299.
60. Lux A, Yu X, Scanlan CN, Nimmerjahn F. Impact of immune complex size and glycosylation on IgG binding to human FcγRs. J Immunol. 2013;190(8):4315–4323. doi:10.4049/jimmunol.1200501.
61. White AL, Beers SA, Cragg MS. FcγRIIB as a key determinant of agonistic antibody efficacy. Curr Top Microbiol Immunol. 2014;382:355–372. doi:10.1007/978-3-319-07911-0_16.
62. White AL, Chan HT, French R, Willoughby J, Mockridge C, Roghanian A, Penfold C, Booth S, Dodhy A, Polak M, et al. Conformation of the human immunoglobulin G2 hinge imparts superagonistic properties to immunostimulatory anticancer antibodies. Cancer Cell. 2015;27(1):138–148. doi:10.1016/j.ccell.2014.11.001.
63. Heckel F, Turaj AH, Fisher H, Chan HTC, Marshall MJE, Dadas O, Penfold CA, Inzhelevskaya T, Mockridge CI, Alvarado D, et al. Agonistic CD27 antibody potency is determined by epitope-dependent receptor clustering augmented through fc-engineering. Commun Biol. 2022;5(1):229. doi:10.1038/s42003-022-03182-6.
64. Oostindie SC, Lazar GA, Schuurman J, Parren P. Avidity in antibody effector functions and biotherapeutic drug design. Nat Rev Drug Discov. 2022;21(10):715–735. doi:10.1038/s41573-022-00501-8.
65. Wang B, Yang C, Jin X, Du Q, Wu H, Dall'acqua W, Mazor Y. Regulation of antibody-mediated complement-dependent cytotoxicity by modulating the intrinsic affinity and binding valency of IgG for target antigen. MAbs. 2020;12(1):1690959. doi:10.1080/19420862.2019.1690959.
66. Mazor Y, Yang C, Borrok MJ, Ayriss J, Aherne K, Wu H, Dall'acqua WF. Enhancement of immune effector functions by modulating IgG's intrinsic affinity for target antigen. PLOS ONE. 2016;11(6):e0157788. doi:10.1371/journal.pone.0157788.
67. Cleary KLS, Chan HTC, James S, Glennie MJ, Cragg MS. Antibody distance from the cell membrane regulates antibody effector mechanisms. J Immunol. 2017;198(10):3999–4011. doi:10.4049/jimmunol.1601473.
68. Bakalar MH, Joffe AM, Schmid EM, Son S, Podolski M, Fletcher DA. Size-dependent segregation controls macrophage phagocytosis of antibody-opsonized targets. Cell. 2018;174(1):131–142.e113. doi:10.1016/j.cell.2018.05.059.
69. Hatterer E, Chauchet X, Richard F, Barba L, Moine V, Chatel L, Broyer L, Pontini G, Bautzova T, Juan F, et al. Targeting a membrane-proximal epitope on mesothelin increases the tumoricidal activity of a bispecific antibody blocking CD47 on mesothelin-positive tumors. MAbs. 2020;12(1):1739408. doi:10.1080/19420862.2020.1739408.
70. Uppal H, Doudement E, Mahapatra K, Darbonne WC, Bumbaca D, Shen B-Q, Du X, Saad O, Bowles K, Olsen S, et al. Potential mechanisms for thrombocytopenia development with trastuzumab emtansine (T-DM1). Clin Cancer Res. 2015;21(1):123–133. doi:https://doi.org/10.1158/1078-0432.Ccr-14-2093.
71. Hansel TT, Kropshofer H, Singer T, Mitchell JA, George AJ. The safety and side effects of monoclonal antibodies. Nat Rev Drug Discov. 2010;9(4):325–338. doi:https://doi.org/10.1038/nrd3003.
72. Suthanthiran M, Fotino M, Riggio RR, Cheigh JS, Stenzel KH. OKT3-associated adverse reactions: mechanistic basis and therapeutic options. Am J Kidney Dis. 1989;14:39–44.
73. Wing MG, Moreau T, Greenwood J, Smith RM, Hale G, Isaacs J, Waldmann H, Lachmann PJ, Compston A. Mechanism of first-dose cytokine-release syndrome by CAMPATH 1-H: involvement of CD16 (FcgammaRIII) and CD11a/CD18 (LFA-1) on NK cells. J Clin Invest. 1996;98(12):2819–2826. doi:https://doi.org/10.1172/jci119110.
74. Suntharalingam G, Perry MR, Ward S, Brett SJ, Castello-Cortes A, Brunner MD, Panoskaltsis N. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. N Engl J Med. 2006;355(10):1018–1028. doi:https://doi.org/10.1056/NEJMoa063842.
75. Winkler U, Jensen M, Manzke O, Schulz H, Diehl V, Engert A. Cytokine-release syndrome in patients with B-cell chronic lymphocytic leukemia and high lymphocyte counts after treatment with an anti-CD20 monoclonal antibody (rituximab, IDEC-C2B8). Blood. 1999;94(7):2217–2224. doi:10.1182/blood.V94.7.2217.419k02_2217_2224.
76. Chenoweth AM, Esparon S, Wines BD, Schuurman J, Labrijn AF, Hogarth PM. Mutation of the TGN1412 anti-CD28 monoclonal antibody lower hinge confers specific FcγRIIb binding and retention of super-agonist activity. Immunol Cell Biol. 2023;101(7):657–662. doi:https://doi.org/10.1111/imcb.12646.
77. Hussain K, Hargreaves CE, Roghanian A, Oldham RJ, Chan HTC, Mockridge CI, Chowdhury F, Frendéus B, Harper KS, Strefford JC, et al. Upregulation of FcγRIIb on monocytes is necessary to promote the superagonist activity of TGN1412. Blood. 2015;125(1):102–110. doi:https://doi.org/10.1182/blood-2014-08-593061.
78. Ball C, Fox B, Hufton S, Sharp G, Poole S, Stebbings R, Eastwood D, Findlay L, Parren PWHI, Thorpe R, et al. Antibody C region influences TGN1412-like functional activity in vitro. J Immunol. 2012;189(12):5831–5840. doi:https://doi.org/10.4049/jimmunol.1201795.
79. Hale G, Davy AD, Wilkinson I. Systematic analysis of fc mutations designed to enhance binding to fc-gamma receptors. MAbs. 2024;16(1):2406539. doi:https://doi.org/10.1080/19420862.2024.2406539.