Author/s:
Chenoweth, AM;Wines, BD;Anania, JC;Mark Hogarth, P

Title:
Harnessing the immune system via FcγR function in immune therapy: a pathway to next-gen mAbs

Date:
2020-04-01

Citation:
Chenoweth, A. M., Wines, B. D., Anania, J. C. & Mark Hogarth, P. (2020). Harnessing the immune system via FcγR function in immune therapy: a pathway to next-gen mAbs. Immunology and Cell Biology, 98 (4), pp.287-304. https://doi.org/10.1111/imcb.12326.

Persistent Link:
https://hdl.handle.net/11343/252353

Immunology & Cell Biology 2020; **98**: 287–304
www.wileyonlinelibrary.com/journal/icb

SPECIAL FEATURE REVIEW

OPEN

Harnessing the immune system *via* FcγR function in immune therapy: a pathway to next-gen mAbs

Alicia M Chenoweth[1,2,3], Bruce D Wines[1,2,4], Jessica C Anania[1,2,5] & P Mark Hogarth[1,2,4]

1 Immune Therapies Laboratory, Burnet Institute, Melbourne, Australia
2 Department of Immunology and Pathology, Central Clinical School, Monash University, Melbourne, Australia
3 St John's Institute of Dermatology, King's College, London, UK
4 Department of Clinical Pathology, University of Melbourne, Parkville, Australia
5 Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

Keywords
ADCC, Fc receptors, immune therapy, monoclonal antibodies, phagocytosis, SARS-CoV-2

Correspondence
P Mark Hogarth, Burnet Institute, GPO Box 2284, Melbourne, VIC 3001, Australia.
E-mail: mark.hogarth@burnet.edu.au

Present address
Alicia M Chenoweth, King's College, London, UK
Jessica C Anania, Uppsala University, Uppsala, Sweden

Received 28 October 2019; Revised 7 March 2020; Accepted 10 March 2020

doi: 10.1111/imcb.12326

Immunology & Cell Biology 2020; **98**: 287–304

Abstract

The human fragment crystallizable (Fc)γ receptor (R) interacts with antigen-complexed immunoglobulin (Ig)G ligands to both activate and modulate a powerful network of inflammatory host-protective effector functions that are key to the normal physiology of immune resistance to pathogens. More than 100 therapeutic monoclonal antibodies (mAbs) are approved or in late stage clinical trials, many of which harness the potent FcγR-mediated effector systems to varying degrees. This is most evident for antibodies targeting cancer cells inducing antibody-dependent killing or phagocytosis but is also true to some degree for the mAbs that neutralize or remove small macromolecules such as cytokines or other Igs. The use of mAb therapeutics has also revealed a "scaffolding" role for FcγR which, in different contexts, may either underpin the therapeutic mAb action such as immune agonism or trigger catastrophic adverse effects. The still unmet therapeutic need in many cancers, inflammatory diseases or emerging infections such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) requires increased effort on the development of improved and novel mAbs. A more mature appreciation of the immunobiology of individual FcγR function and the complexity of the relationships between FcγRs and antibodies is fueling efforts to develop more potent "next-gen" therapeutic antibodies. Such development strategies now include focused glycan or protein engineering of the Fc to increase affinity and/or tailor specificity for selective engagement of individual activating FcγRs or the inhibitory FcγRIIb or alternatively, for the ablation of FcγR interaction altogether. This review touches on recent aspects of FcγR and IgG immunobiology and its relationship with the present and future actions of therapeutic mAbs.

INTRODUCTION

The regulatory approval of the first therapeutic monoclonal antibodies (mAbs) in the 1980s ushered in the modern era of immune therapy. Since then, mAbs have become one of the most clinically successful therapeutic modalities across a diverse array of diseases. They have revolutionized the treatment of chronic inflammatory diseases and of some cancers including otherwise incurable malignancies.[1] They are commercially important and in 2017, five mAbs collectively grossed $45.6 billion in sales, placing them in the top ten drugs globally.[2] MAb development is expanding rapidly with over 100 mAbs approved for clinical use or in late-stage clinical trials and over 600 in various stages of clinical development.[1]

The therapeutic actions of mAbs can take many forms—neutralization of the target such as cytokines in autoimmune disease, clearance of the target such as virus in infection or immunoglobulin (Ig)E in allergy, induction of innate effector cell activation that leads to target destruction by direct killing or the induction of apoptosis and the induction of adaptive

immunity. Most therapeutic mAbs are IgG in origin and the heavy-chain subclass determines many of their biological properties including their long plasma half-life[3]; complement activation, which is important in the action of some cytotoxic mAbs[4-6] and importantly engagement by their fragment crystallizable (Fc) region with specific cell surface receptors, called FcγR, the subject of this review.

In normal homeostatic immunity, there is a balance between IgG immune complex activation of proinflammatory responses through the activating-type FcγRs—which leads to the destruction of opsonized pathogens—and of the modulation of these destructive effector responses by the inhibitory-type FcγR, thereby avoiding injury to the host. Thus, therapeutic mAbs powerfully exploit these opposing activities, making them versatile drugs whose therapeutic potency can be improved by specific engineering of Fc–FcγR interactions.[7]

Many therapeutic mAbs depend, to varying degrees, on FcγR function (Figure 1, Table 1) for their mechanism of action (MOA) and/or their pharmacokinetic properties. For some mAbs interaction with FcγR is central to their MOA, such as the destruction of a target cell by antibody-dependent cell-mediated cytotoxicity (ADCC; Figure 1a) or antibody-dependent cell-mediated phagocytosis (phagocytosis or ADCP; Figure 1b). This also includes mAbs that may harness the inhibitory action of FcγRIIb to modulate the proinflammatory responses of immunoreceptor tyrosine activation motif (ITAM)-dependent receptor signaling complexes (Figure 1c). For other mAbs, FcγR may play a secondary role, such as the removal or "sweeping" of all immune complexes formed by cytokine or virus-specific neutralizing antibodies or of opsonized fragments of lysed target cells which in antigen-presenting cells may also feed the antigen into the antigen-presentation pathways (Figure 1d). In addition, FcγRs, particularly FcγRIIb (Figure 1e), are also key participants in the MOA of immune-stimulating agonistic mAbs or apoptotic mAbs by acting as a scaffold for the additional cross-linking of mAbs already bound to a cellular target, thereby inducing a signal in the target cell.

This review focuses on the cell-based effector functions that arise from the interaction of IgG with the classical human leukocyte FcγR.[7] Although beyond the scope of this review, it should be noted that the IgG-Fc portion dictates other aspects of an antibody's biology, including its serum half-life mediated by the neonatal FcR (FcRn),[3] the activation of complement C1,[8] antiviral protection *via* the intracellular receptor TRIM21[9] and interactions with the Fc receptor-like family.[10]

HUMAN FcγR GENERAL PROPERTIES

The human leukocyte receptors fall into two functional groups, namely, proinflammatory, activating-type receptors (FcγRI, FcγRIIa, FcγRIIc, FcγRIIIa and FcγRIIIb, which are also known as CD64, CD32a, CD32c, CD16a and CD16b, respectively) and the anti-inflammatory, inhibitory-receptor group (FcγRIIb also called CD32b) which was the first immune checkpoint described.

These FcγRs are high-avidity sensors of immune complexes which initiate, and then modulate, cell responses. In the context of normal immune physiology, opsonized target molecules can engage various FcγRs and induce a spectrum of effector responses which can be harnessed by many therapeutic mAbs (Figure 1, Table 1). These responses are not mutually exclusive and one therapeutic mAb may initiate various responses *via* different FcγRs and *via* different cell types.

Understanding the importance of cell-based effector functions in the MOA of therapeutic mAbs requires an appreciation of FcγR biology (Tables 1–3) which also underpins future efforts to tailor new mAbs for the exploitation-specific effector responses. In this review, we address only key aspects of the extensive knowledge of the human leukocyte FcγR family as it relates to effector functions. A number of other reviews more comprehensively explore FcγR biology physiology, biochemistry, genetics and structure.[7,11-14] Notwithstanding the recognized differences between the immunobiology of human FcγR and of rodents or nonhuman primates, animal models of FcR effector function *in vivo* have helped shape the strategies for the development of current therapeutic mAbs and are well reviewed.12,15 Furthermore, humanized FcγR models will provide even greater insights into the future.[16]

FcγR expression on hemopoietic cells

The tissue distribution of the human leukocyte FcγR is well documented and reviewed comprehensively elsewhere.[7,11,17] In the context of effector functions harnessed by therapeutic mAbs, several aspects of the cellular distribution (Table 2) should be emphasized.

FcγR expression profiles differ between cell lineages but almost all mature human leukocytes, and platelets, express at least one FcγR (Table 2). It should also be appreciated that the cellular expression levels and receptor diversity as will be described later is also influenced by the activation state of the cells, anatomical location and the cytokine environment which modulates FcγR expression, particularly for FcγRI and FcγRIIb.[18] For example, resting monocyte subpopulations may express only FcγRIIa but activated macrophages express FcγRI, FcγRIIa and FcγRIIIa and/or FcγRIIb.[7]

Thus, specific characteristics of leukocyte FcγR expression are summarized as follows:

FcγRI is not usually expressed until induced by cytokines such as interferon-γ on monocytes, neutrophils, macrophages, microglial cells in the brain, dendritic cells

and mast cells. The sensitivity of FcγRI to interferon-γ suggests that its *in vivo* activity is closely tied to immune activation events, and mouse studies have suggested that it has a critical role early in immune responses.[19,20] Its role in the MOA of antibodies may vary with anatomical location.[21]

FcγRIIa is expressed only in primates and shows the broadest expression of all FcγRs, being present on all innate

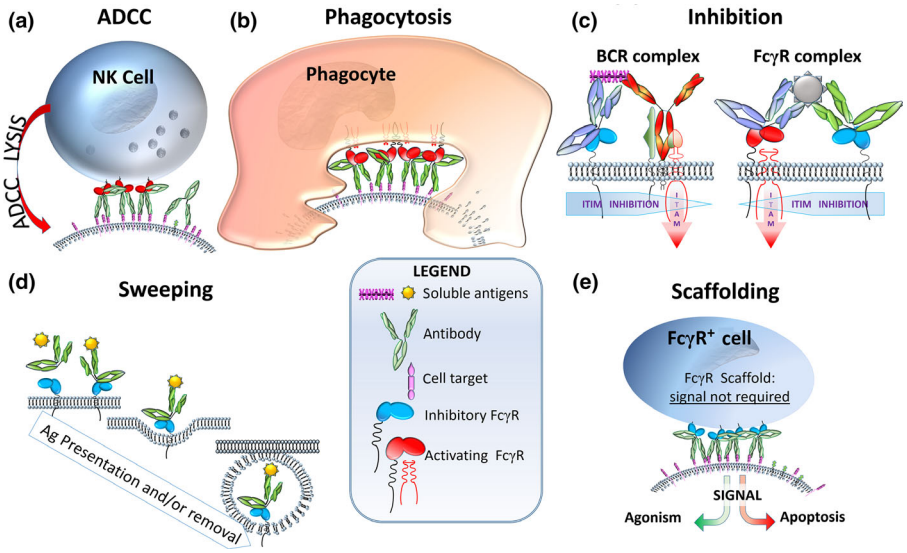

Figure 1. Graphical representation of the FcγR effector functions. **(a)** Natural killer cell antibody-dependent cell-mediated cytotoxicity via FcγRIIIa. **(b)** Antibody-dependent cell-mediated phagocytosis, and/or trogocytosis of large immune complexes, by professional phagocytes via activating FcγR such as FcγRIIIa and FcγRIIa. Biological sequelae include the destruction of the ingested complexes which may also feed antigen into antigen-presentation pathways of antigen-presenting cells (APCs). **(c)** Inhibition of cell activation by FcγRIIb. The immunoreceptor tyrosine activation motif (ITAM)-mediated signaling of B-cell antigen receptors (left) or of activating FcγR (right) on innate immune cells such as macrophages and basophils is inhibited by IgG Fc-mediated co-cross-linking of these activating receptors with the inhibitory FcγRIIb. This leads to phosphorylation of the FcγRIIb immunoreceptor tyrosine-based inhibitory motif (ITIM) and consequently recruits the phosphatases that modulate the ITAM-driven signaling responses leading to diminished cell responses. **(d)** Sweeping or internalization of small immune complexes leading to their removal and, in APC, to enhanced immune responses. **(e)** Scaffolding in which the FcγRs play a passive role. Typically involving FcγRIIb, no signal is generated in the effector cell but "super-cross-linking" of the opsonizing antibody by the FcγR on one cell generates a signal in the conjugated target cell, for example, induction of apoptosis or activation in agonistic expansion of cells and/or their secretion of cytokines. In extreme cases, this leads to life-threatening cytokine storm. ADCC, antibody-dependent cell-mediated cytotoxicity; Ag, antigen; BCR, B-cell receptor; Ig, immunoglobulin; NK, natural killer.

Table 1. FcγR responses relevant to therapeutic monoclonal antibodies (mAbs).

FcγR-mediated mechanism of action	Effector responses	Action	Dominant receptor
Activation	Antibody-dependent cell-mediated cytotoxicity	Direct killing of target cell	FcγRIIIa
	Antibody-dependent cell-mediated phagocytosis, trogocytosis	Direct killing of target cell	FcγRIIIa, FcγRIIa, FcRI
	Antigen presentation	Vaccine-like immunity post-mAb therapy	FcγRIIa, FcγRI, FcγRIIIa
Inhibition	Reduce B-cell proliferation or innate cell activation by antibody complexes	Inhibition of ITAM cell activation (i.e. BCR) or activating-type FcR (i.e. FcγR, FcεRI, FcαRI). Note that the FcγRIIb must be co-cross-linked with the ITAM activating receptor.	FcγRIIb
Sweeping	Internalization	Removal of small immune complexes	FcγRIIb[a]
Scaffolding	Target agonism or apoptosis	Passive "super-cross-linking" of mAb on opsonized target cell, for example, CD40, CD28, CD20, by FcγR on an adjacent cell	FcγRIIb; also FcγRIIa, FcγRI?

BCR, B-cell receptor; ITAM, immunoreceptor tyrosine activation motif.
[a]Activating FcγR can also contribute to removal of complexes.

Table 2. Properties of FcγR.

Receptor	Affinity	IgG specificity	Cell distribution
FcγRI	High	IgG1, IgG3, IgG4	Induced by interferon-γ on monocytes, neutrophils, macrophages, dendritic cell subpopulations; mast cells
FcγRIIa	Low	IgG1, IgG3, but IgG2 binding limited to the FcγRIIa-H^{131} form, ~70% people)	All leukocytes and platelets except T and B lymphocytes
FcγRIIc[a]	Low	IgG1, IgG3, IgG4	NK cells
FcγRIIIa	Low	IgG1, IgG3. Binding avidity reduced by Phe at position 158	NK cells, macrophages, subpopulation of circulating monocytes, myeloid dendritic cells, neutrophils at very low levels
FcγRIIIb	Low	IgG1, IgG3	Neutrophils
FcγRIIb	Low	IgG1, IgG3, IgG4	B lymphocytes, some monocytes (can be upregulated); basophils; eosinophils? Plasmacytoid and myeloid dendritic cells; NK cells only of individuals with FcγRIIIb gene copy number variation Airway smooth muscle, LSEC, placenta, follicular dendritic cell

Ig, immunoglobulin; NK cell, natural killer cell.
[a]Expressed in 20% of people.

leukocytes. It is also present on platelets but its role in effector functions is not established but it is important in certain immune thrombocytopenias. A polymorphic form of this receptor is the only human receptor for human IgG2. This, together with its limited species expression and unique ITAM-containing cytoplasmic tail (reviewed by Anania *et al.* [11]), suggests a unique function in human leukocytes. Interestingly, polymorphism of the receptor is associated with systemic lupus erythematosus and resistance to Gram-negative organisms.[11] A rare, hyper-responsive form is a risk factor for neutrophil-driven anaphylaxis in Ig replacement therapy.[22]

FcγRIIc is an activating FcγR whose expression is regulated single nucleotide polymorphism that permits expression in approximately 20% of humans and in whom it is present at low levels on natural killer (NK) and B cells.[11] It has arisen by gene duplication/recombination resulting in an extracellular region derived from FcγRIIb, which binds IgG4, and with an ITAM-containing cytoplasmic tail related to the activating receptor FcγRIIa. Thus FcγRIIc provides IgG4 with an activation receptor pathway and confers a new biology of IgG4 in these individuals. Its low frequency in the population may also confound *in vivo* mAb clinical testing or use, but as yet there is no evidence for this.

FcγRIII forms are two highly related gene products, FcγRIIIa and FcγRIIIb. The FcγRIIIa is expressed on NK cells and professional phagocytes, particularly macrophages. It is only recently apparent that FcγRIIIa is expressed on neutrophils, albeit at low levels, but plays a role in their function.[23] FcγRIIIb is unique to humans and unlike other FcγRs it is attached to cell membrane *via* a glycophosphatidyl anchor. It is expressed, predominantly and abundantly, on human neutrophils.[7] Its effector function depends in part on its coexpression with FcγRIIa. The lack of FcγRIIIb on macaque neutrophils appears to be compensated for by an increase in FcγRIIa expression.[15]

FcγRIIbs are the inhibitory-type FcγR and arise from a single gene. They lack intrinsic proinflammatory signaling and are instead immune checkpoints. They provide feedback regulation by antibodies, in the form of immune complexes, to inhibit B-cell activation by specific antigen. They also control activating-type FcγR function on innate cells. Two major splice variant forms of FcγRIIb exist with differential tissue expression profiles. FcγRIIb1 preferentially expressed on B lymphocytes contains a 20-amino acid cytoplasmic insertion necessary for membrane retention and cocapping with the B-cell antigen receptor (BCR). FcγRIIb2 is the predominant inhibitory receptor found on basophils and neutrophils, as well as on subpopulations of mast cells, dendritic cells and some monocytes/macrophages. FcγRIIb2 lacks the cytoplasmic insertion of FcγRIIb1 and consequently can internalize rapidly including with the activating FcR when they are co-cross-linked.[11] It is not clear which form is present on human T cells.

One additional comment on tissue distribution is that FcγR expression on T cells has been difficult to establish unequivocally. However, there is increasing evidence that T-lymphocyte populations express FcγR. Some γδ T cells express FcγRIIIa and αβ T cells reportedly express FcγRIIa, FcγRIIb or FcγRIIIa but the significance with respect to effector function mediated by antibody is presently unclear.[24-28]

Expression on nonhemopoietic cells

The immunobiology of FcγR is studied and understood almost exclusively in the context of hematopoietic cell

Table 3. Unique features of IgG subclass Fc and hinge.

IgG subclass	FcγR specificity	Light-chain attachment	Hinge characteristics	Fc stability	Comment
IgG1	All FcγR	Upper hinge	Light-chain attachment Stable core hinge	Stable	Fc is >100× times more stable than IgG4 and IgG2.
IgG2	FcγRIIa His^{131}	CH1 of Fab and/or upper hinge	Stable core hinge with additional inter H-chain disulfide bonds in the upper hinge.	Unstable CH3:CH3	Alternative light-chain attachment creates distinct conformers. Unlike IgG4, the CH3:CH3 instability does not lead to half-molecule exchange as a result of stable core and upper hinge disulfides.
IgG4	FcγRI, FcγRIIb, FcγRIIc	CH1 of Fab	Labile core hinge	Unstable CH3:CH3	Combined instability of core hinge and CH3:CH3 permits half-IgG molecule exchange

Ig, immunoglobulin.

function but relatively recent investigations have identified and explored FcγR expression on nonhemopoietic cells. These studies suggest important roles in normal immune function and in the MOA of some therapeutic mAbs. The most extensively characterized receptor expression is FcγRIIb which is expressed on follicular dendritic cell, airway smooth muscle and liver endothelium. Its abundant expression on liver sinusoidal endothelial cells (LSECs) is estimated to represent the majority of *in vivo* FcγRIIb expression.[17,29-31] As FcγRIIb lacks intrinsic proinflammatory signaling function, its role on these nonhemopoietic cells involves immune complex handling without the danger of, or the need for, induction of local tissue destructive inflammatory responses. On LSEC its major role appears to be immune complex sweeping, a process of removal of small immune complexes such as opsonized virus or macromolecules.[17] This scavenging role by FcγRIIb on LSEC can be exploited in principle by mAbs forming small soluble complexes with their targets such as antiviral, anticytokine or similar antibodies.

FcγR activating or inhibitory signaling

Effector functions that are initiated *via* the activating-type FcγR occur by signaling *via* the ITAM pathway of immune receptors. This well-characterized pathway is used by BCR and T-cell antigen receptors, the IgE receptor FcεRI and IgA receptor FcαRI (reviewed extensively by Hogarth and Pietersz [7] Anania *et al.* [11] and Getahun and Cambier[32]) Induction of an activating signal requires the aggregation of activating FcγR by immune complexes, or by antigen in the case of the BCR. This aggregation at the cell membrane results in specific tyrosine phosphorylation of the ITAM by Src kinases, thus initiating the activation cascade.32-34

The inhibitory-type FcγRs, FcγRIIb1 and FcγRIIb2, whose expression is cell lineage restricted, modulate the ITAM signaling of the BCR or the activating-type FcγRs, respectively.[11] Their function is dependent on the immunoreceptor tyrosine inhibition motif in their cytoplasmic tail.[32,33] This checkpoint action requires that FcγRIIbs are coaggregated with the tyrosine-phosphorylated ITAM-signaling receptor complex which results also in immunoreceptor tyrosine inhibition motif tyrosine phosphorylation and consequential recruitment of lipid or protein tyrosine phosphatases that powerfully dampen the ITAM-induced cell activation.

FcγR-DEPENDENT EFFECTOR RESPONSES

Not all opsonized targets are equal: size, distance, valency and Fc geometry affect potency

To understand the immunobiology of FcγR effector responses particularly in the therapeutic mAb context, it is important to appreciate that the quality and potency of such effector responses is greatly affected by the nature of the IgG immune complex and/or the state of potential effector cells.

First, opsonization, *per se,* of a target is not necessarily sufficient to ensure FcγR interaction in a way that initiates an effector response. Although it is the IgG Fc that interacts with and clusters the FcγR to induce a response, the nature of the Fab interaction with its epitope can strongly influence the likelihood or potency of FcγR effector responses by influencing the density of appropriately presented Fc portions.[35] and also the size of the immune complex.[36] Furthermore, the display/orientation and geometry of the Fc portions, as a consequence of the fragment antigen-binding (Fab)

interaction with the target epitope, can result in effector responses such as ADCC that differ substantially in potency, presumably because the orientation of the Fc makes FcγR engagement more, or less, accessible.[37,38]

Second, in innate effector cells at rest, the largely linear actin cytoskeleton and the extracellular glycosaminoglycan glycocalyx regulate function by interacting with large glycoproteins, such as CD44, arranging these into ordered "picket" fences.[39,40] These corral receptors, including the FcγRs, and sterically inhibit their interaction with ligands. Upon cell activation, cytoskeletal remodeling is associated with the loss of the receptor corrals, allowing FcγRs and other receptors to freely diffuse, engage ligand, cluster and signal.[39] The influence of such surface constraints on receptors and effector cell function helps explain some of the observed epitope distance requirements for optimal mAb function,[39,41] which were apparent in a comparative study of ADCC and ADCP.[42] ADCC was optimal when the epitope was displayed close, 0.3 nm "flush" or 1.5 nm, to the target membrane where close conjugation of effector and target by the mAb presumably facilitates the delivery of pore-forming proteins to the target membrane as required by ADCC. Interestingly, complement-dependent cytotoxicity which also utilizes pore-forming proteins for its cytotoxicity has similar distance constraints. By contrast, ADCP was poor when targeting epitopes displayed close or "flush" to the target cell membrane (within ~0.3 nm) but ADCP activity was restored when the epitope was displayed 1.5 nm off the membrane, demonstrating different optimal epitope distance requirements for ADCC and ADCP.[42]

Although the action of agonistic/antagonistic mAbs is mechanistically distinct to those eliciting cytotoxicity and ADCP, the distance segregation between target and $FcγR^+$ cells is also important. Indeed, the membrane proximal epitopes of CD28 and CD40 are important for the FcγR function in the complex MOA of these mAbs.[43,44]

Clearly, the effects of immune complex valency, Fc density, presentation and geometry together with FcγR organization in the cell membrane suggest that the development of mAbs to certain targets will be heavily influenced by the context of use. Thus, improved mAb potency may not necessarily be achieved by engineering of the Fc polypeptide or its glycan alone. A more function-oriented approach early in mAb selection and development by, for example, application of rapid screening technologies that select for effector potency,[34] followed by Fc engineering may be more productive.

ADCC and phagocytosis

ADCC and ADCP are the most widely appreciated FcγR-dependent effector functions (Figure 1a, b) and are, respectively, mediated primarily via FcγRIIIa on NK cells and professional phagocytes such as macrophages. These effector functions, particularly NK cell ADCC, are believed to be major components of the MOA of cytotoxic therapeutic mAbs used in cancer therapy. In addition, ADCP can also occur *via* FcγRIIa and FcγRI,[45] but the extent to which cytotoxic anticancer therapeutic mAbs depend on these for their MOA in patients is unclear. The improvement in clinical utility of mAbs engineered for selectively increased FcγRIII binding suggests that FcγRIIa and FcγRI may be less important *in vivo* in cell killing effects but perhaps are more important in other aspects of therapeutic efficacy—discussed later.

Inhibition of cell activation by FcγRIIb

FcγRIIb is an immune checkpoint[46,47] and its splice variants are potent modulators of ITAM-dependent signaling (Figure 1c). This modulatory function occurs only when FcγRIIb is coaggregated with an ITAM signaling receptor. Thus, B-cell activation by the binding of the antigen in the immune complex to the BCR is regulated by the simultaneous binding of the Fcs of the immune complex to FcγRIIb1 on the same cell. In innate leukocytes, the activating-type FcR (i.e. FcγRI, FcγRIIa, FcγRIIc, FcγRIII) and the high-affinity IgE receptor, FcεRI, and the IgA receptor, FcαRI, are all modulated by immune complex co-engagement with FcγRIIb2. The inhibitory function contributes to the MOA of therapeutic antibodies that target cell-activating molecules where the target cells also express the inhibitory FcγRIIbs such as the BCR (discussed later). Thus, B-cell activation is modulated by the simulatenous binding of the antigen in the immune complex to the BCR and the binding of the Fcs, also in the immune complex, to FcγRIIB1 on the same cell.

Sweeping: clearance of small immune complexes

The removal of immune complexes in humans depends primarily on the complement receptor pathway and to a lesser degree the FcγR. Among the FcγRs, it has been widely believed that immune complex removal only occurs by phagocytosis/endocytosis of activating-type FcγR. Surprisingly, the inhibitory FcγRIIb, which lacks intrinsic activating function, plays a major role in clearance, and rapidly "sweeps" away small complexes from the circulation (Figure 1d).[48,49] A major tissue involved in the clearance is likely to be the LSEC, where FcγRIIb is expressed abundantly in mice and humans. This role is potentially important in resistance to viruses and toxins but may also be key to optimal performance of therapeutic IgG mAbs whose primary MOA is believed

to be only neutralization of soluble macromolecules, for example, cytokines or IgE.

FcγR uptake of antigen: antibody complexes and shaping the immune response

Monoclonal antibody therapy is a form of passive immunization. Indeed, longer-term vaccine-like or vaccinal immunity has been demonstrated in anti-CD20-treated mice *via* FcγRIIa[50,51] and *in vitro* recall memory responses from CD20-treated patients.[52] Although this is dependent on FcγR and anti-CD20, the mechanism by which long-term anti-tumor response is established remains unclear.

Nonetheless, the active involvement of FcγR in the enhancement of antigen-specific immunity by uptake of immune complexes through FcγR is historically well documented in experimental systems where FcγRs bind immune complexes and thereby feed antigens into the antigen-presentation pathways.[53] This has been demonstrated *in vivo* for small immune complexes *via* human FcγRI on human antigen-presenting cells[54] and in mice.[19] Similarly, the capacity of FcγRIIbs to bind and rapidly internalize antigen–antibody complexes suggests that it too may significantly influence feeding antigens into professional antigen-presenting cells of hematopoietic origin such as dendritic cells and possibly B lymphocytes.

Although not a classical major histocompatibility complex-dependent antigen presentation, FcγRIIb on the stroma-derived follicular dendritic cells influences antibody immunity by recycling antigen–antibody complexes to the cell surface for presentation of intact antigen to B cells.[55]

Although somewhat speculative, FcγRIIb's rapid internalization/sweeping of complexes by the abundant LSEC, which interact with lymphocytes and can present antigen,[56] may have a significant role in shaping immune responses.

Scaffolding of cell-bound mAbs by FcγR$^+$ cells

FcγR-expressing cells can be critical, but passive, participants in the MOA of some mAbs (Figure 1e). In FcγR scaffolding, IgG mAb molecules that have opsonized the cell surface of a target cell are additionally cross-linked by their Fc portions engaging the FcγRs that are arrayed on the surface of a second cell. This "super-cross-linking" of the target-bound mAb by the FcγR lattice or "scaffold" on the adjacent cell greatly exceeds the target cross-linking by the mAb alone, thereby inducing a response in the target cell. Scaffolding was originally identified as the basis of T-cell mitogenesis induced by anti-CD3 mAb.[57,58] The CD3 mAbs alone were poor mitogens but the "super-cross-linking" of the T-cell-bound CD3 mAb by the membrane FcγR on adjacent cells, particularly by monocytes, induced rapid T-cell expansion and cytokine secretion but did not require activation of FcγR-expressing cells.[57] Regrettably, FcγR scaffolding came to prominence and clinical relevance because of its causal role in the catastrophic adverse events resulting from the administration of anti-CD3[57] and anti-CD28 (TGN1412)[59] mAbs.

Nonetheless, FcγR scaffold-based induction of intracellular responses in a target cell can also lead to beneficial therapeutic effects. Such examples are the induction of apoptotic death in a target cell, which is likely part of the MOA of daratumumab in multiple myeloma[60] or the controlled agonistic expansion of cells, for example, *via* CD40 mAb agonism.[43]

IgG subclasses: specificity and affinity for FcγR

Most FcγRs (Table 2) are weak, low-affinity receptors (affinities in the micromolar range) for IgG-Fc, irrespective of whether the IgG is uncomplexed, monomeric or when it is complexed with antigen (i.e. an immune complex). The very avid binding of immune complexes to an effector cell surface that displays an array of FcγR molecules is the result of the collective contributions of the low-affinity interactions of each Fc of the IgGs in the complex with an FcγR. This avidity effect is necessary as the FcγRs operate *in vivo* in environments of high concentrations of uncomplexed monomeric IgG (normally 3–12 g L^{-1}). Thus, the avid multivalent binding of the complex out competes uncomplexed, monomeric IgG. The notable exception to this is the enigmatic FcγRI. This receptor shows high, nanomolar affinity for uncomplexed monomeric IgG and thus would be expected to be constantly occupied *in vivo* by the normal circulating monomeric IgG. However, IgG dissociation permits engagement with immune complexes. Furthermore, FcγRI is not expressed or expressed poorly on resting cells, requiring interferon-γ for induction of its expression, presumably at sites of inflammation.

Although the human IgG heavy-chain constant domains have greater than 90% identity, key amino acid differences confer each subclass with unique structural and functional properties. IgG1 and IgG3 are "universal" ligands, that is, they bind to all FcγRs. Formal measurement of the weak, micromolar K_D interactions of the low-affinity receptors with monomeric IgG1 also revealed differing affinities between the low-affinity FcγRs, with inhibitory FcγRIIb generally having the lowest affinity and FcγRIII the higher, sometimes referred to as a "moderate" affinity receptor.[7,61]

The strength of IgG1 interaction can also be affected by FcγR polymorphism and in the context of therapeutic mAbs, variation in FcγRIIIa is particularly important. The most common and possibly clinically significant polymorphism is phenylalanine/valine variation at position 158 in the IgG-binding site, wherein FcγRIIIa-F^{158} binds IgG1 less well than the FcγRIIIa-V^{158} form.

IgG4 and IgG2 have more restricted FcγR specificity. IgG4 has low affinity ($K_A = \sim 2 \times 10^5\ M^{-1}$) for the inhibitory FcγRIIb, but is also a high-affinity ligand for FcγRI ($K_A = \sim 4 \times 10^8\ M^{-1}$). IgG2 exhibits a highly restricted specificity, showing functional activity with only one polymorphic form of FcγRIIa (binding affinity $K_A = \sim 4.5 \times 10^5\ M^{-1}$) which is permitted by the presence of histidine at position 131 of its IgG-binding site. This FcγRIIa–H^{131} form is expressed in approximately 70% of the population, whereas IgG2 has no functional activity on the other common allelic form, FcγRIIa-R^{131}, which contains arginine at position 131.[11,61]

IgG SUBCLASSES: STRUCTURE AND PROPERTIES

The molecular basis of IgG and FcγR interactions

The extracellular regions of the FcγR are structurally similar. Each low-affinity FcγR has two ectodomains, whereas the high-affinity FcγRI has a third domain but this is not directly involved in IgG binding.[62]

The interaction between the IgG subclasses and the FcγR is most comprehensively defined for human IgG1 by both X-ray crystallographic[7,62,63] and mutagenesis structure/function analyses.[64-66] These studies defined key regions of the IgG sequence required for interaction with their FcγRs.

Crystallographic analyses of the human IgG1-Fc complexed with FcγRI, FcγRII or FcγRIII show that these interactions are similar in topology, and asymmetric in nature. The second extracellular domain of the FcγR inserts between the two heavy chains. Here it makes contacts with the lower hinge of both H chains and with residues of the adjacent BC loop of one CH2 domain and the FG loop of the other. The N-linked glycan at asparagine 297 (N^{297}) of the heavy chain is essential for the structural integrity of the IgG-Fc by affecting the spacing and conformation of the CH2 domains. Indeed, its removal ablates FcγR binding.[67] Of particular relevance to therapeutic mAb development is that the normal low-affinity IgG interaction with FcγRIIIa is profoundly increased by the removal of the core fucose from the N^{297} Fc oligosaccharide.[68]

No crystallographic data are available for IgG2 or IgG4 Fc in complex with FcγR, but mutagenesis studies of the Fc and the FcγR revealed general similarity, but with critical differences, in the interaction of these subclasses with their cognate FcγR.

Unique features of the IgG2 and IgG4 subclasses

In IgG1, the stable interaction of the two heavy chains results from the combined effects of stable covalent inter H-chain disulfide bonds and strong noncovalent interaction of the two CH3 domains (Table 3). In stark contrast, in IgG2 and IgG4 the interaction of the CH3 domains of each H-chain is weak. Residues 392, 397 and 409 (Eu numbering) profoundly affect the stability of these interactions. The difference at position 409 (R^{409} in IgG4 and K^{409} in IgG1) confers a 100-fold decrease in stability of the interface between the two CH3 domains of IgG4 compared with that of IgG1 (Table 3).[69]

Furthermore, the core hinge of IgG4 differs from IgG1 at position 228 (P^{228} in IgG1 and S^{228} in IgG4), resulting in unstable inter-heavy-chain disulfide bonds. This, together with the destabilizing amino acids in the CH3, confers the unique property of half-antibody (Fab arm) exchange between different IgG4 antibodies,[69] thereby creating monovalent, bispecific IgG4 antibodies *in vivo*.[69,70] The similarly unstable interactions between the CH3 domains in IgG2 are conferred by the interface residue M^{397}; however, the stable inter-H-chain disulfide bonds of the core and upper hinge prevent half-molecule exchange (Table 3).[69]

In addition, IgG2 uniquely has three disulfide bond conformers (Table 3). The distinct conformers are formed when (1) each light chain is attached to the Cys^{131} residue of CH1 in the heavy chain (IgG2-A conformer), (2) both light chains attach to the upper hinge (IgG2-B) or (3) one light chain is attached to the CH1 Cys^{131} and one to the upper hinge of the other heavy chain (IgG2-AB).[71] This results in distinct positioning of the Fabs relative to the Fc portions in the different conformers, which has implications for the interaction with antigen and the capacity of IgG2 to cross-link target molecules in the absence of FcγR binding, for example, in an agonistic mAb setting.[72]

It should also be noted that IgG3 has not been used in therapeutic mAbs despite its unique biology. The main impediment to its use are its physicochemical properties such as susceptibility to proteolysis and propensity to aggregate that present challenges to industry-scale production and stability but protein engineering is attempting to overcome these hurdles.[73]

Therapeutic antibody design: improving mAb potency

Many factors affecting FcγR-dependent responses in vivo come into play during mAb therapy. The

experience of three decades of clinical use of mAbs taken together with our extensive, albeit incomplete, knowledge of IgG and FcγR structure and immunobiology provides a war chest for the innovative development of new and highly potent mAbs through the manipulation of their interaction with the FcγR.

Therapeutic mAb engineering strategies are directed by many factors including the biology of the target, the nature of the antigen, the desired MOA and possibly the anatomical location of the therapeutic effect,[21] and thus to optimize potency for a desired response, the context of use is critical.

The nature of the IgG isotype

Different capabilities for the recruitment and activation of the different immune effector functions are naturally found in the Fc regions of the human IgG subclasses. Thus, to achieve a desired MOA, the different IgG subclasses are important starting points for the selection and engineering of the optimal mAb Fc. IgG1 is, in many ways, a proinflammatory or "effector-active" subclass, as it can initiate the complement cascade and is a "universal" FcγR ligand.[74] Notwithstanding it is also a ligand for the inhibitory FcγRIIb, IgG1 elicits proinflammatory responses through all activating-type FcγRs, including ADCC, ADCP and cytokine release.

Because of their more restricted FcγR-binding profile, IgG2 and IgG4 have offered some choice in potentially avoiding FcR effector function without the need for Fc engineering. They have been used as the backbone for therapeutic mAbs either because recruitment of patients' effector functions was unlikely to be necessary for the primary MOA of the mAb or is possibly detrimental to the desired therapeutic effect.[75] However, the use of these unmodified "inert" subclasses is not without consequences and underscores the need for Fc engineering to modify FcγR interactions—See the "Attenuating and ablating FcγR related functions of IgG" section.

Thus, the choice of IgG subclass for therapeutic mAb engineering is an important first step for engineering of novel mAbs of improved specificity, potency and safety.

Fc engineering for enhanced anticancer therapeutics

IgG1 is the predominant subclass used in the development of cytotoxic mAbs where induction of an activation-type response, ADCC or phagocytosis, is considered desirable.[45,76,77]

Cytotoxic mAb cancer therapeutics can control disease progression by one or more mechanisms. Their MOAs include direct induction of apoptotic cell death of the cancer cell (anti-CD20, anti-CD52) or blocking receptor signaling (anti-HER2, anti-EGFR). They may also harness FcγR effector functions, including ADCC in the tumor microenvironment.[78] The approved mAbs, rituximab (anti-CD20), trastuzumab (anti-HER2) and cetuximab (anti-EGFR), are formatted on a human IgG1 backbone and all require activating-type FcγR engagement for optimal therapeutic activity.[79,80] This presents an example where context of therapeutic use is critical for therapeutic mAb design. IgG1 antibodies bind both the activating FcγR (e.g. FcγRIIIa) and the inhibitory FcγRIIb. In some environments effector cells will coexpress FcγRIIb together with FcγRI, FcγRIIa and FcγIIIa, as may occur on a tumor-infiltrating macrophage. Therapy with an IgG1 anti-cancer cell mAb may then be compromised by the inhibitory action of FcγRIIb upon the ITAM signaling of the activating FcγR as both types of receptor would be coengaged on such an effector cell by the mAb bound to the target cell. This leads to reduced therapeutic mAb potency. Thus, the relative contributions of the activating (A) and inhibitory (I) FcγR to the response by an effector cell, the A-to-I ratio, may be an important determinant in clinical outcome of therapeutic mAb activity,[76,81,82] that is, the higher the A-to-I ratio, the greater the proinflammatory response induced by the therapeutic mAb or conversely the lower the A-to-I ratio, the greater the inhibition or dampening of the proinflammatory response.

Thus, the challenge for the development of more potent FcγR effector mAbs is to overcome three major obstacles. First, improving activation potency by selectively enhancing interaction with activating-type FcγR, particularly FcγRIIIa owing to its predominant role in ADCC-mediated killing of tumor cells. Second, reducing binding interactions with the inhibitory FcγRIIb. These two approaches improve the FcγR A-to-I ratio of cytotoxic IgG1 mAbs. Third, overcoming the significant affinity difference in the interaction with the main FcγRIII allelic forms of FcγRIIIa-V^{158} and FcγRIIIa-$F^{15876,83,84}$ which appears to be an important source of patient variability in responses to therapeutic mAb treatment of cancer.

At the time of writing, some mAbs with improved potency are coming into clinical use. Their improved action has been achieved by modifying the N-linked glycan or the amino acid sequence of the heavy-chain Fc (Table 4).

Modification of the Fc glycan

The typical complex N-linked glycan attached to N^{297} of the heavy chain includes a core fucose.[85] Antibodies that lack this fucose have approximately 50-fold improved binding to FcγRIIIa and FcγRIIIb and importantly retain

Table 4. Fc or hinge-engineered monoclonal antibodies (mAbs) approved or in advanced clinical development.

mAb name	Target	IgG backbone	Fc modification	Effect on mAb	Therapy area	Most advanced development stage
Andecaliximab	Matrix Metalloproteinase 9 (MMP9)	IgG4	$S^{228}P$	Stabilize core hinge	Oncology	Phase III
Anifrolumab	Interferon alpha/beta receptor 1	IgG1	$L^{234}F$; $L^{235}E$; $P^{331}S$	Mimic IgG4 hinge and its CH2/F/G loop; plus ablate FcγR binding	Immunology	Phase III
Atezolizumab	PD-L1	IgG1	Aglycosylated ($N^{297}A$)	Ablate FcγR binding	Oncology	Marketed
Benralizumab	Interleukin 5	IgG1	Afucosylated	Selectively enhance FcγRIII interaction	Respiratory dermatology; ear nose throat disorders; gastrointestinal; hematology; immunology;	Marketed
Durvalumab	PD-L1	IgG1	$L^{234}F$; $L^{235}E$; $P^{331}S$	Mimic IgG4 hinge and its CH2 F/G loop; plus ablate FcγR binding	Oncology	Marketed
Evinacumab	Angiopoietin-related protein 3	IgG4	$S^{228}P$	Stabilize core hinge	Metabolic disorders	Phase III
Inebilizumab	CD19	IgG1	Afucosylated	Selectively enhance FcγRIII interaction	Central nervous system; oncology	Phase III
Ixekizumab	Interleukin 17A	IgG4	$S^{228}P$	Stabilize core hinge	Dermatology; immunology; musculoskeletal disorders	Marketed
Margetuximab	HER2	IgG1	$F^{243}L$; $L^{235}V$; $R^{292}P$; $Y^{300}L$; $P^{396}L$	Selectively enhance FcγRIII interaction	Oncology	Phase III
Mogamulizumab	C–C chemokine receptor type 4 (CCR4)	IgG1	Afucosylated	Selectively enhance FcγRIII interaction	Central nervous system; oncology	Marketed
Tafasitamab (MOR208 XmAb 5574)	CD19	IgG1	$S^{239}D$; $I^{332}E$	Selectively enhance FcγRIII interaction	Oncology	Phase III
Nivolumab	PD-1	IgG4	$S^{228}P$	Stabilize core hinge	Infectious disease; oncology	Marketed
Obinutuzumab	CD20	IgG1	Afucosylated	Selectively enhance FcγRIII interaction	Immunology; oncology	Marketed
Ocaratuzumab	CD20	IgG1	$P^{247}I$; $A^{339}Q$	Selectively enhance FcγRIII interaction	Oncology	Phase III
Pembrolizumab	PD-1	IgG4	$S^{228}P$	Stabilize core hinge	Infection; oncology	Marketed
Roledumab	Rhesus D	IgG1	Afucosylated	Selectively enhance FcγRIII interaction	Hematological disorders	Phase III
Spesolimab (BI-655130)	IL-36R	IgG1	$L^{234}A$; $L^{235}A$	Ablate FcγR binding	Gastrointestinal; immunology	Phase III
Teplizumab	CD3	IgG1	$L^{234}A$; $L^{235}A$	Ablate FcγR binding	Metabolic disorders	Phase II
Tislelizumab	PD-1	IgG4	$S^{228}P$; $E^{233}P$; $F^{234}V$; $L^{235}A$;	Stabilize core hinge; mimic IgG2 lower hinge for restricted	Oncology	Phase III

(*Continued*)

Table 4. Continued.

mAb name	Target	IgG backbone	Fc modification	Effect on mAb	Therapy area	Most advanced development stage
			$D^{265}A$; $L^{309}V$; $R^{409}K$	FcγR specificity; ablate FcγR binding; stabilize CH3 interaction		
Toripalimab (JS 001)	PD-1	IgG4	$S^{228}P$	Stabilize core hinge	Oncology	Phase III
Ublituximab	CD20	IgG1	Afucosylated	Selectively enhance FcγRIII interaction	Central nervous system; oncology	Phase III

Ig, immunoglobulin.

the weak, low-affinity binding to the inhibitory FcγRIIb. Furthermore, this glycoengineering increased binding affinity of the modified IgG1 mAb for both FcγRIIIa V^{158} and F^{158} allelotypes.[86-88] Afucosyl versions of the tumor targeting mAbs such as anti-HER2, anti-EGFR and anti-CD20 had greater antitumor effects and increased survival,[68,88,89] which is a reflection of the greatly increased, and selective, FcγRIII binding. Compared with their unmodified counterparts, the afucosyl mAbs showed dramatic improvement of FcγRIII-related effector responses such as stronger NK cell-mediated ADCC, or enhanced neutrophil-mediated phagocytosis through FcγRIIIb and/or FcγRIIIa.[23] However, certain neutrophil functions *via* FcγRIIa may be compromised.[90,91]

There are six afucosylated antibodies in late-stage clinical trials or approved for treatment (Table 4). Notable is obinutuzumab, an afucosyl anti-CD20 mAb which nearly doubles progression-free survival in chronic lymphocytic leukemia patients as compared with the fucose-containing rituximab.[68] This dramatic improvement in clinical utility reinforces the value of glycan engineering specifically and of Fc engineering generally in anticancer treatments.

Mutation of the Fc amino acids

Alteration of the amino acids in the heavy-chain Fc can alter IgG specificity and affinity for activating FcγRs. The anti-CD19 antibody MOR208 (XmAb 5574) is currently in phase III trials for the treatment of chronic lymphocytic leukemia.[92] It contains two mutations in its IgG1 Fc, $S^{329}D$ and $I^{332}E$, which increases affinity to FcγRIIIa, particularly the "lower-affinity" FcγRIIIa F^{158} allele. The mAb shows increased FcγRIII-mediated ADCC and phagocytosis *in vitro*, and reduced lymphoma growth in mouse models.

Margetuximab is an ADCC-enhanced IgG1 Fc-engineered variant of the approved anti-HER2 mAb trastuzumab in phase III for HER2-expressing cancers.[66,93] Alteration of five amino acids ($L^{235}V$, $F^{243}L$, $R^{292}P$, $Y^{300}L$ and $P^{396}L$) enhanced binding to FcγRIIIa which also had the additional effect of decreasing binding to the inhibitory FcγRIIb, and thereby increased its A-to-I FcγR ratio. This was apparent when compared with unmodified trastuzumab the margetuximab showed enhanced ADCC against $HER2^+$ cells *in vitro* and demonstrated superior antitumor effects in an HER2-expressing tumor model in mice.

The anti-CD20 ocaratuzumab is an Fc-engineered IgG1 mAb in late-stage clinical trials for the treatment of a range of cancers, including non-Hodgkin lymphoma and chronic lymphocytic leukemia.[94] Two Fc mutations, $P^{247}I$ and $A^{339}Q$, conferred about 20-fold increase in binding to both major allelic variants of FcγRIIIa and elicited sixfold greater ADCC than unmodified IgG1.

Thus, the engineering of the Fc domain or glycan for improved FcγRIIIa binding is a powerful tool to create more potent and clinically effective anticancer mAbs.

Attenuating and ablating FcγR-related functions of IgG

There are circumstances where binding to FcγR is unnecessary or undesirable in the MOA of a therapeutic mAb. Unmodified IgG irrespective of its subclass or intended therapeutic effect has the potential to engage an FcγR which may lead to suboptimal therapeutic performance or to unexpected and catastrophic consequences.[57,59] Clearly reducing or eliminating FcγR interactions, when they are not required for therapeutic effect, may be desirable. Indeed, this had been addressed by the choice of IgG subclass or by modifying the Fc region. Indeed, most efforts in Fc engineering mAbs that have translated to an approved drug have focused on the reduction or elimination of FcγR interactions (Table 4).

One approach to minimize interactions with the activating FcγR has been the use of IgG4 or IgG2 backbones, which show a more restricted specificity for the activating FcγR and consequently have been traditionally, and simplistically, viewed as "inert" IgG subclasses. Notwithstanding the unexpected, and FcγR-dependent, severe adverse reaction induced by the IgG4 TGN1412 mAb, the IgG4 or IgG2

backbones have been successfully used in many settings. Indeed, checkpoint inhibitors, such as mAbs targeting CTLA-4 or the PD-L1/PD-1 interaction for the suppression of inhibitory signals that contribute to immune tolerance in the tumor microenvironment, are formatted on an IgG4 backbone. Pembrolizumab, nivolumab and cemiplimab are all anti-PD-1 antibodies currently used for cancer therapy and have been formatted on an IgG4 backbone[95-97] with a stabilized core hinge ($S^{228}P$) to prevent half-IgG4 exchange. Similarly, the checkpoint inhibitor tremelimumab is an anti-CTLA-4 antibody formatted on an IgG2 backbone to avoid potential ADCC killing of target cells.[98]

However, the use of IgG2 and IgG4 as "inert" subclasses is problematic. Both bind to the activating receptors FcγRIIa-H^{131} and FcγRI, respectively (Table 2), and initiate effector functions such as neutrophil activation and apoptosis induction.[75,99] Interestingly, in experimental systems, cross-linking of anti-PD-1 IgG4-based mAb by FcγRI switched its activity from blocking to activatory.[10] Moreover, IgG4 binds to FcγRIIb, which may scaffold the therapeutic mAb. Although scaffolding may be beneficial in some contexts, for example, in immune agonism,[43] it can be disastrous and unexpected in others as it was for the anti-CD28 TGN1412 mAb.[59] Thus, the IgG2 and IgG4 subclasses are not the optimum choice for "FcR-inactive" mAbs, and so modifying the Fc is a more direct approach.

The complete removal of the heavy-chain N-linked glycan is well known to ablate all FcγR binding by dramatically altering the Fc conformation.[36,67,101,102] Atezolizumab, an IgG1 anti-PD-L1 checkpoint inhibitor mAb, utilizes this strategy and eliminates FcγR and also complement activation.[13]

Modification to the Fc amino acid sequence of the FcγR-contact regions can also be used to reduce FcγR binding. A widely used modification of IgG1 is the substitution of leucine 234 and 235 in the lower hinge sequence (L^{234} L^{235} G^{236} G^{237}) with alanine ($L^{234}A$ $L^{235}A$). It is often referred to as the "LALA mutation" and effectively eliminates FcγR binding by more than 100 fold14,105 and is used in teplizumab and spesolimab (Table 4).

A separate strategy has used combinations of amino acid residues from the FcγR-binding regions of IgG2 and IgG4, which have restricted FcγR specificity, together with other binding-inactivating mutations. The lower hinge amino acids of the IgG1 mAbs durvalumab (anti-PD-L1) and anifrolumab (anti-interferon-α receptor; Table 4) were modified to mimic the lower hinge of IgG4 ($L^{234}F$). They additionally incorporated $L^{235}E$ in the lower hinge and $P^{331}S$ in the F/G loop of the CH2 domain to ablate FcγR binding by disrupting two major FcγR contact sites[7] and also coincidently decreasing C1q activation.[16]

IgG4 mAbs have been similarly engineered to eliminate their interaction with FcγRI and FcγRIIb. The IgG4 anti-PD-1 antibody tislelizumab has had its FcγR contact residues in the lower hinge E^{233}, F^{234}, L^{235} substituted with the equivalent residues of IgG2 P, V, A ($E^{233}P$, $F^{234}V$, $L^{235}A$) as well as the additional $D^{265}A$ mutation which disrupts a major FcγR contact in CH2. It also has substitutions in the core hinge $S^{228}P$ and the CH3 $L^{309}V$ and $R^{409}K$ to stabilize the H-chain disulfides and CH3 interactions, respectively, thereby preventing half-Ig exchange characteristic of natural IgG4. Collectively, these modifications create a stable IgG4 with no FcγR binding nor complement activation.[17]

Thus, Fc engineering is an effective way to remove FcγR effector functions and may be preferable to using unmodified IgG2 or IgG4 backbones that have a more restricted repertoire of FcγR interactions but which are still able to induce certain effector functions.

Improving FcγRIIb interactions

Preferential or specific Fc engagement of FcγRIIb over the activating FcγR offers several potential therapeutic advantages for new mAbs in distinct therapeutic settings.

Improved recruitment of FcγRIIb immunoreceptor tyrosine inhibition motif-dependent inhibitory function

Harnessing the physiological inhibitory function of FcγRIIb by mAbs that target ITAM receptors has the potential to shut down ITAM-dependent signaling pathways of major importance in antibody pathologies.[32,108] Such ITAM signaling receptors include the BCR complex on B cells which is active in systemic lupus erythematosus, the FcεRI on basophils and mast cell subsets in allergies or the activating-type FcγR on a variety of innate leukocytes in antibody-mediated tissue destruction. In such scenarios, the ITAM signaling receptor complex that is targeted by the therapeutic mAb must be co-expressed on the cell surface with the inhibitory FcγRIIb. This permits coengagement with ITAM signaling receptor by the Fab of the mAb and inhibitory FcγRIIb by its Fc which is the critical requirement in the inhibitory MOA for such therapeutic mAbs (Figure 1).

Obexelimab (also known as XmAb5871; Table 4), currently in early clinical testing in inflammatory autoimmune disease, is an IgG1 mAb that targets CD19 of the BCR complex.[19] It contains two Fc modifications, $S^{267}E$ and $L^{328}F$ (also known as "SELF" mutations), that selectively increased FcγRIIb binding by 400-fold to about 1 nM, which results in powerful suppression of BCR signaling and the proliferation of primary B cells.[19]

The anti-IgE mAb omalizumab is an IgG1 mAb approved for the treatment of allergic disorders.[110,111] A similar but Fc-engineered IgG1 mAb XmAb7195, currently in early clinical testing, contains the affinity-enhancing SELF modifications.[112] Both mAbs sterically neutralize the interaction between IgE and its high-affinity receptor FcεRI to prevent basophil and mast cell activation.[113,114] However, XmAb7195 exhibited more efficient removal (sweeping; discussed later) of circulating IgE and also inhibited B-cell IgE production, presumably by binding to the IgE BCR on the B-cell surface and coclustering with FcγRIIb *via* its affinity-enhanced Fc domain.[112] Thus, XmAb7195's selective modulation of IgE production by IgE^+ B cells in addition to its enhanced clearance of IgE may offer significantly improved therapeutic benefits in allergy therapy beyond simple IgE neutralization.[112] The "SELF" mutations have also been used in agonistic mAbs (discussed later).

One cautionary note is that the arginine 131 (R^{131}) of the IgG-binding site in FcγRIIb is critical for the enhanced affinity binding of "SELF"-mutated Fcs but it is also present in the activating-type "high responder" FcγRIIa-R^{131}. Thus, antibodies modified with "SELF" have very-high-affinity binding to FcγRIIa-R^{131}[115] with a potentially increased risk of FcγRIIa-dependent complications in patients expressing this allelic form, although, so far, none have been reported in clinical trials. However, an alternative set of six Fc mutations, termed "V12" ($P^{238}D$, $E^{233}D$, $G^{237}D$, $H^{268}D$, $P^{271}G$ and $A^{330}R$), potently enhanced FcγRIIb binding without increasing FcγRIIa–R^{131} interaction.[115]

Enhancing the sweeping of small immune complexes

The expression of FcγRIIb on LSEC and its action in the "sweeping" or removal of small immune complexes has opened up new possibilities for the application of FcγRIIb-enhancing modifications.[17] Antibodies or Fc fusion proteins, whose primary MOA is the neutralization of soluble molecules such as IgE or cytokines, are particularly attractive candidates for this approach. Proof-of-concept for this strategy has been demonstrated in experimental models.[48] Indeed, this may be a significant component of the rapid disappearance of IgE from the circulation of patients treated with the anti-IgE XmAb7195 containing the FcγRIIb enhancing "SELF" modifications, as described previously.

Immune agonism through FcγR scaffolding

Agonistic mAbs induce responses in target cells by stimulating signaling of their molecular target. Typically, this is to either enhance antitumor immunity by engaging costimulatory molecules on antigen-presenting cells or T cells (i.e. CD40, 4-1BB, OX40) or promote apoptosis by engaging death receptors on cancer cells (i.e. DR4, DR5, Fas).[116]

The role of FcγR in the action of these types of mAbs appears to be primarily as a scaffold. FcγRIIb is often the predominate receptor involved and the extent of its involvement is complex. In the case of CD40, the degree of FcγRIIb scaffolding potency is linked to the epitope location of the targeting mAb with greater potency seen for membrane proximal epitopes.[43,117] It is also noteworthy that depending on the epitope location, the scaffolding of anti-CD40 mAbs may convert antagonist mAbs to agonistic.

Engineering of the IgG1 Fc region for enhanced and/or specific binding to FcγRIIb can greatly improve agonistic function.[72,118-120] Such mutations induced significantly greater agonistic activity in an anti-DR5 model through increased induction of apoptotic death and decreased tumor growth compared with unmodified IgG1.[121] The "SELF" modifications that dramatically and selectively increase affinity for FcγRIIb have also been used to enhance immune agonism in an anti-OX40 model.[122]

The incorporation of the "V12" Fc mutations into IgG1 specifically enhance FcγRIIb interaction 200-fold, conferring the enhanced agonistic activity of an anti-CD137 antibody and an anti-OX40 mAb.[115,122]

FUTURE ENGINEERING STRATEGIES

Monoclonal antibodies are potent therapeutics in a number of chronic or once incurable diseases. However, there is still extensive unmet clinical need as well as considerable room for improvement in many existing therapeutics.

Further understanding of how antibody structure affects FcγR function is essential for future development of more potent and effective mAbs. Already, engineering of the IgG Fc and its glycan has proved a potent and effective approach for increasing the clinical effectiveness, functional specificity and safety of therapeutic mAbs and is an emerging pathway to the development of the "next-gen" therapeutics.

Future directions in the development and use of therapeutic antibodies should increasingly mimic normal protective antibody responses, which are polyclonal and elicited in the context of innate receptor engagement which includes the FcR as well as other powerfully responsive systems including the Toll-like receptors and complement receptors. Furthermore, the mixed subclass nature of these normal antibody responses suggests that circumstances may arise in therapeutic strategies where there is value in having distinctly modified Fcs for the nuanced engagement of different FcγR family members.

Treatments comprising multiple mAbs and immune stimulants are under investigation in infectious disease for neutralization coverage of variant strains. Indeed, such an approach may be most effective in emerging infectious disease such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. The use of multiple mAbs tailored for distinct effector functions and targeting different epitopes will maximize the opportunity for cocktailing of effector functions in different types of diseases. Indeed, in a small but contemporary example outside of infectious disease, the FDA-approved combination in adenocarcinoma therapy uses a cocktail of two mAbs, pertuzumab and trastuzumab, against Her2.[123]

Rather than one type of Fc to conquer all, the combined use of appropriately selected mAbs whose individual components are enhanced for the engagement of different FcγR members may utilize multiple components of the spectrum of effector responses on offer by the immune system. Such "next-gen" biologics will begin to realize the full potential of FcγR-mediated antibody immune therapeutics and offer transformational change for the treatment of intractable and incurable diseases.

ACKNOWLEDGMENTS

We thank Halina Trist for assistance with the manuscript. We also thank NHMRC, Janina and Bill Amiet Trust, Margaret Walkom Trust and Nancy E Pendergast Trust, Genmab, for their support.

CONFLICT OF INTEREST

None.

REFERENCES

1. Kaplon H, Muralidharan M, Schneider Z, Reichert JM. Antibodies to watch in 2020. *mAbs* 2020; **12**: 1703531.
2. Urquhart L. Market watch: top drugs and companies by sales in 2017. *Nat Rev Drug Discov* 2018; **17**: 232.
3. Ward ES, Ober RJ. Targeting FcRn to generate antibody-based therapeutics. *Trends Pharmacol Sci* 2018; **39**: 892–904.
4. Scott AM, Wolchok JD, Old LJ. Antibody therapy of cancer. *Nat Rev Cancer* 2012; **12**: 278–287.
5. Newsome BW, Ernstoff MS. The clinical pharmacology of therapeutic monoclonal antibodies in the treatment of malignancy; have the magic bullets arrived? *Br J Clin Pharmacol* 2008; **66**: 6–19.
6. Brezski RJ, Georgiou G. Immunoglobulin isotype knowledge and application to Fc engineering. *Curr Opin Immunol* 2016; **40**: 62–69.
7. Hogarth PM, Pietersz GA. Fc receptor-targeted therapies for the treatment of inflammation, cancer and beyond. *Nat Rev Drug Discov* 2012; **11**: 311–331.
8. Derer S, Cossham M, Rosner T, *et al*. A complement-optimized EGFR antibody improves cytotoxic functions of polymorphonuclear cells against tumor cells. *J Immunol* 2015; **195**: 5077–5087.
9. Foss S, Bottermann M, Jonsson A, Sandlie I, James LC, Andersen JT. TRIM21-from intracellular immunity to therapy. *Front Immunol* 2019; **10**: 2049.
10. Rostamzadeh D, Kazemi T, Amirghofran Z, Shabani M. Update on Fc receptor-like (FCRL) family: new immunoregulatory players in health and diseases. *Expert Opin Ther Targets* 2018; **22**: 487–502.
11. Anania JC, Chenoweth AM, Wines BD, Hogarth PM. The human FcγRII (CD32) family of leukocyte FcR in health and disease. *Front Immunol* 2019; **10**: 464.
12. Bruhns P, Jonsson F. Mouse and human FcR effector functions. *Immunol Rev* 2015; **268**: 25–51.
13. Daeron M. Fc receptors as adaptive immunoreceptors. *Curr Top Microbiol Immunol* 2014; **382**: 131–164.
14. Hargreaves CE, Rose-Zerilli MJ, Machado LR, et al. Fcγ receptors: genetic variation, function, and disease. *Immunol Rev* 2015; **268**: 6–24.
15. Trist HM, Tan PS, Wines BD, *et al*. Polymorphisms and interspecies differences of the activating and inhibitory FcγRII of *Macaca nemestrina* influence the binding of human IgG subclasses. *J Immunol* 2014; **192**: 792–803.
16. Casey E, Bournazos S, Mo G, et al. A new mouse expressing human Fcγ receptors to better predict therapeutic efficacy of human anti-cancer antibodies. *Leukemia* 2018; **32**: 547–549.
17. Ganesan LP, Kim J, Wu Y, *et al*. FcγRIIb on liver sinusoidal endothelium clears small immune complexes. *J Immunol* 2012; **189**: 4981–4988.
18. Rosales C. Fcγ receptor heterogeneity in leukocyte functional responses. *Front Immunol* 2017; **8**: 280.
19. Barnes N, Gavin AL, Tan PS, Mottram P, Koentgen F, Hogarth PM. FcγRI-deficient mice show multiple alterations to inflammatory and immune responses. *Immunity* 2002; **16**: 379–389.
20. Gavin AL, Barnes N, Dijstelbloem HM, Hogarth PM. Identification of the mouse IgG3 Receptor: implications for antibody effector function at the interface between innate and adaptive immunity. *J Immunol* 1998; **160**: 20–23.
21. Gordan S, Albert H, Danzer H, Lux A, Biburger M, Nimmerjahn F. The immunological organ environment dictates the molecular and cellular pathways of cytotoxic antibody activity. *Cell Rep* 2019; **29**: 3033–3046.e4.
22. Anania JC, Trist HM, Palmer CS, et al. The rare anaphylaxis-associated FcγRIIa3 exhibits distinct characteristics from the canonical FcγRIIa1. *Front Immunol* 2018; **9**: 1809.
23. Golay J, Valgardsdottir R, Musaraj G, Giupponi D, Spinelli O, Introna M. Human neutrophils express low levels of FcγRIIIA, which plays a role in PMN activation. *Blood* 2019; **133**: 1395–1405.
24. Morris AB, Farley CR, Pinelli DF, et al. Signaling through the inhibitory Fc receptor FcγRIIB induces $CD8^+$ T cell apoptosis to limit T cell immunity. *Immunity* 2020; **52**: 136–150.e6.

25. Descours B, Petitjean G, Lopez-Zaragoza JL, *et al*. CD32a is a marker of a CD4 T-cell HIV reservoir harbouring replication-competent proviruses. *Nature* 2017; **543**: 564–567.
26. Engelhardt W, Matzke J, Schmidt RE. Activation-dependent expression of low affinity IgG receptors FcγRII (CD32) and FcγRIII(CD16) in subpopulations of human T lymphocytes. *Immunobiology* 1995; **192**: 297–320.
27. Holgado MP, Sananez I, Raiden S, Geffner JR, Arruvito L. CD32 ligation promotes the activation of $CD4^+$ T cells. *Front Immunol* 2018; **9**: 2814.
28. Mantzioris BX, Berger MF, Sewell W, Zola H. Expression of the Fc receptor for IgG (Fc gamma RII/CDw32) by human circulating T and B lymphocytes. *J Immunol* 1993; **150**: 5175–5184.
29. Heesters Balthasar A, Chatterjee P, Kim Y-A, *et al*. Endocytosis and recycling of immune complexes by follicular dendritic cells enhances B cell antigen binding and activation. *Immunity* 2013; **38**: 1164–1175.
30. Xia YC, Schuliga M, Shepherd M, *et al*. Functional expression of IgG-Fc receptors in human airway smooth muscle cells. *Am J Respir Cell Mol Biol* 2011; **44**: 665–672.
31. Anderson CL, Ganesan LP, Robinson JM. The biology of the classical Fcγ receptors in non-hematopoietic cells. *Immunol Rev* 2015; **268**: 236–240.
32. Getahun A, Cambier JC. Of ITIMs, ITAMs, and ITAMis: revisiting immunoglobulin Fc receptor signaling. *Immunol Rev* 2015; **268**: 66–73.
33. Daëron M, Lesourne R. Negative Signaling in Fc Receptor Complexes. In: Alt FW, Austen KF, Honjo T, Melchers F, Uhr JW, Unanue ER, eds. Advances in Immunology. Vol **89**. Academic Press; 2006: 39–86.
34. Wines BD, Vanderven HA, Esparon SE, Kristensen AB, Kent SJ, Hogarth PM. Dimeric FcγR ectodomains as probes of the Fc receptor function of anti-influenza virus IgG. *J Immunol* 2016; **197**: 1507–1516.
35. Derer S, Lohse S, Valerius T. EGFR expression levels affect the mode of action of EGFR-targeting monoclonal antibodies. *Oncoimmunology* 2013; **2**: e24052.
36. 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**: 4315–4323.
37. Acharya P, Tolbert WD, Gohain N, *et al*. Structural definition of an antibody-dependent cellular cytotoxicity response implicated in reduced risk for HIV-1 infection. *J Virol* 2014; **88**: 12895–12906.
38. Anand SP, Prevost J, Baril S, *et al*. Two families of Env antibodies efficiently engage Fc-gamma receptors and eliminate HIV-1-Infected Cells. *J Virol* 2019; **93**.
39. Freeman SA, Vega A, Riedl M, *et al*. Transmembrane pickets connect cyto- and pericellular skeletons forming barriers to receptor engagement. *Cell* 2018; **172**: 305–317.e10.
40. Mylvaganam SM, Grinstein S, Freeman SA. Picket-fences in the plasma membrane: functions in immune cells and phagocytosis. *Semin Immunopathol* 2018; **40**: 605–615.
41. 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**: 131–142.e13.
42. Cleary KLS, Chan HTC, James S, Glennie MJ, Cragg MS. Antibody distance from the cell membrane regulates antibody effector mechanisms. *J Immunol* 2017; **198**: 3999–4011.
43. Yu X, Chan HTC, Orr CM, *et al*. Complex interplay between epitope specificity and isotype dictates the biological activity of anti-human CD40 antibodies. *Cancer Cell* 2018; **33**: 664–675.e4.
44. Paluch C, Santos AM, Anzilotti C, Cornall RJ, Davis SJ. Immune checkpoints as therapeutic targets in autoimmunity. *Front Immunol* 2018; **9**: 2306.
45. Minard-Colin V, Xiu Y, Poe JC, et al. Lymphoma depletion during CD20 immunotherapy in mice is mediated by macrophage FcγRI, FcγRIII, and FcγRIV. *Blood* 2008; **112**: 1205–1213.
46. Hibbs ML, Walker ID, Kirszbaum L, *et al*. The murine Fc receptor for immunoglobulin: purification, partial amino acid sequence, and isolation of cDNA clones. *Proc Natl Acad Sci USA* 1986; **83**: 6980–6984.
47. Phillips NE, Parker DC. Fc-dependent inhibition of mouse B cell activation by whole anti-mu antibodies. *J Immunol* 1983; **130**: 602–606.
48. Iwayanagi Y, Igawa T, Maeda A, et al. Inhibitory FcγRIIb-mediated soluble antigen clearance from plasma by a pH-dependent antigen-binding antibody and its enhancement by Fc engineering. *J Immunol* 2015; **195**: 3198–3205.
49. Mates JM, Yao Z, Cheplowitz AM, *et al*. Mouse liver sinusoidal endothelium eliminates HIV-Like particles from blood at a rate of 100 million per minute by a second-order kinetic process. *Front Immunol* 2017; **8**: 35.
50. Abes R, Gelize E, Fridman WH, Teillaud JL. Long-lasting antitumor protection by anti-CD20 antibody through cellular immune response. *Blood* 2010; **116**: 926–934.
51. DiLillo DJ, Ravetch JV. Differential Fc-receptor engagement drives an anti-tumor vaccinal effect. *Cell* 2015; **161**: 1035–1045.
52. Hilchey SP, Hyrien O, Mosmann TR, *et al*. Rituximab immunotherapy results in the induction of a lymphoma idiotype-specific T-cell response in patients with follicular lymphoma: support for a "vaccinal effect" of rituximab. *Blood* 2009; **113**: 3809–3812.
53. Guilliams M, Bruhns P, Saeys Y, Hammad H, Lambrecht BN. The function of Fcγ receptors in dendritic cells and macrophages. *Nat Rev Immunol* 2014; **14**: 94–108.
54. Heijnen IA, van Vugt MJ, Fanger NA, *et al*. Antigen targeting to myeloid-specific human Fc gamma RI/CD64 triggers enhanced antibody responses in transgenic mice. *J Clin Invest* 1996; **97**: 331–338.
55. Bergtold A, Desai DD, Gavhane A, Clynes R. Cell surface recycling of internalized antigen permits dendritic cell priming of B cells. *Immunity* 2005; **23**: 503–514.
56. Mehrfeld C, Zenner S, Kornek M, Lukacs-Kornek V. The contribution of non-professional antigen-presenting cells to immunity and tolerance in the liver. *Front Immunol* 2018; **9**: 635.

57. Alegre ML, Collins AM, Pulito VL, *et al*. Effect of a single amino acid mutation on the activating and immunosuppressive properties of a "humanized" OKT3 monoclonal antibody. *J Immunol* 1992; **148**: 3461–3468.
58. Tax WJ, Willems HW, Reekers PP, Capel PJ, Koene RA. Polymorphism in mitogenic effect of IgG1 monoclonal antibodies against T3 antigen on human T cells. *Nature* 1983; **304**: 445–447.
59. Suntharalingam G, Perry MR, Ward S, *et al*. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *N Engl J Med* 2006; **355**: 1018–1028.
60. Overdijk MB, Jansen JH, Nederend M, et al. The therapeutic CD38 monoclonal antibody daratumumab induces programmed cell death via Fcγ receptor-mediated cross-linking. *J Immunol* 2016; **197**: 807–813.
61. Bruhns P, Iannascoli B, England P, et al. Specificity and affinity of human Fcγ receptors and their polymorphic variants for human IgG subclasses. *Blood* 2009; **113**: 3716–3725.
62. Kiyoshi M, Caaveiro JM, Kawai T, et al. Structural basis for binding of human IgG1 to its high-affinity human receptor FcγRI. *Nat Commun* 2015; **6**: 6866.
63. Sondermann P, Szymkowski DE. Harnessing Fc receptor biology in the design of therapeutic antibodies. *Curr Opin Immunol* 2016; **40**: 78–87.
64. Duncan AR, Woof JM, Partridge LJ, Burton DR, Winter G. Localization of the binding site for the human high-affinity Fc receptor on IgG. *Nature* 1988; **332**: 563–564.
65. Shields RL, Namenuk AK, Hong K, *et al*. High resolution mapping of the binding site on human IgG1 for FcγRI, FcγRII, FcγRIII, and FcRn and design of IgG1 variants with improved binding to the FcγR. *J Biol Chem* 2001; **276**: 6591–6604.
66. Stavenhagen JB, Gorlatov S, Tuaillon N, *et al*. Fc Optimization of therapeutic antibodies enhances their ability to kill tumor cells *in vitro* and controls tumor expansion *in vivo* via low-affinity activating Fcγ receptors. *Cancer Res* 2007; **67**: 8882–8890.
67. Krapp S, Mimura Y, Jefferis R, Huber R, Sondermann P. Structural analysis of human IgG-Fc glycoforms reveals a correlation between glycosylation and structural integrity. *J Mol Biol* 2003; **325**: 979–989.
68. Goede V, Fischer K, Engelke A, *et al*. Obinutuzumab as frontline treatment of chronic lymphocytic leukemia: updated results of the CLL11 study. *Leukemia* 2015; **29**: 1602–1604.
69. Rispens T, Davies AM, Ooijevaar-de Heer P, *et al*. Dynamics of Inter-heavy chain interactions in human immunoglobulin g (igg) subclasses studied by kinetic fab arm exchange. *J Biol Chem* 2014; **289**: 6098–6109.
70. van der Neut Kolfschoten M, Schuurman J, *et al*. Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. *Science* 2007; **317**: 1554–1557.
71. Wypych J, Li M, Guo A, *et al*. Human IgG2 antibodies display disulfide-mediated structural isoforms. *J Biol Chem* 2008; **283**: 16194–16205.
72. Xu Y, Szalai AJ, Zhou T, *et al*. FcγRs modulate cytotoxicity of anti-Fas antibodies: implications for agonistic antibody-based therapeutics. *J Immunol* 2003; **171**: 562–568.
73. 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**: 900–909.
74. Brekke OH, Michaelsen TE, Aase A, Sandin RH, Sandlie I. Human IgG isotype-specific amino acid residues affecting complement-mediated cell lysis and phagocytosis. *Eur J Immunol* 1994; **24**: 2542–2547.
75. Konitzer JD, Sieron A, Wacker A, Enenkel B. Reformatting rituximab into human IgG2 and IgG4 isotypes dramatically improves apoptosis induction *in vitro*. *PLoS One* 2015; **10**: e0145633.
76. Uchida J, Hamaguchi Y, Oliver JA, *et al*. The innate mononuclear phagocyte network depletes B lymphocytes through Fc receptor–dependent mechanisms during anti-CD20 antibody immunotherapy. *J Exp Med* 2004; **199**: 1659–1669.
77. Shi Y, Fan X, Deng H, et al. Trastuzumab triggers phagocytic killing of high HER2 cancer cells *in vitro* and *in vivo* by interaction with Fcγ receptors on macrophages. *J Immunol* 2015; **194**: 4379–4386.
78. Herlyn D, Koprowski H. IgG2a monoclonal antibodies inhibit human tumor growth through interaction with effector cells. *Proc Natl Acad Sci USA* 1982; **79**: 4761–4765.
79. Isaacs JD, Greenwood J, Waldmann H. Therapy with monoclonal antibodies. II. The contribution of Fcγ receptor binding and the influence of CH1 and CH3 domains on *in vivo* effector function. *J Immunol* 1998; **161**: 3862–3869.
80. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate *in vivo* cytotoxicity against tumor targets. *Nat Med* 2000; **6**: 443–446.
81. Nimmerjahn F, Bruhns P, Horiuchi K, Ravetch JV. FcγRIV: a novel FcR with distinct IgG subclass specificity. *Immunity* 2005; **23**: 41–51.
82. Butchar JP, Mehta P, Justiniano SE, *et al*. Reciprocal regulation of activating and inhibitory Fcγ receptors by TLR7/8 activation: implications for tumor immunotherapy. *Clin Cancer Res* 2010; **16**: 2065–2075.
83. Tedder TF, Baras A, Xiu Y. Fcγ receptor-dependent effector mechanisms regulate CD19 and CD20 antibody immunotherapies for B lymphocyte malignancies and autoimmunity. *Springer Semin Immunopathol* 2006; **28**: 351–364.
84. Montalvao F, Garcia Z, Celli S, *et al*. The mechanism of anti-CD20-mediated B cell depletion revealed by intravital imaging. *J Clin Invest* 2013; **123**: 5098–5103.
85. Baković MP, Selman MHJ, Hoffmann M, *et al*. High-throughput IgG Fc N-glycosylation profiling by mass spectrometry of glycopeptides. *J Proteome Res* 2013; **12**: 821–831.
86. Shields RL, Lai J, Keck R, *et al*. Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human FcγRIII and antibody-dependent cellular toxicity. *J Biol Chem* 2002; **277**: 26733–26740.
87. Herbst R, Wang Y, Gallagher S, et al. B-Cell depletion *in vitro* and *in vivo* with an afucosylated anti-CD19 antibody. *J Pharmacol Exp Ther* 2010; **335**: 213–222.

88. Junttila TT, Parsons K, Olsson C, et al. Superior *in vivo* efficacy of afucosylated trastuzumab in the treatment of HER2-amplified breast cancer. *Cancer Res* 2010; **70**: 4481–4489.
89. Mossner E, Brunker P, Moser S, *et al*. Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell-mediated B-cell cytotoxicity. *Blood* 2010; **115**: 4393–4402.
90. Derer S, Glorius P, Schlaeth M, *et al*. Increasing FcγRIIa affinity of an FcgammaRIII-optimized anti-EGFR antibody restores neutrophil-mediated cytotoxicity. *mAbs* 2014; **6**: 409–421.
91. Treffers LW, van Houdt M, Bruggeman CW, *et al*. FcγRIIIb restricts antibody-dependent destruction of cancer cells by human neutrophils. *Front Immunol* 2018; **9**: 3124.
92. Horton HM, Bernett MJ, Pong E, et al. Potent *in vitro* and *in vivo* activity of an Fc-engineered anti-CD19 monoclonal antibody against lymphoma and leukemia. *Cancer Res* 2008; **68**: 8049–8057.
93. Nordstrom JL, Gorlatov S, Zhang W, et al. Anti-tumor activity and toxicokinetics analysis of MGAH22, an anti-HER2 monoclonal antibody with enhanced Fcγ receptor binding properties. *Breast Cancer Res* 2011; **13**: R123.
94. Forero-Torres A, de Vos S, Pohlman BL, *et al*. Results of a phase 1 study of AME-133v (LY2469298), an Fc-engineered humanized monoclonal anti-CD20 antibody, in FcγRIIIa-genotyped patients with previously treated follicular lymphoma. *Clin Cancer Res* 2012; **18**: 1395–1403.
95. Yang X, Wang F, Zhang Y, et al. Comprehensive analysis of the therapeutic IgG4 antibody pembrolizumab: hinge modification blocks half molecule exchange *in vitro* and *in vivo*. *J Pharm Sci* 2015; **104**: 4002–4014.
96. Guo L, Zhang H, Chen B. Nivolumab as programmed death-1 (PD-1) inhibitor for targeted immunotherapy in tumor. *J Cancer* 2017; **8**: 410–416.
97. Burova E, Hermann A, Waite J, *et al*. Characterization of the anti-PD-1 antibody REGN2810 and its antitumor activity in human PD-1 knock-in mice. *Mol Cancer Ther* 2017; **16**: 861–870.
98. Ribas A, Hanson DC, Noe DA, *et al*. Tremelimumab (CP-675,206), a cytotoxic T lymphocyte-associated antigen 4 blocking monoclonal antibody in clinical development for patients with cancer. *Oncologist* 2007; **12**: 873–883.
99. Holland M, Hewins P, Goodall M, Adu D, Jefferis R, Savage CO. Anti-neutrophil cytoplasm antibody IgG subclasses in Wegener's granulomatosis: a possible pathogenic role for the IgG4 subclass. *Clin Exp Immunol* 2004; **138**: 183–192.
100. Zhang T, Song X, Xu L, *et al*. The binding of an anti-PD-1 antibody to FcγRI has a profound impact on its biological functions. *Cancer Immunol* 2018; **67**: 1079–1090.
101. Walker MR, Lund J, Thompson KM, Jefferis R. Aglycosylation of human IgG1 and IgG3 monoclonal antibodies can eliminate recognition by human cells expressing Fc gamma RI and/or Fc gamma RII receptors. *Biochem J* 1989; **259**: 347–353.
102. Nesspor TC, Raju TS, Chin CN, Vafa O, Brezski RJ. Avidity confers FcγR binding and immune effector function to aglycosylated immunoglobulin G1. *J Mol Recognit* 2012; **25**: 147–154.
103. Inman BA, Longo TA, Ramalingam S, Harrison MR. Atezolizumab: a PD-L1-blocking antibody for bladder cancer. *Clin Cancer Res* 2017; **23**: 1886–1890.
104. Herold KC, Bundy BN, Long SA, *et al*. An anti-CD3 antibody, Teplizumab, in relatives at risk for type 1 diabetes. *N Engl J Med* 2019; **381**: 603–613.
105. Woodle ES, Xu D, Zivin RA, *et al*. Phase I trial of a humanized, Fc receptor nonbinding OKT3 antibody, huOKT3γ1 (Ala-Ala) in the treatment of acute renal allograft rejection. *Transplantation* 1999; **68**: 608–616.
106. Stewart R, Morrow M, Hammond SA, *et al*. Identification and Characterization of MEDI4736, an antagonistic anti-PD-L1 monoclonal antibody. *Cancer Immunol Res* 2015; **3**: 1052–1062.
107. Li K, Zhang T, Song J, Xu L, Liu Q, Peng H. Anti-PD1 Antibodies and Their Use as Therapeutics and Diagnostics. George Town, KY: Beigene, Ltd; 2014.
108. Franks SE, Getahun A, Hogarth PM, Cambier JC. Targeting B cells in treatment of autoimmunity. *Curr Opin Immunol* 2016; **43**: 39–45.
109. Chu SY, Vostiar I, Karki S, et al. Inhibition of B cell receptor-mediated activation of primary human B cells by coengagement of CD19 and FcγRIIb with Fc-engineered antibodies. *Mol Immunol* 2008; **45**: 3926–3933.
110. Kourtis G, Rucco A, Loconte F, Caiaffa M, Macchia L. Omalizumab in severe allergic asthma: efficacy assessment by comparison of symptom score and peak expiratory flow values before and after therapy. *J Invest Allergol Clin Immunol* 2010; **20**: 451–452.
111. Shields RL, Werther WR, Zioncheck K, *et al*. Anti-IgE monoclonal antibodies that inhibit allergen-specific histamine release. *Int Arch Allergy Immunol* 1995; **107**: 412–413.
112. Chu SY, Horton HM, Pong E, et al. Reduction of total IgE by targeted coengagement of IgE B-cell receptor and FcγRIIb with Fc-engineered antibody. *J Allergy Clin Immunol* 2012; **129**: 1102–1115.
113. Milgrom H, Fick RB, Su JQ, *et al*. Treatment of allergic asthma with monoclonal anti-IgE antibody. *N Engl J Med* 1999; **341**: 1966–1973.
114. Hochhaus G, Brookman L, Fox H, *et al*. Pharmacodynamics of omalizumab: implications for optimised dosing strategies and clinical efficacy in the treatment of allergic asthma. *Curr Med Res Opin* 2003; **19**: 491–498.
115. Mimoto F, Katada H, Kadono S, et al. Engineered antibody Fc variant with selectively enhanced FcγRIIb binding over both FcγRIIa(R131) and FcγRIIa(H131). *Protein Eng Des Sel* 2013; **26**: 589–598.
116. Croft M, Benedict CA, Ware CF. Clinical targeting of the TNF and TNFR superfamilies. *Nat Rev Drug Discov* 2013; **12**: 147–168.

117. White AL, Dou L, Chan HTC, *et al*. Fcγ receptor dependency of agonistic CD40 antibody in lymphoma therapy can be overcome through antibody multimerization. *J Immunol* 2014; **193**: 1828–1835.
118. White AL, Chan HTC, Roghanian A, *et al*. Interaction with FcγRIIB is critical for the agonistic activity of anti-CD40 monoclonal antibody. *J Immunol* 2011; **187**: 1754–1763.
119. White AL, Beers SA, Cragg MS. FcγRIIB as a key determinant of agonistic antibody efficacy. *Curr Top Microbiol Immunol* 2014; **382**: 355–372.
120. White Ann L, Chan HTC, French Ruth R, *et al*. Conformation of the human immunoglobulin G2 hinge imparts superagonistic properties to immunostimulatory anticancer antibodies. *Cancer Cell* 2015; **27**: 138–148.
121. Li F, Ravetch JV. Apoptotic and antitumor activity of death receptor antibodies require inhibitory Fcγ receptor engagement. *Proc Natl Acad Sci USA* 2012; **109**: 10966–10971.
122. 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**: 27134–27146.
123. Baselga J, Cortés J, Kim S-B, *et al*. Pertuzumab plus Trastuzumab plus docetaxel for metastatic breast cancer. *N Engl J Med* 2011; **366**: 109–119.