Fc-Receptor Interactions Regulate Both Cytotoxic and Immunomodulatory Therapeutic Antibody Effector Functions

David J. DiLillo and Jeffrey V. Ravetch

Abstract

Antibodies are now recognized as key therapeutic tools to combat most forms of malignancy. Although the first wave of therapeutic antibodies that emerged over two decades ago directly target tumor cells for killing, a new class of antibody therapies targeting immunoregulatory pathways to boost antitumor immune responses by activating the immune system is poised for clinical success. A notable common characteristic of both classes of therapeutic antibodies is the importance of the IgG Fc domain, which connects the fine specificity of an antibody with immune cells that mediate antibody-triggered effector functions through their engagement of Fc receptor (FcR) family members. It is now clear that multiple variables, including the nature of the target molecules, the local presence of effector cells, and the expression patterns of FcRs, will dictate whether and how an antibody will necessitate interactions with FcRs to mediate optimal therapeutic effects. Thus, through careful in vivo mechanistic analyses of individual therapeutic antibodies, Fc domains engineered for optimal engagement of the appropriate cellular FcRs must be designed to maximize clinical success.

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Learning Objectives

Antibodies have been used successfully to target either tumor cells for direct killing or immunoregulatory pathways to boost antitumor immune responses. The immunoglobulin constant region, termed the Fc domain, connects the fine-specificity of the antibody with immune cells that mediate antibody-triggered effector functions. The nature of the target molecules, the local presence of immune effector cells, and the expression patterns of Fc-receptor (FcR) family members dictate whether and how an antibody will interact with an FcR to mediate optimal therapeutic effects. Upon completion of this activity, the participant should gain a basic knowledge of FcR biology and the various classes of antibodies being developed for cancer immunotherapy.

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Introduction

Antibodies have become a major therapeutic tool for the treatment of all classes of malignancy. A well-defined but often-overlooked characteristic of antibodies is their bifunctional nature. The variable Fab region of an antibody mediates specificity and dictates to what antigen and with what affinity the antibody will bind its target. However, antibodies also contain a constant region, termed the Fc domain, which engages a diversity of cellular receptors, thereby triggering antibody-mediated effector functions. The Fc domain acts as a bridge between the specificity dictated by the Fab region and cells of the innate and adaptive immune system.

The first therapeutic antibodies designed almost three decades ago targeted tumor antigens, and were developed based on their intrinsic activity to mediate tumoricidal effects by Fab-mediated cross-linking of target molecules to trigger proapoptotic signaling...
cascades or through inhibition of autocrine loops. Little consideration was placed on the potential contributions from the Fc domains and Fc receptors (FcR). However, it is now clear that the Fc domain plays an instrumental role in the effector mechanisms elicited by multiple classes of therapeutic antibodies, whether they directly target tumor cells or alternatively target the immune system, modulating either positive or negative regulatory pathways. In this Masters of Immunology primer, we discuss FcR biology and the mechanisms of action of cytotoxic antitumor antibodies as well as newer classes of agonistic/antagonistic immunomodulatory antibodies, with an emphasis on how they differentially engage various members of the FcR family to mediate their effector functions. New data demonstrating that cytotoxic antitumor antibodies can stimulate long-term antitumor T-cell memory responses through an FcR-dependent "vaccinal effect" are also discussed. Through a thoughtful analysis of their respective mechanisms of action, engineering Fc domains of therapeutic antibodies for optimal engagement of appropriate members of the FcR family will lead to next-generation therapies with enhanced antitumor activities capable of sustained responses.

**Fc Receptor Expression and Function**

Upon binding their cognate antigens, IgG antibodies mediate downstream effector functions by interacting with either type I or type II FcRs. Type I FcRs are members of the immunoglobulin (Ig) superfamily and include the canonical FcRs for IgG (FcγRI, FcγRII, FcγRIII, FcγRIIIA, FcγRIIB, FcγRIIC, FcγRIIIB, FcγRIIIA, FcγRIIIB, FcγRIV) (Fig. 1; ref. 1). Type II FcRs are members of the C-type lectin receptor family and currently include CD209 (also known as DC-SIGN, which is homologous to SIGN-R1 in mice) and CD23. Whether type I or type II FcRs are engaged by an antibody is determined by the conformational state of its Fc domain, which is regulated by the complex, biantennary N-linked glycan attached to Asn297 (1, 2). Thus, differences in Fc glycosylation at Asn297 can lead to modulated affinities for FcRs: sialylated Fc domains adopt a more flexible, "closed" conformation that allows engagement of type II FcRs and reduces binding to type I FcRs (2, 3). By contrast,
nonsialylated Fc domains assume an "open" conformation, thereby allowing binding to type I FcRs and preventing engagement of type II FcRs. Detailed biochemical mechanisms regulating type I and type II FcR engagement, as well as the anti-inflammatory downstream consequences of type II FcR engagement, have been reviewed recently (1, 4, 5). Engagement of type II FcRs by sialylated IgG typically results in suppression of antibody- and T cell–mediated inflammation (6), as exemplified by the use of intravenous immunoglobulin (IVIG) in patients suffering from various autoimmune diseases. By contrast, engagement of type I FcRs results in antibody-dependent cellular cytotoxicity and phagocytosis (ADCC and ADCP), demonstrating the ability of an IgG to bridge target cells/pathogens and FcγR-expressing effector cells to mediate cytotoxicity or phagocytosis (7). Type I FcRs also engage antigen–antibody immune complexes (ICs) and mediate their downstream immunomodulatory effects on antigen-presentation cells (APC) and B cells. Immunomodulatory effects mediated by ICs are observed on dendritic cells (DC), where ICs can enhance antigen uptake and regulate DC maturation in an FcγR-dependent fashion, thereby shaping T-cell responses (8). ICs can also regulate affinity maturation of B cells in the germinal center, thus setting thresholds for B-cell activation (1, 9).

Type I FcR family members comprise the canonical FcγRs, which can be classified into two functionally defined subclasses: activating and inhibitory FcγRs (Fig. 1). The activating FcγRs, which include murine FcγRI, FcγRII, and FcγRIII, as well as human FcγRI, FcγRIIA, and FcγRIIB, initiate cellular activation through their intracellular immunoreceptor tyrosine-based activation motif (ITAM), while inhibitory signaling pathways are triggered by the intracellular immunoreceptor tyrosine–based inhibitory motifs (ITIM) of the mouse and human inhibitory FcγR, FcγRIIB (10). Both activating and inhibitory FcγRs are expressed by cells of the innate immune system. Mouse natural killer (NK) cells exclusively express FcγRII; and human NK cells primarily express FcγRIIIA; B cells of both species exclusively express the inhibitory FcγRIIB. Murine DCs express the full array of activating and inhibitory FcγRs, while human DCs only express a single activating FcγR, huFcγRIIA, and the inhibitory huFcγRIIB (Fig. 1; ref. 7). With the exception of FcγRI, type I FcRs are of intermediate or low affinity and thus will not bind monomeric IgG at normal physiologic concentrations. These receptors have evolved to engage immune complexes or IgG-coated targets, resulting in receptor cross-linking and triggering of cellular responses. The cellular outcome of IgG interactions with FcγRs is governed by the affinity of the Fc for the specific FcγR and the expression pattern of those receptors on the effector cells.

Because most effector cells coexpress activation and inhibitory FcγRs, it is the relative ratio of the binding affinities of a specific IgG Fc to these receptors that will determine the outcome of the IgG–FcγR interaction. These binding affinities are determined by the amino acid sequences of the different IgG Fc subclasses and the N-linked glycan patterns of the IgG Fc domains. Thus, the IgG Fc composition can dramatically influence the in vivo outcome of an antibody–antigen complex engaging FcγRs on an innate cell, by directing the cell into either a proinflammatory or an anti-inflammatory state. This is best exemplified by isotype-specific activity in the murine FcγR system. Mouse (ms)IgG2a demonstrates a 2-log greater affinity for the activating msFcγRIV receptor as compared to the inhibitory msFcγRIIB receptor, while msIgG1 preferentially engages the inhibitory msFcγRIIB receptor (11). An activating inhibitory (A:I) ratio can be assigned to each antibody isotype by creating a ratio of the Fc's affinity for the relevant activating FcγR versus the Fc's affinity for the inhibitory FcγR. Thus, msIgG2a antibodies have an A:I ratio of approximately 70, whereas msIgG1 antibodies show an A:I ratio of 0.1. The in vivo activities of msIgG2a and msIgG1 antibodies tightly correlate with their A:I ratios, as antitumor antibodies of the msIgG2a subclass potently activate effector cells to kill msIgG2a antibody-opsonized target cells, whereas msIgG1 antibodies demonstrate little tumoricidal activity in vivo (11). Human IgG isoforms also demonstrate preferential engagement of FcγRs, but to a lesser degree than the murine FcγR system, and without selective engagement of either activating or inhibitory receptors. Human (hu)IgG2 and IgG4 interact poorly with huFcγRs, whereas huIgG1 and huIgG3 interact more strongly (12). Further, allelic variants of huFcγRs exist in the human population that can considerably affect binding affinities to the huIgG1 and huIgG3 subclasses. For example, the FcγRIIa158V allele has a higher affinity for huIgG1 than the FcγRIIa158F allele, and the FcγRIIa131F allele demonstrates increased binding to huIgG1 than FcγRIIa131V (13). The in vivo consequences of these allelic variants are clearly evident, as patients with cancer carrying the FcγRIIa158V and FcγRIIa131F alleles show greater responses to antitumor monoclonal antibody (mAb) therapies (14, 15). This is discussed in more detail below.

Monoclonal Antibody Therapy for Malignancies

Antibodies have become an important tool in the battle against all forms of malignancy, and the number of therapeutic antibodies in clinical use or under investigation has dramatically increased in recent years. There are several classes of therapeutic antibodies, each with the ultimate effect of priming either the innate or the adaptive arm of the immune system to target tumor cells for destruction: (i) antitumor antibodies that directly engage tumor antigens and target the tumor for destruction by the innate immune system; (ii) agonistic immunomodulatory antibodies that target immune system costimulatory molecules to potentiatantitumor immune responses; and (iii) antagonistic immunomodulatory antibodies that target immune system regulatory molecules to "release the brakes" on antitumor immune responses (Fig. 2).

Antitumor antibodies

The fine specificity demonstrated by mAbs has enabled the targeting of tumor-specific or tumor-associated antigens (TAA) restricted to malignant cells, thereby allowing for precise targeting of tumor cells. Although radionuclide-conjugated or toxin-conjugated antibodies have demonstrated therapeutic activities, “naked” Fc-engineered antibodies have the potential to recruit immune effector pathways that may amplify and extend the activity of antitumor therapies already in clinical use. Several potential mechanisms had been proposed by which antitumor mAbs mediate their tumoricidal effects in vivo, including cross-linking–mediated activation of signaling cascades that lead to cell death, blockade of ligands required for tumor cell survival, recruitment of the complement cascade, or recruitment of FcγR+ cytotoxic or phagocytic effector cells (16). Rituximab, an anti-CD20 antibody, was the first mAb to be approved to fight cancer in lymphoma patients, and its mechanism of action has been
studies been extensively. Although \textit{in vitro} studies indicated that rituximab is capable of activating complement and inducing apoptosis, \textit{in vivo} studies, both in animal models and patients undergoing treatment, have clearly demonstrated that engagement of FcRs on innate cell populations is crucial for this mAb to mediate its antitumor cytotoxic effects (1, 17, 18).

Experiments in immunodeficient mice with xenographs of huCD20\textsuperscript{+} human tumor cells were the first \textit{in vivo} studies to demonstrate that expression of murine activating FcRs is required for the clearance of lymphoma cells by rituximab (17). Subsequent studies in immunocompetent mice treated with anti-msCD20 mAbs further demonstrated that macrophages expressing activating FcRs mediated clearance (ADCC/ADCP) of CD20\textsuperscript{+} B cells or syngeneic lymphoma cells (18, 19). No contributions by FcR\textsuperscript{+} NK cells, complement pathways, or the induction of apoptotic signaling pathways could be detected \textit{in vivo}. These results are consistent with results from studies in humans demonstrating that functional polymorphisms in huFcRIIHA correlate with response rates in lymphoma patients treated with rituximab (14, 15). Investigators have begun to perform therapeutic studies utilizing FcR-humanized mice, in which the full array of huFcRs is expressed in fully immunocompetent mice that lack all murine FcRs (20). These mice fully recapitulate huFcR expression patterns and function, and demonstrate that huIgG1 mAb-mediated ADCC of tumor cells or other cellular populations is mediated by Fc–FcR interactions \textit{in vivo} (20, 21). Specifically, huFcRIIHA expressed by monocytes/macrophages is both necessary and sufficient for huIgG1 mAb-mediated clearance of lymphoma cells (21).

The requirement for Fc–FcR interactions during the cytotoxic clearance of tumor cells by antitumor mAbs \textit{in vivo} has been confirmed for other antitumor mAbs. The anti-Her2 mAb trastuzumab requires Fc–FcR interactions to mediate cytotoxicity of xenographs of Her2\textsuperscript{+} tumor cells in mice (17). Accordingly, functional polymorphisms in huFcRIIHA also correlate with efficacy in breast cancer patients treated with trastuzumab (22). In addition to blocking downstream epidermal growth factor receptor (EGFR) signaling, the efficacy of the anti-EGFR mAb cetuximab also correlates with huFcRIIA and FcγRIIIA polymorphisms (23). Thus, multiple antitumor mAbs mediate their cytotoxic antitumor effects through engaging FcRs expressed by innate cell populations, including monocytes and macrophages.

\textbf{Fc-optimization of cytotoxic antitumor antibodies}

Because both activating and inhibitory FcRs are coexpressed by the majority of innate immune cells that mediate antitumor ADCC/ADCP upon engagement of cell-bound mAbs or immune complexes, a threshold for immune cell activation exists. Thereby, modulation of an antibody's relative engagement of activating versus inhibitory FcRs (the A:I ratio) represents a key approach for augmenting the \textit{in vivo} activity of a therapeutic mAb. This...
rationale is demonstrated by studies in which genetic deletion of the inhibitory FcγR, FcγRIIB, results in augmented mAb-mediated cytotoxicity (17, 19). Alternatively, two routes for engineering the huIgG Fc region for selectively enhanced engagement of activating FcRs are under investigation in both preclinical and clinical studies: (i) modification of Fc–FcγR interactions through manipulating the Fc glycan at Asn297; and (ii) modification of Fc–FcγR interactions through the introduction of Fc domain point mutants.

Although N-linked glycosylation of the IgG Fc is absolutely required for FcR binding (1), modification of specific carbohydrates, such as fucose and branching GlcNAc, can modulate the Fc’s affinity for FcγR. For example, afucosylated IgG demonstrates enhanced affinity specifically for huFcγRIIA, thereby increasing the A1 ratio and resulting in increased ADCC/ADCP effector function both in vitro and in vivo (24). Thus, the next-generation antitumor mAb therapeutics lacking fucose residues for enhanced effector functions are exploiting this technique (25, 26). A remarkable example of the in vivo consequences of selectively engaging activating FcRs and increasing the A1 ratio of a mAb’s Fc is the clinical success of the FDA-approved anti-CD20 mAb obinutuzumab (GA101). Obinutuzumab’s N-linked Fc glycan has been engineered to contain a bisecting N-acetylgalactosamine (GlcNAc), which precludes the addition of fucose residues. Thus, Fc-optimized, afucosylated obinutuzumab plus chemotherapy extended progression-free survival by almost a full year and showed markedly higher rates of complete responses in patients with chronic lymphocytic leukemia compared with treatment with unmodified anti-CD20 rituximab plus chemotherapy (27). Preclinical studies with an afucosylated anti-EGFR antibody also demonstrated increased efficacy compared with unmodified cetuximab (28). Therefore, it is likely that many of the next-generation Fc-optimized, afucosylated antitumor antibodies now under examination in clinical trials, including mAbs targeting CD70, c-Met, EGFR, M1 of IgE, CD157, CCR4, or EphA3, will demonstrate significant efficacy against the tumors they target (Table 1).

Afucosylation of mAbs offers a limited approach to Fc-engineering, because this class of mAb shows augmented engagement of only huFcγRIIA. By contrast, the introduction of Fc domain point mutations may be used to manipulate interactions with any and/or multiple FcγR family members. For example, known mutations are capable of allowing the Fc domain to selectively engage huFcγRIIA, huFcγRIIB, huFcγRIIA, or both huFcγRIIA/RIIB simultaneously (20, 21). Animal studies using FcγR-humanized mice have demonstrated that appropriate Fc point mutations can dramatically modulate the in vivo efficacy of anti-melanoma and anti-CD20 mAbs (20, 21), as well as antibodies that neutralize pathogens (29–31). Preclinical studies have also demonstrated that an anti-CD19 mAb Fc-optimized using point mutations showed augmented antitumor activity in vivo (32). Thus, the next generation of point-mutated, Fc-optimized antitumor mAbs now in clinical testing, including mAbs reactive with B7-H3, HER2, CD30, CD19, and CD20, are likely to show superior efficacy in patients (Table 1).

### Immunomodulatory Antibodies

Tumors have the unique ability to shield themselves from attack from the immune system through a variety of immunosuppressive mechanisms that occur in the tumor microenvironment (33). Thus, a major focus in cancer research is to understand the underlying mechanisms of this immunosuppression and design therapies to “release the brakes” on antitumor immune responses. Therapeutic success has been demonstrated in both preclinical and clinical studies of antibodies targeting not the tumors but the immunoregulatory pathways mediated by molecules expressed by cells of both the innate and adaptive immune system. Initially, it was assumed that using agonistic mAbs to

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**Table 1. Next-generation antitumor antibodies optimized for Fc-mediated effector function**

<table>
<thead>
<tr>
<th>Name</th>
<th>Target</th>
<th>Indication</th>
<th>Company</th>
<th>Fc optimization strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGA271</td>
<td>B7-H3</td>
<td>B7-H3+ carcinomas (solid tumors)</td>
<td>MacroGenics</td>
<td>Fc domain point mutants</td>
</tr>
<tr>
<td>MGAH22 (margetuximab)</td>
<td>HER2</td>
<td>Breast cancer</td>
<td>MacroGenics</td>
<td>Fc domain point mutants</td>
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<tr>
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<td>CD30</td>
<td>Hodgkin lymphoma</td>
<td>Xencor</td>
<td>Fc domain point mutants</td>
</tr>
<tr>
<td>XmAb-5571</td>
<td>CD19</td>
<td>Leukemia/lymphoma (CLL)</td>
<td>Xencor</td>
<td>Fc domain point mutants</td>
</tr>
<tr>
<td>AME-1536 (ocaratuzumab, LY2469298)</td>
<td>CD20</td>
<td>Leukemia/lymphoma (NHL: CLL, FL)</td>
<td>Mentrik/Eli Lilly</td>
<td>Fc domain point mutants</td>
</tr>
<tr>
<td>Obinutuzumab (GA101)</td>
<td>CD20</td>
<td>Leukemia/lymphoma (NHL: CLL, FL)</td>
<td>Roche</td>
<td>Afucosylated Fc domain</td>
</tr>
<tr>
<td>GA201 (RG7160)</td>
<td>EGFR</td>
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<td>Roche</td>
<td>Afucosylated Fc domain</td>
</tr>
<tr>
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<td>CD19</td>
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<tr>
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<td>GD3 ganglioside</td>
<td>Melanoma</td>
<td>Life Science</td>
<td>Afucosylated Fc domain</td>
</tr>
<tr>
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<td>CD70</td>
<td>CD70+ tumors</td>
<td>Argen-x</td>
<td>Afucosylated Fc domain</td>
</tr>
<tr>
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<td>c-Met</td>
<td>c-Met+ tumors</td>
<td>Argen-x</td>
<td>Afucosylated Fc domain</td>
</tr>
<tr>
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<td>M1 of IgE</td>
<td>IgE+ B cells</td>
<td>Genentech</td>
<td>Afucosylated Fc domain</td>
</tr>
<tr>
<td>OBT357 (MEN112)</td>
<td>Bst1 (CD157)</td>
<td>Acute myeloid leukemia</td>
<td>Menarini Group/Oxford</td>
<td>Afucosylated Fc domain</td>
</tr>
<tr>
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<td>Ganglioside GM2</td>
<td>BioTherapeutics</td>
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<tr>
<td>Mogamulizumab (KW-0761)</td>
<td>CCR4</td>
<td>Peripheral and cutaneous T-cell lymphomas</td>
<td>Kyowa Hakko Kirin</td>
<td>Afucosylated Fc domain</td>
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<tr>
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<td>EGFR</td>
<td>Various carcinomas</td>
<td>Glycoptope</td>
<td>Afucosylated Fc domain</td>
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<td>Trai-GEX</td>
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<td>EphA3</td>
<td>Hematological and solid tumors</td>
<td>Kalobios Pharmaceuticals</td>
<td>Afucosylated Fc domain</td>
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Abbreviations: CLL, chronic lymphocytic leukemia; FL, follicular lymphoma; NHL, non-Hodgkin lymphoma.
simply cross-link activator immune molecules (such as costimulatory antitumor antibody). Antitumor mAb opsonizes tumor cells and targets them for killing by FcγR-mediated antibody-dependent cellular cytotoxicity or phagocytosis (ADCC or ADCP), a process that generates antibody–tumor antigen immune complexes (IC). These ICs engage activating FcγRs expressed by DCs, which results in stimulation of DC maturation and presentation of tumor antigens to T cells, thereby leading to long-term antitumor cellular memory formation. Right, tumor cell death by other means may trigger the release of TAAs, which could be captured, processed, and presented to T cells to stimulate antitumor cellular immunity. Blue boxes represent steps at which antibody therapeutics may intervene and augment antibody-mediated cytotoxicity or stimulation of antitumor T-cell memory responses.

**Figure 3.**
Left, mechanism of antitumor vaccinal effect mediated by cytotoxic antitumor antibody. Antitumor mAb opsonizes tumor cells and targets them for killing by FcγR-mediated antibody-dependent cellular cytotoxicity or phagocytosis (ADCC or ADCP), a process that generates antibody–tumor antigen immune complexes (IC). These ICs engage activating FcγRs expressed by DCs, which results in stimulation of DC maturation and presentation of tumor antigens to T cells, thereby leading to long-term antitumor cellular memory formation. Right, tumor cell death by other means may trigger the release of TAAs, which could be captured, processed, and presented to T cells to stimulate antitumor cellular immunity. Blue boxes represent steps at which antibody therapeutics may intervene and augment antibody-mediated cytotoxicity or stimulation of antitumor T-cell memory responses.

**Agonistic immunomodulatory antibodies**

The costimulatory receptor CD40, a member of the tumor-necrosis factor receptor (TNFR) superfamily, has been targeted using agonistic mAbs due to its important role as a positive regulator of innate and adaptive immunity (34). Animal studies have demonstrated an unexpected and absolute requirement for interactions with the inhibitory FcγR, FcγRIIB, for anti-CD40 mAbs to mediate their proinflammatory, T-cell priming, and antitumor effects (35, 36). This finding has been extended to mAbs targeting other members of the TNFR superfamily, including CD95 (Fas), and death receptors (DR)4, and DR5 (37–40). Furthermore, it is now known that anti-TNFR agonistic mAbs function in trans by engaging FcγRIIB on a cell other than the antibody-bound target cell; distinct cellular populations provide FcγRIIB depending on the TNFR target molecule and the local microenvironment (41). Because FcγRIIB signaling is not required for the agonistic effect of these antibodies, engagement of FcγRIIB provides a scaffold to mediate clustering of TNFR molecules to initiate downstream signaling pathways (41). Because clustering of these molecules is absolutely required for the agonistic effect of CD40, Fas, DR4, and DR5 mAbs, reengineering the Fc for augmented engagement of FcγRIIB represents an approach for enhancing the agonistic effect of these mAbs.
Alternatively, clustering can be achieved through Fc-independent means, as has been reported for the human IgG2 subclass that can adopt a "B" form structure that enhances CD40 signaling (42). Indeed, introduction of Fc domain point mutations that selectively enhance the affinity of the Fc for huFcRIIB dramatically augments the antitumor potency of agonistic anti-CD40 mAbs in preclinical FcγR-humanized mouse models (35). Thus, future antibody-based agonistic therapeutics must incorporate ideal Fc domains or other strategies for clustering these trimeric receptors for optimal efficacy.

**Antagonistic immunomodulatory antibodies**

Although most tumors contain some immunogenic antigens, antitumor immune responses are hampered by multiple immunosuppressive mechanisms used by malignant cells in the tumor microenvironment. Immunomodulatory antagonistic mAbs (also known as checkpoint inhibitors) target regulatory molecules expressed by immune cells in order to disrupt their immunoregulatory pathways and promote antitumor immune responses (43). Examples of the regulatory pathways currently being targeted by mAbs include CTLA-4, GITR, OX40, and the PD-1/PD-L1 axis. As with the agonistic mAbs above, it was unexpectedly found that engagement of activating FcγRs is required for optimal antitumor activity in preclinical models using anti–CTLA-4 (44, 45), anti–GITR (46), and anti–OX40 (47) mAbs. Mechanistically, each of these immunomodulatory molecules is highly expressed by intratumoral regulatory T cells (Treg), which, upon opsonization by mAbs, are targeted for cytotoxic depletion by activating FcγRs-expressing innate cells (intratumoral macrophages). The removal of Tregs from the tumor microenvironment thereby enhances the ability of the remaining effector T cells to kill the tumor. By contrast, anti–PD-1 mAbs now in clinical use have been engineered to abrogate interactions with FcγRs, because PD-1 is broadly expressed by T cells, including effector CD8+ T cells that mediate antitumor activities; deletion of this T-cell subset would likely inhibit antitumor immunity and augment tumor growth (Fig. 2). Whether Fc–FcγR interactions are required for the in vivo activity of anti–PD-L1, anti–CD137, anti–CD27, and anti–KIR mAbs now in clinical trials is the subject of active investigation.

The unexpected requirement for interactions with the inhibitory FcγRIIB for agonistic anti-TNF family immunomodulatory antibodies, or with activating FcγRs for some antagonistic antibodies, demonstrates how essential it is to understand the mechanism of action with regard to FcγR engagement of each agonistic or antagonistic immunomodulatory antibody on a case-by-case basis. It is likely that the expression levels of the therapeutic target on effector immune cells, regulatory immune cells, or the tumor itself will dictate whether engagement of FcγRs is necessary to augment or inhibit the effectiveness of a therapeutic antibody in vivo. Expression patterns of activating and inhibitory FcγRs, as well as the presence of the appropriate effector cells in the tumor microenvironment, will further dictate whether and which FcγRs may be involved. Because the complex conditions of the tumor microenvironment cannot be recreated in vitro, careful in vivo mechanistic analyses of each immunomodulatory antibody must be performed to inform the proper engineering of the Fc domain for ideal interaction with the appropriate FcγRs for optimum in vivo efficacy. Thus, future generations of immunomodulatory mAbs will undoubtedly incorporate engineered Fc domains for optimal activity in vivo.

**Antibodies Prime Antitumor Immune Responses: The Vaccinal Effect**

Although most mechanistic studies of antitumor antibodies have focused on their short-term cytotoxic properties, an alternate concept has emerged by which antitumor antibodies may function to activate the immune system to stimulate a potent, long-term antitumor memory immune response. This phenomenon is known as a vaccinal effect.

As early as 1907, Emil von Behring demonstrated that immunization with a mixture of diphtheria toxin and antitoxin (anti-diphtheria antibody) produced safe and lasting immunity to diphtheria in animals and humans (48). We now understand these mixtures to be antigen–antibody immune complexes, which enhance the immunogenicity of soluble molecules. While immune complexes are excellent inducers of humoral immunity (antibody responses), their ability to engage FcγRs expressed by APCs of the innate immune system to prime cellular immunity (T-cell responses) is most relevant in the context of tumor immunity (Fig. 3; ref. 49). DCs, the most potent of the immune system’s APCs, primed with immune complexes are capable of processing and presenting the relevant antigen to both CD4 (through traditional antigen presentation) and CD8 T cells (through cross-presentation; refs. 50, 51). Studies using various antigen–antibody immune complexes have demonstrated that DCs primed with immune complexes cross-present antigens and generate more potent CD8 T-cell responses compared with DCs primed with antigen alone. Activating FcγRs are required for this process, and modulating the balance of activating versus inhibitory FcγR engagement (A:I ratio) toward activating FcγRs augments CD8 immune responses and tumor immunity in vivo (8, 52). Mechanistically, immune-complex engagement of FcγRs leads to DC maturation and upregulation of MHC molecules and costimulatory molecules, including CD80, CD86, and CD40 (52, 53). Thus, immune complexes are potent inducers of cell-mediated immunity.

Passive administration of therapeutic antitumor cytotoxic antibodies likely results in the in vivo generation of immune complexes (Fig. 3), and recent studies have investigated whether such antibodies initiate a vaccinal effect that is characterized by an antitumor cellular immune response. Examples of cytotoxic antibody-induced vaccinal effects from the clinic include patients treated with anti-MUC1 mAbs or anti-HER2/Neu mAbs, who generated MUC1-specific and HER2-specific T-cell responses, respectively (54, 55). Because a single course of treatment with anti-CD20 mAb (rituximab) can result in long-lasting, durable responses, it has been hypothesized that treatment with this mAb may also induce a vaccinal effect in patients with lymphoma (56). In support of this, lymphoma-specific anti-idiotype T-cell responses were detected in some patients treated with anti-CD20 rituximab (57, 58). Anti-CD20 mAb treatment of lymphoma cells in vitro stimulates DC maturation and CD8 T-cell activation (59), and vaccination with huCD20+ tumor cells and anti-huCD20 mAb treatment showed a synergistic antitumor effect in mice (60). Studies using mouse models have also demonstrated that passive administration of anti-CD20 mAbs can initiate antitumor T-cell responses and vaccinal effects (21, 61).

Recent studies have elucidated how an antitumor vaccinal effect could be generated upon treatment with a cytotoxic antitumor mAb using a murine model in which syngeneic EL4 lymphoma cells express huCD20 (EL4-huCD20 cells) as a tumor neoantigen.
effect shed important light on the by antitumor mAbs. Second, immune-complex engagement of immediate cell-mediated cytotoxicity and phagocytosis triggered expressed by monocytes and macrophages is absolutely vital for generating a vaccinal effect memory immune response against the CD20 antigen and subsequently reject rechallenge with wild-type EL4 cells (21). Thus, mice primed with EL4-hCD20 and mAb generate a vaccinal effect memory immune response when rechallenged with EL4-hCD20 cells, but not wild-type EL4 cells. This process relies on both CD4 and CD8 T cells, and adoptive transfer of T cells from mAb/tumor-primed mice transfers the vaccinal effect to naïve animals (61). Using transgenic mice in which activating FcRys are conditionally deleted on CD11c+ cells, it has been demonstrated that FcRγ expression by DCs is required for the generation of the vaccinal effect (21). Collectively, these results demonstrate that cytotoxic clearance (ADCC/ADCP) of tumor cells by anti-huCD20 mAbs induces the generation of huCD20 immune complexes, which engage FcRγ expressed by DCs to initiate a huCD20-specific T-cell vaccinal effect (Fig. 3, left).

Mechanistically understanding the antitumor vaccinal effect in the context of the human FcγR system is vital for designing optimal antitumor antibody therapeutics. As discussed earlier, expression patterns of FcγRs differ between mouse and man. While ADCC-mediating human monocytes/macrophages utilize only huFcRIIA for their cytotoxic effects, vaccinal effect-mediating human DCs only express huFcRIIA (21). To dissect the vaccinal effect mechanistically in the context of the huFcγR system, FcγR-humanized mice were used in combination with huIgG1 anti-huCD20 mAbs, as well as mutant anti-huCD20 mAbs that selectively engage either huFcRIIA, huFcRγIIIA, or both of these receptors. During the primary tumor challenge, engagement of huFcγRIIA was both necessary and sufficient for ADCC-mediated clearance of EL4-huCD20 tumor cells; huFcγRIIA played no role. By contrast, engagement of huFcγRIIB was required for optimal induction of the vaccinal effect in FcγR-humanized mice, consistent with this being the sole activating huFcγR expressed by human antigen-presenting DCs (21). Thus, differential human FcγR requirements exist for antitumor mAb-mediated cytotoxicity versus induction of a memory cellular immune response during the vaccinal effect.

Looking toward the Future in Therapeutic Antibodies in Malignancy

These new mechanistic insights into the antitumor vaccinal effect shed important light on the in vivo effector mechanisms triggered by cytotoxic antitumor antibodies in the context of human IgG and human FcRs. First, Fc engagement of huFcγRIIA expressed by monocytes and macrophages is absolutely vital for immediate cell-mediated cytotoxicity and phagocytosis triggered by antitumor mAbs. Second, immune-complex engagement of huFcγRIIA expressed by human DCs induces DC maturation and upregulation of costimulatory molecules for optimal antigen presentation and cross-presentation to stimulate long-term antitumor T-cell memory (Fig. 3, left). As discussed above, the current collection of next-generation antitumor antibodies are being optimized through glycoengineering or with point mutations for superior huFcγRIIA engagement to augment their cytotoxic effects. However, these new antibodies have not been optimized for huFcγRIIA engagement, as defucosylation does not affect binding to this receptor. Therefore, an ideal antitumor antibody will need to be optimized for both effector functions: optimized huFcγRIIA engagement for augmented immediate cytotoxicity and optimized huFcγRIIA binding for more potent induction of long-term antitumor vaccinal effect T-cell responses.

The next phase of antitumor immunotherapy will not only consist of antitumor and immunomodulatory antibodies with engineered Fc domains for optimal effector functions, but it should also incorporate logical combinations of Fc-optimized antitumor antibodies, Fc-optimized immunomodulatory antibodies, and traditional radiotherapies and chemotherapies (Fig. 3). Because preclinical studies have suggested synergistic effects of immunomodulatory mAbs with distinct mechanisms of action (e.g., combining anti-CTLA-4 and anti-PD-1 checkpoint inhibitors), combinations of various immunomodulatory antibodies are being tested in clinical trials for synergy (62). Combining immunomodulatory mAbs with traditional chemotherapy and radiation treatments has also shown some benefit in early trials, most likely because cell death exposes tumor-specific antigens or neoantigens for recognition by an unemburdened immune system (Fig. 3, right; refs. 63, 64). Combining checkpoint inhibitors with antitumor vaccination may also lead to enhanced results. Thus, a logical extension would be to test cytotoxic antitumor mAbs in combination with immunomodulatory mAbs. One would predict that cytotoxic killing of tumors would generate immune complexes for vaccinal effect induction as well as potentially expose other tumor antigens; memory T-cell responses would likely be boosted in combination with immunomodulatory mAb treatment (Fig. 3, right).

Although multiple classes of therapeutic antibodies are now available to treat the full array of human malignancies as single agents or in logical combinations, one common theme cannot be ignored: The effector functions mediated by the Fc domain of each therapeutic antibody must be carefully evaluated and mechanistically understood. Through thoughtful analysis of each antibody’s mechanism(s) of action in the context of the human FcγR system, more potent therapies can be engineered through optimized engagement of the appropriate FcγRs.

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Fc-Receptor Interactions Regulate Both Cytotoxic and Immunomodulatory Therapeutic Antibody Effector Functions

David J. DiLillo and Jeffrey V. Ravetch


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