

Antibodies, Fc receptors and cancer

Falk Nimmerjahn^{1,2} and Jeffrey V Ravetch¹

Since the first report of the successful use of a monoclonal antibody for the treatment of human B cell lymphoma in 1982, several antibodies have become incorporated into standard treatment protocols for cancer. One of the most important factors that determine antibody activity *in vivo* is the efficient interaction with cellular Fc-receptors on innate immune effector cells. It has become clear that the outcome of this interaction is influenced by several factors, such as the antibody isotype-specific affinity to activating and inhibitory receptors, the level of inhibitory Fc γ RIIB expression, and the composition of the sugar side chain attached to the antibody Fc-portion. These novel insights into antibody FcR interactions might be useful to produce the next generation of improved immunotherapeutic molecules.

Addresses

¹ Laboratory of Molecular Genetics and Immunology, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA

² Laboratory of Experimental Immunology and Immunotherapy, University of Erlangen-Nuremberg, Glueckstr. 6, 91054 Erlangen, Germany

Corresponding author: Ravetch, Jeffrey V (ravetch@rockefeller.edu)

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Introduction

According to WHO estimates, more than 7 million people died of cancer in 2005. For many of these malignancies, surgical removal of the tumor combined with standard chemo- or radiation-therapy is the common treatment. However, complete removal of malignant tissues is not always possible, resulting in reappearance of the tumor. Moreover, many disseminated malignancies, such as leukemia or cancer metastasis, cannot be removed surgically and rely on chemotherapy for elimination.

Owing to their exquisite specificity, antibodies have been in the spotlight as potential therapeutics for treatment of human cancer [1]. The initial use of murine antibodies for human therapy, however, has been complicated because of their strong immunogenicity and inefficient recruitment of human effector pathways. Because of the production of humanized antibodies and mouse strains that

can generate fully human antibodies, many of these obstacles have been overcome [2]. In addition, antibodies have been radio-labeled or conjugated to toxins for specific targeting to the tumor tissue [3]. By genetic engineering, a variety of other antibody-derived molecules such as single-chain Fv and bi-specific antibodies have been generated [4]. Some of the most dramatic clinical responses, however, have been obtained using unmodified antibodies either alone or in combination with chemotherapy.

Among the target antigens recognized by therapeutic antibodies are growth factor receptors, such as members of the epidermal growth factor receptor or vascular endothelial growth factor receptor families, leukocyte antigens, such as CD20 or CD22, death receptors, such as CD30, and molecules that modulate cellular immunity, such as CD40 or CTLA-4 [5]. Indeed, promising results have been obtained using monoclonal antibodies that interfere with inhibitory receptors that regulate T-cell responses, such as CTLA-4 [6,7]. Combining these blocking antibodies with chemotherapy or other tumor-specific antibodies might enhance clinical responses by taking the brakes off cellular immune responses. Furthermore, it has been suggested that blocking antibodies specific for growth factor receptors essential for tumor cell growth and proliferation might be valuable tools to slow down tumor growth [5]. Antibodies that target the epidermal growth factor receptor family, such as cetuximab and panitumumab, have been shown to prevent either ligand binding or dimerization of the growth factor receptors — an absolute requirement for transduction of activating signaling pathways [8,9]. In addition, triggering of pro-apoptotic signals on tumor cells by antibodies specific for death receptors might directly induce tumor cell apoptosis [10]. It has become clear, however, that this modulation of regulatory and survival signaling pathways alone will not be sufficient to eliminate tumor cells, and that the recruitment of secondary immune effector mechanisms is of great importance for the efficiency of antibody therapy (Table 1). Amongst these, antibody-dependent cellular cytotoxicity (ADCC) is emerging as one of the most important factors. During an ADCC reaction, antibodies bound to tumor cells recruit innate immune effector cells that express cellular receptors (Fc receptors [FcRs]) specific for the constant region of the antibody, thereby triggering phagocytosis and the release of inflammatory mediators and cytotoxic substances [11]. Thus, FcRs represent a link between the specificity of the adaptive immune system and the powerful cytotoxic effector responses triggered by the innate effector cells. Although most antibody-based therapeutics are well tolerated, the

Table 1

Target antigens and effector mechanisms of anti-tumor antibodies in cancer therapy.

Cancer type	Species	Antibody target	Mechanism	FcR	Reference
B cell	Mouse	CD20	ADCC	Isotype-specific FcR engagement	[17,18*]
B cell	Mouse	CD19	ADCC	γ -chain dependent	[47]
Melanoma	Mouse	gp-75	ADCC	Isotype-specific FcR engagement	[19**,20,21,43,44]
Breast cancer	Xenograft	Her-2/neu	ADCC	γ -chain dependent	[20]
Large cell lymphoma	Xenograft	CD30	Proliferation block	Independent	[10]
B-cell lymphoma	Xenograft	CD32B	ADCC	FcR-mediated	[50]
T-cell leukemia	Xenograft	CD25	ADCC	γ -chain dependent	[16]
T-cell leukemia	Xenograft	CD2	ADCC	γ -chain dependent	[48]
T-cell leukemia	Xenograft	CD52	ADCC	γ -chain dependent	[49]
Follicular lymphoma	Human	CD20	ADCC	Fc γ RIIIA ^{158V}	[26]
Follicular lymphoma	Human	CD20	ADCC	Fc γ RIIIA ^{158V}	[25]
B-cell lymphoma	Human	Idiotypic vaccination	ADCC	Fc γ RIIIA ^{158V}	[24]

recent use of a superagonistic CD28-specific antibody resulted in a catastrophic reaction, including multi-organ failure, pneumonia and septicaemia, in healthy individuals [12]. This shows the potency of this class of therapeutics and urges for a better pre-clinical evaluation, especially in the case of antibodies that are designed to stimulate rather than to block or delete potent and potentially dangerous effector cells, such as T lymphocytes.

This review will discuss recent mechanistic insights into antibody-mediated triggering of FcRs and how this knowledge could be used to generate optimized therapeutic strategies for the treatment of cancer.

The Fc receptor system

There are two classes of FcRs that can be distinguished functionally: the activating and the inhibitory receptors. Whereas activating FcRs, such as the human Fc γ RIA, Fc γ IIA and Fc γ IIIA and their mouse orthologues Fc γ RI, Fc γ III and Fc γ IV trigger cell activation by way of immunoreceptor tyrosine-based activation motif-dependent signaling pathways, the inhibitory Fc γ RIIB recruits inhibitory signaling pathways by way of an immunoreceptor tyrosine-based inhibitory motif in its cytosolic domain [13].

FcRs are broadly expressed on hematopoietic lineages, such as macrophages, neutrophils, natural killer (NK) cells, mast cells, B cells and dendritic cells (DCs). One of the most important features of the FcR system is the co-expression of activating and inhibitory receptors on the same cell, thereby setting thresholds for cell activation. The only exceptions to this rule are B cells, which only express the inhibitory FcR, and NK cells, which only express the activating Fc γ RIII [11]. Under steady-state conditions, the inhibitory receptor seems to dominate the response, either because of its higher expression level on cells such as DCs or by virtue of its higher affinity to abundant antibody isotypes, thus preventing unspecific activation of the immune system [14,15]. In addition, the low affinity of monomeric antibodies to FcRs

(10^5 – 10^6 M⁻¹) does not allow unspecific binding and cell activation. The exception to this rule is the high affinity Fc γ RI, which binds monomeric IgG with the same affinity as it binds immune complexes (ICs). Thus, Fc γ RI is usually saturated with monomeric IgG and is not available for IC binding, explaining why deletion of this FcR did not result in impaired antibody activity in many model systems [16,17,18*,19**,20]. At high IC concentrations or at local inflamed sites upon cytokine-induced Fc γ RI upregulation this receptor might participate in ADCC reactions *in vivo* [21–23]. By contrast, the low-affinity receptors that have been demonstrated to be crucial for anti-tumor responses in murine [18*,19**,20] and in human [24–26] monoclonal antibody based cancer therapy only interact with ICs [11].

Depending on the receptor and cell type, the fate of the ICs will differ. DCs, for example, which take up ICs via Fc γ RIIB, will recycle them to the cell surface for presentation to B cells [27*]. By contrast, uptake by way of activating FcRs results in proteolytic cleavage of ICs in lysosomal compartments and peptide presentation to cytotoxic and helper T cells [28,29,30*,31,32*]. In addition, ICs have been recognized as potent activators of DCs. This is consistent with the observation that antigen delivered in the form of ICs elicits a stronger immune response than antigen alone [30*,31,33]. At the same time, co-engagement of activating and inhibitory FcRs on DCs will regulate the DC activation state. More recently, it has been suggested that the inhibitory Fc γ RIIB on DCs might also be involved in maintaining peripheral tolerance in the cellular immune system. It was shown that deletion of Fc γ RIIB on DCs resulted in spontaneous cell activation by low levels of immune complexes present in the serum [30*,32*]. Thus, Fc γ RIIB not only sets a threshold for cell activation during inflammatory responses but also is essential to prevent the unspecific activation of DCs under steady-state conditions. Thus, blocking Fc γ RIIB on DCs might be a strategy to enhance anti-tumor immune responses during immunotherapy. As this block will be transient, it might

not lead to the initiation of lasting autoimmune responses; however, more experiments are necessary to address this important point.

Fc receptors determine antibody isotype activity *in vivo*

It has been long appreciated that different antibody isotypes have a different cytotoxic potential *in vivo* [14]. For example, the mouse IgG_{2a} and IgG_{2b} isotypes are more efficient in clearing bacterial or viral infections, in killing tumor cells, or in depleting platelets or red blood cells than their IgG₁ or IgG₃ counterparts [17,18*,19**,34–38,39*,40–42]. It has been speculated that this might be because of the co-engagement of several effector pathways, such as the complement and the FcR systems in parallel.

Several studies have challenged this view recently by demonstrating that the activity of these most potent IgG isotypes is not impaired in mice deficient in components of the classical complement pathway [16,18*,19**,43–45]. By contrast, antibody isotype activity was abrogated in mice deficient in the common signaling subunit of all activating FcRs [14,16,17,19**,43–49] or when antibodies with mutations that abrogate FcR binding were used [20,50]. More importantly, by blocking specific activating FcRs it was demonstrated that individual antibody isotypes mediated their effector functions by triggering selective activating FcRs [18*,19**]. Whereas IgG₁ activity was only determined by co-engagement of FcγRIII and FcγRIIB, IgG_{2a} and IgG_{2b} activity was mostly dependent on the FcγRIV–FcγRIIB pair [18*,19**,39*]. Depending on the tumor load and the amount of therapeutic antibody, the high affinity FcγRI contributed to IgG_{2a} activity [21].

Recently surface plasmon resonance (SPR) analysis was used to measure the actual affinity of the monomeric antibody isotypes for their respective activating and inhibitory FcR pairs [19**,39*]. Using these values, an A/I ratio was assigned to each antibody isotype by dividing the affinity for the relevant activating FcR by the affinity for the inhibitory FcR. Intriguingly, the A/I ratios for the different antibody isotypes closely matched the observed antibody activity *in vivo*. Thus, IgG₁ antibodies have an A/I ratio smaller than one, which suggests that the activity of this isotype is strongly influenced by the inhibitory FcγRIIB. By contrast, IgG_{2a} and IgG_{2b} antibodies have A/I ratios of up to 70, therefore are less-regulated by FcγRIIB. Indeed, in models of platelet depletion or tumor cell killing by IgG switch variants it was shown that deletion of FcγRIIB impacted most strongly on IgG₁ activity [19**,20]. Another important conclusion that can be drawn from these studies is that the role of NK cells in these *in vivo* ADCC reactions is probably limited, because NK cells express neither the inhibitory FcγRIIB nor the activating FcγRIV. Current evidence points to macrophages as the most likely

effector cells, which co-express the relevant FcRs to explain the *in vivo* results.

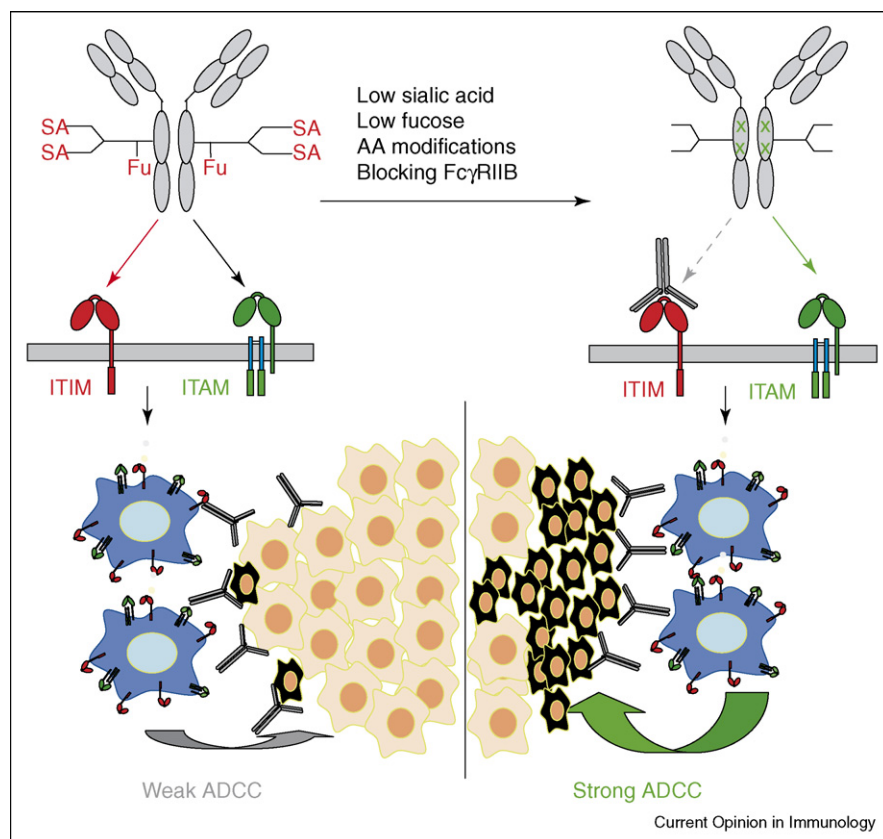
Regarding human IgG isotypes, IgG₁ and IgG₃ have a stronger interaction with FcRs than IgG₂ or IgG₄. In addition, there are allelic variants of activating Fc-receptors that will significantly change the affinity for certain antibody isotypes. Thus, the FcγRIIIA allele that contains a valine in position 158 (FcγRIIIA^{158V}) has a higher affinity for IgG₁ and IgG₃ than the receptor that has phenylalanine (FcγRIIIA^{158F}) at that position, thereby increasing the A/I ratio of these antibody isotypes. Similarly, FcγRIIA^{131H} (which has a histidine at position 131) has a higher affinity for IgG₂ than the 131R allele (which contains an arginine) [11,51]. If FcR-mediated ADCC also contributes to the *in vivo* activity of cytotoxic antibodies in humans, patients undergoing antibody therapy using the high-affinity alleles should respond better. Consistent with this, lymphoma patients treated with an anti-CD20 antibody had a significantly better response to antibody therapy if they were homozygous for the high-affinity allele of FcγRIIIA [25,26]. Moreover, lymphoma patients vaccinated with an anti-idiotypic vaccine responded better if they carried the FcγRIIIA^{158V} allele [24].

Optimizing antibody Fc receptor interactions: taking off the brakes

Given the importance of FcγRIIB as a negative regulator of DC maturation and the activity of cytotoxic antibodies, an obvious strategy to obtain a stronger anti-tumor immune response and to produce antibodies that have higher cytotoxic activity would be to circumvent FcγRIIB activity *in vivo* (Figure 1). Evidence that this might indeed be possible comes from several recent studies.

First, blocking or deleting the inhibitory FcR *in vivo* or *in vitro* on DCs results in enhanced anti-tumor immune responses [15,30*,32*]. Second, several groups have succeeded in generating antibody variants that have selectively increased affinity for the activating FcγRIIIA. To generate these antibody variants, two strategies have been pursued. In the first, antibodies with selected mutations that change the amino acid sequence have been generated either by alanine scanning mutagenesis [52] or by computer-based prediction methods [53*]. With this approach, antibody variants that have increased affinity for the human FcγRIIIA and lower binding to FcγRIIB were identified, which had increased activity *in vitro* and in nude mice injected with human tumor cell lines. In the second, antibody glycosylation variants have been identified that have similar features. The removal of fucose in the sugar moiety attached to Asn-297 resulted in increased cytotoxic activity of mouse and all human IgG antibody isotypes [19**,54–57]. Interestingly, the lack of fucose selectively increased binding to human FcγRIIIA and mouse FcγRIV by 10–50-fold, whereas binding to the other activating FcRs was not

Figure 1



Optimizing antibody-FcR interactions. An important strategy to obtain a stronger anti-tumor ADCC reaction is to optimize the interaction of the antibody Fc-portion with activating FcRs. This can be achieved by blocking the inhibitory FcγRIIB *in vivo* with monoclonal antibodies, or by modifying the primary amino acid sequence (amino acid [AA] modifications) or the sugar moiety of the antibody to obtain selective or enhanced binding to activating FcRs.

changed. At the same time the affinity for the inhibitory receptor did not increase for human FcγRIIB. More recently it was suggested that the reason for this selective increase in affinity for FcγRIIA is the presence of the sugar moiety that is attached to Asn-162 in this receptor [58^{*}]. Deletion of the sugar side-chain abrogated the higher affinity for antibodies that lack fucose. In addition to fucose, it has been demonstrated that the absence of terminal sialic acid residues in the antibody sugar side-chain results in antibodies that have higher cytotoxic activity in humans and mice [59^{••},60]. Whereas most cell lines that are currently used for antibody production do not add significant amounts of terminal sialic acid residues, they do add high levels of fucose. It should be stressed, however, that the lack of fucose will only improve therapeutic activity if FcγRIIA and not FcγRIIB is the dominant FcR that triggers effector cell activation. Thus, careful evaluation of current clinical studies with respect to activating FcR alleles and identification of the relevant activating FcRs is an important issue to be addressed in the future.

Conclusions

Despite promising results in the use of antibodies for therapy of select malignancies, many antibody-based therapeutic interventions have only yielded partial or minor responses. It has become clear that the cytotoxic activity of antibodies *in vivo* is mainly determined by the co-engagement of activating and inhibitory FcRs. With the new insights gained into the factors that influence the interaction of antibodies with FcRs and the role that the inhibitory receptor plays in regulating DC activity, it should now be possible to generate antibodies or tumor vaccines that have improved activity. In the case of anti-tumor vaccines, the use of immune-complexes and the block of the inhibitory FcγRIIB might greatly enhance vaccine activity. Regarding cytotoxic antibodies, the use of antibody glycovariants that have a low amount of sialic acid and fucose and phenotyping of patients with respect to their activating FcR alleles might improve the therapeutic effects. Thus, optimized active or passive immunotherapy, combined with less toxic chemotherapy and if possible surgical removal of solid

tumors, holds great promise for the future therapy of cancer.

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