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New paradigms for functional HIV-specific non-neutralizing antibodies

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Abstract

Purpose of Review—While a large number of novel broadly neutralizing antibodies has been recently described, the induction of such antibodies via vaccination has proven difficult. By contrast, non-neutralizing antibodies arise early during infection and have been repeatedly associated with both protection from infection and disease progression.

Recent findings—We are beginning to gain new insights into the broader landscape of antiviral mechanisms that non-neutralizing antibodies may harness to fight HIV, providing an unprecedented breadth of approaches by which HIV can be blocked and contained.

Summary—In this review we summarize the characteristics of non-neutralizing antibodies, their role in HIV infection, and new paradigm-shifting functions that may be exploited by next generation vaccine approaches aimed at blocking HIV infection.

Keywords

ADCC; HIV; antibodies

Introduction

Recent results from the RV144 vaccine trial [1] and Ad26 vaccination in non-human primates [2] argue that some degree of protection from infection can be achieved in the absence of neutralizing antibodies or cytotoxic T cell responses. In RV144, qualitative differences in the vaccine-induced humoral response, associated with low-levels of IgA and high levels of antibody-dependent cellular cytotoxicity (ADCC), may have contributed to the limited protection observed [3]. This protection, observed in only 30% of vaccinees, waned rapidly after the final boost. Interestingly, compared with other vaccine trials, RV144 induced lower levels of HIV-specific antibody titers, suggesting that (a) increased titers do not necessarily correlate with better protection, (b) qualitative features of non-neutralizing

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antibodies may be a more critical measure of protective efficacy, and (c) vaccine strategies able to increase both the titer and functional potency of antibodies may provide enhanced protection from infection. In this review we will summarize the characteristics of non-neutralizing antibodies, their role in HIV infection, and new paradigm-shifting functions that may be exploited by next generation vaccine approaches aimed at blocking HIV infection.

Two ends of an antibody

Antibodies consist of two antigen-binding domains (variable domains, Fv) and a constant domain (constant domain, Fc). The double antigen-binding domain structure provides high-avidity tethering of the target antigen. Conversely, the Fc provides instructions to the immune system on how the target antigen should be destroyed. While the Fv and Fc domains have largely separable functions, changes in both these ends of the antibody are tightly regulated by a single enzyme, activation-induced cytidine deaminase (AID) [4]. While AID drives somatic hypermutation of the Fv domain, allowing for affinity maturation and diversification of the antibody, AID also drives class switch recombination (CSR) of the Fc domain. Despite its name, the Fc domain varies significantly both in protein sequence, depending on the Ab isotype, and glycosylation, both of which are involved in determining the functional activity of the antibody.

Isotype selection

Upon B cell activation, a naïve B cell can functionally tune its Ab response from the production of IgM to the production of IgG, IgE, or IgA through CSR [5]. As Ab isotypes and subclasses are specialized to have different functional activities and to respond to and eliminate distinct types of pathogens, isotype switching is a highly regulated process largely controlled by cytokines and T helper–provided signals [6] received during B cell priming. For example, IL-4 selectively induces IgG4 and IgE [7, 8], whereas IL-10 and IL-21 induce IgG1 and IgG3 [9]. Furthermore, IgG subclass selection varies by infection and is related both to the inflammatory state induced by the pathogen and the location of the pathogen (intra- vs. extracellular) [10]. Ultimately, differences in functionality are often linked to the Fc affinity for Fcγ receptors (FcγRs) and complement [11, 12] (Figure 1). In general with IgG3 has the highest affinity for FcγRs followed by IgG1 and then IgG2 and IgG4 (Figure 2a and b). Importantly, specific Ab isotypes have been associated with protective humoral immune responses in various diseases, such as malaria where IgG3 levels predict parasite control [10].

Within the human heavy chain locus (*IGH@)*, the IgG3 constant region is the first subtype [13]((Figure 1), suggesting that it is the first IgG subclass that may be selected during an acute immune response. Moreover, the *IGH* subclass sequence follows the FcγR binding affinity, and functional potency, of the subclasses (IgG3>IgG1>IgG2>IgG4), suggesting that IgG subclass selection is evolutionarily programmed to allow for the production of the most functional antibodies early in the immune response, followed by less inflammatory Ab subclasses in subsequent waves. Along these lines, HIV-specific IgG3 antibodies have been proposed as markers of early HIV infection due to their enrichment during the first few weeks of infection [14]. However, IgG3 antibody levels decline rapidly following acute infection and are replaced by a strong IgG1 response in the chronic phase of the disease

[15]. Additionally, spontaneous control of HIV infection (Controllers) in the absence of antiretroviral therapy is associated with the induction of high levels of p24- and gp120-specific IgG1 and the maintenance of gp120-specific IgG3 antibodies [16], whereas progression to AIDS has been associated with increasing levels HIV-specific IgG4 antibodies [17]. Together, these data suggest that spontaneous control is associated with the induction of functionally enhanced antibody isotypes (IgG1 and IgG3), whereas progression to disease is associated with the selection of poorly functional antibodies [16].

Functional tuning by glycosylation

In addition to isotype-associated differences in activity, antibody glycosylation profoundly modulates antibody function. For example, IgG antibodies lacking fucose exhibit increased cytotoxic activity [18–23] as the result of increased affinity to the activating FcγR3A [18] (Figure 2c and d). Therapeutic antibodies optimized to contain low levels of fucose residues are more effective in clearing tumor cells than their fucosylated counterparts [24, 25]. Conversely, increased terminal sialylation imbues antibodies with anti-inflammatory properties [26], and the bioactive fraction of IVIG that is used to treat many inflammatory and autoimmune conditions is thought to be mediated by a fraction of sialylated antibodies within the larger polyclonal pool [27].

In the context of HIV infection, recent data suggest that significant changes occur in both bulk and HIV-specific antibody glycosylation [28, 29]. Specifically, HIV infection is associated with a significant increase in agalactosylated antibodies [29], which are highly inflammatory and typically enriched in patients with inflammatory conditions such as rheumatoid arthritis [27]. Interestingly, the most dramatic enrichment of agalactosylated antibodies was observed among Controllers, suggesting that while these individuals exhibit less overall immune activation [28], B cells in Controllers continue to receive inflammatory signals that drive the secretion of antibodies with glycan structures associated with chronic inflammation. Intriguingly, the glycosylation of HIV-specific antibodies was further skewed towards a more highly inflammatory glycoform, containing less galactose, fucose and sialic acid [28]. The most profound enrichment of afucosylated, agalactosylated antibodies was observed among HIV-specific antibodies in Controllers, who also exhibit enhanced ADCC activity, suggesting that the B cells in these individuals were specifically programmed to make highly functional antibodies that may contribute to persistent viral control. Yet, to date, little is known about the mechanism by which B cells program antibody glycosylation, even though it may offer new alternatives by which antibody effector functions may be actively recruited through vaccination.

Recruitment of innate immune cells through FcγRs

Beyond neutralization, antibodies mediate a broad array of functions including ADCC, antibody-mediated cellular phagocytosis (ADCP), complement-mediated killing (Figure 3A), and antibody-dependent cell-mediated virus inhibition (ADCVI). On a cellular level, the biological functions of IgG are mediated by interactions between Fc and FcγRs, which are found on all innate immune cells.

Six major FcγRs have been identified in humans: FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa, and FcγRIIIb [30] (Figure 1). With the exception of FcγRIIb, which inhibits activation, all other FcγRs are activating receptors, signaling either directly through immune tyrosine activating motifs (ITAMs) in their cytoplasmic tail or through interactions with separate ITAM-containing proteins [30–32]. The genes encoding FcγRIIa and FcγRIIIa contain single nucleotide polymorphisms that result in receptors that differ in their binding affinities for different IgG subclasses [33]. NK cells generally express FcγRIIIa and, in some individuals, FcγRIIc. Other cell types likely to be involved in preventing or controlling HIV infection, such as monocytes and macrophages may express all of the FcγRs with the exception of FcγRIIIb, which is primarily expressed on neutrophils and eosinophils. It should be emphasized that expression of FcγRs on any given cell type may differ according to activation state, cytokine milieu, and location.

Over the past three decades, significant work has pointed toward a role for Fc-FcγR interactions in both infection and vaccine-mediated control of HIV/SIV in vitro and in vivo. While several studies have explored the potential role of FcγR-mediated antibody functions in preventing HIV infection [34–37], only one study directly implicated these functions in prevention [38]. In this study, infusion of IgG1 b12, a monoclonal antibody (mAb) that engages FcγRs, protected macaques from vaginal exposure to SHIV162p3, and this protection was partially lost when the mAb was mutated to abrogate FcγR binding. Although this study directly demonstrated the importance of Fc-FcγR interactions in augmenting protection, the precise antibody function(s) responsible remain unknown. Thus, while Fc-FcγR interactions have been directly identified as a mechanism of protection in a rhesus macaque model of vaginal SHIV infection, the contribution of any single FcγR-mediated antibody function to protective immunity remains unknown.

Function depends on multimerization

While passive transfer strategies using non-neutralizing, functional antibodies alone have failed to definitively demonstrate protection from HIV/SIV infection [35–37, 39], the biology underlying the protective activity of neutralizing and non-neutralizing antibodies is likely different. Therefore, comparisons of passively administered single mAbs may not be informative. Unlike neutralizing antibodies that block a limited number of viral epitopes on the surface of a virus [40], non-neutralizing antibodies must form avid immune complexes that are able to recruit the low-affinity receptors or innate immune proteins necessary for their function [12]. Due to the limited amount of envelope on the surface of HIV [41], non-neutralizing antibodies may have a limited impact on the virus itself; however, because HIV assembles in lipid rafts [42], the concentration of HIV antigens on the surface of cells may allow non-neutralizing antibodies to bind and cluster Fc receptors on effector cells. However, polyclonal non-neutralizing antibodies simultaneously targeting several epitopes on a single envelope may have a better opportunity to form large avid immune complexes than mAbs. Therefore, while previous passive transfer efforts of single, non-neutralizing antibodies exhibited limited success [34], it is likely that cocktails of high-affinity, Fc-enhanced non-neutralizing antibodies that could simultaneously decorate the surface of HIV envelope could mediate sterilizing protection from infection.

Individual antibody functions and their role in HIV infection

Antibodies are able to recruit a spectrum of different antiviral functions including:

ADCC. ADCC occurs when an antibody forms a bridge between antigen-expressing target cells and FcγR-expressing effector cells; the cross-linking of FcγRs on the effector cells results in target cell death. While several studies have explored the role of vaccine-elicited ADCC-inducing antibodies in preventing HIV, SIV, or SHIV infection, there has been no direct demonstration of a protective effect. However, as mentioned above, in secondary analyses of the RV144 data, the combination of low vaccine-elicited plasma anti-gp120 IgA levels and high ADCC antibody activity was associated with a lower infection rate [3]. Furthermore, in macaques, vaccine-induced ADCC activity has been correlated with infection outcomes [37, 39, 43]. For example, a very strong trend towards protection from infection was observed following heterologous prime-boost vaccinated macaques exposed to repetitive SIVmac251 intrarectal challenge[2]. Likewise, ADCC-inducing responses elicited by live, attenuated SIVΔnef correlated with protection from intravenous or vaginal challenge with SIVmac251 [43]. Additionally, ADCC-mediating IgG in cervico-vaginal secretions has been elicited by vaccination of macaques, and it is possible that such ADCC activity plays a role in protection against SHIV162p3 vaginal challenge [44]. Other studies have found either an inverse correlation between ADCC activity prior to challenge and acute or chronic viremia levels or a direct correlation between ADCC activity and the number of challenges required for infection [35, 37, 45], suggesting that these responses may have post-acquisition antiviral effects.

However, no study to date has directly demonstrated that an ADCC-mediating antibody is responsible for preventing HIV/SIV infection. Moreover, ADCC-inducing responses following MVA vaccination were not associated with the risk of SIVsmE660 infection after rectal challenge [46]. Furthermore, a recent mAb passive infusion study has now called into question whether NK cell–mediated ADCC activity is involved in protection against mucosal SHIV challenge [47]. In this study, animals were infused with either IgG1 b12 or a non-fucosylated version of the mAb, designed to increase FcγRIIIa binding and NK cell-mediated ADCC activity. Although a small number of animals were studied, there was no difference in protection against repeated low-dose vaginal SHIV challenge for the two forms of the mAb, suggesting that other FcγR interactions could be more critical in driving antiviral control and clearance, particularly at mucosal surfaces.

Phagocytosis. Phagocytes, such as monocytes, macrophages, and dendritic cells, can internalize antibody-coated virus or antibody-coated infected cells. Like ADCC-inducing antibodies, it is likely that phagocytosis of HIV-1 immune complexes augments the anti-viral activity of neutralizing or non-neutralizing antibodies. However, interestingly, depending on the inflammatory state, phagocytes, and not CD16+ NK cells, are more abundant at mucosal membranes where the large majority of infections occur [48, 49]. Therefore, it is possible that antibodies able to mediate the rapid clearance of the virus or virally infected cells could have a beneficial role in preventing HIV infection [50–53]. Recently, antibodies elicited in healthy human volunteers by an Ad26 vaccine expressing clade A HIV-1 Env were shown to mediate phagocytosis of gp120-coated beads [54].

As with other FcγR-mediated antibody functions, IgG subclass is a determinant of phagocytosis, and it is likely that FcγRIIa or FcγRIIIa genotypes and differential binding to different FcγRs impact phagocytosis in vivo [55, 56]. Subjects possessing the FcγRIIa RR genotype demonstrated reduced internalization of HIV-1 immune complexes than subjects with the RH or HH genotypes, and this reduced phagocytic activity was linked to more a rapid decline in CD4 cells, suggesting that phagocytic clearance of virus and/or virally infected cells plays a role in post-acquisition disease control [57]. Similarly, quantitative differences in the capacity of antibodies to drive phagocytosis were recently associated with qualitative differences in FcγR binding profiles. Specifically, antibodies from Controllers exhibited increased phagocytic activity that was associated with the enhanced capacity of both bulk plasma and HIV-specific IgG to engage FcγRIIa and not FcγR2IIb [56], this altered FcγR binding profile was associated with changes in antibody glycosylation, potentially pointing to new antibody glycoforms that may allow antibodies to prevent infection more robustly at mucosal membranes, where phagocytic cells are more abundant. Moreover, as mentioned above, although the non-fucosylated b12 did not exhibit enhanced protection from infection, it is plausible that modifications, such as those elicited by Controllers that enhance phagocytosis, may offer an alternate mechanism by which passively transferred mAbs may mediate enhanced protection from infection via the utilization of the phagocytic cells that abundantly line mucosal membranes.

ADCVI. ADCVI is not an antibody function itself; rather, it is a measure of net antiviral activity provided by antibody in combination with FcγR-bearing effector cells. The components contributing to the antiviral effect measured in ADCVI assays include ADCC, the production of β chemokines, and phagocytosis [58]. ADCVI antibody activity has been shown to rise early and to correlate with the fall in viremia observed during acute infection [58]. Moreover, ADCVI activity is enriched in Controllers and is associated with the preferential accumulation of agalactosylated antibodies [28]. As with ADCC, ADCVI-inducing antibodies elicited by vaccination or present in passively infused reagents correlate with protection or reductions in viral load in monkeys after various types of challenge with SIV or SHIV [35–38, 59, 60]. In humans, increased ADCVI activity elicited by a recombinant gp120 vaccine in the Vax004 trial was associated with lower infection rates [61]. However, the ADCVI response to primary infection with HIV-1 did not correlate with the risk of superinfection in a cohort of women in Kenya [62].

New mechanisms of protection by non-neutralizing antibodies

Antibodies are present at the majority of the mucosal surfaces exposed to HIV during sexual transmission. In addition to the presence of antibodies within the fluids associated with these barriers, the antibodies may also be able to interact with the mucosal and/or mucus components themselves (Figure 3b). The first barrier encountered by HIV at mucosal epithelial barriers is composed of secreted mucins, which coat the mucosal surfaces of the colorectal tract and the female reproductive tract. Because most HIV infections are caused by a single transmitted founder virus [63], the majority of viruses must get stuck or cleared at the mucosal surface. Therefore, recent efforts have been focused on dissecting the potential properties of antibodies that promote more effective interactions with mucins to

trap the virus, a mechanism that would effectively neutralize the virus by preventing if from reaching potential target cells.

Although the interaction of antibodies and mucus has not been well defined, there have been a number of recent paper that suggest that antibodies and mucus can function together to reduce the pathogenic consequences of microbes. For example, mucus in combination with secretory IgA provided optimal protection from toxin-induced injury in a tissue culture model [64]. These results echo studies published a decade ago suggesting that the secretory component of IgA mediates the protective effects of mucus-bound IgA [65]. Similarly, the deletion of MyD88 in mice decreased mucin 2 and secretory IgA production, leading to compromised antibacterial immunity [66]. IgG may also interact comparably with mucus. For example, the presence of IgG antibodies to *Helicobacter pylori* in cervical mucus has been associated with infertility [67].

Antibodies may also interact with the mucosal surfaces, potentially retaining HIV at the interface of the mucosal epithelium and the lumen. Trapped at the surface, HIV would not be able to penetrate the mucosal epithelial barrier. These interactions could take place between cell-associated mucins (e.g., MUC1, MUC4, and MUC16) and the Fc portions of antibodies. Antibodies have also been observed to accumulate in the superficial aspects of the squamous epithelium of the ectocervix [68, 69]. However, while these observations suggest another level of potential protection provided by non-neutralizing antibodies at the mucosal epithelium, these interactions need to be better defined.

Summary

While a large number of novel broadly neutralizing antibodies has been recently described [70–72] the induction of such antibodies via vaccination has proven difficult [73]. By contrast, non-neutralizing antibodies arise early during infection and have been repeatedly associated with both protection from infection and disease progression. Moreover, we are beginning to gain new insights into the broader landscape of antiviral mechanisms that non-neutralizing antibodies may harness to fight HIV, providing an unprecedented breadth of approaches by which HIV can be blocked and contained. Together with new insights into the biophysical properties that allow antibodies to recruit specific innate effector functions, significant progress is being made in our understanding of the humoral parameters that might be manipulated via vaccination to improve antiviral control of HIV.

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Key Bullet Points

- Beyond neutralization, antibodies are able to recruit a wide array of antiviral activities against HIV through the innate immune system.
- Antiviral activity of non-neutralizing antibodies is programmed through selective antibody subclass selection and antibody glycosylation.
- Beyond traditional antiviral effector mechanisms, antibodies may also trap virus at mucosal membranes thereby preventing HIV infection.

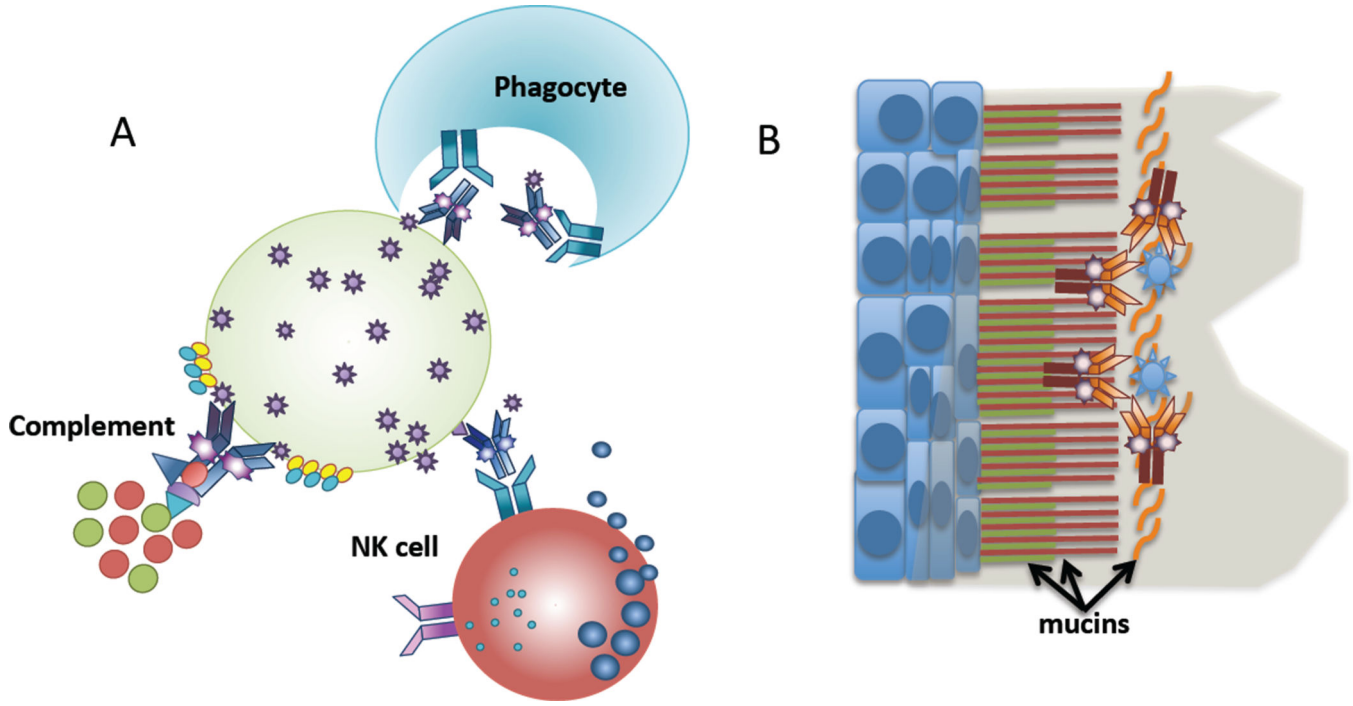


Figure 1. Antiviral properties of non-neutralizing HIV-specific antibodies

(A) HIV-specific antibodies act as beacons aimed at attracting innate immune cells or complement to kill infected cells through various different mechanisms including, but not limited to, ADCC, ADCP, and complement activation. (B) Newer data suggest that HIV-specific antibodies may also act to trap the virus above mucosal membranes, through the interaction with mucus components, preventing infection, and therefore potentially offering a novel mechanism by which vaccine induced antibodies may prevent infection.

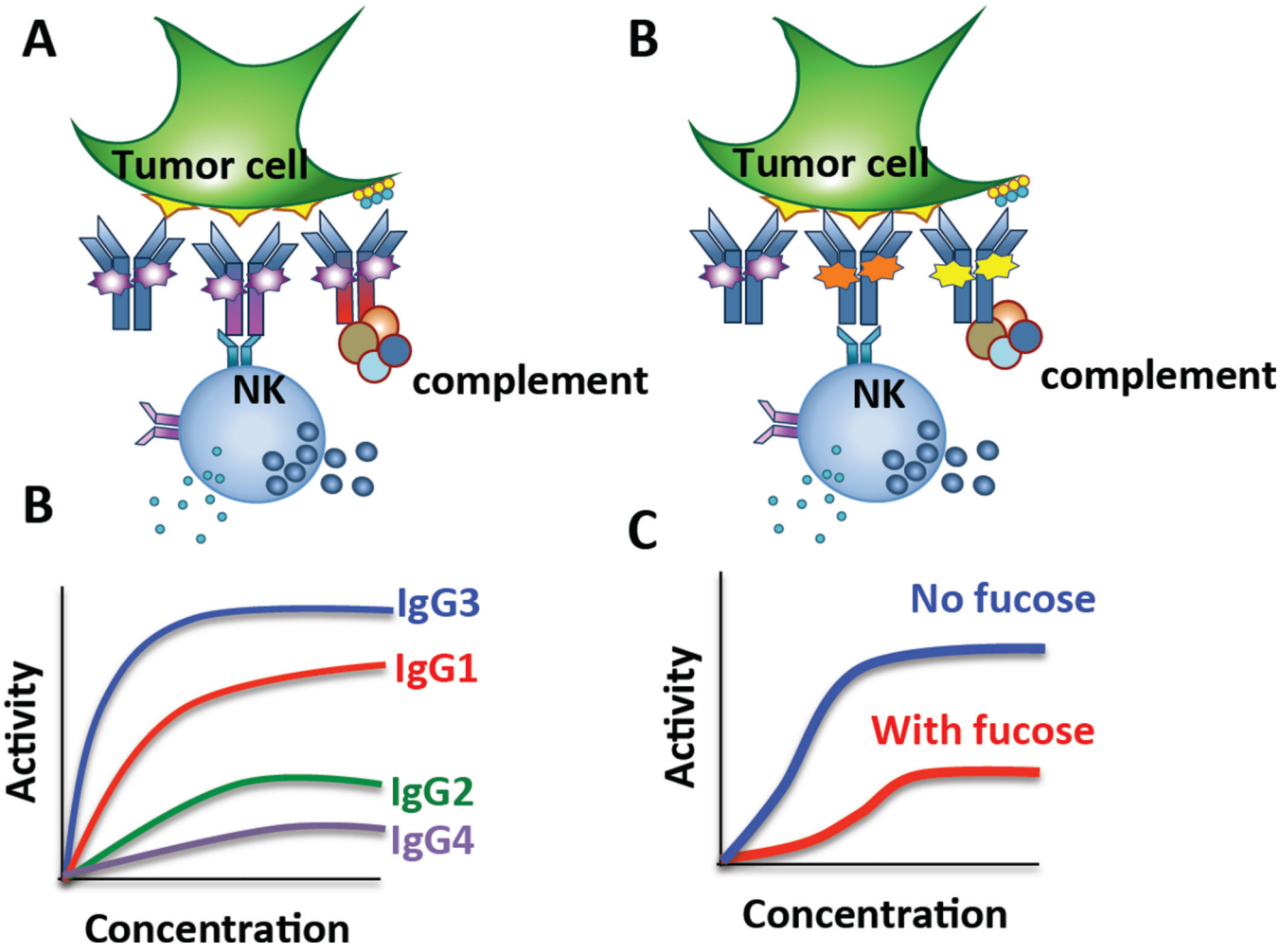
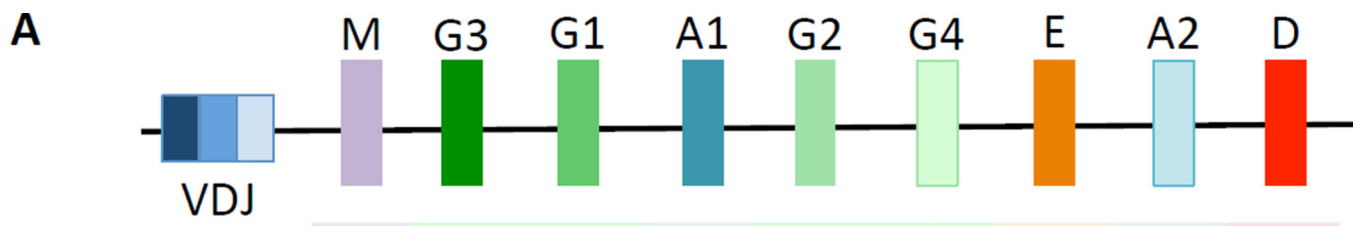


Figure 2. Antibody functionality is tuned through isotype selection and altered glycosylation

(A) The generation of monoclonal therapeutics on difference constant domain isotypes can drive significantly different functions, and (B) induce hierarchically different levels of tumor mediated killing in the presence of NK cells. (C) Similarly, simply altering the glycan structure attached to the same antibody heavy chain car lead to different antibody effector functions, (D) where the removal of a single sugar, fucose, has been shown to improve NK cell mediated tumor clearance dramatically.



B

	M	G3	G1	A1	G2	G4	E	A2	D
Complement activation	+++	+++	++	+	+	-	-	+	-
Phagocytosis	-	+++	++	-	-	+/-	-	-	-
ADCC	-	+++	++	-	-	-	-	-	-
Mast Cell Activation	-	+	+	-	-	-	+++	-	++
FcγR1	-	+++	+++	-	+	+	-	-	-
FcγR2a/b	-	+	+	-	+++	+/-	-	-	-
FcγR3a/b	-	+++	++	-	+/-	-	-	-	-
C1q	+++	+++	++	+	+	-	-	+	-

Figure 3. The *IGH* locus and associated function

(A) depicts a rearranged variable domain (VDJ) (left) followed by the intact sequence of the different constant domain isotypes. (B) summarizes the breadth of functions and protein interaction potencies for each of the antibody constant domain isotypes.