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Opportunities to exploit non-neutralizing HIV-specific antibody activity

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Abstract

Antibodies act as a nexus between innate and adaptive immunity: they provide a means to engage a spectrum of innate immune effector cells in order to clear viral particles and infected cells, and prime antigen presentation. This functional landscape is remarkably complex, and depends on antibody isotype, subclass, and glycosylation; the expression levels and patterns of a suite of Fc receptors with both complementary and opposing activities; and a host of innate immune cells capable of differential responses to opsonized particles and present at different sites. In vivo, even neutralizing antibodies rely on their ability to act as molecular beacons and recruit innate immune effector cells in order to provide protection, and results from both human and macaque studies have implicated these effector functions in vaccine-mediated protection. Thus, while enhancing effector function is a tractable handle for potentiating antibody-mediated protection from HIV infection, success will depend critically on leveraging understanding of the means by which antibodies with specific functional profiles could be elicited, which effector functions could provide optimal protection, and perhaps most critically, how to efficiently recruit the innate effector cells present at sites of infection.

Keywords

antibody; IgG; effector function; ADCC; phagocytosis; FcγR; HIV; vaccine; passive transfer

1. Introduction

The ability to tune the antibody constant domain to better serve as a molecular beacon in recruiting the potent protective functions of a host of innate immune effector cells via interactions with Fc binding receptors presents an opportunity to both augment the protection afforded by neutralizing antibodies, and impart non-neutralizing antibodies with protective activity. Indeed, evidence of the vital importance of antibody effector function has been accumulating in both the setting of monoclonal antibody transfer and vaccination(1). Thus, optimization of antibody effector function represents a complementary approach to improving antigen-binding and neutralization activity, and could enhance antibody-mediated protection from HIV-1 infection. Yet, just as the challenges associated with eliciting

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neutralizing antibodies have proven to be substantial, much work remains to be done in order to effectively amplify the protective activity afforded by the antibody Fc domain: the complex landscape of antigen binding and neutralization is mirrored by the complexity of diverse effector cell types, Fc receptors, and Fc domain isotype, subclass, and glycosylation patterns, each of which can have a profound effect on the fate of opsonized virus or cells. Thus, in order to most productively exploit these antibody activities, the innate immune cells present at sites of transmission and infection, the most robust and protective effector functions, and the antibody features which would most effectively drive these protective innate immune responses, must be identified and characterized (Figure 1).

Numerous reports have highlighted the possible importance of antibody Fc-effector functions in HIV acquisition and progression. In macaque models, antibody effector function has been found to be critical to the protection achieved by a broadly neutralizing antibody(2), to correlate with delayed progression to AIDS(3), and antibody-dependent cellular cytotoxicity (ADCC) activity driven by vaccine-induced antibodies has been found to correlate with protection from infection or reduced viremia(4–9). In humans, ADCC-inducing antibodies are present during acute infection, elevated in subjects who spontaneously control viral replication, and correlate with reduced rate of progression(10–18). Furthermore, in the setting of vaccination, antibodies with ADCC activity, or more broadly, antibody-dependent cellular viral inhibition (ADCVI) activity correlated with reduced risk of infection in the VAX004 trial, were readily induced in the RV144 trial, and in the absence of IgA, correlated with reduced risk of infection(19–21).

Collectively, this data suggests that there are significant differences in the ability of antibodies from HIV+ and vaccinated subjects to recruit the cytotoxic functions of innate immune effector cells. These differences may have relevance to both reduced rate of disease progression and reduced risk of infection, suggesting that enhancing antibody effector function may offer a tractable handle for protection mediated by vaccination or passively transferred antibodies. However, in order to fully leverage this protective activity, it will be important to understand the settings in which antibodies with potent ADCVI activity are naturally induced, and the antibody features, Fc receptors, and innate immune cells associated with protective effector functions.

2. Antibodies

2.1 Combinatorial diversity at both ends

Antibodies represent an impressive feat of molecular evolution—possessing the ability to adapt to recognize virtually any target and to drive the disposition of that target along a number of diverse paths. Neither function is static: rather, signals supplied from helper T cells and antigen-presenting cells (APCs) continually steer the evolution of both antigen binding and Fc domain attributes resulting in the generation of a polyclonal response with staggering combinatorial functional diversity. These changes are made both at the genetic level, where deletions, insertions, and mutation occur(22), and post-translationally, where variant glycosylation is observed(23). Each of these modifications can act to diversify Fv or Fc function.

The variable region, at the tip of both the heavy and the light chains, is unique among B cells, however it is identical for all Abs secreted by a single B cell clone. The variable region of each Ab is extremely heterogeneous within a single individual, conferring the capacity for each antibody to bind a specific antigen. This variability is initially obtained via combinatorial selection of small genetic cassettes (VDJ region recombination)(22), and can subsequently be compounded by excision and insertion of nucleotides, and a process called somatic hypermutation which leads to the incorporation of mutations, creating further diversity(24).

This diversity of Fv sequence and function is complemented by diversity in the antibody constant, or Fc domain. There are 5 classes, or isotypes, of antibodies: IgA, IgD, IgG, IgE, and IgM. They differ in the binding valency they achieve, from monovalent (naturally bispecific IgG4) to decavalent (IgM). They vary by their distribution and concentration, IgG being most common in serum, and IgA and IgM more abundant at mucosal surfaces and in secretions. For some isotypes, functional activity is also modulated among different subclasses(22), genetic allotypes(25), and by post-translational incorporation of specific glycans at conserved glycosylation sites(23).

2.2 Class-switch selection: excision and revision

The Ab isotype and subclass of a given B cell changes following B cell activation. Immature, antigen-inexperienced B cells express the IgM isotype bound to the cell surface(26). Following B cell maturation, a B cell gains the capacity to express both IgM and IgD, which form the B cell receptor (BCR), and permit antigen responsiveness. BCR engagement results in internalization of foreign antigen(27), which is then processed and expressed in MHC class II molecules on the surface of the B cell(28). Antigen-specific CD4⁺ T cells, which recognize peptide-MHC class II complexes on the surface of these activated B cells are able to then release large quantities of cytokines and provide co-stimulatory signals that drive further activation of the presenting B cell(29). This CD4⁺ T cell cross-talk leads to the induction of B cell proliferation, class switching, and consequently somatic hypermutation – which allows B cell clones to generate antibodies with higher affinity for the antigen(30). B cell proliferation results in the generation of a large pool of antigen-specific, Ab-secreting plasma B cells, and a small number of memory B cells that persist after the target antigen is cleared(27). In some situations, B cells may become activated and secrete Abs in a T-cell independent manner as well(31). However in most cases, B cell activation, priming, and memory B cell generation is tightly regulated by antigen-specific helper CD4⁺ T cells.

Following proliferation, some plasma B cells undergo isotype switching, a mechanism that induces Abs to change from IgM or IgD to the other isotypes including IgE, IgA, or IgG. IgM tends to have a lower affinity for the antigen compared to the other isotypes due to the fact that it is the first antibody secreted during the primary response and has therefore undergone limited somatic hypermutation. To overcome this lower affinity, IgM is a pentamer, which increases the avidity of the antibody for the antigen, thereby increasing binding. However, due to its large structure, IgM has a limited ability to penetrate tissues, therefore smaller, higher affinity isotypes are subsequently generated by class switching,

mainly to the IgG isotype. The IgG isotype can be further divided into 4 subclasses, (Table 1(22, 32)), with dramatically variant biophysical properties, serum prevalences, and ability to interact with innate immune cells and receptors. Similarly, IgA may also be secreted following B cell class switching and is involved in mediating mucosal immunity. IgA likewise exists in multiple forms, including IgA1 and IgA2, which differ in disulfide bond structure and in prevalence among different secretions/tissues, and between serum and secretory IgA (sIgA), which is multimeric and gains the capacity to interact with the polymeric Ig receptor. Alternatively, IgE can also be secreted in the context of an allergic reaction, and is predominantly involved in the activation of mast cells(22). These class-switch selections can be made multiple times in repeated germinal center reactions. However, because the gene segments encoding upstream constant domains are excised from the genome when a class-switch decision is made, the opportunity to revise selection in subsequent germinal center reactions is limited to remaining downstream segments at the individual B cell level, but can vary at the population level.

2.3 Programming Fc activity via vaccination

That class switching(33) and Fc glycosylation(34) are regulated by signals B cells receive from CD4+ T cells, APCs and other inflammatory cues, offers the potential to design vaccines that elicit specific functional antibody profiles via rationally steering isotype, subclass, and glycosylation. While general associations have become apparent: IgG4 is associated with chronic antigen stimulation, IgG2 responses are often mounted against polysaccharides, and IgE predominates in response to helminth infections, the specific molecular mechanisms underlying these preferences have not been fully elucidated. Accordingly, further studies aimed at characterizing the signaling pathways that dictate class switch choice and glycosylation offer the opportunity for basic biology to feed into the development of designer vaccines with potentiated antibody activity.

Class-switch selection has been well-characterized in the setting of natural HIV infection, which is marked by generalized systemic immune activation(35). This hyperactivation results in dramatic polyclonal IgG1-biased hypergammaglobulinemia, where only a fraction of the Abs are HIV-specific(36). The virus itself may drive the activation of B cells, and increased expression of B cell activating factor (BAFF) has been described in chronic HIV-1 infection(31). Thus, chronic activation likely contributes to phenotypic and functional alterations in the B cell compartment, driving elevated production of Abs(37–39) as early as acute HIV-1 infection(40). Increased levels of IgE, IgA, IgG1 and IgG3 have been observed, concurrent with reduced levels of IgG2, in the setting of chronic HIV-1 infection(41).

Promisingly, it is possible to induce a remarkably different spectrum of Ab classes to even the same protein antigen via different vaccination regimens. Though both vaccine regimens utilized the same protein antigen, VAX003, in which the AIDSVAX B/E proteins were administered 7 times, and RV144, in which this protein was used as a boost followed a viral vector priming regimen, induced antibodies with dramatically different effector profiles(42). These differences raise important questions: it remains to be determined if the differential protection observed in these trials can be attributed to these striking differences in Fc domain profiles. Additionally, longitudinal profiling of samples from VAX003 may allow

determination to be made as to whether, in the absence of priming, the composition of the B/E monomer vaccine itself, or the number of repeated vaccinations drove the divergent responses observed. Likewise, RV305, a follow up study to RV144 with additional boosts may provide insights as to whether the responses observed in RV144 can be amplified or extended while maintaining a stable, polyfunctional profile. Lastly, though Fc domain glycosylation is known to dramatically impact antibody function, characterization of whether different vaccination regimens also modulate antibody potency via this mechanism remains an open question.

Significantly, considerable in vitro evidence exists that Fc domain characteristics are not homogenized by polyclonality and avidity. Rather than being driven simply by titer, antibody composition clearly plays a role in cellular assays of antibody function including NK cell cytotoxicity and monocyte phagocytosis(43–45). The induction of antibodies with conflicting or complementary activities appears to function to tune the overall activity of polyclonal pools. As examples, the presence of IgG2 antibodies has been found to decrease the activity of polyclonal serum(46), whereas acute HIV infection is associated with an enrichment of IgG3 antibodies (47) coincident with robust ADCC activity (16). Similarly, IgA responses were identified as a correlate of risk in RV144(21), though it remains to be determined whether these antibodies might directly inhibit the protective activity of other antibodies, or if they are simply a marker of subjects who responded differently to the vaccine, potentially resulting from bypassing protective IgG subclass selection. Nonetheless, these studies provide evidence that the distribution of Fc characteristics across even a polyclonal population of Abs is key to activity, that this distribution can be actively influenced by vaccination, and that it may provide a means to enhance vaccine efficacy.

2.4 Fc optimization in the setting of passive monoclonal antibody transfer

It is certainly more straightforward to envision tailoring isotype, subclass, and glycosylation in the setting of passive antibody transfer, where these properties can be controlled in the context of recombinant protein expression systems. Indeed, a wide variety of tools exist for the modulation of recombinantly produced antibodies, including a suite of amino acid point mutations that alter Fc domain recognition by innate immune cells(48, 49) and glycoengineered production hosts(50–53). While Fc modification has led to outstanding gains in some therapeutic settings(54–57), evidence has been mixed in NHP studies of HIV infection. Seminal work by Hessel et al, demonstrated that even in the context of the broadly neutralizing Ab b12, compromised Fc function resulted in reduced sterilizing protection from infection(2). However, when b12 was glycoengineered as an afucosylated variant with improved binding to FcγR3a, enhanced protection was not observed(58). Whether this result indicates that effector functions that depend on other FcγR may be more critical to b12-mediated protection, or whether the effector activity of b12 cannot be enhanced, remains to be determined. Finally, it is possible that ADCC enhancing modifications to b12 could not improve the protective activity of this monoclonal therapeutic due to a lack of relevant effector cells at the site of transmission(58). Whether these results are widely representative of other monoclonal Abs, or polyclonal Ab pools is also an open question. To address some of these questions, work to evaluate the in vivo protection

afforded by a suite of Fc variants with different abilities to interact with Fc γ R is ongoing(59).

These experiments also raise important questions about the site(s) of antibody-mediated protection, which may differ by route of exposure. For example, IgA responses have been identified as a correlate of protection in exposed, uninfected subjects(60, 61), and have been shown to inhibit viral transcytosis and block infection in vitro(62). Optimization of the mucosal trapping activity of HIV-specific IgAs could facilitate topical formulation of antibodies designed to act in concert with microbicides. Alternatively, more efficient transport of IgG to mucosal surfaces may also be improved via rational design. Alternatively, Fc-modifications that could harness alternate Fc-receptors and innate immune cell subsets, that are more abundant at mucosal membranes, could instead have a significant impact on protective immunity. Indeed, the antibody characteristics that provide optimal protection at mucosal surfaces and across different tissues have yet to be fully resolved.

Based on the prospect of preventing infection via passive transfer or in vivo production of neutralizing Abs, a real opportunity for effector enhancement exists. However, researchers will need to be mindful that Fc alterations that are not found naturally, or are only found in a fraction of the population, such as the IgG3 allotype which allows extended plasma circulation(63), are likely to bear increased risk of immunogenicity (64, 65).

3. Fc Receptors

3.1 Diversity in structure, function, and expression

While Abs alone can neutralize pathogens, they are unable to mediate effector functions independently, and depend critically on a set of Fc receptors (FcR) and other innate immune receptors which bind to the Ab constant region, and belong to the immunoglobulin superfamily or lectin families of receptors(66). There are 5 groups of FcRs, each able to bind a single Ab isotype: Fc α R, Fc γ R, Fc μ R, Fc δ R, Fc ϵ R, and the MHC-related FcRn. However within various families of FcR, distinct isoforms may exist, designated by a roman numeral (Fc γ RI, Fc γ RII, Fc γ RIII) and by CD nomenclature (CD64, CD32, and CD16, respectively). Additionally, each subgroup may also encode different variants which are referred to by an alphabetical letter (Fc γ RIIa or Fc γ RIIb) (67), and may also exist in multiple allotypic forms and splice variants.

Humans express four classes of Fc receptors for IgG: the high affinity Fc γ RI (CD64) which binds monomeric IgG, and the low affinity Fc γ RII (CD32) and FcRIII (CD16) that interact with IgG immune complexes(68), and FcRn receptor which functions as an IgG transporter (summarized in Table 2(22, 32, 69)). All Fc γ Rs share a common structural motif encoding two or three (immunoglobulin-like domains extracellularly, a transmembrane domain, and an intracellular C terminal tail. The Fc γ Rs share similar, but not identical extracellular domains that confer specificity in ligand-binding, but differ significantly in their transmembrane and cytoplasmic regions, allowing them to transduce different signals (70).

Within each Fc γ R subclass, distinct isoforms have been identified that mediate different effector functions. The Fc γ RI, RIIa, and RIIIa are activating receptors as they encode

immunoreceptor tyrosine-based activation motifs, either within a cytoplasmic tail or in association with the common gamma chain, an accessory signaling molecule. In contrast, FcγRIIb encodes an immunoreceptor tyrosine based inhibitory motif in its cytoplasmic tail and therefore delivers inhibitory signals(68). In a subset of humans, FcγRIIc, which is comprised of an intracellular domain homologous to FcγRIIa with the extracellular domain of FcγRIIb, is also present. FcγRIIb is gpi-linked and has no transmembrane or intracellular domain, but is thought to have the ability to signal through FcγR2a, the complement receptor CR3, or via its location in lipid rafts(71–73). The critical nature of FcR during the immune response has been demonstrated by experiments in FcR deficient mice. Deletion of activating FcR protects against immune complex-mediated induced inflammation in autoimmune or infectious diseases, while the deletion of inhibitory FcR seems to induce autoimmunity(66, 68, 70). Similarly, polymorphisms of FcγR have been associated with potentiated responses to infectious disease, monoclonal antibody therapy, and increased risk of autoimmunity(74, 75).

FcRs are distributed on distinct subsets of cells, allowing cells to respond specifically following recognition of Ab-opsonized material (Figure 1). Stimulatory FcγR are localized on platelets, monocytes, granulocytes, macrophages, and natural killer cells(22). Because the same receptor(s) may be present on different cell types, capable of different responses to receptor ligation, the effector function triggered by Abs are highly dependent on the cell subset that expresses the receptor. Activating FcγR may lead to phagocytosis, Ab-dependent cellular cytotoxicity (ADCC), degranulation and/or the release of pro-inflammatory cytokines and reactive oxygen species. In DCs, uptake driven by FcγR can lead to antigen presentation on class I as well as class II MHC(76, 77). The only inhibitory FcR, FcγRIIb, is expressed on B lymphocytes and on myeloid cells, which when bound in synchrony with other activating receptors on the B cells or monocytes, acts to raise the threshold required for activation of the effector cell, via the delivery of a strong inhibitory signal(68).

3.2 Effect of FcγR polymorphisms and expression in HIV infection and susceptibility

Disease associated polymorphisms that affect the affinity of FcR for IgG have been identified for FcγRIIa and FcγRIIIa(68). In the case of FcγRIIa, an arginine to histidine substitution at position 131 results in more efficient binding to IgG2. With respect to FcγRIIIa, a polymorphism of a phenylalanine to valine at position 158 results in higher affinity for IgG1 and IgG3. Both of these polymorphisms have been associated with several autoimmune diseases(67). Yet, in the context of HIV infection, conflicting results have come from studies characterizing the role of these polymorphisms. The Fc RIIIA-V/V158 polymorphism was found to both have no effect on disease progression(78) as well as to correlate with rapid progression and lack of antiviral control(79). Recently, this high affinity polymorphism was found to be associated with increased risk of infection in a vaccinated population(80), offering what may be the first clinical evidence of antibody-dependent enhancement in HIV. Similarly, the H131 allele of FcγRIIa, which exhibits high affinity for IgG2 has been found to be protective against progression in adults(78), but associated with perinatal infection(81), implying that distinct mechanisms of phagocytosis may mediate antiviral activity at different barriers. Complicating matters further, Fc (GM) allotype diversity and Fc R allotype diversity appear to interact, as in a recent genetic study

comparing HIV controllers with infected subjects indicated a strong relationship between genetic background and likelihood of spontaneous control(82).

Because these polymorphisms may affect risk of infection and rate of progression in different ways, whether recruitment of Fc R activity is beneficial or detrimental may depend on the stage of infection. Inflammatory and cytotoxic activities may protect from infection but in the setting of chronic infection, they may fuel inflammation and immune exhaustion, resulting in accelerated progression. Such discrepancies are not easily resolved, and are in fact compounded by additional levels of regulation in Fc γ R expression. Additional polymorphisms, resulting in differences in the cell types which express Fc γ R, as well as the level at which they are expressed(83), may also play a role, and because multiple cell types express multiple receptor types, receptor haplotypes rather than particular alleles may also impact activity(84). Likewise, expression of a number of receptors is inducible rather than constitutive(85), and varies with immune status. Chronic HIV infection is known to modulate these expression patterns, which can impair effector function(86).

3.3 Non-canonical FcR

While Fc γ R have been most intensely studied, a number of additional receptors and soluble factors also recognize the Ab Fc domain. These proteins include serum and cell-expressed lectins such as the plasma proteins C1q and Mannose Binding Lectin (MBL) which can initiate the complement cascade(87), DC-SIGN, a sialic acid recognizing lectin present on B cells(88), and Dectin-1, which recognizes galactosylated Fc when ligated to Fc γ R2b(89). Other connections exist between complement proteins, Fc γ R, and Ab, such as the C5a anaphylatoxin, C5aR and CR3, which are known to interact with and alter the downstream effects of Fc γ R signaling(90, 91). The mannose receptor can drive uptake of agalactosylated Ab in dendritic cells and macrophage(92). The intracellular TRIM21 receptor is involved in proteasomal targeting of IgG opsonized virus(93). Other IgG binding proteins, such as as Fc γ binding protein (Fc γ BP), which associates with mucus at epithelial barriers(93, 94), and FcR-like (FcRL) receptors 4,5, and A(95, 96) are all known to bind IgG, but remain poorly characterized. The role these and other IgG receptors may play in determining the disposition of Ab-opsonized virus or infected cells in HIV remains to be determined.

3.4 Crosstalk and competition between FcR

That FcR require avid interactions, yet functionally distinct receptors compete for antibody recognition establishes an interesting dynamic and allows effector cells to set thresholds for activation, and tune the downstream fate of opsonized particles. On the receptor side, expression of the inhibitory Fc γ RIIb receptor sets a threshold for phagocytosis and cytokine release(97); whereas the composition of IgG and degree of opsonization impacts activity on the antibody side(98). Even within the same cell type, phagocytosis driven by Fc γ R2a differs from that driven by complement in terms of resulting in an oxidative burst and inflammatory response(99). FcRn has been found to participate in phagocytosis in neutrophils(100). Fc γ RIIb has been described to synergistically enhance phagocytosis in association with both Fc γ RIIa and CR(101). Yet, in the setting of monoclonal Ab therapy, various effector mechanisms have also been observed to compete: complement fixation has been observed to block NK cell activation and ADCC in vitro, and complement depletion has permitted

enhanced Ab efficacy in vivo(102, 103). Many of these dynamics remain unexplored in understanding HIV infection and pathology.

3.5 Altering Fc γ R expression in the setting of vaccination or passive transfer

Given the role IgG receptors play in antibody effector function, rationally altering the expression levels and pattern of Fc γ R represents a plausible means to tune antibody activity. Efforts to increase expression of the inhibitory Fc γ R2b receptor using sialylated IgG, to decrease Fc γ R2a expression using a TNFa binding Ab, and to increase activating Fc γ R expression using bacterial lipopolysaccharide have been evaluated(104–106). Because altering effector cell activity is likely to have pleiotropic effects, great caution would be called for in pursuing this avenue to enhance antibody activity in the context of achieving life-long protection from HIV infection, whether by vaccination or by passive Ab transfer. However, tissue-specific alteration of effector cell activity via topical gel or a similar method may represent a clinically feasible means to augment Fc γ R activity only where needed, and as a means to avoid the risks likely associated with systemic modulation of Fc γ R expression and effector cell function.

4. Effector cells

Given that all innate immune cells express Fc-receptors and therefore can be activated via Ab opsonized material, it is likely that vaccine induced antibodies able to specifically recruit antiviral functions that can be rapidly deployed at the site of infection may have a profound impact on protection from infection. As described in Figure 1, innate cells that may mediate antiviral activity include neutrophils, natural killer (NK) cells, monocytes, macrophages, and dendritic cells (DC), but are distributed in widely divergent manners across tissues and compartments in the body. Therefore therapeutic or vaccine approaches aimed at harnessing the antiviral activity of distinct innate immune cells will rely critically on the availability of target effector cells at the sites of viral transmission or replication.

4.1 Neutrophils

Neutrophils are the first line defense against incoming pathogens and represent 50% to 75% of total circulating nucleated white blood cells. Importantly, these innate effectors are the first to arrive to sites of infection, arriving within minutes due to their remarkable chemotactic and adhesive properties, and they play a critical role in the acute inflammatory response to foreign invaders or trauma. Neutrophils are best known for their role in phagocytosis and killing of extracellular bacteria, however they can provide protection against a diverse set of pathogens, and do so by performing a variety of different functions (reviewed in(107, 108)). These functions include tissue remodeling, antigen presentation, recruiting other blood cells, and polarizing T cell responses(109–112). While neutrophils are characterized for their ability to phagocytose, these cells also induce reactive oxygen species, cytokines/chemokines, lipid mediators, and release copious amounts of antimicrobials and enzymes. Neutrophils can be activated directly through cytokine stimulation, TLR signaling, as well as indirectly by complement receptor activation[14], or FcR-induced activation upon encounter with an immune complex. Moreover, upon deposition of antibody complexes inside of tissues, neutrophils are the first cells recruited

and primed to amplify the ongoing inflammatory response. Neutrophils are a remarkably heterogeneous population, where 75–85% express Fc γ R and 29–40% express Fc α R. Among the Fc γ Rs, neutrophils co-express Fc γ R2a (CD32A) and Fc γ R3b (CD16B), however Fc γ R2a is only responsive after neutrophil activation, suggesting that the dominant FcR on this innate immune cell subset is Fc γ R3b. Moreover, upon cytokine stimulation, neutrophils may additionally upregulate Fc γ R1, potentially aimed at enhancing IC clearance upon activation.

Neutropenia has been repeatedly associated with differential HIV infection risk from mother to child and disease progression(113), suggesting that these cells play a critical role in antiviral control. However, neutrophil activity decreases with progressive infection, potentially resulting in compromised immunity(114). Moreover, recent work suggests that neutrophils may facilitate antiviral control through the formation of extracellular traps(115). Additionally, bispecific monoclonal therapeutics, able to recruit the cytotoxic activity of neutrophils, were able to inhibit viral replication(116). However, whether neutrophil recruiting antibodies are enriched in subjects with durable protection of HIV/SIV infection, and whether such antibodies can be elicited by vaccination to control and clear the virus more aggressively is uncertain. Yet due to their numbers and speed, neutrophils represent an understudied and potentially attractive effector cell population that could provide a remarkable arsenal to combat the incoming virus.

4.2 Natural Killer cells

Like neutrophils, NK cells participate in our first line defense against infection, but unlike neutrophils, these cells only represent ~15% of circulating lymphocytes, yet are ideal effectors for the rapid removal of antibody opsonized material due to their potent cytotoxic activity and ability to lyse target cells without the need for prior antigen sensitization. NK cells can be divided into two subsets of cells based on their surface expression of CD56 (117). The minor population of circulating NK cells belongs to the CD3^{neg}CD56^{bright}CD16^{neg} NK cells, do not mediate ADCC, are poorly cytolytic, but are able to secrete large amounts of pro-inflammatory cytokines such as interferon- γ (IFN- γ), tumor necrosis factor- α (TNF- α), macrophage inflammatory protein-1 β (MIP-1 β), and granulocyte macrophage colony-stimulating factor (GM-CSF), etc., upon activation. However, the bulk of the circulating NK cell population belongs to the highly cytolytic CD3^{neg}CD56^{dim}CD16^{pos} population that can robustly mediate ADCC, contain large quantities of perforin and granzyme, and secrete moderate levels of the aforementioned cytokines. Moreover, because the cytolytic CD3^{neg}CD56^{dim} NK cells express high levels of Fc R3a, these cells play a critical role in responding to ICs, and are centrally involved in the elimination of antibody coated targets via ADCC. Furthermore, NK cells may also express Fc R2c, Fc α R, and Fc R1 following cytokine activation.

Several monoclonal therapeutics have been optimized to recruit more effective ADCC, including rituximab. This is achieved via the production of the antibody in a cell line that cannot add fucose to the antibody glycan, resulting in higher affinity interaction with Fc γ R3a expressed on NK cells. Interestingly, while neutralizing antibodies are rarely induced in HIV infected patients, ADCC inducing antibodies are enriched in the plasma of subjects with non-progressive disease (7, 14–18, 118–120), are detectable as early as a few

weeks post-infection(13, 118), clearly belong to the IgG isotype, and are dependent on the Fc portion of the antibody(121), and it's glycosylation state(45). However inconsistent results have been obtained regarding the importance of these types of humoral immune responses in HIV disease progression, likely related to the small groups of subjects, often unbalanced for age/sex, inconsistent target and effector cells used in distinct ADCC studies, and differences in gp120 molecules/viruses utilized(18, 120, 122–125). Thus larger studies are needed to evaluate the critical nature of NK cell mediated ADCC responses, their specificities, to gain critical insights into the specific mechanisms by which NK cell mediated ADCC may contribute to antiviral control.

While both passive transfer and vaccine induced non-neutralizing ADCC inducing antibodies have largely failed to provide protection from SIV challenge, NK cell mediated ADCC inducing antibodies have been implicated in post-acquisition antiviral control(4–6, 8, 9, 126). However the critical nature of NK cell mediated ADCC responses was most clearly shown in the context of compromised protection from SHIV challenge using an Fc point mutant of the neutralizing b12(2), which lacked the capacity to interact with Fc-receptors. Thus while both the wild type and complement-non-binding variant antibodies provided robust protection from infection, the FcR knockout resulted in reduced protection from infection, suggesting that sterilizing protection from infection is likely mediated by a combination of antibody activities including antibody mediated recruitment of the innate immune response through FcRs. However, interestingly, altered glycosylation of the Fc domain to enhance ADCC mediated activity via NK cells, did not augment protective efficacy(58), suggesting that either b12 mediated NK cell protective activity is already maximized or that the mechanism of b12 action may be through alternate cellular subsets, particularly related to those present at mucosal sites where the majority of infections occur worldwide.

It is becoming clear that multiple NK cell subsets exist in different immunological compartments. It is uncertain whether these NK cells differentiate in these tissues to respond to environmental needs, or whether they traffic differentially to these sites. The distribution of the 2 peripheral NK cell subsets is reversed in most tissues, including the lymphnode (LN), gut, and female reproductive tract (FRT) where the majority of the cells belong to the cytokine secreting CD3^{neg}CD56^{bright} NK cell subset and only 10% of the cells belong to the CD3^{neg}CD56^{dim} cytolytic compartment of NK cells(127). Moreover, few tissue cells express FcγR3a, typically expressed on peripheral CD56^{dim} NK cells, required for mediating ADCC. The exclusion of cytolytic KIR+CD56^{dim} NK cells may be intended to protect inductive sites from potential immunopathology as these sites are often subject to dramatic inflammation, however this may also allow HIV to replicate unabated in the absence of early antiviral pressure by these cytolytic effectors.

Similarly, NK cells represent a large fraction of intraepithelial lymphocytes (IEL) that circulate in the gastrointestinal (GI) tract, surveying for tumor or infected cells(128). GI NK cells, similar to uterine NK cells, resemble the CD56^{bright} blood NK cell compartment, and express low levels of CD16(129, 130). Moreover, recent data suggest that NK cells may also traffic to the lamina propria, but neither the IEL or LP NK cells expand during viremic HIV infection (131). Instead, gut NK cells only expand in subjects with poor immunological

reconstitution following anti-retroviral therapy initiation, yet still express low levels of CD16. Thus, while NK cells may reside in lymphoid and mucosal compartments, these cells are not poised to mediate ADCC, due to low basal FcR expression within tissues. However, cytolytic FcR expressing NK cells are among the first cells to arrive to the site of infection to aid in the rapid containment/clearance of pathogens.

4.3 Monocytes/Macrophages/Dendritic cells

While ADCC has been shown to play a crucial role in better disease outcome in HIV-1 infection, further evidence suggests that additional antibody functions, including the capacity to induce phagocytosis, ADCC, may also be responsible for antiviral control through the rapid clearance of immune complexes. Among the phagocytes, monocytes represent approximately 5% of circulating leukocytes, and typically patrol the periphery for a few days before moving into tissues, where they can mature into macrophages or dendritic cells, specialized for protection of the particular organ in which they take residence. While these cells specialize in phagocytosis, monocytes/macrophages/DCs secrete large quantities of cytokines upon activation and are centrally involved in presenting the antigens that they take up to the immune system to prime effective adaptive immunity.

Monocytes/macrophage/DC mediated phagocytosis can occur directly through the recognition of pathogens through innate pattern recognition receptors or through Fc-receptors that decorate the surface of these cells. Importantly, while the majority of immature circulating monocytes only express Fc γ R1 and Fc γ R2a, activation and differentiation of these cells can lead to the upregulation of additional FcRs including Fc γ R3a, Fc γ R2c, Fc α R, and Fc γ R2b on DCs. Thus depending on the combination of different FcRs expressed and engaged on a given cell, a diverse array of different immune responses may be induced.

Previous work by Holl et al. showed that blockade of Fc γ RI and Fc γ RII on HIV-infected macrophages or HIV-infected immature DCs, respectively, resulted in reduced antibody-mediated control of HIV viral replication (132). However, a number of myeloid cell functions are impaired following HIV-1 infection *in vivo* as well as *in vitro*, including chemotaxis (133, 134), phagocytosis (135–138), intracellular killing (139), and cytokine production (140), potentially contributing to poor antiviral control (141–143). Moreover, FcR expression is perturbed throughout the course of infection, likely impacting the functional quality of FcR mediated activation of these cells. For example Fc γ RI is highly upregulated on monocytes in acute HIV infection, potentially contributing productively to the rapid clearance of ICs upon antibody production to help establish viral set point, whereas progressive infection is associated with a loss of Fc γ R2a and Fc γ R3a potentially resulting in poor antiviral control. Moreover, recent data suggests that antibodies from different subject classes do indeed possess variable capacity to drive phagocytosis(43). Thus given the abundance of phagocytic cells (macrophages/DCs) in tissues, of both the female reproductive tract mucosa and gastrointestinal tract, therapeutic interventions including both passively transferred monoclonal antibodies and/or vaccine induced antibodies able to harness this innate immune effector function may afford the greatest level of protection against infection.

4.4 Potential to exploit: Vaccination

The window of opportunity to prevent HIV is remarkably short; from the moment the first virus enters the body to the point where it infects the first cell, due to the fact that once inside a cell, HIV has evolved intricate means by which it is able to hide from the immune system. Therefore vaccine or therapeutic strategies aimed at preventing infection must act aggressively to clear the very first infected cells, and may not be able to rely on traditional vaccine-induced immune responses that require cellular proliferation, differentiation, and homing to the site of infection. As described above, NK cells and neutrophils only constitute a small fraction of total cells in tissues, macrophages and DCs are abundantly present at these sites. Thus approaches aimed at boosting NK cell mediated activity exclusively to enhance vaccine-mediated protection may only confer limited protection. This may have accounted for the inability of Fc-optimized b12(58) to provide additional protection from infection upon SHIV challenge. In contrast, phagocytic antibodies can promote disposition of immune complexes in either the absence of activation, or involving the release of copious amounts of inflammatory modulators that can promote more effective recruitment of other innate effector cells such as NK and neutrophils. This mechanism may also promote more effective antigen-delivery to DCs resulting in more potent presentation of foreign antigens to adaptive immunity. Thus these results have led to speculation that perhaps the enhanced ability of RV144 vaccine antibodies to mediate phagocytosis(42) may have afforded a fraction of vaccines with the additional ability to resist infection upon exposure. Thus next generation vaccine efforts should place some emphasis on generation antibodies that not only enhance ADCC but also ADCP.

4.5 Could enhanced effector function help effect a cure?

Beyond protection from infection via vaccination, accumulating data suggests that eliminating viral infection may be possible. Two cases of human “functional” cure have been reported, including a myeloablative bone marrow transplant with CCR5d32 bone marrow that led to “cure” in an adult (144) and antiviral therapy induced blunting of viral spread and dissemination in an infant in Mississippi (145). However following infection, HIV establishes a latent reservoir in CD4+ T cells and other immune cells. Because latency is linked to transcriptional silencing of the integrated provirus by histone acetylation and other modifications, several histone deacetylase inhibitors (HDACi) have now been tested as a mechanism to potentially derepress the latent reservoir. Interestingly, inhibition of HDACs has now been shown to lead to reactivation of both cell-associated viral RNA (146–153) and virion release (146, 148, 154, 155), strongly suggesting that the reservoir may be reactivatable. However, this reactivation alone does not lead to eradication of the reservoir, and therefore, additional directed destructive mechanisms are critically needed to rapidly deplete cells that are reactivated.

Among the potential “shock and kill” strategies (156), activated T cells have been recently shown to efficiently kill reactivated cells (157). However, T cell-based strategies are limited by potential archived viral escape mutants, potential irreversible T cell exhaustion resulting in compromised killing activity, qualitative differences in vaccine-induced immunity by polymorphic major histocompatibility complex (MHC) class I alleles, and issues related to T cell homing to sites of viral latency. Conversely, beyond T cells, cytolytic HIV-specific

antibodies may also induce the rapid destruction of reactivated cells by directing the cytotoxic and antiviral activity of the innate immune system. Moreover, Fc-optimizing principles developed by the monoclonal antibody (mAb) therapeutics community for the rapid and effective clearance of tumor or autoimmune cellular targets may be applied to HIV-specific monoclonal therapeutics to enhance destruction of reactivated cells. These potent HIV-specific killer antibodies, may be optimized to take advantage of the arsenal of innate immune cells present at distinct reservoir sites (brain, lymphnodes, gut, etc). These therapeutics may be administered alone or in cocktails aimed at covering oligoclonal epitope arrays on reactivated cells, in combination with reactivation regimens to rapidly destroy reactivated cells and drive the “kill” required to attain a functional cure.

5. Conclusion

The innate immune response represents the body’s first line defense against infection, and possesses a remarkable repertoire of cells that are able to aggressively attack and contain most pathogens. However, vaccine and passive transfer efforts to date have not had the opportunity to optimally harness the cytolytic power of this first line of defense. While the complex landscape of antibody, Fc γ R, and innate immune cell activity poses challenges to study, it also provides a number of means by which rational vaccine design or antibody engineering could impact the HIV epidemic. Because each of the diverse innate effector cell types, Fc receptors, and Fc domain isotype, subclass, and glycosylation patterns can have a profound effect on the fate of opsonized virus or cells, each can be considered a possible means to enhance the protective function of antibodies.

Thus the potential exists to design vaccines that elicit specific functional antibody profiles via rationally steering antibody Fc isotype, subclass, and glycosylation. Results from studies of both natural infection and vaccination indicate that this distribution can be actively influenced, and that doing so may provide a means to enhance antibody activity. While finer-grained analysis of IgG subclasses and glycosylation have begun to provide significant insights into the dynamic range of antibody activities, similarly fine resolution of IgA composition could likewise provide valuable insights given the signatures observed for IgA in a number of studies. Likewise, factors that influence FcR expression level and innate immune cell trafficking and activity hold considerable promise. Furthermore, antibody-mediated protection must take advantage of the cells that are present at the site of infection. Understanding the primary sites of infection at which antibodies could provide maximal benefit, whether the gut, lymph nodes, female reproductive tract, or other, will be key to translating the studies summarized here into candidate vaccine strategies. A wealth of data from natural infection, passive transfer, and human and macaque vaccine studies are consistent with the ability of innate immune recruiting antibodies to provide protection from infection or progression. What remains is to identify and characterize the innate immune cells present at sites of transmission and infection, the most robust and protective effector functions, and the antibody features which would most effectively drive these protective innate immune responses, in order to most productively exploit these antibody activities.

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Figure 1. Antibody-mediated innate immune recruitment

A diverse set of innate immune effector cells, as well as soluble factors, can interact with antibody-opsonized virus or infected cells. These include C1q and MBL, which can initiate the complement cascade, resulting in formation of the membrane attack complex as well as feeding forward to recruit other innate effectors that express complement receptors. Phagocytic cells such as DC, monocytes, and macrophage express a number of antibody as well as pattern-recognition receptors that can drive phagocytic uptake, reactive oxygen bursts, or secretion of cytokines, chemokines, and other soluble factors. Neutrophils can be induced to release an NO respiratory burst, while NK cells can degranulate. Most of these innate immune cells express a combination of FcR and the specific set of receptors ligated will drive differential effector functions and outcomes.

Table 1

Properties of IgG subclasses

	IgG1	IgG2	IgG3	IgG4
Molecular weight (kDa)	146	146	170	146
Hinge length (# of aa)	15	12	62	12
Serum concentration (mg/ml); (%)	7 (60%)	3.8 (30%)	0.51 (~5%)	0.56 (~5%)
Halflife (days)	21	21	7–21	21
Specificity to	proteins	polysaccharides	proteins	proteins, allergens

Table 2

Canonical IgG receptors and their properties

	FcγR1	R2a	R2b	R3a	R3b	Rn
Affinity	nM	μM	μM	nM-μM	μM-nM	pH dependent
polymorphisms		H131/R131		V158/F158	NA1/NA2	
Expression pattern						epithelial cells
Macrophage	X	X	X	X		X
Neutrophils	X	X	X		X	X
Basophils		X	X		X	
Eosinophils	X	X	X	X		
Platelets		X				
Mast cells			X	X		
Dendritic cells	X					X
B cells			X			X
NK cells				X		
Intracellular domain	γ chain	ITAM	ITIM	γ chain	gpi linked CR3 FcγR2a lipid rafts	β2m
function	uptake, killing	uptake, killing	inhibition	killing		transport
Binding preference	IgG1/3 IgG4 IgG2	IgG1/3 IgG4 *IgG2 bound by H13	IgG1/3/4 IgG2	IgG1/3 afucosylated, bisected glycans IgG4 IgG2	IgG1/3 afucosylated, bisected glycans IgG4 IgG2	IgG1 IgG4 IgG3 IgG2