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Roles of Fc-Mediated Effector Functions in Broadly Neutralizing Antibody Activity Against HIV

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Abstract

Purpose of Review: 'Broadly neutralizing antibodies' (bNAbs), are rare HIV-specific antibodies which exhibit the atypical ability to potently neutralize diverse viral isolates. While efforts to elicit bNAbs through vaccination have yet to succeed, recent years have seen remarkable pre-clinical and clinical advancements of passive immunization approaches targeting both HIV prevention and cure. We focus here on the potential to build upon this success by moving beyond neutralization to additionally harness the diverse effector functionalities available to antibodies via Fc-effector functions.

Recent Findings: Recent studies have leveraged the ability to engineer bNAb Fc domains to either enhance or abrogate particular effector functions to demonstrate that activities such as antibody dependent cell-mediated cytotoxicity contribute substantially to *in vivo* antiviral activity. Intriguingly, recent studies in both non-human primates and in humans have suggested that passive bNAb infusion can lead to durable immunity by enhancing virus-specific T-cell responses through a 'vaccinal effect'.

Summary: The combination of antibody engineering strategies designed to enhance effector functions, with the broad and potent antigen recognition profile of bNAbs, has the potential to give rise to powerful new therapeutics for HIV. We aim to provide a timely review of recent advances to catalyze this development.

Keywords

HIV; broadly neutralizing antibodies; Fc effector function; immunotherapy; vaccinal effect

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Introduction

Human immunodeficiency virus (HIV)-specific antibody (Ab) responses against the viral Envelope (gp120, gp41) are typically detectable within a few weeks of infection, and increase over the course of disease (1–3). However, the majority of these antibodies are non-neutralizing (4). Broadly neutralizing antibodies (bNAbs) are defined as Abs with the ability to neutralize highly variable viral pathogens. In the case of HIV, although the natural emergence of bNAbs is rare, substantial efforts have led to the isolation of an array of bNAbs which achieve varying neutralization potencies and breadths across the vast diversity of HIV clades by binding to vulnerable sites on the Envelope glycoprotein (5). The development of vaccine approaches capable of eliciting bNAbs remains a 'holy grail' in the field, towards which progress continues. In the absence of a vaccine, however, the passive infusion of bNAbs holds potential both for the prevention and treatment of HIV, through direct neutralization of the HIV virions and by boosting different components of the immune system to control or eliminate infection (5). Building off of success in non-human primate simian-human immunodeficiency virus (SHIV) models of HIV infection (6, 7), the passive transfer of several different bNAbs to HIV-infected individuals has been shown to transiently suppress viremia (8, 9**). Beyond neutralization, a critical aspect of Abs in general – which extends to bNAbs - is their ability to exert diverse effector functionalities by virtue of interactions mediated through their 'Fragment crystallizable' (Fc) domains (10). These Fc domains of bNAbs mediate the opsonization of virions or infected cells by complement components, which leads directly to lysis (11, 12). By engaging with Fc receptors on effector cells, bNAbs can also enhance the killing of infected $CD4^+$ T cells by natural killer (NK) cells, and increase the phagocytosis of infected cells by macrophages and neutrophils (13, 14). Here, we summarize the current knowledge about Fc-mediated functions of bNAbs, and technical advancements that have led to improved efficacy of these antibodies by modifying their Fc domains.

Antibody-dependent complement-mediated lysis (ADCML)

The classical pathway of the complement system activates by the attachment of C1q to the Fc domain of antibodies that have bound to pathogens or to infected cells. C1q activates C1r and C1s serine proteases that initiate the recruitment of other complement factors. This proteolytic cascade leads to the formation of membrane attack complex (MAC) and lysis of the pathogen or infected cell (Figure 1A) (15). IgG1 and IgG3 subclasses are the most efficient activators of complement (16). Mujib *et al* tested a large panel of bNAbs and concluded that anti- V1/V2/glycan bNAbs such as PG9, PG16, and PGT145 bound to HIV Envelope on the surface of primary HIV-infected cells and induced ADCML (17). 2F5, 4E10, 2G12, VRC01, and 3BNC117 were not able to enhance the clearance of virus-infected cells by ADCML in their experiments (17). Complement activation and its lysis effect can be prevented by specific surface molecules that are called regulators of complement activation (RCA) (18, 19). RCAs like CD46, CD55, and D59 prevent the generation of MAC, which is the final step of all three pathways of complement activation (18, 19). RCAs can be transferred to virions from infected cells, and therefore, be present on both the cell-free virus and the $CD4^+$ infected cells (20, 21). Saifuddin *et al* showed that HIV virions hijacked and expressed human CD46, CD55, and CD59 on their surfaces to escaped

ADCML (20). Functional blockade of CD59 significantly enhanced the ADCML activity of 2F5, 4E10, and 2G12 bNAbs against three HIV laboratory strains (R5, X4, and R5/X4), six primary isolates, and provirus-activated ACH-2 cells (21). Similarly, Hu *et al* reported that a recombinant protein of the bacterial toxin intermedilysin inhibited human CD59 function, enabling efficient ADCML of virions and infected cells (22). In this context, blockade of RCAs by anti-RCA-antibodies, inhibitory peptides, or other potential small molecules may boost the function of bNAbs and facilitate the neutralization of the cell-free virions or infected cells by ADCML.

Antibody-dependent cellular cytotoxicity (ADCC)

NK cells bind to antibody-coated infected cells by their Fc receptors and induce them to undergo apoptosis, or other mechanisms of death, in a process called ADCC (Figure 1B). Disease progression has been reported to be slower in HIV-infected individuals that exhibit potent ADCC activity (23–28). NK cells use FcγRIIIA (CD16), a low affinity receptor, for binding to clustered IgG molecules on the surface of coated cells. Upon binding, NK cells release perforin and granzymes that trigger the target cell to die (13, 25–27, 29). There are thus two critical, and separable components of Ab function that determine ADCC potency: i) degree of binding to infected cells – determined by the Fab Ab region, and ii) degree of binding to CD16 on NK cells – determined by the Fc Ab region.

With respect to Fab activity, the relationship between the ability of an Ab to neutralize virus, and its ability to bind to infected cells correlate only in a non-reciprocal way. That is, Abs that exhibit potent virus neutralization *generally* are also effective at binding to infected cells while, on the other hand, it is common to identify antibodies that are able to bind to infected cells but cannot neutralize virus. This discrepancy arises from the fact that neutralization of a viral particle specifically requires binding to the functional viral trimers (or “spikes”) that mediate binding to the CD4 receptor and CCR5 or CXCR4 co-receptor, and subsequent fusion to infected cells. While such functional trimers will be present at some level on infected cells, these cells will also be decorated by a variety of non-functional forms of Envelope, including gp41 ‘stumps’, gp120/gp41 monomers, and other species – which commonly expose epitopes that are not present on functional trimers. Antibodies that target such non-functional forms of Envelope can therefore bind to infected cells, and target these cells for ADCC, but do not neutralize viral particles. When Env is in open conformation, antibodies against conserved CD4-induced (CD4i) epitopes can be generated. These anti-CD4i Abs have shown to be strong inducers of ADCC (29–32), however both the Vpu and Nef proteins of HIV have been shown to attenuate ADCC by anti-CD4i Abs (24, 33, 34).

While it is therefore possible to use non-neutralizing antibodies in therapeutic strategies aimed at inducing ADCC, there are three main advantages for utilizing bNAbs. First, there is extensive clinical experience with several bNAbs from passive infusion studies. Second, there are advantages to harnessing neutralizing activity alongside ADCC in an antibody therapeutic. Third, the neutralizing activity against a broad array of viral isolates that defines a bNAb is likely to translate into equally broad ADCC activity. Supporting this, we recently demonstrated, using a panel of clade B virus reactivated from latent reservoirs, that the

ability to neutralize a given virus generally correlated with the ability to bind to corresponding infected cells – a critical pre-requisite for ADCC (35*).

Several other studies have assessed and compared ADCC activity across different bNAbs. Bruel *et al* investigated the CD16 signaling and the killing activity of NK cells, using a panel of ten bNAbs and found that the most active bNAbs were those that induced the strongest CD16 stimulation (36). NIH45–46 and 3BNC117 antibodies, which target the CD4bs, anti-glycan/V3 antibodies 10–1074 and PGT121, and 10E8 anti-gp41 antibody induced strong ADCC, while anti-glycan/V3 antibody PG16 and anti-CD4bs VRC01 were less active (36). In contrast, the anti-CD4bs antibody 12A12, anti-gp120/gp41 interface antibody 8ANC195, and anti-MPER antibody 4E10 were not observed to induce ADCC (36).

For bNAbs that bind equivalently to infected cells, the second factor that will determine ADCC potency is the degree to which these bind to CD16 on NK cells. Of the subclasses of IgG, the affinity for CD16 is highest for IgG3 - followed by IgG1, IgG4 and IgG2. Fc glycosylation variations, and polymorphic differences between individuals, can also affect the binding strength of IgG molecules to CD16 (37*). It has been shown that afucosylated IgG1 at asparagine (N)-297 position increased the binding affinity of IgG1 to CD16 on NK cells by 50 folds, which led to enhanced ADCC activity (38). Fucosylation of N-297 has been suggested to limit the conformational flexibility of IgG1 through its steric interaction with N162 on CD16, as truncation of N162 reverses the decreased binding affinity (39). A number of mutations have been discovered which enhance ADCC activity by augmenting CD16 binding, as reviewed below.

Antibody-dependent phagocytosis (ADP)

Granulocytes, monocytes, and macrophages are professional phagocytes express which express a diverse set of FcγRs Fc receptors on their surfaces, which mediate elimination of infected cells through ADP (Figure 1C) (40). Neutrophils are abundant in the circulation and rapidly migrate to sites of infection and perform effector functions (41). However, they have mainly been considered as anti-bacterial and anti-fungal immune cells, and their protective role in HIV infection is not well characterized (42). Saitoh *et al* reported that neutrophil extracellular trap (NETs) capture HIV by first detecting virus via TLR7 and TLR8, and then eliminating the virus through myeloperoxidase and α-defensin, but did not study the effect of anti-HIV antibodies and the roles of Fc receptors (43). Sips *et al* reported evidence supporting that in certain mucosal sites, ADP may be the dominant mode by which Abs engage effector cells. Contrary to low expression of CD16 on NK cells and their weak engagement in ADCC, macrophages and neutrophils expressed high levels of CD16 and CD32 on their surface and showed effective phagocytic clearance of immune complexes. Fc-engineered variants of the bNAb VRC01 bearing each the following sets of mutations all enhanced macrophage phagocytosis: S267E/H268F/S324T (SEHFST), S239D/I332E/G236A (SDIEGA), S239D/I332E (SDIE) and S239D/I332E/A330L (SDIEAL), with all except SEHFST increasing neutrophil phagocytosis as well (14). In addition to FcγRs, mucosal macrophages and monocytes express FcαR that may be involved in IgA2-mediated phagocytosis (14). The length of the hinge between the Fab and Fc may play an important role in increasing the magnitude of ADP (44–46). Chu *et al* used THP-1 monocytic cell line

and constructed hinge variants of HIV-specific antibody subclasses. They found that native IgG3 subclasses of VRC01 (anti-CD4bs) and 447–52D (anti-loop 3) bNAbs had higher phagocytic activity compared to their corresponding native IgG1 subclasses. They also showed that IgG1, and IgG3 variants of both bNAbs with extended hinges elicited stronger phagocytic activity compared to their native forms (47*). Although there are many studies about the biology of macrophages in response to HIV, details of their roles in ADP clearance of HIV are not well known. The interaction of granulocytes with HIV, including their potential to reduce HIV reservoirs through ADP (ex. mediated by bNAbs) also requires further study.

HIV bNAb isotypes and subclasses

The majority of bNAbs which have been isolated thus far possess the IgG isotype Fc domain, and many of these are of the IgG1 subclass, however this may not be optimal for the engagement of effector functionalities. Richardson *et al* demonstrated that an IgG3 bNAb that is specific for V2 loop of the Envelope (VRC026), mediated enhanced ADCC and neutralization potency compared to the corresponding IgG1 variant (48). Of note, the *in vivo* half-life of IgG3 is shorter than IgG1, which might necessitate shorter intervals between administration in a therapeutic setting. Hypothetically, the elicitation or infusion of bNAbs in a prophylactic setting may benefit from a combination of anti-HIV IgG and IgA isotypes, to achieve protective mucosal immunity (49). IgG has multiple advantages in terms of engaging effector functions: it is the main isotype involved in complement fixation and thus a potent driver of ADCML virions (17), IgG is also a potent inducer of ADCC by NK cells to induce cell death in infected cells, as well as of engulfment of infected cells through ADP by monocytes, macrophages and neutrophils (49–51). IgA, however, is the dominant isotype in mucosal secretions, and the mucosa is the natural site of most HIV transmissions. Thus, IgA may be more effective in blocking transcytosis of HIV, preventing the infection of $CD4^+$ T cells at mucosal surfaces (52–56), and protecting DCs from infection, which is of great importance as well (57, 58), whereas IgG may complement these functions by enabling clearance of cells that do become infected. In two studies, anti-gp41 IgA protected HIV transmission in IgG seronegative individuals that were highly exposed to HIV (56–59). Tudor *et al* have investigated the role of CH1 domain by constructing a 2F5 IgA2 bNAb and comparing it with 2F5 IgG1. They demonstrated that 2F5 IgA2 bound to gp41 with a higher affinity and blocked CCR5-tropic HIV transcytosis across epithelial cells more efficiently (60). It is interesting to note that the dominant subtype of IgA in mucosal tissues is IgA2, whereas IgA1 is more prevalent in the periphery (46, 60), where its role in HIV-inhibition is not well studied. A distinct potential advantage of IgA1 with respect to harnessing bNAbs for HIV cure, is its ability to engage the IgA1 receptor, FcαRI (CD89) which is expressed on monocytes, macrophages and granulocytes (61), and can drive potent elimination of HIV infected cells by ADP (62). Thus, whereas all antibodies currently in the clinic or in clinical trials – for any indication, ex. rituximab for lymphoma – have been IgG, exciting opportunities exist to explore alternative isotypes of bNAbs.

While bNAbs can hypothetically be produced as a variety of different Ig isotypes and subclasses, with the goal of leveraging the various advantages of each – either alone, or in combinations – the possibility for negative synergies should also be considered. This can be

illustrated using the RV144 HIV vaccine trial, in which both: i) IgG antibodies that bound to the variable regions 1 and 2 of Envelope and ii) ADCC activity correlated inversely with the rate of infection amongst vaccines, whereas levels of binding of plasma IgA antibodies to these same sites correlated directly with the rate of infection (63, 64). This has been suggested to indicate that ADCC mediated through IgG may have offered protection, which was inhibited by competition with IgA – an effect that could be observed *in vitro* (65). In moving forwards to explore alternative isotypes of bNAbs, investigators should be mindful for the potential for such competitive interactions.

Engineered Neutralizing antibodies

The density of HIV Envelope on the surfaces of virions and infected cells is sufficiently low that two such molecules are rarely in close enough proximity to be recognized by the Fabs of a single antibody, potentially limiting ADCC (66, 67). To overcome this limitation, Ramadoss *et al* constructed a bispecific bNAb antibody, using VCR01 as the backbone that had a high affinity single chain variable fragment (scFv) to target CD16 on NK cells. The affinity of this recombinant bNAb to gp41 was equal to original VCR01, and its affinity to CD16 was increased. This recombinant bNAb bound to infected primary $CD4^+$ T cells and boosted NK cell killing activity (68*). The scarcity of Envelope glycoprotein may also be addressed by making bi- or tri-specific antibodies, which can increase both avidity and the neutralization breath (69). It has been suggested that increased avidity of multivalent bNAbs also enhances other functionalities such as ADCML, ADCC, and ADP, but this needs to be investigated in more detail (10, 70, 71). Bourzanos *et al* generated Fc domain variants of 3BNC117, 10–1074 and PG16 IgG1 bNAbs including G236A/S239D/A330L/I332E (GASDALIE) and G236R/L328R (GRLR) and compared these with wild type bNAbs. While no differences in binding to gp120 and neutralization of viruses were observed, GASDALIE versions of these bNAbs showed increased binding to FcγRs and enhanced antiviral activity *in vivo*, while binding was abrogated for GRLR variants (72). Next, they developed a bi-specific anti-Envelope bNAbs of IgG1 subclass and generated a modified version of this antibody as well. A hinge domain of IgG3 was added to the Fc domain of the modified version to increase Fab domain flexibility. They observed increased neutralization activity of the modified version compared to the parental Ab *in vitro* and in HIV infected humanized mice (73). Improving antibody half-life is another aspect of bNAb engineering, as LS mutant (M428L and N434S) of VRC01 showed enhanced binding to neonatal Fc receptor (FcRn), which led to increased half-life, but did not affect its ADCC activity in a cynomolgus macaque model of SHIV (74). The LS mutant of VRC01 was shown to be safe in a phase one clinical trial, and exhibited a 4-fold longer half-life than the parental Ab (75). As FcRn-enhancing mutations may decrease ADCC activity, one study investigated the combinational effect of QL, LS, A, AAA and YTE mutations that increase FcRn binding, with DE and DLE mutations that increase ADCC. While the gp120 binding affinities of all combinations were similar to the original VRC01, only DE-LS and DLE-LS mutants enhanced both epithelial transcytosis as an indication of half-life, and ADCC in vitro (76). Recently, Kerwin *et al* replaced potential destabilizing residues in the variable region of 10–1074 IgG1 bNAb. They replaced the heavy chain residue T108 with R108 at the base of the CDR3 loop which allowed for the formation of a nascent salt bridge with heavy chain

residue D137. They also induced three additional mutations to increase conformational stability (77). We also have shown in a novel humanized mouse model that Fc domain of 10–1074 antibody was essential for viral control – where treatment of these mice with 10–1074 bNAb significantly reduced the viral load compared to 10–1074-FcR^{null} (78*). As is evident in the above, there is tremendous potential to employ protein engineering approaches to enhance multiple aspects of bNAbs in order to fully leverage the potential of these as therapeutics.

Vaccinal effect of bNAbs

In the use of tumor-targeting Abs to treat cancer, there have been multiple reports of anti-tumor activity has persisted after Abs have cleared. This has led to the postulation of a 'vaccinal effect' whereby transient treatment with these Abs is thought to have primed adaptive immune responses (79, 80). How might such a vaccinal effect arise, and could this be of benefit in the HIV setting?

Immature DCs sense pathogens through pattern recognition receptors and initiate a proinflammatory immune response by secretion of cytokines. They develop to mature DCs after the phagocytosis of pathogens and become professional antigen presenting cells upon migration to secondary lymphoid tissues (81). DCs can uptake HIV virions and infect CD4$^+$ T cells through infectious synapse, and therefore contribute to pathogenesis of HIV. Interestingly, some bNAbs like 10–1074 and PGT121 have been shown to accumulate at virological synapses and block the transfer of viral material to uninfected T cells (82). However, the main role of DCs is to prime both cellular and humoral immunity. The use of new generation of bNAbs that show increased neutralization breath, and the presence of FcγRs on DCs, may contribute to efficient uptake of neutralized virus that is not infectious for DCs anymore. This may allow for the development of a similar vaccinal effect that has been proposed in the cancer field, which needs to be investigated in detail. It has been shown that treatment of SHIV-infected cynomolgus macaques with 3BNC117 and 10–1074 bNAbs for two weeks led to undetectable viral loads that were maintained for two to six months. Almost half of the animals behaved as controllers with undetectable viral loads, and the rest maintained a very low level of viremia for two years. Infusion of an anti-CD8β depleting antibody to controller animals resulted in specific decline of CD8$^+$ T cells and subsequent viral rebound, suggesting the existence of a vaccinal effect by these two potent bNAbs (83**). In line with this study, HIV-infected individuals with undetectable viral load were treated with 3BNC117 and 10–1074 bNAbs after analytical antiretroviral (ART) interruption (ATI). All participants developed enhanced Gag-specific CD8$^+$ T cell responses, and eight of nine participants had increased specific CD4$^+$ T cell responses (84**). The mechanism of generation of the adjuvant effect of these bNAbs at the presence of natural antigen and its effectiveness in long term control of HIV infection needs be investigated in more details, including the consideration of whether this can be modulated through the use of alternative Fc domains.

Conclusion

The absence of an effective vaccine has made bNAbs highly appealing in the field of HIV, as these antibodies can be used prophylactically to prevent infection. Several studies indicate that bnAbs may be effective as a prophylactic treatment but there is not yet a licensed product to this effect. The effect of a single dose of these antibodies can last for several months, which makes them a potentially attractive alternative to daily ART. bNAbs also hold potential to contribute to curing HIV infection, or achieving ART-free remission, as a result of targeting various effector cells to eliminate reservoir-harboring cells - an outcome that depends on the binding activities of both the Fab and Fc domains. New advances have allowed for the modification of Fab to generate multivalent antibodies, and the modification of Fc domains to substantially increase the binding of these antibodies to their corresponding Fc receptors. The use of one highly potent bNAb alone, will lead to temporary viral control and then viral escape mutation. Similar to the excellent effectiveness of combinational ART, the use of multiple engineered antibodies might be a new path towards the eradication of the HIV reservoir or durable suppression of viral replication.

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Key points

- The role of bNAbs in HIV reservoir control is not limited to neutralization as their Fc domains are involved in diverse effector functionalities.
- The use of alternative isotypes, or engineered Fc domains can enhance multiple functional aspects of bNAbs toward their potential application as prophylactic or therapeutic agents.
- The potential for passive administration of bNAbs to enhance virus-specific T-cell responses through a vaccinal effect holds promise as a strategy to achieve HIV remission.

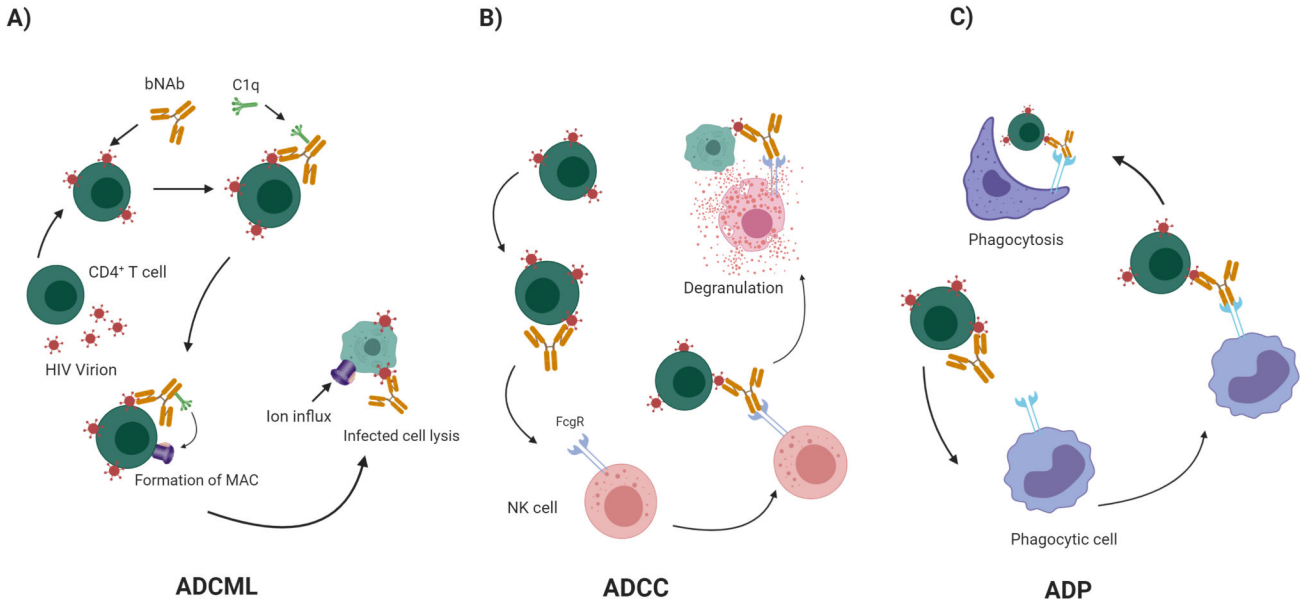

Figure 1: Antibody-dependent killing of infected CD4$^+$ T cells is mediated by bNAbs.

bNAbs initiate several effector functions through their Fc domain. A) Attachment of C1q to the Fc domain of bNAbs that have bound to HIV infected cells initiates the complement cascade, which leads to formation of MAC and lysis of the infected cells by ADCML. B) bNAbs bind to the Env glycoprotein on the surface of HIV-infected CD4$^+$ T cells. NK cells recognize infected cells and bind to Fc domain of the Ab through their FcγR. This binding allows their activation and degranulation, which leads to ADCC killing of the infected cells. B) Professional phagocytes express a diverse set of FcγRs on their surfaces that can bind to bNAb-coated infected cells, deriving them to eliminate infected cells by ADP.