Anti-HIV-1 antibody-dependent cellular cytotoxicity: is there more to antibodies than neutralization?

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Purpose of review
An increasing body of evidence suggests that nonneutralizing Fc effector functions including antibody-dependent cellular cytotoxicity (ADCC) contribute to protection against HIV-1 acquisition. We discuss recent advances in anti-HIV-1 ADCC research with a particular focus on ADCC mediated by Env-specific antibodies *in vitro* and *in vivo*, the curative potential of HIV-1-specific ADCC antibodies and the mechanisms of HIV-1 resistance to ADCC.

Recent findings
ADCC activities of broadly neutralizing and nonneutralizing monoclonal antibody panels were recently characterized *in vitro* against several lab-adapted and primary isolates of HIV-1. ADCC activity of these monoclonal antibodies generally correlated with binding to infected cells and were greater against the lab-adapted strains compared with primary HIV-1 isolates. Several recent studies in mouse and macaque models of HIV-1 infection suggest Fc-mediated effector functions contribute to the protective efficacy of broadly neutralizing antibodies and exert immune pressure on HIV-1 *in vivo*.

Summary
An increasing body of evidence suggests that ADCC-mediating antibodies, particularly when combined with neutralizing functions, can facilitate prevention and control of HIV-1. The precise mechanisms of partial protection conferred by nonneutralizing antibodies *in vivo* remain unclear and will need to be fully investigated in order to realize their full potential for HIV-1 vaccines.

Keywords
antibody-dependent cellular cytotoxicity, broadly neutralizing antibody Fc-mediated effector functions, nonneutralizing

INTRODUCTION

Research on humoral immunity to HIV-1 has largely focused on generating broadly neutralizing antibodies (bNAbs), which can recognize a diverse range of HIV-1 strains and block infection through binding to crucial 'sites of vulnerability' on the HIV-1 Env trimer [1,2]. Passive transfer of bNAbs can reliably protect macaques from chimeric simian–human immunodeficiency virus (SHIV) challenge and humanized mice from challenges with HIV-1 [3–10]. The induction of bNAbs is considered the 'holy grail' of HIV-1 vaccine research but has proven difficult to achieve by vaccination. In contrast, protection from HIV-1 acquisition in the modestly successful RV144 vaccine trial (31% efficacy) was not associated with bNAbs, but correlated with nonneutralizing antibodies against the variable 1 and 2 (V1V2) regions of Env [11,12]. Reduced risk of infection among vaccinees also correlated with anti-Env antibodies capable of mediating antibody-dependent cellular cytotoxicity (ADCC) in the presence of low plasma titers of anti-Env IgA. These immune correlates have spurred renewed interest in fragment crystallizable (Fc)-mediated nonneutralizing functions of HIV-1-specific antibodies.

HIV-1-specific ADCC involves the binding of the antigen-binding fragment (Fab) of antibodies to Env expressed on infected cells and the subsequent cross-linking of Fc gamma receptors (FcγR) on innate effector cells including natural killer (NK)

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KEY POINTS

- Broadly neutralizing and nonneutralizing monoclonal antibodies can mediate ADCC *in vitro* against cells infected with lab-adapted and primary isolates of HIV-1.
- Fc effector functions may contribute to the protective efficacy of broadly neutralizing antibodies against cell-free and cell-associated HIV-1 challenge in murine and nonhuman primate models.
- HIV-1 can evade ADCC by downregulating the expression of Env glycoproteins on the surface of infected cells and by concealing CD4-induced epitopes on Env that are highly targeted by antibodies induced in HIV-1 infection.

cells and monocytes [13,14]. The crosslinking of FcγRs activates these innate effector cells to secrete a series of cytokines and chemokines, and induces degranulation of cytotoxic granules containing perforin and granzyme B.

This review discusses recent advances in anti-HIV-1 ADCC research and highlights ADCC research presented at the 9th IAS Conference on HIV Science (IAS 2017). We present a particular focus on ADCC mediated by Env-specific monoclonal antibodies (mAbs) *in vitro* and *in vivo*, discuss the curative potential of HIV-1-specific ADCC antibodies and the mechanisms of resistance to ADCC.

ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY MEDIATED BY ENV-SPECIFIC MONOCLONAL ANTIBODIES *IN VITRO*

Over the years, an array of Env-specific mAbs have been isolated from HIV-1-infected patients [15]. These mAbs can generally be classified into neutralizing antibodies, which can bind to Env on HIV-1 virions and block infection, or nonneutralizing antibodies. Although the neutralization capacity of mAbs has been very well characterized with the standardized TZM-Bl neutralization assay against a large panel of HIV-1 strains, the ADCC potential of mAbs has been less well defined with the lack of a standardized ADCC assay across different research groups. It is still not known which ADCC assays are the most physiologically relevant or the most predictive of *in vivo* efficacy. Assays to measure ADCC activity vary greatly depending on a multitude of factors including the type of target cell used, conformation of Env, isotype subclass of the antibody and the type of effector cell used [13,16]. In particular, the conformation of Env, whether in its native closed trimeric state or open CD4-bound conformation with CD4i epitopes exposed, will govern whether many mAbs can bind and mediate ADCC.

It is important to note that neutralization and Fc-mediated effector functions are not mutually exclusive and can in fact overlap substantially. Von Bredow *et al.* [17] compared the ADCC and neutralization activities of a large panel of neutralizing and non-neutralizing mAbs directed against a range of different epitopes on HIV-1 Env. Although ADCC and neutralization were largely correlated, some mAbs were able to mediate ADCC in the absence of neutralization activity, and vice versa. ADCC in the absence of neutralization was observed particularly against cells infected with lab-adapted viruses, where certain Env epitopes that are absent on the HIV-1 virion were exposed on the surface of infected cells.

Another study characterized the in-vitro ADCC activity of a broad range of bNAbs against cells infected with the lab-adapted NLAD8 and NL4.3 strains and several primary transmitted/founder (T/F) HIV-1 isolates [18▪]. The ADCC activity of these bNAbs correlated positively with binding to infected cells and neutralization. The authors reported that the binding of bNAbs to cells infected with the primary T/F isolates was much lower compared with cells infected with the lab-adapted HIV-1 strains. The combination of five bNAbs targeting multiple different epitopes on Env improved binding and resulted in ADCC against cells infected with most of the T/F isolates, albeit at a lower level compared with cells infected with the lab-adapted strains. In line with a previous study showing that a combination of bNAbs improved neutralization potency and breadth against T/F viruses [19], these results suggest that bNAb combinations targeting different epitopes can enhance the potency and breadth of the ADCC response as well. In a similar study, Bruel *et al.* [20] compared the ADCC activity of nine nonneutralizing mAbs and five potent bNAbs. The authors found that although the nonneutralizing mAbs could mediate ADCC against cells infected with lab-adapted HIV-1 strains, they inefficiently recognized and killed cells infected with primary T/F isolates and ex-vivo reactivated cells from HIV-1^{+} patients. On the contrary, Mayr *et al.* [21] examined a different panel of nonneutralizing mAbs directed against the V1V2 region of Env and demonstrated that these nonneutralizing V1V2 mAbs mediated greater ADCC against cells infected with the primary HIV-1 isolate SF162 compared with several bNAbs tested. These V1V2 nonneutralizing mAbs shared similar specificities with antibodies that correlated with lowered risk of infection in the RV144 trial. The authors further demonstrated that the V1V2 mAbs could mediate ADCC against cells infected with

several subtype B and C primary HIV-1 isolates and two subtype B T/F isolates.

ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY MEDIATED BY ENV-SPECIFIC MONOCLONAL ANTIBODIES *IN VIVO*

Although ADCC activity can be relatively easily determined *in vitro*, the protective efficacy of ADCC is much more complicated to determine *in vivo*, where it might be difficult to separate Fc-dependent effects from Fab-dependent neutralizing functions. A seminal study published in 2007 demonstrated that FcγR engagement was important in bNAb-mediated protection against mucosal SHIV challenge in macaques [4]. The passive transfer of the LALA variant of b12, with mutations that abrogate FcγR-binding, resulted in significantly reduced protection against SHIV challenge compared with the wildtype b12 antibody. A further study using a more physiological low dose repeated SHIV challenge recapitulated these results and showed that b12-treated macaques remained protected from SHIV infection after nearly twice as many challenges compared with macaques treated with the LALA variant [22]. Parsons *et al.* [23▪] recently showed that the bNAb PGT121 was partially protective against cell-associated SHIV challenge in macaques. Cell-associated virus may be a significant mechanism of virus transmission [24,25] and ADCC effector functions may be important in preventing infection by this route (Fig. 1). Although Parsons *et al.* did not formally show that Fc effector functions contributed to this partial efficacy *in vivo*, *in vitro* experiments demonstrated that PGT121 could engage macaque FcγRs and activate macaque NK cells. Macaque effector peripheral blood mononuclear cells (PBMCs) were also able to mediate ADCC against HIV-1-infected target cells using PGT121. One of us (Kent) presented this work at IAS 2017 and noted the implications for the ongoing antibody-mediated prevention (AMP) efficacy trials (NCT02568215 and NCT02716675) [26]. It is possible that efficacy in these trials may be reduced if cell-associated transmissions of HIV-1 can evade the VRC01 bNAb infusions, as it has been demonstrated that VRC01 has reduced efficacy against cell-to-cell transmission of HIV-1 *in vitro* [25,27]. Further macaque experiments are planned to determine whether Fc effector functions can contribute to protection against cell-associated SHIV challenge by using the non-FcγR-binding LALA variant of PGT121.

The importance of FcγR-binding for bNAb-mediated protection *in vivo* has also been demonstrated in several studies utilizing mouse models of HIV-1 infection. Bournazos *et al.* [28] first showed that mice given the bNAb 10-1074 with the GASDALIE mutation, which improves affinity towards activating FcγRs, had lower viral loads following adoptive transfer of HIV-1-infected $CD4^+$ T cells compared with mice administered bNAbs with the GRLR mutation that abrogates FcγR-binding. In a similar study, ART-treated mice were administered with the bNAbs 3BNC117 and 10-1074 or the GRLR variants before

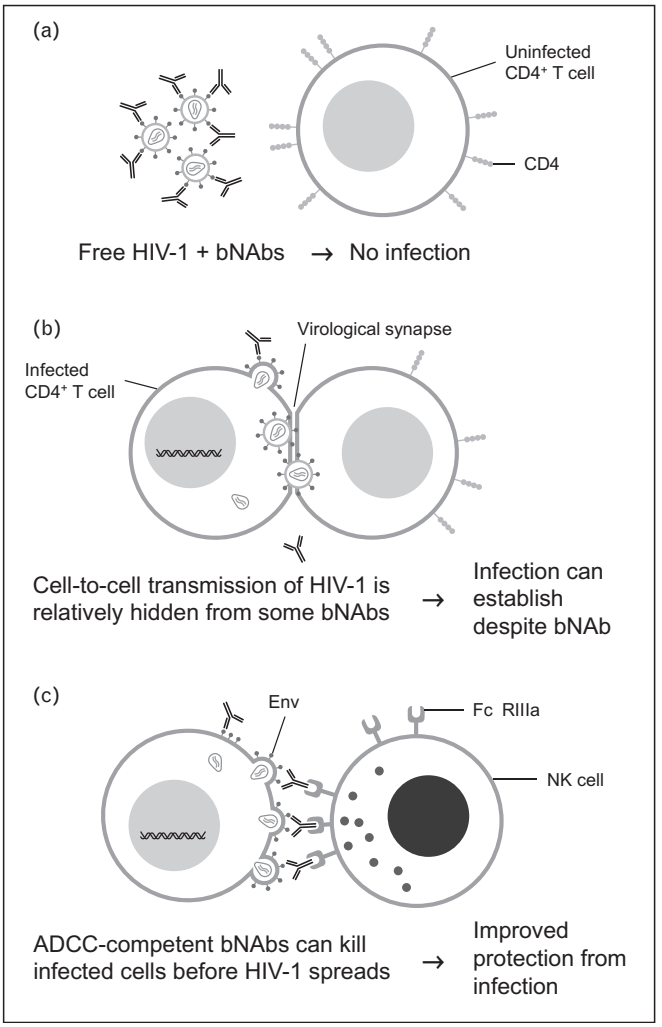

FIGURE 1. Mechanisms of protection mediated by broadly neutralizing antibodies. (a) bNAbs can prevent HIV-1 infection by directly neutralizing cell-free virions. (b) Cell-to-cell transmission of HIV-1 is a significant mode of viral transmission and is relatively hidden from some bNAbs [25]. bNAbs will need to access the virological synapse and neutralize virions to block cell-mediated spread of HIV-1. (c) ADCC-competent bNAbs might play a role in preventing cell-associated spread of HIV-1 by targeting Env on the surface of infected cells and killing the infected cells by engaging effector cells including NK cells. ADCC, antibody-dependent cellular cytotoxicity; bNAbs, broadly neutralizing antibodies; NK, natural killer.

being adoptively transferred with HIV-1-infected cells [29▪▪]. There was a significantly lower percentage of HIV-1 Gag^+ infected cells in the mice treated with wildtype bNAbs compared with mice receiving the GRLR variants. The authors provided further evidence that the bNAb-mediated depletion of infected cells *in vivo* was FcγR-dependent as this clearance was diminished when the mice were treated with FcγR-blocking antibodies. In a postexposure prophylaxis model, where humanized mice were treated with a triple mix of bNAbs 4 days after challenge with HIV-1, only 1 of 21 mice exhibited rebound in viremia in the group receiving wildtype bNAbs compared with 9 of 15 mice in the group receiving bNAbs with the GRLR mutation [30].

A recent murine study examined the *in vivo* ADCC activity of a broadly neutralizing CD4-antibody fusion protein that constituted of a subunit of the CD4 protein and an antibody domain specific for the gp120 coreceptor-binding site on each arm [31]. This fusion protein had improved binding to the neonatal FcR for increased half-life *in vivo* and a defucysolated IgG_1 Fc domain for increased affinity toward FcγRIIIa. This fusion protein eliminated more than 80% of HIV-1-infected cells *in vivo* in an infected humanized mouse model. Further experiments demonstrated that this *in vivo* ADCC was NK cell-dependent as clearance of infected cells was substantially diminished following depletion of $CD56^+$ NK cells.

The modest level of protection observed in the RV144 trial correlated with nonneutralizing antibodies against the V1V2 region of Env [12]; however, more definitive evidence of the protection conferred by these nonneutralizing antibodies *in vivo* is needed. Passive transfer of nonneutralizing mAbs has previously showed minimal or no protective efficacy against mucosal SHIV challenge and there has been limited direct evidence that these nonneutralizing antibodies can exert substantial immune pressure on HIV-1 *in vivo* [32–34]. A recent study by Horwitz *et al.* [35▪▪] showed that passive transfer of nonneutralizing antibodies could select for resistant viruses *in vivo* in an Fc-dependent manner. The nonneutralizing mAb 246D, directed against the gp41 stump, was able to modestly clear adoptively transferred $HIV\text{-}1_{YU2}$-infected cells compared with a control mAb. Although unable to suppress viremia in humanized mice with established $HIV\text{-}1_{YU2}$ infection, wildtype 246D was able to select for viruses with a recurring mutation that limited exposure of its gp41 epitope compared with the 246D-GRLR variant, which had abrogated FcγR-binding. Similar escape mutations were observed when a primary transmitted/founder isolate of HIV-1 was used. These results provide more conclusive evidence that nonneutralizing antibodies can select for viral escape mutants in an Fc-dependent manner.

HIV-1 CURE: POTENTIAL ROLE FOR ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY ANTIBODIES

Current HIV-1 treatment regimens are not curative as they do not eliminate the reservoir of long-lived latently infected cells that harbor quiescent but replication-competent proviruses [36]. Most HIV-1 cure research has focused on the 'shock and kill' cure approach that aims to reactivate HIV-1 expression in latently infected cells and subsequently kill the reactivated cells through immune-mediated mechanisms [37]. Several candidate latency-reversing agents (LRA) have been examined *in vivo* but none of them have reduced the size of the latent reservoir [38–40]. This suggests that additional immune interventions might need to be harnessed to help eliminate reactivated cells. An issue in utilizing autologous ADCC responses is that HIV-1-specific ADCC antibodies decline in patients on long-term ART [41,42]. A recent study also showed that treatment with panobinostat, a candidate LRA, did not provide sufficient antigenic stimulus to boost autologous anti-HIV-1 ADCC antibodies [43]. If autologous ADCC responses are insufficient to eliminate reactivated cells, the passive transfer of ADCC-competent bNAbs in conjunction with latency reversal might be a more promising approach.

Indeed, ex-vivo reactivated cells from ART-suppressed HIV-1-infected patients can serve as targets for ADCC mediated by bNAbs. Bruel *et al.* [18▪,20] show that a mixture of bNAbs or several potent bNAbs individually could mediate ADCC against reactivated HIV-1-infected $CD4^+$ T cells derived from $HIV\text{-}1^+$ donors. Although these results seem promising, the patient-derived $CD4^+$ T cells had to be stimulated with phytohemagglutinin and cultured for 7–12 days before HIV-1 Gag expression could be detected. Such high levels of reactivation may not be attainable *in vivo* with the current generation of LRAs.

The only demonstration of *in vivo* efficacy for the shock and kill approach using bNAbs has been in an HIV-1-infected humanized mouse model. In the study by Halper-Stromberg *et al.* [30], there was a decreased proportion of mice exhibiting viral rebound in the group treated with a combination of bNAbs (3BNC117, 10-1074 and PG16) and a combination of LRAs (vorinostat, I-BET151 and anti-CTLA4), compared with mice receiving bNAbs alone or mice receiving bNAbs and a single LRA. These results suggest that a combination of bNAbs

and sufficient reactivation of viral expression might be able to reduce the size of the latent reservoir. Further studies in nonhuman primate models or human clinical trials will need to be conducted to determine if this approach is feasible with a larger and more genetically diverse latent reservoir. Sufficient Env expression on the surface of reactivated cells will likely be necessary for bNAbs to target and eliminate the reactivated cells via ADCC.

POTENTIAL MECHANISMS OF RESISTANCE TO ANTI-HIV-1 ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY

Evans [44] summarized the various mechanisms of HIV-1 resistance to ADCC at IAS 2017. An important determinant of ADCC against HIV-1-infected cells is the antigen density of Env on the surface of infected cells for antibody-binding [45]. The expression of Env on the surface of HIV-1-infected cells is tightly regulated for the optimal level of Env incorporation into budding virions and for subsequent HIV-1 infectivity. The gp41 glycoprotein within Env contains a membrane-proximal endocytosis motif in its cytoplasmic tail that regulates the surface expression of Env prior to virus assembly [46]. Von Bredow *et al.* [47] showed that amino acid substitutions within this endocytosis motif results in accumulation of Env on the surface of the infected cell, increasing binding of Env-specific antibodies and directly increasing susceptibility to ADCC. In addition, HIV-1 Vpu can protect infected cells from ADCC by antagonizing tetherin, an interferon-inducible restriction factor that tethers budding virions to the surface of the infected cell. By blocking tetherin, Vpu prevents the accumulation of virions on the surface, thereby reducing the density of Env glycoproteins available for antibody-binding and ADCC [48,49].

Apart from directly reducing Env antigen density on the surface of infected cells, HIV-1 is able to limit the exposure of epitopes on Env for antibody-binding. Several studies now show that CD4-downregulation by Vpu and Nef prevents the interaction of Env and CD4 on the surface of infected cells, thereby reducing exposure of the CD4i inner domain epitopes that are highly targeted by ADCC antibodies present in HIV-1^+ sera [50,51]. Alsahafi *et al.* [52,53] demonstrated that Nef isolates from HIV-1 elite controllers more poorly downregulate CD4 from the surface of infected cells compared with HIV-1 progressors, resulting in increased exposure of CD4i epitopes and increased susceptibility to ADCC mediated by HIV-1^+ sera. The authors suggested that this enhanced ADCC against the viral isolates with impaired Nef could be a contributing factor to the prolonged suppression of viremia in the absence of ART for these elite controllers.

A potential way to overcome the concealment of CD4i ADCC epitopes on Env is to utilize small molecule CD4 mimetic compounds (CD4mc) that engage Env in a similar manner to soluble CD4 and trigger conformational changes that reveal the inner domain CD4i epitopes [54]. These CD4mc have been shown to enhance binding of antibodies within HIV-1^+ sera, breast milk and cervicovaginal fluids to infected cells and enhance ADCC against ex-vivo-amplified primary $CD4^+$ T cells using autologous effector cells and serum antibodies [55,56].

BREADTH OF ANTIBODY RECOGNITION AND FRAGMENT CRYSTALLIZABLE EFFECTOR FUNCTIONS AS IMPORTANT DETERMINANTS OF PROTECTIVE IMMUNITY

A clear limitation of some bNAbs in macaque passive transfer studies is whenever the bNAbs are not sufficiently broad to protect against the HIV-1 strain employed for challenge [57▪]. This has been mirrored in humans with passive administration of a bNAb therapeutically, where preexisting resistant viruses reduced the therapeutic efficacy of the bNAb VRC01 [58]. This issue of breadth of coverage will presumably also translate to the efficacy of antibodies that mediate nonneutralizing Fc effector functions but direct evidence is lacking. Using novel assays of Fc receptor binding, McLean *et al.* [59▪▪] recently found FcγR-binding antibodies from RV144 vaccinees had fairly modest recognition of a diverse panel of Env proteins. In contrast, HIV-1 infected patients who naturally control HIV-1 have been shown to have broad recognition of HIV-1 Env proteins by antibodies capable of engaging Fcγ receptors [60].

Antibodies can initiate a wide array of Fc-mediated functions through diverse innate effector cells. This subject, termed 'systems serology' was reviewed in the context of HIV-1 by Alter [61] at IAS 2017. For the RV144 trial, both Yates *et al.* [62] and Chung *et al.* [63] showed HIV-1-specific IgG3 was a key driver of Fc-mediated functions. Chung *et al.* [63] subsequently identified broad patterns in Fc-functional antibodies that correlated with protection from infection and the broad principle of this work was also applicable to antibodies against mycobacterium tuberculosis [64].

CONCLUSION

An increasing number of studies are dissecting the contributions of Fc-mediated effector functions to

antibody-mediated immunity against HIV-1. New methods developed to systematically dissect and analyze the humoral response towards HIV-1 may reveal new insights into the roles functional antibodies may play in vaccine-mediated protection against HIV-1 [59▪▪,63,65,66]. Both Fab and Fc arms of anti-HIV-1 antibodies will likely have important and complementary roles to play in HIV-1 prevention, therapeutic and curative strategies.

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Conflicts of interest

There are no conflicts of interest.

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