Natural Killer (NK) Cell Education Differentially Influences HIV Antibody-Dependent NK Cell Activation and Antibody-Dependent Cellular Cytotoxicity

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Immunotherapy using broadly neutralizing antibodies (bNAbs) endowed with Fc-mediated effector functions has been shown to be critical for protecting or controlling viral replication in animal models. In human, the RV144 Thai trial was the first trial to demonstrate a significant protection against HIV infection following vaccination. Analysis of the correlates of immune protection in this trial identified an association between the presence of antibody-dependent cellular cytotoxicity (ADCC) mediated by immunoglobulin G (IgG) antibodies (Abs) to HIV envelope (Env) V1/V2 loop structures and protection from infection, provided IgA Abs with competing specificity were not present. Systems serology analyses implicated a broader range of Ab-dependent functions in protection from HIV infection, including but not limited to ADCC and Ab-dependent NK cell activation (ADNKA) for secretion of IFN-γ and CCL4 and expression of the degranulation marker CD107a. The existence of such correlations in the absence of bNAbs in the RV144 trial suggest that NK cells could be instrumental in protecting against HIV infection by limiting viral spread through Fc-mediated functions such as ADCC and the production of antiviral cytokines/chemokines. Beside the engagement of FcyRIIIa or CD16 by the Fc portion of anti-Env IgG1 and IgG3 Abs, natural killer (NK) cells are also able to directly kill infected cells and produce cytokines/chemokines in an Ab-independent manner. Responsiveness of NK cells depends on the integration of activating and inhibitory signals through NK receptors, which is determined by a process during their development known as education. NK cell education requires the engagement of inhibitory NK receptors by their human leukocyte antigen ligands to establish tolerance to self while allowing NK cells to respond to self cells altered by virus infection, transformation, stress, and to allogeneic cells. Here, we review recent findings regarding the impact of inter-individual differences in NK cell education on AB-dependent functions such as ADCC and ADNKA, including what is known about the HIV Env epitope specificity of ADCC competent Abs and the conformation of HIV Env on target cells used for ADCC assays.

Keywords: natural killer cells, antibody-dependent cellular cytotoxicity, antibody-dependent natural killer cell activation, natural killer cell education, CD16, inhibitory natural killer cell receptors, non-neutralizing antibodies, broadly neutralizing antibodies
INTRODUCTION

There is great interest in developing an effective vaccine against HIV infection. It is generally acknowledged that inducing broadly neutralizing antibodies (bNAbs) would be a desirable goal for prophylactic HIV vaccines. The most potent bNAbs have been shown to protect against virus infection or to suppress viral replication in humanized mouse models and in rhesus macaques (1–6). Clinical trials conducted in HIV-infected humans, using the bNAbs VRC01 and 3BNC117, reduced HIV viral load by up to 2.5 logs (7, 8) and delayed viral rebound after antiretroviral therapy (ART) interruption (9, 10). However, there are still significant challenges to inducing such antibodies (Abs) through vaccination. BNAbs are rarely elicited in natural HIV infection and many exhibit high levels of affinity maturation (11–15). Despite this, progress has been made producing bNAbs in animal models using sequential cycles of boosting with defined immunogens (16). It is interesting to note that bNAbs able to protect humanized mice or rhesus macaques against challenge with HIV or simian/human immunodeficiency virus (SHIV) require an Fc region able to interact with Fc receptors (FcRs) on innate immune cells (17–19). One of these FcRs, FcyRIIIa, also known as CD16, is found on natural killer (NK) cells, macrophages, and monocyte subsets (20–22).

The HIV vaccine tested in the RV144 Thai trial is the only vaccine to date that conferred modest (approximately 31%) but significant protection against HIV infection (23). Protection was not associated with the presence of bNAbs or cytotoxic T cell responses (24). Rather, protection from HIV infection in trial participants was associated with the presence of anti-HIV envelope (Env) specific immunoglobulin G (IgG) non-neutralizing Abs (nNAbs) able to mediate Ab-dependent cellular cytotoxicity (ADCC) provided no potentially competitive IgA Abs were present (24–27). Follow-up analyses using systems serology approaches confirmed findings from correlation analyses and identified links between anti-Env V1/V2-specific IgG, IgG3, and IgG1, and Ab-dependent functions such as ADCC, Ab-dependent cellular phagocytosis, Ab-dependent complement deposition, and Ab-dependent NK cell activation (ADNKA) for secretion of IFN-γ and CCL4, and expression of CD107a in recipients of the RV144 vaccine (28). This raised the possibility that anti-HIV Env-specific nNAbs able to mediate ADCC and ADNKA activity may play a protective role against HIV infection.

Natural killer cells can be activated through Ab-dependent pathways that involve CD16 engagement by the Fc region of IgG1 and IgG3 Abs (29–35). They can also be activated by Ab-independent missing self recognition mechanisms based on how they were educated during development. Activating NK cells by either mechanism leads to secretion of chemokines and cytokines and to the release of cytotoxic granules that lyse target cells. ADNKA is the term used to describe the activation of NK cells for chemokine/cytokine secretion and degranulation by Ab-dependent stimuli. ADCC, on the other hand, denotes the lysis of target cells by NK cells in the presence of an Ab bridge. In the literature, these two activities have often been incorrectly referred to as ADCC. NK cells are important effector cells for these two Ab-dependent functions. Here, we will review recent findings on Ab-dependent functions mediated by NK cells and explore what is known regarding the influence of NK cell education on ADNKA and ADCC.

NK CELL EDUCATION

Tolerance to self and the state of activation of NK cells is determined by an ontogenic process termed education. NK cell education requires the interaction of inhibitory NK receptors (iNKRs) with their cognate human leukocyte antigen (HLA) ligands on neighboring cells (36, 37). The education of NK cells determines how these cells will respond to infected, transformed, stressed, and allogeneic cells in an Ab-independent fashion. Education is a complex process whereby functionality is tuned by the number of iNKRs engaged, the strength of interactions between iNKRs and their ligands, and whether activating NK cell receptors are also engaged (38–44). NK cells lacking iNKRs for self-HLA ligands remain uneducated and hyporesponsive (45). iNKRs involved in NK cell education include NKG2A and the killer immunoglobulin-like receptors (KIR)3DL1, KIR2DL1, KIR2DL2, and KIR2DL3 (see Table 1). NKG2A is a C-type lectin receptor that forms a heterodimer with CD94 (46, 47). It interacts with non-classical major histocompatibility complex class I (MHC-I) HLA-E antigens presenting 9-mer peptides cleaved from the leader sequence of several MHC-I proteins (48, 49). Both NKG2A and HLA-E have limited sequence variability and their effects on NK cell education were initially reported to be similar from one person to another (50). The inhibitory KIRs (iKIRs) recognize subsets of HLA antigens together with peptides (51). KIR3DL1 interacts with a subset of HLA-A and -B antigens belonging to the HLA-Bw4 (Bw4) group (52–54). Bw4 antigens differ from the remaining HLA-Bw6 (Bw6) HLA-B variants at amino acids 77–83 of the HLA heavy chain (55). Bw6 isoforms do not interact with KIR3DL1 receptors such that KIR3DL1+ NK cells from individuals carrying no Bw4 alleles are not educated through this receptor. KIR2DL3 and KIR2DL2 are encoded at the same locus and interact with HLA-C group 1 (C1) variants that have an asparagine at position 80 of the HLA heavy chain (56–58). The remaining HLA-C variants, belonging to the C2 group, have a lysine at this position and are ligands for KIR2DL1 (56). The KIR2DL3 receptor can also bind certain C2 variants, though with a lower affinity than either KIR2DL1 or KIR2DL2 (57, 59, 60). Therefore, KIR2DL3+ NK cells from individuals expressing a C1 ligand are educated, but remain uneducated or modestly educated through this receptor in individuals who are negative for C1 ligands. By contrast, KIR2DL1+ NK cells require the expression of a C2 ligand for education.

Genome-wide association studies (GWAS) confirm that genes influencing HIV viral load set point map to the MHC-I region on chromosome 6 (61, 62). MHC-I antigens encoded in this region form complexes with peptides, which are recognized by the T cell receptors on CD8+ T cells (63). It is well established that CD8+ T cells play an important role in HIV viral control (64–66). However, NKG2A and iKIR on NK cells also recognize MHC-I peptide complexes (48, 49, 52, 53, 56). Both epidemiological and functional studies have implicated iKIRs, particularly KIR3DL1, in combination with certain Bw4 variants in protection from HIV
TABLE 1 | Inhibitory natural killer (NK) cell receptors involved in NK cell education.

| Receptor | Ligand | aa at position 80 of the human leukocyte antigen (HLA) heavy chain | Effect on education when ligand is present | Ligand levels in HIV-infected cells | Reference |
|----------|--------|---------------------------------------------------------------|-----------------------------------------------|--------------------------------------|-----------|
| NKG2A    | HLA-E + leader peptide from HLA-A, -B, -C, and -G | Enhanced | Maintained | (48, 49) |
| Killer immunoglobulin-like receptors (KIR3DL1) | HLA-B*Bw4, HLA-A*23, 24, and 32 | Isoleucine (*80I) or threonine (*80T) | Enhanced | Downmodulated | (52-54) |
| KIR2DL1  | HLA-C2 | Lysine | Enhanced | Maintained or downmodulated depending on HIV isolate | (56-60) |
| KIR2DL2  | HLA-C1 (some HLA-C2) | Asparagine | Enhanced | Maintained or downmodulated depending on HIV isolate | (56-60) |
| KIR2DL3  | HLA-C1 (some HLA-C2) | Asparagine | Enhanced | Maintained or downmodulated depending on HIV isolate | (56-60) |

The influence of NK cell education in ADNKA

Antibody-dependent NK cell activation measures NK cell activation following incubation with Ab opsonized targets cells. Even though ADNKA depends on the presence of Ab, NK cell education can also influence NK cell activation through ADNKA. Many of the earlier reports describing a role for NK cell education in ADNKA used the CEM.NKr.CCR5 (CEM) cell line coated with recombinant HIV Env gp120 as target cells (76). CEM cells express the CCR5 co-receptor for HIV entry and are resistant to direct NK cell killing (77–79). CEM cells are negative for Bw4 and C2 antigens but express C1 antigens (80).

A higher frequency of KIR3DL1+, than KIR3DL1− NK cells, from carriers of KIR3DL1/Bw4 genetic combinations secrete IFN-γ and express CD107a in responses to anti-HIV Ab opsonized gp120-coated CEM. This differential activation of KIR3DL1+ and KIR3DL1− NK cell populations also occurs when the stimulus is HIV-infected or gp120-coated allogeneic primary CD4+ T cells (76). As well, a higher frequency of KIR2DL1+ than KIR2DL1− NK cells from carriers of educating KIR2DL1/HLA-C2 combinations secrete IFN-γ in response to HIV-infected autologous targets and gp120-coated CEM cells in the presence of anti-HIV Env-specific Abs in plasma from HIV+ individuals (81). By contrast, if NK cells are from carriers of the non-educating KIR/HLA pair KIR2DL1/C1 hmnz, KIR2DL1+ and KIR2DL1− NK cells respond to anti-HIV Ab-dependent stimulation equivalently (81). These observations implicate NK cell education in NK cell responses to anti-HIV Ab opsonized gp120-coated CEM cells, infected allogeneic CEM cells, and gp120-coated primary CD4+ T cells. CD16 engagement is also important in ADNKA activity as NK cell activation is always higher in the presence versus absence anti-HIV-specific Abs.
NK cell populations respond more robustly than their uneducated counterparts to HIV-infected cells that secrete cytotoxic granules that can lyse HIV-infected target cells (83). It is plausible that ADCC activity directed at allogeneic HIV-infected cells contributed to the modest protection conferred by the RV144 HIV vaccine trial, in which ADCC competent anti-HIV Env-specific Abs were generated and to the protection conferred to infants who remain uninfected despite exposure to breast milk from HIV-infected mothers (24, 84).

There is a lack of consensus regarding whether educated NK cell populations respond more robustly than their uneducated counterparts to stimulation with anti-HIV oposzed autologous gp120-coated cells. KIR3DL1+ and KIR2DL1+ NK cells from carriers of KIR/HLA combinations able to support education through these receptors have been reported to respond better that their uneducated counterparts to HIV Ab-dependent activation (81, 85). These findings are consistent with results reported by Lang et al. (86). These observations have been interpreted as evidence that Ab-dependent activation of NK cells can overcome inhibitory signals mediated by the interaction of HLA ligand binding to self iKIR. However, this is not a general finding in that others have noted that ligands on autologous target cells to iNKR on educated NK cells suppress the activity of educated NK cells compared to that of their uneducated counterparts (87, 88). Further research is needed to understand what accounts for these discrepant results.

The experiments describing ADNKA in this section have used an inclusive gating strategy to compare how NK cell populations expressing, or not, one iNKR respond to anti-HIV oposzed target cells. When NK cells are stained inclusively for the presence of a single iNKR, the targeted population includes NK cells expressing other iNKRs not stained for. These other iNKRs could influence NK cell responses to HIV Ab oposzed target cells depending on which iNKR/HLA receptor ligand pairs contributed to the education of the NK cells studied. By using an Ab panel detecting KIR3DL1, KIR2DL1, KIR2DL3, and NKG2A on CD3+CD56dim NK cells, it would be possible to focus on NK cell populations expressing one of these iNKRs to the exclusion of the others. Such Ab panels that also detect multiple NK cell functions using Abs conjugated with different fluorochrome have been designed (89, 90). In future studies, these Ab panels should be used to exclusively gate on NK cell populations expressing single iNKRs that detect functions induced by anti-HIV Ab oposzed target cells. Such an experimental approach will allow for a more precise definition of NK cell responses within population expressing single educating receptors to activation through missing self recognition of the ligands for these iKIR on allogeneic CEM cells in addition to signals received via ligation of CD16 (91).

The frequency of NK cells responding to stimulation in ADNKA assays displays inter-individual variation. One possible mechanism underlying the range of NK cell effector responses in ADNKA assays is likely related to inter-individual differences in iNKR/HLA ligand effects on NK cell education. KIR3DL1 allotypes differ in their cell surface expression levels, with high, low, and null expression allotype groups (69, 92–95). These KIR3DL1 allotypes also differ in their affinity for particular HLA-B allotypes (44, 96). KIR2D receptors differ in their affinity for C1 and C2 antigens (57, 60). HLA-A, -B, and -C antigens also differ in their cell surface expression levels (44, 72, 97). Thus, these factors, the number of iNKR/HLA pairs participating in NK cell education in each study subject, and the presence of ligands on CEM cells that provide, or that fail to provide, inhibitory signals to NK cells may all influence NK cell activation levels in ADNKA assays. Several authors have tested expression levels for HLA-B and C allotypes and have examined the avidity of interactions of high and low expression KIR3DL1 receptor groups for HLA-B antigens with either an isoleucine or a threonine at position 80 of the HLA heavy chain (44, 69, 72, 98, 99). The putative influence of inter-personal immunogenetics on ADNKA activity could be explored by correlating ADNKA activation levels with KIR3DL1/HLA-B, KIR2DL1/HLA-C2, and KIR2DL3/HLA-C1 affinity and expression levels as has been described by Boudreau et al. (44). For ADNKA, activation through education-dependent missing self-recognition and CD16 signaling influence NK cell activation while for ADCC the effect of education-dependent missing self-recognition is minimized due to the low frequency of single positive (SP) iKIR+ NK cells positive for CD16. The comparison of assay results where one or more of these receptor ligand interactions is blocked may provide further insights into the role of signaling through iNKR or CD16 in ADNKA and ADCC.

**MEASURING ADCC ACTIVITY**

As opposed to ADNKA, ADCC measures target cell phenomena arising from the bridging of effector and target cells by an Ab whose Fc portion binds CD16 on effector cells and whose Fab portion recognizes an antigen on target cells. In the context of ADCC function-directed HIV Env gp120-coated target cells, the target antigens recognized by ADCC competent Abs are HIV Env (30, 78, 100). ADCC activity directed to HIV infected may also recognize Tat (100).

Early versions of anti-HIV ADCC assays measured 51Chromium release from target cells (101–103). These have been replaced by flow cytometry-based assays using either CEM cells coated with gp120 or gp140, HIV-infected CEM or HIV-infected primary CD4+ T cells as target cells. Primary HIV-infected target cells have included reactivated CD4+ T cells from HIV-infected subjects or CD4+ T cells infected with transmitted/founder (T/F) HIV isolates (104–106). The GranToxiLux ADCC (GTL-ADCC) assay measures the delivery of granzyme B (GzB) to target cells, an early step in the pathway leading to target cell lysis (83, 107, 108). In the GTL-ADCC assay, target cells are labeled with fluorescent and viability dyes before incubation with effector cells, either peripheral blood mononuclear cells (PBMCs) or NK cells in the presence of HIV-specific ADCC competent Abs and a GzB substrate. If ADCC is induced following incubation with HIV-specific Abs, effector cells will release GzB that will enter target cells and hydrolyze the GzB substrate, activating its fluorescence, which can be detected by flow cytometry. Thus, the GTL-ADCC assay provides an estimate of ADCC activity.
by measuring the number of viable targets that are positive for proteolytically active GzB.

Read outs for ADCC assays include the loss of target cells loaded with a fluorescent marker, infected with green fluorescent protein-tagged HIV, luciferase tagged HIV, or Gag p24+ cells (105, 106, 108–113). The lactate dehydrogenase (LDH) release ADCC assay measures the loss LDH from dying target cells by ELISA (76, 114). The widely used rapid and fluorometric ADCC has been shown to not measure ADCC but rather the uptake of the membrane dye PKH-26 used to label target cells by monocyte-mediated trogocytosis (115, 116).

**THE SPECIFICITY OF ANTI-HIV ADCC COMPETENT Abs**

Both bNAb and nNAb can mediate ADCC activity provided they can stably bind to target cells (105, 106, 113, 117–121). HIV Env epitopes targeted by nNAb include the immunodominant region of gp41 (122) and CD4-induced (CD4i) epitopes exposed by CD4 ligation of HIV Env on infected cells (111, 123, 124). Examples of prototypic anti-Env-specific Abs specific for a CD4i epitope is A32, which belongs to the anti-cluster A Ab group targeting the C1/C2 region and 17b, which recognizes the co-receptor binding site (CoRBS) (119, 125). Other nNAb have been reported to recognize the CD4bs and the V3 loop of gp120, which are also targeted by bNAb, though the nNAb bind these epitopes in a manner that does not prevent HIV entry (126–130). At least some of the epitopes targeted by ADCC competent nNAb are poorly exposed on CD4 unliganded cell surface Env trimers. This is mainly due to accessory proteins Nef and Vpu that downregulate cell surface CD4 making CD4i epitopes unavailable for Ab recognition (111, 120, 131, 132). Bruel et al. found that CEM cells infected with two laboratory-adapted HIV strains bound Abs from several classes of bNAb and nNAb epitope specificity. If binding occurred, these Abs usually also mediated ADCC activity against these infected cells (106). However, when reactivated, HIV-infected cells from the reservoir of ART-treated HIV+ individuals or CD4+ T cells infected with T/F strains were used as target cells, several monoclonal nNAb bound a lower frequency of infected cells with a lower affinity than did bNAb. Furthermore, nNAb, compared to bNAb, exhibited poor ADCC activity against infected with such primary HIV strains (105, 106, 113, 117, 133). This phenomenon is likely related to the inability of nNAb to access epitopes in the closed unliganded conformation of HIV Env (134).

Non-neutralizing Abs, particularly those specific for CD4i epitopes, preferentially bind HIV-infected bystander cells present in cultures with HIV+ CD4+ T cells (106, 135, 136). HIV-infected CD4+ T cells can shed HIV Env gp120 leaving behind gp41 stumps (136). The shed gp120 binds CD4 on the surface of uninfected bystander CD4+ T cells. This interaction has the potential to open the closed Env conformation exposing CD4i epitopes, making bystander enhanced targets for CD4i-specific ADCC competent Abs.

Strategies to improve the targeting of the open Env conformation by ADCC competent nNAb has prompted exploring the use of CD4 mimetics to increase the susceptibility of HIV-infected cells to ADCC (106, 135, 137, 138). Richard et al. worked with CD4 mimetics that were unable to enhance the recognition of HIV-infected cells to A32 Abs by themselves (138). However, these small molecules initiated the opening of Env trimers enough to permit the binding of Abs such as 17b with specificity for a conserved epitope overlapping the CoRBS. Once 17b bound, the trimeric Env structure opened sufficiently to allow binding of A32 and susceptibility to ADCC activity (138).

It should be noted that most studies measuring anti-HIV ADCC activity have used gp120- or gp140-coated CEM cells as targets. While such targets are easy to prepare and convenient to use, the HIV Env on coated cells is monomeric and differs quantitatively and conformationally from trimeric Env found on the surface of HIV-infected cells. On coated cells, CD4 remains on the target cell surface while it is downregulated on infected cells unless Nef and/or Vpu HIV deletion mutants are used for infection. This needs to be kept in mind when interpreting the results of studies using coated cells as targets.

**THE INFLUENCE OF NK CELL EDUCATION ON ADCC ACTIVITY**

The GTL-ADCC assay using gp120-coated CEM cells as targets was used to show that education of effector populations through KIR3DL1 had no significant effect on the percent of GzB+ (%GzB+) target cells generated in a GTL-ADCC assay (139). There may be several explanations for this observation. One possibility is that NK cells are not the main effector cell in the GTL-ADCC assay. A drawback of using PBMCs as effector cells in ADCC assays is that it is difficult to draw conclusions regarding which effector population is responsible for GzB delivery to the target cells. Several Fcy receptor-expressing cell types, including NK cells, monocytes/macrophages, and γδ T cells, are capable of mediating ADCC (107, 115, 140–144). To confirm that NK cells are the source of ADCC activity in the GTL-ADCC assay, Pollara et al. used effector PBMCs depleted of CD56−CD16+ NK cells and observed that ADCC responses declined by over 66% (145, 146). Purified NK cells and PBMCs from the same donors produced similar %GzB+ target cells (107). Together, these findings indicate that the GTL-ADCC assay is measuring NK cell-mediated ADCC responses.

In the GTL-ADCC assay, PBMC effector cells are a heterogeneous population that includes NK cells educated through 1, 2, or more iKIR and/or NKG2A. An Ab panel detecting KIR3DL1, KIR2DL1, KIR2DL3, and NKG2A on CD3−CD56dim NK cells was used to gate exclusively on SPiNKR+ NK cells. NK cells SP for iKIR had significantly lower frequencies of CD16− cells than did SPNKG2A+ or NKG2A−iKIR− NK cells (147). iKIR+ NK cells are educated if they develop in a setting in which the iKIR’s ligand is co-expressed. The implication of this observation is that educated SPiKIR+ NK cells would be poor ADCC effector cells as a median of <5% of them are CD16+ (Figure 1). This could account for the lack of an effect of KIR3DL1-mediated NK cell education on the %GzB+ target cells generated in the GTL-ADCC assay (139, 147). Thus, it would be expected that NKG2A+ NK cells are superior...
to iKIR+NKG2A− NK cells as effector cells in the GTL-ADCC assay. NKG2A/HLA-E interactions educate NKG2A+ NK cells and these receptor ligand pairs are widely expressed with limited inter-individual variation. Their influence on NK cell education would have limited between-subject variation. If ADCC activity is an important correlate of protection against HIV, these findings suggest that inter-individual variation in NK effector cell education based on which iKIR/HLA receptor/ligand pairs are present would have a minimal impact on ADCC potency at the level of HIV-infected target cell lysis or suppression of replication. Together, these findings illustrate that the potency of NK cell education and functional activation of NK effector cells does not predict the %GzB+ generated by ADCC.

In summary, factors important in determining ADNKA and ADCC activity differ from each other. The role of NK cell education in ADCC activity is limited by the low frequency of CD16+ NK cells among SPiKIR+ NK cells that have the potential to be educated through iKIR/HLA ligand interactions. Thus, a higher frequency of either uneducated NK cells or NK cells educated through NKG2A than those educated through iKIR are CD16+ and able to mediate ADCC. On the other hand, both CD16 engagement and missing self-recognition contribute to ADNKA. The consequences of these findings for HIV vaccines is that NK cell education should contribute minimally to inter-individual differences in target cell lysis by ADCC. Furthermore, NK cell activation by Ab-dependent HIV-infected cell stimuli will vary depending on how NK cells are educated, the nature of the stimulatory cell and effect of HIV infection on cell surface MHC-I expression (90, 148).

CONCLUDING REMARKS

Arguing for a role for anti-HIV ADNKA and/or ADCC activity in protection from infection are the findings from the RV144 vaccine trial, which identified ADCC activity as a correlate of protection that was frequently linked to ADNKA activity (24, 27, 28). Moreover, antigenic drift from ADCC targeting Env epitopes has been documented, highlighting a role for ADCC being able to exert anti-HIV immune pressure (149). Of note, it is unlikely that bNAbs contributed to either of these findings as neither RV144 vaccinated individuals (24) nor most HIV+ persons make HIV-specific bNAbs. Suppression of HIV viral load in HIV-infected persons receiving the bNAb 3BNC117 is likely not solely due to virus neutralization as this treatment also appears to clear infected cells (133). Also, the beneficial effect of treatment with several bNAbs depends on IgG Fc region effects (17–19). On the other hand, several attempts to show that nNAds can protect against infection in rhesus macaques infected with SHIV have failed, though passive transfer of these Abs may have suppressed viremia or restricted the number of T/F viruses in some cases (122, 150–152). By contrast, the passive transfer of the most active bNAds mediates sterilizing protection in primate models (1–6). The protective role of anti-HIV nNAds and/or how to manipulate the ability of these Abs to protect from HIV infection or how to use them therapeutically is an active area of research with several questions left to answer.

AUthOR CONTRIBUTIONS

Substantial contributions to the conception or design of the work (NB, ZK, AT-M, CL, SK, and FD). Drafting the work or revising it critically for important intellectual content (NB, ZK, AT-M, CL, SK, and FD). Final approval of the version to be published (NB, ZK, AT-M, CL, SK, and FD). Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved (NB, ZK, AT-M, CL, SK, and FD).

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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