Obstacles to ideal anti-HIV antibody-dependent cellular cytotoxicity responses

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Abstract

A safe and effective vaccine against HIV is a global health priority. Large-scale phase III clinical vaccine trials based on neutralizing antibodies and cytotoxic T-lymphocytes have failed to provide protection, highlighting the lack of understanding of basic immune correlates of protection against HIV. The partial success of the RV144 vaccine trial, however, sparked an intense research effort to identify and describe the protective potential of non-neutralizing antibodies. Correlates of protection analyses have identified antibodies that induced antibody-dependent cellular cytotoxicity (ADCC) as potentially important. Despite the attractiveness of utilizing ADCC antibodies for HIV vaccine design, it is important to note that effective ADCC responses are contingent on many factors. As discussed in this review, these factors are important considerations for determining the feasibility of designing an optimal ADCC antibody-inducing vaccine construct. Important determinants of ADCC responses include characteristics of the antibody, such as isotype and subclass, antigen-specificity, titer, durability and glycosylation of the constant region. Second, ADCC immune responses are highly contingent on the natural killer (NK) cell effectors. This review will describe the current state of knowledge regarding the ontogeny of NK cells, highlighting the continuous "education" they undergo that determines their functional potential upon stimulation. Other important NK cell factors, such as constant region receptor polymorphisms, cellular exhaustion, and the effects of the cytokine milieu on cellular function, will also be covered. Finally, an exciting, but yet untested, role for NK cell-mediated ADCC lies in its potential ability to eliminate latently infected cells, which harbor the viral reservoir. The review will address the potential of a two-pronged attack, where latently infected cells are induced to express HIV antigens and then eliminated by NK cells via an ADCC mechanism, with the goal of inducing a cure.

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1. Introduction

Human immunodeficiency virus (HIV) infection is a global health concern. There are currently over 30 million HIV-infected people, and United Nations AIDS (UNAIDS) reports over two million new infections annually [1]. Due to these staggering numbers, there is an urgent need for novel prophylactic vaccine constructs and innovative therapies for eliminating viral reservoirs from HIV-infected people. This review will discuss the evidence behind designing antibody (Ab)-based vaccines/therapies against HIV. The difficulties in eliciting anti-HIV neutralizing Abs and the protective potential of non-neutralizing Abs will be addressed. The evidence identifying Ab-dependent cellular cytotoxicity (ADCC) as an important non-neutralizing Ab-triggered effector function against HIV, as well as the factors that determine the strength of ADCC responses will be considered. Lastly, the review will integrate the many factors influencing ADCC for the design of therapeutics to eliminate reactivated latent HIV or for the design of prophylactic vaccines.

2. Antibody approaches to HIV vaccines

The most promising research direction for designing an effective vaccine against HIV has been broadly neutralizing antibodies (BnAbs). These Abs are capable of neutralizing a wide array of viral isolates, both within and across HIV subtypes [2]. The production of Abs with this breadth of neutralization potential occurs in approximately 20% of infected individuals [3]. A growing number of monoclonal prototypes for these BnAbs have been discovered, purified and mass-produced from a subset of such HIV-infected individuals. These Abs are directed against an array of epitopes on the HIV envelope (Env), which consists of homotrimers of the non-covalently associated gp120 and gp41, including the CD4 binding-site of gp120 [4], the glycan shield [5], the membrane proximal external region of gp41 [6] and epitopes dependent on trimer formation [2]. The efficacy of BnAbs in preventing infection has been demonstrated in primate models utilizing the chimeric simian-human immunodeficiency virus (SHIV) [7–10]. Passive transfer of BnAbs to rhesus macaques prior to challenge with SHIV has repeatedly been demonstrated to provide sterilizing immunity. Given this remarkable observation, much consideration has been given to designing vaccines capable of eliciting anti-HIV BnAbs through vaccination. Unfortunately, a BnAb-inducing vaccine has yet to be designed. It is generally thought that novel vaccination constructs and strategies will be required to elicit Abs with the extensive somatic hypermutation observed on BnAbs [11].

3. ADCC antibodies against HIV

Despite the lack of success in designing a BnAb-inducing vaccine there has been a recent surge of interest in Abs capable of targeting HIV via neutralization-independent mechanisms. In particular, there have been significant studies on Abs capable of soliciting the help of cells of the innate immune system, such as natural killer (NK) cells, to detect and respond to Ab bound to viral antigens on infected cells via a mechanism termed ADCC. This interest has mainly been driven by the results of the recent RV144 HIV vaccine efficacy trial in Thailand, which revealed modest protection against HIV infection in the absence of strong or broad neutralizing Ab or cytotoxic T-lymphocyte responses [12]. The vaccine regimen, however, did induce Abs capable of activating NK cells for ADCC [13,14]. Furthermore, the presence of ADCC Abs in vaccinees was associated with protection from infection, as long as the Ab carriers also had low levels of anti-Env IgA [15], which has the potential to inhibit ADCC by blocking access to Env epitopes by anti-viral IgG [16].

While the potential role of ADCC in the efficacy of the RV144 trial provides some of the most robust evidence that this immune mechanism could be useful for prophylactic vaccination against HIV, there is also a large body of literature demonstrating the potential of ADCC to target HIV and result in positive outcomes. Indeed, Abs capable of mediating anti-HIV ADCC have been observed in highly exposed seronegative individuals (HESN) [17], higher titers of ADCC Abs have been reported in elite controllers [18], ADCC Abs that target a wider array of antigens have also been observed in slow progressors [19], and immunodeficiency virus-infected macaques that have slow progressing infections sustain higher ADCC responses than animals with more rapid disease progression [20]. Furthermore, and perhaps most impressive, elegant experiments have revealed that some of the protection conferred by passively transferred BnAbs may be a result of ADCC. Indeed, modification of the anti-CD4 binding site BnAb, b12, so it could not initiate NK cell-mediated ADCC resulted in a diminished capability of the Ab to protect macaques from SHIV infection [21].

Despite the assortment of positive data regarding the potential of ADCC as a prophylactic anti-HIV immune response, many questions remain about how to most appropriately and successfully induce and maintain potent ADCC-based immunity against HIV. There are numerous factors that can influence the NK cell effector functions elicited by anti-viral Abs. The ability of Abs to activate NK cells for ADCC is determined by characteristics of the Abs themselves, exogenous factors that influence the functional potential of NK cells, as well as several intrinsic features of NK cells. One pertinent issue is that vaccines against HIV that induce anti-viral Abs are notorious for their inability to maintain sufficient Ab titers [22,23]. Indeed, Ab titers induced by the RV144 regimen dropped off rapidly within 6 months of the last vaccination, an occurrence that may be related to the reduction in efficacy over time [22,24]. Further concerns are raised by the prejudice of the ADCC functional potential of NK cells by genetic determinants through an ontological process termed education [25–28]. As such, it is uncertain that all individuals carry NK cells capable of mediating sufficient Ab-dependent effector functions to provide protection against incoming viral infections via this immune response. Nonetheless, much research is being conducted to determine the factors necessary for the occurrence of an ideal ADCC response, which could one day be implemented as ammunition against HIV.

4. Ab factors determining ADCC

4.1. Ab isotype and subclass

The mediation of ADCC by NK cells occurs through the ligation of the low affinity receptor for the constant region (Fc) of IgG (i.e.,
CD16 or FcγRIIIa) by antigen-bound Abs. The CD16 receptor recognizes subclasses within the IgG isotype with differing affinities (i.e., IgG3 > IgG1 > IgG4 > IgG2) [29]. Consistent with these differing affinities, IgG1 and IgG3 Abs activate NK cells to mediate more robust ADCC [30]. In terms of anti-HIV ADCC, IgG1 Abs are the most potent inducers of ADCC [31].

It should be noted that a small number of studies have demonstrated that NK cells also express receptors that bind the constant regions of IgM and IgA [32,33]. Furthermore, the receptor for the constant region of IgA triggers Ab-dependent signaling upon ligation [34]. The contribution of this receptor to NK cell-mediated ADCC, however, is less understood/established than the contribution of CD16. In addition, IgA can compete with IgG for antigen binding and reduce the observed ADCC [16]. Given the conflicting data regarding the role of IgA in triggering NK cell-mediated ADCC and the robust data regarding the ability of IgG to trigger NK cell-mediated ADCC, future vaccine constructs may benefit from inducing IgG-biased Ab responses. Furthermore, as IgG subclasses that are poor inducers of ADCC (i.e., IgG2 & IgG4) could theoretically also compete for antigen binding sites and inhibit ADCC mediated by IgG subclasses that induce robust ADCC (i.e., IgG1 and IgG3), vaccine constructs designed to bias the Ab response towards IgG1 and IgG3 production are desired.

4.2. Titer and clonal nature of Ab response

Although most individuals infected with HIV carry at least some Abs capable of activating NK cells through CD16 [34], positive outcomes in the context of HIV infection are usually observed in individuals with high titers of such Abs [18]. Assessments of the role of ADCC in protection from disease progression and protection of infants from mother to child transmission via breastfeeding have revealed that ADCC Ab titers and/or ADCC response magnitudes are directly associated with protective outcomes [18,35]. Corroborating this evidence is the demonstration that the titers of ADCC-competent Env-binding IgG and/or magnitudes of ADCC responses in primate models of HIV infection have been shown to correlate with higher CD4+ T-cell counts and protection from infection [20,36]. Further, Smalls-Mantey et al. recently demonstrated that the magnitude of ADCC observed is proportional to the quantity of anti-Env Ab bound to the surface of HIV ADC target cells [37]. In conjunction with data suggesting high ADCC titers are desirable, the work by Smalls-Mantey et al. suggests that it may be desirable for these high titers to consist of a polyclonal Ab population. This would allow the clustering of Abs directed against different Env epitopes on the limiting number of viral spikes on the cell surface. Indeed, experiments comparing purified polyclonal IgG to polyclonal anti-HIV Abs demonstrated the importance of polyclonal anti-viral Ab responses for effective ADCC.

Coinciding with the evidence suggesting polyclonal ADCC-competent Ab levels, important for the magnitude of ADCC observed, are several features of the ADCC responses observed in RV1414 trial vaccine recipients. The vaccine regimen induced ADCC-competent Abs against several Env epitopes [14]. The ADCC responses were primarily directed to conformational epitopes recognized by the A32 monoclonal Ab to the C1 region of Env, but also included non-A32 recognized epitopes. Furthermore, the titers of Abs in the sera of these vaccine recipients declined quickly after the final immunization [22]. This decrease in Ab titer with duration since final immunization coincides with decreased vaccine efficacy with time since last immunization [24]. Although the RV1414 trial was reported to have mediated 31% efficacy over a follow up of 42 months, if the trial had been powered to study efficacy at 12 months after the first immunization (6 months after the last immunization), 60% efficacy could have been reported [24]. It has been speculated that the vaccine may have lost its protective capacity as the induced Ab titers waned [38]. Combined with the demonstration that vaccinated individuals developed polyclonal anti-Env Ab responses directed against an array of epitopes [14], it appears as if maintaining high titers of ADCC Abs and/or high ADCC responses, which is associated with positive outcomes during chronic infection and childhood exposure to HIV through breast milk [18,35], was also important in RV144-induced ADCC responses.

The notion that sustained high titers of ADCC-competent Abs is important for immune-mediated protection from immunodeficiency virus infection has also been supported in primate studies. Indeed, a recent report by Alpert et al. demonstrated that protection of macaques vaccinated with attenuated SIV was linked with the level of Abs capable of mediating ADCC [36]. Furthermore, the level of ADCC Abs was higher and more sustained in the macaques vaccinated with the attenuated virus compared to macaques vaccinated with a non-persistent SIV vaccine construct. These observations corroborate the previously mentioned association of lower efficacy of the RV144 vaccine with duration from last immunization [24], which coincided with the waning of Ab titers [22]. Cumulatively, these results highlight the importance of the titer of ADCC-competent Abs for elicitation of NK cell-mediated ADCC responses, and suggest that the efficacy of vaccines inducing ADCC Abs is dependent upon sustaining high titers of these Abs post immunization.

4.3. Ab Fc glycosylation

Further to variable region features, the glycosylation profile of the constant region of an Ab can greatly alter the response elicited by interaction with the innate immune system. Glycosylation of asparagine 297 residues impacts the structure of the Ab, as well as influences Fc-dependent effector functions [39]. The exact composition of the glycovariants, however, determines the particular innate effector cell populations utilized and the inflammatory or anti-inflammatory activity produced by the Ab. The sera IgG of healthy individuals can contain between 30 and 40 of these glycovariants, and it has been demonstrated that dramatic alteration of IgG glycosylation profiles is linked to disease activity in a number of autoimmune conditions, such as rheumatoid arthritis and osteoarthritis [40–42]. The presence, absence, or quantity of three major sugar residues (i.e., fucose, galactose and sialic acid) within the glycans has been shown to alter ADCC activity.

4.4. Galactose

There have been conflicting reports regarding the effect of galactosylation of Ab Fcs, with some groups reporting enhanced ADCC activity while others not observing this effect [43–46].

4.5. Fucose

Fucosylation of Ab glycans has been linked to pro-inflammatory Ab functions. Though it is the removal of fucose residues that has been shown to increase the affinity of IgG for the activating receptor FcyRIIIa and increase ADCC activity up to a 100-fold [46,47].

4.6. Sialic acid

The addition of certain sugar residues can hinder the ADCC function of Abs. For example, the addition of sialic acid to the glycan residues confers an anti-inflammatory function to the Ab. Sialylation of IgGs hinders their FcyRIIIa binding and therefore ADCC mediating activity [48].

A number of studies have explored glycoengineering of IgGs for enhanced effector functions in the context of immunotherapy [49,50]. Changes in serum IgG glycosylation is observed during
pregnancy, ageing, as well as during a number of disease states. For this reason modulation of IgG glycosylation is thought to be an active process [51,52]. The process of Ab glycosylation, however, is poorly understood and the elicitation of Abs with specific glycosylation profiles by vaccination will require further research into the control of this process.

4.7. Ideal ADCC Abs

Collectively, the reviewed literature constructs a biochemical definition of the ideal ADCC Ab. As highlighted in Fig. 1, Abs best suited to mediate ADCC: (1) are specific for antigens present on the surface of putative target cells; (2) carry the constant region of the IgG1 or IgG3 subclass [30,31]; (3) lack either sialic acid or fucose glycans [46–48]. Furthermore, ADCC Abs should induce more robust responses when they are present as high titer polyclonal populations containing clones specific for numerous epitopes on the antigen [37].

5. NK cell factors determining ADCC

5.1. “Education” of NK cells to enhance their ability to mediate ADCC

The ability of NK cells to mediate effector functions upon encountering putative target cells is determined through a two-tier vetting process (Fig. 2). The first stage of this procedure, termed education, is initiated during early NK cell ontogeny and continues throughout the lifespan of the cell [53]. This education process determines whether the cell will be conferred with the potential to mediate effector functions, including direct and Ab-dependent cytolysis/degranulation and secretion of cytokines [25–27,54,55]. During this process the NK cell, through its activating and inhibitory receptors, interacts with the normal healthy self-environment. In general, it has been demonstrated that NK cells that carry inhibitory receptors for self-ligands are conferred with the ability to mediate enhanced effector functions [25–27,54,55]. Alternatively, NK cells that carry activating receptors that recognize self-ligands are less able to mediate effector functions [56]. Cumulatively, this is thought to regulate NK cells and prevent them from amassing the ability to mediate anti-self immune responses. The process, however, does equip NK cells to respond to malignant or virus-infected cells, which often upregulate stress-induced ligands for activating NK cell receptors and downregulate class I major histocompatibility complexes (MHC-I or HLA-I) that serve as ligands for many inhibitory NK cell receptors [57–60].

Evidence of the occurrence of NK cell education in vivo has been provided by several studies in mice and humans. Indeed, mice that lack MHC-I exhibit hypofunctional NK cells [61]. When such mice are used to create transgenic strains, with single MHC-I genes, a gain in NK cell functionality is observed, and this functionality is restricted to the NK cell subset that expresses an inhibitory receptor that interacts with the newly expressed MHC-I. A role for activating NK cell receptors reducing the functional potential of NK cells has also been illustrated in mice. Artificial overexpression of ligands for activating receptors renders NK cells expressing these receptors hypofunctional [62,63].

Similar to studies in mice, an abundance of evidence suggests NK cell education is an important determinant of NK cell functional potential in humans. Numerous groups have shown that human NK cell education is a result of a subset of receptors termed killer immunoglobulin-like receptors (KIR) interacting with their HLA-I ligands. Indeed, individuals that carry combinations of the inhibitory KIR3DL1 and its HLA-Bw4 ligand, or combinations or inhibitory KIR2DL1/2/3 receptors and their HLA-C ligands, have been shown to have higher NK cell responsiveness to stimulation with HLA-I-devoid target cells, as well as allogeneic or autologous ADCC target cells [25–28,54,55]. Furthermore, this function is mostly observed in the NK cells expressing the inhibitory KIR, which have been educated through interactions with their HLA-I...
ligands. Also coinciding with the role of activating receptors on NK cell education in mice, human NK cells expressing the activating KIR2DS1 have been shown to be hypofunctional to in vitro stimulation when they are obtained from individuals that carry its HLA-C2 ligand [56].

The evidence discussed above may create the appearance that NK cell function potential is regulated in an off/on manner through the interaction of inhibitory and activating receptors with self-ligands. The process of NK cell education, however, is a much more complicated process, which involves the tuning of functional potential on the basis of the cumulative signals received through activating and inhibitory receptors [64–67]. Research in both mice and humans support this notion. The isolation and in vitro stimulation of NK cells from both species has revealed that the NK cells that are most likely to respond, and respond with the widest array of effector functions, are those NK cells that carry numerous inhibitory NK cell receptors for which the donor carries the MHC-I ligands. Indeed, NK cells that receive low cumulative inhibitory signals during education are conferred with the ability to mediate degranulation of cytotoxic granules. With increasing levels of cumulative inhibitory signals NK cells gain the ability to produce and secrete cytokines, such as IFNγ and TNFα.

An additional layer of complexity regarding the process of NK cell education is that the functional potential of the NK cells is not set during the initial interaction of the NK cells with the self-environment, but that education is a continuous ontological process throughout the lifespan of the cell. Indeed, elegant studies in mice have demonstrated that the transfer of NK cells from MHC-I-devoid mice into MHC-I-competent mice results in the conferral of function to the cells that were beforehand hypofunctional [68]. Similarly, the transfer of NK cells from MHC-I-competent mice to MHC-I-devoid mice results in the educated NK cells losing their ability to respond to stimulation [69]. These observations likely also have relevance during viral infections. Indeed, many viruses, such as HIV, have the ability to downregulate HLA-I expression [57]. Furthermore, in areas of high viral replication, such as lymph nodes, the repertoire of peptides presented on HLA-I molecules would be altered. As KIR are involved in NK cell education and their interactions with HLA-I are influenced by the groove-bound peptide [70], viral infections may have the potential to alter the NK cell education process in areas of high viral replication that contain NK cells.

As mentioned previously, and of relevance to this review, several research groups have demonstrated that NK cell education is important for deciding the functional potential of NK cells to mediate effector functions after stimulation by Ab-coated target cells. Indeed, NK cells educated through KIR2DL1/2/3 and KIR3DL1 mediate higher effector functions after stimulation by non-self ADCC target cells than do non-educated NK cells [25,27,28].
Furthermore, KIR3DL1 educated NK cells have been demonstrated to mediate higher anti-HIV ADCC against autologous targets than non-educated NK cells [26]. This observation suggests that ADCC can overcome the inhibitory potential of KIR/HLA-I interactions and utilize the functional potential conferred through the education process. It should be noted, however, that research on the ability of educated NK cells to be activated for ADCC by cancer monoclonal Ab therapies has revealed that KIR3DL1-expressing NK cells mediate enhanced function in the absence of HLA-Bw4, but they are inhibited in the presence of HLA-Bw4 [28]. The different functionalities of KIR3DL1-expressing NK cells in the presence of HLA-Bw4 in anti-HIV and anti-cancer ADCC are intriguing. As ADCC against HIV usually involves the utilization of polyclonal Ab mixtures, and is indeed more efficient in the presence of polyclonal Abs, we hypothesize that the reason, KIR3DL1-expressing NK cells are more easily inhibited in anti-cancer ADCC, is that the NK cells are not maximally stimulated by the monoclonal Ab treatments. Regardless, the presently available data suggests that NK cell education is a determinant of NK cell ADCC functional potential, and this phenomenon should be taken into consideration when interpreting studies investigating HIV vaccines and therapies that utilize ADCC responses.

5.2. Receptor repertoire and regulation of activation

The second tier of regulation for NK cell functionality, alluded to in Fig. 2 and in the NK cell education section above, is the generation of a cumulative signal during the interaction of activating and inhibitory NK cell receptors with ligands on the surface of putative target cells. The activation of NK cells to mediate effector functions requires that a cumulative positive signal be delivered from these surface interactions [71]. Furthermore, the strength of this activating signal has been demonstrated to determine the exact effector functions that are mediated by the NK cell [72]. Lower cumulative activating signals induce NK cells to degranulate cytotoxic granules and secrete chemokines (i.e., CCL3, CCL4, & CCL5). Larger cumulative activating signals allow NK cells to secrete cytokines, such as IFNγ and TNFα.

The majority of research investigating the impact of cumulative receptor signaling on NK cell activation has been conducted using Ab-independent forms of stimulation. A role for activating and inhibitory receptors other than CD16, however, has been observed during the stimulation of NK cells via ADCC. Indeed, Bryceson et al. recently demonstrated that ligation of co-stimulatory receptors, such as 2B4 and NKG2D, acts synergistically with ligation of CD16 during NK cell activation [73]. Similarly, several investigators have demonstrated that inhibitory KIR receptors, such as KIR2DL1, KIR2DL2, KIR2DL3, and KIR3DL1, can inhibit ADCC responses [28,74]. With regards to anti-HIV ADCC, KIR that interact with HLA-C and the HLA-E-binding inhibitory NKG2A receptor dramatically reduce ADCC [74]. Interestingly, KIR3DL1-expressing NK cells mediate anti-HIV ADCC against autologous target cells. As each of these inhibitory receptors contributes to NK cell education, these results represent somewhat of a conundrum: Why only some educated NK cells are capable of mediating ADCC against targets expressing the cognate antigen for their educating inhibitory receptor? Although more research is needed to elucidate the reason for these observations, perhaps the most likely factor at play here is that the activating signal received through interaction with the ADCC target cells is stronger than the inhibitory signals being mediated through KIR3DL1, but not those being transduced through the inhibitory NKG2A or KIR2DL1/2/3 receptors. As such, the KIR3DL1-expressing NK cells are able to utilize the functional advantage conferred through the education process, whereas the function conferred through NKG2A or KIR2DL1/2/3-mediated education is inaccessible.

Although many questions remain regarding the reasons for the observed anti-HIV ADCC functionalities of NK cells with different phenotypes, it is well established that the receptor repertoire of NK cells greatly influences the ability of NK cells to respond to Ab-coated target cells. As such, the NK cell receptor repertoire, and the interpersonal differences in this repertoire, represents another factor that is necessary to consider when designing vaccines and immunotherapies utilizing ADCC.

5.3. NK cell “exhaustion”

Chronic viral infections such as HIV and hepatitis C virus (HCV), as well as some malignancies, have been demonstrated to alter the phenotype, functionality and subset distribution of NK cells. These disease entities can decrease the prevalence of the highly cytolytic CD56dimCD16+ NK cell subset and increase the frequency of the less functional CD56−CD16− subset [75,76]. Furthermore, these illnesses have been associated with reductions in the expression of the natural cytotoxicity receptors (NCRs, i.e., Nkp30, Nkp44, & Nkp46) [77–79], which has been linked to decreased activation of NK cells through these important receptors. The expression of CD16 is also reduced in patients with these conditions [80,81]. Lastly, NK cells from these patients are less functional upon ex vivo stimulation [79,82,83].

Although the exact mechanism(s) for NCR and CD16 expression alterations has not yet been determined, we hypothesize that these phenotypes are reached as a result of chronic activation and exhaustion. This hypothesis is supported by the demonstration that in vitro activation of NK cells produces similar alterations in phenotype and functionality as are observed in chronic infection. Indeed, activation by either MHC-I-devoid or ADCC target cells results in NK cell downregulation of CD16 and CD56, reduces functionality upon secondary stimulation, and reduces the expression of the activating NCRs [84–87].

Collectively, these observations raise concerns about the desirability of continued NK cell activation during chronic viral infections. A robust correlation between high ADCC titers and slower HIV disease progression has been demonstrated [18]. This observation is difficult to reconcile with the demonstrations that activation of NK cells for direct or Ab-dependent effector functions alters the cellular phenotype in manner similar to that observed in progressive HIV infection and AIDS [75–77,79,81,84–87]. This conundrum highlights the necessity for studies with more relevance regarding the in vivo role of ADCC in HIV disease progression. It is necessary to carry out long-term studies evaluating the potential of autologous NK cells to mediate ADCC against autologous infected targets in the presence of autologous Abs from patients with different disease progression patterns. Given the complicated interplay of activating and inhibitory receptors in educating NK cells for ADCC, as well as determining the activation of the same NK cells during interactions with putative target cells, it is notable that many published studies are confounded by assessing ADCC with cell lines or mixtures of Abs and effector cells from different donors [14,18,34]. Admittedly, it is difficult to disqualify a role for NK cells and ADCC in slowing HIV disease progression, given the observations of higher frequencies of function-conferring KIR3DL1/HLA-Bw4 combinations in HIV-infected slow progressors [55,88–90], as well as the demonstration of higher titers of ADCC Abs in elite controllers [18]. We raise the hypothesis that anti-HIV ADCC responses may be most beneficial during the early stages of HIV infection when NK cells remain most functional. This may “set the scene” for later disease-progression profiles. Indeed, ADCC Abs are observed during early HIV infection and NK cells expressing KIR3DL1 are expanded at this time also [91]. Collaboratively, these NK cells and Abs may eliminate infected cells and reduce the viral load set point, ultimately reducing the level of continuous
stimulation of NK cells required, as well as the general level of immune activation.

Aside from the issues activation-induced NK cell exhaustion raises about the role of ADCC and general NK cell activation in protection from disease progression, this phenomenon is also an important consideration for the goal of utilizing ADCC for therapeutic and prophylactic purposes. Indeed, if ADCC Abs were induced by vaccines or injected in the format of an immunotherapy, the efficacy of these Abs may be drastically reduced in individuals carrying exhausted NK cells resulting from another chronic infection or malignancy. For individuals carrying a non-exhausted NK cell repertoire, however, it is likely that these NK cells would be fully capable of utilizing vaccine-induced anti-viral Abs to eliminate desired cells via ADCC.

5.4. The influence of FcR polymorphisms on ADCC immunity

A critical component of the NK cell for its ADCC potential is the CD16 or FcγRIIIa receptor. This is the low avidity Fc receptor, which recognizes immobilized IgG bound to its cognate antigen. A very important polymorphism has been observed in this receptor. This polymorphism at position 158 determines the degree of expression of the CD16 receptor, its affinity for antigen-bound IgG and the magnitude of the ADCC responses elicited via signaling through the receptor [92]. Individuals that carry a valine (V) at position 158 have CD16 variants that are expressed at a higher surface density, bind with a higher affinity to antigen-bound IgG and trigger higher ADCC responses upon ligation; whereas, the opposite is true for each of these observations for CD16 from individuals with a phenylalanine (F) at position 158.

Most work characterizing the differences in CD16 polymorphisms at position 158 has been conducted in vitro; however, polymorphisms at position 158 have also been demonstrated to be of clinical relevance. Indeed, individuals carrying the function-enhancing V158 polymorphism have better outcomes upon initiation of monoclonal Ab cancer therapies [93]. Given this clinical relevance, CD16 polymorphisms have also been hypothesized to be of importance for anti-HIV ADCC, and potentially of relevance for understanding disease progression and/or vaccine-induced protection. Counterintuitive to the notion that ADCC is important for protection from HIV infection and disease progression, individuals carrying the more highly functional V158 polymorphism have been shown to be more likely to contract HIV infection and exhibit a progressive infection [94]. Similarly, and also counterintuitive to the notion that ADCC is involved in protection from HIV infection in vaccine recipients, low-risk VAX004 vaccinees homozygous for the V158 polymorphism have been shown to be more likely to become HIV infected than those carrying the F158 polymorphism [95]. These observations are concurrent with the notion that continued high levels of ADCC in chronic viral infection may be undesirable, but are at odds with the view that ADCC could be protective if ADCC–competent Abs are present prior to HIV infection. It should be noted, however, that the CD16 V158 polymorphism has also been associated with higher rates of infection in non-vaccinated individuals [94]. As these individuals most likely do not have Abs capable of mediating ADCC against HIV-infected target cells, it is highly unlikely that the CD16 polymorphism is increasing the rate of infection in an Ab-dependent manner. Thus, the relationship of the CD16 receptor to increased infection is most likely explainable due to a non-Ab-dependent effector function or through genetic linkage to some unidentified marker. As such, the interpretation of the association of the CD16 V158 polymorphism with enhanced infection in the VAX004 trial remains open to debate.

Notwithstanding the open issues of debate regarding the role of CD16 polymorphisms in determining susceptibility to HIV infection, the polymorphisms at position 158 are clearly important for determining the expression and function of the receptor. As such, analysis of these polymorphisms will be essential to take into consideration for vaccine and therapeutic design.

6. Exogenous factors that influence ADCC immunity

6.1. Cytokines

Exogenous soluble factors, such as cytokines, have great potential to alter the nature of many immune responses. Indeed, a role for many cytokines in adjusting the function of NK cells for direct and Ab-dependent effector functions has been demonstrated. Several independent investigations have illustrated the ability of IL-2, IL-10, IL-12, IL-15, IL-18 and IL-22 to enhance the cytolytic potential of NK cells [96]. Similarly, several groups have noted suppressive roles for both IL-4 and TGF-β.

Aside from demonstrations of the effects of cytokines on NK cells in vitro, the plasma levels of these molecules in individuals with chronic viral infections have been linked to ex vivo NK cell function. For example, heightened levels of TGF-β have been linked with reduced functionality. Blockade of TGF-β has even been shown to increase the function of NK cells isolated from Hepatitis B virus (HBV)-infected patients [97].

Although the demonstration that plasma cytokines can influence NK cell functionality during chronic viral infection may be important for understanding the immunopathology of chronic viral infections, perhaps the most important potential of the effects of cytokines on NK cell functionality is their ability to prime NK cells for therapeutic purposes. Certainly, priming of NK cells with cytokines, such as IL-2, has been shown to increase the functionality of NK cells and increase their efficacy for attacking cancer cells when re-administered to animals or humans with malignancies [98]. Such ex vivo cytokine priming of NK cells could be of much interest for immunotherapies directed at attacking reactivated latent HIV reservoirs.

7. Potential integration of factors to eradicate latent HIV

For years it has been demonstrated that HIV establishes a chronic latent viral reservoir in resting memory CD4+ T-cells and probably other cells that become infected, such as NK cells [99,100]. This reservoir remains stable throughout treatment of HIV infection with combined antiretroviral therapy (cART), prevents the eradication of virus from the body and is the reason lifelong cART is required for infected individuals. Recently, there have been several publications demonstrating latent HIV is reactivated by histone deacetylase (HDAC) inhibitors in vitro and in vivo [101–103]. This observation has raised hope that reactivated virus, and the cells harboring this virus, can be eliminated, producing a functional cure that would allow patients to live without the need for lifelong cART. One of the most essential questions raised by this new possibility is how to best eliminate the infected cells identified upon reactivation of virus.

As activation of NK cells through CD16 induces the cytolysis of target cells baring antigen-bound Abs [27], we propose that ADCC could be utilized to eliminate the reactivated latently infected CD4+ T-cells [104]. Clinical studies involving the reactivation of latently infected T-cells, however, have not induced the spontaneous clearance of reactivated infected cells [101], suggesting that the in vitro ADCC responses available on cART at this time are insufficient to clear these cells.

As explained in the above sections, several reasons exist that may explain the inability of NK cells to respond sufficiently to clear latently infected cells. First, although cART undoes some of the functional exhaustion of NK cells in HIV-infected subjects [79,105],
NK cells from infected individuals on cART still exhibit more baseline activation than NK cells from uninfected individuals [106]. An accumulation of too many exhausted NK cells could reduce the ability of the NK cell pool to mediate sufficient ADCC responses to clear reactivated infected cells. Second, NK cell education has been demonstrated to be important for NK cell-mediated ADCC [25–28]. With regards to anti-HIV ADCC education of NK cells through KIR3DL1/HLA-Bw4 combinations seem to be of importance [26]. Indeed, KIR3DL1-educated NK cells mediate robust ADCC against autologous target cells; whereas, NK cells expressing the education-competent KIR2DL1/2/3 or NKG2A receptors have been demonstrated to be inhibited by their ligands that are present on autologous HIV ADCC target cells [74]. Interestingly, KIR3DL1-educated NK cells lose their functional advantage for ADCC in HIV-infected individuals, regardless of cART [26]. As such, it is possible that individuals could have too few functional educated NK cells after the reactivation of latent viral reservoirs, and reduced ability of NK cells to clear reactivated infected cells.

Deficits in NK cell functional potential in chronically HIV-infected individuals potentially represent a major hurdle to overcome for utilization of NK cell-mediated responses to clear reactivated latent infection CD4+ T-cells. Implementation of in vivo cytokine therapy, or re-administration of NK cells activated ex vivo with cytokines, could represent methodologies to overcome these problems. Previous studies have demonstrated that IL-2 and IL-15 can increase the function of NK cells isolated from HIV-infected individuals [107,108]. This suggests that the cytokines can allow the NK cells to overcome the exhaustion they exhibit when utilized directly ex vivo. Alternatively, treatment of NK cells with cytokines may allow NK cells to garner further education, which would increase their functional potential. Indeed, it has recently been demonstrated that one of the mechanisms behind the increased NK cell functionality upon treatment with IL-2 or IL-15 is increased expression of inhibitory KIR, which bind to self HLA-I [109].

Several possibilities for Ab deficits could also explain the inability of patients to spontaneous clear reactivated infected cells. As previously mentioned, the amount of ADCC-competent Abs is an important indicator of the ability to elicit NK cell-mediated anti-HIV ADCC responses [37]. The titers of anti-HIV Abs tend to be reduced in chronically infected individuals after suppression of virus with cART [110]. Our lab has recently replicated this observation and demonstrated that after prolonged suppression of viral replication with cART anti-HIV ADCC Abs are reduced (unpublished data). Another possible Ab-related problem for clearing reactivated latent virus is that the reactivated virus may exhibit escape mutations to the available Abs. Indeed, the ability of HIV to escape ADCC pressure has previously been demonstrated by our research group [111].

Overcoming deficits in the anti-HIV Ab response also presents a major potential hurdle for clearing reactivated latently infected cells. The problem of lower Ab titers in individuals that have been successfully treated with cART may be easily overcome with a booster immunization against autologous virus prior to reactivation. The problem of viral escape mutants to circulating ADCC Abs, however, represents a more nefarious setback. Several lines of research suggest Ab responses to HIV may be subject to a phenomenon termed repertoire freeze, where Abs most well suited to targeting earlier viral variants for destruction are sufficiently cross-reactive with contemporary autologous viruses to prevent the induction of more efficient novel Ab responses [112,113]. As such, attempts to immunize individuals in a manner that would create novel Ab specificities may fail due to the ability of the currently available Abs and B-cells to recognize the new antigen, be boosted, and mop-up antigen. One possibility to overcome this potential problem would be to eliminate the presently available anti-viral humoral immune response, and memory B-cells. Indeed, it has previously been shown in macaques that idiotypic-based suppression of anti-viral Abs in the context of an active infection allows for the development of novel Ab specificities [114,115]. This mechanism of regulating the humoral immune response to HIV may also be relevant for increasing the presence of useful ADCC Abs in chronically infected individuals prior to viral reactivation.

The potential to reactivate and eliminate the latent viral reservoirs of HIV-infected individuals represents an exciting and promising avenue of research into curing HIV infection. This area of research is still very much in its infancy, and many questions remain about the best strategies and potential pitfalls. Although we focus on utilizing ADCC to eliminate reactivated virally infected cells, it should be noted that other cytolytic immune responses, such as those mediated by cytotoxic CD4+ or CD8+ T-lymphocytes, could also provide a mechanism to eliminate reactivated infected cells. The observation that individuals with reactivated virus do not spontaneously clear infected cells, however, suggests that immunological roadblocks are in existence for all of these cytolytic responses [101]. Indeed, HIV-specific CD8+ T-cells require pre-activation to mediate robust cytolysis of reactivated latently infected cells [116]. As such, the roadblocks to immune-mediated clearance of reactivated latently infected cells need to be elucidated and immune modulating strategies designed to increase the chances of an effective functional cure against HIV. A successful protocol for eliminating reactivated latently infected cells will likely utilize more than one method of immune-mediated clearance.

8. Potential integration of factors for vaccination against HIV

The search for an effective prophylactic HIV vaccine has been the holy grail of HIV research since the identification of the viral agent as the cause of AIDS in 1984 [117–120]. Traditional attempts to design prophylactic HIV vaccines have focused on eliciting neutralizing Abs or CD8+ cytotoxic T-lymphocyte (CTL) responses [121]. The recent success of the RV144 trial, however, has suggested that vaccine-induced ADCC Abs may provide a pathway to protection from HIV infection [15,16]. Nevertheless, as alluded to in the previous sections of this review, there are several hurdles to utilizing ADCC Abs as a prophylactic vaccine.

Perhaps the foremost problem facing an HIV vaccine that utilizes Abs is that these proteins wane with duration from final immunization [22,23]. Diminishing Ab titers are associated with decreased vaccine efficacy overtime, suggesting it is necessary to add components to the vaccine to maintain high Ab titers. Vaccination of non-human primates with live-attenuated SIV has proven effective at sustaining sufficient Ab levels to provide long-term protection against challenge with pathogenic viral variants [36]. However, live-attenuated viruses have the potential to revert to pathogenic forms [122], the utilization of live-attenuated viruses for the vaccination of humans is not up for consideration. As such, future investigations need to evaluate alternative methods to sustain high titers of anti-viral ADCC Abs. Such methods could include prolonged immunization regimens, unique adjuvants and/or the implementation of long-lived vaccine vectors that lack pathogenic potential. For example, in a recent review one of us suggested studying the potential of anti-idiotypic Abs, directed against a non-antigen binding site of HIV Abs, to mediate an interaction that mutually sustains both anti-viral and anti-anti-viral B-cell populations [113].

A major concern regarding utilization of HIV vaccines that exploit ADCC Abs is that the potential of NK cells to mediate ADCC is prejudiced by the process of NK cell education, which is determined by genetic factors. As reviewed above, the NK cell populations that have the greatest functional potential upon successful direct or Ab-dependent activation are those that have been educated through the interaction of inhibitory receptors with
their HLA-I ligands [25–28,54]. Indeed, NK cells educated through the interaction of KIR3DL1 and HLA-Bw4 exhibit a functional advantage when stimulated with autologous HIV ADCC target cells [26]. It is currently thought that other education competent receptor/ligand combinations (i.e., KIR2DL1/HLA-C2, KIR2DL2 or KIR2DL3/HLA-C1, & NKG2A/HLA-E) do not produce NK cell populations with functional advantages for mediating anti-HIV ADCC. These educated NK cell populations are largely inhibited during HIV ADCC assays [74]. Because of this role for NK cell education in determining the ADCC potential of NK cells, individuals lacking the educating KIR3DL1/HLA-Bw4 combination may have less potential to eliminate early infected autologous CD4+ cells shortly after HIV exposure. The potential bias of ADCC Ab inducing vaccines to work more efficiently in individuals carrying genetic combinations of educating receptor/ligand combinations represents a difficult problem to overcome. For example, techniques to turn on non-educated NK cells, which lack inhibitory receptors for self, could be undesirable, as these cells may have increased potential to mediate autoimmune responses.

Lastly, considerations of utilizing anti-HIV ADCC for vaccine design need to take into consideration the mechanism of HIV transmission. It is frequently assumed that HIV is transmitted primarily as a free virus within seminal and vaginal fluids. There is, however, much evidence that HIV can be transmitted via infected allogeneic lymphocytes present in semen or vaginal fluids [123–127]. Indeed, animal models have revealed that the levels of infected lymphocytes required to obtain infection are more reflective of the amount of HIV-infected cells present in bodily fluids than are the levels of free virions required to obtain infection. If infected allogeneic lymphocytes are indeed involved in the transmission of HIV, they add an extra layer of complexity to efforts to design vaccines utilizing ADCC. Due to interindividual variability infected lymphocytes from different individuals will express different HLA-I combinations, as well as different peptides bound within the grooves of these molecules. These complexes of HLA-I and peptide will have the potential to interact differently with different inhibitory KIR, potentially rendering the effectiveness of an ADCC–utilizing vaccine dependent upon a very complex interaction of the KIR repertoire and educational status of the NK cells of the recipient of the infected cells and the HLA-I repertoire of the infected cell donor. Although this represents a major potential problem for vaccines utilizing ADCC, it is notable that some educated NK cells are capable of overcoming inhibition to mediate ADCC in the presence of their ligands [26]. The mechanism behind this phenomenon needs to be elucidated. If this is due to an antibody factor, such as the polyclonal nature of the response, it may be possible to design vaccines that can render this concern irrelevant.

9. Conclusions

Long abandoned as an uninteresting or irrelevant anti-viral immune response, recent evidence has implicated ADCC as a potent anti-viral immune response associated with slower progression towards AIDS and potentially important for preventing infection [15,17,18,21]. Indeed, many investigators are now studying the prospect of harnessing the power of ADCC responses to design therapeutics and vaccines.

Attempts to design vaccines against HIV have long focused on the induction of broad neutralizing Ab responses and/or robust CTL [121]. The recent RV144 efficacy trial revealed protection from infection despite not sufficiently eliciting either of these immune responses [12,15]. Instead, the protection conferred by the RV144 regimen has been linked to non-neutralizing binding IgG. Furthermore, in the absence of anti-viral IgA, which can inhibit IgG-mediated ADCC, the ADCC potential of the anti-viral IgG has been correlated with the observed protection [15,16]. As highlighted in this review, many factors are known to influence ADCC responses. A collective synthesis of the current knowledge regarding these factors is required to assist the design of future vaccine constructs attempting to utilize anti-viral ADCC. Furthermore, intense research efforts are needed to answer the major questions being raised by the current state of knowledge. Central to the design of a credible ADCC-utilizing HIV vaccine are techniques to sustain vaccine-induced Abs, as well as a more robust understanding of the mechanisms involved in the mucosal transmission of HIV.

Conflict of interest statement

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