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SUPPLEMENT ARTICLE

Humoral and Innate Antiviral Immunity as Tools to Clear Persistent HIV Infection

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Human immunodeficiency virus (HIV) type 1 uses the CD4 molecule as its principal receptor to infect T cells. HIV-1 integrates its viral genome into the host cell, leading to persistent infection wherein HIV-1 can remain transcriptionally silent in latently infected $CD4^+$ T cells. On reactivation of replication-competent provirus, HIV-1 envelope glycoproteins (Env) are expressed and accumulate on the cell surface, allowing infected cells to be detected and targeted by endogenous immune responses or immune interventions. HIV-1 Env-specific antibodies have the potential to bind HIV-1 cell surface Env and promote elimination of infected $CD4^+$ T cells by recruiting cytotoxic effector cells, such as natural killer cells, monocytes, and polymorphonuclear cells. Harnessing humoral and innate cellular responses has become one focus of research to develop innovative strategies to recruit and redirect cytotoxic effector cells to eliminate the HIV-1 latently infected $CD4^+$ T-cell reservoir.

Keywords. Antibodies; innate immunity; bispecific antibodies; HIV-1; ADCC; latency; cure.

Cellular and humoral adaptive immune responses partially control virus replication during natural human immunodeficiency virus (HIV) type 1 infection and affect the virus set point [1, 2]. HLA class I–restricted $CD8^+$ T-cell responses effect decline in virus load after acute infection [3, 4], and polyfunctional $CD8^+$ T cells potently select for virus escape mutants [5–7]. HIV-1 antibodies comprise 2 major classes, neutralizing antibodies (NAbs) that can prevent the infection of target cells by binding to virion envelope glycoproteins (Env), and nonneutralizing antibodies (non-NAbs) that are unable to bind to virion Env and prevent infection but are able to recognize HIV-1 Env on the surface of HIV-1–infected cells at the time of virus entry or virus assembly/budding and mediate antiviral activity through Fc effector functions [8, 9]. NAbs apply immune pressure to select virus escape mutants and, like $CD8^+$ T cells, shape the virus repertoire in chronic HIV-1 infection [10]. These Env antibody virus escape mutations can also select antibody responses with broader neutralizing activity [10–13]. In contrast, the role of non-NAbs in inducing virus escape mutations during acute infection has been less studied, but mutations selected by non-NAbs can be detected in a small number of individuals because of the ability of non-NAbs to mediate antibody-dependent cellular cytotoxicity (ADCC) [14].

Innate immune responses are also engaged early in acute HIV-1 infection (AHI), as indicated by the release of proinflammatory cytokines, defined as the "cytokine storm," which has been described as a hallmark of AHI by Stacey et al [15]. Of interest, included among the first observed plasma cytokine responses are increased levels of the interferons and interleukin 15, cytokines with important roles in activating natural killer (NK) cells [16, 17]. NK cells undergo tissue redistribution during AHI [18] and help control virus replication through recognition of infected cells via killer immunoglobulin receptors and killer immunoglobulin receptor–like molecules [19–21].

Thus, both antibody and cellular immune responses can contribute to limiting HIV acquisition, controlling viremia, and eliminating HIV-infected cells. Integration of antibody and cellular immunity occurs through Fc receptor (FcR)–mediated effector functions [8, 22–24], including phagocytosis and ADCC. Naturally produced antibodies mediate these antiviral effector functions by recruiting canonical FcR-bearing innate effector cells, including monocytes, NK cells, and polymorphonuclear neutrophils. In addition, HIV-specific antibodies can be engineered into antibody-based molecules that can specifically recruit other populations of cytotoxic effector cells, such as $CD8^+$ T cells, to further promote infected cell eradication and to reach additional tissues or immune compartments. Thus, harnessing both $CD8^+$ T-cell and NK cell effector functions to target HIV-1–infected cells is a key experimental strategy to reduce the size of, or eliminate, the HIV-1 latently infected cell pool. In this article, we discuss the principles guiding the development and use of antibody and antibody-based molecules to treat HIV-1 infection.

HURDLES IN ELIMINATING THE LATENT RESERVOIR

During AHI, HIV-1 replicates in $CD4^+$ T cells and causes profound immune activation and $CD4^+$ T-cell depletion [15, 25, 26].

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After acute infection, HIV-1–specific CD4+ T cells become the main targets of virus infection during the transition from naive to effector and memory T cells [27, 28]. In addition, cytokines and high levels of viremia are responsible for elevated levels of immune activation in AHI [15], resulting in infection of both naive and memory CD4+ T cells [28]. Thus, HIV-1 integrates into the genome of a diverse population of activated proliferating CD4+ T cells. After immune control of virus replication immune activation declines and proviral CD4+ T cells return to a resting phase while harboring the provirus that can persist in this silent stage for the remaining of the life span of these cells [29]. The wide variety of CD4+ T cells among the latent reservoir, and their differential susceptibility to activation stimuli, represent significant hurdles to their elimination by immune responses or by combination antiretroviral therapy (cART) [30].

One mechanism that has been proposed to eradicate the latent reservoir relies on the administration of latency-reversing agents [31, 32] that can reactivate provirus for viral antigen expression, followed by elimination of the infected cell either through direct cytolytic effects of the virus life cycle or with endogenous immune responses. However, this strategy, originally called "shock and kill" [31, 32], has proved difficult to implement because of (1) low levels [33] and impaired function [34–36] of HIV-1–specific CD8+ T cells; (2) insufficient activation of HLA class I–restricted antigen-specific CD8+ effector T cells [30, 33, 37]; (3) inadequate expression of HIV-1 antigens by reactivated CD4+ T cells [38]; (4) localization of reactivated CD4+ T cells in protected sites, such as lymph node B cell follicles [39, 40]; and (5) viral escape mutants that limit virus-infected T-cell clearance by CD8+ cytotoxic T cells [41, 42].

To overcome the natural limitations of the immune system in targeting the chronic phase of HIV-1 infection and the latent reservoir, new strategies have been proposed that harness the antiviral activities of NAb or non-NAb monoclonal antibodies (mAbs); mAbs can be selected based on their ability to target conserved regions of HIV-1 Env on virus-infected CD4+ T cells (ie, non-Nabs) or on their breadth of neutralization of HIV-1 isolates (ie, broadly neutralizing antibodies [bNAbs]) [8, 43]. Non-NAbs capable of mediating ADCC by recruiting Fcγ receptor (FcγR) IIIa–bearing cells such as, NK cells, are of interest as immune therapies to reduce the size of the latent reservoir because these antibodies can target Env on the surface of primary virus–infected cells [44]. The use of mAb-based molecules that can recognize and promote infected cell killing combined with cART is a promising new strategy for treating HIV-1 infection [45–47]. Moreover, mAbs and derivative molecules could be used in combination with latency-reversing agents to augment endogenous immune effector functions for the elimination of latently infected cells on reactivation of the provirus.

NEUTRALIZING ANTIBODIES

The bNAbs bind to 1 of 6 sites on the virion Env and prevent virion infection (Figure 1). These sites of neutralization vulnerability include the gp41 membrane external proximal region [48], the gp120-gp41 interface [49], the CD4-binding site (CD4bs) [50–53], variable regions 1 and 2 (V1/V2) glycan [54], the fusion domain [55], and variable region 3 (V3) glycan [56, 57]. Although the development of NAb in HIV-1–infected patients is rarely associated with control of disease progression [58], passive administration of bNAbs in nonhuman primates individually or in combination can prevent simian-human immunodeficiency virus (SHIV) infection [59–67]. The protective effect of bNAbs also requires Fc-mediated effector functions [68–73]. Thus far, no vaccine to date has been successful at eliciting bNAbs, and this failure seems to be linked to certain antibody characteristics, such as long and hydrophobic heavy chain complementarity determining region 3 loops, high degrees of somatic hypermutation, and/or autoreactivity—all traits of antibodies controlled by immune tolerance mechanisms [74–76]. bNAbs do arise in about 50% of HIV-1–infected individuals, but only after 2–4 years of infection [77]. It has been demonstrated that virus diversity in infected individuals arises because of pressure exerted by the immune system and the ability of HIV-1 to mutate, leading in turn to the ability of the immune response to develop more bNAb responses [10, 13, 78]. This cycle of events may explain the delay in the appearance of HIV-1 bNAb responses.

In the humanized mouse model (see the review by Nixon et al [79] in this special issue for the discussion of animal models

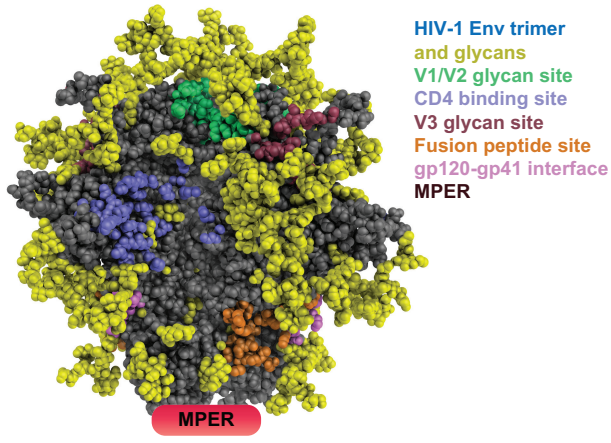

Figure 1. Sites of vulnerability on the human immunodeficiency virus (HIV) type 1 envelope glycoprotein (Env) spike. The structure of a HIV-1 prefusion trimer is displayed and the 6 major sites of vulnerability targeted by broadly neutralizing antibodies discussed in this review are indicated: the variable regions 1 and 2 (V1/V2) loop (*green*), the base of the variable region 3 (V3) loop (*maroon*), the CD4-binding site (*lavender*), the interface between gp120 and gp41 proteins (*magenta*), the fusion peptide region (*orange*), and the membrane proximal external region (MPER). Because of the limited structural information, the MPER near the base of the Env trimer is represented by a red cylinder.

for HIV-1 latency), passively administered postexposure prophylaxis with a combination of CD4bs bNAb 3BNC117 [53], V3 glycan bNAb 10–1074 [56], and V1/V2 glycan bNAb PG16 [54] decreased viremia in about 50% of the mice and substantially delayed virus rebound compared to cART treated animals [80]. Moreover, the investigators observed a decline in the level of cellular-associated DNA in the aviremic mice treated with the 3-mAb cocktail. In addition, it was demonstrated that critical mutations that nullified the interactions of their Fc domains with FcγRs on effector cells attenuated the therapeutic potential of the mAbs. The majority (60%) of mice treated with the FcR^{null} antibody cocktails showed early rebound viremia after treatment, whereas early rebound viremia was observed in only 5% of the mice treated with the cocktail of antibodies with unmutated Fc regions. Therefore, bNAbs can significantly affect both plasma viremia and the pool of latently infected cells through recognition of HIV-1 Env on the host cell membrane [80] and engagement of FcγR-bearing cells, as shown elsewhere [23].

Several preclinical studies conducted in nonhuman primates support the observation in the humanized mouse model that bNAbs can not only successfully reduce the level of plasma viremia during chronic infection [81–83] but might also reduce levels of proviral DNA. The passive administration of the V3 glycan bNAb, PGT121 [57], and the CD4bs bNAbs 3BNC117 [53] and b12 [52] in combination reduced SHIV proviral DNA levels in the gut-associated lymphoid sites, lymph nodes, and peripheral blood [82]. Moreover, the VRC07/PGT121 combination administered during acute SHIV infection in rhesus macaques not only induced a lower level of peak viremia and reduced the pool of latently infected cells [84] but could also control virus replication and prevent the establishment of latency [85]. Whether the observed reduction of provirus DNA in tissue compartments can be sustained needs to be further investigated (see also the review by Henrick et al [86] on the measurement of the latent reservoir in this special issue).

Recent human trials have evaluated the safety and efficacy of passively infused bNAbs as HIV therapeutics. Infusion of 3BNC117 bNAb reduced plasma viremia by 1 log [87]. Moreover, longitudinal observation of the individuals enrolled in this study revealed that some of the participants were capable of developing broader NAb responses than those present before the infusion of the 3BNC117 mAb, suggesting bNAb selection of new virus variants that drove endogenous NAb breadth [88]. Therefore, one positive aspect of bNAb-based immunotherapy resides in the possibility that it may positively alter the natural humoral responses. The VRC01 CD4bs bNAb was also tested in HIV-1–infected individuals [89], and the administration of a single dose of mAb induced a plasma viremia reduction of almost 2 $\log_{10}$. However, in both studies, resistant virus isolates were either observed before treatment, hampering the therapeutic effect of the mAb [89], or appeared after infusion of the bNAbs during analytical treatment interruption [90]. The appearance of virus escape mutants should be regarded as a cautionary aspect of the administration of individual bNAbs, and advocates for the search of more potent antibodies or evaluations of bNAb combinations that target multiple epitopes to minimize the potential for virus escape.

NONNEUTRALIZING ANTIBODIES

The ALVAC/AIDSVAX B/E RV144 vaccine trial conducted in Thailand showed an estimated 31.2% vaccine efficacy [91]. An immune correlates analysis indicated that non-NAbs targeting the V2 region that were capable of mediating ADCC were correlated with decreased transmission risk [92]. ADCC antibody responses in HIV-1 infection may also control virus replication [93–95] and delay disease in adults [96, 97] and children [95]. Although non-NAbs have failed to protect from infection in nonhuman primate challenge studies, they limit simian immunodeficiency virus [98] or SHIV [64, 73] replication and limit the number of transmitted/founder viruses [99].

During the course of study of RV144 immune correlates, it was found that the ADCC-mediating antibodies, while nonneutralizing, bound to the surface of transmitted/founder virus–infected $CD4^+$ T cells [9]. Thus, these types of antibodies were well suited for use in developing therapeutic antibodies that can target virus-infected $CD4^+$ T cells in the setting of chronic HIV-1 infection [45].

The therapeutic usage of mAb and mAb-derived molecules to eliminate latently infected cells on spontaneous or pharmacologically induced reactivation of replication competent provirus would rely on the ability of these molecules to recognize HIV-1 envelope as soon as it is expressed on the surface of infected cells. Among the non-NAbs, it has been reported that those directed against the CD4-inducible constant regions 1 and 2 of the gp120 could be among the first to bind to the surface of infected cells [100, 101]. These observations have prompted interest in developing antibody-derived molecules that will be further discussed. Of note, the epitope recognized by these mAbs is also one of the most conserved sequences of the HIV-1 envelope, providing a unique target for broad recognition of circulating HIV-1 isolates [45, 102].

BISPECIFIC ANTIBODIES

To treat cancer and other human diseases, new immune therapeutics have been developed by combining 2 or more antigen-binding variable fragments of immunoglobulins into a single molecule termed bispecific (bsAb) or trispecific antibodies. These antibodies can either bind to antigens presented on the surface of an individual cell, or on 2 distinct cells [103]. In general, the bsAbs can be divided in 2 major categories based on the presence or absence of the Fc region. Its presence can be beneficial during the production and purification of these molecules, but it also provides better solubility, stability, effector functions

related to the ability to recruit FcR-bearing cytotoxic effector cells and complement, and half-life by binding to the FcR neonatal. In contrast, bsAbs lacking the Fc region rely exclusively on their ability to bind specific antigens for their biological effects.

Novel bsAbs based on bNAbs targeting neutralization-sensitive regions of the HIV-1 envelope have been successfully developed and tested in vitro for their neutralization potency [104] (Figure 2A); bsAbs were found to retain the potency and the breadth of the parental individual mAbs and were also able to neutralized HIV-1 isolates that were resistant to the individual bNabs. The same studies demonstrated that the pharmacokinetic properties of the bsAbs were equal to those of the parental antibodies. These data were encouraging and indicated that further evaluation should be conducted in animal models to validate their activity and determine bispecific bNAb safety and efficacy. Alternative strategies for bsAbs have been explored and led to the design of iMabm36 [105] and $10E8_{v2.0}$/iMab [106] (Figure 2A). One immunoglobulin arm in these bsAbs is specific for human CD4 (iMab), which blocks the gp120-binding site on the CD4 molecule, and the second arm is specific for HIV-1 gp120 (m36 or $10E8_{v2.0}$). Thus, these novel bsAbs use a dual mode of action to prevent virus entry by interacting both with the virus itself and with the host cell surface receptor required for infection. The latter bsAb ($10E8_{v2.0}$/iMab) [106] has also been tested in the humanized mouse model and was demonstrated to both reduce virus load and provide protection from virus challenge. These findings support further evaluation of the therapeutic efficacy of bsAbs in clinical trials.

BISPECIFIC T-CELL ENGAGERS AND DUAL-AFFINITY RETARGETING MOLECULES

A further technological development of the bsAb concept was to engineer molecules that consisted of 2 single-chain variable fragments (scFvs) from different antibodies joined by a single polypeptide linker [108]. These new molecules were designed with the specific goals of improving the size, valency, flexibility, half-life, and biodistribution. The first-generation molecule included 1 scFv that bound to $CD3^+$ T cells ($CD4^+$ or $CD8^+$) via the CD3 receptor, and the other scFv was specific for the B6.2 molecule expressed on tumor cells; these molecules were first defined as bispecific T-cell engagers (BiTEs). Of note, they can redirect T-cell killing in an antigen-specific manner that is independent of major histocompatibility class I recognition of the antigen-bearing cells and the presence of costimulatory molecules [109, 110]. Subsequent work was performed to improve the stability, potency, and manufacturability of the BiTEs, which led to the generation of dual-affinity retargeting (DART) molecules [111]. In DART molecules, the variable domains of the 2 specificities are incorporated into a disulfide-linked heterodimer in which short linkers between the variable light chain and variable heavy chain segments promote a "diabody"-type association, with the disulfide bond stabilizing the structure [112–114].

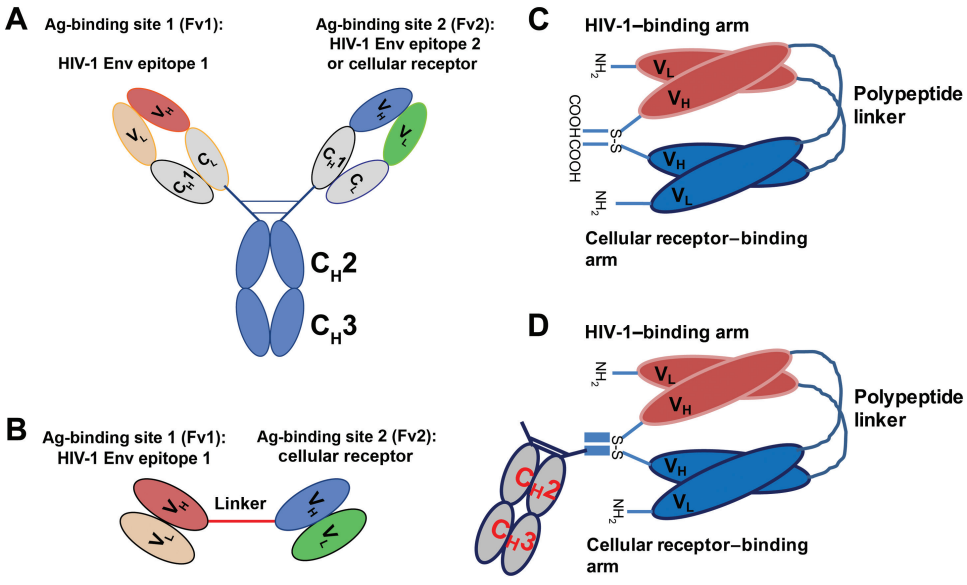

Figure 2. Anti–human immunodeficiency virus (HIV) type 1 envelope (Env) antibody-derived molecules. *A,* Bispecific antibodies combine 2 antigen (Ag)–binding site variable fragments (Fvs) into a single immunoglobulin. The 2 Fvs (Fv1 and Fv2) can recognize 2 different antigenic regions of the HIV-1 Env [104*], or the HIV-1 Env and cellular receptors involved in virus entry [105*, 106*] or in cytotoxic functions [107*]. *B,* Bispecific T-cell engagers (BiTEs), which are generated by using a single polypeptide linker to join 2 single-chain Fvs (scFvs) with different antigen specificities. The first-generation HIV BiTE was designed to bind the HIV-1 envelope CD4-binding site and recruit cytotoxic T cells by engaging CD3 [47]. *C, D,* Dual-affinity retargeting (DART) molecules. The variable domains of the 2 scFvs are not only linked by a polypeptide linker to create a "diabody"-type structure but also stabilized into a disulfide-linked heterodimer with different antigen specificity. One arm can bind the HIV-1 envelope, and the other can bind cytotoxic effector cells [45]. DART molecules can also be designed to include the antibody Fc region to improve the half-life of the molecules [46].

Both BiTEs and DART strategies have been explored to develop novel classes of therapeutics that can be used to treat chronic HIV-1 infection with the specific goal of eliminating latently infected cells on the reactivation of the provirus. Pegu et al [47] developed a BiTE molecule capable of recruiting CD3 cytotoxic T cells and redirecting their killing by virtue of the anti–HIV-1 arm based on the VRC07 [51] mAb targeting the HIV-1 CD4bs envelope region (Figure 2B). The molecule was named VRC01-αCD3 and, as previously observed for the other BiTEs, it was shown to induce activation of $CD8^+$ or $CD4^+$ T cells only in presence of target cells expressing the HIV-1 envelope. One peculiar characteristic of this molecule was its ability to reduce the frequency of proviral DNA positive $CD4^+$ T cells in 5 of 8 subjects during a 2-day in vitro tissue culture system, suggesting that this molecule could be effective when used as a component of HIV cure treatment strategies.

As further development of the DART molecules to treat HIV-1 infection, Sung et al [45] explored the ability of novel HIV-specific DART molecules to eliminate HIV-infected cells (Figure 2C). The DART molecules used in this study were composed of a CD3-specific arm for recruitment of cytotoxic effector T cells and an HIV-specific arm based on the CD4-inducible constant regions 1 and 2 and gp41 cluster 1 non-NAbs A32 and 7B2, respectively, for recognition of HIV-1 envelope on the surface of infected cells. These molecules were demonstrated to be able to redirect the killing of HIV-1–infected cells and reduce the amount of virus recovered from virus outgrowth assays performed with cell cultures from antiretroviral therapy–treated patients on incubation with the latency-reversing agent vorinostat.

Similar results were observed by Sloan et al [46], who developed DART molecules with the HIV-1 arm based not only on the A32 and 7B2 non-NAbs but also on bNAbs that target the N332 glycan (PGT121) [57], V1/V2 (PGT145) [115], CD4bs (VRC01) [50], and MPER (10e8) [48]. Most of these DART molecules had 50% effective concentrations in the picomolar range, suggesting that they are suitable for clinical applications [45]. Of note, when DART molecules specific for different HIV envelope regions were evaluated in combination, the investigators did not observe either antagonistic nor synergistic effects for their cytotoxic activity, suggesting that it may be possible to combine DART molecules to expand the breadth of activity against the diversity of HIV-1 isolates present in clinical settings.

Interestingly, both BiTEs and DART molecules were demonstrated to be capable of redirecting normal resting cytotoxic T cells for killing of HIV-infected cells, bypassing the need of effector cell preactivation, which has been described elsewhere as a hurdle in the elimination of the latent reservoir by shock-and-kill strategies because endogenous HIV-1–specific HLA class I-restricted $CD8^+$ T cells require preactivation for effective cytolysis of reactivated latently infected cells [33].

FUTURE DIRECTIONS

Novel treatment strategies that will rely on the use of antibody-based immune therapies can overcome the hurdles thus far identified for the HIV-1–specific immune responses to eliminate latently infected cells alone or in combination with latency-reversing agents and antiretroviral therapy. However, more work is needed to determine the clinical impact that these molecules may have in treating latent HIV-1 infection. The minimum amount of Env antigen expressed on the surface of HIV-1–infected $CD4^+$ T cells that will be needed for antibody recognition is not known. Moreover, it will be crucial to determine whether new molecules such as BiTEs and DART molecules may have a superior biodistribution profile compared with other bsAbs, which would allow them to penetrate and recruit effector cells in the anatomic sites harboring the latently infected cells [39, 115].

Passive administrations of antibody have relied on the parenteral administration of these molecules and their derivatives. Therefore, a pressing challenge resides in the design of new molecules that have a longer half-life to require less frequent administration regimens while retaining the specificity of the parental mAb. In the case of DART molecules, for instance, there has already been an initial attempt at increasing the half-life of the molecules by engineering them to express a Fc region that can bind to the FcR neonatal and provide increased recirculation of the molecule without promoting other Fc-related effector functions [46] (Figure 2D). Moreover, recombinant adeno-associated viruses have been engineered to deliver fusion proteins that resemble antibodies and antibody-based molecules. Gardner et al [116] have been able to deliver a CD4-immunoglobulin fusion protein in combination with a small CCR5 mimetic sulfopeptide that provided protection from challenge with SHIV-AD8 for up to 40 weeks after infusion, without adverse effects. It is important to perform human clinical trials with these agents to evaluate their safety, immunogenicity and, ultimately, efficacy.

Finally, considering the advances in cancer therapy obtained by using mAbs that target immune checkpoints [117], and the fact that some of the same checkpoints have been demonstrated to be involved in the exhaustion of anti-HIV-specific cellular immune responses, such as the expression of PD-1 [34–36], efforts should be devoted to the antibody-mediated rescue of exhausted effector T cells by interfering with the interaction of programmed cell death protein 1 (PD-1) and/or cytotoxic T-lymphocytes antigen 4 (CTLA-4) with their ligands [118–120].

CONCLUSIONS

From the work of understanding the immune correlates of the RV144 vaccine trial and the immunobiology of bNAb development have come new non-NAb and bNAb reagents that—either as whole molecules or as engineered bispecific or trispecific antibody molecules—constitute a new class of therapeutic reagents for treating HIV-1. The field is presently in the early stages of

evaluation of these new reagents for treatment of HIV-1 infection, for targeting the latently infected pool of CD4$^+$ T cells, and to address their safety, immunoregulatory functions, and immunogenicity. Additional ongoing work is aimed at improving antibody-based reagent half-life and potency to increase the anti–HIV-1 effects of these antibodies. The hope is that new antibody formulations, in combination with cART and latency-reactivating agents, can promote NK or CD8$^+$ T-cell killing of infected CD4$^+$ T cells, thus adding a new class of drugs for the treatment, and eventual cure, of HIV-1 infection.

Notes

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