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Polyfunctionality of broadly neutralizing HIV-1 antibodies

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Abstract

Purpose of review

The discovery of broadly neutralizing HIV-1 antibodies (bNAbs) has provided a framework for vaccine design and created new hope toward an HIV-1 cure. These antibodies recognize the HIV-1 Envelope and inhibit viral fusion with unprecedented breadth and potency. Beyond their unique neutralization capacity, bNAbs also activate immune cells and interfere with viral spread through non-neutralizing activities. Here, we review the landscape of bNAbs functions and their contribution to clinical efficacy.

Recent findings

Parallel evaluation of bNAbs non-neutralizing activities using in vivo and in vitro models have revealed how their importance varies across antibodies and strains. Non-neutralizing bNAbs functions target both infected cells and viral particles, leading to their destruction through various mechanisms. Reservoir targeting and prevention in context of suboptimal neutralization highly depends on bNAbs polyfunctionality. We recently showed that bNAbs tether virions at the surface of infected cells, impairing release and forming immune complexes, with consequences that are still to be understood.

Summary

Non-neutralizing activities of bNAbs target infected cells, virions, and immune complexes, promoting viral clearance and possibly improving immune responses. We review how these functions participate to the efficacy of bNAbs and how they can be manipulated to improve bNAbs therapies.

Keywords

Broadly neutralizing antibodies, non-neutralizing activities, Fc-effector functions,

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Introduction

Anti-retroviral therapies (ART) have transformed HIV-1 into a chronic disease. Daily use of ART dampens viral replication to undetectable levels, preventing acquired immunodeficiency syndrome (AIDS) and transmission. These successes depend on ART accessibility, which is limited by politic and economic factors. Thus, in the absence of a vaccine, HIV-1 continues to spread in the human population at a pandemic level. Furthermore, a cure remains needed for the nearly 40 million of individuals that are currently living with the virus.

The identification of broadly neutralizing antibodies (bNAbs) offered new hope in both vaccine and cure research [1]. Infusion of bNAbs can prevent HIV-1 infection of naïve individuals and suppress viral replication in those already infected, provided that the virus is sensitive [2–4]. Importantly, an increasing body of evidence is suggesting that bNAbs add to existing ART, by providing long-lasting protection, by targeting the viral reservoir and potentially by stimulating endogenous immune responses [5–8].

Clinical successes of bNAbs have motivated a global research effort to characterize their mechanisms of action, with the aim of optimizing their utilization. These studies have confirmed that neutralization potency is key in bNAbs efficacy, but have also revealed that bNAbs are polyfunctional. Indeed, non-neutralizing effector functions mediated by the fragment crystallizable (Fc) domain are required for optimal efficacy. Each of the different classes of antibodies (IgG, IgA...) correspond to a unique Fc. In a seminal study, Ravetch and colleagues demonstrated that the Fc of bNAbs assist neutralization to limit HIV-1 replication in various mouse models [9]. Engineering the Fc to increase its interaction with Fc Receptors (FcRs, and FcγR for IgGs) improves bNAbs therapeutic efficacy [9]. Consistently, the Fc-mediated functions of a non-neutralizing antibody triggered viral evolution by killing infected cells [10]. In non-human primate models, the contribution of the Fc varies depending on antibody and context. In pre-exposure prophylaxis, PGT121 prevents infection independently of its Fc [11], while B12 requires it [12]. In therapeutic settings, it was estimated that 21% of viral clearance elicited by a VRC01 is mediated by its Fc domain [13].

While demonstrating the requirement of the Fc domain for optimal efficacy of bNAbs, these observations did not inform about the pathways involved. FcRs are diverse (either activating or inhibiting), widely expressed by immune and non-immune cells, and possess various affinities for Fc regions [14]. Antibody subtypes (IgG in the case of bNAbs) and glycosylation further modulate interactions between antibodies and FcRs [15]. This diversity leads to a large landscape of possible non-neutralizing antiviral functions and creates opportunities to leverage Fc engineering to improve bNAbs efficacy [16]. In vitro studies have revealed that bNAbs exert a wide range of activities, including elimination of infected cells and virions, creation of immune complexes, and protection of mucosal surfaces (see figure). In the following sections, we review these activities and discuss their importance in therapeutic settings.

Elimination of infected cells

The most studied non-neutralizing activities of bNAbs, is the antibody-dependent cellular cytotoxicity (ADCC). It consists in the activation of the cytotoxic potential of NK cells by antibodies. It requires FcγRIIIa (CD16) expression on NK cells and cell surface antibody binding on target cells. ADCC induction is not limited to bNAbs [17]. HIV-1 viral accessory proteins Vpu and Nef evade ADCC of non-neutralizing antibodies by modulating Env quantity and conformation at the surface of infected cells (review in [18]). These viral countermeasures also limit ADCC mediated by bNAbs, but infected cells remain largely sensitive [19,20]. bNAbs can elicit ADCC against cells infected with relevant viral strains, such as transmitted-founder virus or strains isolated from the viral reservoir [21,22]. ADCC by bNAbs strongly correlates to their neutralization potency, suggesting recruitment of NK cells is more influenced by the intrinsic capacity to bind Env, rather than the epitope targeted [19–21].

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Antibody-decorated cells may also be eliminated by other pathways, such as complement activation or phagocytosis [16]. We have observed that a subset of bNAbs activates the complement [23]. In contrast to ADCC, complement activation by bNAbs is restricted to some epitopes, namely the CD4 binding site (CD4bs) and the V3 loop. This may be explained by the structural basis of complement activation, which involves recognition of hexametric IgG structures by the complement component C1q [24]. Therefore, epitopes with high binding stoichiometry, and/or localized on the side of the trimer (allowing for the Fc to lie in parallel to the plasma membrane) may be favored. Complement activation by bNAbs further differ from ADCC by its outcome. Elimination of infected cells by complement is slow and observed in vitro only after a few days of culture, while ADCC occurs in a few hours [23,25]. The underlying mechanism remains cryptic. Whether complement modulates infected cell metabolism, as observed when complement receptors are engaged at the surface of activated T cell remains to be elucidated [26]. Alternatively, sub-optimal membrane-attack complex (MAC) deposition may trigger CA^{2+} flux, inflammasome activation and potentially cell death [27].

Phagocytic cells may also be activated by antibodies. This phenomenon is commonly called antibody-dependent cellular phagocytosis (ADCP) or antibody-dependent phagocytosis (ADP). Various mechanisms are involved, depending on the nature of the phagocytic cell. Monocytes, macrophages, dendritic cells and neutrophils are all capable of phagocytosis, but harbor different combinations of FcγRs, which vary within each of these cell types according to their activation status and localization [28]. Forthal and colleagues observed that cells covered with viral particles are susceptible to phagocytosis by a monocytic cell line in the presence of various monoclonal antibodies [29]. Env conformation in virions-opsonized cells largely differs from that of infected cells [30]. Whether phagocytic cells can engulf infected cells opsonized by bNAbs remains an open question that deserves further investigation.

Various immune pathways for infected cell elimination exist and HIV-1 infected cells are diverse. Beyond CD4 T cells, HIV-1 infects macrophages and dendritic cells. They exist under various polarization and activation status. When compared to T cells, HIV-1 infected macrophages are poorly susceptible to bNAbs-mediated ADCC, despite similar levels of Env accessibility [31]. This observation suggests that active mechanisms protect macrophages from ADCC and provides a proof-of-concept that some cellular compartments may act as sanctuaries for HIV-1 under ADCC pressure. CD4 T cells in the reservoir do not express viral proteins, but the observation of clonal persistence raises the question of Env expression during proliferation [32]. Overall, how cellular diversity in HIV-1 infected cells influence bNAbs-mediated ADCC-, complement- and phagocytosis-mediated clearance deserves more investigation.

Destruction of virions

Binding of antibodies to viral particles is often a pre-requisite of neutralization and may also trigger non-neutralizing antibody functions. This includes the complement system, which upon activation eventually leads to MAC deposition, membrane rupture and disintegration of viral particles [33]. This process is called virolysis. It has been suggested to contribute to the control of viremia during primary infection [34]. Consistently, HIV-1 encapsulates the regulatory complement proteins CD59 and CD55 within virions to evade complement attack [35]. Complement may be a double-edge sword, as its chemoattractant and stimulatory properties may also promote viral spread. This phenomenon most likely occurs when neutralization is lacking or sub-optimal. bNAbs are capable of eliciting virolysis in vitro, but in vivo evidence for a contribution of complement to bNAbs efficacy are missing [36]. These studies have demonstrated that a careful in vitro evaluation of bNAbs properties is needed prior to conclude of pathways elicited in vivo [37]. Thus, further work is needed to determine whether bNAbs capable of virolysis activate these functions in vivo and whether they participate to clinical efficacy.

Complement activation may also promote phagocytosis by activating Complement Receptors (CRs). Thus, antibodies and complement may synergize at the surface of viral particles

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to trigger phagocytosis, a phenomenon that has been reported for hexameric antibodies [38]. Whether bNAbs themselves can activate phagocytic cells to clear viral particles is a matter of debate. In vitro assays using Env-coated beads clearly demonstrated their phagocytic activity [39]. In the context of viral particles, contradictory results have been reported. On the one side, Env levels may be too low to allow sufficient signaling through FcγR and subsequent phagocytosis [29]. On the other side, the phagocytic activity of antibodies correlates to vaccine efficacy [40]. Phagocytic capacity strongly differs across cell types and activation status, and the relevance of in vitro assays remains to be determined.

Overall, bNAbs can target viral particles for neutralization and other effects, but the underlying mechanisms and their contribution to in vivo efficacy in unclear. This is in part due to the lack of tools to reliably analyze viral particles in vitro. Future technological advances in single viral particle analysis will be key to uncover how bNAbs target virions by other means than neutralization.

Tethering of viral particles

In addition to physical destruction of infected cells and viral particles, antibodies may impair viral spread by interfering with viral assembly or limiting viral release from cells.

For instance, antibody binding to the membrane-express Chikungunya virus (CHIKV) Env can hinder recruitment of viral structural proteins, thereby blocking viral budding and accumulating viral material into cells [41]. Virions may also accumulate at the plasma membrane as immune complexes (ICs), as reported for Influenza, Marburg virus, and, more recently, for HIV-1 [42–44]. Viral assembly is not impaired and virions are produced, however, upon release in the extra-cellular space, viral particles are sequestered by antibodies in large immune complexes that form between virions and cell membrane-anchored viral proteins. This trap accumulates virions as they are produced, reducing the amount of viral material released in the extracellular space. This phenomenon is reminiscent of tetherin, a restriction factor that inserts into membranes to create bridges between viral particles and infected cells, dramatically reducing HIV-1 spread [45]. In the case of HIV-1, this tetherin-like activity of antibodies has been reported only with a subset of the most potent bNAbs [44]. Consistently, viruses trapped in membrane-associated ICs are fully neutralized [44]. Therefore, the impact on viral replication in dense areas such as lymph nodes is probably limited as compared to neutralization alone, but this phenomenon may be of outmost importance to generate immunomodulatory ICs. bNAbs are studied for their capacity to promote endogenous immune responses (the so-called “vaccinal effect”) via Env-containing immune complexes (for a review see [46]). However, the origin of these immunogens is unknown. HIV-1 containing-ICs are difficult to produce in vitro, likely due to the limited number of Env incorporated into virions [47]. Thus, bNAb-mediated tethering of virions at the membrane of infected cells may constitute an important source of ICs. It has been reported that HIV-1 membrane aggregates created by tetherin activate NF-κB and trigger immune response [48]. Whether bNAbs-meditated formation of ICs at the plasma membrane participate in the initiation of the vaccinal effect deserves to be investigated.

FcRn-dependent bNab functions

In addition to FcγR, IgGs interact with the neonatal FcR (FcRN). Recognition of Fc by FcRn differs from that of FcγR by its stoichiometry (one Fc binds 2 FcRns) and its requirement for an acidic pH [49]. This latter is responsible for the most studied functions of FcRn, which is to recycle IgGs from endosomes to limit their degradation and expand their half-life. This activity is often manipulated by Fc-engineering to improve the biodisponibility of therapeutic mAbs (YTE and LS mutations), allowing to reduce doses and space injections [50]. In the context of HIV-1, the LS mutation has been introduced into 10-1074, 3BNC117 and VRC01 mAbs [51,52], allowing to control viral replication with a single injection for a median of 20 weeks in non-human primates [51].

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Interestingly, the ability of the Fc to interact with FcRn is not solely responsible for the pharmacokinetic of IgG as determinants in the Fab region also modulate FcRn affinity [53].

Beyond recycling, FcRn mediates transcytosis of IgG across epithelia. This is of outmost importance, as mucosal immunity is key in the prevention of HIV-1. The LS mutation increases antibody transfer across cellular monolayers in vitro and promotes accumulation of infused IgGs bearing the LS mutation in the lumen of the gastrointestinal tract [54]. More importantly, improved FcRn affinity ameliorates immune protection upon a rectal challenge performed after infusion of a sub-optimal antibody dose [54]. While the opposite was not tested (i.e an antibody lacking FcRn interaction), data from the literature indicates that FcRn participates in the accumulation of WT IgG in the mucosa and contributes to protection upon mucosal challenge [55]. FcRn also mediates passive fetal humoral immunity by transferring IgG across the placenta [49]. Altogether, these data suggest that FcRn is a key contributor of the efficacy of bNAbs to prevent sexual HIV-1 infection and possibly maternofetal transmission. How Fc mutations influence the biodistribution of bNAbs beyond serum half-life deserves further investigations.

More recently, the vaccinal effect has emerged as a possible mechanism underlying the efficacy of bNAbs therapies, and as a mean to improve and extend their utilization (for a review see [46]). FcRn engagement in the lysosome of dendritic cells is required to target engulfed antibodies to cross-presentation pathways [56]. FcRn also allows for the discrimination of small and large ICs, which are either recycled or loaded on MHC-A, respectively [57]. Understanding how FcRn participates to presentation of viral antigens during HIV-1 bNAbs therapy and how this is modulated by Fc mutations increasing their half-life will provide new keys to understand and improve antibody functions.

Conclusions

Polyfunctionality is integral to bNAbs activities. Non-neutralizing activities include clearance of infected cells and viral particles, as well as formation and uptake of immune complexes (see figure). During therapy, these functions may assist neutralization to expand its breadth and allow for reservoir targeting. It may be achieved through direct (i.e clearance of infected cells and viruses) or indirect mechanisms (i.e immunomodulation). As pan-HIV-1 neutralization is not yet elicited by vaccination and is not sufficient to cure HIV-1, it is mandatory to better understand the polyfunctionality of bNAbs to combat HIV-1.

Key points

- How cell types and activation status influence Env expression and subsequent clearance of infected and reservoir cells by bNAbs?
- Which non-neutralizing antibody functions impose immune pressure on the virus?
- Is bNAbs-mediated trapping of viral particles at the cell surface involved in their immunomodulatory properties?
- To which extent Fc-engineering impacts biodistribution and polyfunctionality of bNAbs?
- Is it possible to design drugs that synergize with bNAbs by boosting their polyfunctionality?

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Conflict of interest

The authors declare no conflict of interest.

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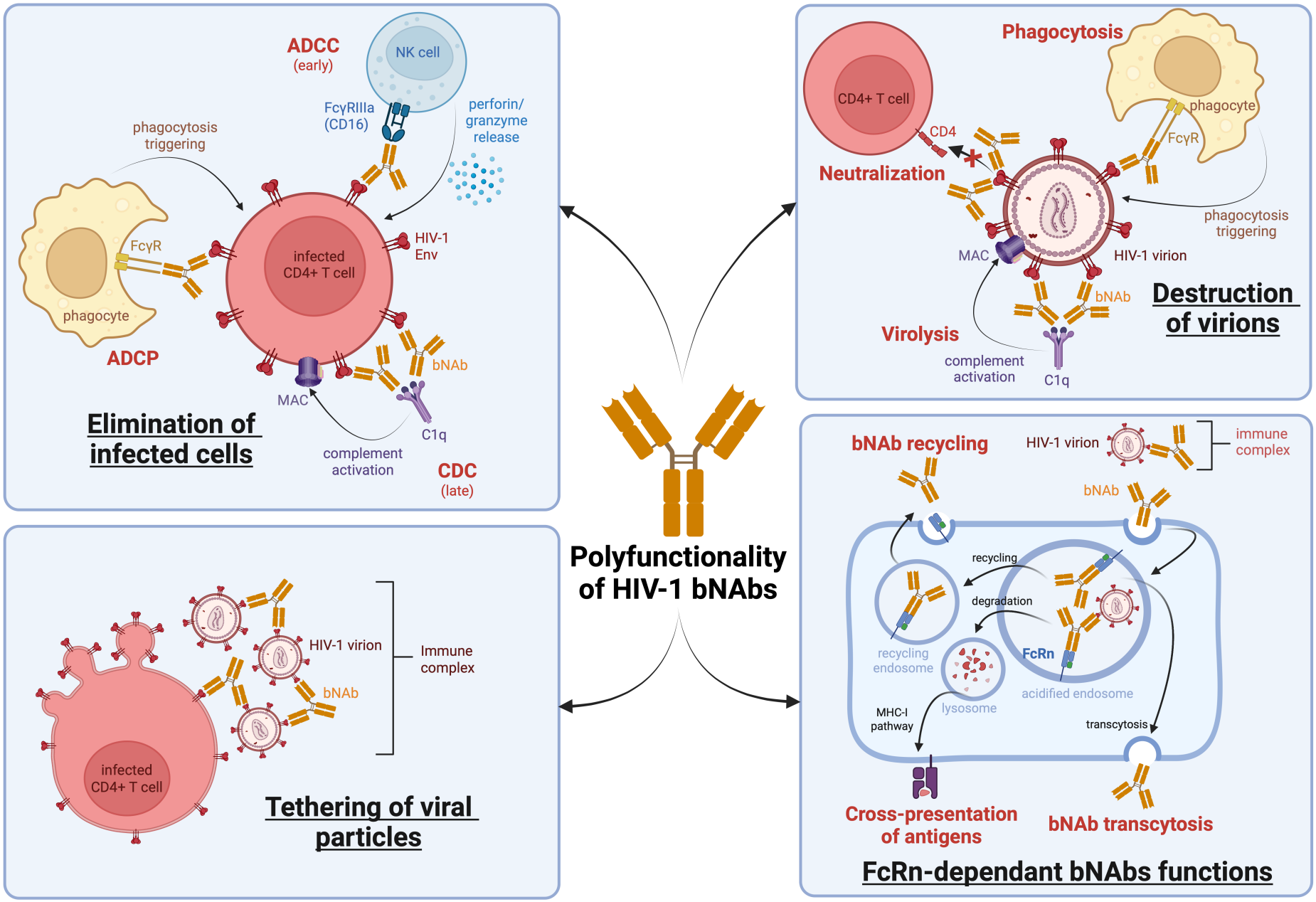
ADCC
(early)
NK cell
FcγRIIIa
(CD16)
perforin/
granzyme
release
phagocytosis
triggering
FcγR
infected
CD4+ T cell
HIV-1
Env
phagocyte
ADCP
bNAb
MAC
C1q
complement
activation
CDC
(late)
Elimination of
infected cells
Phagocytosis
CD4+ T cell
CD4
phagocyte
FcγR
Neutralization
phagocytosis
triggering
MAC
HIV-1 virion
bNAb
Destruction
of virions
Virolysis
complement
activation
C1q
Polyfunctionality
of HIV-1 bNAbs
HIV-1 virion
Immune
complex
bNAb
infected
CD4+ T cell
Tethering of viral
particles
bNAb recycling
HIV-1 virion
immune
complex
bNAb
recycling
degradation
recycling
endosome
FcRn
acidified endosome
lysosome
MHC-I
pathway
transcytosis
Cross-presentation
of antigens
bNAb transcytosis
FcRn-dependant bNAbs functions

Figure Legend

Schematic representation of bNAbs polyfunctionality. Beyond neutralization, bNAbs target infected cells and viral particles for clearance via activation of immune effector mechanisms (upper left and right). Opsonization of infected cells may also lead to tethering of viral particles, limiting viral release and forming large immune complexes (lower left). Most non-neutralizing activities are elicited by gamma-chain Fc Receptor (FcγR), but IgGs also interact with neonatal FcR (FcRn), with consequences that are yet not fully elucidated in the context of HIV-1 bNAbs (lower right).

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