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Human monoclonal antibodies and engineered antibody domains as HIV-1 entry inhibitors

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Abstract

Purpose of review—To summarize the in vivo efficacy of neutralizing human monoclonal antibodies (nhmAbs) against HIV-1, to discuss the recent finding that an engineered human antibody VH domain (domain antibody, dAb) exhibits exceptionally potent and broadly cross-reactive neutralizing activity against HIV-1 primary isolates by targeting a hidden conserved epitope that is not accessible by larger antibodies, and to suggest the possibility of developing a novel class of potent HIV-1 inhibitors based on human dAbs.

Recent findings—HIV-1 has evolved a number of strategies to evade humoral immunity including protecting highly conserved and important structures from the access of antibodies generated by the immune system. We have recently demonstrated that a human dAb (size ~15 kDa), m36, targets a highly protected structure on the HIV-1 envelope glycoprotein (Env), gp120, and exhibits exceptionally potent neutralizing activity against HIV-1 primary isolates with potency on average higher than those of the broadly cross-reactive nhmAb scFv m9 and the inhibitory peptide C34.

Summary—The efficacy of the anti-HIV-1 therapy is significantly compromised by resistance to the currently used FDA-approved antiretroviral drugs suggesting an urgent need to develop novel classes of potent inhibitors. Several bcnhmAbs are highly effective against HIV-1 infection in vitro but their administration to HIV-1-infected humans has only resulted in modest antiviral effects. Engineered human antibody fragments, dAbs, could be more potent because of their small size (about 10-fold smaller than that of an IgG) which allows targeting of highly conserved structures on the HIV-1 Env that are not accessible by full-size antibodies and relatively efficient penetration into the densely packed lymphoid environment where HIV-1 mostly replicates and spreads.

Keywords

HIV-1; monoclonal antibody; domain antibody; neutralization; human

Introduction

Antibodies have been very successful in protecting animals and humans from infection by many viruses [1–3]. However, treatment of HIV-1-infected patients with broadly cross-reactive neutralizing human monoclonal antibodies (bcnhmAbs) conferred, at best, only transient reduction in viral loads most likely due to the rapid emergence of resistant viruses [4,5*] and the inability of antibody to eliminate virus-producing cells especially in the densely packed lymphoid environment [6]. Here we review the in vivo efficacy of nhmAbs against HIV-1

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Note added in proof

Recently, an article was published in *J Virol* (82:12069, 2008) where potent cross-reactive HIV-1-neutralizing single domain antibodies from llama were described that target the CD4 binding site.

(Table 1) and discuss the recent finding that an antibody as an isolated VH domain (which is about 10-fold smaller than an IgG) can access highly conserved structures on the HIV-1 gp120 and exhibits exceptionally potent neutralizing activity against HIV-1 primary isolates which could open an avenue for developing a novel class of potent HIV-1 inhibitors based on human dAbs [19**].

Neutralization of HIV-1 by full-size mAbs

A majority of the anti-HIV-1 mAbs have been characterized in an IgG1 format with size of about 150 kDa (Fig. 1). They are directed mostly against the Envs; some of them target cell surface receptors that are vital for virus infection [20,21,22*].

One of the best characterized and very potent nhmAbs targeting the CD4 binding site (CD4bs) on gp120 is b12 which was selected by phage-display of an antibody library constructed from the bone marrow of an HIV-1-infected donor [23]. B12 has been shown to neutralize a wide range of HIV isolates from different clades [24]. Intravenous infusion of b12 provided dose-dependent protection to macaques vaginally challenged with the R5 virus SHIV_{162P4} [7] (Table 1). Further, it has been found that vaginal administration of b12 can partially protect macaques from vaginal SHIV transmission [8]. These observations support the concept that nhmAbs can help prevent the sexual transmission of HIV-1 to humans. A recent study [20**] looked into the role of antibody Fc-mediated effector functions in protection of macaques against vaginal challenge. A significant decrease in the ability of b12 to protect macaques against SHIV challenge was observed when Fc receptor and complement binding activity were engineered out of the antibody while no loss of antibody protective activity was associated with elimination of complement binding alone suggesting the importance of antibody activity against both free viruses and virus-infected cells for effective protection. Unfortunately, b12 has not been tested in human clinical trials. Another CD4bs nhmAb, F105, was evaluated in a phase I clinical trial [9]. Although it was safe and nontoxic as a single intravenous injection, there was no obvious anti-HIV-1 activity observed.

2G12, 2F5 and 4E10 are identified to be three of the most potent nhmAbs against HIV-1 in vitro [25]. 2G12 [26] is a unique carbohydrate-binding nhmAb binding to a particular glycan arrangement in gp120 while 2F5 and 4E10 [27,28] target the membrane-proximal external region (MPER) of gp41. In vitro studies demonstrated that these three antibodies could synergize in their neutralizing potency [29] so they were evaluated mainly in combination in animal models and in HIV-infected persons. When administered alone, neither 2F5 nor 2G12 completely protected macaques from intravenous challenge although treated animals displayed a less profound drop in CD4⁺ T cells [10]. One of three macaques treated with a combination of 2F5 and 2G12 was apparently protected while the other two had marked reductions in viral loads. A phase I clinical trial with the 2F5/2G12 combination was conducted in seven HIV-1-infected patients [11]. The antibodies were safe and well tolerated. Transient reductions in viral loads and increases in CD4⁺ T cell counts and CD4⁺/CD8⁺ ratios were observed in five of seven and all individuals, respectively. However, virus escape from 2G12 occurred. The nhmAb 4E10 was added to the regimen to result in a cocktail of three antibodies including 2F5 and 2G12 [30]. A phase I evaluation in seven individuals again showed no drug-related adverse effects. During the phase II clinical trial [12], eight chronically and six acutely infected individuals who were on antiretroviral treatment (ART) before antibody administration were recruited. The capacity of the antibodies to suppress or delay the viral rebound caused by the ART was evaluated. Four of six acutely infected individuals showed a pronounced delay of viral rebound while the delay was observed in only two of eight chronically infected ones suggesting more significant impact of antibody treatment among acutely infected individuals. Resistance to 2G12 but not to 2F5 or 4E10 was observed in 12 of 14 individuals suggesting that strong immune pressure and escape were induced by 2G12 [5*]. Further evaluation

indicated that the in vivo activities of these three antibodies were likely due to direct neutralization and/or Fc receptor-mediated mechanisms such as phagocytosis and ADCC but not to complement-mediated virolysis [31*], in agreement with the previous study [20**].

V3 on gp120 is one of the principle neutralizing determinants of virus and elicits many antibodies in vivo. C β 1 is a chimeric mouse-human mAb [13]. Chimpanzees intravenously treated with C β 1 either 24 h before or 10 min after intravenous challenge with HIV-1_{IIIB} remained free of virus infection and did not present any signs of persistent infection. Recently, a humanized mAb, KD-247, has been shown capable of providing macaques with sterilizing protection against SHIV_{C2/1} [14]. CGP 47,439, a chimeric mouse-human mAb, was tested in a human (phase I/IIA) trial [15]. Intravenous infusion of CGP 47,439 resulted in a reduction in plasma viral RNA and p24 antigen in seven of twelve patients. Another reagent, hNM01, is a humanized rat mAb [16]. In a phase I study, three of four patients receiving hNM01 had a decrease in plasma viral loads.

The primary receptor CD4 and the coreceptor CCR5 are vital for infection by most HIV isolates and are therefore attractive targets. A single dose of TNX-355 (humanized-mouse anti-CD4 mAb) [17] or PRO 140 (humanized-mouse anti-CCR5 mAb) [18*] led to dose-dependent reduction in plasma HIV-1 RNA loads and increases in CD4+ T cell counts in HIV-infected patients. Although their use could be limited by the possibility of interference with the immunologic functions of these receptors and to deplete their expressing cells in vivo [21], study on PRO 140 [18*] showed no drug-related serious adverse events.

Inhibition of HIV-1 infection by antibody fragments - Fab and scFv

It has been proposed that engineered antibody fragments (e.g. scFv and Fab) (Fig. 1) may be better able to control virus replication because they are much smaller so that they may be capable of avoiding certain defense mechanisms such as steric occlusion that HIV has developed against antibodies generated by host immune mechanisms [21]. A finding [32] in line with this proposal is that the scFvs (~25 kDa) and Fabs (~50 kDa) targeting the epitopes on gp120 induced by CD4 binding (CD4i) neutralize virus better than their IgG forms (~150 kDa). According to a previously published model [32], size-dependent neutralization could be due to steric restriction for antibody access to CD4i epitopes. One of the reasons could be that after CD4 binds to the virus, the available space between the virus and the target cell surface is not sufficient to accommodate a whole antibody molecule but is adequate for antibody fragments. Thus, most of the CD4i mAbs that have been in development for use as potential therapeutics as single molecules or fusion proteins such as 17b [32], X5 [33], m9 [34] and m16 [35] are in scFv or Fab format. An encouraging result is that such antibodies are exceptionally potent and broad as has been demonstrated for scFv m9, which exhibits superior neutralizing activity compared to known HIV-1 bcnhmAbs (b12, 4E10, 2F5, 2G12, X5) when tested against more than 100 isolates (Zhang M et al., unpublished work). However, before these engineered antibody fragments can be suited for human clinical trials, several issues should be addressed including their short half-life and lack of biological effector functions due to the absence of Fc. These issues will be discussed in the following section.

Engineered human dAbs as a novel class of inhibitors

Isolated stable human antibody variable domains have been previously described [36,37**] and are promising candidate therapeutics against cancers and autoimmune diseases (<http://www.arana.com>; <http://www.ablynx.com>). These smallest antibody fragments with size ranging from 11 to 15 kDa are called “domain antibodies” or “dAbs” (Fig. 1). The absence of the VL or VH domain means that the paratope is concentrated over a smaller area so that the dAbs provide the capability of interacting with novel epitopes that are inaccessible to conventional VH-VL pairs and penetrating into solid tissues even better than Fab and scFv.

Analysis of the structures of HIV-1 Envs shows that most of their surface is hidden from humoral immune responses by glycosylation and oligomeric occlusion [38,39]. The CD4 binding site and the coreceptor binding site represent two major targets on gp120 for nhmAbs. The CD4 binding site is formed as a recessed pocket with highly conserved residues centered and surrounded by variable residues [38]. This pocket is flanked by V1/V2 loop structures which may further limit access by large antibodies [40]. The steric occlusion also takes place in the CD4i epitopes overlapping with the coreceptor binding site which are formed and/or exposed after CD4 binding [32,38,39]. The available space between the CD4-anchored viral spike and the proximal target membrane may not be enough to accommodate full size antibodies that might bind these epitopes [32]. The CD4i epitopes are also masked by the flanking V2 and V3 loops [38]. These physical constraints require that antibodies are small and comprise protruding paratopes to access these regions as has been demonstrated for CD4i antibodies 17b and X5 which exhibit better neutralization activity as Fabs or scFvs and have long protruding heavy chain CDR3s [32,39,41]. Thus, we have hypothesized [19**] that antibody fragments even smaller than scFvs such as dAbs could be exceptionally potent against HIV-1 by binding to conserved structures that are inaccessible or partially accessible by full-size antibodies generated by the human immune system. It has been recently demonstrated [19**] that a human dAb, m36, targeting such highly protected structures on HIV-1 gp120 is broadly cross-reactive against HIV-1 primary isolates and has potency on average 2–3 fold higher than that of scFv m9 which has superior neutralizing activity compared to other known HIV-1 bcnmAbs (b12, 4E10, 2F5, 2G12, X5) when tested against more than 100 isolates (Zhang M et al, unpublished work), presenting supporting evidence for our hypothesis. We have also speculated that such dAbs could be more resistant to HIV-1 escape because their hidden epitopes could be more conserved and invariable as required for the maintenance of substantial binding to receptor or coreceptor. Moreover, dAbs may have better binding kinetics (e.g. increased association rate) while recognizing and anchoring the physically obstructed epitopes on HIV-1 Envs so that they may neutralize virus faster to efficiently prevent establishment of infection. This may be especially relevant in the context of acute infection.

The human clinical trials described above indicate that the existing HIV-1 nhmAbs of IgG format are unable to confer favorable antiviral activity. In particular, viral rebound occurs in all patients receiving either high or low doses of these nhmAbs. Importantly, the impact of antibody treatment seems to be less pronounced among chronically infected individuals [12] suggesting that these nhmAbs can be less effective in established HIV-1 infection. Since HIV-1 replicates and spreads in the densely packed lymphoid environment such as spleen, gut and lymph nodes, it is reasonable to predict that nhmAbs of large size may have difficulty in eliminating virus and virus-infected cells in these tissues perhaps due to their limited ability to penetrate. Because of their small molecular size, dAbs may penetrate even better than scFvs and Fabs, which have been shown to move much faster in solid tumors than IgG [42,43*].

Two major issues need to be addressed before dAbs can be suited for *in vivo* use. These include their short half-life in circulation and lack of biological effector functions as has been described for other antibody fragments including scFvs and Fabs. Our finding [19**] that a fusion protein of dAb with a human serum albumin binding peptide (HSAbp), which still has very small size (~15–20 kDa) (Fig. 1), retains about the same neutralizing activity as unconjugated dAb indicates a possibility to improve the antibody half-life *in vivo*. Direct fusion to HSA [44*] and PEGylation [45*] are alternative strategies to enhance the antibody pharmacokinetics. However, such molecules have relatively large size that could lead to decreased inhibitory activity. Attractively, dAbs can be fused to human IgG1 Fc (Fig. 1) to retain biological effector functions and long half-life while remaining smaller than an IgG (~75 kDa, about half of the size of an IgG). Although some of these strategies bring dAbs back to medium molecular weight (~85 and 75 kDa for fusion proteins with HSA and human IgG1 Fc, respectively) agents, they could still promise more efficient penetration than full-length antibodies (~150 kDa).

Importantly, fusion proteins of dAb could preserve better ability of targeting certain hidden conserved epitopes such as CD4bs epitopes than full size antibodies; such epitopes could be accessed by dAbs that have smaller size and in general smaller paratopes than the Fabs of full-size antibodies. The half-life and effector functions may not be of significant concern when antibodies are applied vaginally as a topical microbicide [8]. In all cases the small size of dAbs allows for higher molar quantities per gram of product; this should provide a significant increase in potency per dose and a reduction in overall manufacturing cost (<http://www.domantis.com>).

Conclusion

HIV-1 has evolved a number of strategies to escape host immune surveillance, prominently by modifications to the Envs. Thus, naturally occurring whole antibodies to HIV-1 Env may not have favorable inhibitory activity against viral infection, replication and disease progression, as evidenced by the lack of sustained significant effect in several clinical treatment trials. This is most likely due to the rapid generation of resistant viruses and the presumably limited or lack of antibody infiltration of the lymphoid environment where HIV replicates and spreads. The engineered smallest antibody fragments, dAbs, may have properties that may evade mechanisms used by HIV to escape neutralization better than current nhmAbs can although only *in vivo* experiments in animals and humans can definitely prove this hypothesis.

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Figure 1.
Schematic representation of human antibody as an IgG1, antibody fragments and their derivatives. Their estimated molecular size is indicated.

Table 1

Representative nmAbs against HIV-1 used in vivo

Epitope	Antibody	Format	Animal/Human	Virus	Infusion route	Challenge route	Efficacy	References
CD4-binding site on gp120	b12	IgG1	Macaque	SHIV _{162P4}	Intravenously	Vaginally	4/4 protected	[7]
	b12	IgG1	Macaque	SHIV _{162P4}	Vaginally	Vaginally	9/12 protected	[8]
	F105	IgG1	Human	NA	Intravenously	NA	No obvious inhibitory activity	[9]
Carbohydrate on gp120	2G12	IgG1	Macaque	SHIV _{89.6PD}	Intravenously	Intravenously	High-level plasma viremia; Less decrease in CD4+ T cells	[10]
gp41	2F5	IgG1	Macaque	SHIV _{89.6PD}	Intravenously	Intravenously	High-level plasma viremia	[10]
	2F5/2G12	IgG1	Macaque	SHIV _{89.6PD}	Intravenously	Intravenously	1/3 with transient infection; 2/3 with marked reductions in viral loads	[10]
	2F5/2G12	IgG1	Human	NA	Intravenously	NA	5/7 with transient reductions in viral loads; 7/7 with transient increase in CD4+ T cells	[11]
V3 on gp120	2F5/2G12/4E10	IgG1	Human	mostly clade B	Intravenously	NA	2/8 chronically and 4/6 acutely infected individuals with delay in viral rebound	[12]
	Cβ1	IgG1	Chimpanzee	HIV-1 _{IIIB}	Intravenously	Intravenously	1/1 protected	[13]
	KD-247	IgG1	Macaque	SHIV _{C21}	Intravenously	Intravenously	2/2 protected	[14]
	CGP 47,439	IgG1	Human	NA	Intravenously	NA	7/12 with reductions in plasma viral RNA and p24	[15]
	hNM01	IgG1	Human	NA	Intravenously	NA	3/4 with decrease in plasma viral loads	[16]
CD4	TNX-355	IgG4	Human	NA	Intravenously	NA	Dose-related reductions in plasma viral loads in 30 individuals	[17]
CCR5	PRO 140	IgG1	Human	NA	Intravenously	NA	Dose-related reductions in plasma viral loads in 39 individuals	[18*]

NA, not applied or not available.