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Epitope Target Structures of Fc-mediated Effector Function During HIV-1 Acquisition

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Abstract

Purpose of review—This review analyzes recent studies suggesting that highly conserved epitopes in the HIV-1 Env trimer are targets of potentially protective non-neutralizing antibodies that mediate antibody-dependent cellular cytotoxicity (ADCC).

Recent findings—Recent studies in both non-human primates and humans, suggest that non-neutralizing antibodies play a role in blocking infection with SHIV/SIV or HIV-1 by Fc-mediated effector function, in particular ADCC. Further, several studies implicate highly conserved epitopes in the C1 region of gp120 as targets of these antibodies. However, these suggestions are controversial, as passive immunization studies do not indicate that such antibodies can block acquisition in non-human primates. Potential reasons for this discrepancy are discussed in the structural context of potent ADCC epitopes on target cells during the narrow window of opportunity when antibodies can block HIV-1 acquisition.

Summary—Cumulative evidence suggests that in addition to virus neutralization, Fc-mediated effector responses to highly conserved epitopes in the HIV-1 trimer play distinct as well as overlapping roles in blocking HIV-1 acquisition. Evidence will be discussed whether non-neutralizing antibodies specific for epitopes on the HIV-1 Env trimer that become exposed during viral entry contribute significantly to blocking HIV-1 acquisition.

Keywords

Fc-mediated effector function; HIV; antibody; epitope

Introduction

NHP passive immunization studies show that neutralization is an important mechanism for blocking HIV-1 acquisition [1–4•]. The recent discovery of broadly neutralizing monoclonal

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antibodies (mAbs) offers hope that vaccines can elicit broadly protective responses [5–9], although this is an elusive goal currently. This is because high levels of somatic hypermutation required for neutralization breadth [10•] and clonal deletion of self-reactive B cells that recognize the membrane proximal epitope region (MPER) [11•–14]. By contrast, a large number of natural history studies in humans spanning over twenty-five years a similar NHP studies strongly imply Fc-mediated effector function contributes to post-infection viral control (reviewed in [15•–19]). These correlations are strong but they occur post-infection, leaving open the question of whether Fc-mediated effector function alone can block HIV-1 acquisition.

In regard to this point, NHP vaccination elicits ADCC ([20, 21••], in preparation) or phagocytic [22••] responses that correlate inversely with reduced risk of SHIV/SIV acquisition. Similarly, secondary analyses of the VAX004 [23] and RV144 [24••] vaccine trials found inverse correlations between Fc-mediated effector function and reduced risk of infection in volunteer subpopulations. Further RV144 analyses implicated the highly conserved non-neutralizing A32 epitope of gp120 as the target of ADCC responses correlating with reduced infection risk [25, 26••]. This epitope is a major ADCC target in HIV-1 infected individuals [27] where it is subject to immune escape early in infection [28]. Recently, we reported that the A32 epitope is a member of a cluster of at least three non-neutralizing epitopes (Epitope Cluster A) on the gp41-interactive face of gp120 that mediate potent ADCC [29••]. Collectively, these correlative studies implicate a role for Fc-mediated effector function alone in blocking HIV-1 acquisition. This hypothesis is also supported by a natural history study where breast milk ADCC titers correlated inversely with infant transmission [30••].

On the other hand, passive immunization studies using non-neutralizing antibodies with Fc-mediated effector function have largely failed to confirm this hypothesis. An early passive immunization study suggested that non-neutralizing antisera with Fc-mediated effector function could block SIV acquisition in NHPs [31] but protection was not observed later in the same system [32]. Similarly, two recent NHP studies using non-neutralizing gp41 mAbs found post-infection control of SHIV162p3 [3, 33]... In [33], these mAbs were selected on the basis of potent ADCC. It should be noted that in [3], two of the five NHPs in the anti-gp41 treated group remained uninfected while five of five NHPs in the control group were infected, suggesting that this mAb might have blocked acquisition, although it was not statistically significant. By contrast, all NHPs in the group receiving similar anti-gp41 mAbs were infected [33], suggesting that the protection against acquisition in [3] is more apparent than real. In both studies, neutralizing mAbs blocked acquisition. Thus, either the correlations cited above are not causal or the NHP models in their current configuration underestimate the ability of non-neutralizing antibodies to block acquisition by Fc-mediated effector function.

As argued elsewhere [15•], in vivo dose-response behavior unique to Fc-mediated effector function, prozones in particular, possibly underestimate acquisition blocking due to the high doses of mAbs such as those used when only post-infection control was observed [3, 33]. Emerging data that high non-neutralizing mAb doses can modestly reduce the number of transmitted SHIV variants supports this possibility [34]. As discussed in [15•], prozone

behavior is determined by antibody specificity by affecting the quality of antigen-antibody complexes to trigger Fc-mediated effector function. We showed recently that specificity is a key element of ADCC potency in vitro [29] and argued that it is likely to be so in vivo [15•]. Accordingly, this review will focus on epitope categories that are likely to be targets of potent Fc-mediated effector function by non-neutralizing antibodies during HIV-1 acquisition. Both passive immunization and anti-retroviral studies (reviewed in [16]) show that there is at most a twenty-four hour “window of opportunity” for an antibody to block acquisition. This sets the temporal stage for considering which epitope targets are extant on target cells for Fc-mediated effector function during the window of opportunity. For simplicity, discussion will be limited to effector functions that require the interaction between an effector cell and a target cell, such as ADCC.

Two Classes of Epitope Targets for Fc-mediated Effector Function During HIV-1 Acquisition

Although it is common practice to use infected cells as target cells in ADCC assays, it is unlikely that there will be many infected cells budding virions early in the twenty-four window of opportunity. Classical single step infection studies show that *env* mRNA only appears at eight hours post-infection and becomes maximal only after twenty-four hours [35, 36]. Thus, there should be very little trimeric Env on infected cell surfaces during the first twelve to sixteen hours after in vivo exposure that can be targeted by Fc-mediated effector function. Further, this “null” period is probably longer because in vivo infections are unlikely to be synchronous. This led us to consider whether epitopes exposed during viral entry, rather than assembly and release, can be potent ADCC targets. This hypothesis stemmed also from our earlier observation that the highly conserved A32 epitope persists on target cell surfaces during Env-mediated cell fusion [37], which led to the hypothesis that it would be a potent ADCC target during viral entry [38]. Our recent study confirmed and extended this hypothesis to other Cluster A epitopes associated with the gp41-interactive face of gp120 [29]. Thus, we propose two classes of epitope targets that are distinguished by whether they are on virions entering target cells (entry targets) or on target cells that are infected and budding virions (release targets). They are distinguished further by their temporal appearance during the twenty-four hour window of opportunity as diagrammed in Figure 1. Entry targets will be prevalent during the early part of the window whereas release targets will appear later (Figure 1). Figure 2 shows the predicted Env structures on entry and release targets.

Entry Targets

As shown in Figure 2, upper panel, there are two epitope categories likely to be extant on target cells during viral entry. The first includes epitopes on native trimers that have not been triggered by binding to cell surface CD4 and, subsequently, co-receptors. It is envisioned that these trimers are distal on the attached virion to those binding CD4. These epitopes should be highest in concentration shortly after binding, decreasing thereafter due to conformational rearrangement of the trimer during viral entry. This is consistent with our observation [39] that PG9 is less effective at ADCC than mAbs specific for epitopes that

become exposed selectively during viral entry (CD4i epitopes). Thus, CD4i epitopes constitute the second epitope category during viral entry.

CD4i epitopes include structures that are exposed consequent to binding CD4 and subsequent co-receptor interactions during viral entry. An excellent review was published recently detailing mAb specificities that mediate ADCC and they include both neutralizing and non-neutralizing mAbs recognizing most of the known epitope regions of gp120 and gp41 (Table 1 in [18•]). Because that list was drawn from many studies, it is not possible to rank order the different specificities for potency. Accordingly, our analysis of entry target epitopes will be limited to the most potent CD4i mAbs that we rank ordered in plots of EC50 versus % plateau cytotoxicity in [29]. Over a large series of studies, we have found that mAbs recognizing epitopes associated with the gp41-interactive face of gp120 are consistently highly potent in that they achieve 100% plateau cytotoxicity with EC50s ranging from approximately 5 pM to 5 nM ([29], and in preparation). These mAbs define Epitope Cluster A, which is comprised of at least three different sub-groups defined by competition with mAbs A32 and C11 for binding to CD4 triggered gp120 [29]. One sub-group only competes A32, the second only competes C11, and the third competes both. Mutagenesis studies have mapped the A32 epitope sub-region to mobile layers 1 and 2 in the C1/C2 region [40, 41] and the C11 epitope sub-region to the 7-stranded β -sandwich [42]. We have confirmed and extended this finding for the A32 sub-region by x-ray crystallography and for the C11 region by docking of the C11 Fab crystal structure to gp120 and by mutagenesis (in preparation). As shown in Figure 3, these regions are buried in the highest resolution Env trimer structure available to date where they are in apparent contact with gp41 [43, 44••] as previously predicted [45]. Using fluorescence correlation spectroscopy we have directly shown that these epitopes are not exposed on virions in solution [46•]. Curiously, both earlier studies [45] and ours [46] show that soluble CD4 does not expose these regions on free virions whereas they are exposed and become strong ADCC targets during viral entry on CD4+ CCR5+ cells [29]. This is supported also by the demonstration that soluble CD4 induces a metastable conformation of the trimer that is less prevalent with cell surface CD4 [47]. Because Epitope Cluster A requires trimer binding to cell surface CD4, the structural basis for potency of this region on trimeric Env is inferential and likely to remain so until high resolution methods to characterize structures on viable cell surfaces are brought to bear on the problem. There is an additional, unexpected, observation that hints at the nature of ADCC target structures during viral entry.

We have observed that mAbs specific for the conserved principal immunodominant domain (PID) including the intra-chain disulfide loop (DSL) of gp41 are potent mediators of ADCC against entry targets using our sensitization method and rank order algorithm [29] (in preparation). As reviewed in [18•] it has been known for some years that this region is an ADCC target on infected cells, and a recent study confirmed that two mAbs to this region exhibit considerable ADCC potency against release targets [33]. It is interesting to note that the two most potent sets of entry epitope targets localize to regions of both gp120 and gp41 that have been known for many years to interact in stable trimers [48–55]. PID epitopes are of particular interest as entry targets. It was shown many years ago that this region is becomes more exposed on infected cells by the interaction with soluble CD4 [56, 57] and it is also becomes exposed during Env-mediated cell fusion [58]. By contrast, mAbs specific

for this region can bind virions [3, 33, 46, 59, 60] although binding can be increased in some cases by soluble CD4 [46]. Virion binding in the absence of CD4 is probably due to gp41 “stumps” on the virion surface that are variable byproducts of viral biosynthesis [60]. Both our studies [46, 58] and the recent trimer structure (see Figure S7 in [43••]) suggest that this region is inaccessible until interactions with CD4 and, possibly, the co-receptor. Thus, there are two possible targets of anti-PID mAbs during viral entry, a variable population extant on the free virion surface and a population that becomes exposed during viral entry. We do not know whether the former population exists on transmitted variants but it is highly likely that the latter population does. Our data suggest that in the context of entering virions, that the latter population is proximal to the exposed Cluster A epitope region that is a potent ADCC target. This hypothesis is being pursued using super-resolution microscopy, as it cannot be answered via structural studies such as x-ray crystallography using truncated Env proteins.

Release Targets

Surprisingly, release targets are also comprised of two epitope categories and possibly more. First, by definition, infected cells produce infectious viral particles that have native trimers on their surfaces. In principle, any epitope that is exposed on native trimer can be an ADCC target. As reviewed in [18] and illustrated in Figure 4, broadly neutralizing mAbs specific for epitopes in the immune site of vulnerability on gp120 [61], the CD4 binding site (CD4bs) [62, 63], and the MPER of gp41 [33, 64], can mediate ADCC against release targets. Further, in the case of the CD4bs mAb, b12, there is evidence that both neutralization and Fc-mediated effector function contribute to protection of NHPs against vaginal challenges with SHIV162p3 [1, 2]. These predictions are based on literature and the recent trimer structures that are missing the MPER [43, 44••]. In addition, release targets can theoretically express gp41 “stumps” as discussed above.

Further, it is key keep in mind that on virions the trimers interact with the matrix protein via their cytoplasmic tails, which is necessary for their incorporation into budding virions (reviewed in [65•]). The nature of the cytoplasmic tail can affect the CD4-dependence of infectivity as well as epitope stable CD4i exposure on native trimers [66, 67]. Additionally, whether the matrix protein is cleaved from the p55^{Gag} precursor determines fusogenicity and epitope exposure on the trimer ectodomain [68]. Thus, release targets are almost certain to be antigenically more complex than predicted by the recent soluble trimer structures. A full understanding of the potential for mAbs to exert Fc-mediated effector function requires careful documentation of epitope exposure during various stages of virus maturation at the infected cell surface using super resolution microscopy.

Second, release targets can also express CD4i epitopes, and Epitope Cluster A in particular. This was shown first in studies of HIV-1 infected individuals where the A32 sub-region is a major ADCC target of antibodies from infected individuals [27]. This result has been confirmed and extended recently to the C11 epitope sub-region as well [29, 69••]. In a very recent study [69••], it was shown that exposure of the Cluster A epitope region was modulated by Nef and Vpu, which control the levels of CD4 on the surface of the infected target cell. This suggests the possibility that Nef and Vpu evolved as viral defenses against the exposure of highly conserved CD4i epitope targets during virion release as proposed in

[69••]. From a structural standpoint, the issues raised above for entry targets will also pertain to this category of release targets. It will be important to determine whether this category of release targets is extant in vivo and whether their structures are accurately reflected in our in vitro systems.

Conclusions

The studies reviewed above suggest that Fc-mediated effector function by non-neutralizing antibodies remains a viable possibility for protection against HIV-1 acquisition. This hypothesis is supported by a significant body of correlative evidence from both natural history and vaccine studies in NHPs and humans. By contrast, direct tests of this hypothesis in NHPs have largely been negative, though non-neutralizing mAbs with Fc-mediated effector function do contribute to post-infection control in two recent studies. Thus, either this hypothesis is wrong or passive immunization studies in NHPs underestimate the ability of Fc-mediated effector function alone to protect against HIV-1 acquisition. As argued elsewhere [15•, 16], the latter possibility is viable due to differences in dose-response curve behaviors for neutralization and Fc-mediated effector function and, perhaps, because effector cell capacity is exceeded in high-dose SHIV/SIV challenges. The discussion above focuses on an additional issue, the structures of ADCC epitopes that are present on target cells during the narrow window of opportunity when antibodies can block HIV-1 acquisition. A distinction is made between epitopes on target cells during viral entry (entry targets) and epitopes on target cells during viral assembly and release (release targets). Entry epitopes are proposed to be the predominant targets during the window of opportunity where CD4i epitopes will be the prevalent structures.

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Key Points

- Distinct ADCC target epitopes are exposed during window of opportunity.
- Fc-mediated effector function recognizes both native and CD4i epitopes.
- Passive immunization studies should take both epitopes into account.

Figure 1.

Epitope target categories during the twenty-four hour “window of opportunity” during which acquisition can be blocked by antibodies. Entry targets are comprised of epitopes extant during viral entry, prior to productive infection of a target cell. Release targets are epitopes exposed on infected cells producing virions.

Figure 2.

Epitopes exposed on entry targets and release targets. Both entry targets and release targets can express epitopes found on un-triggered Env trimers as well as CD4i epitopes that become exposed due to receptor interactions. The known epitope specificities associated with entry and release targets are discussed in their respective sections of the manuscript.

Figure 3.

Stereo depiction of Epitope Cluster A and its proximity to gp41 in the native Env trimer. The top panel is the 4.7Å resolution crystal structure from 4nco.pdb. gp120 is grey, gp41 is blue, and carbohydrates are shown colored by their elements. The middle panel shows secondary and tertiary structures for trimeric Env. Coloring is as in the top panel except that mobile layer 1 is green, mobile layer 2 is red, and the 7-stranded-beta-sandwich is yellow. The bottom panel shows the relationship between gp41 and the Cluster A epitope region on one gp41-gp120 subunit. Residues implicated by published mutagenesis studies in the A32 [40, 41] and C11 [41, 42] epitope sub-regions of Epitope Cluster A are shown as Cory-Pauling-Koltun (cpk) spheres and colored according to their locus in mobile layers 1 and 2, as well as the 7-stranded β -sandwich. The trimer structures are derived from a stabilized, soluble, cleaved trimer lacking the MPER region derived from the clade A founder strain BG505. Both PGT128 and PGV04 are broadly neutralizing antibodies.

Figure 4.

Stereo depiction of major epitope targets on 4.7 Å native Env trimers from 4nco.pdb. The binding of PGT122 Fab fragments in 4nco.pdb (light green and dark green for light and heavy chain, respectively), indicate the supersite of immune vulnerability [70••]. This site includes elements of the glycan shield, V1, V2, V3 and CoRBS regions. The CD4bs is identified by the binding of PGV04 Fab (magenta and purple for light and heavy chain, respectively), which was overlaid onto the 4nco.pdb structure from 3j5m.pdb. The MPER is missing from these structures, its approximate position is indicated by the lettering.. The trimer structures are derived from a stabilized, soluble, cleaved trimer lacking the MPER region derived from the clade A founder strain BG505. Both PGT128 and PGV04 are broadly neutralizing antibodies.