JOE DOUPE LECTURE

Exposing HIV-1 Env: Implications for therapeutic strategies

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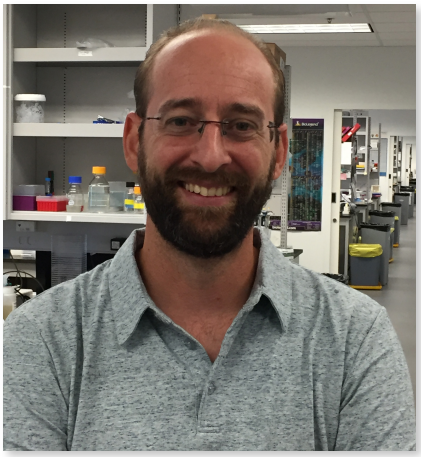

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Abstract

The human immunodeficiency virus (HIV-1) envelope glycoprotein trimer (Env) is exposed on the surfaces of both virions and infected cells. Thus, Env is the principal target for neutralizing antibodies and antibodies able to mediate antibody-dependent cellular cytotoxicity (ADCC). The HIV-1 Env is a flexible molecule known to exist in at least three different conformational states: states 1, 2 and 3. Before interacting with the primary receptor, CD4, Env preferentially adopts a compact, "closed" conformation (state 1) that is largely antibody-resistant. The CD4 binding "opens" Env increasing the vulnerability of infected cells to ADCC mediated by non-neutralizing antibodies, as these easily-elicited antibodies preferentially recognize epitopes exposed in the open conformational states (states 2/3). These antibodies include the anti-co-receptor binding site and the anti-cluster A families of antibodies that, in combination with small CD4-mimetic compounds, stabilize a new asymmetric Env conformation (state 2A) that is vulnerable to ADCC. Approaches aimed at stabilizing this "open" conformation represent new interventional approaches to fight HIV-1 infection.

The human immunodeficiency virus (HIV-1) envelope glycoprotein trimer (Env) is a metastable flexible molecule. Following interaction with CD4, Env transitions from its unliganded "closed" high-energy conformation (state 1) to an intermediate "partially open" conformation (state 2) and then to an "open" CD4-bound low-energy conformation (state 3) [1,2].

The unliganded Env of most primary HIV-1 isolates, in the closed state 1 conformation, is mostly resistant to antibody attack [2], but can be recognized by broadly neutralizing antibodies (bnAbs). The CD4 binding changes the conformation of Env by reorganizing the V1V2 and V3 loops resulting in the adoption of the "open" CD4-bound conformation, referred to as state 3 [1-3]. This conformation is vulnerable to antibody-dependent cellular cytotoxicity (ADCC), mediated by CD4i antibodies easily elicited by vaccination or present in the sera from HIV-1-infected individuals [4,5]. To avoid the exposure of these vulnerable epitopes, Env tightly controls its transition from state 1 to states 2 and 3 with elements located in the V1V2 and V3 loops [1,4,6,7]. Additional elements participate in keeping Env in state 1, including the Phe 43 cavity, a highly conserved ~150 Å3 pocket located at the interface between the inner and outer domain of gp120, which allows interaction with CD4 [8].

Env from circulating HIV-1 strains shield CD4i epitopes

The vast majority of circulating HIV-1 strains express functional Nef and Vpu accessory proteins, which downregulate CD4 and thus limit the exposure of CD4i Env epitopes by keeping Env in its closed (state 1) conformation at the surface of infected cells [5,9-12] (Figure 1A). Accordingly, Nef and Vpu deletion results in CD4 accumulation at the cell surface, resulting in CD4-Env interaction driving the exposure of CD4i epitopes and sensitization of HIV-1-infected cells to ADCC (Figure 1D) [5,9,10,12].

HIV-1 Env residue 375 modulates sensitivity to CD4i antibodies

Most HIV-1 group M Envs have an empty Phe 43 cavity due to the presence of a small residue such as serine at position 375 (S375), which favors a closed Env conformation [13,14]; however, CRF01_AE isolates have a naturally occurring histidine (H375) at this position [15]. The substitution of S375 with bulky hydrophobic residues fills the Phe 43 cavity and predisposes Env to adopt more "open" conformations [13,15,16]. Accordingly, histidine or tryptophan substitutions at this position (S375H or S375W) increase the susceptibility of cells infected with HIV-1 clade B (or other clades) to ADCC mediated by CD4i antibodies such as the cluster A antibody A32 (Figure 1B) [16]. Conversely, replacement of histidine with serine at position 375 (H375S) in HIV-1 CRF01_AE decreases the susceptibility of infected cells to ADCC [16]. CRF01_AE is the predominant HIV-1 strain circulating in Thailand, where the HIV-1 vaccine "Thai" RV144 trial took place. In this trial, which presented a modest degree of protection, a non-significant trend towards a lower risk of HIV-1 infection was observed among vaccine recipients with higher ADCC responses [17]. These observations raise the intriguing possibility that the presence of His 375 in the circulating strain contributed to the observed vaccine efficacy [16].

Small CD4-mimetic compounds (CD4mc) expose Env CD4i epitopes and sensitize HIV-1-infected cells to ADCC

Theoretically, agents driving Env to sample the CD4-bound conformation should expose CD4i epitopes that are readily recognized by ADCC-mediating antibodies, thus sensitizing infected cells to ADCC despite Nef and Vpu activities (Figure 1C). The CD4mc are small-molecules (~320–520 Daltons) that bind within the conserved HIV-1 gp120 Phe43 cavity that is located at the CD4-binding site [18-20]. The CD4mc block the gp120-CD4 interaction and induce conformational changes in gp120 that are similar, but not identical, to those induced by CD4 binding [21,22]. It is now well established that this strategy works at least *in vitro* and *ex*

FIGURE 1. Susceptibility of HIV-1-infected cells to ADCC

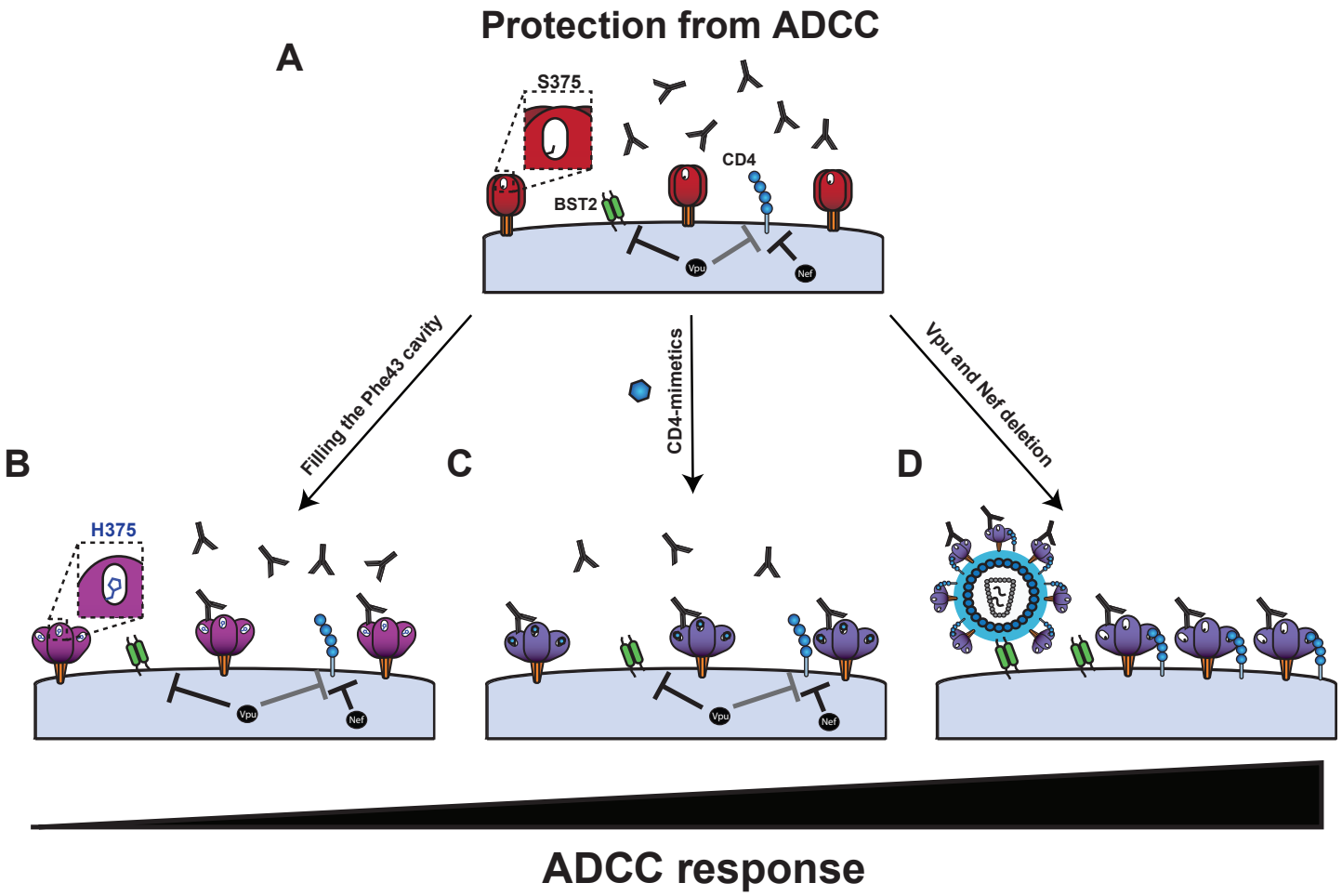

Figure 1: ADCC-mediating Abs present in the sera from HIV-1-infected individuals preferentially recognize Env in its "open" CD4-bound conformation. (A) To limit the exposure of Env CD4i epitopes, HIV-1 has evolved protective mechanisms to downregulate CD4 via Nef and Vpu activities. It also counteracts the host restriction factor BST-2 through Vpu-mediated downregulation. (B) Residue 375, located within the Phe43 cavity, modulates the transition of Env to the CD4-bound conformation. The presence of a small amino acid at this position keeps Env in its unbound "closed" conformation; the presence of a bulky residue at this position, such as the naturally-occurring histidine at position 375 (H375) in CRF01_AE strains, shifts Env conformation to a state closer to the CD4-bound state, enhancing susceptibility of infected cells to ADCC. (C) Small CD4-mimetic compounds (CD4mc), in conjunction with anti-CoRBS and anti-cluster A antibodies, sensitize HIV-1-infected cells to ADCC by stabilizing the ADCC-vulnerable state 2A conformation. (D) Deletion of Vpu and Nef results in accumulation of Env-CD4 complexes at the cell-surface, enhancing the susceptibility of HIV-1-infected cells to ADCC by exposing CD4i epitopes.

vivo settings. The CD4mc have been shown to sensitize HIV-1-infected cells to ADCC mediated by antibodies present in sera, cervicovaginal fluids and breast milk from HIV-1-infected individuals [22-28], as well as sera from gp120-vaccinated non-human primates [29,30]. This strategy was also shown to work against primary CD4 T cells isolated from HIV+ individuals using their own (autologous) sera [27,28].

Sequential opening of Env is required to expose ADCC-mediating epitopes

From the different CD4i antibodies present in HIV+ sera that recognize the CD4-bound conformation, those

recognizing the gp120 inner domain cluster A region present the most potent ADCC-mediating activity [5,31]. Interestingly, CD4mc are unable to enhance recognition of HIV-1-infected cells by anti-cluster A Abs in the absence of co-receptor binding site (CoRBS) antibodies (such as 17b) [22,24]. These results indicate that CD4mc initially open the trimeric Env enough to allow the binding of CoRBS antibodies but not anti-cluster A antibodies. The CoRBS antibody binding further opens the trimeric Env, allowing anti-cluster A antibody interaction and sensitization of infected cells to ADCC. Therefore, ADCC responses mediated by cluster A antibodies in HIV+ sera involve a sequential opening of the Env trimer on the surface of HIV-1-infected cells [22]. It was recently shown that CD4mc, anti-CoRBS and anti-cluster A antibodies stabilize a new, ADCC-vulnerable, asymmetric state 2A conformation [23]. Whether stabilizing this conformation in vivo has an impact on the size of the viral reservoir or disease progression remains to be evaluated.

Conclusion

The new understanding of the link between Env conformation and ADCC responses described above suggests that all the elements required to eliminate infected cells are already present in HIV-1-infected individuals, provided that their antibodies recognize Env at the surface of infected cells. The CD4mc bring this last piece to the puzzle, by opening Env and allowing recognition of infected cells by ADCC-mediating antibodies and subsequent recruitment of effector cells. It is possible that increasing the potency and breath of CD4mc might increase their efficacy. The recent identification and development of a new family of CD4mc, having a close proximity to the highly-conserved D368 residue involved in CD4 binding, represents a step in this direction [32].

While using CD4mc to eliminate infected cells appears to be a promising tool for HIV-1 eradication, their ability to reduce the size of the viral reservoir in vivo remains to be proven. Testing this concept in animal models, such as HIV-1-infected humanized mice or SHIV-infected non-human primates, is required to establish the value of this auspicious therapeutic approach.

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References

1. Herschhorn A, Ma X, Gu C, et al. Release of gp120 restraints leads to an entry-competent intermediate state of the HIV-1 envelope glycoproteins. *mBio*. 2016;7(5).
2. Munro JB, Gorman J, Ma X, et al. Conformational dynamics of single HIV-1 envelope trimers on the surface of native virions. *Science*. 2014;346(6210):759-763.
3. Ma X, Lu M, Gorman J, et al. HIV-1 Env trimer opens through an asymmetric intermediate in which individual protomers adopt distinct conformations. *eLife*. 2018;7.
4. Prevost J, Richard J, Ding S, et al. Envelope glycoproteins sampling states 2/3 are susceptible to ADCC by sera from HIV-1-infected individuals. *Virology*. 2018;515:38-45.
5. Veillette M, Coutu M, Richard J, et al. The HIV-1 gp120 CD4-bound conformation is preferentially targeted by antibody-dependent cellular cytotoxicity-mediating antibodies in sera from HIV-1-infected individuals. *J Virol*. 2015;89(1):545-551.
6. Kwon YD, Finzi A, Wu X, et al. Unliganded HIV-1 gp120 core structures assume the CD4-bound conformation with regulation by quaternary interactions and variable loops. *Proc Natl Acad Sci U S A*. 2012;109(15):5663-5668.
7. Liu J, Bartesaghi A, Borgnia MJ, Sapiro G, Subramaniam S. Molecular architecture of native HIV-1 gp120 trimers. *Nature*. 2008;455(7209):109-113.
8. Kwong PD, Wyatt R, Robinson J, Sweet RW, Sodroski J, Hendrickson WA. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature*. 1998;393:648-659.
9. Richard J, Prevost J, Baxter AE, et al. Uninfected bystander cells impact the measurement of HIV-specific antibody-dependent cellular cytotoxicity responses. *mBio*. 2018;9(2).
10. Veillette M, Desormeaux A, Medjahed H, et al. Interaction with cellular CD4 exposes HIV-1 envelope epitopes targeted by antibody-dependent cell-mediated cytotoxicity. *J Virol*. 2014;88(5):2633-2644.
11. Alsahafi N, Ding S, Richard J, et al. Nef Proteins from HIV-1 elite controllers are inefficient at preventing antibody-dependent cellular cytotoxicity. *J Virol*. 2016;90(6):2993-3002.
12. Prevost J, Richard J, Medjahed H, et al. Incomplete downregulation of CD4 expression affects HIV-1 env conformation and antibody-dependent cellular cytotoxicity responses. *J Virol*. 2018;92(13).

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13. Xiang SH, Kwong PD, Gupta R, et al. Mutagenic stabilization and/or disruption of a CD4-bound state reveals distinct conformations of the human immunodeficiency virus type 1 gp120 envelope glycoprotein. *J Virol.* 2002;76(19):9888-9899.
14. Finzi A, Xiang SH, Pacheco B, et al. Topological layers in the HIV-1 gp120 inner domain regulate gp41 interaction and CD4-triggered conformational transitions. *Mol Cell.* 2010;37(5):656-667.
15. Zoubchenok D, Veillette M, Prevost J, et al. Histidine 375 modulates CD4 binding in HIV-1 CRF01_AE envelope glycoproteins. *J Virol.* 2017;91(4).
16. Prevost J, Zoubchenok D, Richard J, et al. Influence of the envelope gp120 Phe 43 cavity on HIV-1 sensitivity to antibody-dependent cell-mediated cytotoxicity responses. *J Virol.* 2017;91(7).
17. Haynes BF, Gilbert PB, McElrath MJ, et al. Immune-correlates analysis of an HIV-1 vaccine efficancy trial. N Engl J Med. 2012;366(14):1275-88. doi: 10.1056/NEJMoa1113425.
18. LaLonde JM, Kwon YD, Jones DM, et al. Structure-based design, synthesis, and characterization of dual hotspot small-molecule HIV-1 entry inhibitors. *J Med Chem.* 2012;55(9): 4382-4396.
19. Lalonde JM, Le-Khac M, Jones DM, et al. Structure-based design and synthesis of an HIV-1 entry inhibitor exploiting X-ray and thermodynamic characterization. *ACS Med Chem Lett.* 2013;4(3):338-343.
20. Madani N, Schon A, Princiotto AM, et al. Small-molecule CD4 mimics interact with a highly conserved pocket on HIV-1 gp120. *Structure.* 2008;16(11):1689-1701.
21. Schon A, Madani N, Klein JC, et al. Thermodynamics of binding of a low-molecular-weight CD4 mimetic to HIV-1 gp120. *Biochemistry.* 2006;45(36):10973-10980.
22. Richard J, Pacheco B, Gohain N, et al. Co-receptor binding site antibodies enable CD4-mimetics to expose conserved anti-cluster A ADCC epitopes on HIV-1 envelope glycoproteins. *EBioMedicine.* 2016.
23. Alsahafi N, Bakouche N, Kazemi M, et al. An asymmetric opening of HIV-1 envelope mediates antibody-dependent cellular cytotoxicity. *Cell Host Microbe.* 2019;25(4):578-587 e575.
24. Anand SP, Prevost J, Baril S, et al. Two families of env antibodies efficiently engage Fc-gamma receptors and eliminate HIV-1-infected cells. *J Virol.* 2019;93(3).
25. Lee WS, Richard J, Lichtfuss M, et al. antibody-dependent cellular cytotoxicity against reactivated HIV-1-infected cells. *J Virol.* 2015;90(4):2021-2030.
26. Richard J, Prevost J, von Bredow B, et al. BST-2 Expression modulates small CD4-mimetic sensitization of HIV-1-infected cells to antibody-dependent cellular cytotoxicity. *J Virol.* 2017;91(11).
27. Richard J, Veillette M, Brassard N, et al. CD4 mimetics sensitize HIV-1-infected cells to ADCC. *Proc Natl Acad Sci U S A.* 2015;112(20):E2687-2694.
28. Richard J, Veillette M, Ding S, et al. Small CD4 mimetics prevent HIV-1 uninfected bystander CD4 + T cell killing mediated by antibody-dependent cell-mediated cytotoxicity. *EBioMedicine.* 2016;3:122-134.
29. Ding S, Verly MM, Princiotto A, et al. Small molecule CD4-mimetics sensitize HIV-1-infected cells to ADCC by antibodies elicited by multiple envelope glycoprotein immunogens in non-human primates. *AIDS Res Hum Retroviruses.* 2016.
30. Madani N, Princiotto AM, Mach L, et al. A CD4-mimetic compound enhances vaccine efficacy against stringent immunodeficiency virus challenge. *Nat Commun.* 2018;9(1): 2363.
31. Ding S, Veillette M, Coutu M, et al. A highly conserved residue of the HIV-1 gp120 Inner domain is important for antibody-dependent cellular cytotoxicity responses mediated by anti-cluster A antibodies. *J Virol.* 2015;90(4):2127-2134.
32. Ding S, Grenier MC, Tolbert WD, et al. A new family of small-molecule CD4-mimetic compounds contact the highly conserved aspartic acid 368 of HIV-1 gp120 and mediates ADCC. *J Virol.* 2019.

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