Epitope targeting and the antibody-dependent cellular cytotoxicity response against HIV-1

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Abstract

Antibody-dependent cellular cytotoxicity (ADCC) has been linked with protection against HIV-1. Non-neutralizing epitopes in the gp120 C1 region have been implicated as potential targets for protective antibody responses elicited by vaccination, although antibody-binding titers against these epitopes do not consistently correlate with protection. We hypothesized that differences in the mode of epitope recognition may be responsible for large differences in ADCC activities between antibodies targeting the gp120 C1 region. Here, we report crystal structures of two antibodies that differ 75-fold in their ADCC potency. Our results provide the first atomic level definition of A32-like epitopes and provide an understanding of how precise epitope location and mode of binding can dramatically affect the potency of Fc-mediated effector function.

Introduction

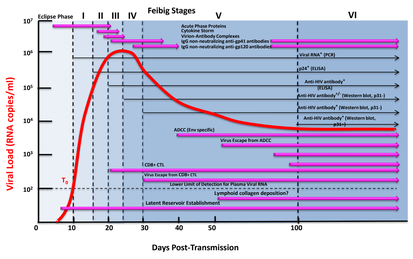


Figure 1: Timeline of events following HIV-1 transmission. HIV-1 envelope directed ADCC is detected about 40 days post infection, and is followed closely by the emergence of virus escape variants.

- Existing evidence suggests that protective antibody responses can involve neutralizing activity as well as Fc receptor-dependent processes, such as ADCC[1].
- ADCC escape variants emerging in HIV-1 infected people[2] and ADCC responses correlating with reduced risk of infection in the RV144[3] vaccine trial point toward non-neutralizing epitopes in the C1 region of gp120 as relevant targets for potentially protective antibodies.

Goal

- To understand the structural basis for ADCC activity of antibodies targeting the C1-region of HIV-1 gp120.

Strategy

- Select monoclonal antibodies that target the gp120 C1 region, yet differ in their ADCC-mediating potencies.
- Determine structures, in complex with gp120, of these antibodies and analyze the structural basis for the differences in biological function.

Results

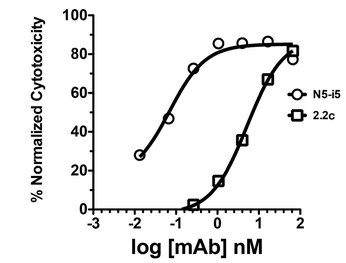


Figure 2: Representative ADCC curves for mAbs N5-i5[4] and 2.2c. ADCC assays were carried out using CEM-NKr-CCR5 target cells sensitized with recombinant gp120 from the HIV-1_{Ba-L} isolate.

Mab N5-i5 shows 75-fold higher ADCC potency than mab 2.2c

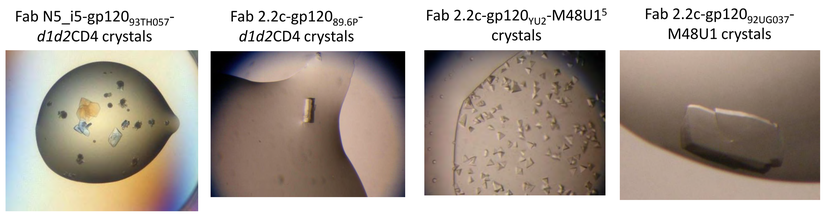


Figure 3: Crystallization of N5-i5 and 2.2c in complex with HIV-1 gp120. Mabs N5_i5 and 2.2c were crystallized in complex with gp120 in the CD4-trigerred conformation.

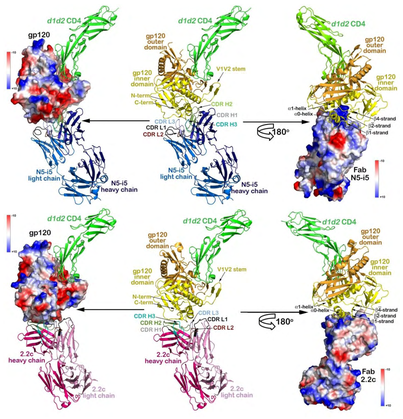


Figure 4: Epitope definition of Mabs N5-i5 and 2.2c. (A) Crystal structures of N5-i5 (top panel) and 2.2c (bottom panel) bound to gp120-*d1d2*CD4. Left left and right panels show electrostatic potential displayed on molecular surfaces.

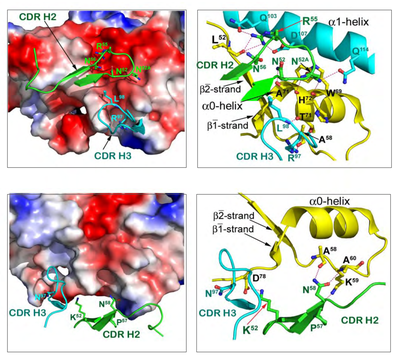


Figure 5: Binding interfaces of CDR H2 and CDR H3 loops of mAb N5-i5 (top) and mAb 2.2c (bottom) with gp120. Antibody complementary determining region residues engaged in gp120 binding are shown as balls-and-sticks. The electrostatic potential of gp120 is displayed over its molecular surface. H-bonds are shown as dashes in magenta.

Mabs N5-i5 and 2.2c bind overlapping but also distinct epitopes on the gp120 inner domain, at different angles via juxtaposed antibody heavy and light contact surfaces . While N5-i5 engages helices α0 and α1, 2.2c forms its complex with no binding contact to α1 of gp120 (Fig. 5).

A

	mAb N5-i5	mAb 2.2c	fold*
K_d (M)	$2.7\pm1.6 \times 10^{-10}$	$4.9\pm0.5 \times 10^{-10}$	1.8
k_a (1/Ms)	$6.7\pm0.5 \times 10^{5}$	$11.5\pm1.3 \times 10^{5}$	1.7
k_d (1/s)	$19.6\pm6.2 \times 10^{-5}$	$55.6\pm12.5 \times 10^{-5}$	2.8

* fold difference

B

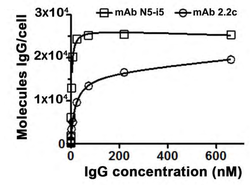


Figure 6: Binding of Mabs N5_i5 and 2.2c to (A) monomeric gp120 and **(B)** gp120 sensitized CEM-Nkr-CCR5+ target cells.

Although N5-i5 and 2.2c bound monomeric gp120 with similar affinities (Fig. 6A), binding to target cells revealed ~18-fold greater binding of N5-i5 than 2.2c (Fig. 6B) indicating that N5-i5 has greater ability to cross-link gp120-CD4 complexes on the cell surface.

Binding models (Fig.7A) suggested that the 2.2c binding mode on the cell surface results in suboptimal positioning of Fc domain for interaction with Fcγ receptors on the effector cell membrane. To test this geometric hypothesis we switched the Fv domains of both mAbs from heavy to light chains and vice-versa and measured ADCC activities of new Fv-swapped hybrids (Fig. 7).

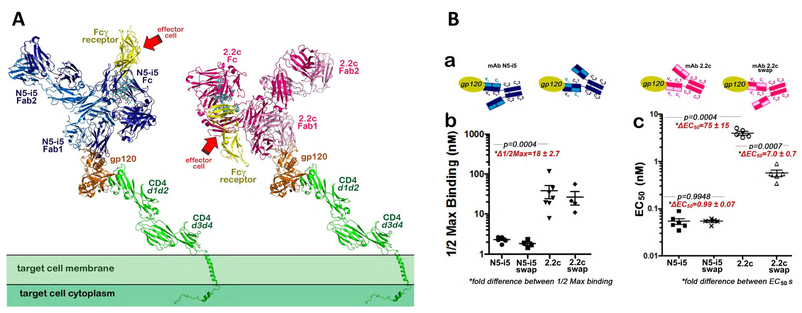


Figure 7: Role of antibody orientation in ADCC potency. (A) Model of putative ADCC immune-complexes formed by mAb N5-i5 and 2.2c at the target/effector cell interface. **(B)** Effect of swapping heavy and light chain orientation on ADCC potency and cell-surface binding.

ADCC of Fv domain-swapped 2.2c improved ~7-fold (Fig. 7B) suggesting that the 2.2c epitope is poorly accessible on the target cell surface, as judged by cell surface binding and saturation studies, and ADCC potency at this epitope could be increased by changing the binding mode to more optimally position the CH2 domains for interaction with Fc receptors on effector cells.

Conclusions

- Our studies provide atomic level definition of A32-like epitope footprints and provide the structural basis for the differences in ADCC responses to the A32-like epitopes.
- Both the ability of an antibody to cross-link antigen on the target cell and positioning of its CH2 domain for optimal Fc receptor binding contribute to ADCC potency. These studies shed new light on the structural basis of ADCC and point toward the importance of epitope fine specificity and mode of antibody attachment as dominant elements affecting ADCC potency.

References: 1) Bonsignori, M. *et al* (2012) Journal of Virology 86(21):11521-32. 2) Chung, A. W., et al (2011) Proc Natl Acad Sci U S A 108, 7505-7510. 3) Haynes, B. F., *et al* (2011) N Engl J Med 366, 1275-86. 4) Guan, Y.*et al* (2013) PNAS 110, E69-78. 5) Acharya, P., *et al* (2013) Structure 21, 1018-1029. **Acknowledgements:** The authors thank N. Gohain, X. Wu, T. Liu, W. Huang, C.C. Huang, R. K. Louder, T.S. Luongo, J. S. McLellan, M. Pancera, Y. D. Kwon, Y. Yang, B. Zhang, R. Flinko, J. S. Foulke, M. M. Sajadi and R. Kamin-Lewis, for contributing to the structural and functional analyses. Support for this work was provided by the Intramural Research Program of the VRC, National Institute of Allergy and Infectious Diseases, NIH, by the Bill and Melinda Gates Foundation, and by the National Institute of Allergy and Infectious Diseases.