Antibody-Dependent, FcγRI-Mediated Neutralization of HIV-1 in TZM-bl Cells Occurs Independently of Phagocytosis

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We previously showed that expression of human FcγRI on TZM-bl cells potentiates neutralization by gp41 membrane-proximal external region (MPER)-specific antibodies. Here we show that lysosomotropic reagents known to block phagocytosis do not diminish this effect. We also show that FcγRI occasionally potentiates neutralization by antibodies against the V3 loop of gp120 and cluster I of gp41. We conclude that FcγRI provides a kinetic advantage for neutralizing antibodies against partially cryptic epitopes independent of phagocytosis.

Many cells of the immune system express Fc receptors that bind immune complexes through the constant region of immunoglobulins to mediate a variety of innate and acquired immune defense mechanisms (1, 2). In the case of cell-free HIV-1, FcγRs have been implicated in both infection enhancement (3) and virus destruction by phagocytosis (4, 5, 6). To study the impact of the four major Fcγ receptors on cell-free HIV-1 in greater detail, we stably expressed each of the cDNAs for the human FcγRs on TZM-bl cells. Using these new cell lines, we assayed a panel of HIV-1-positive sera and monoclonal antibodies (MAbs) for neutralization potency against several strains of HIV-1. We showed that expression of FcγRI/CD64 had a profound effect on HIV-1 neutralization by the gp41 membrane-proximal external region (MPER)-specific MAbs 2F5 and 4E10 (7). In the presence of FcγRI expression, neutralization titers for 4E10 and 2F5 were increased as much as 5,000-fold depending on the virus used. Given that both 2F5 and 4E10 have been shown to prevent viral infection in a macaque model (8) and that both MAbs are broadly neutralizing, an understanding of their neutralizing mechanism may be beneficial for vaccine development.

As we proposed previously, at least two mechanisms might account for the observed positive effect of FcγRI on HIV-1 neutralization. One would involve the pre-positioning of Abs at the cell surface, thereby giving them a kinetic advantage in accessing epitopes that are more-readily exposed after virus attachment. This mechanism is supported by the finding that cell-free HIV-1 immune complex formation was not required prior to Ab-FcγRI engagement in order for FcγRI to augment the neutralization activities of 2F5 and 4E10 (7).

A second mechanism that may potentially facilitate HIV-1 neutralization is phagocytosis. HeLa cells, from which the TZM-bl cell line was developed, are known to exhibit properties of non-professional phagocytes (9, 10, 11). Several mammalian cells have been shown to acquire phagocytic properties upon expression of FcγRs (12, 13, 14). Thus, surface expression of FcγRs may, in principle, turn TZM-bl cells into professional phagocytes.

Macrophages, dendritic cells, and neutrophils use phagocytosis to internalize particles larger than 0.5 μm. This process can be triggered by diverse membrane components, such as scavenger receptors, complement receptors, and Fc receptors (13, 14). During phagocytosis, internalized particles are trafficked into a complex of membrane-bound structures known as phagosomes (15).

At the end of the phagocytic process, particle degradation requires phagosome maturation, a process by which these vesicles become increasingly acidified by acquiring different proteins and eventually fuse with acidic lysosome structures (15).

The antibiotic bafilomycin A₁ and the weak base chloroquine are well-characterized compounds known to block acidification and protein degradation in lysosomes (16, 17, 18). We used these drugs to test whether phagocytosis facilitates HIV-1 neutralization in cells expressing FcγRI. The drugs were used at the highest concentrations that were nontoxic to TZM-bl and TZM-bl/FcγRI cells. To verify that the drugs were capable of blocking lysosomal acidification, we tested the ingestion of pHrodo *Escherichia coli* BioParticles (Invitrogen, Carlsbad, CA) by TZM-bl/FcγRI cells in the presence and absence of each drug. In this phagocytosis assay, the fluorescence of the pHrodo dye dramatically increases as the pH of the surroundings becomes acidic (15). Cells were treated for 4 h with either 1 nM bafilomycin A₁ or 50 μM chloroquine at 37°C (Sigma-Aldrich, St. Louis, MO), trypsinized, washed with phosphate-buffered saline, and incubated with pHrodo *E. coli* at 37°C for 15 min. Cells were then washed, fixed with 1% formaldehyde, and analyzed by flow cytometry using a BD FACSCalibur analyzer (BD Biosciences). Compensation and analysis were performed by using FlowJo software (Tree Star). Approximately 50% of the TZM-bl/FcγRI cells were positive in the absence of drug, whereas only 25% were positive in the presence of drug, demonstrating the capacity of these lysosomotropic agents to block phagocytosis in this cell line.

To test for an effect on neutralization, TZM-bl cells and TZM-bl/FcγRI cells were pretreated for 4 h with either 1 nM bafilomycin A₁ or 50 μM chloroquine at 37°C and used in standard neutralization assays (7) in the continued presence of the drugs. Two HIV-1 subtype B Env-pseudotyped viruses (6535.3 and QH0692.42) were assayed with 2F5, 4E10, and IgG1b12. As shown in Fig. 1, expression of FcγRI dramatically improved the neutral-

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FIG 1 Effect of lysosomotropic agents on HIV-1 neutralization in T2M-bl and T2M-bl/Fc γ RI cells. Cells were pretreated with either 1 nM bafilomycin A₁ or 50 μ M chloroquine as described in the text and then used to test neutralization of HIV-1 subtype B Env-pseudotyped viruses 6535.3 and QH0692.42 (both produced in 293T cells) by 2F5, 4E10, and IgG1b12 in the continued presence of the lysosomotropic agents. T2M-bl/Fc γ RI cells that were untreated or treated with either chloroquine or bafilomycin are shown as closed circles, closed squares, or closed triangles, respectively. Corresponding conditions with T2M-bl cells are shown as open circles, open squares, and open triangles, respectively.

izing activity of 2F5 and 4E10 against both viruses in the absence of lysosomotropic agents. Moreover, neither lysosomotropic agent showed any evidence of reversing the Fc γ RI-mediated effect on 2F5 and 4E10. As reported previously, Fc γ RI had no effect on the neutralizing activity of IgG1b12 (7), and the activity of this MAb was unaltered by the lysosomotropic agents.

The results presented here indicate that phagocytosis is not a mechanism responsible for the enhanced neutralizing activity of MPER-specific antibodies in T2M-bl/Fc γ RI cells. Though the lysosomotropic agents inhibited the phagocytosis of pHrodo *E. coli* BioParticles by only 50% under the conditions used (conditions that were clearly free of toxic effects to the cells), we saw no reduction in HIV-1-neutralizing activity in their presence. It remains possible that immune complexes may be trapped at the cell membrane in partial phagocytic cups induced by the incomplete activation of the Fc γ Rs (19).

A recent study showed that activation of FcRs by immune com-

plexes blocked the replication of HIV-1 and related primate lentiviruses in human macrophages (20). This activation restricted HIV-1 reverse transcription and integration but not viral entry, nuclear import, or gene expression from integrated proviruses and was not due to enhanced degradation of incoming viral proteins. This FcR-mediated restriction of lentiviruses also appeared to involve the induction of the cyclin-dependent kinase inhibitor p21^{Cip1/WAF1}. It is unlikely that a similar mechanism may be responsible for the efficient neutralization of HIV by MPER MAbs because this phenomenon does not occur with every immune complex, as was observed in the FcR-mediated restriction of lentiviruses in macrophages (20).

A more likely mechanism for enhanced Ab-mediated neutralization of HIV-1 in T2M-bl/Fc γ RI cells is that the Fc receptor pre-positions Abs at the virus surface, thereby breaching the steric barrier at the virus-cell interface (21) for Abs whose epitopes are not fully revealed until the Env glycoprotein spike changes con-

TABLE 1 Occasional effect of FcγRI expression on the neutralizing activity of MAbs against the V3 loop of gp120 and cluster I of gp41 in TZM-bl cells

MAb	Epitope	Reference	IC ₅₀ (μg/ml) in TZM-bl and TZM-bl/FcγRI cells of virus ^a :			
			6535.3	QH0692.42	SC22.3C2.LucR	RHPA.LucR
1361	V2	29	>25/ 13.8	>25/>25	>25/>25	>25/>25
1393A	V2	30	16.8/16.3	>25/>25	>25/>25	>25/>25
1357D	V2	29	>25/>25	>25/>25	>25/>25	>25/>25
697-30D	V2	31	>25/>25	>25/>25	>25/>25	>25/>25
830A	V2	30	>23/>23	>23/>23	>23/>23	>23/>25
2297	V2	32	>25/>25	>25/>25	>25/>25	>25/>25
2219	V3	33	<u>1.1/0.07</u>	<u>13.2/0.45</u>	>25/>25	<u>>25/0.4</u>
3869	V3	34	<u>2.0/0.3</u>	<u>21.9/6.2</u>	>25/>25	<u>>25/0.9</u>
447-52D	V3	35	<u>0.3/0.05</u>	<u>7.8/0.9</u>	>25/>25	>25/ 11.8
3074	V3	36	>25/ 10.6	>25/ 17.7	>25/>25	>25/>25
654-30D	CD4bs	37	>25/ 22.3	>25/>25	>25/>25	>25/>25
1008-30D	CD4bs	38	22.0 />25	>25/>25	>25/>25	>25/>25
729-30D	CD4bs	39	16.8/14.6	>25/>25	>25/>25	>25/>25
1331-160A	C5	29	>25/>25	>25/>25	>25/>25	>25/>25
670-30D	C5	39	>25/>25	>25/>25	>25/>25	>25/>25
858-30D	C5	39	>22.5/>22.5	>22.5/>22.5	>22.5/>22.5	>22.5/>22.5
240-D	gp41 cluster I	40	<u>>25/0.27</u>	>25/>25	<u>>25/1.0</u>	>25/ 23.2
246-D	gp41 cluster I	40	>25/>25	>25/>25	>25/ 11.9	>25/>25
50-69D	gp41 cluster I	41	<u>>25/0.78</u>	>25/>25	<u>>25/6.4</u>	>25/>25
181D	gp41 cluster I	40	>25/>25	>25/>25	>25/>25	>25/>25
126-7D	gp41 cluster II	40	>25/>25	>25/>25	>25/>25	>25/>25
167D	gp41 cluster II	40	>25/>25	>25/>25	>25/>25	>25/>25

^a Results are shown as IC₅₀ for TZM-bl cells/IC₅₀ for TZM-bl/FcγRI cells. Positive neutralization is shown in boldface type. Differences that were >3-fold between TZM-bl and TZM-bl/FcγRI cells are underlined (3-fold differences exceed the normal variation of the assay). Viruses 6535.3 and QH0692.42 were used as Env-pseudotyped viruses. Viruses SC22.3C2 and RHPA were used as infectious molecular clones that expressed the entire Env ectodomain of the designated HIV-1 strain (Env.IMC.LucR viruses) (42).

formation upon receptor and coreceptor binding (22–25). We note that CD89 (FcαR1) expression on TZM-bl cells does not potentiate the neutralizing capacity of the IgA version of 2F5 (our unpublished results). This finding suggests that the structure of IgG and the characteristics of its interaction with FcγRI are most favorable for the pre-positioning effect. The unusual very long third complementarity-determining region of the heavy chain, CDR H3, of these MAbs may contribute to this property (26).

The FcγRI effect on 2F5 and 4E10 has been seen with every HIV-1 isolate we have tested that is sensitive to these MAbs, and we have seen a similar effect with other MPER MAbs, including CAP206-CH12 (27) and m66 (28). Previously, however, we had not seen an effect with Abs against other epitopes (7). We have now tested a larger number of MAbs against four additional tier 2 viruses and show that FcγRI can occasionally augment the neutralizing activity of MAbs against the V3 loop of gp120 and against cluster I epitopes in gp41 (Table 1), although not with the same intensity and frequency seen with MPER MAbs. The V3-specific MAb 447-52D and the gp41 cluster I-specific MAbs 240-D, 256-D, and 50-69 have been shown to inhibit replication of HIV-1 strains BaL, Bx08, and TV1 in human macrophages and immature dendritic cells expressing FcγRs but not in human peripheral blood mononuclear cells (5, 6).

Given these results, we believe that FcγRI can augment the conventional entry-inhibiting neutralizing activity of Abs against multiple epitopes on gp120 and gp41 that tend to be poorly exposed prior to virus attachment. In this regard, the TZM-bl/FcγRI cell line appears to be an interesting tool for mechanistic studies of HIV-1 neutralization as well as for monitoring vaccine-elicited neutralizing Ab responses. It remains to be determined whether

FcγRI-augmented neutralizing activity of HIV-1-specific Abs *in vitro* has potential value for vaccine protection.

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REFERENCES

1. Nimmerjahn F, Ravetch JV. 2006. Fcγ receptors: old friends and new family members. *Immunity* 24:19–28.
2. Nimmerjahn F, Ravetch JV. 2008. Fcγ receptors as regulators of immune responses. *Nat. Rev. Immunol.* 8:34–47.
3. Montefiori DC. 1997. Role of complement and Fc receptors in the pathogenesis of HIV-1 infection. *Springer Semin. Immunopathol.* 18:371–390.
4. Ackerman ME, Moldt B, Wyatt RT, Dugast A-S, McAndrew E, Tsoukas S, Jost S, Berger CT, Sciaranghella G, Liu Q, Irvine DJ, Burton DR, Alter G. 2011. A robust, high-throughput assay to determine the phagocytic activity of clinical antibody samples. *J. Immunol. Methods* 366:8–19.
5. Holl V, Peressin M, Schmidt S, Decoville T, Zolla-Pazner S, Aubertin A-M, Moog C. 2006. Efficient inhibition of HIV-1 replication in human immature monocyte-derived dendritic cells by purified anti-HIV-1 IgG without induction of maturation. *Blood* 107:4466–4474.
6. Holl V, Peressin M, Decoville T, Schmidt S, Zolla-Pazner S, Aubertin A-M, Moog C. 2006. Nonneutralizing antibodies are able to inhibit human immunodeficiency virus type 1 replication in macrophages and immature dendritic cells. *J. Virol.* 80:6177–6181.
7. Perez LG, Costa MR, Todd CA, Haynes BF, Montefiori DC. 2009. Utilization of immunoglobulin G Fc receptors by human immunodeficiency virus type 1: a specific role for antibodies against the membrane-proximal external region of gp41. *J. Virol.* 83:7397–7410.

8. Hessel AJ, Rakasz EG, Tehrani DM, Huber M, Weisgrau KL, Landucci G, Forthal DN, Koff WC, Poignard P, Watkins DI, Burton DR. 2010. Broadly neutralizing monoclonal antibodies 2F5 and 4E10 directed against the human immunodeficiency virus type 1 gp41 membrane-proximal external region protect against mucosal challenge by simian-human immunodeficiency virus SHIV_{Ba-L}. *J. Virol.* 84:1302–1313.
9. Byrne GI, Moulder JW. 1978. Parasite-specific phagocytosis of *Chlamydia psittaci* and *Chlamydia trachomatis* by L and HeLa cells. *Infect. Immun.* 19:598–606.
10. Clerc P, Sansonetti PJ. 1987. Entry of *Shigella flexneri* into HeLa cells: evidence for directed phagocytosis involving actin polymerization and myosin accumulation. *Infect. Immun.* 55:2681–2688.
11. Guzmán-Verri C, Chaves-Olarite E, von Eichel-Streiber C, López-Goñi I, Thelestam M, Arvidson S, Gorvel J-P, Moreno E. 2001. GTPases of the Rho subfamily are required for *Brucella abortus* internalization of nonprofessional phagocytes. *J. Biol. Chem.* 276:44435–44443.
12. Giodini A, Rahner C, Cresswell P. 2009. Receptor-mediated phagocytosis elicits cross-presentation in nonprofessional antigen-presenting cells. *Proc. Natl. Acad. Sci. U. S. A.* 106:3324–3329.
13. Hunter S, Kamoun M, Schreiber A. 1994. Transfection of an Fc receptor cDNA induces T cells to become phagocytic. *Proc. Natl. Acad. Sci. U. S. A.* 91:10232–10236.
14. Indik Z, Kelli C, Chien P, Levinson AI, Schreiber AD. 1991. Human FcγRII, in the absence of other Fcγ receptors, mediates a phagocytic signal. *J. Clin. Invest.* 88:1766–1771.
15. Kinchen JM, Ravichandran KS. 2008. Phagosome maturation: going through the acid test. *Nat. Rev. Mol. Cell. Biol.* 9:781–795.
16. Matsuzawa Y, Hostetler KY. 1980. Inhibition of lysosomal phospholipase A and phospholipase C by chloroquine and 4,4'-bis(diethylaminoethoxy)α,β-diethyldiphenylethane. *J. Biol. Chem.* 255:5190–5194.
17. Ohkuma S, Poole B. 1978. Fluorescence probe measurement of the intralysosomal pH in living cells and the perturbation of pH by various agents. *Proc. Natl. Acad. Sci. U. S. A.* 75:3327–3331.
18. Yoshimori T, Yamamoto A, Moriyama Y, Futai M, Tashiro Y. 1991. Bafilomycin A₁, a specific inhibitor of vacuolar-type H⁺-ATPase, inhibits acidification and protein degradation in lysosomes of cultured cells. *J. Biol. Chem.* 266:17707–17712.
19. Zhang Y, Hoppe AD, Swanson JA. 2010. Coordination of Fc receptor signaling regulates cellular commitment to phagocytosis. *Proc. Natl. Acad. Sci. U. S. A.* 107:19332–19337.
20. Bergamaschi A, David A, Le Rouzic E, Nisole S, Barré-Sinoussi F, Pancino G. 2009. The CDK inhibitor p21^{Cip1/WAF1} is induced by FcγR activation and restricts the replication of human immunodeficiency virus type 1 and related primate lentiviruses in human macrophages. *J. Virol.* 83:12253–12265.
21. Labrijn AF, Poignard P, Raja A, Zwick MB, Delgado K, Franti M, Binley J, Vivona V, Grundner C, Huang CC, Venturi M, Petropoulos CJ, Wrin T, Dimitrov DS, Robinson J, Kwong PD, Wyatt RT, Sodroski J, Burton DR. 2003. Access of antibody molecules to the conserved coreceptor binding site on glycoprotein gp120 is sterically restricted on primary human immunodeficiency virus type 1. *J. Virol.* 77:10557–10565.
22. Bou-Habib DC, Roderiquez G, Oravec T, Berman PW, Lusso P, Norcross MA. 1994. Cryptic nature of envelope V3 region epitopes protects primary monocytotropic human immunodeficiency virus type 1 from antibody neutralization. *J. Virol.* 68:6006–6013.
23. Frey G, Peng H, Rits-Volloch S, Morelli M, Cheng Y, Chen B. 2008. A fusion-intermediate state of HIV-1 gp41 targeted by broadly neutralizing antibodies. *Proc. Natl. Acad. Sci. U. S. A.* 105:3739–3744.
24. Gustchina E, Bewley CA, Clore GM. 2008. Sequestering of the prehairpin intermediate of gp41 by peptide N36Mut(e,g) potentiates the human immunodeficiency virus type 1 neutralizing activity of monoclonal antibodies directed against the N-terminal helical repeat of gp41. *J. Virol.* 82:10032–10041.
25. Wyatt R, Sodroski J. 1998. The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. *Science* 280:1884–1888.
26. Zwick MB, Komori HY, Stanfield RL, Church S, Wang M, Parren PWHI, Kunert R, Katinger R, Wilson IA, Burton D. 2004. The long third complementarity-determining region of the heavy chain is important in the activity of the broadly neutralizing anti-human immunodeficiency virus type 1 antibody 2F5. *J. Virol.* 78:3155–3161.
27. Morris L, Chen X, Alam M, Tomaras G, Zhang R, Marshall DJ, Chen B, Parks R, Foulger A, Jaeger FM, Donathan M, Bilska M, Grey ES, Abdool Karim SS, Kepler TB, Whitesides J, Montefiori D, Moody MA, Liao H-X, Haynes BF. 2011. Isolation of a human anti-HIV gp41 membrane proximal region neutralizing antibody by antigen-specific single cell sorting. *PLoS One* 6:e23532. doi:10.1371/journal.pone.0023532.
28. Zhu Z, Qin HR, Chen W, Zhao Q, Shen X, Schute R, Wang Y, Ofek G, Streaker E, Prabakaran P, Fouda GG, Liao H-X, Owens J, Louder M, Yang Y, Klaric K-A, Moody MA, Mascola JR, Scott JK, Kwong PD, Montefiori D, Haynes BF, Tomaras GD, Dimitrov DS. 2011. Cross-reactive HIV-1-neutralizing human monoclonal antibodies identified from a patient with 2F5-like antibodies. *J. Virol.* 85:11401–11408.
29. Gorny MK, VanCott TC, Williams C, Revesz K, Zolla-Pazner S. 2000. Effects of oligomerization on the epitopes of the human immunodeficiency virus type 1 envelope glycoproteins. *Virology* 267:220–228.
30. Nyambi PN, Mbah HA, Burda S, Williams C, Gorny MK, Nadas A, Zolla-Pazner S. 2000. Conserved and exposed epitopes on intact, native, primary human immunodeficiency virus type 1 virions of group M. *J. Virol.* 74:7096–7107.
31. Gorny MK, Moore JP, Conley AJ, Karwowska S, Sodroski J, Williams C, Burda S, Boots LJ, Zolla-Pazner S. 1994. Human anti-V2 monoclonal antibody that neutralizes primary but not laboratory isolates of HIV-1. *J. Virol.* 68:8312–8320.
32. Gorny MK, Pan R, Williams C, Wang XH, Volsky B, O'Neal T, Spurrier B, Sampson JM, Li L, Seaman MS, Kong XP, Zolla-Pazner S. 2012. Functional and immunochemical cross-reactivity of V2-specific monoclonal antibodies from human immunodeficiency virus type 1-infected individuals. *Virology* 427:198–207.
33. Gorny MK, Williams C, Volsky B, Revesz K, Cohen S, Polonis VR, Honnen WJ, Kayman SC, Krachmarov CP, Pinter A, Zolla-Pazner S. 2002. Human monoclonal antibodies specific for conformation-sensitive epitopes of V3 neutralize HIV-1 primary isolates from various clades. *J. Virol.* 76:9035–9045.
34. Gorny MK, Wang XH, Williams C, Volsky B, Revesz K, Witover B, Burda S, Urbanski M, Nyambi P, Krachmarov C, Pinter A, Zolla-Pazner S, Nadas A. 2009. Preferential use of the VH5-51 gene segment by the human immune response to code for antibodies against the V3 domain of HIV-1. *Mol. Immunol.* 46:917–926.
35. Gorny MK, Xu J-Y, Karwowska S, Buchbinder A, Zolla-Pazner S. 1993. Repertoire of neutralizing human monoclonal antibodies specific for the V3 domain of HIV-1 gp120. *J. Immunol.* 150:635–643.
36. Gorny MK, Williams C, Volsky B, Revesz K, Wang XH, Burda S, Kimura T, Koning FA, Nadas A, Anyangwe C, Nyambi P, Krachmarov C, Pinter A, Zolla-Pazner S. 2006. Cross-clade neutralizing activity of human anti-V3 monoclonal antibodies derived from the cells of individuals infected with non-B clades of HIV-1. *J. Virol.* 80:6865–6872.
37. Nyambi PN, Gorny MK, Bastiani L, van der Groen G, Williams C, Zolla-Pazner S. 1998. Mapping of epitopes exposed on intact human immunodeficiency virus type 1 (HIV-1) virions: a new strategy for studying the immunologic relatedness of HIV-1. *J. Virol.* 72:9384–9391.
38. Jeffs SA, Gorny MK, Williams C, Revesz K, Volsky B, Burda S, Wang XH, Bandres J, Zolla-Pazner S, Holmes H. 2001. Characterization of human monoclonal antibodies selected with a hypervariable loop-deleted recombinant HIV-1 (IIIB) gp120. *Immunol. Lett.* 79:209–213.
39. Zolla-Pazner S, O'Leary J, Burda S, Gorny MK, Kim M, Mascola J, McCutchan FE. 1995. Serotyping of primary human immunodeficiency virus type 1 isolates from diverse geographic locations by flow cytometry. *J. Virol.* 69:3807–3815.
40. Xu J-Y, Gorny MK, Palker T, Karwowska S, Zolla-Pazner S. 1991. Epitope mapping of two immunodominant domains of gp41, the transmembrane protein of human immunodeficiency virus type 1, using ten human monoclonal antibodies. *J. Virol.* 65:4832–4838.
41. Gorny MK, Gianakakos V, Sharpe S, Zolla-Pazner S. 1989. Generation of human monoclonal antibodies to HIV. *Proc. Natl. Acad. Sci. U. S. A.* 86:1624–1628.
42. Edmonds TG, Ding H, Yuan X, Wei Q, Smith KS, Conway JA, Wiczorek L, Brown B, Polonis V, West JT, Montefiori DC, Kappes JC, Ochsenbauer C. 2010. Replication competent molecular clones of HIV-1 expressing Renilla luciferase facilitate the analysis of antibody inhibition in PBMC. *Virology* 408:1–13.