Short Communication: Virion Aggregation by Neutralizing and Nonneutralizing Antibodies to the HIV-1 Envelope Glycoprotein

Marina R. Alexander,¹ Rogier W. Sanders,^{1,2} John P. Moore,¹ and Per Johan Klasse¹

Abstract

We used dynamic light scattering to detect aggregation of HIV-1 virions by antibodies (IgG) to the viral envelope glycoprotein (Env). Virions of different strains were inactivated by 2,2'-dithiodipyridine (AT-2), a procedure that abrogates infectivity but preserves the native antigenic structure of Env. Neutralizing antibodies directed to a V3-base- and glycan-dependent epitope on gp120 and to the apex of the Env trimer, as well as nonneutralizing antibodies to the epitope cluster I on the gp41-ectodomain, aggregated virions, but in markedly narrow concentration ranges. In contrast, the neutralizing antibody 2G12, which is specific for a composite glycan-dependent epitope on gp120 and functionally monovalent because of its unusual domain-swap structure, was nonaggregating. These results have potentially complex implications for vaccine development.

AGGREGATION OF VIRIONS BY ANTIBODY (Ab) has an intricate relationship to virus neutralization. Although neutralization has been defined so as to exclude aggregation,¹ aggregated virions show reduced infectivity in quantal assays.² Conversely, the nonneutralized or persistent fraction of infectious virus has been attributed to aggregation.³ Furthermore, aggregation and neutralization differ in their Ab concentration dependencies.^{4–10} Because an Ab valency of at least two is needed to cross-link virions, the extent of aggregation usually has a dome-shaped relationship to the Ab concentration, declining at higher occupancies when it becomes improbable that a free paratope of an Ab molecule that is bound to one virion will find a free epitope on a second virion.^{11–13}

Both neutralizing Abs (NAbs) and nonneutralizing Abs (non-NAbs) can capture HIV-1 virions onto a solid phase.^{14–17} A non-NAb to a gp41 cluster-I epitope, F240, captures HIV-1 virions particularly well.¹⁸ Furthermore, topically delivered F240 may have protected macaques marginally from vaginal SHIV transmission.¹⁸ F240 is nonneutralizing because its epitope is exposed only on nonfunctional gp41 stumps lacking gp120. Hence, non-NAbs like F240 do not interfere directly with Env-mediated virus–cell fusion but can capture and potentially also cross-link virions, thereby causing them to aggregate. We now report that IgGs, whether NAbs directed to gp120 or non-NAbs to gp41 epitopes, can aggregate virions but only in markedly narrow concentration ranges.

Aggregates of Abs and virions were formed as follows. IgG stock solutions were centrifuged at $10,000 \times g$ for 5 min to pellet insoluble material; IgG was then serially diluted in

11 steps in the range 4–30 $\mu\text{g/ml}$ in 50 μl of phosphate-buffered saline (PBS) in 96-well plates, the eight last wells serving as controls without Ab. Virions were obtained by culturing the T cell lines H9, SupT1, or A66-R5 infected with the genetically heterogeneous viruses MN,¹⁹ BaL,²⁰ and ADA-M.²¹ Virus in the supernatants was inactivated with 2,2'-dithiodipyridine (Aldrithiol-2, AT-2) and sucrose gradient fractionated.^{22,23} A 5- μl aliquot of virion suspension was then added to each well, and the plate was shaken gently (300 rpm) for 2 h at 37°C. Total protein and Gag (p24) concentrations in the different preparations are given in Table 1. Finally, aggregates were detected by dynamic light scattering (DLS) in a Malvern Zetasizer μV instrument. Particles were illuminated with a laser beam at 25°C and the scattered light was detected at an angle of 90° over 30 s, as triplicate measurements.

In control experiments, we first tested the anti-gp41 non-NAb F240 on microvesicles released from uninfected H9 cells and AT-2-treated identically to the virion preparations. As expected, the microvesicles varied widely in particle diameter (200–1,000 nm²⁴) independently of the F240 IgG concentration and similarly with the Fab present, results that together demonstrate lack of nonspecific aggregation (Fig. 1, Table 1).

In contrast, HIV-1 MN virions produced in H9 cells were effectively aggregated by F240 IgG at concentrations around 15 $\mu\text{g/ml}$, but again not by the F240 Fab (Fig. 1, Table 1). BaL virions produced in SupT1 cells also gave a sharp aggregation peak with F240. The PGT128 bNAb, directed to a

¹Department of Microbiology and Immunology, Weill Cornell Medical College, Cornell University, New York, New York.

²Department of Medical Microbiology, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands.

TABLE 1. AGGREGATION OF HIV-1 VIRIONS BY ANTI-GP120 AND ANTI-GP41 MONOCLONAL ANTIBODIES

Particles	Ab (IgG ^a)	R ²	Aggregation	Size [nm] ^b
Microvesicles H9	F240	—	No	NA ^c
Protein ^d = 3.0	F240 Fab	—	No	NA
Gag ^d = NA				
MN H9	F240	0.91	Yes	7,300
Protein = 6.4	F240 Fab	—	No	NA
Gag = 930				
BaL SupT1	F240	1.0	Yes	3,200
Protein = 2.4	PG9	—	No	NA
Gag = 350	PGT128	0.96	Yes	3,400
	2G12	—	No	NA
ADA-M SupT1	F240	0.41	ND ^e	NA
Protein = 2.5	PG9	0.60	Yes	1,400
Gag = 470	PGT128	0.49	Yes	780
	2G12	0.0029	No	NA
ADA-M A66-R5	F240	0.71	Yes	1,800
Protein = 6.3	PG9	0.95	Yes	7,300
Gag = 730	PGT128	0.93	Yes	5,200
	2G12	—	No	NA
	246-D 1	0.35	Yes	1,100
	246-D 2	0.42	Yes	1,600
	246-D 3	0.32	Yes	1,100

^aAntibodies were analyzed as IgG except where Fab is stated.

^bThe estimated hydrodynamic diameter of aggregates in nm was reported as the largest peak-mean intensity above 250 nm. The values are approximate, particularly above 1 μ m.

^cNA, not applicable.

^dTotal protein concentrations (mg/ml) were determined by the DC Protein Assay (Bio-Rad); Gag capsid protein (p24) concentrations (μ g/ml) were measured by densitometry after SDS-PAGE electrophoresis and comparison with an HPLC-purified p24 standard. For the H9 and SupT1 cell lines the total protein concentration was approximately 2-fold higher for the virion than the microvesicle-only preparations (microvesicles from A66-R5 were not measured). If all p24 is contained in mature virions, 1 μ g/ml in the viral preparation will give $\sim 10^9$ virions/ml in the final virus-Ab mix.

^eND, not determined; the modeling failed to identify a peak.

composite glycan and V3-base epitope, gave a lower peak, but none (only a marginal elevation at the highest Ab concentration) was detected for PG9, a bNAb recognizing a quaternary-structural epitope at the Env-trimer apex.^{25,26} ADA-M virions, also from SupT1 cells, were aggregated by PGT128 and PG9. But the data for this virus preparation mixed with the non-NAb F240 were ambiguous, merely indicating a possible particle-size increase at high Ab concentrations. ADA-M virions produced in A66-R5 cells were aggregated by PG9, PGT128, and F240; aggregates of different sizes arose at distinct Ab concentrations. The non-NAb D-246, like F240 directed to a gp41 epitope in cluster I, also aggregated ADA-M virions from A66-R5 cells (Fig. 1, Table 1). The Nab 2G12, which is functionally monovalent because of its unusual domain-swap structure,²⁷ consistently failed to aggregate the MN, BaL, and ADA-M virions (Fig. 1, Table 1).

Taken together, the above results confirm the theoretically predicted dependence of virion aggregation on Ab concentration as described by an approximately Gaussian or distinctly peaked curve¹¹ (Fig. 1). Both NAb (PG9 and PGT128) and non-NAb (F240 and D-246) were capable of

aggregating virions, just as both NAb and non-NAb can capture them.^{14–17} We also investigated whether the neutralizing capacity of PGT128 and PG9 agreed with their partly differential aggregation of BaL and ADA-M virions. The bNAbs were titrated in a Tzm-bl infectivity assay²⁸ against infectious virus preparations of BaL and ADA-M, obtained in parallel from the same SuPT1–CCR5 cl.30 cells as the AT-2-treated virus and purified identically. PGT128 neutralized BaL with an IC₅₀ of $0.054 \pm 0.0053 \mu$ g/ml (mean $\pm$ SEM from three experiments), i.e., 100-fold lower than the Ab concentration that gave peak aggregation; but PG9 only neutralized BaL to a maximum of $\sim 30\%$. Thus a uniformly low degree of binding to BaL virions would explain both the poor neutralization and the lack of clearly detectable aggregation by PG9 within the concentration range tested.

The two Abs differed less between them against ADA-M, but neither was sufficiently potent to allow determinations of IC₅₀ and maximum degree of neutralization, whereas pseudovirus with clonal ADA-Env (Cl. AD8²⁹) was readily neutralized by both, PGT128 plateauing near 100% and PG9 at $\sim 80\%$ neutralization. Cultured, genetically heterogeneous virus is expected to be relatively resistant to neutralization, and to yield low slopes of the inhibition curves.²⁸ It should be noted though that whereas neutralization approaching completeness requires the absence of virions with poorly antigenic functional Env, aggregation merely depends on the presence of antigenic virions at the optimal ratio to Ab. Hence, aggregation at an Ab concentration lower than the IC₅₀, as occurred with ADA-M, is explicable. The relationship of aggregation to neutralization may also differ among viruses because of the amount of Env on the virions and its degree of functionality.^{2,30} And aggregating-to-neutralizing concentration ratios for Abs may be affected by the stoichiometry of binding: the number of potential epitopes for PGT128 is three times higher than for PG9 on intact, native spikes. Furthermore, PGT128 would be better able than PG9 to bind to decayed or more open spikes.^{13,31} Therefore, the conditions favoring monovalent engagement of the Ab on one virion, and thereby allowing the cross-linking of another virion, would differ both among Abs and virion preparations.¹¹

We note that not only uninfected cells release nonviral vesicles, but such particles also contaminate virion preparations.^{32,33} Although the sucrose-gradient-purified virions used here contained more protein than did the microvesicles, and were smaller and more uniform in size, they would also contain significant amounts of virion-sized microvesicles. If shed gp120 had stuck to the microvesicles, or some Env trimers stochastically had been incorporated into them, that could possibly have allowed them to be aggregated. Although we cannot entirely rule out this possibility, we note that the gp120 content in microvesicles cosecreted with virions is negligible.³² The virion and nonvirion contents of aggregates might be explored electron-microscopically or by confocal microscopy with fluorescently labeled Gag.^{34,35} The aggregation of noninfectious virions, however, particularly by the nonneutralizing gp41-specific Abs, quite plausibly contributes to the aggregates, which would thus comprise mixtures of infectious and inert particles.^{14,35}

Our results differ to some extent from those of a recent study by Stieh *et al.* describing the aggregation of HIV-1 virions induced by dimeric IgA, but not by IgG, specific for

FIG. 1. HIV-1 virion aggregation by neutralizing antibodies (NABs) and non-NABs to Env. The diagrams show the approximate diameter (nm) of the particles or aggregates on the y-axes as a function of the monoclonal Ab (mAb) concentration ($\mu\text{g/ml}$) on the x-axes. Brownian motion influences how the intensity of scattered light fluctuates; since the size of colloid particles determines the velocity of their motion, the particle diameter can be derived from an analysis of the light scattering profile.⁴⁷ Diameter values, particularly $>1\ \mu\text{m}$, should nevertheless be regarded as highly approximate; furthermore, aggregates are unlikely to be uniformly spherical. *Top row:* Microvesicles released from H9 cells were not aggregated by the IgG of F240 or its Fab (*left*); HIV-1 MN virions produced in H9 cells were aggregated by F240 IgG but not by its Fab (*right*). *Middle row:* BaL virions produced in SupT1 cells were aggregated by F240 and PGT128 (*left*); ADA-M virions produced in SupT1 cells were aggregated by PG9 and PGT128; aggregation by F240 possibly occurred at a too high Ab concentration to be revealed by the curve fit (*right*). *Bottom row:* ADA-M virions produced in A66-R5 cells were aggregated by PG9, F240, and PGT128 (*left*), as well as by the anti-gp41 non-NAB 246-D (three replicate experiments, *right*). The functionally monovalent 2G12 bNAb did not aggregate any of the virions. Note that the y-axis scales are adjusted to the peak heights.

Env; pentameric IgM, however, was only weakly aggregating as was monomeric IgA of one monoclonal Ab to Env.³⁶ The experimental conditions of DLS detection of aggregation were largely similar to ours: Stieh *et al.* studied AT-2-inactivated HIV-1 BaL, which was included in our panel, and the final concentrations of virion-contained Gag were close ($25\text{--}35\ \mu\text{g/ml}$); they tested Ab-virus incubation at 37°C over 1–6 h; we used 2 h incubations at 37°C , but we subsequently performed the DLS at the reduced temperature of 25°C .

Hence, the main difference was in the Abs used and the smaller size of the dilution steps in our study.³⁶

The aggregates we detected had markedly larger diameters ($>700\ \text{nm}$ vs. increases in size detected by Stieh *et al.* of $30\text{--}75\ \text{nm}$). These diameter differences would translate into orders of magnitude of differences in volume and hence in virion content of the aggregates. But we refrain from those calculations, partly because of instrument imprecision for apparent diameters $>1\ \mu\text{m}$, and partly because aggregates

might not be compactly assembled and might assume elongated or flat as well as more spherical shapes and thereby vary greatly in the number of particles they comprise even at similar apparent diameters. Furthermore, we did not study IgM or secretory IgA forms of NAb and non-NAb, but it is plausible that these pentameric and dimeric molecules would aggregate virions more effectively than monomeric IgG does.³⁶ Of note, though, is the vast excess of IgG over IgA in the female genital tract, which may render our observations biologically relevant.³⁷

The study by Stieh *et al.* also indirectly addressed the hypothesis that the virion-aggregating capacity of anti-gp120 Abs contributed to the marginal protection conferred by the RV144 vaccine, which induced no broadly active NAb.^{38,39} IgA purified from sera of RV144 participants, although these Abs would be largely monomeric, significantly increased the DLS-measured diameters of particles when incubated with virions of one of the vaccine strains (A244); still, the increase in diameter was modest (from on average around 210 to 240 nm).³⁶ Somewhat paradoxically, virion-aggregate size was negatively correlated with IgA Env-binding titers, which have previously been associated with increased risk of infection in the RV144 trial.^{38,39} In summary, these indirect investigations, though intriguing, are suggestive rather than directly supportive of a protective role for HIV-1 aggregation. Our results, however, suggest that IgG induced by HIV-Env immunization might sometimes aggregate virions, even in the female genital tract where it would be relevant to protection.³⁷

Although merely speculative, it seems worth considering how aggregation might affect infectivity. In principle, HIV-1 aggregation could be weakly protective *in vivo* by slowing the diffusion of virions, particularly through mucus in the female genital tract⁴⁰; there it might conceivably also prevent percolative penetration through defects in mucosal linings.^{40–42} Furthermore, macrophages phagocytose and degrade Ab-opsonized virions, an activity that would be promoted by the larger size of Ab-virion aggregates.^{43,44} But the net effect of aggregation on HIV-1 transmission is still uncertain.

First, at the portal of entry, how effectively would aggregated virions infect, if they do reach target cells? Whether they would be more or less infectious than individual virions might largely depend on the neutralizing capacity of the aggregating Abs. Aggregates formed by NAb would have low or no infectivity, in accordance with the degree of Ab binding, whereas those formed by non-NAb might be highly infectious through enhanced attachment and the delivery of augmented infectious doses to the target cells reached. In this regard, it seems pertinent that the effects of semen-derived enhancer of virus infection (SEVI), partly attributable to virion aggregation by SEVI fibrils, differ drastically between cell culture, where HIV-1 attachment is promoted, and the female genital tract, where percolative diffusion across epithelial defects is prevented.⁴⁵ Antibody-induced aggregation at virion concentrations that may occur in genital mucosae deserve further investigations. Furthermore, although aggregating Abs would be much easier than bNAb to induce, the narrow operative ranges of Ab concentrations required for aggregation undermine any practical value of virion-aggregating Abs to vaccine development. It is possible, however, that polyclonal Abs would induce broader and more composite aggregation peaks.

In conclusion, Env-specific IgG can aggregate HIV-1 virions, but the relevance of this finding to protection from infection is uncertain. Passive mucosal immunization with bNAb protects from viral acquisition in the macaque model of HIV-1 infection. In contrast, one of the non-NAb shown here to aggregate HIV-1 virions effectively, F240, did not protect significantly when administered vaginally to macaques; only a marginal effect was observed.⁴⁸ But methods are now available to determine under what conditions aggregates are formed *in vivo* and thus to explore what the net effects on infectivity are.⁴⁵ When mediated by neutralizing Abs, aggregation might enhance protection or else be redundant, or it might be counterproductive, by enhancing the persistent fraction of infectivity.^{2,13} Notably, the functionally monovalent bNAb 2G12, which as demonstrated here lacks aggregating capacity, is particularly potent *in vivo* in relation to its *in vitro* efficiency, compared with other bNAb.⁴⁶ Whether the principally different aggregation mediated by non-NAb has any relevance to protection also remains uncertain. In practice, however, when vaccinees are exposed to HIV-1 virions, the conditions favoring mixed aggregates, comprising NAb and non-NAb, as well as infectious and noninfectious virions, are likely to arise, at least in some anatomical locales.

Acknowledgments

We thank Julian Bess and Jeff Lifson of the AIDS and Cancer Virus Program at the Frederick National Laboratory, Frederick, MD, for providing purified AT-2-inactivated HIV-1 virus and microvesicle preparations as well as the sister preparations of infectious virus. The AIDS and Cancer Virus program is supported with federal funds from the National Cancer Institute and National Institutes of Health under contract HHSN261200800001E. We are also grateful to Jeff Lifson for helpful comments on the manuscript. We thank Gargi Debnath and Thomas Ketas for performing neutralization assays. This work was supported by NIH grant R37 AI36082.

Author Disclosure Statement

No competing financial interests exist.

References

1. Mandel B: Neutralization of animal viruses. *Adv Virus Res* 1978;23:205–268.
2. Klasse PJ and Sattentau QJ: Occupancy and mechanism in antibody-mediated neutralization of animal viruses. *J Gen Virol* 2002;83:2091–2108.
3. Wallis C and Melnick JL: Virus aggregation as the cause of the non-neutralizable persistent fraction. *J Virol* 1967;1:478–488.
4. Brioen P, Dekegel D, and Boeye A: Neutralization of poliovirus by antibody-mediated polymerization. *Virology* 1983;127:463–468.
5. Colonno RJ, Callahan PL, Leippe DM, *et al.*: Inhibition of rhinovirus attachment by neutralizing monoclonal antibodies and their Fab fragments. *J Virol* 1989;63:36–42.
6. Dowd KA, Jost CA, Durbin AP, *et al.*: A dynamic landscape for antibody binding modulates antibody-mediated neutralization of West Nile virus. *PLoS Pathog* 2011;7:e1002111.

7. Nemerow GR and Cooper NR: Isolation of Epstein Barr-virus and studies of its neutralization by human IgG and complement. *J Immunol* 1981;127:272–278.
8. Thomas AA, Vrijnsen R, and Boeye A: Relationship between poliovirus neutralization and aggregation. *J Virol* 1986;59:479–485.
9. Wetz K, Willingmann P, Zeichhardt H, and Habermehl KO: Neutralization of poliovirus by polyclonal antibodies requires binding of a single IgG molecule per virion. *Arch Virol* 1986;91:207–220.
10. Wohlfart C: Neutralization of adenoviruses: Kinetics, stoichiometry, and mechanisms. *J Virol* 1988;62:2321–2328.
11. Vos Q, Klasen EA, and Haaijman JJ: The effect of divalent and univalent binding on antibody titration curves in solid-phase ELISA. *J Immunol Methods* 1987;103:47–54.
12. Alexander MR, Ringe R, Sanders RW, *et al.*: What do chaotrope-based avidity assays for antibodies to HIV-1 envelope glycoproteins measure? *J Virol* 2015;89:5981–5995.
13. Yasmeen A, Ringe R, Derking R, *et al.*: Differential binding of neutralizing and non-neutralizing antibodies to native-like soluble HIV-1 Env trimers, uncleaved Env proteins, and monomeric subunits. *Retrovirology* 2014; 11:41.
14. Liu P, Williams LD, Shen X, *et al.*: Capacity for infectious HIV-1 virion capture differs by envelope antibody specificity. *J Virol* 2014;88:5165–5170.
15. Liu P, Yates NL, Shen X, *et al.*: Infectious virion capture by HIV-1 gp120-specific IgG from RV144 vaccinees. *J Virol* 2013;87:7828–7836.
16. Nyambi PN, Gorny MK, Bastiani L, *et al.*: 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* 1998;72: 9384–9391.
17. Poignard P, Moulard M, Golez E, *et al.*: Heterogeneity of envelope molecules expressed on primary human immunodeficiency virus type 1 particles as probed by the binding of neutralizing and nonneutralizing antibodies. *J Virol* 2003;77:353–365.
18. Burton DR, Hessel AJ, Keele BF, *et al.*: Limited or no protection by weakly or nonneutralizing antibodies against vaginal SHIV challenge of macaques compared with a strongly neutralizing antibody. *Proc Natl Acad Sci USA* 2011;108:11181–11186.
19. Gallo RC, Salahuddin SZ, Popovic M, *et al.*: Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science* 1984;224:500–503.
20. Gendelman HE, Orenstein JM, Martin MA, *et al.*: Efficient isolation and propagation of human immunodeficiency virus on recombinant colony-stimulating factor 1-treated monocytes. *J Exp Med* 1988;167:1428–1441.
21. Gartner S, Markovits P, Markovitz DM, *et al.*: The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science* 1986;233:215–219.
22. Lifson JD, Rossio JL, Piatak M Jr, *et al.*: Evaluation of the safety, immunogenicity, and protective efficacy of whole inactivated simian immunodeficiency virus (SIV) vaccines with conformationally and functionally intact envelope glycoproteins. *AIDS Res Hum Retroviruses* 2004;20: 772–787.
23. Rossio JL, Esser MT, Suryanarayana K, *et al.*: Inactivation of human immunodeficiency virus type 1 infectivity with preservation of conformational and functional integrity of virion surface proteins. *J Virol* 1998;72:7992–8001.
24. Andreu Z and Yanez-Mo M: Tetraspanins in extracellular vesicle formation and function. *Front Immunol* 2014; 5:442.
25. Walker LM, Huber M, Doores KJ, *et al.*: Broad neutralization coverage of HIV by multiple highly potent antibodies. *Nature* 2011;477:466–470.
26. Walker LM, Phogat SK, Chan-Hui PY, *et al.*: Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. *Science* 2009;326: 285–289.
27. Calarese DA, Scanlan CN, Zwick MB, *et al.*: Antibody domain exchange is an immunological solution to carbohydrate cluster recognition. *Science* 2003;300: 2065–2071.
28. Ketas TJ, Holuigue S, Matthews K, *et al.*: Env-glycoprotein heterogeneity as a source of apparent synergy and enhanced cooperativity in inhibition of HIV-1 infection by neutralizing antibodies and entry inhibitors. *Virology* 2012; 422:22–36.
29. Theodore TS, Englund G, Buckler-White A, *et al.*: Construction and characterization of a stable full-length macrophage-tropic HIV type 1 molecular clone that directs the production of high titers of progeny virions. *AIDS Res Hum Retroviruses* 1996;12:191–194.
30. Klasse PJ and Moore JP: Quantitative model of antibody- and soluble CD4-mediated neutralization of primary isolates and T-cell line-adapted strains of human immunodeficiency virus type 1. *J Virol* 1996;70:3668–3677.
31. Julien JP, Lee JH, Cupo A, *et al.*: Asymmetric recognition of the HIV-1 trimer by broadly neutralizing antibody PG9. *Proc Natl Acad Sci USA* 110:4351–4356.
32. Bess JW Jr, Gorelick RJ, Bosche WJ, *et al.*: 1997; Microvesicles are a source of contaminating cellular proteins found in purified HIV-1 preparations. *Virology* 2013;230: 134–144.
33. Gluschkof P, Mondor I, Gelderblom HR, and Sattentau QJ: Cell membrane vesicles are a major contaminant of gradient-enriched human immunodeficiency virus type-1 preparations. *Virology* 1997;230:125–133.
34. Bhattacharya B, Weiss RA, Davis C, *et al.*: Detection and quantitation of human immunodeficiency virus type-1 particles by confocal microscopy. *J Virol Methods* 2004;120:13–21.
35. Klasse PJ: Molecular determinants of the ratio of inert to infectious virus particles. *Prog Mol Biol Transl Sci* 2015;129:285–326.
36. Stieh DJ, King DF, Klein K, *et al.*: Aggregate complexes of HIV-1 induced by multimeric antibodies. *Retrovirology* 2014;11:78.
37. Klasse PJ, Sanders RW, Cerutti A, and Moore JP: How can HIV-type-1-Env immunogenicity be improved to facilitate antibody-based vaccine development? *AIDS Res Hum Retroviruses* 2012;28:1–15.
38. Haynes BF, Gilbert PB, McElrath MJ, *et al.*: Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N Engl J Med* 2012;366:1275–1286.
39. Klasse P and Moore JP: Good CoP, bad CoP? Interrogating the immune responses to primate lentiviral vaccines. *Retrovirology* 2012;9:80.
40. Hope TJ: Moving ahead an HIV vaccine: To neutralize or not, a key HIV vaccine question. *Nat Med* 2011;17: 1195–1197.

41. Carias AM, McCoombe S, McRaven M, *et al.*: Defining the interaction of HIV-1 with the mucosal barriers of the female reproductive tract. *J Virol* 2013;87:11388–11400.
42. Forthal D, Hope TJ, and Alter G: New paradigms for functional HIV-specific nonneutralizing antibodies. *Curr Opin HIV AIDS* 2013;8:393–401.
43. Dugast AS, Stamatatos L, Tonelli A, *et al.*: Independent evolution of Fc- and Fab-mediated HIV-1-specific antiviral antibody activity following acute infection. *Eur J Immunol* 2014;44:2925–2937.
44. Lux A, Yu X, Scanlan CN, and Nimmerjahn F: Impact of immune complex size and glycosylation on IgG binding to human FcγR3. *J Immunol* 2013;190:4315–4323.
45. Allen SA, Carias AM, Anderson MR, *et al.*: Characterization of the influence of semen-derived enhancer of virus infection (SEVI) on the interaction of HIV-1 with female reproductive tract tissues. *J Virol* 2015;89:5569–5580.
46. Hessel AJ, Rakasz EG, Poignard P, *et al.*: Broadly neutralizing human anti-HIV antibody 2G12 is effective in protection against mucosal SHIV challenge even at low serum neutralizing titers. *PLoS Pathog* 2009;5:e1000433.
47. Hassan PA, Rana S, and Verma G: Making sense of Brownian motion: Colloid characterization by dynamic light scattering. *Langmuir* 2015;31:3–12.

Address correspondence to:

Per Johan Klasse

Department of Microbiology and Immunology

Weill Cornell Medical College

Cornell University

1300 York Avenue, Box 62

New York, New York 10065

E-mail: pek2003@med.cornell.edu