

# Neutralizing Antibody Response and Antibody-Dependent Cellular Cytotoxicity in HIV-1–Infected Individuals from Guinea-Bissau and Denmark

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## Abstract

The development of therapeutic and prophylactic HIV vaccines for African countries is urgently needed, but the question of what immunogens to use needs to be answered. One approach is to include HIV envelope immunogens derived from HIV-positive individuals from a geographically concentrated epidemic with more limited viral genetic diversity for a region-based vaccine. To address if there is a basis for a regional selected antibody vaccine, we have screened two regionally separate cohorts from Guinea-Bissau and Denmark for neutralizing antibody activity and antibody-dependent cellular cytotoxicity (ADCC) against local and nonlocal circulating HIV-1 strains. The neutralizing activity did not demonstrate higher potential against local circulating strains according to geography and subtype determination, but the plasma from Danish individuals demonstrated significantly higher inhibitory activity than that from Guinea-Bissau individuals against both local and nonlocal virus strains. Interestingly, an opposite pattern was observed with ADCC activity, where Guinea-Bissau individual plasma demonstrated higher activity than Danish plasma and was specifically against the local circulating subtype. Thus, on basis of samples from these two cohorts, no local-specific neutralizing activity was detected, but a local ADCC response was identified in the Guinea-Bissau samples, suggesting potential use of regional immunogens for an ADCC-inducing vaccine.

## Introduction

**A**N EFFECTIVE PROPHYLACTIC HIV-1 vaccine will preferably induce antibodies with broad neutralizing activity and antibodies that mediate efficient antibody-dependent cellular cytotoxicity (ADCC). Passive administration of monoclonal neutralizing antibodies to macaques and subsequent protection against SHIV challenge are strong evidence for the protective effect of vaccine-elicited neutralizing antibodies.<sup>1,2</sup> The neutralizing antibodies have the ability to inhibit the viral transmission if present at the time of infection. These antibodies have been studied extensively in

*in vitro* studies, and common characteristics, such as long HCDR3s, have been identified.<sup>3</sup> However, eliciting broadly neutralizing antibodies seems very difficult since only 10%–30% of infected individuals develop such antibodies<sup>4–7</sup> after long maturation and somatic hypermutation processes.<sup>8,9</sup> The ADCC-mediating antibodies have gained more attention since the Thai RV144 vaccine efficacy trial demonstrated that the observed protection was correlated with low plasma levels of IgA envelope (Env) antibodies in association with a high level of nonneutralizing IgG antibodies with ADCC activity,<sup>10,11</sup> which highlights the importance of also non-neutralizing antibodies. These types of antibodies have also

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been revealed to occur in elite controllers,<sup>12</sup> and it has been suggested that control of viremia is associated with a broader ADCC response.<sup>13</sup> The two different functions of antibodies, ADCC and neutralization, have been demonstrated to coincide with some characterized monoclonal antibodies.<sup>14,15</sup>

Whether the vaccine-induced antibodies are neutralizing or nonneutralizing, they should most likely be targeting the HIV-1 envelope (Env). Specific regions of the Env trimer are known as the targets for broadly neutralizing anti-HIV antibodies, and extensive attempts have been undertaken to construct immunogens to direct antibodies to these areas.<sup>16</sup> However, the growing knowledge of neutralizing epitope structures on the HIV-1 Env has not automatically translated into the generation of improved immunogens,<sup>16</sup> emphasizing the importance of continuing all approaches in the search for HIV-1 vaccine immunogens. The main challenge of the tremendous genetic diversity of globally circulating HIV-1 strains<sup>17,18</sup> remains unsolved, and it is still unclear which vaccine antigen to use to address this hurdle.

The two different approaches when designing new immunogens are to elicit region-specific and broadly targeting immune responses. The RV144 trial used the region-specific approach when the immunogens used matched the local circulating strains.<sup>19</sup> It is unlikely that the developed response would have a protection against other subtypes found elsewhere in the world.<sup>20</sup> No vaccine candidate tested to date has demonstrated a sufficient, potent, and broad immune response. Another promising attempt tested immunization with several different Env subtypes, which indeed induced broad multi-subtype anti-Env-binding antibodies in a phase IIa clinical trial.<sup>21</sup> However, a following phase IIb trial, HVTN 505, with the same immunization regime was halted prematurely due to lack of efficacy.<sup>22</sup> Future vaccine candidates may benefit from the recent development of stabilized soluble Env trimers,<sup>23,24</sup> which mimic the native envelope spike and could be useful both in DNA<sup>25</sup> and antigen vaccines. Despite the recent discovery of a large number of broadly neutralizing antibodies, it is unknown how to elicit such antibodies. To determine if there is any basis for a local vaccine, we have tested the antiviral activity in two different patient cohorts from two different geographical regions. As a model for this study, Guinea-Bissau and Denmark were chosen as these two relatively small and distinct regions harbor different circulating HIV-1 strains with subtypes A and CRF02\_AG dominating in Guinea-Bissau<sup>26,27</sup> and subtype B dominating in Denmark.<sup>28,29</sup> Neutralization and ADCC activities against circulating HIV-1 subtypes in Guinea-Bissau and Denmark and against a subtype not found in these regions were evaluated in these two cohorts. A distinct pattern of crossclade neutralization was particularly apparent in the plasma of Danish HIV-1-positive individuals, whereas neutralization in the Guinea-Bissau plasma was less potent. However, the ADCC activity demonstrated a different pattern, with the Guinea-Bissau plasma having a higher potency than the Danish plasma and was specifically against Env of the local subtype A origin.

## Materials and Methods

### Study subjects

Plasma samples from Guinea-Bissau were obtained from HIV-1-infected individuals participating in the Bissau HIV Cohort<sup>30,31</sup> based at the Hospital Nacional Simão Mendes,

Bissau. The recruited individuals were enrolled for a clinical phase I study of a therapeutic HIV-1 vaccine (www.ClinicalTrials.gov id: NCT01141205).<sup>32</sup> The HIV status of the enrolled individuals was confirmed at the National Public Health Laboratory, Bissau. Preimmunization plasma samples from 13 ART-naïve individuals were selected for this study (Table 1), none of them coinfecting with HIV-2. The plasma samples from Denmark were selected from a group of HIV-1-infected individuals enrolled for a clinical phase I therapeutic HIV-1 vaccine study (www.ClinicalTrials.gov id: NCT 01009762).<sup>33</sup> Preimmunization plasma samples from 10 ART-naïve individuals were selected (Table 1). The studies were approved by the National Committee for Health Research Ethics of the Danish Ministry of Health (H-D-2008-063), the Danish Medicines Agency (Journal no. 2612–3785), and UCEPS, the National Ethics Committee of Guinea-Bissau (Parecer NCP/No.15/2007). All the patients provided written and informed consent.

### Genomic sequencing and subtype determination

Subtype determination of Guinea-Bissau material was based on sequencing of the envelope C2-V3 region, as described by Palm *et al.*<sup>26</sup> In brief, viral RNA was extracted from plasma samples and amplified by reverse transcription-polymerase chain reaction (PCR), and the env C2-V3 region was sequenced. The sequences were aligned in Mega 5,<sup>34</sup> with A3 and CRF02\_AG used as reference sequences, and A1 sequences were used as the outgroup sequences (a detailed description of the reference sequences is described by Palm *et al.*<sup>26</sup>). Maximum likelihood phylogenetic trees were generated, and the subtypes were identified by the BootScan analysis using SimPlot v3.5.<sup>35</sup> The sequences determined to be A3/CRF02\_AG recombinants, as described by Palm *et al.*,<sup>26</sup> will be referred to as A3/02 recombinants. The subtype determination of Danish material was conducted by an analysis of partial *pol* sequences of 1,200 base pairs containing the protease and partial reverse transcriptase; the sequences were generated through population-based sequencing using ViroSeq HIV-1 genotyping System v. 2 (Abbott Diagnostics, Foster City, CA).

### Cloning of the HIV-1 envelope

To include viral variants from Guinea-Bissau in the neutralization assay, two cloned gp160 genes from two different HIV-1-infected individuals, ViFU288 and ViFU262, were used for pseudovirus production. The extracted viral RNA (MagNa Pure 96 DNA and Viral NA Small Volume Kit; Roche Diagnostics, Mannheim, Germany) from plasma samples was reverse transcribed with SuperScript III (Invitrogen, Carlsbad, CA), followed by env amplification with the Expand Long Template PCR System (Roche Applied Science, Basel, Switzerland) and gel purification. The env regions were cloned into the pcDNA<sup>TM</sup>3.3-TOPO<sup>®</sup> vector (Invitrogen) and were transformed into JM109 (Stratagene, La Jolla, CA). The bacterial colonies were screened by PCR for the correct insert, and the selected plasmids were recovered using an S.N.A.P. MiniPrep Kit (Invitrogen) and were sequenced for confirmation.

### Pseudoviruses

The virus panel used in the TZM-bl neutralization assay consisted of HIV-1 pseudovirus from subtypes A (ITM1-4,

TABLE 1. CLINICAL DATA FOR STUDY INDIVIDUALS AT THE TIME OF SAMPLING

Country	Study individuals	Sex	HIV-1 subtype	HIV-1 plasma viral load (copies/ml)	CD4 <sup>+</sup> T-cell count (cells/ $\mu$ l)
Guinea-Bissau	ViFU1 111	F	A3/02	n.a.	496
	ViFU1 207	M	A3/02	16,500	284
	ViFU1 235	M	A3	n.a.	456
	ViFU1 238	M	A3	720	609
	ViFU1 250	F	CRF02_AG	251,000	354
	ViFU1 261	F	A3	n.a.	529
	ViFU1 282	F	CRF02_AG	10,338	541
	ViFU1 284	M	CRF02_AG	642,000	281
	ViFU1 285	F	A3/02	733,000	266
	ViFU1 288	F	A3/02	135,280	701
	ViFU1 290	F	CRF02_AG	6,782	851
	ViFU1 295	F	CRF02_AG	12,746	530
	ViFU1 299	F	A3/02	45,386	506
Denmark	PV01	M	B	2,060	542
	PV03	M	B	29,518	430
	PV04	M	B	128,543	320
	PV05	M	B	21,180	660
	PV06	M	B	16,468	530
	PV07	M	B	70,898	380
	PV08	M	B	27,862	570
	PV09	M	B	458,300	520
	PV10	M	B	28,123	570
	PV16	M	B	3,200	457

M, male; F, female; n.a., not available.

Q461), B (SF162, Bx08, BaL, QH0692, AC10.0.29), C (92Br025), CRF02\_AG (VI1090, ViFU262), and A3/02 (ViFU 288), and the panel included both neutralization-sensitive<sup>36</sup> and more resistant variants. Pseudoviruses were produced by cotransfecting HEK293T cells<sup>37</sup> with a plasmid containing the viral gp160 gene and the pNL4.3LucR-E- plasmid containing a luciferase reporter gene. The pNL4.3LucR-E- plasmid was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: pNL4.3LucR-E- from Dr. Nathaniel Landau.<sup>38,39</sup> Forty-eight hours after transfection, the pseudovirus stocks were harvested and tested for infectivity using TZM-bl target cells.<sup>40</sup> The pseudoviruses were diluted to titers optimal for use in neutralization assays, which were  $\sim 10^5$  relative light units.

#### Neutralization assay

Plasma neutralizing activities were measured by the luciferase-based assay in TZM-bl cells, as previously described.<sup>41,42</sup> Briefly, heat-inactivated plasma, which was threefold diluted in six steps starting at 1:20, was mixed with pseudovirus and incubated for 1 h at 37°C before adding TZM-bl cells. The ability of plasma to neutralize virus infection was assessed by measuring luciferase activity 48 h after virus inoculation and comparing the results to a control infection without any plasma. TriMab, a mix of three mAbs (b12, 2F5, and 2G12) (obtained from the Centre for AIDS Reagents, NIBSC, United Kingdom), was used in parallel in every neutralization assay as a strongly neutralizing control. The neutralization titers are expressed as the reciprocal of the plasma dilution that inhibited virus infection by 50% (ID<sub>50</sub>). Plasma samples that did not reach the ID<sub>50</sub> at the 1:20 dilution were assigned a value of 20 for the geometric mean titer calculations.

#### Anti-Env antibody enzyme-linked immunosorbent assay

Envelope-specific IgG titers were determined by enzyme-linked immunosorbent assay (ELISA) according to a previously described method,<sup>25</sup> which uses recombinant gp120<sub>BaL</sub> protein (Immune Technology, New York, NY) as the coating antigen. Total Env-specific IgG was determined with conjugate rabbit anti-human IgG (cat. no. P214; Dako A/S, Glostrup, Denmark), and the four different IgG subclasses were determined with mouse anti-human IgG1, IgG2, IgG3, and IgG4 (clone HP6069, HP6014, HP6047, and HP6025; Life Technologies, Carlsbad, CA, respectively).

#### ADCC assay

ADCC activity was detected using PanToxiLux (OncoImmune, Gaithersburg, MD) according to the previously described ADCC-GranToxiLux procedure.<sup>43,44</sup> Env-coated CEM.NKR<sub>CCR5</sub> cells<sup>45</sup> were used as target cells and coated 90 min at 4°C with recombinant Env. Env-coated target cells have previously been demonstrated to be equally effective in the ADCC assay as infected target cells,<sup>44</sup> which we confirmed in a separate experiment (data not shown). Recombinant Env was derived from *envelopes* of subtype B, BaL (Immune Technology), and subtype A, UG37 (Polymun Scientific, Klosterneuburg, Austria). Targets cells were labeled with TFL4 and NFL1 (target cell marker and viability marker, respectively). The PBMC from two HIV-1-seronegative donors were pooled and used as the source of NK effector cells. Targets cells and effector cells were counted for viability, adjusted to the effector-to-target cell (E:T) ratio of 30:1, and incubated with granzyme B (GzB)

substrate in 96-well plate for 5 min at room temperature before antibodies were added. The plasma samples were tested as fivefold serial dilutions starting at 1:300 and incubated at room temperature for 15 min, followed by 60-min incubation at 37°C. Samples were acquired using BD LSRII instrument and analyzed using FlowJo (Tree Star version 8.8.7, Ashland, OR), see gating strategy in Supplementary Figure S1 (Supplementary Data are available online at [www.liebertpub.com/aid](http://www.liebertpub.com/aid)). A pool of 17 in-house HIV-negative plasma was used as negative control and a standard antibody HIVIG (obtained from NIH AIDS Research and Reagent Program) as positive control. A threshold for positive ADCC activity was set to the mean percent GzB activity mediated by HIV-negative plasma samples plus three standard deviations, which corresponded to >9.53% of effector cells positive for active GzB for rgp120<sub>BaL</sub> and >6.77% for rgp140<sub>UG37</sub>. The final results are reported as the endpoint dilution, that is, the highest reciprocal of the plasma dilution that was positive for ADCC activity.<sup>10</sup> The results are also demonstrated in Supplementary Figure S2 as percent GzB activity, that is, percentage of cells positive for proteolytically active GzB out of the total viable target cell population.

*Statistical analysis*

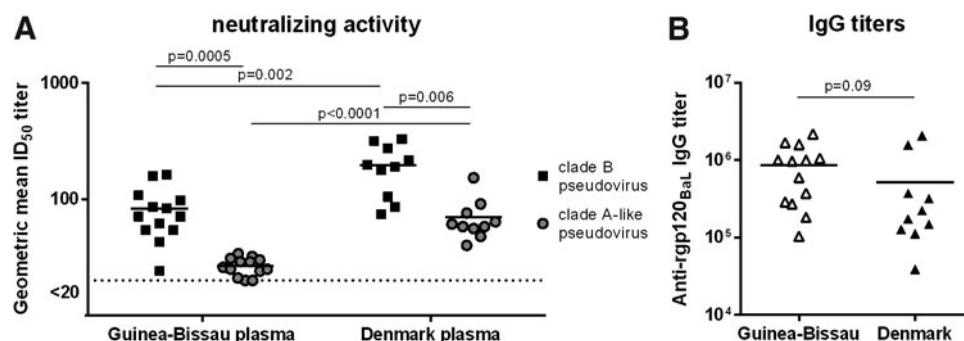
The differences between Guinea-Bissau and Denmark plasma samples in neutralization and ADCC and IgG titers were evaluated for statistical significance by the nonparametric Mann–Whitney *U* test. When comparing neutralization and ADCC titers by the same samples but against different subtypes, Wilcoxon’s matched pairs test was used. The data were analyzed with Prism v.5.0 (GraphPad Software, Inc., San Diego, CA).

**Results**

*Crossclade neutralizing antibodies differ in HIV-1-infected individuals from Guinea-Bissau and Denmark*

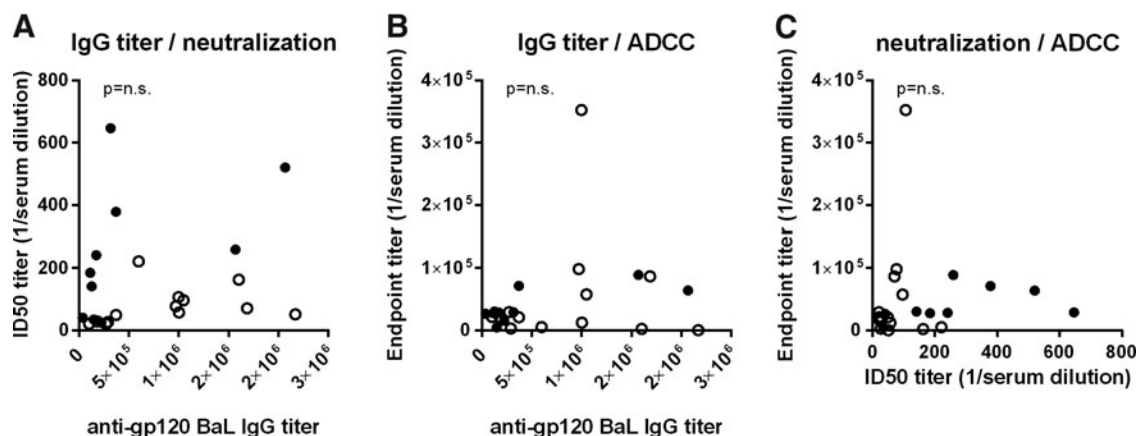
We studied plasma from 13 and 10 HIV-1-infected individuals living in Guinea-Bissau and Denmark, respectively, to determine intra- and crossclade HIV-1 neutralizing activity. The subtype/CRF distribution in the two groups was

similar to previous estimates,<sup>26–29</sup> including A3, CRF02\_AG, and A3/02 subtypes in the Guinea-Bissau group and only subtype B in the Danish group (Table 1). The median CD4<sup>+</sup> T-cell count was similar in the two groups, 506 cells/ $\mu$ l blood [interquartile range (IQR) 319–575] in Guinea-Bissau and 525 cells/ $\mu$ l blood (IQR 417–570) in Denmark, and the median plasma viral load was comparable at 30,943 (IQR 9,449–348,750) and 27,993 (IQR 13,151–85,309) copies/ml, respectively. Plasma samples were screened for neutralizing activity using the TZM-bl assay against a pseudotyped virus panel that represented several different subtypes, including Danish subtype B and the Guinea-Bissau subtypes A, CRF02\_AG, and A3/02. To compare the intra- and crossclade neutralization between the two different geographical groups, a geometric mean ID<sub>50</sub> neutralizing antibody titer was calculated for both groups against the regional local subtypes. Interestingly, the Danish samples displayed significantly higher neutralizing antibody titers against both subtype B and subtypes A, CRF02\_AG, and A3/02 pseudoviruses than the Guinea-Bissau plasma samples (Fig. 1A). To determine if the lower neutralizing activity in the Guinea-Bissau samples reflected lower HIV-1 Env-specific IgG, the levels of anti-rgp120<sub>BaL</sub> antibody titers in the plasma samples were measured in ELISA. The levels of Env-specific IgG were similar in Guinea-Bissau and Danish individuals (Fig. 1B) despite the fact that the rgp120<sub>BaL</sub> used in the ELISA is a subtype B envelope, which could favor the antibody titers in the Danish samples. The individual neutralizing titers for each individual plasma–virus combination are shown in a heat map (Fig. 2). All viruses in the panel could be neutralized by some of the plasma samples. In addition to the regional subtypes, a Tier 1 subtype C virus, 92Br025, was included in the neutralization assay to test a nonregional subtype for both cohorts. Plasma from both groups showed neutralizing activity against the subtype C virus, but the Danish samples had significantly higher neutralizing titers (Mann–Whitney *U* test, *p* = .0009). The subtype B pseudoviruses were overall more sensitive to neutralization; however, this result may be expected as the three Tier 1 subtype B viruses used have previously been demonstrated to be highly sensitive to neutralization, whereas the subtypes A, AG, and A3/02 have been shown to be generally more resistant.<sup>36</sup> To summarize, there appears to be no enhancement of regional intraclade neutralization in our



**FIG. 1.** Heterologous neutralizing activities in plasma from Guinea-Bissau (*n* = 13) and Denmark (*n* = 10). (A) The potency of neutralizing activity was determined by the geometric mean ID<sub>50</sub> titer for each individual across the five subtype B pseudoviruses and the five subtype A-like pseudoviruses. Individual plasma titers of <20 were assigned a value of 20 for the geometric mean titer (GMT) calculations. (B) The gp120<sub>BaL</sub>-specific antibody response was determined by ELISA. The values are mean ± standard error of the mean. ELISA, enzyme-linked immunosorbent assay.





**FIG. 4.** ADCC activity of plasma does not correlate with neutralization or IgG binding. The gp120<sub>BaL</sub>-specific antibody response for each individual was compared to the neutralizing titer of BaL pseudovirus (A) and the ADCC activity against target cells coated with Env<sub>BaL</sub> (B). The neutralizing titer of BaL pseudovirus was also compared to ADCC activity against target cells coated with Env<sub>BaL</sub> (C). *Open circles*, Guinea-Bissau plasma; *filled circles*, Danish plasma.

Env-specific IgG of the IgG1 and IgG3 subclasses, which are the two IgG subclasses with the strongest affinity for the NK-cell Fc receptor CD16<sup>47</sup> because ELISA levels of rgp120<sub>BaL</sub>-specific IgG1 and IgG3 were similar in plasma from both regions (Fig. 3B). Rgp120<sub>BaL</sub>-specific IgG2 and IgG4 also demonstrated similar levels between the two groups (data not shown). Thus, we detected ADCC activity, including cross-clade, in nearly all samples. However, the intraclade activity was higher in the plasma samples from Guinea-Bissau compared to crossclade activity ( $p = .0005$ ) and was higher than the ADCC activity observed in the Danish samples ( $p = .044$ ).

Finally, no correlation between the ability of plasma to neutralize the subtype B virus BaL to mediate ADCC activity against the same virus or the presence of Env<sub>BaL</sub>-specific IgG ELISA was observed (Fig. 4).

**Discussion**

The primary goal of this study was to evaluate the basis for the construction of a regionally tailored HIV-1 antibody-based vaccine. Given the high diversity of HIV-1 (Env), we hypothesized that an antibody-based vaccine component may have to be based on regional immunogens of the same subtype. This approach could facilitate a more precisely focused immunological response to a more limited pool of similar circulating strains. We have characterized intra- and cross-clade neutralizing and ADCC activities in plasma from HIV-1-infected individuals from Guinea-Bissau and Denmark harboring circulating viral strains of different subtypes. The two cohorts were matched for viral load and CD4 cell count to eliminate factors that may influence the neutralizing and ADCC activities.<sup>48–51</sup> Interestingly, we found that plasma from Danish individuals was more potent in neutralizing activity regardless of the virus subtype than that from Guinea-Bissau individuals. The greater neutralizing titers of the Danish plasma were seen for all 11 pseudoviruses tested and surprisingly most pronounced for a Tier 1 CRF02\_AG virus (VI1090). However, the ADCC activity in the same plasma samples demonstrated a different pattern. The Guinea-Bissau samples revealed a higher intraclade than crossclade ADCC activity, which was a pattern that was not observed in the

Danish samples. In addition, the ADCC activity against the subtype A Env was higher in the Guinea-Bissau samples than in the Danish samples. These differences in inhibitory activity were not due to simply higher titers of anti-HIV-1 Env IgG as the levels were similar in the two cohorts. Moreover, a concern that the samples from the two regions have been treated differently, which may influence the antibody quality, is ruled out because the Guinea-Bissau plasma, which had lower neutralizing activity, demonstrated high ADCC activity compared with the Danish plasma, and the ELISA antibody titers were the same in both groups. Rather, the antibody specificity may differ perhaps by exposure of crossreactive neutralizing antibody epitopes or ADCC antibody epitopes. The neutralizing and ADCC activities of the two groups could not be correlated with gender (data not shown), which is in agreement with Mata *et al.*<sup>52</sup> The Guinea-Bissau-specific subtypes A3, CRF02\_AG, and A3/02 have previously been demonstrated to differ in disease progression,<sup>26</sup> but this did not reflect any variations in neutralization or ADCC activity in our assays (data not shown).

The observed higher neutralizing titers and broadness of plasma samples from subtype B-infected individuals were unexpected but agree with Seaman *et al.*,<sup>36</sup> who demonstrated that pools of the subtype B plasma in general demonstrated higher levels of neutralizing potency than other plasma pools, such as A or CRF02\_AG. However, the observed superiority of the subtype B plasma to additionally neutralize A, CRF02\_AG, and A3/02 pseudoviruses over the Guinea-Bissau plasma samples is in contrast with Jacob *et al.*,<sup>53</sup> who reported high intraclade neutralization of CRF02\_AG plasma samples. An explanation for the broader neutralization by the Danish samples could be that an infection with subtype B viruses elicits broader antibody responses due to the more sensitive phenotype of the subtype B viruses, as observed in Figure 1. Brown *et al.*<sup>54</sup> support the theory that neutralization-sensitive viruses can have a greater exposure of neutralizing epitopes and thus elicit a broader and more potent immune response in individuals infected with such viruses. Indeed, the large classification of 107 viruses conducted by Seaman *et al.*<sup>36</sup> ordered many of the subtype B viruses into more sensitive categories and ordered the

CRF02\_AG subtype viruses to relatively higher neutralization-resistant categories. The idea of using a viral strain for immunization, such as the subtype B isolate Bx08, which exposes common epitopes for neutralizing antibodies and is known to be commonly recognized by immune sera from a variety of HIV-1-infected individuals,<sup>55</sup> has previously been tested, although with a limited neutralizing humoral response against primary virus.<sup>56–58</sup>

We have not examined if there is an overlap between the antibodies responsible for the neutralizing and ADCC activities in this study, but ADCC-mediating antibodies have previously been shown to be both neutralizing and non-neutralizing.<sup>14,59,60</sup> Nevertheless, we found no association between ADCC and neutralizing activity when comparing the response from both assays and while using the same viral strain in the assays, the subtype B HIV<sub>BaL</sub>. However, such a correlation was not expected, and the broad neutralizing capacity of plasma has previously been demonstrated to not necessarily translate into ADCC activity and vice versa.<sup>12,61</sup>

We found ADCC activity against both subtypes B and A Env in nearly all plasma samples, which is consistent with previous reports of crossclade ADCC activity in HIV-infected individuals.<sup>46,52,61</sup> The Guinea-Bissau plasma samples had increased ADCC activity against the subtype A Env, and we denote this as intraclade activity, as the subtypes A3, CRF02\_AG, and A3/02 contain mainly the subtype A sequence in the *env* region.<sup>62</sup> Augmented intraclade-specific ADCC activity has been previously noted for the subtype B plasma,<sup>46,52</sup> but surprisingly in our study, the subtype B Danish samples did not show any enhanced ADCC activity against the subtype B Env. The higher ADCC activity in samples from Guinea-Bissau was not due to the concentrations of the primary IgG1 and IgG3 ADCC-mediating antibodies<sup>47</sup> because a similar IgG antibody subclass distribution was observed in both groups. Rather, a difference in antibody specificities to ADCC epitopes, which differ from the broadly neutralizing epitopes, is a possibility. There are several epitopes within gp120 that can bind monoclonal antibodies to both neutralizing and ADCC activities; however, there are also epitopes that only bind nonneutralizing antibodies.<sup>14</sup> These epitopes are both CD4 inducible and present on the surface of gp120. Alterations of these gp120 epitopes for different clade viruses, resulting in nonneutralizing antibodies, could explain the ADCC activity variation we see in the Guinea-Bissau and Danish plasma.

The data presented in this study may contribute to the selection of empiric candidate vaccines in limited test of concept clinical studies in Denmark and/or West Africa. Although broad neutralizing and ADCC responses may additionally depend on individual factors, it is still the particular virus Env to which they were exposed that induced the antibody specificity in that individual. We believe that the elicited response stems from viral variants with specific envelope properties, such as harboring neutralizing and nonneutralizing epitopes. Hopefully, these responses can be elicited again in human or animal models if formulated into an appropriate vaccine immunogen, or cocktails of immunogens, and with the right delivery strategy.<sup>25,63</sup>

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#### Sequence Data

Env sequences of ViFU288 and ViFU262 were deposited into GenBank under accession numbers KR709173 and KR709172.

#### Author Disclosure Statement

No competing financial interests exist.

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