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Neutralizing Antibody Responses in Acute Human Immunodeficiency Virus Type 1 Subtype C Infection▽

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The study of the evolution and specificities of neutralizing antibodies during the course of human immunodeficiency virus type 1 (HIV-1) infection may be important in the discovery of possible targets for vaccine design. In this study, we assessed the autologous and heterologous neutralization responses of 14 HIV-1 subtype C-infected individuals, using envelope clones obtained within the first 2 months postinfection. Our data show that potent but relatively strain-specific neutralizing antibodies develop within 3 to 12 months of HIV-1 infection. The magnitude of this response was associated with shorter V1-to-V5 envelope lengths and fewer glycosylation sites, particularly in the V1-V2 region. Anti-MPER antibodies were detected in 4 of 14 individuals within a year of infection, while antibodies to CD4-induced (CD4i) epitopes developed to high titers in 12 participants, in most cases before the development of autologous neutralizing antibodies. However, neither anti-MPER nor anti-CD4i antibody specificity conferred neutralization breadth. These data provide insights into the kinetics, potency, breadth, and epitope specificity of neutralizing antibody responses in acute HIV-1 subtype C infection.

Neutralizing antibodies (NAbs) against the human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein develop relatively slowly compared to HIV-specific CD8 T cells, which develop within weeks of infection (6, 16). This has led to the notion that antibody responses are less relevant to viral control, at least during the acute phase of infection. Recent technological advances in measuring NAb responses have shown that in some HIV-1-infected individuals, potent autologous responses can develop within a few months of infection, although others fail to develop such antibodies until much later (8, 13, 19). The fact that the envelope gene undergoes significant genetic variation which enables the virus to escape NAbs is testimony to the pressure exerted by these early autologous NAbs (19). Antibodies capable of neutralizing viruses other than the autologous virus take considerably longer to develop (8, 13), and only a few individuals develop truly broadly cross-reacting sera. These observations suggest that while there may be many targets for NAbs, few are located in highly conserved sites that might serve as suitable epitopes for inclusion in a vaccine immunogen.

The study of the antibody specificities of sera from HIV-1-infected individuals and of the relationship of those specificities to the breadth and potency of responses has become a topic of substantial interest, since this information may inform vaccine design (R. Wyatt, presented at the AIDS Vaccine 2005 International Conference, Montreal, Canada). Antibodies to CD4-induced (CD4i) epitopes are frequently found in HIV-1-infected individuals (1) and are thought to primarily target the coreceptor binding site, which includes the bridging sheet and, possibly, parts of the V3 region (20, 21). These polyclonal HIV-1-elicited antibodies, as well as a large number of different human monoclonal antibodies (MAbs) to HIV-1 CD4i epitopes (11, 21), can potently neutralize both HIV-1 and HIV-2 when they are pretreated with soluble CD4 (sCD4), indicating that the CD4i coreceptor binding surface is highly conserved antigenically among different subtypes of HIV-1 and the very divergent HIV-2 lineage (1).

Another site that has gained considerable attention recently as a target for NAbs is the membrane-proximal region (MPER), a linear stretch of 34 amino acids in gp41. MAbs targeting this region, such as 2F5 and 4E10, cross-neutralize a large fraction of HIV-1 isolates, and the MPER is therefore considered an important target for vaccines. However, antibodies with 2F5 or 4E10 binding specificity are rarely found in plasmas of HIV-1-infected individuals (23; J. M. Decker et al., presented at the Keystone Symposium on HIV Vaccines, Keystone Resort, Keystone, CO, 2006), possibly due to their cross-reactivity with autoantigens, which results in clonal deletion of B cells with these specificities (4). The use of an HIV-2 chimeric envelope containing the HIV-1 MPER, however, has greatly facilitated our ability to study responses to epitopes throughout the MPER, and we have observed that approximately one-third of HIV-1-infected individuals develop such NAb responses (F. Bibollet-Ruche et al., presented at the Keystone Symposium on HIV Vaccines, Keystone Resort, Keystone, CO, 2006).

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In this study, we explore the evolving NAb response over the first year of infection with HIV-1 subtype C. In addition to analyzing the autologous and heterologous NAb responses by conventional assays, we examined epitope-specific NAbs to CD4i and MPER epitopes in early infection in an effort to understand how such antibodies might contribute to neutralization breadth.

MATERIALS AND METHODS

A cohort of 245 high-risk, HIV-negative women was established in 2004 in Durban, South Africa, for follow-up and subsequent identification of HIV seroconversion. Detection of HIV infection was based on two HIV-1 rapid antibody tests (Determine [Abbott Laboratories, Tokyo, Japan] and Capillus [Trinity Biotech, Jamestown, NY]) performed monthly. Pooled PCR testing (Amplicscreen v1.5; Roche Diagnostics, Rotkreuz, Switzerland) for HIV-1 RNA was done on all antibody-negative samples. All positive samples identified through the pooling assay were confirmed using a quantitative RNA test and an HIV enzyme immunoassay (BEP 2000; Dade Behring, Marburg, Germany) on the same and subsequent samples. Women from this HIV-negative cohort, as well as other seroincidence cohorts, who had a reactive HIV antibody test within 3 months of a previously negative result or had detection by HIV-1 RNA PCR (Roche Amplicor v1.5) in the absence of HIV antibodies were enrolled in this study (CAPRISA 002). The timing of infection was determined as the midpoint between the last antibody-negative test and the first antibody-positive test or 14 days before the participant was PCR positive and antibody negative. Intense clinical follow-up and sample collection were done at enrollment, weekly for 3 weeks, fortnightly until 3 months, monthly until 12 months, and quarterly thereafter. CD4 T-cell counts were assessed using a FACSCalibur flow cytometer, and viral loads were measured using the COBAS AMPLICOR HIV-1 Monitor test, v1.5 (Roche Diagnostics). Plasmas, which were collected in EDTA, and sera were stored at −70°C until use. Written informed consent was obtained from all participants. This study received ethical approval from the University of the Witwatersrand, University of KwaZulu-Natal, and University of Cape Town.

Plasma samples, sCD4, and viruses. Plasma samples from HIV-1 subtype C-infected blood donors (BB8, BB12, BB28, BB55, BB70, and BB106) were purchased from the South African National Blood Service and previously described by Li et al. (9). Recombinant sCD4 was purchased from R&D Systems (Minneapolis, MN). The envelope clone SF162.LS was obtained from Leonidas Stamatatos (Seattle Biomedical Research Institute, Seattle, WA) (17). HIV-2 7312A (1) and derived chimeras were obtained from George Shaw (University of Alabama, Birmingham, AL).

Cell lines. The JC53-bl cell line, engineered by John Kappes and Xiaoyun Wu, was obtained from the NIH AIDS Research & Reference Reagent Program. 293T cells used for transfection were obtained from George Shaw (University of Alabama, Birmingham, AL) and David Montefiori (Duke University School of Medicine, Durham, NC). Both cell lines were cultured in Dulbecco's modified Eagle's medium (Gibco BRL Life Technologies) containing 10% heat-inactivated fetal bovine serum and 50 μg/ml gentamicin (Sigma). Cell monolayers were disrupted at confluence by treatment with 0.25% trypsin in 1 mM EDTA.

Cloning of envelope genes. Long-template HIV-1 cDNA transcripts were generated from viral RNAs extracted from plasma. Whole genomes were amplified from cDNA by using a modified limiting dilution nested PCR assay as described by Rousseau et al. (14). First-round whole-genome products were used as templates to amplify full-length envelope genes. The 3-kb PCR fragments, generated using the envA and envM primers (2), were cloned into the pCDNA 3.1-TOPO vector (Invitrogen) and screened as previously described (3). Env-pseudotyped viruses were obtained by cotransfecting the Env plasmid with pSG3deltaEnv (19), using Fugene transfection reagent (Roche).

Neutralization assay. Neutralization was measured as a reduction in luciferase gene expression after a single round of infection of JC53-bl cells with Env-pseudotyped viruses (10). Titers were calculated as the inhibitor concentrations (IC_{50}) or reciprocal plasma/serum dilutions (ID_{50}) causing a 50% reduction of relative light units.

CD4i and MPER neutralization assays. CD4i and MPER neutralization assays were performed as described by Decker et al. (1), using the HIV-2 virus 7312A and the HIV-2/HIV-1 MPER chimeras described in Fig. 9. Briefly, 2,000 IU of virus was incubated with fivefold dilutions of plasma/serum (starting dilution, 1:20). After 1 hour, the mixture was added to 40% confluent JC53-bl cells, which had been seeded the day before in a 96-well plate. Infection was measured 48 h later by evaluating the luciferase activity. To evaluate the CD4i antibody response, the virus was preincubated for 1 h with sCD4 at a concentration equal to the IC_{50} for each virus strain before adding the diluted plasma/serum.

gp160 sequencing. Cloned *env* genes were sequenced using an ABI PRISM Big Dye Terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA) and then resolved on an ABI 3100 automated genetic analyzer. The full-length gp160 sequences were assembled and edited using Sequencher v. 4.0 software (Genecodes, Ann Arbor, MI). The number of potential N-linked glycosylation sites (PNGS) was determined using the software N-glycosite (http://www.hiv.lanl.gov/content/hiv-db/GLYCOSITE/glycosite.html). Multiple alignments were performed using Clustal X (version 1.83) and were edited with BioEdit (version 5.0.9). Phylogenetic analysis was performed with MEGA, version 2.1. A neighbor-joining tree was constructed with distances calculated using Kimura's two-parameter model, with reference sequences obtained from the Los Alamos Sequence Database (http://hiv-web.lanl.gov). Bootstrap values are the result of 1,000 resamplings.

Statistical analysis. Correlation analysis was performed by using Graphpad Prism 4.0 software to perform Spearman's nonparametric rank test. Correlations were considered statistically significant when P values were ≤ 0.05.

Nucleotide sequence accession numbers. The GenBank database accession numbers for the *env* clones described in this study are EF203957 to EF203989.

RESULTS

Development of autologous NAb response against early virus. Fourteen female sex workers with acute HIV-1 infection were identified prospectively as part of the CAPRISA 002 study. These women were estimated to have been infected for a median of 5 weeks (range, 2 to 8 weeks) at the time of enrollment (Table 1). Plasma RNA from the enrollment sample was used to amplify the envelope gene for each participant. The resultant *env* amplicons were cloned into an expression vector and cotransfected with a subtype B backbone to generate Env-pseudotyped viruses. Sequence analysis indicated that these envelope clones grouped together with the population sequence from each individual, and all sequences clustered significantly with HIV-1 subtype C reference sequences (data not shown).

The development of the autologous NAb response was examined, using one to three clones per participant and serum samples collected at roughly bimonthly intervals over 12 months (Fig. 1). The neutralization sensitivities of the multiple clones derived from each sample were very similar, and analysis of the sequences showed that there was minimal genetic diversity at this time point (data not shown). The Env-pseudotyped viruses were examined for coreceptor usage, using GHOST-3 cells expressing CD4 and either CCR5 or CXCR4. All Env-pseudotyped viruses used the CCR5 coreceptor, not the CXCR4 coreceptor. Examination of the V3 sequences using the subtype C position-specific scoring matrix (5) also predicted CCR5 usage.

Analysis of the autologous NAb response against the early envelopes showed a large degree of variation between participants in the kinetics and magnitude of the response (Fig. 1). Most women showed an increase in titer over the first 6 months, with a median time to first detection of 19 weeks, with titers reaching a plateau at between 6 and 12 months. Eight women developed potent NAb responses, with titers of >1:1,000, by 12 months, while two, CAP61 and CAP210, developed weak responses within 12 months of infection. One of these (CAP210) was a rapid progressor, while the other (CAP61) was a controller (Table 1). There was no correlation between the patient's clinical status and the magnitude or kinetics of the autologous NAb response.

TABLE 1. Clinical data for 14 acutely HIV-1 subtype C-infected individuals

Patient no.[a]	Date of blood draw	Time (wk) postinfection	Viral load (RNA copies/ml)	CD4 count (cells/μl)	Clinical status[b]
002-10-0008	17 May 2005	3	373,000	360	
002-10-0045	11 May 2005	5	236,000	974	Controller
002-10-0061*	20 December 2004	8	610	389	Controller
002-10-0063	19 January 2005	4	277,000	414	Rapid progressor
002-10-0084	28 February 2005	3	9,140	636	
002-10-0085	22 June 2005	5	621,000	419	
002-10-0088*	17 February 2005	5	29,400	963	
002-10-0206	12 July 2005	8	368,000	365	Rapid progressor
002-10-0210	25 May 2005	5	127,000	461	Rapid progressor
002-10-0228	11 May 2005	7	3,840	867	Controller
002-10-0239	10 August 2005	5	95,800	845	
002-10-0244*	23 May 2005	8	19,200	557	
002-10-0255*	21 June 2005	8	196,000	693	
002-10-0256	5 September 2005	6	56,500	689	

[a] *, patients from the seroincidence cohort. The rest of the patients were from the sex worker cohort.
[b] Controller, individual with a CD4 count of >350 cells/ml and a viral load of <2,000 copies/ml on at least two consecutive measurements after 6 months of infection; rapid progressor, individual with a CD4 count of <350 cells/ml and a viral load of >100,000 copies/ml on at least two consecutive measurements after 6 months of infection.

Envelope sensitivity to autologous neutralization depends on length and N-linked glycosylation of the variable loops. To assess whether the neutralization titers correlated with the genetic characteristics of the envelope gene, we analyzed the length of variable loops and the number of PNGS. The length of the V1-to-V5 region, as well as the number of PNGS in this region, correlated inversely with autologous NAb titers at 12 months postinfection (Fig. 2a and d). The correlation of titer

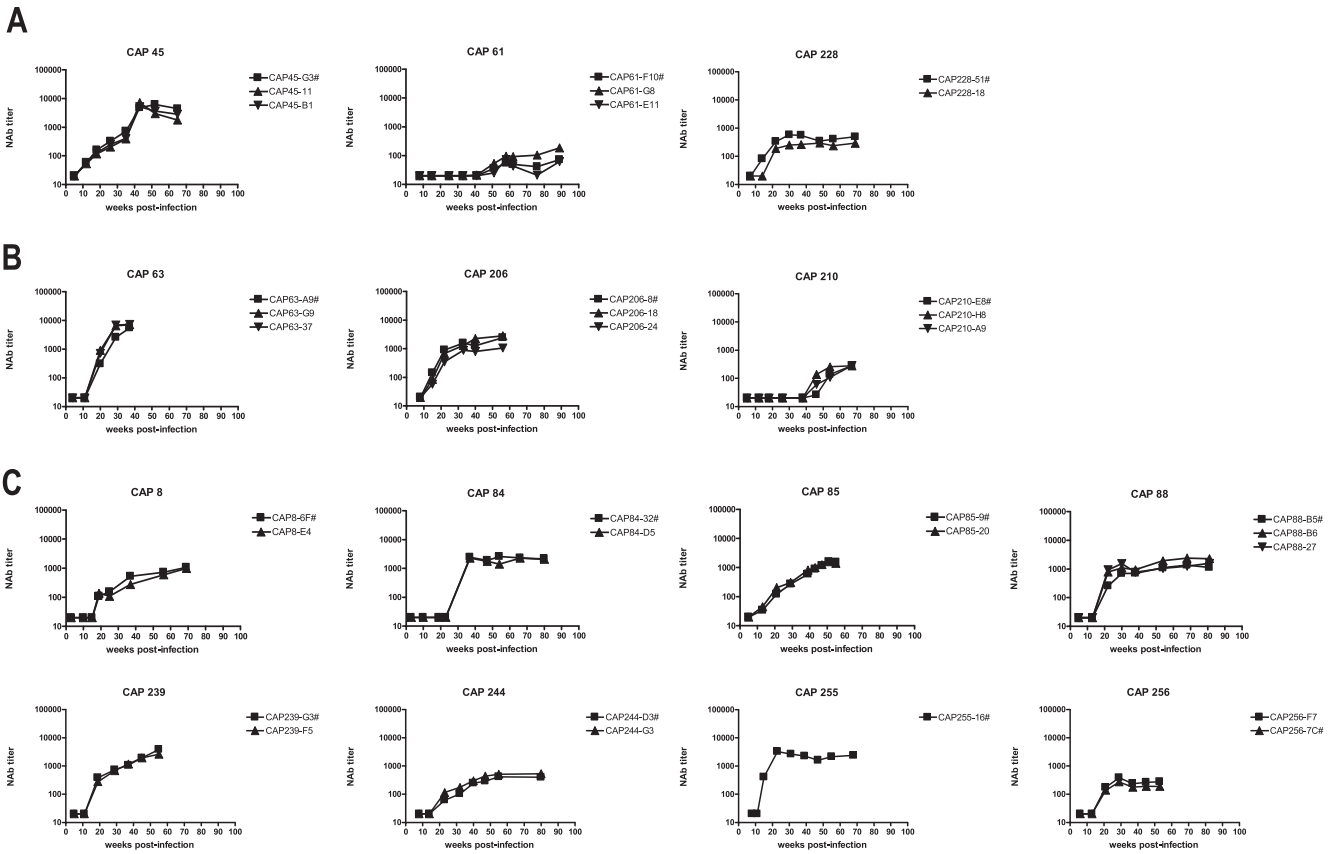

FIG. 1. Comparison of autologous neutralization sensitivities of multiple envelope clones from individual patients. One to three functional envelope clones at a single time point (as indicated in Table 1) for each of 14 acutely infected patients were tested against autologous serum in an Env-pseudotyped virus neutralization assay. Results are shown as IC_{50} values (neutralization titers) over time among participants defined as controllers (A), rapid progressors (B), or normal progressors (C) according to criteria listed in Table 1. The clones marked with a pound sign were used in all subsequent experiments.

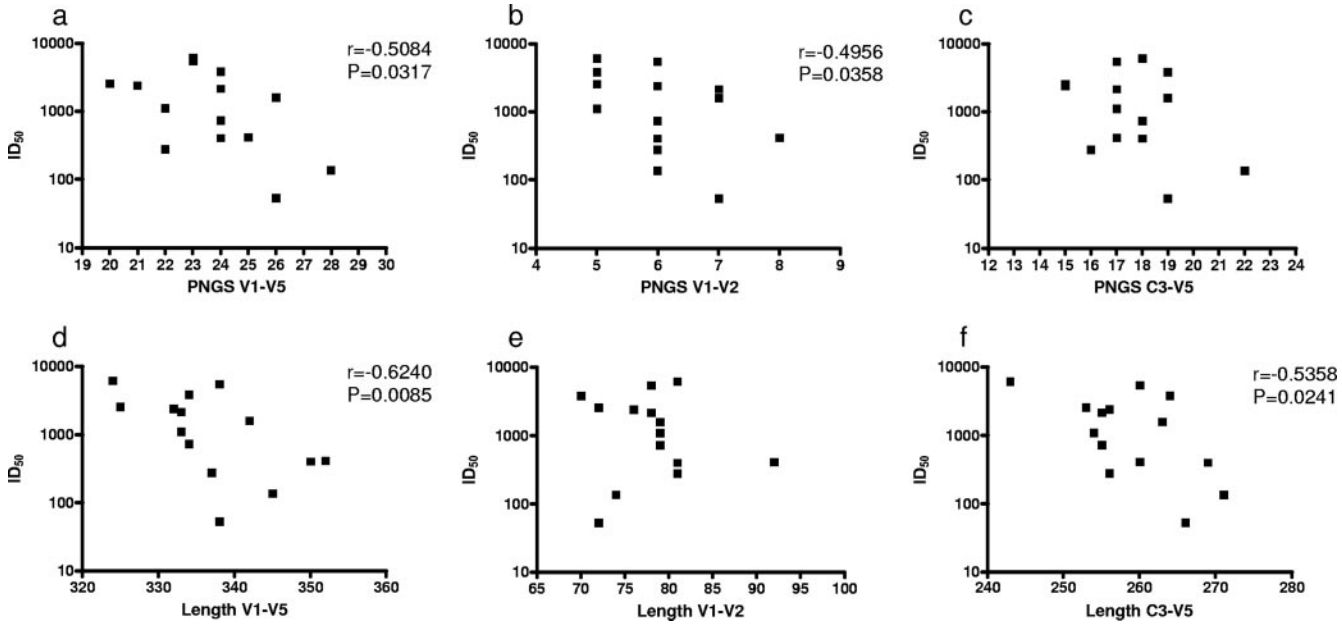

FIG. 2. Correlation between number of N-linked glycosylation sites, variable loop length, and autologous neutralization titer. The autologous neutralization titer at 12 months of infection for each of the 14 study subjects was plotted against the numbers of PNGS in the V1-to-V5 (a), V1-V2 (b), and C3-to-V5 (c) regions or the lengths of the V1-to-V5 (d), V1-V2 (e), and C3-to-V5 (f) regions of the matched viral clone. The Spearman *r* coefficient and the *P* value are shown when the correlation is significant ($P < 0.05$).

with V1-to-V5-region length ($P = 0.0085$) was stronger than that with the number of PNGS ($P = 0.0317$). Analysis of the V1-V2 and C3-to-V5 regions independently demonstrated that the number of PNGS in the V1-V2 loop, but not the length of the V1-V2 loop, was associated with resistance to autologous neutralization (Fig. 2b and e).

Early NAbs are isolate specific. The potency of the early autologous responses prompted us to examine the ability of the

6th months serum	Enrolment virus													
	CAP 8	CAP 45	CAP 61	CAP 63	CAP 84	CAP 85	CAP 88	CAP 206	CAP 210	CAP 228	CAP 239	CAP 244	CAP 255	CAP 256
CAP 8	**155**	<20	**34**	<20	<20	**136**	<20	**37**	<20	<20	<20	**29**	<20	<20
CAP 45	<20	**327**	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
CAP 61	<20	<20	<20	<20	**27**	**66**	<20	<20	<20	<20	<20	<20	<20	<20
CAP 63	<20	<20	<20	**2572**	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
CAP 84	**22**	<20	<20	<20	**2443**	**21**	<20	<20	<20	<20	<20	<20	<20	<20
CAP 85	<20	<20	<20	<20	<20	**273**	<20	<20	<20	<20	<20	<20	<20	<20
CAP 88	**27**	<20	<20	<20	<20	<20	**257**	<20	<20	<20	<20	<20	<20	<20
CAP 206	<20	<20	<20	<20	<20	**82**	<20	**1577**	<20	<20	<20	<20	<20	<20
CAP 210	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
CAP 228	<20	<20	**29**	<20	**27**	**36**	<20	<20	<20	**576**	<20	<20	<20	<20
CAP 239	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	**731**	<20	<20	<20
CAP 244	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	**103**	<20	<20
CAP 255	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	**2668**	<20
CAP 256	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	**272**

12th months serum	Enrolment virus													
	CAP 8	CAP 45	CAP 61	CAP 63	CAP 84	CAP 85	CAP 88	CAP 206	CAP 210	CAP 228	CAP 239	CAP 244	CAP 255	CAP 256
CAP 8	**733**	<20	**40**	<20	**29**	**248**	<20	**37**	<20	<20	<20	**29**	**23**	<20
CAP 45	<20	**6199**	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
CAP 61	<20	<20	**54**	<20	**145**	**84**	<20	<20	<20	<20	**32**	<20	<20	<20
CAP 63	<20	<20	<20	**5490**	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
CAP 84	<20	<20	<20	<20	**2585**	**21**	<20	<20	<20	<20	<20	<20	<20	<20
CAP 85	**29**	<20	<20	**113**	<20	**1697**	<20	<20	<20	<20	<20	<20	<20	<20
CAP 88	<20	<20	<20	<20	<20	**33**	**1097**	<20	<20	<20	<20	<20	<20	<20
CAP 206	<20	<20	<20	<20	<20	**236**	<20	**2425**	<20	<20	<20	<20	<20	<20
CAP 210	<20	<20	<20	<20	<20	<20	<20	<20	**138**	<20	<20	<20	<20	<20
CAP 228	<20	**36**	<20	<20	**27**	**41**	<20	**51**	<20	**405**	<20	<20	<20	<20
CAP 239	<20	<20	**37**	<20	**28**	<20	<20	<20	<20	<20	**3841**	<20	<20	<20
CAP 244	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	**416**	<20	<20
CAP 255	<20	<20	**96**	<20	<20	**78**	<20	<20	<20	<20	**67**	<20	**2163**	<20
CAP 256	<20	**195**	**27**	<20	<20	<20	<20	<20	<20	<20	**85**	<20	<20	**188**

FIG. 3. Heterologous neutralization of enrollment viruses by sera obtained at 6 and 12 months postinfection. Sera obtained at 6 and 12 months postinfection from all 14 patients were tested for neutralization against a representative Env-pseudotyped virus clone from each patient. The reciprocal ID_{50} values are shown, and those in bold show where neutralization was observed. The highlighted (boxed) cells represent autologous neutralization.

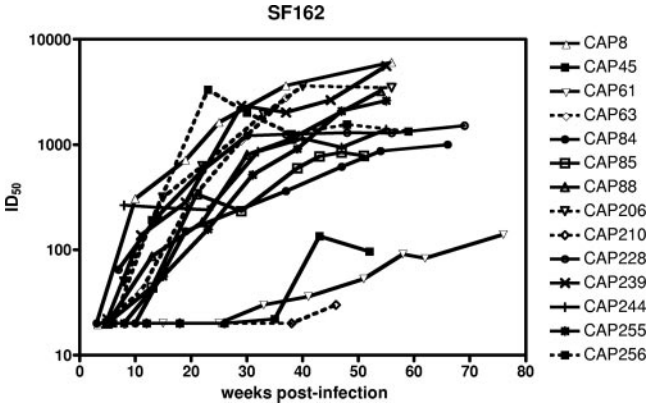

FIG. 4. Neutralization of SF162, a neutralization-sensitive subtype B virus. Sequential serum samples from 14 HIV-1 subtype C-infected patients were tested for neutralizing activity against SF162. Results are shown as the ID_{50} values (neutralization titers) over time.

sera to cross-neutralize other envelopes within the CAPRISA cohort. Sera collected at 6 months and 12 months postinfection showed very little heterologous neutralization (Fig. 3). Thus, while the matched serum-envelope combinations showed high titers, this was not the case for nonmatched serum-envelope combinations. We did observe a slight increase in breadth at 12 months postinfection, which suggests that the capacity for cross-neutralization will likely increase over time. Of particular interest was patient CAP61, whose serum neutralized an unmatched envelope (from CAP84) better than the autologous envelope.

Despite the absence of cross-neutralizing antibodies for primary viruses in these women, most of them were able to neutralize the highly sensitive subtype B virus SF162 (Fig. 4). However, three participants, two of whom also had low autologous NAbs (CAP61 and CAP210), had much lower titers against SF162 than did the rest of the cohort. In contrast, although patient CAP45 developed a strong autologous NAb response (Fig. 1A), this individual failed to neutralize SF162 or any other of the viruses tested, suggesting a highly type-specific neutralization response.

Sensitivity of early HIV-1 subtype C envelopes to antibody neutralization. The neutralization sensitivities of the envelope clones used in this study were tested using six broadly cross-reactive subtype C plasma samples (Fig. 5). Most envelopes were relatively resistant to neutralization, similar to the subtype C reference panel (9) and as expected for primary viruses. There was no correlation between the sensitivity to heterologous neutralization and clinical status. The most sensitive envelopes were those of the CAP84 and CAP85 clones (geometric mean titer [GMT], 489 and 780, respectively). In addition to having an unusually high GMT when tested against subtype C plasma, the CAP85 clone showed the highest sensitivity to neutralization by heterologous CAPRISA sera (Fig. 3). This suggests a neutralization-sensitive phenotype for this envelope clone. However, unlike many neutralization-sensitive viruses, it was not sensitive to sCD4 (IC_{50}, 15.5 μg/ml) (data not shown). The clones most resistant to heterologous neutralization were those from patients CAP8 and CAP256 (GMT, 101 and 82, respectively). The two clones that were relatively resistant to neutralization by the autologous sera (from patients CAP61 and CAP210) displayed a mid-range GMT value. This suggests that the failure of autologous sera to neutralize these viruses is not simply the result of an envelope with a neutralization-resistant phenotype.

We evaluated whether the lengths of the variable regions and the number of PNGS correlated with the sensitivity to heterologous neutralization of each envelope clone, excluding the CAP85 clone. Overall sensitivity, as assessed by the number of CAPRISA sera able to neutralize each clone, was correlated with the length of the V1-V2 region (Fig. 6A). Furthermore, the GMT for a heterologous panel of subtype C-infected plasmas also correlated significantly with V1-V2 length (Fig. 6B). Correlations of heterologous neutralization sensitivity with the length of the C2-to-V5 region and the number of PNGS were not significant (data not shown).

Antibodies to CD4i epitopes. Antibodies to CD4i epitopes were measured using an HIV-2 envelope, as described by Decker et al. (1). We found that 12 of 14 HIV-1 subtype C-infected participants developed CD4i antibodies and that these antibodies were generally characterized by variable kinetics and their early appearance following infection (Fig. 7). We observed a weak correlation between the magnitude of the CD4i antibody response and the number of heterologous viruses neutralized ($P = 0.04$). Interestingly, three women, including two rapid progressors, had high titers of CD4i antibodies at the earliest time tested. Earlier, preinfection samples

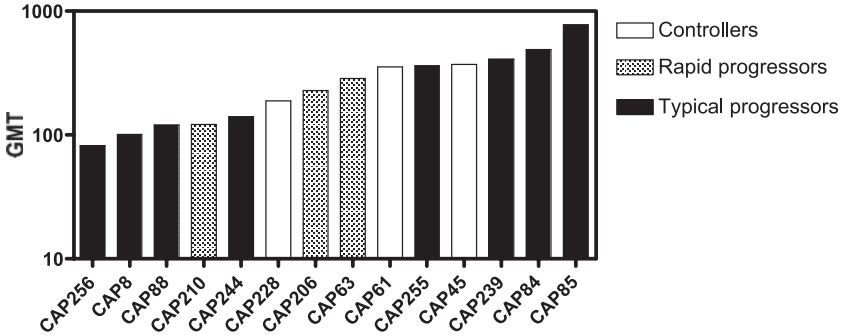

FIG. 5. Neutralization sensitivities of early envelope clones from HIV-1 subtype C-infected patients. The neutralization sensitivities of 14 Env-pseudotyped viruses are represented as the GMTs for neutralization by six HIV-1-positive plasma samples (BB8, BB12, BB28, BB55, BB70, and BB106). The bars indicate the clinical status of the patients from whom the clones were derived.

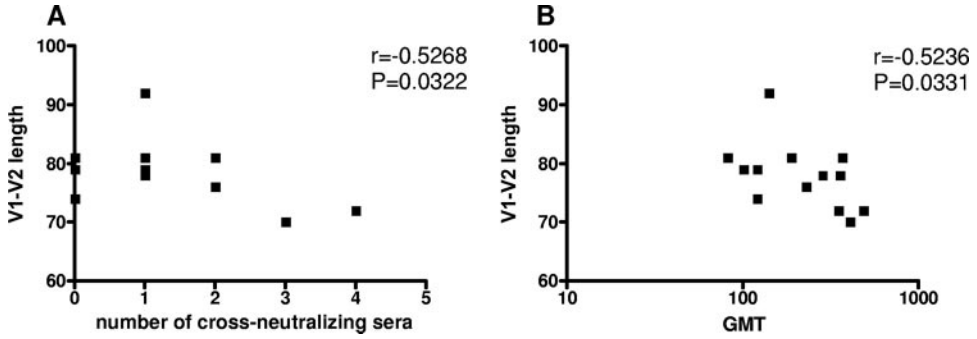

FIG. 6. Correlation between neutralization sensitivity and genetic characteristics of the envelope. The length of the V1-V2 region of each envelope clone tested for heterologous neutralization was plotted against the number of cross-neutralizing sera (derived from Fig. 3) or the GMT of six HIV-1-positive plasma samples (Fig. 5). The Spearman *r* coefficient and the *P* value are depicted for each correlation.

collected from two of these women were negative for CD4i antibodies, confirming that these antibodies were indeed induced by HIV-1 infection (Table 2). The appearance of these CD4i antibodies before the autologous strain-specific NAb response gave us the opportunity to test the capacity of these Abs to neutralize the autologous virus in the presence of sCD4. No neutralization was detected when the autologous virus was preincubated with sCD4 at its IC_{50} (Table 2), which is consistent with earlier work where we found the HIV-1 CD4i coreceptor binding surface to be less accessible than that on HIV-2, even in the presence of sCD4 (1).

Antibodies to gp41 MPER epitopes. Anti-MPER antibodies were measured using the HIV-2 7312A envelope bearing a subtype C MPER, referred to as C1C. Two of the 14 women (CAP85 and CAP206) developed high titers of anti-MPER antibodies within 6 months of infection (peaking at 40 weeks postinfection and reaching titers of approximately 1:1,000), with three additional women developing lower titers later in infection (Fig. 8). In order to map more precisely the region within the MPER targeted by these antibodies, sera from CAP85 and CAP206 were tested against additional chimeric mutants that carry small regions or only point mutations of the MPER, as shown in Fig. 9. Two specific constructs, 7312A-C3 and 7312A-C6, allow the detection of 2F5- and 4E10-like antibodies, respectively (Decker et al., presented at the Keystone Symposium on HIV Vaccines, Keystone Resort, Keystone, CO, 2006). For both CAP85 and CAP206, sera contained antibodies to the 7312A-C1, 7312A-C8, and 7312A-C4GW chimeras, suggesting that these anti-MPER NAbs target the C-terminal region of the MPER. Interestingly, the lack of neutralization of the 7312A-C4 chimera indicated that W670 is important for recognition. While these antibodies recognized a region that overlaps the 4E10 epitope, they were not 4E10-like because they failed to neutralize the 7312A-C6 construct (Bibollet-Ruche et al., presented at the Keystone Symposium on HIV Vaccines, Keystone Resort, Keystone, CO, 2006).

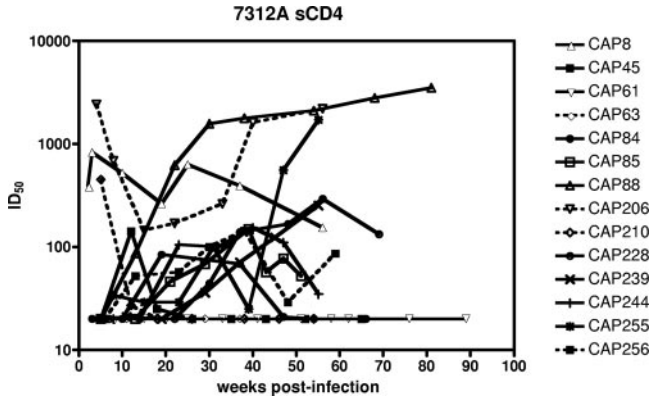

FIG. 7. CD4i NAb responses in acute HIV-1 subtype C infection. Sera were assayed using an HIV-2 (7312A) envelope in the presence of 9 nM sCD4. Titers are shown as ID_{50} values over time. No neutralization was observed in the absence of sCD4 (data not shown).

DISCUSSION

In this study, we report that individuals infected with HIV-1 subtype C developed a potent autologous NAb response between 3 and 12 months after infection, with a median time of 19 weeks. However, these antibodies were highly type specific and rarely neutralized heterologous viruses from the same cohort. Antibodies to CD4i epitopes were present in most participants, often earlier than the autologous antibodies, and in some within weeks of infection. NAbs to the MPER developed much later and in fewer individuals. Collectively, these data provide important insights into the early autologous antibody responses in HIV-1 subtype C infection.

Previous studies of HIV-1 subtype B-infected individuals have shown that autologous NAbs develop within months of infection (13, 19). While the times to peak titer were shown to be similar for a subtype C-infected cohort, with a range of 7 to 24 months, titers were, on average, 3.5-fold higher (8). We also report high titers in this subtype C-infected cohort, with 8 of 14 women reaching titers in excess of 1:1,000 within the first year of infection, though in some cases they had not yet peaked. In a study by Li and coworkers, an analysis of the genetic characteristics of recently transmitted viruses showed that subtype C envelopes were more compact, with shorter V1-to-V4 regions, than their subtype B counterparts and that this inversely correlated with the magnitude of the autologous neutralization response (8). Thus, the higher titers of autologous antibodies in subtype C infection may be more related to a generally neutralization-sensitive phenotype than with higher antibody titers per se (9). In this study, we also found that the length of

TABLE 2. Detection of CD4i antibodies in two patients prior to and after HIV-1 infection[a]

Patient no.	Date of blood draw	HIV-1 enzyme-linked immunosorbent assay result	Pooled RNA PCR result	Time (wk) postinfection	Viral load (copies/ml)	CD4 T-cell count (cells/μl)	Autologous neutralization[b]		Neutralization of 7312A[b]	
							Without sCD4	With sCD4	Without sCD4	With sCD4
002-10-0008	28 September 2004	Negative	Negative	−30	NA	756	ND		20	20
	6 January 2005	Negative	Negative	−16	NA	ND	ND		20	20
	3 March 2005	Negative	Negative	−8	NA	ND	ND		20	20
	11 May 2005	Positive	ND	2	207,000	ND	20	20	20	393
	17 May 2005	Positive		3	373,000	360	20	20	20	823
	6 July 2005			10	368,000	467	20		20	521
	7 September 2005			19	73,100	329	108		20	263
	19 October 2005			25	98,400	343	90		20	631
	11 January 2006			37	27,600	227	176		20	392
	23 May 2006			56	39,300	332	619		20	154
002-10-0206	21 April 2005	Negative	Negative	−4	NA	1,643	ND		20	20
	15 June 2005	Positive	ND	4	196,000	ND	20	20	20	2,429
	12 July 2005			8	368,000	365	20	20	20	687
	30 August 2005			15	113,000	292	145		20	145
	20 October 2005			22	127,000	ND	906		20	170
	3 January 2006			33	138,000	325	1,577		20	263
	21 February 2006			40	252,000	290	1,291		20	1,619
	14 June 2006			56	210,000	297	2,425		20	2,204

[a] Dates shown in bold are the times of enrollment sample collection. NA, not applicable; ND, not done.
[b] Reciprocal serum dilution that gives 50% neutralization.

the V1-to-V5 region inversely correlated with the autologous NAb titer. In addition, we also found an inverse correlation between the number of PNGS and the autologous neutralization titer. These data suggest that increased variable loop lengths and N-linked glycosylation may protect the vulnerable areas of the envelope from antibody recognition.

Despite the potency of the autologous NAb response, these antibodies had limited breadth even after 12 months of infection. This is similar to what was reported recently by Li et al. for subtype C infection, which is in contrast to the higher degree of breadth reported for subtype B-infected individuals (8). The extremely narrow specificity of the early NAb response in subtype C infection suggests that these antibodies may be targeted to variable regions, which would include V1-V2, V4, and V5. Interestingly, we found that the sensitivities of early subtype C envelopes to heterologous neutralization inversely correlated with the length of the V1-V2 region. This inverse correlation suggests that the epitope(s) involved in cross-reactivity may be occluded by this loop, consistent with multiple previous studies (12, 15, 18). This is in contrast to the correlation we observed with autologous responses where increased glycosylation in the V1-V2 region, but not V1-V2 length, was associated with less potent responses. Glycosylation may offer protection of the V1-V2 region from antibodies, and therefore increased numbers of PNGS may result in reduced autologous NAb titers. Taken together, these correlations suggest that the V1-V2 region occludes more-conserved epitopes involved in cross-reactivity, whereas the autologous response may be targeted more to type-specific areas, possibly V1-V2 itself.

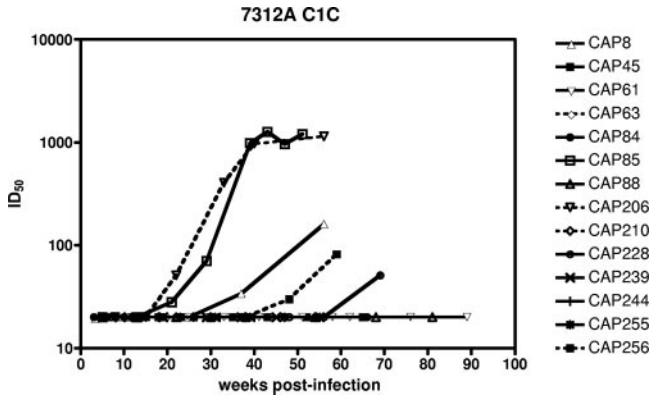

FIG. 8. MPER NAb responses in acute HIV-1 subtype C infection. Serum samples from 14 acutely infected individuals were tested against an HIV-2 envelope containing an HIV-1 MPER optimized for HIV-1 subtype C sequences (7312A CIC). Results are shown as ID_{50} values over time.

The envelope protein from patient CAP85 was the most sensitive to neutralization by both sera from within the CAPRISA cohort and other heterologous plasma samples and was excluded from the analysis shown in Fig. 6. The virus sensitivity profile was more akin to that of the primary viruses in the subtype C panel that had been cultured than to those of viruses cloned directly from plasma (9). Analysis of the gp160 sequence from the virus obtained from CAP85 demonstrated a number of changes at highly conserved positions, including A219T, M434V, and P437N. These mutations were present in all four functional clones obtained from this individual, which were all highly sensitive to neutralization (data not shown). The residue M434 is embedded in the bridging sheet of gp120 (7), and substitutions at this position have major effects on the sensitivity to CD4i antibodies (1, 22). This suggests that the M434V substitution in the CAP85 virus may expose the coreceptor binding site, making it especially sensitive to neutralization. This virus, however, did not have a general neutralization-sensitive phenotype, as it was not particularly sensitive to sCD4.

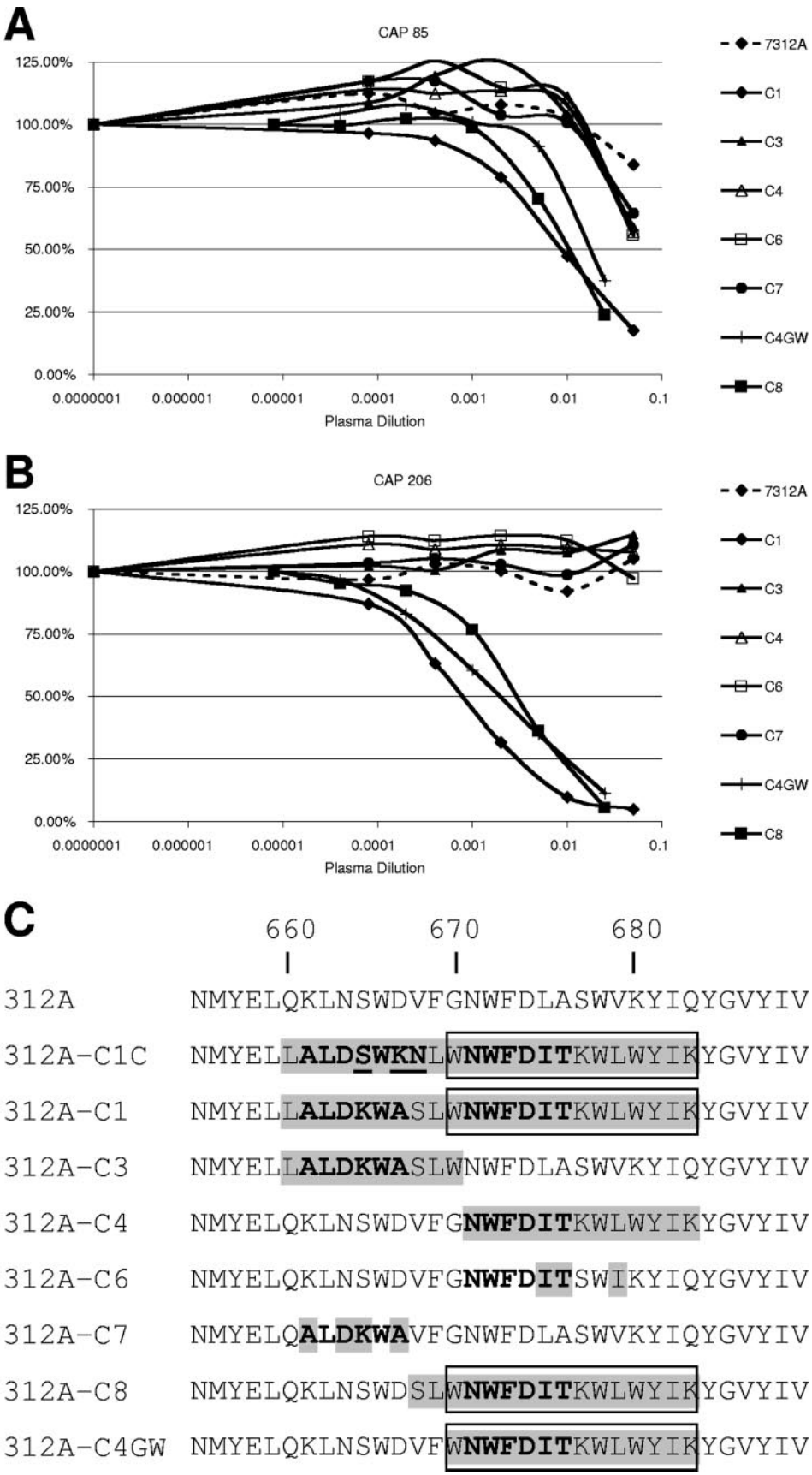

FIG. 9. Mapping of anti-MPER neutralizing activity in two patients. Serum samples from patients CAP85 (A) and CAP206 (B) were tested against eight HIV-2 chimeric viruses containing HIV-1 MPER fragments (C) plus the 7312A control virus. Results are shown as percentages of inhibition. The inserted fragments or mutated residues are shaded for each construct. The amino acids involved in the 2F5 and 4E10 epitopes are represented in bold. The common regions among the constructs where activity was found are boxed.

CD4i antibodies developed within a median of 12 weeks in most participants, often before the appearance of an autologous response. In three women, CD4i antibodies were detected very early, within 2 to 5 weeks of infection. Interestingly, all three women had low CD4 T-cell counts, and two were classified as having rapid disease progression, suggesting that these antibodies were not controlling viral replication. Surprisingly, these antibodies failed to neutralize their autologous envelopes in the presence of sCD4. It would be of interest to examine earlier envelope clones, if possible, to determine if they showed sensitivity to CD4i antibodies. If so, this could suggest that CD4i antibodies function to constrain the virus to remain CD4 dependent, as proposed previously (1). Overall, the contribution of CD4i antibodies to breadth remains unclear, as we found only a weak correlation between the magnitude of this response and the ability to neutralize heterologous viruses.

Antibodies to the MPER developed in a much smaller proportion of women than did CD4i antibodies. Only two women (CAP85 and CAP206) developed high titers, both within 6 months of infection. Epitope mapping demonstrated that these antibodies were not 4E10-like, despite their epitopes overlapping the 4E10 epitope. Both of these sera were unusual in their ability to neutralize the heterologous virus COT6, which has been shown to be highly sensitive to the gp41 MAb 4E10 (E. Gray, unpublished). Since sensitivity to 4E10 may be due to a more-exposed MPER, these data suggest that the neutralization of COT6 by CAP85 and CAP206 sera may be due to anti-MPER antibodies. However, other than COT6, these sera were not especially cross-reactive to heterologous viruses, suggesting that these anti-MPER antibodies do not generally confer breadth of activity, at least during the first year of infection.

In summary, the results reported here confirm previous data indicating that the autologous neutralizing response in HIV-1 subtype C-infected individuals develops to a high titer within months of infection but remains strain specific even after 1 year. The correlation between V1-V2 length and virus sensitivity to heterologous but not autologous neutralization suggests that strain-specific antibodies target areas distinct from those targeted by cross-neutralizing antibodies. The specificities of these early autologous antibody responses remain to be determined.

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