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Epitopes for broad and potent neutralizing antibody responses during chronic infection with human immunodeficiency virus type 1

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ABSTRACT

Neutralizing antibody (nAb) response is sporadic and has limited potency and breadth during infection with human immunodeficiency virus type 1 (HIV-1). In rare cases, broad and potent nAbs are actually induced in vivo. Identifying specific epitopes targeted by such broad and potent nAb response is valuable in guiding the design of a prophylactic vaccine aimed to induce nAb. In this study, we have defined neutralizing epitope usage in 7 out of 17 subjects with broad and potent nAbs by using targeted mutagenesis in known neutralizing epitopes of HIV-1 glycoproteins and by using in vitro depletion of serum neutralizing activity by various recombinant HIV-1 glycoproteins. Consistent with recent reports, the CD4 binding site (CD4BS) is targeted by nAbs in vivo (4 of the 7 subjects with defined neutralizing epitopes). The new finding from this study is that epitopes in the gp120 outer domain are also targeted by nAbs in vivo (5 of the 7 subjects). The outer domain epitopes include glycan-dependent epitopes (2 subjects), conserved nonlinear epitope in the V3 region (2 subjects), and a CD4BS epitope composed mainly of the elements in the outer domain (1 subject). Importantly, we found indication for epitope poly-specificity, a dual usage of the V3 and CD4BS epitopes, in only one subject. This study provides a more complete profile of epitope usage for broad and potent nAb responses during HIV-1 infection.

Introduction

Human immunodeficiency virus type 1 (HIV-1) infection only in rare cases induces potent neutralizing antibody (nAb) responses that can effectively neutralize diverse primary strains of HIV-1 (Deeks et al., 2006; Dhillon et al., 2007; Li et al., 2007, 2009; Sather et al., 2009; Shen et al., 2009). Even in such cases, serum neutralizing titers are often low, with IC_{50} values in the dilution range of 1:10 to 1:100. The nature of such broad and potent nAbs to HIV-1 envelope glycoproteins (Envs) is of interest for vaccine development, as passive immunization with broad and potent nAbs can prevent virus infection in the rhesus macaque model of SHIV infection (Mascola et al., 1999; Parren et al., 2001; Shibata et al., 1999; Veazey et al., 2003). The existence of nAbs with high neutralizing potency and breadth in vivo has been demonstrated by the characterization of monoclonal antibodies (mAbs) 2G12, 2F5, and 4E10, generated by hybridoma formation or EBV transformation of B lymphocytes (Muster et al., 1993; Stiegler et al., 2001; Trkola et al., 1996; Zwick et al., 2001). Of note, the broad and potent neutralizing mAb-b12 was produced using recombinant phage technology, whereas mAbs PG9 and PG16 were produced using recombinant techniques based on near-clonal B-cell cultures (Burton et al., 1994; Walker et al., 2009). Many mAbs either have limited potency but relatively good breadth or have high potency but are strain-specific in neutralization (reviewed in Wyatt and Sodroski, 1998; Zolla-Pazner, 2005). Hypothetically, broad and potent nAb responses in vivo may also be composed of a high concentration of antibodies with limited potency/good breadth or a large number of antibodies with limited breadth/high potency, or some combination of these extremes.

HIV-1 Envs exist on the virion surface as a trimer of gp120/gp41 heterodimers, representing the only viral target for Env-

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specific nAbs. Primary sequences of HIV-1 gp120 have five conserved (C1–C5) and five variable (V1–V5) regions, and the gp41 ectodomain is well-conserved among HIV-1 variants (Gaschen et al., 2001; Kuiken, Korber, and Shafer, 2003; Modrow et al., 1987; Myers et al., 1992; Starcich et al., 1986; Willey et al., 1986). The gp120 variable regions are exposed on the mature Env trimer and are heavily glycosylated, protecting the conserved gp120 structures from nAbs (Modrow et al., 1987; Starcich et al., 1986; Wyatt et al., 1998). Most known broad nAbs to HIV-1 Envs target epitopes in five structural regions. (1) The CD4BS is the binding target for b12, a broad and potent nAb, as well as some broad but less-potent nAbs, such as F105, F91 and 1.5e (Burton et al., 1994; Moore et al., 1994; Posner et al., 1993; Robinson et al., 1990). Typical CD4BS epitopes lie between the inner and outer domains of gp120, thus comprising elements of both domains. Both recombinant gp120 monomer and gp140 trimer contain such epitopes (Yang et al., 2000b, 2002). Interestingly, the b12 epitope is largely made of elements from gp120 outer domain and is capable of binding the recombinant form of gp120 outer domain known as OD1, in addition to gp120 and gp140 (Yang et al., 2004; Zhou et al., 2007). (2) The CD4-induced (CD4i) site is the gp120 core structure binding to the coreceptor, either CCR5 or CXCR4 (Moore and Sodroski, 1996; Thali et al., 1993; Xiang et al., 2003). It is poorly exposed before CD4 engagement and virus attachment, thus is a poor target for nAbs (Decker et al., 2005; Li et al., 2006). The V3 loop is adjacent to this site and contributes significantly to coreceptor binding. V3-specific nAbs are mostly strain-specific, but a few anti-V3 antibodies have also been described to have somewhat greater neutralizing breadth (Gorny et al., 1997, 2002; Stanfield et al., 2004). The V3 region is a part of the gp120 outer domain. The OD1 protein, but not its V3-deleted version (ODΔV3), can bind V3-specific antibodies that are conformation-dependent and neutralizing but not those that target the linear V3 sequences and are nonneutralizing (Yang et al., 2004). (3) 2G12 is the only nAb targeting the glycan mass on the surface of gp120 outer domain, known as the "silent face" (Trkola et al., 1996). 2G12 efficiently binds to recombinant HIV-1 glycoproteins including gp140 trimer, gp120 monomer, and OD1. (4) The V2 loop is recently identified as the target for mAbs PG9 and PG16 which neutralize almost all group M HIV-1 strains with great strength (Walker et al., 2009). The epitope for PG16 also involves the V3 structure. (5) The membrane proximal external region (MPER) of the gp41 ectodomain contains two well-characterized neutralizing epitopes—2F5 and 4E10 (Muster et al., 1993; Zwick et al., 2001). The gp140 trimer contains the sequences covering these epitopes, but not the gp120 monomer (Yang et al., 2002). Interestingly, the gp120 outer domain contributes major binding surfaces for the first 3 groups of nAbs, making it a high-density target for nAb responses (Kwong et al., 1998; Yang et al., 2004; Zhou et al., 2007).

Recent efforts have demonstrated the use of CD4BS in a few individuals in vivo by using recombinant HIV-1 gp120 and its epitope-specific mutant derivatives to bind and deplete nAbs from antisera containing broad and potent nAbs (Binley et al., 2008; Dhillon et al., 2007; Gray et al., 2009b; Li et al., 2007, 2009; Sather et al., 2009). The gp41 MPER epitopes have also been reported as targets of nAbs with various neutralizing breadth and potency (Gray et al., 2009a, 2009b; Shen et al., 2009). In this report, we have characterized nAb responses in chronic HIV-1 infections using a panel of HIV-1 Env mutants to detect the change of sensitivity to neutralization due to alterations in known immunoepitopes. Complemented by the commonly used technique of depleting neutralizing activities from antiserum using recombinant HIV-1 Env proteins, we found that in addition to the CD4BS site, epitopes located in the gp120 outer domain, including glycan-dependent epitopes and conserved elements of the V3 region, are also targeted by broad and potent nAbs developed in chronic HIV-1 infection.

Results

Neutralizing antibody responses in people with chronic HIV-1 infection

We collected serum or plasma samples from 244 subjects with chronic HIV-1 infection in 3 separate cohorts with diverse genetic backgrounds. Neutralizing activity in samples was titrated against a panel of tier-2 HIV-1 strains (6 each from clades B and C) by a neutralization assay based on the single-round entry of pseudotyped viruses into the TZM-bl luciferase reporter cells as described (Li et al., 2006). Samples from 14 (≈6%) of 244 subjects neutralized 10 of the 12 tier-2 HIV-1 strains with an IC_{50} of 1:100 dilution or higher (Table 1). For our study, this level of neutralizing activity was defined as "broad and potent nAb responses in vivo." These 14 samples did not inhibit the entry of SVA-MLV, indicating that the virus-inhibiting

Table 1
Neutralization titers of sera from selected subjects against Tier-2 HIV-1 strains.[a]

Subjects	Clade B, Tier-2[b]						Clade C, Tier 2						SVA-MLV
	B1	B2	B3	B4	B5	B6	C1	C2	C3	C4	C5	C6	
C1-211[c]	251	518	58	679	396	748	1059	288	244	1175	<20	228	<20
C1-210	245	315	157	114	257	34	2802	378	102	1692	971	138	<20
C1-343	1827	245	287	1000	156	593	206	238	362	195	48	54	<20
C1-540	151	58	184	1158	22	514	220	163	178	122	305	1262	<20
C1-581	263	39	284	1690	154	735	349	1349	250	136	704	173	<20
C1-090	235	44	34	120	224	397	192	209	3414	136	150	2522	<20
C1-111	124	109	1776	299	458	64	119	120	150	191	132	676	<20
C1-141	216	188	544	141	77	94	250	167	86	234	152	521	<20
C1-259	718	254	161	315	147	83	882	486	184	314	249	938	<20
C1-440	441	288	759	399	155	230	1480	282	190	312	191	369	<20
C8-117	44	190	508	97	118	103	662	163	209	327	<20	295	<20
C8-258	262	196	371	242	57	180	289	123	356	567	625	640	<20
C1-328[d]	68	42	<20	134	68	72	143	88	<20	371	<20	82	<20
UAB-B	721	157	224	123	315	160	106	208	183	44	116	51	22
UAB-M	525	255	756	1568	93	367	169	68	77	355	248	99	<20
UAB-D[d]	83	105	349	845	69	121	372	35	95	206	362	330	<20
UAB-S[d]	163	195	106	263	30	79	50	64	96	87	56	29	<20

[a] All IC_{50} values were obtained once and reported as it is.
[b] HIV-1 strains: B1-B6 are 6535, QH0692.42, SO422661.8, RHPA4259.7, AC10.0.29, and PVO.4, and C1-C6 are Du156.12, Du172.17, Du422.1, ZM197M.PB7, ZM214M.PL15, and CAP45.2.x.
[c] Subjects' name with "C1-xxx," "C8-XXX," and "UAB-x" were from cohorts CHAVI001, CHAVI008, and UAB, respectively.
[d] Subjects not fitting the selection standards, i.e., with more than two IC_{50} values below 1:100.