Available online at www.sciencedirect.com

SCIENCE DIRECT

Virology 337 (2005) 136 – 148

VIROLOGY

www.elsevier.com/locate/yviro

Neutralization sensitivity of HIV-1 Env-pseudotyped virus clones is determined by co-operativity between mutations which modulate the CD4-binding site and those that affect gp120–gp41 stability

Simon Beddows[a,b,*], Natalie N. Zheng[c], Carolina Herrera[b], Elizabeth Michael[b], Kelly Barnes[b], John P. Moore[b], Rod S. Daniels[c], Jonathan N. Weber[a]

[a]*Jefferiss Research Trust Laboratories, Wright Fleming Institute, Imperial College School of Medicine, London W2 1PG, UK*
[b]*Department of Microbiology and Immunology, Weill Medical College, Cornell University, New York, NY 10021, USA*
[c]*Virology Division, National Institute for Medical Research, The Ridgeway, London NW7 1AA, UK*

Received 4 January 2005; returned to author for revision 22 February 2005; accepted 28 March 2005
Available online 26 April 2005

Abstract

Adaptation of antibody neutralization-resistant human immunodeficiency virus type I (HIV-1) to growth in vitro generally results in the acquisition of a neutralization-sensitive phenotype, an alteration of viral growth kinetics, and an array of amino acid substitutions associated with these changes. Here we examine a panel of Env chimeras and mutants derived from these neutralization-resistant and -sensitive parental Envs. A range of neutralization and infectivity phenotypes was observed. These included a modulation of the CD4 binding site (CD4bs) towards recognition by neutralizing and non-neutralizing CD4bs-directed antibodies, resulting in a globally neutralization-sensitive Env; alterations which affected Env complex stability; and interactions which resulted in differential infectivity and CCR5/CXCR4 usage. This range of properties resulted from the complex interactions of no more than three amino acids found in key Env locations. These data add to a growing body of evidence that dramatic functional alterations of the native oligomeric Env protein complex can result from relatively minor amino acid substitutions.

Keywords: HIV; Envelope; gp120; gp41; Antibody; Neutralization; Mutants; CD4 binding site

Introduction

The generation of a broadly neutralizing antibody response remains a major goal in the development of a human immunodeficiency virus type 1 (HIV-1) vaccine, in addition to other immune effector mechanisms (Garber et al., 2004; Klausner et al., 2003). However, natural envelope polymorphisms, poor presentation, and/or immunogenicity of defined neutralizing antibody epitopes, the potential for 'escape' from neutralizing antibodies, and neutralization resistance in general have all greatly hampered vaccine strategies to date (Burton et al., 2004). Neutralizing monoclonal antibodies (MAbs) have been characterized as interfering with attachment to CD4; disrupting gp120/CD4/co-receptor interactions; or preventing fusion with the target cell membrane (Burton et al., 1994, 2004; Conley et al., 1994; Muster et al., 1993; Thali et al., 1991a, 1993; Trkola et al., 1996; Zwick et al., 2001). While antibodies with these specificities are reactive against a diverse range of isolates (Binley et al., 2004; D'Souza et al., 1997; Trkola et al., 1995), they appear to be rarely generated during natural infection. Broadly cross-neutralizing antibodies can be generated during natural infection, albeit to low titer (Moore et al., 1996; Nyambi et al., 1996; Weber et al., 1996), but their specificity has rarely, if ever, been fully defined. Encouragingly, passive transfer studies using MAbs or polyclonal antisera provide a proof of principle that pre-existing neutralizing antibodies can significantly protect against HIV/SHIV chal-

* Corresponding author. Department of Microbiology and Immunology, Weill Medical College, Cornell University, New York, NY 10021, USA. Fax: +1 212 746 8340.
E-mail address: sib2005@med.cornell.edu (S. Beddows).

0042-6822/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2005.03.033

lenge (Conley et al., 1996; Mascola et al., 1999, 2000; Shibata et al., 1999; Veazey et al., 2003).

In one approach to defining novel neutralizing antibody epitopes for vaccine exploitation, several groups are trying to define viral envelope alterations that confer differential neutralization sensitivity or resistance. Such alterations arise from passage of laboratory-adapted or clinical HIV-1 isolates in vitro or in vivo, in some cases under antibody selection pressure. They have been reported to affect neutralization sensitivity of HIV-1 strains to individual MAbs or more globally to a range of antibodies directed to diverse epitopes (Cayabyab et al., 1999; Cheng-Mayer et al., 1999; Follis et al., 1998; Mo et al., 1997; Park et al., 1998; Pugach et al., 2004; Reitz et al., 1988; Sawyer et al., 1994; Watkins et al., 1996; Wrin et al., 1995). While some of these alterations were found in defined neutralizing antibody epitopes and affect antibody binding directly, many were found at distant residues and are presumed to confer neutralization sensitivity or resistance indirectly, possibly by changing the overall structure of the HIV-1 envelope.

In a previous study, we described the differential neutralization sensitivity of a peripheral blood mononuclear cell (PBMC)-derived R5X4 HIV-1 strain (W61D) and its T-cell line adapted (TCLA) counterpart to HIV-1-specific MAbs, HIV-1 patient sera, and envelope-homologous vaccinee sera, despite the absence of any changes in V1, V2, and V3 (Beddows et al., 1999). We have now generated full-length *env* clones from the PBMC and TCLA virus stocks, in addition to the construction of 5 chimeras and 10 mutants, to elucidate the mechanisms of this differential neutralization sensitivity. We demonstrate that the altered neutralization phenotypes for HIV-1_{W61D} during T-cell line adaptation are due to conformational changes within the native Env oligomer rather than alteration of specific antibody epitopes. Structure–function studies such as these, especially where co-operativity between Env domains are implicated, should help to elucidate how neutralization resistance occurs, and contribute to our understanding of critical targets for vaccine development.

Results

Parental Env variants display a range of neutralization sensitivities to HIVIg

We generated parental *env*-gene clones from acutely infected PBMC (HIV-$1_{W61D/PBMC}$) and SupT1 (HIV-$1_{W61D/SupT1}$) cells using the same virus stocks that have been previously described to display differential neutralization sensitivities to a range of anti-Env antibodies (Beddows et al., 1999). In all, seven full-length functional *env*-genes were rescued; four from the HIV-$1_{W61D/PBMC}$-infected cells (7.1, 7.4, 7.12, and 7.15) and three from HIV-$1_{W61D/SupT1}$-infected cells (6.7, 6.14, and 6.24). Env-pseudotyped virus bearing the encoded Envs were able to infect both CXCR4- and CCR5-expressing U87.CD4 cells, recapitulating the parental R5X4 biological phenotype (data not shown). In order to assess whether the parental Env variants also displayed the differential neutralization sensitivity apparent in the virus stocks from which they were derived, we first tested Env-pseudotyped virus stocks against the Ig-purified polyclonal reagent, HIVIg (Fig. 1). Overall, the SupT1-derived clones were more neutralization sensitive ($P < 0.01$; Mann–Whitney U test) than the PBMC-derived clones, though the distribution of neutralization titers between the two groups did overlap. For example, two of the three SupT1-derived clones (6.7 [mean ± SD IC_{50}, 6 ± 4 μg/ml; $n = 4$] and 6.14 [71 ± 8 μg/ml]) and one PBMC-derived clone (7.15 [137 ± 98 μg/ml]) were relatively neutralization sensitive, while one PBMC clone (7.12 [923 ± 117 μg/ml]) was particularly neutralization resistant. Indeed, Env-pseudotyped virus expressing the envelope from clone 6.7 was 154-fold more sensitive to neutralization than the pseudovirus derived from clone 7.12. The remaining three clones (6.24, 7.1, and 7.4) were of intermediate sensitivity, displaying a 2- to 3-fold greater sensitivity to HIVIg than clone 7.12.

env-gene sequencing analysis

Each parental *env*-gene clone was fully sequenced and Env translation products were aligned with the amino acid sequence of the neutralization resistant clone, 7.12, to allow a better assessment of what polymorphisms may have accounted for the observed differential neutralization sensitivity (Fig. 1). One substitution, E440G (numbered according to the HXB2 Env sequence), was present in 3/3 SupT1 clones but 0/4 PBMC clones, suggesting it had arisen during T cell line adaptation. Three changes, D241N, N636D, and K805Q, were found in all clones except 7.12, suggesting that one or more of them may modulate neutralization sensitivity in the remaining Envs. However, the 1–2 $\log_{10}$ increase in neutralization sensitivity to HIVIg displayed by clones 6.7, 6.14, and 7.15 was unlikely to be due to these polymorphisms alone. Three of the Env substitutions (N188K, D241N, and N616K) would be expected to alter potential N-linked (NXS/T) glycosylation sites. The neutralization-sensitive clone 6.7 had a substitution (D457G) in a position involved in gp120 binding to CD4 (Kwong et al., 1998), a substitution in the V3 domain (A316T), and a substitution in the leucine zipper-(LZ)-like domain in gp41 (H564N) (Douglas et al., 1997). This latter substitution was also found in clone 6.24, which displayed intermediate neutralization sensitivity to HIVIg. Indeed, all three SupT1-derived clones and the neutralization sensitive PBMC-derived clone, 7.15, had one or more substitutions in, or adjacent to, this gp41 domain.

Env glycoprotein determinants of neutralization sensitivity

In an attempt to delineate the impact that one or more of these amino acid substitutions may have on the overall neutralization sensitivity of the W61D Env, we initially