Published in final edited form as:

J Virol Methods. 2011 January ; 171(1): 183–189. doi:10.1016/j.jviromet.2010.10.022.

A Versatile Vector for the Production of Pseudotyped Viruses Expressing gp120 Antigens from Different Clades of Primary HIV-1 Isolates

Zheng Wang^{1,2}, Mingshun Zhang³, Yan Wang¹, Yanmei Jiao⁴, Lu Zhang³, Lin Li², Zuhu Huang³, Hao Wu⁴, Jingyun Li², Shan Lu^{1,3,*}, and Shixia Wang^{1,3}

¹Laboratory of Nucleic Acid Vaccines, Department of Medicine, University of Massachusetts Medical School, Worcester, MA 01655, USA

²Department of HIV/AIDS Research, State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing 100071, China

³China-USA Vaccine Research Center and Jiangsu Province Key Laboratory in Infectious Disease, Department of Infectious Diseases, the First Affiliated Hospital, Nanjing Medical University, Nanjing 210029, China

⁴Department of Infectious Diseases, Youan Hospital, Capital Medical University, Beijing 100069, China

Summary

A novel HIV-1 Env expression vector (SF162-Z) was developed by introducing two new cloning sites on the backbone of an existing vector that produces a full length Env from HIV-1 SF162 isolate. These sites facilitate the swapping of the gp120 portion of the SF162 Env with matching gp120 antigens from HIV-1 isolates of different genetic clades. Final production of functional pseudotyped viruses will express chimeric Env antigens, including gp41 of the parental SF162 and gp120 from other primary isolates. This system is useful for testing the neutralizing sensitivity of partial *env* gene products frequently identified in viral quasi species in patients infected with HIV or when only partial gp120 gene products are available.

Keywords

HIV-1; Envelope protein; gp120; pseudotyped virus; neutralizing antibody

1. Introduction

Understanding protective antibody responses is essential for the development of effective vaccines against Human Immunodeficiency Virus Type I (HIV-1). The introduction of the pseudotyped virus system (Polonis et al., 2008; Richman et al., 2003) was a key technical development that allowed for a more precise measurement of neutralizing activities by a

© 2010 Elsevier B.V. All rights reserved.

*Correspondence to Shan Lu Department of Medicine University of Massachusetts Medical School 364 Plantation Street, LRB 304 Worcester, MA 01605, USA Tel. 1-508-856-6791 Shan.lu@umassmed.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

protective antibody or animal serum against a cloned HIV-1 Env antigen that is expressed on the surface of pseudotyped viruses before infecting a target cell line. In contrast, other neutralizing antibody tests, using uncloned HIV-1 viral stocks, are not able to provide the molecular details of such an antibody-antigen relationship. The pseudotyped virus system also allows for high reproducibility and high through-put processing of a large number of antibodies against different primary HIV-1 Env antigens in a more consistent fashion.

Because the full-length gp160 Env glycoprotein is required for the production of HIV-1 Env-expressing pseudotyped viruses, it is not feasible to use the current pseudotyped virus system as a means to study partially cloned HIV-1 Env antigens. Given the strong replication potential of HIV-1, along with its high mutation rate, there is a high degree of viral diversity in patients infected with HIV, some existing as viral quasi species. Env protein mutations in such quasi species may significantly contribute to their ability to escape neutralizing activities that are often developed during the early phase of HIV-1 infection (Deeks et al., 2006; Frost et al., 2005; Moore et al., 2009; Rong et al., 2009; Wei et al., 2003). However, cloning of full length *env* genes for all viral isolates is not always feasible or successful. Developing a system that can test the neutralizing sensitivity of partial Env sequences is clearly needed.

The full length gp160 HIV-1 Env antigen is cleaved into gp120 and gp41 subunits: gp41 is anchored to the surface of viruses or cells via its transmembrane domain while gp120 is non-covalently linked to gp41 (Earl et al., 1990). In general, the gp120 subunit is prone to more mutations than gp41. Within a subtype, mutations occur in high levels at the gp120 domain but less so for gp41. Therefore, a significant number of studies have focused on the impact of gp120 mutations on antibody neutralizing activity. Furthermore, many partially cloned *env* genes are also in the form of gp120. In the current report, a simple system was developed that can easily subclone isolated gp120 antigens into an existing Env-expressing viral vector that can then be used to produce pseudotyped viruses that express these individual gp120 antigens on a common gp41 backbone. This system should facilitate the study of neutralizing antibody responses against a wide range of primary HIV-1 gp120 antigens.

2. Materials and Methods

2.1 Production of SF162-Z vector

The mammalian expression vector pCAGGS, expressing HIV-1 Env from SF162 isolate (Vaine et al., 2008; Vaine et al., 2010; Wei et al., 2003), was used as the template for further molecular manipulation. Two sets of primers, gp120-NheI-1F/gp120-NheI-1R and gp120-SphI-1F/gp120-SphI-1R (Table 1), were designed to introduce NheI site at the C1 (amino acids 22-23 from the start of C1) and SphI site at the C5 (amino acids 458-459 from the start of C1) regions of SF162 Env insert in pCAGGS/SF162. Two runs of site-directed mutagenesis using the above primers were conducted with the QuikChange Site-Directed Mutagenesis Kit from Invitrogen (Cat #200518; La Jolla, CA 92037). The final mutated SF162 Env clone with these two new cloning sites in pCAGGS vector was designated as SF162-Z.

2.2 Production of CNHN24-Z and C1-Z vectors

Two gp120 gene inserts from HIV-1 clade B isolate CNHN-24 (Wang et al., 2009) and clade C isolate 92BR025 (Gao et al., 1996a) were PCR-amplified from the partial gp140 and gp160 genes, respectively, by using the primers listed in Table 1. These two gp120 inserts were then subcloned separately into the SF162-Z vector at NheI and SphI sites to replace the

equivalent segment in SF162 Env, leading to the two chimeric Env-expressing clones, CNHN24-Z and C1-Z.

2.3 Detection of Env expression by modified and chimeric Env vectors

The expression of Env proteins from the parental clone SF162, modified SF162 Env clone SF162-Z, and two chimeric Env clones CNHN24-Z and C1-Z was verified in transiently transfected 293T cells, as described previously (Wang et al., 2006). Briefly, cells were harvested at 72 hours post-transfection, and the cell lysates and supernatants were subjected to SDS-PAGE and blotted onto PVDF membrane. Blocking was done with 0.1% I-Block (Tropix, Bedford, MA). The membrane was incubated with broadly reactive gp120-specific rabbit serum RC29-1 at 1:1000 dilution for 1h and reacted subsequently with AP-conjugated goat anti-rabbit IgG at 1:5000 dilution for 30 min. RC29-1 serum was produced by a 5-valent HIV-1 Env vaccine (Vaine et al., 2008). Membranes were washed with blocking buffer after each step. Western-light substrate was then applied to the membrane for 5 min. X-ray films were exposed to the membrane and developed by a Kodak processor.

2.4 Production of HIV-1 pseudotyped viruses

The DNA plasmids expressing different HIV-1 *env* genes (SF162, SF162-Z, CNHN24-Z, C1-Z, and other primary isolates JRFL or QH0692) were separately co-transfected with the pSG3ΔEnv backbone vector into 293T cells to produce HIV-1 pseudotyped viruses. The transfection culture media were harvested at 48 hours after the transfection and the TCID₅₀ of the pseudotyped viruses on Tzm-bl cells was determined, as described previously (Li et al., 2005).

2.5 Antibodies and human sera

Neutralizing monoclonal antibodies (mAb), 2G12 and b12, were obtained from the NIH AIDS Research & Reagent Program. Rabbit sera immunized with CNHN24 gp120 DNA prime – protein boost was produced previously (Wang et al., 2009); and serum from a patient infected with HIV was collected at the AIDS Clinic of Youan Hospital, Beijing, China.

2.6 Neutralization assays

A neutralization assay was performed using a single round of infection in Tzm-bl cells in 96-well plates, as previously described (Montefiori, 2004; Vaine et al., 2008). Pseudoviruses were added at 200 TCID₅₀/well and incubated with mAbs, heat-inactivated rabbit or human sera at 37°C for 1 hour. Tzm-bl cells were then seeded at 10,000 cells/well in a final concentration of 20 µg/mL DEAE dextran. Plates were incubated at 37°C for 48 hours and developed with luciferase assay reagent per the manufacturer's instructions (Promega). Neutralization was calculated as a percent reduction in luciferase activity in the presence of rabbit or human sera compared to the luciferase activity induced by the virus in the absence of antibody $\{1 - [\text{luciferase} + \text{immune sera}] / [\text{luciferase} + \text{preimmune sera}]\} \times 100$ (Vaine et al., 2008; Vaine et al., 2010). The reciprocal neutralizing antibody titers of rabbit and human sera or microgram of mAb that achieved 50% reduction in luciferase activity (IC₅₀) were also determined.

3. Results

3.1 Construction of a modified SF162 Env-expressing (SF162-Z) vector

Env antigen from the primary HIV-1 B clade isolate SF162 is well-characterized in the literature. This primary Env is highly sensitive to a wide range of animal sera and monoclonal antibodies and has frequently been used as a positive control in the pseudotyped

virus system to evaluate neutralizing antibody activities for animal and human immune sera and Env-specific monoclonal antibodies. Therefore, in the current report, the SF162 Env antigen was chosen as the backbone to design a novel vector that can express heterologous gp120 genes from various HIV-1 primary isolates for the production of pseudotyped viruses.

Based on the sequence analyses of *env* genes available in the HIV sequence database, two restriction enzyme sites, *Nhe*I and *Sph*I, were selected as these rarely appear in the gp120 region of the primary HIV-1 *env* genes. Two pairs of primers (gp120-*Nhe*I-1F/gp120-*Nhe*I-1R and gp120-*Sph*I-1F/gp120-*Sph*I-1R), as shown in Table 1, were used to introduce *Nhe*I and *Sph*I sites into the C1 (nucleotides 160-165) and C5 (nucleotides 1468-1473) regions of SF162 *env* gene, respectively, in the pCAGGS vector, which contains the original full length SF162 *env* gene (Fig 1). The substitution of the original nucleotide sequences with these two new sites did not affect the overall sequences of SF162 Env. Cleavage at these two sites will remove most of SF162 gp120 coding sequence, leaving only 22 amino acids in C1 region and 11 amino acids upstream of the gp120/gp41 cleavage site. The sequence alignments showed that the sequences in C1 and C5 regions surrounding the new *Nhe*I and *Sph*I sites were relatively conservative across different clades (Fig 1B and 1C). Based on the analysis of 2331 HIV-1/SIVcpz *env* DNA sequences in HIV gene database (Year 2009) from Los Alamos National Laboratory, there were 130 (5.5 %) *env* sequences containing *Nhe*I site and 140 (6.0 %) *env* sequences containing *Sph*I site. Therefore, these 2 sites were rare in HIV *env* genes, and the locations of these two sites at the N-terminal or C-terminal ends would allow a close to complete swap of SF162 gp120 with other primary gp120 antigen genes (see below). After mutagenesis, the sequences in the modified SF162 *env* clone (named as SF162-Z) were confirmed by both restriction enzyme digestion and DNA sequencing (data not shown).

3.2 Env expression by SF162-Z vector

To examine whether the modified SF162 *env* clone SF162-Z could express the Env protein properly, 293T cells were transfected transiently with the SF162-Z clone. For this clone, along with the original SF162 clone as the positive control and a mock DNA vector as the negative control, the expression of Env protein in 293T cells was examined by Western blot using a broadly reactive gp120-specific rabbit serum RC29-1 (Vaine et al., 2008). The results indicated that 293T cells transfected with either SF162-Z or SF162 had very comparable levels of Env production and some of the full length gp160 was further cleaved into gp120 in a similar pattern (Fig 2). The gp41 band did not appear in Fig 2 because the rabbit immune serum used was gp120-specific.

3.3 Production of functional pseudotyped viruses by SF162-Z vector

In order to examine whether the modified SF162-Z Env was functional, pseudotyped viruses were produced by co-transfection of 293T cells using SF162-Z vector and pGS3-dEnv. The cell culture supernatants were harvested at 48 hours after transfection and pseudotyped SF162-Z viral stocks and the TCID₅₀ of these viral stocks were determined in Tzm-bl cells (Montefiori, 2004). Viral stocks were also prepared with the original SF162 Env vector as the control. The results demonstrated that the infection titers of viral stocks produced by using the SF162-Z vector were similar to that of the viral stocks produced by the original SF162 vector: both stocks reached titers of 25,000~30,000 TCID₅₀ (Fig 3-A).

Sensitivities of both viral stocks to neutralization were then evaluated by using broadly reactive neutralizing rabbit serum, R490, which was generated by a HIV-1 SF162 gp120 DNA prime – 5 valent gp120 protein boost as an immunization strategy that has been described previously (Vaine et al., 2008; Wang et al., 2008). The neutralization curves with serially diluted rabbit serum showed similar sensitivities for both SF162 and SF162-Z

pseudotyped viral stocks (Fig 3-B), and the average neutralizing antibody titers were very close at about ~1:1400 (Fig. 3-C). The above results demonstrated that the SF162-Z *env* vector functioned in a similar manner to the original SF162 *env* vector, as demonstrated by their Env antigen expression, production of viral stocks with similar infectivity, and neutralizing antibody sensitivity.

3.4 Generation of functional pseudotyped viruses expressing the chimeric Env incorporating heterologous gp120 antigens

The purpose of SF162-Z is to facilitate the swapping of gene sequences coding for the majority of the SF162 gp120 antigen with similar segments from other primary HIV-1 gp120 antigen genes. In the current report, two primary HIV-1 gp120 genes, CNHN24 from a clade B' isolate (Wang et al., 2009) and 92BR025 (C1) from a clade C isolate (Gao et al., 1996a; Wang et al., 2006), were tested for the utility of SF162-Z vector. The C1-C5 region of these two gp120 genes were PCR-amplified individually and subcloned into the SF162-Z vector to replace the *NheI-SphI* segment at the C1-C5 region in the SF162-Z vector, leading to the production of CNHN24-Z and C1-Z vectors in the same pCAGGS backbone. These two new vectors express the chimeric HIV-1 Env antigens, including the C1-C5 region from either CNHN24 or C1 gp120 plus the common gp41 subunit from SF162. Both new vectors were verified by DNA sequencing.

The expression of chimeric CNHN24-Z and C1-Z Envs were confirmed by transfection of 293T cells and Western blot analyses of transiently-expressed antigens. Both CNHN24-Z and C1-Z gp160 were expressed well and could be further processed into the gp120 subunit (Fig 4-A). Both CNHN24-Z and C1-Z pseudoviruses were generated by co-transfection of 293T cells with one of the new chimeric Env vectors and the pGS3-dEnv vector. The infectivity of pseudoviruses expressing either the chimeric CNHN24-Z or the C1-Z Env antigens was examined in Tzm-bl cells. Both CNHN24-Z and C1-Z pseudoviruses showed high TCID₅₀ at 25,000 - 50,000 range, similar to that produced by the parental SF162 pseudovirus stock (Fig. 4-B). These results showed that the SF162-Z vector can be used to effectively produce functional chimeric Env pseudoviruses that incorporated gp120 antigens from different primary isolates and the same gp41 from SF162.

3.5 Use of chimeric CNHN24-Z pseudoviruses for the study of neutralizing activities in either rabbit immune sera or sera from HIV-1 infected patients

While standard pseudovirus panels are useful to screen antibody responses to determine those with high breadth or potency, these viruses cannot provide specific information on the antigen specificity of neutralizing activities and in particular, the relationship between immune sera and viral isolates circulating in a particular region and the emergence of new viral isolates that may escape existing neutralizing antibodies. On the other hand, quick and easy production of pseudotyped viruses expressing such new emerging Env antigens will provide useful tools to study the relationship between a neutralizing antibody and the target HIV-1 Env antigen. Two studies are included below to demonstrate the utility of chimeric Env pseudoviruses for such analyses.

In the first study, a rabbit immunization study was conducted to test the immunogenicity of Env from a newly isolated HIV-1 clone from a Chinese patient, CNHN24. It is well known in the literature that it is difficult to produce a full length Env as a soluble recombinant protein subunit antigen due to the transmembrane domain of the Env protein. When a DNA vaccination approach was used, the immunogenicity of gp160 was found to be worse than that of the gp120 antigen (Lu et al., 1998). More importantly, it has also been demonstrated recently that gp120 can function as a powerful antigen when used in the form of DNA prime-protein boost (Wang et al., 2006). Therefore, a similar approach was used to study the

immunogenicity of CNHN24 Env in the form of gp120 antigen. A DNA vaccine (HN24) expressing CNHN24 gp120 was constructed and its expression by this DNA vaccine vector was confirmed by Western blot using supernatant that was transiently expressed in 293T cells (Fig 5-A). Rabbits were immunized with either a DNA prime-protein boost approach or protein immunization alone after receiving the mock immunization of empty DNA vector without HIV-1 Env antigen, as reported (Wang et al., 2009). Animal studies were conducted with the review and approval by the University of Massachusetts Medical School (UMMS) Institutional Animal Care and Use Committee (IACUC) according to accepted international animal welfare regulations. Immune rabbit sera from both groups produced similar titers of HN24 gp120-specific IgG responses, based on ELISA (Fig 5-B). However, the neutralizing activities from these two types of rabbit sera were different. Greater neutralizing antibody responses were detected against the sensitive SF162 pseudoviruses with the DNA prime-protein boost approach when compared to protein alone approach (Fig. 5-C). Similarly, when the more resistant CNHN24-Z viruses were tested, only the DNA prime – protein boost rabbit immune sera induced low but positive titers of neutralizing antibody against CNHN24-Z virus (Fig 5-D). These results were consistent to previous reports that DNA prime-protein boost was more effective in eliciting neutralizing antibody responses than protein alone (Vaine et al., 2009; Vaine et al., 2008; Vaine et al., 2010; Wang et al., 2005; Wang et al., 2006). The findings in this study showed that the unique CNHN24-Z chimeric virus was useful to evaluate neutralizing antibody responses induced by a homologous gp120 antigen.

A second study used CNHN24-Z pseudotyped viruses, along with other clade B viruses such as SF162, JRFL, and QH0692 to evaluate the spectrum of neutralizing antibody responses in 20 sera from patients infected with HIV (NJ001 ~ NJ020, Table 2) collected from the AIDS clinic at Youan Hospital, Beijing, China. Human sera at 1:20 dilution were tested against different viruses and the percent of neutralization was determined. Table 2 is a sample result showing positive neutralizing antibody detected in 15 out of 20 patient serum samples examined. A diverse neutralizing antibody profile was identified with only two sera neutralizing all four pseudoviruses tested. Pseudoviruses SF162, JRFL, and QH0692 all express Env antigens collected from other parts of the world. Therefore, the inclusion of pseudovirus CNHN24-Z allowed us to examine antigen specificity against Env antigens circulating in the same region where the sera were collected, a critical step for the development of vaccines capable of preventing HIV-1 circulating in different parts of the world. Using SF162-Z vector as a tool, more pseudoviruses can be produced that express additional gp120 antigens collected from China to provide even more complete picture of the neutralizing antibody profile in patients infected with HIV.

4. Discussion

Measurement of neutralizing antibodies is important for the understanding of the mechanisms used by HIV-1 to escape the host's immune system and provides critical information on the relative effectiveness of candidate vaccines. A number of neutralizing antibody assay systems have been developed, including the more traditional use of uncloned viral stocks to infect human PBMCs and more recent development of pseudotyped viruses to infected standard target cell lines (Mascola, 2004; Mascola et al., 2005; Montefiori, 2004; Montefiori et al., 1998a; Montefiori et al., 1996; Montefiori et al., 1998b; Ochsenbauer and Kappes, 2009; Polonis et al., 2008; Polonis et al., 2009; Schweighardt et al., 2007). The pseudotyped virus system greatly improved the reproducibility of such assays and more importantly, provided better understanding on the antigen-antibody relationship between HIV-1 Env and neutralizing antibodies.

In the current report, a modified HIV-1 Env expression vector was developed for the production of chimeric Env proteins that incorporated various primary gp120 antigens on a common gp41 background from the SF162 isolate. These results demonstrated that this modified vector, SF162-Z, was effective in expressing two heterologous primary gp120 antigens, one from clade B and one from clade C. Pseudotyped viruses incorporating these chimeric Env antigens were able to infect targeted TZM-B1 cells and these viruses are useful in the study of neutralizing antibody activities of animal immunized sera and sera from patients infected with HIV.

Previous studies have also produced chimeric Env expression vectors, such as the chimeric antigens expressing key HIV-1 Env epitopes on the backbone of HIV-2 Env (Wei et al., 2003) and the SF162 Env-based chimeric vector expressing V3 epitopes from Env of different clades (Krachmarov et al., 2006). However, neither of these systems can be used to accommodate the cloning of near full sequence primary gp120 antigens as is possible in the system described in the current report.

The availability of this Env-expression system should be a useful tool to produce more pseudotyped viruses that cover a broader spectrum of primary gp120 antigens. The system reported here was mainly developed for the purpose of studying neutralizing antibodies against gp120 but it does not ignore the importance of Nab targeted at gp41 region. Using a common gp41, such as that from SF162, will still allow for the study of neutralizing activities against gp41, such as those similar to mAb, 4E10, and 2F5; however, naturally occurring neutralizing antibodies against gp41 are very rare (Verkoczy et al., 2009) and more importantly, there are fewer mutations in the gp41 region compared to the gp120 region within each clade. For studies where the goal is to characterize gp41, a gp160 pseudotyped virus can be produced using the full length Env sequences, as described previously (Li et al., 2005; Montefiori, 2004).

The data in the current report also demonstrated that heterologous gp120 and gp41 pairs can be used to produce functional Env as part of a pseudotyped virus. Based on the analysis of HIV-1 gp120 gene sequences available in database, the two restriction sites used to produce SF162-Z were very rare, thus potentially allowing many primary gp120 antigens to take advantage of the system reported here. However, only two examples from clade B and clade C were tested in this pilot study. Further observation is needed once more chimeric Env antigens are produced by using this system to understand if gp120 antigens from additional clades can also be used in this system.

In summary, this new SF162-Z vector should offer a convenient tool for the production of more pseudotyped viruses, particularly for gp120 antigens identified from many field studies. Information obtained from using this technique will provide more information on neutralizing antibody responses during natural infection, which can guide further the development of effective vaccines against HIV-1.

Acknowledgments

This work was supported in part by NIH grants AI065250, AI082274, & AI082676, China Major Infectious Research Projects 2008ZX10001-002, 2008ZX10001-012, 2008ZX10001-006 and 2008ZX1001-1010 as well as the National Natural Science Foundation of China (No. 30700706). Core resources supported by the NIH Diabetes Endocrinology Research Center grant DK032520 were also used. The authors would like to thank Dr. Jill M. Grimes Serrano for her critical reading and editing of this manuscript.

References

Deeks SG, Schweighardt B, Wrin T, Galovich J, Hoh R, Sinclair E, Hunt P, McCune JM, Martin JN, Petropoulos CJ, Hecht FM. Neutralizing antibody responses against autologous and heterologous

viruses in acute versus chronic human immunodeficiency virus (HIV) infection: evidence for a constraint on the ability of HIV to completely evade neutralizing antibody responses. *J Virol* 2006;80:6155–64. [PubMed: 16731954]

- Earl PL, Doms RW, Moss B. Oligomeric structure of the human immunodeficiency virus type 1 envelope glycoprotein. *Proc Natl Acad Sci U S A* 1990;87:648–52. [PubMed: 2300552]
- Frost SD, Wrin T, Smith DM, Kosakovsky Pond SL, Liu Y, Paxinos E, Chappey C, Galovich J, Beauchaine J, Petropoulos CJ, Little SJ, Richman DD. Neutralizing antibody responses drive the evolution of human immunodeficiency virus type 1 envelope during recent HIV infection. *Proc Natl Acad Sci U S A* 2005;102:18514–9. [PubMed: 16339909]
- Gao F, Morrison SG, Robertson DL, Thornton CL, Craig S, Karlsson G, Sodroski J, Morgado M, Galvao-Castro B, von Briesen H, Beddows S, Weber J, Sharp PM, Shaw GM, Hahn BH. Molecular cloning and analysis of functional envelope genes from human immunodeficiency virus type 1 sequence subtypes A through G. The WHO and NIAID Networks for HIV Isolation and Characterization. *J Virol* 1996a;70:1651–67. [PubMed: 8627686]
- Gao F, Morrison SG, Robertson DL, Thornton CL, Craig S, Karlsson G, Sodroski J, Morgado M, Galvao-Castro B, von Briesen H, et al. Molecular cloning and analysis of functional envelope genes from human immunodeficiency virus type 1 sequence subtypes A through G. The WHO and NIAID Networks for HIV Isolation and Characterization. *J Virol* 1996b;70:1651–67. [PubMed: 8627686]
- Li M, Gao F, Mascola JR, Stamatatos L, Polonis VR, Koutsoukos M, Voss G, Goepfert P, Gilbert P, Greene KM, Bilska M, Kothe DL, Salazar-Gonzalez JF, Wei X, Decker JM, Hahn BH, Montefiori DC. Human immunodeficiency virus type 1 env clones from acute and early subtype B infections for standardized assessments of vaccine-elicited neutralizing antibodies. *J Virol* 2005;79:10108–25. [PubMed: 16051804]
- Lu S, Wyatt R, Richmond JF, Mustafa F, Wang S, Weng J, Montefiori DC, Sodroski J, Robinson HL. Immunogenicity of DNA vaccines expressing human immunodeficiency virus type 1 envelope glycoprotein with and without deletions in the V1/2 and V3 regions. *AIDS Res Hum Retroviruses* 1998;14:151–5. [PubMed: 9462925]
- Mascola JR. Neutralizing antibody quantification by flow cytometry. *Methods Cell Biol* 2004;75:709–16. [PubMed: 15603449]
- Mascola JR, D'Souza P, Gilbert P, Hahn BH, Haigwood NL, Morris L, Petropoulos CJ, Polonis VR, Sarzotti M, Montefiori DC. Recommendations for the design and use of standard virus panels to assess neutralizing antibody responses elicited by candidate human immunodeficiency virus type 1 vaccines. *J Virol* 2005;79:10103–7. [PubMed: 16051803]
- Montefiori DC. Evaluating neutralizing antibodies against HIV, SIV and SHIV in luciferase reporter gene assays.. In: Coligan, AMKJE.; Margullies, DH.; Shevach, EM.; Strober, W., editors. *Current Protocols in Immunology*. Vol. 12. John Wiley; Hoboken, NJ: 2004. p. 1-5.
- Montefiori DC, Collman RG, Fouts TR, Zhou JY, Bilska M, Hoxie JA, Moore JP, Bolognesi DP. Evidence that antibody-mediated neutralization of human immunodeficiency virus type 1 by sera from infected individuals is independent of coreceptor usage. *J Virol* 1998a;72:1886–93. [PubMed: 9499040]
- Montefiori DC, Pantaleo G, Fink LM, Zhou JT, Zhou JY, Bilska M, Miralles GD, Fauci AS. Neutralizing and infection-enhancing antibody responses to human immunodeficiency virus type 1 in long-term nonprogressors. *J Infect Dis* 1996;173:60–7. [PubMed: 8537683]
- Montefiori DC, Reimann KA, Wyand MS, Manson K, Lewis MG, Collman RG, Sodroski JG, Bolognesi DP, Letvin NL. Neutralizing antibodies in sera from macaques infected with chimeric simian-human immunodeficiency virus containing the envelope glycoproteins of either a laboratory-adapted variant or a primary isolate of human immunodeficiency virus type 1. *J Virol* 1998b;72:3427–31. [PubMed: 9525675]
- Moore PL, Ranchobe N, Lambson BE, Gray ES, Cave E, Abrahams MR, Bandawe G, Mlisana K, Abdool Karim SS, Williamson C, Morris L. Limited neutralizing antibody specificities drive neutralization escape in early HIV-1 subtype C infection. *PLoS Pathog* 2009;5:e1000598. [PubMed: 19763271]
- Ochsenbauer C, Kappes JC. New virologic reagents for neutralizing antibody assays. *Curr Opin HIV AIDS* 2009;4:418–25. [PubMed: 20048706]

- Polonis VR, Brown BK, Rosa Borges A, Zolla-Pazner S, Dimitrov DS, Zhang MY, Barnett SW, Ruprecht RM, Scarlatti G, Fenyo EM, Montefiori DC, McCutchan FE, Michael NL. Recent advances in the characterization of HIV-1 neutralization assays for standardized evaluation of the antibody response to infection and vaccination. *Virology* 2008;375:315–20. [PubMed: 18367229]
- Polonis VR, Schuitemaker H, Bunnik EM, Brown BK, Scarlatti G. Impact of host cell variation on the neutralization of HIV-1 in vitro. *Curr Opin HIV AIDS* 2009;4:400–7. [PubMed: 20048704]
- Richman DD, Wrin T, Little SJ, Petropoulos CJ. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. *Proc Natl Acad Sci U S A* 2003;100:4144–9. [PubMed: 12644702]
- Rong R, Li B, Lynch RM, Haaland RE, Murphy MK, Mulenga J, Allen SA, Pinter A, Shaw GM, Hunter E, Robinson JE, Gnanakaran S, Derdeyn CA. Escape from autologous neutralizing antibodies in acute/early subtype C HIV-1 infection requires multiple pathways. *PLoS Pathog* 2009;5:e1000594. [PubMed: 19763269]
- Schweighardt B, Liu Y, Huang W, Chappey C, Lie YS, Petropoulos CJ, Wrin T. Development of an HIV-1 reference panel of subtype B envelope clones isolated from the plasma of recently infected individuals. *J Acquir Immune Defic Syndr* 2007;46:1–11. [PubMed: 17514017]
- Vaine M, Lu S, Wang S. Progress on the induction of neutralizing antibodies against HIV type 1 (HIV-1). *BioDrugs* 2009;23:137–53. [PubMed: 19627166]
- Vaine M, Wang S, Crooks ET, Jiang P, Montefiori DC, Binley J, Lu S. Improved induction of antibodies against key neutralizing epitopes by human immunodeficiency virus type 1 gp120 DNA prime-protein boost vaccination compared to gp120 protein-only vaccination. *J Virol* 2008;82:7369–78. [PubMed: 18495775]
- Vaine M, Wang S, Hackett A, Arthos J, Lu S. Antibody responses elicited through homologous or heterologous prime-boost DNA and protein vaccinations differ in functional activity and avidity. *Vaccine* 2010;28:2999–3007. [PubMed: 20170767]
- Verkoczy L, Moody MA, Holl TM, Bouton-Verville H, Searce RM, Hutchinson J, Alam SM, Kelsoe G, Haynes BF. Functional, non-clonal IgM-restricted B cell receptor interactions with the HIV-1 envelope gp41 membrane proximal external region. *PLoS One* 2009;4:e7215. [PubMed: 19806186]
- Wang S, Arthos J, Lawrence JM, Van Ryk D, Mboudjeka I, Shen S, Chou TH, Montefiori DC, Lu S. Enhanced immunogenicity of gp120 protein when combined with recombinant DNA priming to generate antibodies that neutralize the JR-FL primary isolate of human immunodeficiency virus type 1. *J Virol* 2005;79:7933–7. [PubMed: 15919951]
- Wang S, Kennedy JS, West K, Montefiori DC, Coley S, Lawrence J, Shen S, Green S, Rothman AL, Ennis FA, Arthos J, Pal R, Markham P, Lu S. Cross-subtype antibody and cellular immune responses induced by a polyvalent DNA prime-protein boost HIV-1 vaccine in healthy human volunteers. *Vaccine* 2008;26:3947–57. [PubMed: 18724414]
- Wang S, Pal R, Mascola JR, Chou TH, Mboudjeka I, Shen S, Liu Q, Whitney S, Keen T, Nair BC, Kalyanaraman VS, Markham P, Lu S. Polyvalent HIV-1 Env vaccine formulations delivered by the DNA priming plus protein boosting approach are effective in generating neutralizing antibodies against primary human immunodeficiency virus type 1 isolates from subtypes A, B, C, D and E. *Virology* 2006;350:34–47. [PubMed: 16616287]
- Wang Z, Wang SX, Liu SY, Bao ZY, Zhuang DM, Li L, Zhang CH, Zhang L, Li JY, Lu S. Immunogenicities of Env glycoproteins from circulating HIV-1 isolates in China focusing on the strategy of “DNA prime plus protein boost”. *Chin Med J (Engl)* 2009;122:2339–45. [PubMed: 20079137]
- Wei X, Decker JM, Wang S, Hui H, Kappes JC, Wu X, Salazar-Gonzalez JF, Salazar MG, Kilby JM, Saag MS, Komarova NL, Nowak MA, Hahn BH, Kwong PD, Shaw GM. Antibody neutralization and escape by HIV-1. *Nature* 2003;422:307–12. [PubMed: 12646921]

Fig 1.

(A). A diagram of HIV-1 SF162 Env gene inserts. The upper and lower panels respectively show the original SF162 gp160 gene, and the modified SF162 gp160 (SF162-Z) gene following the introduction of NheI and SphI restriction enzyme sites at the 5' and 3' ends of the gp120 coding region. The numbers above the SF162 gene indicate the nucleotide positions. Both SF162 and SF162-Z gp160 proteins being coded by the above genes contain the same natural cleavage site between gp120 and gp41 subunits. (B). Alignment of nucleotide sequence in Env C1 region surrounding SF162-Z-NheI site and covered by primers gp120-NheI-1F and gp120-NheI-1R with parental SF162 and representative *env* sequences of HIV-1 primary isolates from various clades. (C). Alignment of nucleotide sequence in Env C5 region surrounding SF162-Z-SphI site and covered by primers gp120-SphI-1F and gp120-SphI-1R with parental SF162 and representative *env* sequences of HIV-1 primary isolates from various clades: 92UG037.8 (clade A), 92US715.6 (clade B), 92BR025.9 (clade C), 92UG021.16 (clade D), 93TH976.17 (clade E), 93BR020.17 (clade F) and 92UG975.10 (clade G) (Gao et al., 1996b).

Fig 2.

Western blot analysis of the HIV-1 Env proteins expressed by SF162 and SF162-Z in pCAGGS vector, plus empty pCAGGS vector as a negative control. Transfected 293T cell supernatant (S) or lysate (L) samples (10 μ l/sample) were loaded to each lane in SDS-PAGE. HIV-1 gp120 immune rabbit serum (RC29-1) was used as the detection antibody at 1:1000 dilution.

Fig 3.

Viral infectivity and neutralizing sensitivity of pseudotyped viruses produced from co-transfection of pCAGGS vector plasmid and either the original SF162 or modified SF162-Z vectors with pSG3^{ΔEnv} HIV-1 backbone plasmid in Tzm-bl cells. (A). The median tissue culture infection doses (TCID₅₀) titers for both SF162 and SF162-Z viral stocks were determined. Data are shown as the mean TCID₅₀ titers with standard deviations from six preparations of each pseudotyped virus stock. (B). Neutralization of SF162 and SF162-Z pseudotyped viruses by a gp120-specific rabbit immune serum R490. Sample neutralization curves are shown as results collected from triplicate wells using 200 TCID₅₀ as the infection dose. (C). Neutralizing antibody titers (IC₅₀) of rabbit serum R490 against either SF162 or SF162-Z pseudotyped viruses. The results shown are the mean IC₅₀ titers from six independent assays.

Fig 4.

(A). Western blot analysis of HIV-1 Env proteins expressed by either C1-Z Env or CHHN24-Z Env gene inserts in pCAGGS vector, plus empty pCAGGS vector as control. Transfected 293T cell supernatant (S) or lysate (L) samples (10 μ l/sample) were loaded to each lane. Broadly reactive HIV-1 gp120-specific rabbit immune serum (RC29-1) was used as the detection antibody at 1:1000 dilution. (B). Analysis of viral infectivity of pseudotyped viruses produced from co-transfection of empty pCAGGS vector plasmid, original SF162 Env vector, modified C1-Z or CHHN24-Z vectors with pSG3 Δ Env HIV-1 backbone plasmid in Tzm-bl cells. The median tissue culture infection doses (TCID₅₀) titers are shown with standard deviations from six preparations of pseudotyped virus stocks for each virus.

Fig 5.

(A). Western blot analysis of gp120 protein expressed by gp120 DNA vaccine HN24 or empty DNA vector pJW4303, in transfected 293T cell supernatant. HIV-1 gp120-specific rabbit immune serum (RC29-1) was used as the detection antibody at 1:1000 dilution. (B). HN24 gp120-specific IgG titers in rabbit immunized with either HN24 gp120 DNA prime-protein boost or protein alone approaches. (C). and (D). Neutralizing antibody titers (IC_{50}) against HIV-1 pseudotyped virus SF162 and CNHN24-Z (HN24-Z), respectively, in rabbit sera immunized with either HN24 gp120 DNA prime-protein boost or protein alone approaches. The gp120-specific IgG titers in (B) and neutralizing antibody titers in graphs (C) and (D) are the means of triplicate assays with standard deviations.

Table 1

Primers to introduce 2 new cloning sites

Primer	Sequence
gp120-NheI-1F	TTTTGT GCTAGC _{NheI} GATGCTAAAGCCTATG
gp120-NheI-1R	AGCATC GCTAGC _{NheI} ACAAAATAGAGTGGTG
gp120-SphI-1F	ACCAAG GCGATGC _{SphI} AGAAGAGTGGTGCAGAG
gp120-SphI-1R	TCTTCT GCGATGC _{SphI} CTGGTGGGTGCTAC

Table 2

Neutralizing antibody responses in HIV-1 patient sera

Antibodies	Clade B			Clade B'		MuLV
	SF162	JRFL	QH0692	HN24-Z		
<i>Patient sera</i>						
NJ001	65	-	-	-	-	-
NJ002	79	-	-	-	-	-
NJ004	94	60	-	53	-	-
NJ005	95	-	-	-	-	-
NJ006	79	-	-	-	-	-
NJ007	60	-	-	-	-	-
NJ008	97	59	51	78	-	-
NJ009	97	71	63	51	-	-
NJ010	93	71	69	-	-	-
NJ012	92	-	-	-	-	-
NJ013	90	-	-	-	-	-
NJ014	-	-	-	-	-	-
NJ015	-	-	-	-	-	-
NJ016	-	-	-	-	-	-
NJ017	83	-	-	-	-	-
NJ018	-	-	-	-	-	-
NJ019	91	73	-	91	-	-
NJ020	-	-	-	-	-	-
<i>Control mAb</i>						
2G12	89	99	75	87	-	-
IgG1 b12	93	87	92	85	-	-