SHORT COMMUNICATION

Design of possibly useful vaccines against human immunodeficiency virus 1

Eduardo A. Padlan

The Marine Science Institute, College of Science
University of the Philippines Diliman
Quezon City 1101, Philippines

Possibly useful vaccines against human immunodeficiency virus 1 are designed based on the interaction of the A/E 93TH057 gp120 core, variant V65C/S115C, with antibody NIH45-46, a broadly neutralizing antibody. The antigenicity of this gp120 core is modified by replacing all the solvent-accessible residues with amino acids that are less reactive, except those residues which constitute the epitope of NIH45-46. The antibodies elicited by these vaccines are expected to be focused on this epitope and should also be broadly neutralizing.

INTRODUCTION

To aid in the development of a vaccine against human immunodeficiency virus 1 (HIV-1), the complexes of gp120, the envelope glycoprotein of the virus, with antibodies that can neutralize a broad class of the virus are being studied (see, e.g., Diskin et al. 2011, Wu et al. 2011, Zhou et al. 2010). One antibody, NIH45-46, has been shown to be particularly broadly neutralizing and its binding to gp120 has been analyzed (Diskin et al. 2011). That structural information is used here to design possibly useful vaccines against the virus. The strategy used in the design is one that reduces the antigenicity of the surface of an antigen except for a chosen site on the molecule, thereby accentuating the antigenicity of that site; this is proposed to cause the focusing of the antibody response to the chosen site (Padlan 2010). The vaccines that are proposed are based on the A/E 93TH057 gp120 core, variant V65C/S115C, which is the antigen in the NIH45-46 study (Diskin et al. 2011), and the site, whose antigenicity is emphasized, is the epitope of NIH45-46. Briefly, antigenicity reduction is achieved by replacing surface residues with amino acids whose physicochemical properties, e.g., hydrophilicity, size and polarizability, and electronic properties (Sandberg et al. 1998, De Genst et al. 2002), make them less reactive and thereby contribute less to antigenicity. In addition, in order to preserve the tertiary structure of the antigen for proper presentation of the NIH45-46 epitope to the immune system, the replacement amino acids are those which have the same propensity for secondary structure as the original residues. The replacement rules, reproduced from Padlan (2010), are presented in Table 1. Preservation of tertiary structure is ensured by maintaining the interior of the molecule, i.e., by replacing only the residues that are exposed to solvent, and by keeping residues that may be crucial to the three-dimensional structure of the molecule, e.g., prolines and cysteines.

* Corresponding author
Email Address: eduardo.padlan@gmail.com
Submitted: February 6, 2012
Revised: June 1, 2012
Accepted:June 3, 2012
Published: July 20, 2012
Editor-in-charge:Leodevico L. Ilag
Reviewers:
Leodevico L. Ilag
Vijay S. Reddy

MATERIALS AND METHODS

Sequence and structural data

The amino-acid sequence of the A/E 93TH057 gp120 core, variant V65C/S115C, and the crystal structure of its complex with antibody NIH45-46 (Diskin et al. 2011) were obtained from the Protein Data Bank (PDB) entry 3U7Y. The amino-acid sequence of this gp120 core is presented in Table 2. Several residues were not observed in the crystal structure and the missing residues were obtained by homology modeling using the online facility, SWISS-MODEL (Arnold et al. 2006, Kiefer et al. 2009, Peitsch 1995) (implemented at http://swissmodel.expasy.org/), and using the crystal structure of the gp120 core in PDB entry 3NGB (Zhou et al. 2010) as template. The gp120 residues, which have at least one atom within 4.0 Angstroms of an atom in the NIH45-46 antibody, were designated as comprising the NIH45-46 epitope. The residues in the NIH45-46 epitope are indicated in Table 2.

Design of the vaccines

The solvent accessibilities of the amino-acid side chains of the modeled gp120 core were computed by the method of Connolly (1983), using programs developed by Sheriff et al. (1985) and adapted by the author. The secondary structure of the modeled gp120 core was predicted using the program DSSP (Kabsch and Sander 1983). Amino-acid replacements designed to reduce the antigenicity of the parts outside the NIH45-46 epitope were then made on the basis of solvent exposure and secondary structure using the rules presented in Table 1.

Table 1. Replacement rules for reducing reactivity while preserving secondary structure.

Amino acid	Helix	If in Sheet	Coil	Turn
		Change to:		
Arg	Ala	Thr	Ala	Ala
Asn	Ala	Thr	Ser	Gly
Asp	Ala	Thr	Ser	Gly
Gln	Ala	Thr	Ala	Thr
Glu	Ala	Thr	Ala	Thr
His	Ala	Thr	Thr	Thr
Lys	Ala	Thr	Thr	Thr
Phe	Ala	Thr	Ala	Ala
Trp	Ala	Thr	Ala	Val
Tyr	Ala	Thr	Ala	Thr

Ala, Cys, Gly, Ile, Leu, Met, Pro, Ser, Thr, and Val are not replaced.

Reproduced from Padlan (2010)

Two exposure levels were used in deciding which gp120 residues to replace. In the first case, residues whose side chains are at least 40% accessible to solvent were replaced; in the second case, those with at least 25% exposure were replaced. The 40% and 25% exposure levels are reasonable estimates of the solvent accessibility of amino-acid side chains.

Antigenicity comparisons

Relative antigenicities were calculated as described elsewhere (Padlan 2010) for the A/E 93TH057 gp120 core, variant V65C/S115C, and the two mutated molecules. Briefly, a value for the antigenicity is assigned to each residue (alpha-carbon) position, obtained by combining the physicochemical variables (Sandberg et al. 1998, De Genst et al. 2002) of all the residues within 22 Angstroms of the position, weighted by the fractional solvent accessibilities of the residues.

RESULTS

Shown in Table 2 are the A/E 93TH057 gp120 core, variant V65C/S115C, and the two modified gp120 sequences. The mutated sequence, in which the residues with at least 40% exposure had been replaced, is labeled "mut". The one, in which the residues with at least 25% exposure had been replaced, is labeled "mut2". In both the "mut" and "mut2" sequences, the NIH45-46 epitope is preserved. The "mut" sequence is 78.2% identical to the unmodified sequence, while "mut2" is 72.2% identical. Both of these levels of sequence homology are generally accepted as indicative of close similarity in tertiary structure. The additional disulfide bond formed between the engineered cysteines at positions 65 and 115 (Diskin et al. 2011) should contribute to the stability of the tertiary structure. The "mut" and "mut2" sequences are proposed to represent possibly useful vaccines against HIV-1.

Antigenicity plots for the A/E 93TH057 gp120 core, variant V65C/S115C, and the two molecules resulting from the proposed mutations are presented in Figure 1.

DISCUSSION

The idea of focusing the antibody response to a chosen region of a protein antigen was first suggested by Hopp and Woods (1981) using peptide fragments as immunogens.
However, since antibody epitopes are most probably conformational (Benjamin et al. 1984, Davies et al. 1988), it is unlikely that a peptide would elicit a strong antibody response to that part of the antigen from which the peptide immunogen was derived, especially since peptides can assume many different structures in solution. A procedure that focuses the antibody response to a chosen part of an intact antigen is more likely to succeed.

The idea of shifting the antibody response from one epitope to other epitopes by the judicious replacement of amino acids

was first suggested by Fazekas de St. Groth (1977). Attempts to shift the antibody response to others parts of a protein antigen have been made (see, for example, Scheerlinck et al. 1993, Temoltzin-Palacios et al. 1994, Garrity et al. 1997, Cleveland et al. 2000, émy Sadeyen et al. 2003), but to this author's knowledge no attempt has been made to focus the response to a specific part.

The method proposed earlier by the author (Padlan 2010) attempts to focus the antibody response to a particular part of a protein antigen while maintaining the three-dimensional structure of the molecule. This is accomplished by reducing the antigenicity of all the exposed regions of the antigen, except that of the chosen part. The region to which the antibody response is focused could be the part that is used for binding to a receptor, the active site of the molecule, the epitope of a neutralizing antibody, or a part that is critical to the function of the molecule. The method is used here to design an immunogen that could elicit an antibody response to the epitope of an antibody that is known to be neutralizing. The antibodies that are elicited will likely be neutralizing also.

The antigen is the core of gp120, the molecule on the surface of HIV-1 which binds to the CD4 of the target lymphocyte to gain entry into the cell. HIV-1 is highly variable, but antibodies elicited by the gp120 core have been shown to neutralize a wide spectrum of HIV-1 variants. One such antibody is NIH45-46; its epitope is known (Diskin et al. 2011)

Table 2. Sequence of the A/E 93TH057 gp120 core, variant V65C/S115C, and those of the proposed possibly useful vaccines against HIV-1

```
                10        20        30        40        50        60
                 |         |         |         |         |         |
3U7Y  VWKDADTTLFCASDAKAHETECHNVWATHACVPTDPNPQEIHLENVTENFNMWKNNMVEQ
mut   VTTTASTTLTCASSATATETECHNVWATAACVPTSPGPAAITLTGVTETFTMWKNNMVEQ
mut2  VTTTASTTLTCASSATATATECHNVWATAACVPTSPGPAAITLTGVTTTFTMWKNNMVEA

                70        80        90        100       110
                 |         |         |         |         |
3U7Y  MQEDVISLWDQCLQPCVKLTG...GSVIKQACPKISFDPIPIHYCTPAGYVILKCNDKNF
mut   MQAAVISLWATCLAPCVTLTG...GSVITAACPTISFSPIPIHYCTPAGYVILKCNSTGF
mut2  MQAAVISLWATCLAPCVTLTG...GSVITAACPTISFSPIPITYCTPAGTVILTCSSTGF

       120       130       140       150       160       170
        |         |         |         |         |         |
3U7Y  NGTGPCKNVSSVQCTHGIKPVVSTQLLLNGSLAEEEIIIRSENLTNNAKTIIVHLNKSVE
mut   SGTGPCTGVSSVTCTHGIKPVVSTQLLLGGSLATTTIIIRSENLTNNAKTIIVHLTTSVT
mut2  SGTGPCTGVSSVTCITGITPVVSTQLLLGGSLATTTIIIRSTNLTNNAKTIIVHLTTSVT

       180       190       200       210       220       230
        |         |         |         |         |         |
3U7Y  INCTRPSN...ggsgsggDIRKAYCEINGTKWNKVLKQVTEKLKEHFNN...KTIIFQPP
mut   ITCTRPSS...GGSGSGGSIATATCTITGTAWNAVLAAVTAKLTTTFGG...TTIIFAPP
mut2  ITCTRPSS...GGSGSGGSIATATCTITGTAWAAVLAAVTAALTTTFGG...TTIIFAPP

               240       250       260       270       280
                |         |         |         |         |
3U7Y  SGGDLEITMHHFNCRGEFFYCNTTQLFNNTCIg...netmkGCNGTITLPCKIKQIINMW
mut   SGGDLEITMHHFNCAGEFFYCSTTALFSGTCIG...SETMTGCSGTITLPCTIKQIITMW
mut2  SGGDLAITMHTFNCAGEFFYCSTTALFSGTCIG...SETMTGCSGTITLPCTITTIITMW

       290       300       310       320       330       340
        |         |         |         |         |         |
3U7Y  QGTGQAMYAPPIDGKINCVSNITGILLTRDGGANNTSNETFRPGGGNIKDNWRSELYKYK
mut   QGTGQAMYAPPIGGTITCVSTITGILLTRDGGANGTSNETFRPGGGNIKDNWRSELYKYK
mut2  QGTGQAMAAPPIGGTITCVSTITGILLTRDGGANGTSNETFRPGGGNIKDNWRSALYTYT

       350
        |
3U7Y  VVQIE
mut   VVQIA
mut2  VVTIA
```

The amino acid sequence of the A/E 93TH057 gp120 core, variant V65C/S115C, is from PDB entry 3U7Y. The sequence mutated to replace residues whose side chains are at least 40% exposed to solvent is labeled "mut" and that in which the residues replaced are at least 25% exposed is labeled "mut2". The numbering scheme is sequential. The gp120 core in PDB entry 3U7Y starts at residue position 44 and ends at position 492. The segments which had been excised to produce the core (residues 125-197, 302-317, 356, and 398-403 in the 3U7Y numbering scheme) are indicated by the dots. The residues which could not be located in the crystal structure are indicated by italicized, lower-case letters. The gp120 residues in contact with the NIH45-46 antibody are shown bold and underlined. The amino acid replacements are shown bold and in red.

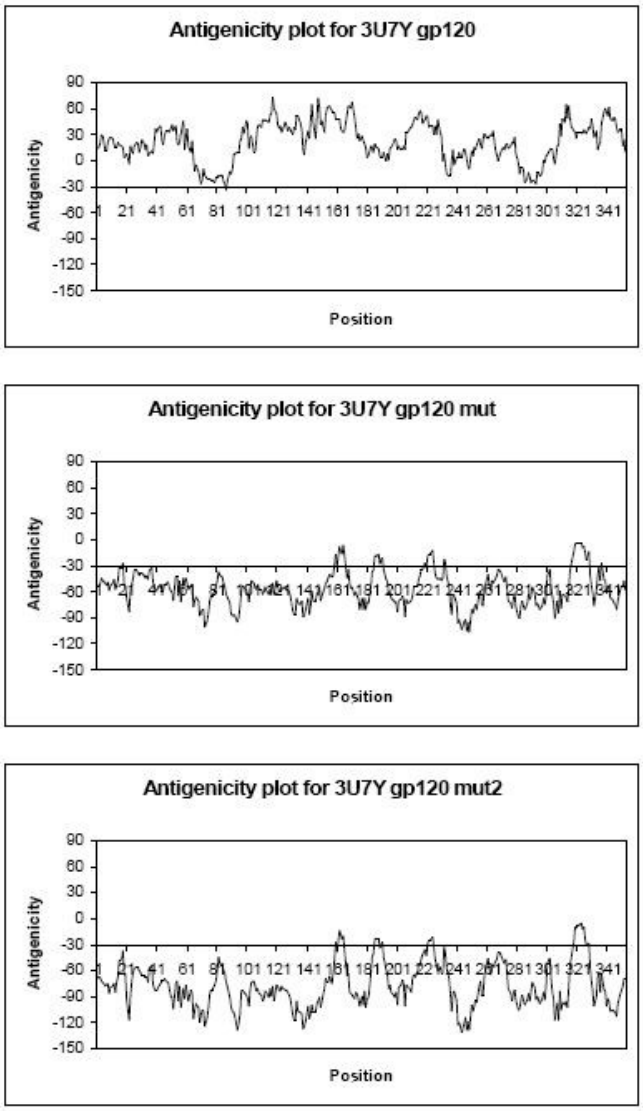

Figure 1. Plots depicting the relative antigenicity of the A/E 93TH057 gp120 core, variant V65C/S115C, and the mutated sequences plotted against residue position. Numbering follows that in Table 2.

and is indicated in Table 2. Note that the NIH45-46 epitope is composed of amino acids from several segments of the amino-acid sequence. It is this epitope that is the target of the procedure used here. Two possible vaccines were designed and their sequences are presented in Table 2.

An attempt to illustrate the accentuation of the antigenicity of the NIH45-46 epitope as a result of the procedure is presented in Figure 1, where a measure of antigenicity is plotted against residue position. In the absence of a structure for the antibody-antigen complex, it is not possible to accurately delineate an antibody epitope since antigens are usually not regular in shape and antibody combining sites show great variation. The NIH45-46 epitope is elongated and a radius of 22 Angstroms is needed to include all the side-chain atoms in the epitope. That radius was used in the computations of the relative antigenicities. The potential contribution of each residue to the relative antigenicity of an epitope is simply correlated with its reactivity, as expressed by the physicochemical descriptors derived by Sandberg et al. (1998) and used by De Genst et al. (2002).

In Figure 1, a general reduction in antigenicity is seen except at certain positions as shown by peaks. Notably, the two most prominent peaks correspond to the longest stretches, around residues 163 and 322, in the NIH45-46 epitope (Table 2). The peaks around positions 186 and 225 are probably artifacts in view of the deletions at those parts of the molecule.

CONCLUSION

It is proposed that the "mut" and "mut2" sequences in Table 2, which are the result of a purposeful focusing of the antibody response to the epitope of the broadly neutralizing NIH45-46 antibody, represent possibly useful vaccines against HIV-1. A more faithful preservation of the gp120 core structure would be expected with the "mut" sequence. On the other hand, a greater accentuation of the NIH45-46 epitope would be expected with the "mut2" sequence. Which of the two would make a better vaccine can only be known by actual testing.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

REFERENCES

Arnold K, Bordoli L, Kopp J, Schwede T. The SWISS-MODEL Workspace: A web-based environment for protein structure homology modelling. Bioinformatics 2006; 22:195-201.

Benjamin DC, Berzofsky JA, East IJ, Gurd FRN, Hannum C, Leach SJ, Margoliash E, Michael JG, Miller A, Prager EM, Reichlin M, Sercarz EE, Smith-Gill SJ, Todd PE, Wilson AC. The antigenic structure of proteins: a reappraisal. Annu Rev Immunol 1984; 2:67-101.

Cleveland SM, Buratti E, Jones TD, North P, Baralle F, McLain L, McInerney T, Durrani Z, Dimmock NJ. Immunogenic and antigenic dominance of a nonneutralizing epitope over a highly conserved neutralizing epitope in the gp41 envelope glycoprotein of human immunodeficiency virus type 1: its deletion leads to a strong neutralizing response. Virology 2000; 266:66-78.

Connolly ML. Solvent-accessible surfaces of proteins and nucleic acids. Science 1983; 221:709-713.

Davies DR, Sheriff S, Padlan EA. Antibody-antigen complexes. J Biol Chem 1988; 263:10541-10544.

De Genst E, Areskoug D, Decanniere K, Muyldermans S, Andersson K. Kinetic and affinity predictions of a protein-protein interaction using multivariate experimental design. J Biol Chem 2002; 277:29897-29907.

Diskin R, Scheid JF, Marcovecchio PM, West AP Jr, Klein F, Gao H, Gnanapragasam PN, Abadir A, Seaman MS, Nussenzweig MC, Bjorkman PJ. Increasing the potency and breadth of an HIV antibody by using structure-based rational design. Science 2011; 334:1289-1293.

émy Sadeyen J-R, Tourne S, Shkreli M, Sizaret P-Y, Coursaget P. Insertion of a foreign sequence on capsid surface loops of human papillomavirus type 16 virus-like particles reduces their capacity to induce neutralizing antibodies and delineates a conformational neutralizing epitope. Virology 2003; 309(1):32-40.

Fazekas de St. Groth S. Antigenic, adaptive and adsorptive variants of the influenza haemagglutinin. In Topics in Infectious Diseases. Vol. 3 (R.G. Laver, H. Bachmayer, and R. Weil, eds) (Springer-Verlag, Vienna) 1977; pp 25-48.

Garrity RR, Rimmelzwaan G, Minassian A, Tsai WP, Lin G, de Jong JJ, Goudsmit J, Nara PL. Refocusing neutralizing antibody response by targeted dampening of an immunodominant epitope. J Immunol 1997; 159: 279-289.

Hopp TP, Woods KR. Prediction of protein antigenic determinants from amino acid sequences. Proc Natl Acad Sci USA 1981; 78:3824-3828.

Kabsch W, Sander C. Dictionary of protein secondary structure: pattern recognition of hydrogen-bonded and geometrical features. Biopolymers 1983; 22(12):2577-2637.

Kiefer F, Arnold K, Künzli M, Bordoli L, Schwede T. The SWISS-MODEL Repository and associated resources. Nucl Acids Res 2009; 37:D387-D392.

Padlan EA. A method for designing molecules for use in directing the antibody response to a chosen region of a protein antigen. Phil Sci Letts 2010; 3(2):36-47.

Peitsch MC. Protein modeling by E-mail Bio/Technology 1995; 13:658-660.

Sandberg M, Eriksson L, Jonsson J, Sjostrom M, Wold S. New chemical descriptors relevant for the design of biologically active peptides. A multivariate characterization of 87 amino acids. J Med Chem 1998; 41:2481-2491.

Scheerlinck JP, DeLeys R, Saman E, Brys L, Geldhof A, De Baetselier P. Redistribution of a murine humoral immune response following removal of an immunodominant B cell epitope from a recombinant fusion protein. Mol Immunol 1993; 30: 733-739.

Sheriff S, Hendrickson WA, Stenkamp RE, Sieker LC, Jensen LH. Influence of solvent accessibility and intermolecular contacts on atomic mobilities in hemerythrins. Proc Natl Acad Sci USA 1985; 82(4):1104-1107.

Temoltzin-Palacios F, Thomas DB. Modulation of immunodominant sites in influenza hemagglutinin compromise antigenic variation and select receptor-binding variant viruses. J Exp Med 1994; 179: 1719-1724.

Wu X, Zhou T, Zhu J, Zhang B, Georgiev I, Wang C, Chen X, Longo NS, Louder M, McKee K, O'Dell S, Perfetto S, Schmidt SD, Shi W, Wu L, Yang Y, Yang ZY, Yang Z, Zhang Z, Bonsignori M, Crump JA, Kapiga SH, Sam NE, Haynes BF, Simek M, Burton DR, Koff WC, Doria-Rose NA, Connors M; NISC Comparative Sequencing Program, Mullikin JC, Nabel GJ, Roederer M, Shapiro L, Kwong PD, Mascola JR. Focused evolution of HIV-1 neutralizing antibodies revealed by structures and deep sequencing. Science 2011; 333:1593-1602.

Zhou T, Georgiev I, Wu X, Yang ZY, Dai K, Finzi A, Do Kwon Y, Scheid JF, Shi W, Xu L, Yang Y, Zhu J, Nussenzweig MC, Sodroski J, Shapiro L, Nabel GJ, Mascola JR, Kwong PD. Structural basis for broad and potent neutralization of HIV-1 by antibody VRC01. Science 2010; 329:811-817.