Arch Virol (2010) 155:2029–2034
DOI 10.1007/s00705-010-0785-y

BRIEF REPORT

Comparative reactivity of serum samples from Argentinean HIV-infected patients with V3 peptides from subtype B or BF recombinants

J. Ambrosioni · G. Andreani · C. Acuipil · R. Barral · R. Rabinovich · Liliana Martinez Peralta

Received: 11 December 2009 / Accepted: 30 July 2010 / Published online: 11 September 2010

Abstract To analyze humoral cross-reactivity to V3 peptides from subtype B and BF recombinant forms, plasma samples from 50 HIV-1-infected patients were characterized by sequencing fragments of the *env* and *pol* genes. An in-house EIA was performed using peptides corresponding to the 15 central amino acids of the V3 loop of gp120 from subtypes B (MN, SF2) and F1 and a consensus peptide from Argentinean BF recombinants. No differences were found with respect to the infecting subtype, but significant differences were found among the peptides. Reactivity was higher against the MN and BF peptides in both groups infected with subtype B ($n = 28$) and BF ($n = 22$) recombinants than against subtype F1 and SF2 peptides.

HIV-1 is divided into three groups: M, N and O. The M group has the highest distribution, and at least nine subtypes have been identified [1], as well as many other circulating recombinant forms containing sequences of two or more different HIV-1 subtypes [1–3].

The presence of BF recombinants was detected in Argentina already in the 1990s [4–6]. Later, the predominance of BF recombinants in the heterosexual population as well as in injecting drug users (IDUs) and of subtype B in men who have sex with men (MSM) was described [7–9]. Because of this complex pattern of HIV subtype distribution in Argentina, it is important to study the cross-reactivity of antibodies to subtype B and BF recombinants [10].

Neutralizing antibodies are crucial in preventing viral infections. Less clear is their role in the containment of viral replication in infected individuals. However, evidence is accumulating that neutralizing antibodies may prevent or delay progression to AIDS [11]. Many neutralizing epitopes have been described in the envelope glycoproteins gp120 and gp41. The third hypervariable (V3) loop of the HIV-1 envelope glycoprotein gp120 has been recognized as one of the most important epitopes, since it is partially exposed during various stages of the disease. It is also immunogenic in essentially all HIV-infected subjects and capable of inducing antibodies able to neutralize a broad array of primary isolates [12]. Therefore, V3 peptides were selected here as a prior study to neutralizing capacity.

The aim of this study was to analyze the cross-reactivity against V3 peptides among samples from Argentinean patients infected with subtype B or BF recombinants of HIV strains.

Informed consent was obtained, and blood samples were collected from 50 HIV-infected patients. The median age was 36 (1–63) years, and 67% were male. All of the patients were undergoing antiretroviral treatment with virological failure. CD4 T cell count was available for 42 patients (84%) at the time of sampling. The median CD4 T cell count was 280 T cells/mm^3 (range 91–930). Four patients had fewer than 200 CD4 T cells/mm^3. None of them showed signs of any opportunistic infection at the time of the study.

J. Ambrosioni · G. Andreani · C. Acuipil · R. Barral · R. Rabinovich · L. Martinez Peralta
National Reference Center for AIDS, Microbiology Department, School of Medicine, University of Buenos Aires, Buenos Aires, Argentina

L. Martinez Peralta (✉)
Microbiology Department, University of Buenos Aires, Paraguay 2155, C1121ABG Buenos Aires, Argentina
e-mail: lilimp@fmed.uba.ar

Partial sequences of *pol* and e*nv* (C2C3) genes were analyzed. Plasma samples were grouped according to their *env* sequences. A partial HIV *pol* gene fragment containing the RT and protease region and the C2C3 *env* region were amplified by RT-PCR. RNA was extracted using a Qiagen Viral RNA Kit (Valencia, CA, USA). The outer primers for the *pol* fragment were RT3474R (5′ GAATCTCTCTGTT TTCTGCCAGTTC) and PRO5F (5′ AGAAATTGCAGG GCCCCTAGGAA), and the inner primers were PRORT (5′ TTTCCCCACTAACTTCTGTATGTCATTGACA 3′) and PRORT (5′ AGANCAGAGCCAACAGCCCCACCA 3′). The outer primers for the *env* fragment were ED5 (5′ ATGGGATCAAAGCCTAAAGCCATGTG 3′) and ED12 (5′ AGTGCTTCCTGCTGCTCCCAAGAACCCAAG 3′), and the inner primers were ES7 (5′ tgtaaaacgacggccagt CTGTTAAATGGCAGTCTAGC 3′) and ES8 (5′ cagga aacagctatgaccCACTTCTCCAATTGTCCCTCA 3′).

For direct sequencing, these PCR products were analyzed in an automated DNA sequencer (ABI 3100, Applied Biosystems). All sequences were assembled using the Sequencher software (Genecodes Inc., Ann Arbor, MI, USA). Sequence alignment and comparison of nucleotide sequences were performed using CLUSTAL X. Pairwise evolutionary distances were estimated (DNADIST, PHYLIP) using the Kimura two-parameter method (ts/tv = 2). Phylogenetic analysis was then conducted using the neighbor-joining (NJ) method (MEGA4). The reliability of the groups was evaluated by performing 1,000 bootstrap replicates. Sequences were aligned with reference sequences from the Los Alamos HIV sequence database and Argentine sequences, including sequences from the same geographical region. Analysis of recombinant forms was done with Simplot and Bootscannig in Simplot version 2.5. Molecular characterization of *env* was performed to classify the samples as subtype B or BF recombinant.

Synthetic peptides of 15 amino acids from the central part of the V3 loop were used for the analysis. The peptides corresponded to subtype B (MN and SF2 strains), subtype F (provided by the NIH, USA) and a BF peptide constructed according to the amino acid sequences of samples from our laboratory (Table 1): MN peptide sequence, KRIHIGPGRAFYTTK; SF2 peptide sequence, KSIYIGP GRAFHTTG; F1 peptide sequence, KSIHLGPGQAFY ATG; developed BF peptide sequence, RKSIQIGPG RAFYAT (synthesized by Eurogentec SA, Herstal, Belgium). Consensus amino acid sequences were obtained from the Los Alamos GenBank database (Available at http://www.hiv.lanl.gov/cgibin/CONSENSUS_DOWNLOAD/ConsensusDownloader.cgi).

An in-house EIA was performed. Multiple plasma dilutions and peptide concentrations were evaluated to optimize the assay. Finally, peptides were used at a concentration of 1 μg/ml in a 0.1 M carbonate/bicarbonate buffer and attached to *Polysorp* plates (NUNC). Plasma samples were used at a dilution of 1:200 in PBS with 3% (m/v) powdered milk. A commercial anti-IgG labeled using a peroxidase kit (Dakopatts, Sweden) was used in a dilution of 1:2,000. Plates were read using a spectrophotometer with a 492-nm filter. Reactivities were expressed as log 10 of the absorbances.

Table 1 Developed V3 BF peptide, and aminoacid sequences of 13 BF recombinants used for the construction

Sample	Amino acid sequence	Accession no.
1	CTRPNNNTRKSIRIGPGQTFYATGEIIGNIRKAHC	AY037283
2	CTRPNNNTRKSIQLGPGRAFYATGEIIGDIRKAHC	AY037281
3	CTRPNNNTRKSIQLGPGRAFYATGDIIGDIRKAHC	AF385936
4	CTRPNNNTRKSIQLGPGRAFYTTGNIIGNIRKAHC	AY037271
5	CTRPSNNTRKSIQIGPGRAFYTTGDIIGDIRKAHC	AY037280
6	CTRPSNNTRKGIQMGWGRAFYTTKDIIGDIRQAHC	AY037267
7	CTRPSNNTRKSIHIGPGRAFYTTGKIIGDIRQAHC	AY037276
8	CTRPNNNTRTSIRIGPGQAFYATGDIIGDIRKAHC	AY037278
9	CTRPNNNTRTSIQIGPGRAFYATGDIIRDIRQAHC	AY037273
10	CERPNNNTRKSINIGPGRAFYATGDIIGDIRQAHC	AY037275
11	CKRPNNNTRKSIHIGPGRAFYATGDIIGDIRQAHC	AY037277
12	CTRPNNNTRKSITIGPGRAFYATGAIIGNIRKAHC	AY037272
13	CTRPNNNTRKGIHIGPGRAFYATGEIIGDIRRAHC	AY037266
Peptide designed:	**RKSIqIGPGRAFYAT**[a]	
F1 consensus	RKSIHLGPGQAFYAT	
B consensus	RKSIHIGPGRAFYTT	

[a] Capital letter: amino acid present in more than 50% of samples. Lower-case letters: amino acid present in less than 50% of samples

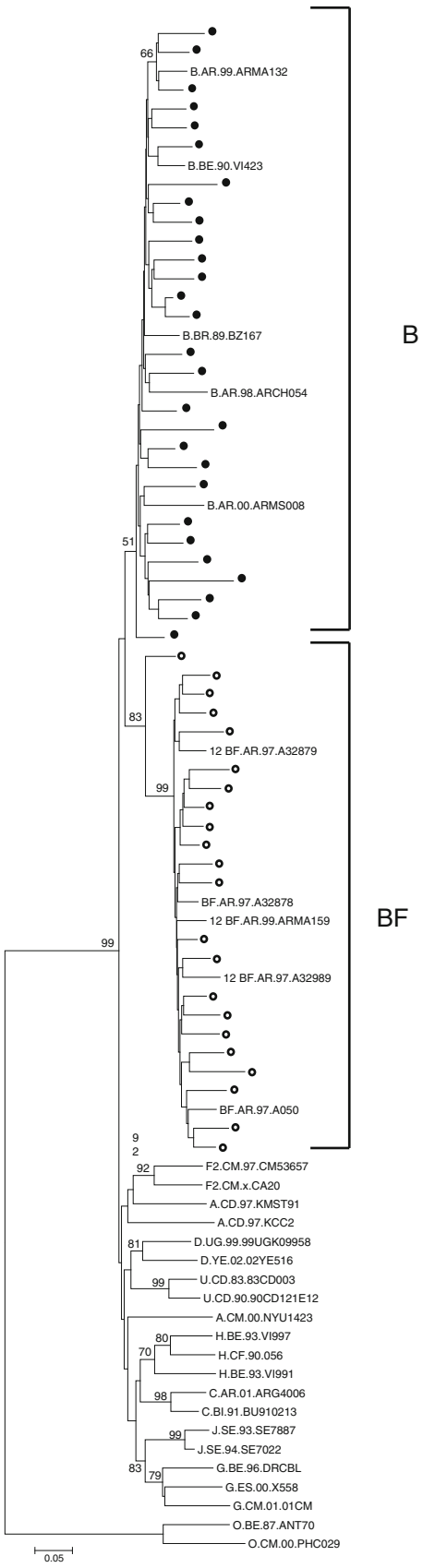

Fig. 1 Phylogenetic tree of the HIV-1 *env* region of the sequences analyzed and HIV-1 reference sequences. The tree was constructed by the neighbor-joining method using Kimura two-parameter model (ts/tv = 2/1). Bootstrap values (1,000 resamples) greater than 50 and the scale are indicated on the tree

An ANOVA test was used to compare reactivities. Data were analyzed using Statistix software.

A consensus BF peptide was developed according to the amino acid sequences of 13 characterized samples from Argentinean patients infected with BF recombinants. The amino acid sequence of the consensus BF peptide was RKSIqIGPGRAFYAT. Capital letters represent amino acids that are present in more than 50% of the samples, and lower-case letters represent the most frequent amino acids, although the latter were present in less than 50% of the samples studied (Table 1). The peptide that was designed differed by three amino acids from the F1 consensus sequence, by two from the B consensus sequence, by three from the SF2 peptide, and by three from the MN peptide. Only one amino acid difference was found with respect to the CRF_012 BF recombinant consensus at position 5 (Q instead of H). According to *env* sequences, samples from 28 patients (median age 41 years, 86% male) were characterized as subtype B, and samples from 22 patients (median age 34 years, 56% male) were characterized as BF recombinants (Fig. 1). The differences in median age are due to the inclusion of 5 infants among the BF patients, and the differences in gender are due to the predominance of subtype B in MSM, and BF in the heterosexual and IDU populations. Four samples (14.30%) characterized as B in *env* were BF recombinants in *pol*. Three samples (13.63%) characterized as BF in *env* were subtype B in *pol*.

The reactivity of plasma samples from patients infected with subtype B or BF recombinants against different peptides and a comparison of reactivities according to the infecting subtype are shown in Table 2. A pool of serum samples with high reactivity was used as a positive control, and seronegative samples were used as negative controls. No differences in reactivity against MN, BF, F and SF2 peptides were found between the groups of samples. The reactivity of samples with discordant subtypes between *pol* and *env* was comparable with those corresponding to the same *env* group. The reactivity of samples from pediatric patients was comparable with the reactivity of samples from adult patients. A comparison of the reactivities of the peptides is shown in Table 3. Both groups of samples showed a stronger response against the MN and BF peptides. The reactivity against the MN peptide was significantly higher than against the F and SF2 peptides. Regarding the BF peptide, significant differences were found only against SF2. No differences in reactivity were

Table 2 Reactivity of serum samples from 50 HIV-infected patients with V3 peptides and comparison with the infecting subtype

Plasma samples	Peptide			
	MN	BF	F	SF2
Patients infected with subtype B virus ($n = 28$)	2.26	2.03	1.85	1.84
Patients infected with recombinant BF virus ($n = 22$)	2.16	1.99	1.88	1.81
	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$

Table 3 Reactivity of serum samples from 50 HIV-infected patients with V3 peptides

Peptide	Reactivity	Peptide	Reactivity	*p*
MN	2.21	BF	2.01	0.197
		F	1.86	0.000
		SF2	1.82	0.000
BF	2.01	MN	2.21	0.197
		F	1.86	0.093
		SF2	1.82	0.008
F	1.86	MN	2.21	0.000
		BF	2.01	0.093
		SF2	1.82	0.256
SF2	1.82	MN	2.21	0.000
		BF	2.01	0.008
		F	1.86	0.256

Peptides correspond to 15 central amino acids of the V3 loop of gp120 of subtypes B (MN, SF2), F1 and a consensus BF recombinant peptide. Reactivity is expressed as log 10 of the average of the absorbance values. An ANOVA test was used to compare reactivity

found between MN and BF, or between the F and SF2 peptides. Twenty-two out of 28 (78.57%) samples from patients infected with subtype B viruses reacted more strongly with the MN peptide. On the other hand, in the group of samples from patients infected with BF recombinants, only 6 out of 22 (27.27%) reacted more strongly with the BF peptide. Among the 50 samples studied, 3 of them demonstrated extremely high and comparable reactivity against MN and BF, but not against SF2 or F. Other three samples demonstrated high and comparable reactivity against the four peptides studied. Among these six samples, two exhibited an amino acid deletion at position 25 of the V3 loop; one sample exhibited a GGG amino acid sequence in the central position of the V3 loop, and the other three samples did not exhibit any particular characteristics. For the rest of the samples, the only particular characteristic was an APG amino acid sequence instead of GPG in the central position of the V3 loop for seven samples. These changes in amino acid sequences had no impact on cross-reactivity.

The coexistence of subtype B and BF recombinants is clearly established in South America, and subtype F is also found in Brazil [2, 4–6, 8, 13, 14]. Variants of non-B subtypes have slowly been introduced into the HIV-1 epidemic in other Western Countries [15]. Moreover, cases of infection with BF recombinants have also been described in Europe [16].

Even though subtype B and BF recombinants are closely related, case reports of patients who are coinfected with both subtype B and BF recombinants have been described (Andreani et al., unpublished data), which may represent a failure in heterotypic immunity. Although several neutralizing domains of HIV have been described in gp120 and gp41, the V3 loop remains one of the most important [12]. Some monoclonal antibodies, such as M ab 2219, have been reported to cross-react with the V3 loop of several different subtypes, including subtypes B, A and F [17, 18]. In our study, the MN peptide was used to evaluate the response against subtype B. This work showed that both groups of patients produced especially strong immune responses to this peptide. However, the response against BF peptide was similar. Other studies have also shown stronger responses against the subtype B amino acid sequences and broader neutralizing antibody production [19].

Studies of the reactivity of samples against V3 peptides have been performed previously in Argentina, showing higher reactivity against the MN peptide. However, the samples were not characterized according to the predominant subtype [20]. In samples separated according to their *env* sequences, comparable results were obtained, such as a higher response against MN in both groups of patients.

Glycosylation at specific sites on gp120 is believed to play an important role in virus-neutralizing epitopes [21, 22], but this topic was not investigated in this paper, since an assay using peptides of 15 amino acids in length was developed.

Because of their importance in HIV-1 neutralization, HIV vaccine candidates using different V3 loop approaches are continuously being developed and studied [23, 24]. Current data support the hypothesis that the V3 region of gp120 can induce broadly reactive, cross-neutralizing antibodies and, as such, they should constitute a prominent target for a immune response induced by an HIV vaccine [12, 25]. In a recently published clinical trial, a gp120-based vaccine candidate was associated with a reduced risk of HIV infection in a community-based population [26].

Our results suggest that subtype B sequences may be included in the development of a potential vaccine candidate for the region. Because of the subtle differences observed in this study, it still needs to be studied whether BF sequences also need to be considered. Six out of 50 of our samples exhibited high and comparable reactivity against both MN and BF peptides. In addition, three of them also reacted broadly against F and SF2 peptides. An in-depth study of these sequences could be useful for identifying candidates for the development of a local vaccine in South America. Overall reactivity against the F1 peptide was weak. Although subtype F is rare in Argentina, it has been reported much more frequently in Brazil, and genetic differences among subtypes may be on the increase [24]. To the best of our knowledge, this is the first study that compares cross-reactivity against subtypes and recombinant forms. Further studies need to be conducted on the V3 loop and other important neutralizing domains within gp120 and gp41, among related subtypes, especially BF recombinants and subtype F, and with different strains within the same subtype in this area of the world.

Acknowledgments Peptides (MN, SF2, F) were obtained through the AIDS Research and Reference Reagent Program, AIDS Program, NIAID, NIH: HIV-1V3 peptides were obtained from the Division of AIDS, NIAID. This work was supported by grants to L.M.P. from the University of Buenos Aires (M043) and the National Agency for Promotion of Science and Technology (PICT 2007-1021).

References

1. McCutchan FE (2000) Understanding the genetic diversity of HIV-1. AIDS 14(Suppl 3):S31–S44
2. Sabino EC, Shpaer EG, Morgado MG, Korber BT, Diaz RS et al (1994) Identification of human immunodeficiency virus type 1 envelope genes recombinant between subtypes B and F in two epidemiologically linked individuals from Brazil. J Virol 68:6340–6346
3. Carr JK, Salminen MO, Koch C, Gotte D, Artenstein AW et al (1996) Full-length sequence and mosaic structure of a human immunodeficiency virus type 1 isolate from Thailand. J Virol 70:5935–5943
4. Marquina S, Leitner T, Rabinovich RD, Benetucci J, Libonatti O et al (1996) Coexistence of subtypes B, F, and as B/F env recombinant of HIV type 1 in Buenos Aires Argentina. AIDS Res Hum Retroviruses 12:1651–1654
5. Fernandez-Medina D, Jansson M, Rabinovich RD, Libonatti O, Wigzell H (1999) Identification of human immunodeficiency virus type 1 subtypes B and F B/F recombinant and dual infection with these subtypes in Argentina. Scand J Infect Dis 31:235–242
6. Comez Carrillo M, Salomon H, Pando MA, Kijak G, Avila MM (2001) Distribution of subtypes and recombinant of HIV. Situation in Argentina. Medicina (B Aires) 61:881–889
7. Carr JK, Avila M, Gomez Carrillo M, Salomon H, Hierholzer J et al (2001) Diverse BF recombinants have spread widely since the introduction of HIV-1 into South America. AIDS 15:F41–F47
8. Avila MM, Pando MA, Carrion G, Peralta LM, Salomon H et al (2002) Two HIV-1 epidemics in Argentina: different genetic subtypes associated with different risk groups. J Acquir Immune Defic Syndr 29:422–426
9. Gomez Carrillo M, Avila M, Hierholzer J, Pando M, Martinez PL et al (2002) Mother-to-child HIV type 1 transmission in Argentina: BF recombinants have predominated in infected children since the mid-1980s. AIDS Res Hum Retroviruses 18:477–483
10. Turk G, Gherardi MM, Laufer N, Saracco M, Luzzi R et al (2008) Magnitude, breadth, and functional profile of T-cell responses during human immunodeficiency virus primary infection with B and BF viral variants. J Virol 82:2853–2866
11. Humbert M, Dietrich U (2006) The role of neutralizing antibodies in HIV infection. AIDS Rev 8:51–59
12. Zolla-Pazner S (2005) Improving on nature: focusing the immune response on the V3 loop. Hum Antibodies 14:69–72
13. Teixeira SL, Bastos FI, Telles PR, Hacker MA, Brigido LF et al (2004) HIV-1 infection among injection and ex-injection drug users from Rio de Janeiro, Brazil: prevalence, estimated incidence and genetic diversity. J Clin Virol 31:221–226
14. Eyer-Silva WA, Couto-Fernandez JC, Morgado MG (2007) Molecular epidemiology of HIV type 1 in inner Rio De Janeiro State, Brazil. AIDS Res Hum Retroviruses 23:303–308
15. Buonaguro L, Tagliamonte M, Tornesello M, Buonaguro FM (2007) Evolution of the HIV-1 V3 region in the Italian epidemic. New Microbiol 30:1–11
16. Sierra M, Thomson MM, Rios M, Casado G, Castro RO et al (2005) The analysis of near full-length genome sequences of human immunodeficiency virus type 1 BF intersubtype recombinant viruses from Chile, Venezuela and Spain reveals their relationship to diverse lineages of recombinant viruses related to CRF12_BF. Infect Genet Evol 5:209–217
17. Stanfield RL, Gorny MK, Zolla-Pazner S, Wilson IA (2006) Crystal structures of human immunodeficiency virus type 1 (HIV-1) neutralizing antibody 2219 in complex with three different V3 peptides reveal a new binding mode for HIV-1 cross-reactivity. J Virol 80:6093–6105
18. Cardozo T, Swetnam J, Pinter A, Krachmarov C, Nadas A et al (2009) Worldwide distribution of HIV type 1 epitopes recognized by human anti-V3 monoclonal antibodies. AIDS Res Hum Retroviruses 25:441–450
19. Krachmarov C, Pinter A, Honnen WJ, Gorny MK, Nyambi PN et al (2005) Antibodies that are cross-reactive for human immunodeficiency virus type 1 clade a and clade B V3 domains are common in patient sera from Cameroon, but their neutralization activity is usually restricted by epitope masking. J Virol 79:780–790
20. Pampuro SE, Calarota SA, Marquina SA, Rabinovich RD, Libonatti OV (1996) Reactivity of Argentine serum samples against synthetic V3-based HIV-1 peptides. J Acquir Immune Defic Syndr Hum Retrovirol 12:527–528
21. Koch M, Pancera M, Kwong PD, Kolchinsky P, Grundner C et al (2003) Structure-based, targeted deglycosylation of HIV-1 gp120 and effects on neutralization sensitivity and antibody recognition. Virology 313:387–400
22. Teeraputon S, Louisirirojchanakul S, Auewarakul P (2005) N-linked glycosylation in C2 region of HIV-1 envelope reduces sensitivity to neutralizing antibodies. Viral Immunol 18:343–353
23. Schilling R, Heil A, Langner K, Pohlmeyer K, Larsen M et al (2006) A multivalent HIV-vaccine: development of a plasmid DNA for the expression of HIV envelope glycoproteins with hypervariable V3-loop domains. Vaccine 24:4648–4650
24. Vaine M, Wang S, Crooks ET, Jiang P, Montefiori DC et al (2008) 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 82:7369–7378

25. Eda Y, Takizawa M, Murakami T, Maeda H, Kimachi K et al (2006) Sequential immunization with V3 peptides from primary human immunodeficiency virus type 1 produces cross-neutralizing antibodies against primary isolates with a matching narrow-neutralization sequence motif. J Virol 80:5552–5562

26. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J et al (2009) Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med 361:2209–2220