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## Maternal neutralizing antibodies against a CRF01\_AE primary isolate are associated with a low rate of intrapartum HIV-1 transmission

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### ABSTRACT

Mother-to-child transmission (MTCT) of HIV-1 provides a model for studying the role of passively acquired antibodies in preventing HIV infection. We determined the titers of neutralizing antibodies (NAbs) against six primary isolates of clades B and CRF01\_AE in sera from 45 transmitting and 45 nontransmitting mothers matched for the main independent factors associated with MTCT in Thailand. A lower risk of MTCT, particularly for intrapartum transmission, was associated only with higher NAb titers against the CRF01\_AE strain, MBA. The envelope glycoprotein of this strain showed an unusually long V2 domain of 63 amino acids, encoding six potential N-linked glycosylation sites. We provided experimental data indicating that the extended V2 domain contributed to the higher level of resistance to neutralization by mothers' sera in this strain. Taken together the data suggest that some primary isolates with specific properties may be useful indicators for identifying protective antibodies.

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### Introduction

Mother-to-child transmission (MTCT) is the leading source of human immunodeficiency virus (HIV) infection in children. In the absence of preventive measures, transmission may occur during pregnancy (*in utero*), labor or delivery (intrapartum) or after birth, through breastfeeding. Previous studies have shown that, despite a heterogeneous viral population in the mother, only homogeneous viral variants were generally transmitted to the infant (Ahmad et al., 1995; Samleerat et al., 2008; Scarlatti et al., 1993; Verhofstede et al., 2003; Wolinsky et al., 1992). Maternal neutralizing antibodies (NAbs) can cross the placental barrier, reaching high levels in the fetus at the end of pregnancy and protecting the infant against infection with numerous pathogens (Englund, Glezen, and Piedra, 1998; Safrit et al., 2004). Maternal antibodies are therefore among the selective host factors that could play a role in limiting transmission of neutralization-sensitive variants. However, conflicting results have been

obtained concerning the role of maternal NAbs in reducing MTCT of HIV (Barin et al., 2006; Bongertz et al., 2001; Hengel et al., 1998; Lathey et al., 1999; Scarlatti et al., 1993). The differences in the results obtained may be due to differences in the methods used or populations studied. Particularly, analyses of the role of passively transferred antibodies should clearly separate cases of intrapartum transmission from *in utero* transmission: only in cases of intrapartum transmission can we be sure that exposure to the virus occurs in the presence of optimal levels of IgGs. Supporting a role of maternal NAbs in limiting MTCT, several recent molecular studies have shown that viruses transmitted are escape variants resistant to autologous maternal plasma (Dickover et al., 2006; Wu et al., 2006).

We previously hypothesized that broadly cross-neutralizing heterologous NAbs would protect babies against intrapartum HIV transmission. We measured NAb titers against primary isolates of various clades in sera from pregnant Thai women, for whom the time of transmission was known (Barin et al., 2006). We identified an association between higher titers of NAbs against a CRF01\_AE primary isolate, MBA — belonging to the predominant clade in Thailand — and lower rates of intrapartum transmission. However, only one isolate per clade was used in this previous study. Here, we extended our previous study using several CRF01\_AE strains in a different Thai

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population, selected on the basis of highly stringent criteria, and we confirmed the association previously observed. We also identified a specific feature of the envelope glycoprotein in this strain, accounting for this association, at least in part.

## Results and discussion

### Neutralizing activity of sera from mothers against six primary isolates

Neutralizing activity was tested against six heterologous primary isolates of different phenotypes in sera from 45 transmitting and 45 nontransmitting Thai mothers. Subjects were matched for baseline viral load and duration of zidovudine prophylaxis, the two main independent factors associated with MTCT in Thailand (Table 1A). Three of these primary isolates, MBA, LEA and C1712, belong to the predominant clade in Thailand, CRF01\_AE. The other three, BIG, CHA and FRO, belong to the less prevalent clade B. None of the 90 sera displayed no neutralizing activity, whereas 15 sera (17%), including both transmitters and non-transmitters, showed neutralizing activity against all six strains. Although cross-clade NABs were detected in several sera, subtype-specific neutralizing antibodies predominated (Table 1B, Fig. 1A). Among the 80 CRF01\_AE-infected mothers, 76 sera (95%) neutralized at least one strain of subtype CRF01\_AE, a higher proportion than observed for strains of subtype B (50 of 80, 63%). The opposite was previously observed when we explored serum samples from long-term non-progressors infected by subtype B variants (Braibant et al., 2006). However, the sensitivity to neutralization differed between CRF01\_AE strains. The MBA strain was more resistant to neutralization than the other two CRF01\_AE strains, LEA and C1712. Only 57 of 90 sera (63%) were able to neutralize MBA, whereas 75 (83%) and 81 (90%) sera neutralized C1712 and LEA, respectively (McNemar's test,  $P < 0.001$  and  $P = 0.001$ , respectively). In contrast, the three subtype B isolates were each neutralized by a similar proportion of sera: BIG was neutralized by 39 (43%), CHA by 35 (39%) and FRO by 38 (42%) (McNemar's test not significant).

**Table 1**

Baseline characteristics of transmitting and nontransmitting mothers (A) and comparison of detectable neutralizing antibodies against the six primary isolates in transmitting and nontransmitting mothers (B).

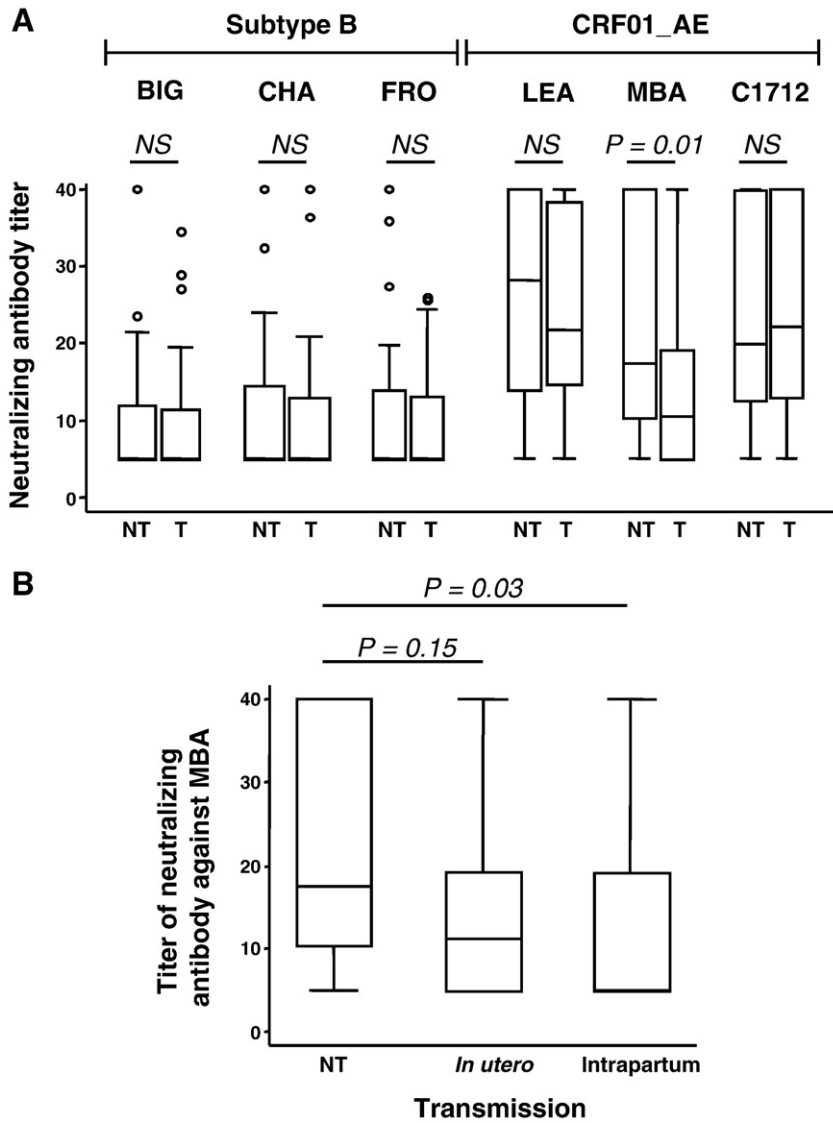
A				
Characteristics	Nontransmitters	Transmitters	p value	
	(n = 45)	(n = 45)	Median (range)	Kruskal–Wallis test
Age (years)	26 (22–30)	25 (23–30)		0.61
CD4 cell count (per mm <sup>3</sup> )	352 (240–434)	320 (220–450)		0.96
Viral load (log <sub>10</sub> copies/ml)	4.38 (3.89–4.75)	4.41 (3.87–4.71)		0.97
Duration of zidovudine prophylaxis (weeks)	6 (4–11)	6 (4–11)		0.92
Hemoglobin level (g/dl)	10.6 (10.1–11.5)	10.4 (9.8–11.2)		0.23
Hematocrit (%)	32 (30–35)	32 (30–34)		0.33
Gestational age at sample acquisition (weeks)	26 (22–26)	26 (21–26)		0.60
Sample acquisition to delivery (weeks)	14 (13–17)	14 (12–19)		0.98
B				
Presence of detectable NABs against	Nontransmitters	Transmitters	p value	
	(n = 45)	(n = 45)	n (%)	Fisher's exact test CLR
BIG (B/R5)	22 (49%)	17 (38%)	0.40	0.30
CHA (B/R5X4)	18 (40%)	17 (38%)	1.00	0.84
FRO (B/X4)	20 (44%)	18 (40%)	0.83	0.67
LEA (CRF01_AE/R5)	41 (91%)	40 (89%)	1.00	0.74
MBA (CRF01_AE/R5X4)	34 (76%)	23 (51%)	0.03	0.02
C1712 (CRF01_AE/X4)	37 (82%)	38 (84%)	1.00	0.78

The percentage of mothers with detectable NABs against the three subtype B isolates and two of the CRF01\_AE isolates (LEA and C1712) was similar between transmitting and nontransmitting mothers (Table 1B). In contrast, NABs against MBA – the most resistant CRF01\_AE strain to neutralization – were detected in 34 of 45 nontransmitting mothers (76%), a proportion significantly higher than that in transmitting mothers (23 of 45, 51%) (Fisher's exact test:  $P = 0.03$ ; conditional logistic regression, CLR:  $P = 0.02$ , odds ratio 3.8; 95% confidence interval: 1.2 to 11.3) (Table 1B). This difference remained significant if the analysis was restricted to the 80 CRF01\_AE-infected mothers (CLR:  $P = 0.01$ , odds ratio 6.5; 95% confidence interval: 1.5 to 28.8). Similarly, the distribution of titers against BIG, CHA, FRO, LEA and C1712 did not differ significantly between transmitting and nontransmitting mothers, whereas higher titers against MBA were observed in nontransmitting mothers (Wilcoxon signed rank,  $P = 0.01$ ) (Fig. 1A). High titers of NABs against MBA remained associated with a lower rate of HIV-1 transmission when the analysis was restricted to CRF01\_AE-infected mothers (Wilcoxon signed rank,  $P = 0.009$ ).

Neutralizing antibodies against MBA were undetectable in 15 of the 29 mothers who transmitted the virus intrapartum (52%) but in only seven of 29 (24%) matched nontransmitting mothers (Fisher's exact test,  $P = 0.06$ ; CRL,  $P = 0.05$ ), and were undetectable in six (43%) of the 14 mothers who transmitted the virus *in utero* but only two of 14 (14%) matched nontransmitting mothers (Fisher's exact test,  $P = 0.21$ ; CRL,  $P = 1$ ). Levels of neutralizing antibodies against MBA were significantly lower in mothers who transmitted the virus intrapartum than in matched nontransmitting mothers (Wilcoxon signed rank,  $P = 0.03$ ). In contrast, they were not statistically different between mothers who transmitted the virus *in utero* and nontransmitting mothers (Wilcoxon signed rank,  $P = 0.15$ ) (Fig. 1B). These findings confirm the results of a previous study of a random sample of 28 transmitting and 62 nontransmitting mothers (Barin et al., 2006). Of the 45 transmitting and the 45 nontransmitting women who participated in the present study, only 14 (31%) and 3 (7%) were also included in the previous study. This indicates that the population was substantially different from the previous study, and therefore strengthens the finding. Such antibodies may thus be associated with a lower risk of MTCT. As intrapartum exposure to HIV resembles a natural challenge in the presence of preexisting antibodies, it might be possible to identify correlates of protection useful for ongoing or future vaccine studies.

### Molecular characteristics of the MBA envelope glycoprotein

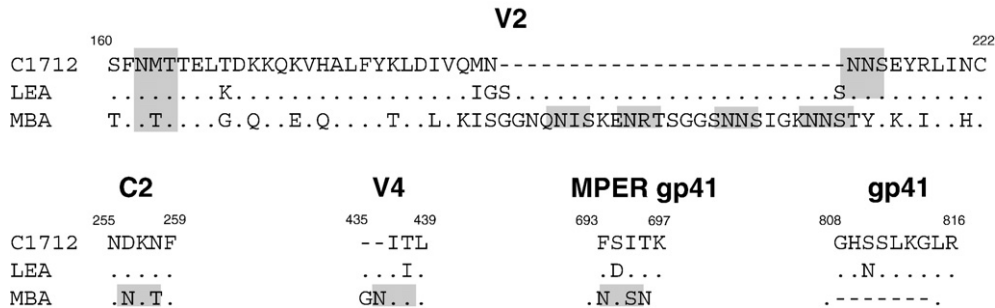
The molecular characteristics of the envelope glycoprotein of MBA may at least partially explain the association between a reduced risk of MTCT and the presence of NABs against this isolate. We thus studied the *env* gene in the three CRF01\_AE strains. Alignment of the Env amino-acid sequences from the three strains revealed a major difference between MBA and the other two strains (Fig. 2). MBA Env had an unusually long V2 domain of 63 amino acids (23 and 25 amino acids longer than that of LEA and C1712, respectively), encoding six potential N-glycosylation sites (PNGS) versus only two PNGS in LEA and C1712. Searches of homology between MBA Env and sequences found in the HIV sequence database, using the HIV Blast program, revealed that this extended MBA V2 domain is unique. A detailed analysis of the V2 domains of the 68 CRF01\_AE strains present in the web-alignment of the full spectrum of HIV sequences (<http://www.hiv.lanl.gov/content/sequence/NEWALIGN/align.html>) from the HIV sequence database, revealed that the longest of these V2 domains was composed of 53 amino acids (accession number U51188) and the V2 domain with the greatest number of potential glycosylation sites encoded five PNGS (accession number AY444805). In addition to this long MBA V2 domain, three PNGS were found in the C2 constant domain, the V4 variable loop and the membrane proximal external



**Fig. 1.** Comparison of neutralizing antibody titers in transmitting (T) and nontransmitting (NT) mothers. (A) Comparison of NAb titers against the six strains in transmitting and nontransmitting mothers. Box plots show the distribution of maternal antibody titers; for each distribution, the horizontal lines represent the lower adjacent 25th, median, 75th, and upper adjacent percentiles. (B) Comparison of NAb titers against the MBA strain in nontransmitting mothers and transmitting mothers according to time of transmission (*in utero* or intrapartum). Nab titers were compared using the Wilcoxon matched-pairs signed-ranks test. NS: not significant.

region (MPER) of the MBA envelope glycoproteins (Fig. 2). We also identified a deletion of seven amino acids in the gp41 cytoplasmic tail in MBA, but not in LEA or C1712. The three PNGS observed in MBA are not specific to this strain. Indeed, two PNGS are present in the C2 and

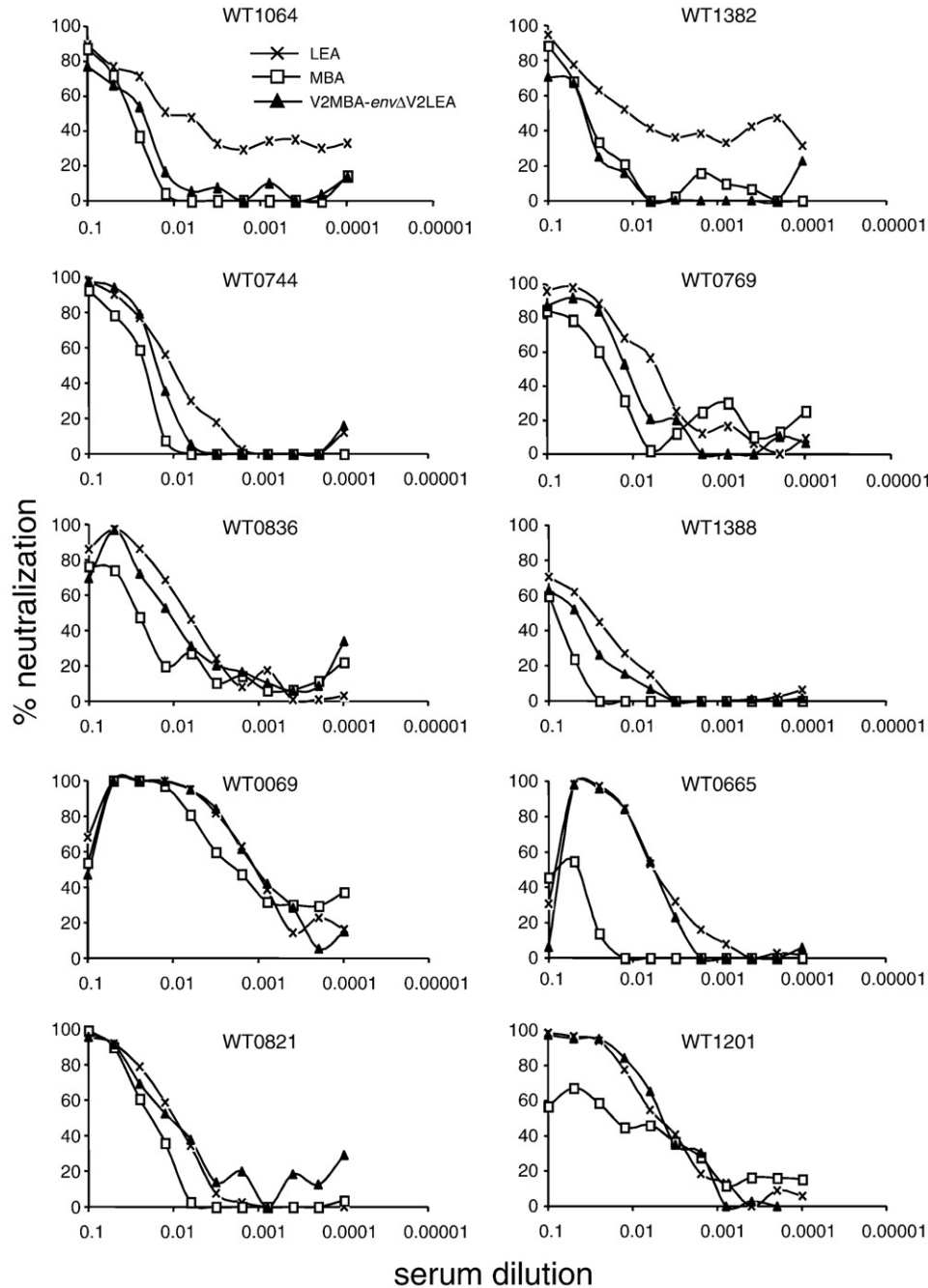
MPER domains in the envelope protein of the strain HxB2, which is highly sensitive to neutralization. Additionally, the envelope proteins of several other CRF01\_AE strains possess the two PNGS in the V4 and MPER domains (data not shown).



**Fig. 2.** Sequence characteristics, and their location, in the MBA envelope glycoprotein. The Env amino-acid sequences of C1712, LEA and MBA were aligned using the BioEdit package version 5.0.9. Only regions showing characteristics specific to MBA are indicated. Amino-acid numbering is based on MBA amino-acid Env sequence. Identical amino acids and insertions are indicated by dots or dashes, respectively. PNGS (NXT or NXS) are highlighted in grey.

Several reports having shown that the V1V2 domain strongly influences sensitivity to neutralization by masking neutralizing targets (Pinter et al., 2004; Rong et al., 2007; Sagar et al., 2006; Shibata et al., 2007; Wolk and Schreiber, 2006), we therefore hypothesized that this unusual V2 domain contributes to the greater resistance to neutralization observed for MBA than that observed for LEA or C1712. To test this hypothesis, we produced pseudotyped viruses expressing the MBA and LEA wild-type Env proteins or two chimeric Env proteins, constructed by swapping the V2 domains (V2LEA–EnvΔV2MBA and V2MBA–EnvΔV2LEA). The chimeric V2LEA–EnvΔV2MBA pseudotyped virus, expressing the V2 domain of LEA in an MBA Env backbone, was not infectious. We therefore compared the neutralization profiles of the three other viruses,

expressing MBA, LEA and V2MBA–EnvΔV2LEA, using 10 serum samples from mothers whose sera were more efficient at neutralizing LEA than MBA (Fig. 3). The MBA V2 domain conferred a higher level of resistance to neutralization to LEA in the presence of six of the 10 sera (Fig. 3; Table 2). Indeed, the chimeric virus displayed neutralization profiles for sera WT1064 and WT1382 similar to those of MBA, but with a level of sensitivity to neutralization approximately three times lower than that displayed by LEA. The neutralization profiles of the chimeric virus in the presence of sera WT0744, WT0769, WT0836 and WT1388 were intermediate between those of LEA and MBA, with LEA displaying a level of sensitivity to neutralization that was between 1.5 and 2.2 times higher. In contrast, the presence of the MBA V2 region did not affect the neutralization profile of the



**Fig. 3.** Effect of the MBA V2 domain on LEA neutralization. Neutralization activity in sera from 10 mothers was determined against viruses pseudotyped with the parental Env proteins from LEA (crosses) and MBA (open squares), and with the chimeric envelope protein V2MBAEnvΔV2LEA (closed triangles). Percentage of neutralization is plotted against serum dilution. The sera used are indicated at the top of each panel.

**Table 2**  
Neutralizing antibody titers in 10 serum samples against LEA, MBA and V2MBA–EnvΔV2LEA pseudotyped viruses.

Serum	LEA	MBA	V2MBA–EnvΔV2LEA
WT1064	100	32.5	44.2
WT1382	95	30.5	28.1
WT0744	98.7	46.8	66.8
WT0769	193.2	54.6	87
WT0836	146.1	38.1	89.8
WT1388	34	12.7	21.6
WT0069	981	572.9	1014.7
WT0665	182.9	22.3	183.2
WT0821	108.7	57.3	93.5
WT1201	217.1	65.1	240.3

chimeric virus in the presence of sera WT0069, WT0665, WT0821 and WT1201. These results indicate that the V2 region of MBA is involved in determining neutralization resistance in this strain, but that other determinants may be involved.

Taken together, our data suggest that the antibodies involved in reducing the risk of MTCT would be potent antibodies targeting either epitopes exposed on the large V2 domain itself or, alternatively, distant epitopes exposed by a particular conformation imposed by this large heavily glycosylated structure. Interestingly, Pinter et al. showed that the V1V2 domain is a global regulator of sensitivity of primary isolates to neutralization by antibodies commonly induced upon infection, but not by rare antibodies able to neutralize a broad range of primary isolates, such as the human monoclonal antibodies 2F5, 2G12 or b12 (Pinter et al., 2004). Further characterization of maternal antibodies with potent neutralizing activity against MBA and associated with a lower risk of MTCT, may therefore allow the identification of key epitopes involved in neutralization and protection.

In conclusion, this study confirms that higher titers of maternal antibodies against a CRF01\_AE primary isolate, MBA, are associated with a lower intrapartum risk of HIV-1 transmission in Thailand, and that the V2 domain of gp120 seems to have a major role in the neutralization process. Our results suggest that certain primary isolates may be indicative of a protective antibody response. Further studies aiming to select strains indicative of neutralization/protection may facilitate the identification of correlates of protection crucial for the development of protective vaccines. The MTCT model could be beneficial for such studies.

## Materials and methods

### Study population and samples collection

Samples were obtained from HIV-1-infected pregnant women enrolled in a clinical trial assessing various zidovudine (ZDV) treatment regimens for the prevention of MTCT in Thailand (Perinatal HIV Prevention Trial, PHPT-1), in which infants were not breastfed (Lallemant et al., 2000). The HIV-1 infection status of the infants was determined by HIV-1 proviral DNA PCR as previously described (Barin et al., 2006; Lallemant et al., 2000). We selected 45 transmitting mothers (cases) and 45 nontransmitting mothers (controls) from the original study. Subjects were matched for baseline maternal viral load and duration of maternal zidovudine prophylaxis, the two main independent factors associated with MTCT (Table 1A). Of the 45 transmitting mothers, 14 transmitted HIV-1 to their infants *in utero* and 29 transmitted the virus intrapartum. The timing of transmission was not specified for two of the mothers. Blood samples were collected at baseline, before the start of ZDV prophylaxis, 4 to 11 weeks before delivery. Maternal viruses were subtyped, by both V3 serotyping (Barin et al., 2006) and phylogenetic analysis of genomic sequences (Brand et al., 2004). Samples

with discrepant results in V3 serotyping and genetic analysis were defined as indeterminate. HIV-1 CRF01\_AE was identified in 80 (89%) and subtype B, in four (4%) of the 90 mothers. Six samples were indeterminate.

### Heterologous primary isolates neutralization assay

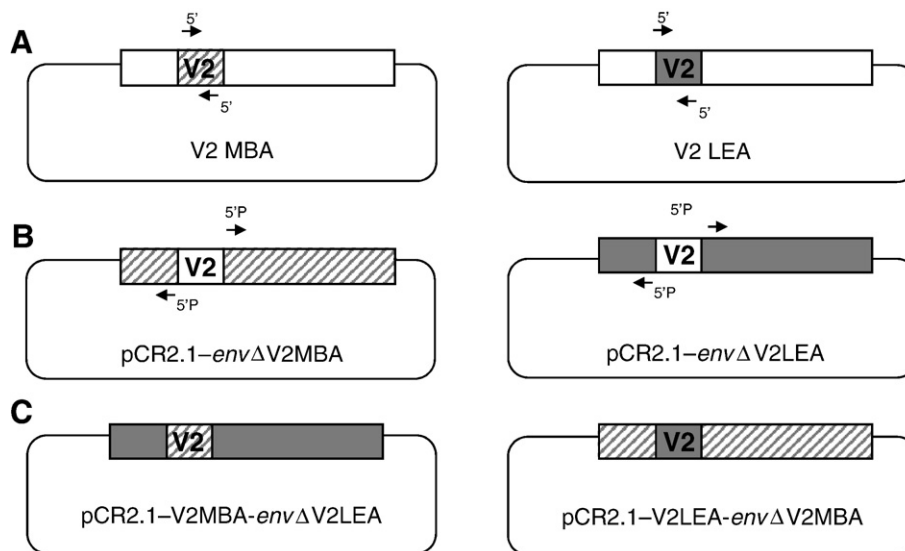
The heterologous primary isolates selected for this study belonged to the two prevalent clades in Thailand (CRF01\_AE and B) and displayed various phenotypes (X4, R5, dual-tropic). There were three clade B strains, FRO (X4), BIG (R5) and CHA (R5X4), and three CRF01\_AE strains, C1712 (X4), LEA (R5) and MBA (R5X4). The titers of NAb against the six primary isolates in each maternal serum sample were determined using the P4P cell assay, as previously described (Barin et al., 2004; Barin et al., 2006; Charneau et al., 1994). The assay was performed in duplicate and results were expressed as mean values. The neutralization titer was defined as the reciprocal of the serum dilution resulting in a 90% decrease in the number of infected cells 2 days after infection with 100 TCID<sub>50</sub> (50% tissue culture infectious dose).

### Amplification, cloning and sequencing of env genes

Genomic RNA from C1712, LEA and MBA viruses was extracted from each virus stock using the QIAamp® viral RNA Mini Kit (Qiagen). Full-length (gp160) *env* gene was amplified by nested RT-PCR using subtype CRF01\_AE *env*-specific primers. The outer primer pair was sensAEext (5'-GGTTARTTVARAGAATAAGAGAAAGAG-3') and asAEext (5'-TRCTTTTGGACCAYTTGCGYCCCAT-3'), and the inner primers pair was sensAEint (5'-AGAAGACAGTGAAATGAGACTGA-3') and asAEint (5'-ATDTTATRSAAAGHCCTTTCDAAGCC-3'). Reverse transcription (RT) was carried out for 30 min at 50 °C. This was followed by the first round of PCR, using the SuperScript™ One-Step RT-PCR for Long Templates kit (Invitrogen) with the following conditions: 2 min at 94 °C, then 35 cycles of 15 s at 94 °C, 30 s at 50 °C and 3 min at 68 °C, and a final extension step of 10 min at 72 °C. MBA *env* gene amplification differed from that of the two other viruses by the number of cycles (40 cycles) and annealing temperature (45 °C). A 5 µl aliquot of the products from the first round of PCR was then used as template for the second round of amplification under the same cycling conditions, with Platinum® PCR SuperMix High Fidelity (Invitrogen). PCR products were cloned into pCR2.1 (TOPO TA cloning® Kit; Invitrogen), before being excised by EcoRI restriction and transferred into the EcoRI site of the pCI expression vector (Invitrogen). PCR products and pCR2.1-*env* clones were sequenced, using a set of *env*-specific internal primers, according to the Dye Terminator cycle sequencing protocol (Applied Biosystems). Potential N-linked glycosylation sites (PNGS) were identified by N-GlycoSite (Zhang et al., 2004). MBA, C1712 and LEA *env* sequences have been submitted to GenBank and assigned accession numbers DQ518410, DQ518411 and DQ218412, respectively.

### Construction of chimeric env genes

Two chimeric *env* genes, V2LEA–*env*ΔV2MBA and V2MBA–*env*ΔV2LEA, were constructed in the pCR2.1 vector. V2 domains of LEA and MBA *env* genes were inverted using a domain exchange strategy, as previously described (Rong et al., 2007). The donor V2 domains of LEA and MBA were amplified by PCR using specific primers annealing at both ends of the V2 domains (Fig. 4A). The primer pairs used for the amplification of the V2 domains were: LEAV2S (5'-TCTTTTAATATGACCACAGAACTAAAAGATAAG-3') and LEAV2AS (5'-ACAATTTATTAACCTATACTACTATTACTACTACTCTT-3') for LEA, and MBV2S (5'-ACTTTTAATACGACACACTAGGAGAT-3') and MBV2AS (5'-ACAATGTATTAATATACTACTACTATAGGACTATTG-TTTTCC-3') for MBA. The recipient *env* backbones (deleted of



**Fig. 4.** Construction of the V2 chimeric Envs. (A) *Env* sequences encoding donor V2 domains (MBA and LEA) were amplified by PCR using specific primers that anneal at both ends of the V2 domain. (B) *Env* sequences encoding recipient *env* backbones within the pCR2.1 vector (pCR2.1-*env*ΔV2MBA and pCR2.1-*env*ΔV2LEA) were amplified by PCR using specific primers that anneal to regions adjacent to the V2 primers. A 5' phosphate group was added to these primers for ligation to the V2 amplicon. (C) Sequences encoding the V2 domains and recipient backbones were blunt-end ligated to generate chimeric *env* genes in pCR2.1 (pCR2.1-V2MBA-*env*ΔV2LEA and pCR2.1-V2LEA-*env*ΔV2MBA).

the V2 domain) within the pCR2.1 vector were amplified by PCR using specific primers that anneal to regions adjacent to the V2 primers (Fig. 4B). A 5' phosphate group (Phos) was added to these primers to allow ligation to the V2 amplicon. The primer pairs used for the amplification of the *env* backbones were: LEAEnvS (5'-Phos-AATACTTCAGTCATTAAGCAGGCTTGTG-3') and LEAEnvAS (5'-Phos-ACAGTTTCTTACTTCATCTGTTATATTTCCTATTT-3') for LEA, and MBAEnvS (5'-Phos-AATTCITTCAGTCATTAAGCAAGCATGTCC-3') and MBAEnvAS (5'-Phos-GCAGTTTCTTACTGATTTTCCATTTTATTGTCT-TAC-3') for MBA.

The PCR amplification conditions for the V2 domains were 30 s at 98 °C, followed by 35 cycles of 10 s at 98 °C, 30 s at 57 °C and 30 s at 72 °C, and a final extension step of 72 °C for 10 min. The same amplification conditions were used for *env* backbones, with an extension time at 72 °C increased to 4 min. PHUSION High Fidelity DNA Polymerase (BioLabs) was used to generate blunt-ended PCR amplicons. PCR amplicons were digested with DpnI to remove contaminating template DNA prior to ligation. Each donor V2 DNA fragment was ligated to its recipient *env* backbone to produce two pCR2.1 constructs containing chimeric *env* genes (Fig. 4C). Chimeric *env* genes were then extracted from pCR2.1 by EcoRI digestion and transferred into the EcoRI site of the pCI expression vector (Invitrogen).

#### *Env*-pseudotyped virus neutralization assay

*Env*-pseudotyped viruses were generated by co-transfecting  $3 \times 10^6$  293T cells with 12 μg of each pCI-*env* plasmid and 8 μg of pNL4.3.LUC.R<sup>-</sup>E<sup>-</sup> (Connor et al., 1995), using calcium phosphate (Invitrogen). After titration, pseudotyped virus stocks were diluted to obtain 600 TCID<sub>50</sub>/ml in growth medium. A volume of 25 μl corresponding to 15 TCID<sub>50</sub> was then incubated for 1 h at 37 °C with 75 μl of two-fold serial dilutions of heat-inactivated serum. The virus/serum mixture was then used to infect 10,000 TZM-bl cells (Platt et al., 1998; Wei et al., 2002) in the presence of 30 μg/ml DEAE-dextran. Infection levels were determined after 48 h, using the Bright-Glo luciferase assay (Promega) and a Centro LB 960 luminometer (Berthold Technologies) to measure luciferase activity in cell lysates. The assay was performed in duplicate and results were expressed as mean values. Neutralizing antibody titers were defined as the

reciprocal of the serum dilution required to reduce relative luminescence units (RLU) by 50%.

#### Statistical analysis

Categorical variables were compared using Fisher's exact test and a conditional logistic regression (CLR) model to take into account the matching of the two groups on maternal baseline viral load and ZDV prophylaxis treatment duration (Barin et al., 2006). Continuous variables were compared using the Wilcoxon matched-pairs signed-ranks test. We used McNemar's chi-squared test for matched case-control data to compare the proportions of patient sera able to neutralize each strain.

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