

# Correlation of Maternal Cytophilic Human Immunodeficiency Virus (HIV)-1 V3 Loop Peptide-Specific Antibodies in Infants with Vertical HIV Transmission

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## ABSTRACT

Approximately 70% of human immunodeficiency virus (HIV)-1-infected pregnant women do not transmit HIV to their offsprings. The identification of factors involved in maternal-child transmission of HIV is important for the design and implementation of therapeutic and prevention strategies. Recently we have developed a modified peripheral mononuclear cell (PBMC) culture system for *in vitro* antibody production (IVAP) by which we can distinguish serum-derived cytophilic anti-HIV-1 antibody from *de novo* synthesized antibody. In this study, we analyzed the presence of antibodies directed to HIV-1 gp160, gp120, gp41, and V3 loop synthetic peptides (C51, C53, and C57 from MN and IIB strains) utilizing the grid-blot method in PBMC cultures of 52 mother-child pairs. Among the mothers (39 nontransmitters and 13 transmitters), presence of serum-derived cytophilic antibodies or *de novo* synthesized HIV V3 loop peptide-specific

antibodies did not correlate with vertical transmission. However, PBMC-associated, cytophilic antibodies of maternal origin reactive with C51, C53, and C57 from MN and IIB strains were identified in cultures of uninfected infants, but not in infected infants. These observations suggest that cytophilic antibodies of maternal origin directed to HIV-1 V3 loop peptides which are bound to infant cells might play a role in preventing vertical transmission. (*Pediatr Res* 38: 384-389, 1995)

### Abbreviations

**PBMC**, peripheral blood mononuclear cells  
**IVAP**, *in vitro* antibody production  
**TBS**, Tris buffered saline  
**gp**, glycoprotein

The V3 loop of the HIV-1 gp120 envelope glycoprotein is a disulfide-linked closed loop of about 30 amino acids which contains the principal neutralizing domain for virus-neutralizing antibodies (1-3). More importantly, binding of V3 loop with a second cell surface receptor is believed to be essential for entry of HIV into CD4<sup>+</sup> cells (4). The *in vitro* characteristics of anti-V3 loop antibodies suggest that this antibody could be itself prevent HIV-1 infection *in vivo* (5, 6). A mouse-human chimeric MAbs specific for the V3 loop domain was found to protect chimpanzees against infection with HIV-1 when administered either before or after challenge (7). Antibodies directed to HIV-1 V3 loop have been associated with lower heterosexual transmission from male to female and slower disease progression in infected individuals (8-10). Thus V3 loop-specific antibodies may play an important role in HIV-1 infection. However, the role of antibodies against V3

loop in protection against vertical transmission of HIV is still controversial (11-19). Recently we have observed that, in HIV-infected individuals, supernatants of cultured PBMC for 1 h contain cytophilic antibodies which are eluted from PBMC. If the PBMC are washed and recultured for 7 d, they secrete *de novo* synthesized antibody designated as *in vitro* antibody production or IVAP. It is therefore possible to distinguish "cytophilic" anti-HIV-1 antibody from "*de novo*" HIV antibody production (20). Maternal derived cytophilic antibodies are identifiable in culture supernatants of PBMC from infants born to HIV-infected women (20). In this study, direct evidence for correlation of anti-V3 loop antibodies with maternal-child transmission was sought in order to determine whether the serum-derived maternal cytophilic antibodies which adhered to the surface of infant's PBMC correlate with occurrence of vertical transmission of HIV. We have analyzed PBMC culture supernatants from 52 mother-infant pairs for the presence of antibodies directed to HIV-1 gp160, gp120, gp41, and V3 loop synthetic peptides C51, C53, and C57 of MN and IIB strains by utilizing the grid-blot method (21). Our findings suggest that the presence of cytophilic antibodies of maternal

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origin on infant PBMC may play a role in preventing transmission of HIV.

## METHODS

**Study group.** This study group consists of 52 pairs of HIV-1-infected mothers and their offspring. The infants were clinically monitored for periods of >18 mo of age and were determined as being infected or not infected according to the Communicable Disease Center criteria for HIV infection (22). Culture supernatants from PBMC were collected in infants at age  $\leq 3$  mo, the mean age at time of sample collecting from HIV-infected infants was  $1.45 \pm 0.65$  mo ( $n = 13$ ) and that of HIV-uninfected infants,  $1.56 \pm 0.73$  mo ( $n = 39$ ). From the mothers, the blood samples were obtained at the same time as the infants. Based on their children's outcome, the mothers were divided into nontransmitting and transmitting groups. A retrospective analyses of antibody determination was performed in culture supernatants as described below.

**PBMC culture procedure.** PBMC were separated by Ficoll-hypaque density gradient centrifugation on Lymphoprep (Nucmed, Oslo, Norway) as previously described (20). The cells were washed three times in Hanks' balanced salt solution and resuspended at a concentration of  $1 \times 10^6$ /ml in RPMI 1640 supplemented with 10% FCS, penicillin, and streptomycin. PBMC were then cultured for 1 h at 37°C, supernatants collected and designated as "1-h supernatants" (containing serum-derived cytophilic antibodies). After washing the PBMC again with PBS, cells were resuspended in RPMI with 10% FCS and cultured for additional 7 d; supernatants were collected and designated as "7-d supernatants" (containing antibodies synthesized *de novo*). The supernatants were stored at -20°C until assayed for presence of HIV antibodies by grid-blot and ELISA.

**HIV-1 envelope glycoproteins and synthetic peptides of V3 loop.** HIV-1 envelope glycoproteins gp160 and gp120 were purified from supernatants of HIV-1<sub>451</sub>-infected clone of Hut 78 cells as described previously (23). Immunodominant peptide from gp41 (JB4 IIIB) and peptides of V3 loop from HIV-1 MN and IIIB strains (C51, C53, C57) were kindly provided by Dr. Paolo Rossi, and the sequences are shown in Table 1. p121 represents a segment of gp41, consisting of 80 amino acids from 566 to 645 (Repligen Corp., Cambridge, MA).

**Grid-blot procedure for antibody determination.** The grid-blot procedure was carried out as described previously (21). Briefly, a wet sheet of nitrocellulose paper previously soaked in Tris buffered saline (TBS) for 20 min was positioned on the

slotted portion of the acrylic plate of a SURF-BLOT apparatus (Idea Scientific Co., Minneapolis, MN). The foam pad followed by the metal plate of the apparatus were then stacked against the back of the nitrocellulose sheet and the apparatus clamped securely. HIV gp160, gp120, and the desired peptides, dissolved in TBS (3  $\mu$ g/ml) were applied to each channel in 100  $\mu$ l volumes. The SURF-BLOT was then incubated for 60 min on a platform shaker. The channels were washed twice with TBS and the apparatus disassembled. The antibody screening was performed by first blocking the nitrocellulose sheet in Blotto (2% non-fat milk in TBS) for 60 min followed by assembly of the sheet in the SURF-BLOT apparatus as described above, except that the sheet was rotated 90° clockwise so that the channels were now oriented at right angles to the row of absorbed specific antibody on the nitrocellulose sheet. One hundred microliters of supernatant were added to each channel. The SURF-BLOT was incubated for 60 min as before, the channels were washed, and the device was disassembled. To visualize the anti-V3 loop IgG, the nitrocellulose sheets were incubated with alkaline phosphatase-conjugated goat-anti-human IgG (1:1000 dilution) for 60 min, washed in TBS, and visualized with the 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium.

**ELISA for detecting anti-HIV-1 gp160 IgG in supernatants.** The method followed was essentially as described previously (20). Briefly, culture supernatants were added in duplicate wells to the plates coated with HIV-1 gp160 and incubated for 1 h at 37°C. Bound anti-gp160 IgG was detected by alkaline phosphatase-conjugated goat F(ab')<sub>2</sub> anti-human IgG antibody (Tago Inc., Burlingame, CA). The OD of absorbance was measured at 405 nm on a microplate reader (Coulter Electronics Inc., Hialeah, FL) and analyzed on the Softmax ELISA program (Molecular Devices, Menlo Park, CA).

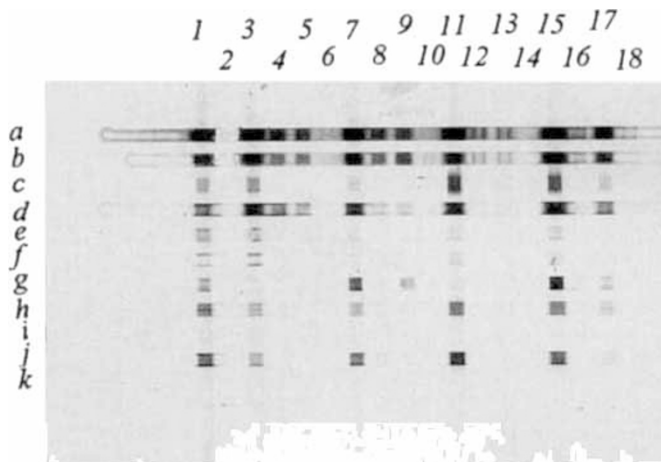
**Statistical analysis.** Comparisons of the OD value from HIV-1 gp160 ELISA were made between the transmitting and nontransmitting groups of mothers and infected and uninfected infants. The data were analyzed using the unpaired *t* test. For determination of differences in reactivity to HIV-1 V3 loop peptides between the groups, Fisher's exact test was performed.

## RESULTS

**Sensitivity of grid-blot.** The grid-blot assay can be adapted to screen antibody reactivities to a wide range of peptides or antigens for study of humoral immunity in serum and/or culture supernatants (21). In this study, the sensitivity was assessed by HIV-1 envelope gp41 and anti-gp41 MAb (p121 and MAb 2A2, respectively): the limitation of detecting antibody was 1.56 ng/ml when P121 antigen (3  $\mu$ g/ml) was loaded on nitrocellulose. One hundred percent sensitivity was obtained when applying this assay to detect anti-HIV IgG in IVAP supernatants from HIV<sup>+</sup> individuals (24). Typical results of grid-blot for detecting IgG antibodies against HIV-1 gp160, gp120, JB4 IIIB, and peptides from V3 loop in IVAP supernatants from maternal-child pairs are shown in Figure 1.

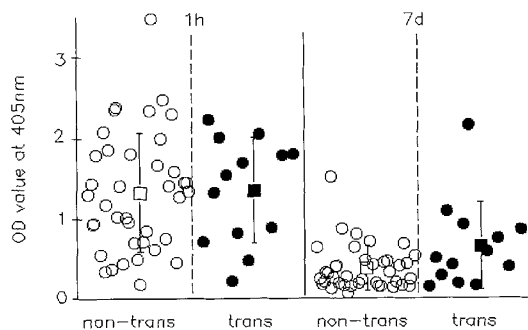
**Table 1.** Synthetic peptides from HIV-1 V3 loop used for this study

Peptide name	First amino acid	Last amino acid	Sequence
C51 MN	294	308	INCTRPNYNTRKRTH
C51 IIIB	294	308	INCTRPNNTRKSIR
C53 MN	306	320	RKRIHIGPGRAFYT
C53 IIIB	304	318	RKSIRIQRGPGRAFV
C57 MN	325	339	TIRQAHCNISRAKWN
C57 IIIB	324	338	GNMRQAHCNISRAKW
JB4 IIIB	598	617	GIWGCSGKLTCTTAVPWNAS



**Figure 1.** Typical result of grid-blot in the modified IVAP supernatants from mother-infant pairs. Row (HIV-1 envelope glycoproteins and V3 loop peptides): a, gp160; b, gp120; c, JB4-IIIB (gp41); d, P121 (gp41); e, C51 MN; f, C51 IIIB; g, C53 MN; h, C53 IIIB; i, C57 MN; j, C57 IIIB; k, TBS. Lane (supernatants): 1, positive control; 2, negative control; 3, 1-h supernatant (sup) from 215 mother; 4, 7-d sup from 215 mother; 5, 1-h sup from 215 infant (HIV-1 infected); 6, 7-d sup from 215 infant; 7, 1-h sup from 221 mother; 8, 7-d sup from 221 mother; 9, 1-h sup from 221 infant (HIV-1 uninfected); 10, 7-d sup from 221 infant; 11, 1-h sup from 220 mother; 12, 7-d sup from 220 mother; 13, 1-h sup from 220 infant (HIV-1 uninfected); 14, 7-d sup from 220 infant; 15, 1-h sup from 224 mother; 16, 7-d sup from 224 mother; 17, 1-h sup from 224 infant (HIV-1 uninfected); 18, 7-d sup from 224 infant. HIV-1 V3 loop C53 MN-, C53 IIIB-, and C57 IIIB-specific antibodies can be detected in 1-h supernatants from HIV-1-uninfected infants 221 (lane 9) and 224 (lane 17).

**Presence of anti-V3 loop antibodies in PBMC supernatants from mothers did not correlate with vertical transmission.** We have demonstrated that the revised culture procedure of PBMC for IVAP could distinguish between serum-derived cytophilic anti-HIV antibodies which adhere to the surface of PBMC and are released into the 1-h supernatants from those synthesized *de novo* and are secreted into the 7-d supernatants (20). When the 1-h supernatants from PBMC of transmitting and nontransmitting mothers are analyzed for antibodies to HIV-1 by ELISA and grid-blot, the results did not show any significant difference between the two groups; the OD values for anti-HIV-1 gp160 IgG ELISA were  $1.32 \pm 0.73$  in non-transmitters and  $1.35 \pm 0.63$  in transmitters (Fig. 2). Reactivity



**Figure 2.** HIV-1 gp160 antibody determination by ELISA in nontransmitting and transmitting mothers. The 1-h supernatants (left) and 7-d supernatants (right) of PBMC cultures from nontransmitting and transmitting mothers were assayed for anti-HIV-1 gp160 IgG by ELISA. The OD values were not significantly different in the two groups of mothers.

to HIV-1 gp160, gp120, gp41, and V3 loop peptides C51, C53, and C57 from MN and IIIB strains in the two groups are shown in Table 2, and no difference was noted between them.

The 7-d supernatants which contain anti-HIV antibodies produced by PBMC *in vitro* and represent a humoral immune response to HIV infection were analyzed by ELISA and grid-blot. No significant differences were observed between the nontransmitter and transmitter groups; the OD values from anti-HIV-1 gp160 IgG ELISA were  $0.37 \pm 0.28$  and  $0.59 \pm 0.54$ , respectively ( $t = 1.91$ ,  $p > 0.05$ ) (Fig. 2). Reactivities to HIV-1 gp160, gp120, gp41, and V3 loop peptides C51, C53, and C57 from MN and IIIB strains from the 7-d supernatants were also not different in the two groups (Table 3).

**Correlation of maternally serum-derived cytophilic anti-V3 loop antibodies in PBMC supernatants of infants with vertical transmission.** We analyzed anti-HIV gp160 IgG and antibodies against HIV V3 loop peptides in 1-h culture supernatants of PBMC from infants. Cytophilic antibodies of maternal origin have been shown previously to be released in these supernatants (20). Results show that antibodies against HIV-1 V3 loop peptides (C51, C53, and C57 from MN and IIIB strains) could be detected only in noninfected infants (Table 4). Of 39 seroreverters, 17 had antibodies to 1 or more V3 loop peptides. In their 1-h supernatants, antibody to C53 MN was the most frequent and was detectable in 1-h supernatants of 14 infants ( $p < 0.05$ ). None of the infected infants had detectable V3 loop peptide antibodies in their 1-h supernatants. The mean OD of anti-HIV gp160 in 7-d supernatants which represent *de novo* antibody production was  $0.04 \pm 0.02$  in noninfected infants, and  $0.132 \pm 0.277$  in infected infants ( $t = 2.13$ ,  $p < 0.05$ ) (Fig. 3). Anti-HIV gp160 IgG in the 1-h supernatants was not different between noninfected and infected infants, the mean OD being  $0.51 \pm 0.44$  and  $0.52 \pm 0.57$ , respectively (Fig. 3). These results were also confirmed in reactivity to HIV gp160 by grid-blot.

## DISCUSSION

Transplacental transfer of maternal antibodies to infants is known to be an important component of protective immunity against a variety of infectious agents. However, the role of anti-HIV antibodies in vertical transmission of HIV is unclear. In this study, we have demonstrated that maternally derived HIV V3 loop directed antibodies adhering to infant PBMC, when present, were associated with a lack of infection in the infants.

We have previously observed (20) that 1-h supernatants from cultured PBMC contain serum-derived cytophilic antibodies which are released from the surface of the PBMC. If these cells were washed and recultured, then no HIV-specific antibody was detected in 24-h supernatants either in infected or uninfected infants; HIV-specific antibody was detectable in 7-d culture supernatants only from HIV-infected infants, representing *de novo* antibody secretion. HIV-1-specific antibodies detectable in 1-h supernatants of PBMC from uninfected infants were demonstrated to be maternal in origin. We investigated the relationship of maternal HIV V3 loop-specific antibodies with vertical

**Table 2.** Presence of cytophilic anti-V3 loop antibodies in PBMC supernatants from mothers did not correlate with vertical transmission

		No. of supernatants reactive by grid-blot to:						
		C51		C53		C57		
gp160	gp120	JB4IIIB	MN	IIIB	MN	IIIB	MN	IIIB
Nontransmitters								
39/39 (100)	36/39 (92)	34/39 (87)	16/39 (41)	13/39 (33)	26/39 (67)	19/39 (49)	13/39 (33)	10/39 (26)
Transmitters								
13/13 (100)	13/13 (100)	13/13 (100)	5/13 (39)	5/13 (39)	6/13 (46)	6/13 (46)	4/13 (31)	6/13 (46)

The V3 loop peptide-specific antibodies in 1-h PBMC supernatants from mothers which contain serum-derived cytophilic antibodies were detected by grid-blot; 39 mothers had HIV-uninfected infants (nontransmitters), 13 mothers had HIV-infected infants (transmitters). Numbers in parentheses indicate percentages showing positive results.

**Table 3.** Presence of *de novo* synthesized specific antibodies to V3 loop peptides in PBMC supernatants from mothers did not correlate with vertical transmission

		No. of supernatants reactive by grid-blot to:						
		C51		C53		C57		
gp160	gp120	JB4IIIB	MN	IIIB	MN	IIIB	MN	IIIB
Nontransmitters								
34/39 (87)	23/39 (59)	18/39 (46)	7/39 (18)	2/39 (5)	5/39 (13)	3/39 (8)	3/39 (8)	4/39 (8)
Transmitters								
11/13 (85)	12/13 (92)	6/13 (46)	3/13 (23)	2/13 (16)	2/13 (16)	2/13 (16)	2/13 (16)	2/13 (16)

The V3 loop peptide-specific antibodies in 7-d PBMC supernatants from mothers which contain *de novo* synthesized antibodies were detected by grid-blot; 39 mothers had HIV-uninfected infants (nontransmitters), 13 mothers had HIV-infected infants (transmitters). Numbers in parentheses indicate percentages showing positive results.

**Table 4.** Anti-V3 loop cytophilic antibodies of maternal origin in PBMC cultures of infants correlated with lack of HIV transmission

		No. of supernatants reactive by grid-blot to:						
		C51		C53		C57		
gp160	gp120	JB4IIIB	MN	IIIB	MN	IIIB	MN	IIIB
HIV-exposed but not infected								
38/39 (97)	30/39 (77)	21/39 (54)	5/39 (13)	2/39 (5)	14/39 (36)*	7/39 (18)	1/39 (3)	4/39 (10)
HIV-infected								
13/13 (100)	10/13 (77)	7/13 (54)	0/13 (0)	0/13 (0)	0/13 (0)	0/13 (0)	0/13 (0)	0/13 (0)

The V3 loop peptide specific antibodies in 1-h PBMC supernatants from infants (<3 mo) which contain cytophilic antibodies of maternal origin were determined by grid-blot; 39 infants seroreverted (not infected), 13 infants were HIV-infected. Numbers in parentheses indicate percentages showing positive results.

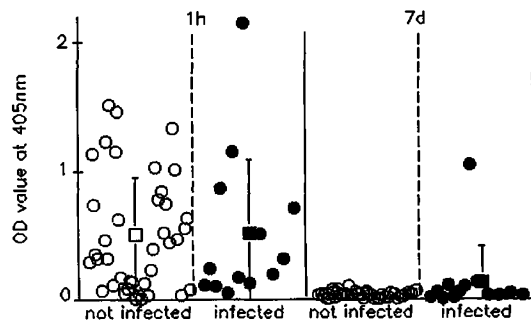
\* A significant difference was noted between not infected and infected infants for anti-C53 MN peptide antibody,  $p < 0.05$ .

transmission in PBMC culture supernatants from mother-infant pairs. The grid-blot method was selected because of the ease with which screening for antibodies to different HIV-1 envelope gp and V3 peptides could be performed using 100  $\mu$ l of the culture supernatant.

In the 52 mothers studied (39 nontransmitter and 13 transmitter), presence of serum-derived cytophilic antibodies or *de novo* synthesized HIV V3 antibodies in maternal PBMC cultures did not correlate with vertical transmission. However, in the PBMC of their infants, serum-derived maternal cytophilic antibodies directed to C51, C53, and C57 from MN and IIIB strains, most prominently C53 MN antibody could be eluted only from cells of uninfected infants, but not of infected infants. These observations suggest that cytophilic antibodies of maternal origin directed to HIV-1 V3 loop peptides are transferred selectively and get bound to infant cells. Whether or not such cytophilic binding of antibody occurs may depend on as yet undetermined characteristics of antibodies, e.g. anti-

body affinity or the presence of viral replication in the infected children which might have resulted in a decrease in HIV-specific antibody available for binding to Fc-bearing cells. The protective mechanism is also speculative; presence of antibody on target cells could, for instance, protect the cells from virus entry.

The reactivity to HIV gp160, gp120, gp41 (JB4IIIB), and V3 loop peptides in 1-h supernatants from mothers in both groups was detected. Although the trend for a higher reactivity to C53 MN in 1-h supernatants was observed in nontransmitting mothers, these results provide evidence that presence of HIV V3 loop-specific antibody in the mothers does not correlate with lower vertical transmission. This finding is consistent with recent reports indicating that HIV V3-specific antibody in mothers did not reveal a correlation with prevention from HIV infection in their offspring (14, 16–19). Additionally, in the present study, humoral immunity to HIV infection in mothers as evaluated by *de novo* antibody synthesis was also not related



**Figure 3.** HIV-1 gp160 antibody determination by ELISA in HIV-infected and uninfected infants. The 1-h supernatants (left) and 7-d supernatants (right) of PBMC cultures from HIV-uninfected and infected infants were assayed for anti-HIV-1 gp160 IgG by ELISA. Cytophilic anti-gp160 antibody (1-h supernatant) was similar in the two groups. As expected, IVAP was detectable only in infected infants ( $0.132 \pm 0.28$ ), not in the uninfected infants ( $0.04 \pm 0.02$ ),  $p < 0.05$ .

with vertical transmission, since the levels were equivalent in transmitting and nontransmitting mothers.

Among previous published reports about maternal HIV V3-specific antibodies with vertical transmission, three studies screened reactivity to V3 loop peptides in serum from infants by ELISA: two studies found that maternal HIV V3 specific antibodies in serum from infants correlated with low vertical transmission (12, 13), whereas one did not observe a significant difference between infected and uninfected infants (19). In our study, maternally derived cytophilic HIV-1 V3 specific antibodies could only be detected in some and not all uninfected infants which suggests that cytophilic antibodies specific for different V3 loop sequence might be present on infant PBMC and/or that other factors are of importance in protection from vertical transmission. Factors which have been shown to play a role in transmission of infection from mother to infants include maternal CD4 count, virus burden in the mothers (25) and HIV-specific immunity in the fetus (26). Factors affecting timing of transmission may have an overriding influence on protective role of humoral immunity, because placental transfer occurs only late during the third trimester and a fraction of infants does appear to get infected early in gestation (27).

In our study, cross-reactivity to V3 loop peptides from MN and IIB strains was observed, although the predominant strain in the present study was MN. The cross-reactivity is a common phenomenon among HIV-1 V3-specific antibodies, and the predominant reactivity to the MN strain is consistent with the high prevalence of HIV MN-related viruses in the United States (28, 29).

Our results suggest that, although the "presence" of maternal HIV V3-specific antibodies in mothers may of itself not correlate with vertical transmission, their adherence to infant PBMC may play a role in prevention of HIV vertical transmission.

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