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Vertical HIV-1 Transmission: Importance of Neutralizing Antibody Titer and Specificity

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Neutralization analyses were carried out with plasma from 132 volunteer human immunodeficiency virus (HIV)-1 infected women (76% pregnant, 24% with infants suspected for HIV-1 infection) collected between 1994 and 1998, against autologous and heterologous primary- and the reference HIV-1 MN isolates. A significantly lower percentage of HIV-1 transmissions was observed after 1996, parallel to a more intense antiretroviral treatment of infected pregnant women. HIV-1 isolation was significantly more frequent from peripheral blood mononuclear cells of mothers of infected children than mothers of uninfected children ($P = 0.0065$). Neutralization of autologous HIV-1 isolates was comparable for HIV-1 transmitters and nontransmitters' plasma, whereas neutralization of the reference isolate HIV-1 MN was more frequent at high titers for pregnant women who did not transmit HIV to their offspring compared to pregnant women who did. Although neutralization of heterologous primary HIV-1 isolates from HIV transmitters and non transmitters by transmitter plasma occurred with similar frequency, neutralization of isolates from transmitters was much more frequent when heterologous plasma from nontransmitters were used. Macrophage-tropic heterologous HIV-1 isolates were neutralized more frequently at higher titers by plasma from nontransmitters than from transmitters. The results obtained indicate that antiretroviral treatment, lack of success of HIV-1 isolation and high titers of antibodies able to neutralize macrophage-tropic viruses appear to be of importance for protection against HIV-1 vertical transmission for the group of patients studied.

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INTRODUCTION

Immune correlates for HIV protection are still not totally defined, although it seems probable that several host and viral factors interact [1]. Induction of suppressor and cytotoxic T lymphocytes (CTL) and of neutralizing antibodies (NAbs) is considered fundamental for a protective immune response to vaccine candidates [2]. The effectiveness of neutralizing antibodies in natural HIV infection is still being discussed. In persons known as 'long-term nonprogressors' [1] neutralizing antibodies get progressively stronger and broader, eventually surpassing most other anti-HIV-1 neutralizing antibody responses [3]. More recent data indicate that the potency of the neutralizing antibody response is of fundamental importance, at least in animal models [4].

Autologous neutralizing antibodies and antibodies able to neutralize a broad range of primary HIV-1 isolates appear to be important for the prevention of vertical mother-to-child HIV-1 transmission [5–7], although some reports deny the importance of autologous neutralizing antibodies [8]. Similarly, some studies report an association between levels of anti-V3 antibodies and the protection of HIV-1 transmission [9, and others], while other studies found no such correlation [10, and others]. Mother-to-child transmission occurs, in absence of chemotherapy, in 13–48% of pregnancies [11], indicating that some factors must exist that affect the rate of vertical HIV-1

transmission so that the majority of children born by untreated HIV-1 infected women do not become infected [8].

Some factors have been identified that appear to correlate to vertical HIV-1 transmission, although contradictory reports can be found about most of them. For example, high viral loads in pregnant women appear to increase the risk of HIV-1 transmission [12–14], although it appears that high viral loads may predict the risk but not the timing of the vertical transmission [15]. In contrast, a recent report found no significant difference in viral loads in blood of transmitting and nontransmitting mothers [7]. The timing of the vertical transmission appears to be highest during labor and delivery, and risk factors may vary according to the time of transmission [16]. Another factor is the phenotype of the virus that may be transmitted vertically. Rapidly replicating isolates capable of inducing syncytia in T cell lines (SI) appear to be more easily transmitted [17]. However, high replication levels in monocyte-derived macrophages appears to be an important characteristic of HIV-1 transmitted vertically [7, 18]. The selective transmission of HIV-1 phenotypes has been studied, and although genetic variants may be nonselectively [19] or selectively transmitted [20], placental trophoblasts of nontransmitters appear not to be infected by HIV-1, although placental lymphocytes are HIV-1 positive [21]. Since the ACTG 076 study in 1994 [22], treatment of HIV-1 infected pregnant women has been strongly recommended. Some indications even suggest that zidovudine reduces paediatric infection independent of the levels of the maternal virus [13, 23].

The present study was carried out in order to collect data on the influence of HIV-1 neutralizing antibodies on vertical transmission (VT) of HIV-1 in Brazil, by the analysis of plasma from pregnant HIV-1 infected women or women having recently given birth to children with unknown infection status. Owing to the free antiretroviral treatment of all pregnant women in Brazil since 1997, the vertical transmission of HIV-1 has declined, although numbers are not yet available [24]. However, the transmission rate of HIV-1 from mother to child is still significant, and the quest for immunological correlates of protection is still of fundamental importance in the search for a protective vaccine or immunotherapy.

MATERIALS AND METHODS

Patients. The study was cleared by the FIOCRUZ Ethical Committee, and informed consent was obtained from each participant. Pregnant volunteer HIV-1 seropositive patients or mothers with children suspected to be infected with HIV-1 were selected from cohorts of the Evandro Chagas Hospital, IOC, FIOCRUZ, from the State Employee Hospital and from the General Hospital of Nova Iguaçu, Rio de Janeiro, RJ, from 1994 (three patients), 1995 (28 patients), 1996 (27 patients), 1997 (61 patients) to 1998 (12 patients). The HIV-1 infection in mothers was diagnosed by standard ELISA methodology, with Western-blot confirmation. Only 67% of the women had been diagnosed as HIV-1 infected before their first visit, explaining the relatively low percentage (67%) under retroviral treatment at sample collection even after 1997. All 132 women included in this study were accompanied at the respective Hospitals for prenatal care. Samples were identified sequentially from TV01 to TV167. The HIV-1 infection of the children was determined by HIV-1 antibody ELISA at 12 and 18 months of age for children born before January 1996 (31 children) and by polymerase chain reaction (PCR) within 1 and 6 months of birth (carried out by Ruth Dickover, Dr K Nielsen's laboratory, Pediatric Infectious Diseases Department, UCLA, Los Angeles, CA, USA) for children born from 1996 onwards. Milk substitute was given to the mothers to prevent postpartum transmission of HIV-1. The clinical information that was collected was: age of the volunteer, gestational data (week of gestation, previous gestations, probable/actual date of birth), dates of first HIV-1 positive serology and last HIV-1 negative serology, clinical staging, probable exposure category, period and identification of antiretroviral treatment. Additional neutralization assays were carried out with two of the 'rapid/high' HIV-1 isolates obtained from patients TV31 and TV68. The TV31 was a sample collected in 1995 from a 34-year-old woman who had given birth 4 months earlier, asymptomatic, with no antiretroviral treatment, infected through heterosexual contacts, with her first AIDS diagnostic test at sample collection, with 203 CD4 lymphocytes/mm^3, and infected with a B subtype HIV-1. The TV68 was a sample obtained in 1996 from a 25-year-old woman in her 6th month of pregnancy, with advanced disease, and with her first AIDS diagnostic test 1 month before sample collection, 346 CD4 lymphocytes/mm^3, infected with a Bbr subtype HIV-1 (Brazilian variant of the B subtype, characterized by the sequence GWGR at the top of the V3 loop), and who had been receiving zidovudine treatment for 2 weeks.

HIV-1 isolation. HIV-1 was isolated using traditional coculture of patient peripheral blood mononuclear cells (PBMC) with preactivated PBMC from healthy individuals [25]. Not more than 3 passages (= addition of fresh cells) were carried out for production of viral stocks. For two isolates (designated TV31 and TV68), an additional expansion was carried out in PBMC using viral stocks frozen at −70 °C. Normal human blood was kindly donated by the Santa Catarina Institute for Hemophiliacs of Rio de Janeiro. Blood donors were tested for HIV infection, HTLV-I, Hepatitis B and C, Syphilis and for Chagas disease. Only buffy coats from seronegative donors were employed.

Neutralization assay. Supernatants from primary cocultures collected between the 7th and 21st day of coculture were used as viral stocks (and from the additional expansion of TV31 and TV68). The standard assay employing the preincubation of viral dilutions (five five-fold dilutions) with heat-inactivated (30 min/56 °C) plasma diluted to 1 : 10 or 1 : 50 for 1 h at 37 °C followed by the addition of Phytohemagglutinin-activated normal human PBMC (10\5 cells per well, mixture of PBMC's from at least two donors), was used [26, 27]. The neutralization was considered positive when a reduction of at least 75% of the viral input was detected as measured by p24 concentration [28]. As TCID 50% were carried out in parallel to neutralization assays, no primary viral stock (and, in some cases, no more plasma) was available to repeat some of the experiments with insufficient data (results not included) or to further evaluate susceptibility to neutralization by additional plasma. The neutralization was evaluated as (+) = ≥ 50% neutralization of viral input by 1 : 10 diluted plasma (less than 50% neutralization was considered negative); + = ≥ 75% neutralization of viral input by 1 : 50 diluted plasma; + + = ≥ 90% neutralization of viral input by 1 : 50 diluted plasma.

Biological characterization. Supernatants from primary cocultures were used to infect triplicate MT2 cells and normal human preactivated PBMCs in parallel [29]. Positivity was determined by p24 assays on

days 7, 10 and 14 and by syncytium observation. Throughout the paper we have used the term 'macrophage tropism' for all tested isolates that did not induce syncytia. Cocultures with p24 positivity before day 10 (by day 6 or 7) were designated 'rapid', titers of more than 50 pg p24/ml at first positivity were designated 'high' (HIV-1 p24 ELISA DuPont NEN Life Science, Boston, MA, USA).

Seroreactivity. Several custom-made synthetic biotinylated peptides (Chiron Mimotopes, San Diego, CA, USA) were used: (a) corresponding to the V3 loop of HIV-1 subtypes: V3B (NTRKSIHIGPGRAFY), the Brazilian consensus peptides V3Bbr (NTRKSIHMGWGRAFY) and V3Br5 (NTRKSIHIGWGRAFY), V3C (KSIRIGPGQTFYAT), V3D (RQRTHIGPGQALYTT), V3F (RKSIHLGPQAFYTT) and the Brazilian V3Fbr (NTRKSIPLGPGRAFY); (b) corresponding to gp120 V2 loops of HIV-1 subtype B isolate MN (IRDKMQKEYALLYKL) and a Brazilian Bbr primary isolate (VKDKELLEYALFYNL), and (c) peptides corresponding to a neutralizing epitope in gp41 (amino acids 735–752 DRPEGIEEEGEKDRDRS) and to the immunodominant epitope in gp41 of the HIV-1 isolate MN (GFWGCSGKLICTTTVPWNAS). Duplicates of sequentially diluted heat-inactivated plasma were incubated with biotinylated synthetic peptides bound to multiwell plates (Maxisorb, Nunc, Roskilde, Denmark) precoated with streptavidin, using a pools of plasma as negative (HIV-1 negative blood donors) and positive (HIV-1 infected AIDS patients) controls. Specific reactivity was assessed (using two 8 M urea washes after antibody incubation) by peroxidase-conjugated antihuman-IgG binding and peroxide/tetrametilbenzidine revelation [30].

HIV-1 clade determination. The determination of the genetic subtype of the HIV-1 isolates was carried out using the heteroduplex mobility assay (HMA) with the primers and technique described by Delwart *et al.* [31]. Identification of the Brazilian B subtype variant Bbr was carried out using the Fok I restriction fragment length polymorphism determination [32].

Viral load. The number of viral RNA copies per milliliter of plasma was determined using the NASBA kit (Organon, Boxtel, the Netherlands).

Statistical analysis. The Mann–Whitney nonparametric unpaired test for two-tailed *P*-values was used for comparative evaluation of frequencies (Instat Program, GraphPad, San Diego, CA, USA).

RESULTS

Clinical and laboratory data

A total of 167 samples were collected from 146 women corresponding to 148 pregnancies, of which 111 pregnancies resulted in noninfected children and 21 pregnancies in HIV-1-infected children, with an overall HIV-1 transmission rate of 16%. The HIV-1 vertical transmission for 16 of the pregnancies could not be determined as a result of the loss of follow-up before birth. A total of 101 (77%) of the samples were obtained from women in their 10th to 36th week of pregnancy (mean 16% in their first, 35% in their second and 49% in their third trimester), and 31 from women between 1 and 18 months postpartum.

The high transmission rate observed was mostly the result of the very high vertical transmission of HIV-1 observed for the group of women infected in 1995 (11/28), which decreased in 1996 (2/27) and 1997 (5/61) to approximately 10% (comparing 1995 and 1997, $P = 0.0177$). This decrease in transmission occurred in parallel to an increase in frequency of antiretroviral treatment as shown in Fig. 1 (comparing 1995 and 1997, $P = 0.0072$). Antiretroviral treatment of the majority (87%) consisted in zidovudine administered according to the ATCG 076 protocol, while the other women under antiretroviral treatment received 2 nucleoside analogues.

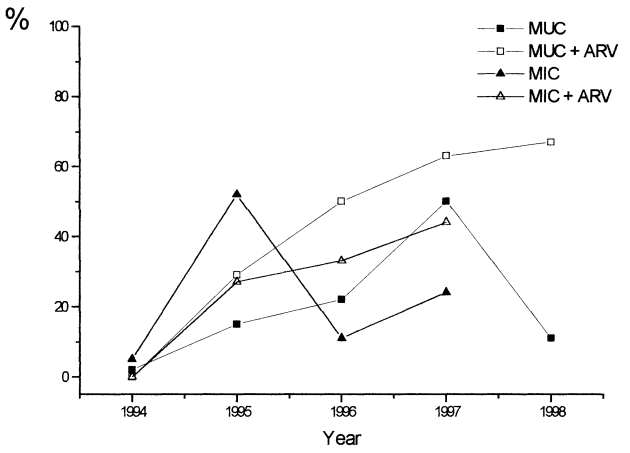

Fig. 1. Percentual distribution of mothers of uninfected children (MUC) and mothers of infected children (MIC) with or without antiretroviral (ARV) treatment between 1994 and 1998.

The majority of the women was between 20 and 34 years old (79%), with no differences observed between the age distribution in the groups of mothers of uninfected children (MUC, mean age 28.0 ± 5.9 SD) and mothers of infected children (MIC, mean age = 28.9 ± 5.9 SD). Infection through heterosexual HIV-1 transmission was reported by the majority of the patients (96% of MUC, 95% of MIC).

As to the disease progression, 56% were asymptomatic at sample collection and 16% were classified as AIDS cases. A slightly higher number of AIDS cases in the group of MIC compared to MUC was observed (15 of 110 MUC versus 6 of 21 MIC). A total of 34% of the women had been seropositive for HIV-1 for more than 1 year, and no significant differences could be observed between MIC and MUC. Number of circulating CD4 lymphocytes was known for 117 of the 167 samples, with 13 samples containing less than 200 CD4/mm^3 (11%) and 46% more than 400 CD4/mm^3. A slightly higher number of MUC (50 of 100) had above 400 CD4/cm^3 than determined for MIC (four of 17, $P = 0.053$).

The HIV-1 subtype was determined for 25 of the isolates obtained, 22 belonging to subtype B (12 for MUC and six for MIC; four of these further identified as the Brazilian Bbr variant: three for MUC, one for MIC) and three to subtype F (one in the MUC and two in the MIC group). However, the number of typed isolates is too small to allow analysis of the significance of subtype distribution among MUC and MIC.

Viral load (VL) was determined for most of the patients giving birth from October 1997 onwards, varying between < 400 (18 of 41 MUC) and 380 000 RNA copies/ml. However, although VL from 41 MUC was available, only two MIC were

Table 1. Autologous neutralization of primary HIV-1 isolates obtained from mothers of uninfected children (MUC) and from mothers of infected children (MIC) and neutralization of the reference strain HIV-1 MN (# isolates neutralized/# isolates tested)

	Autologous neutralization			Neutralization of HIV-1 MN		
	(+)*	+	+ +	(+)	+	+ +
MUC – total	13/16 (81%)	9/16 (56%)	7/16 (44%)	68/96 (71%)	52/96 (54%)	40/96 (42%)
pregnant	11/13 (85%)	9/13 (69%)	7/13 (54%)	63/88 (72%)	41/88 (47%)	31/88 (35%)
MIC – total	5/7	3/7	3/7	12/20 (60%)	6/20 (30%)	4/20 (20%)
pregnant	4/4	2/4	2/4	3/8	0/8	0/8

*(+) = ≥ 50% neutralization, 1 : 10 diluted plasma; + = ≥ 50% neutralization, 1 : 50 diluted plasma; + + = ≥ 90% neutralization, 1 : 50 diluted plasma.

included in the analysis (VL 30 000 and 170 000), not permitting comparative evaluation.

HIV-1 isolation

Isolation of the HIV-1 was more frequently positive from MIC than from MUC: from 45 of 108 MUC and 16 of a total of 20 MIC ($P = 0.0065$). This remained true when the isolation from the blood obtained from pregnant women was compared: 39 of 92 MUC and six of seven MIC ($P = 0.0074$). Treatment with antiretrovirals did not influence the isolation success: eight of 18 MIC from which isolation was achieved were under antiretroviral treatment at sample collection versus 24 of 56 MUC under treatment and 21 of 52 without antiretroviral treatment before sample collection. Similarly, no correlation between the success of HIV-1 isolation with clinical staging of the volunteers was observed.

Biological analysis

Infection of MT2 cells in parallel to preactivated human PBMCs was used to distinguish some of the isolates into T-tropic and M-tropic isolates. Although a slightly greater number of M-tropic isolates was obtained from MUC (eight of 13 versus three of seven MIC isolates), no significant differences with isolates from MIC could be observed. Seven of the MIC isolates could be termed 'rapid/high' (r/h) and three 'slow/low' (s/l), against 15 'r/h' and 10 's/l' MUC isolates, with intermediate characteristics ('r/l' or 's/h') for the remaining isolates. Although five 's/l' isolates were used in neutralization assays, the low titers resulted in low TCID 50%, with a consequent low number of assays and dilutions of plasma analyzed.

Seroreactivity

No statistically significant differences between frequency and titer of binding to synthetic peptides could be observed when plasma from MUC and MIC were compared. Although a slightly higher extent of cross-reactivity for MUC plasma with V3 peptides from different HIV-1 subtypes/variant and between V3-peptides and peptides corresponding to other epitopes was initially observed, these observations could not be verified for the whole group: no statistically significant differences could be observed in the amplitude of peptide binding antibodies between MIC and MUC plasma. Plasma from pregnant women showed a lower percentage of reactivity with these peptides, although statistical significance could not be calculated owing to the limitations presented by the low number of plasma from pregnant MIC available.

Neutralization

Autologous neutralizing antibodies. No statistically significant difference between the frequency or the titer of autologous neutralizing antibodies could be detected in the plasma of MIC (Table 1). However, these results correspond to the whole MUC and MIC groups, including samples from women who were not pregnant at sample collection, and autologous neutralization postpartum may not have been the same during pregnancy. Unfortunately, autologous neutralization assays could be carried out for only four of the MIC- and 13 of the MUC-derived isolates obtained from volunteers which were pregnant at sample collection, and no statistically significant differences were observed between MIC and MUC. No correlation between the detection or the titer of autologous neutralizing antibodies could be established with any of the parameters analyzed (week of pregnancy, antiretroviral treatment, viral load, #CD4/mm^3, date of first seropositive diagnostic assay, clinical staging, isolate phenotype, capacity to neutralize heterologous primary or reference HIV-1).

Neutralization of HIV-1 MN. Comparison of the neutralization potency against a reference HIV-1 strain indicated that although a slightly higher number of plasma from MUC was able to neutralize HIV-1 MN than observed for the plasma from MIC, this difference was more pronounced when only plasma from pregnant volunteers were compared. No statistically significant difference between HIV-1 MN neutralization at 1 : 10 dilutions of plasma from MUC and MIC could be detected ($P = 0.4$), and

Table 2. Comparative neutralization of the primary isolates TV31-V [a T-cell tropic isolate, growing rapidly to high titers (r/h) obtained from a HIV-1 transmitting mother (MIC)] and TV68 [a macrophage-tropic isolate obtained from a pregnant woman who abandoned the Hospital during pregnancy] and the T-cell line adapted (TCLA) isolate HIV-1 MN by plasma from MIC and from non-HIV-transmitting mothers (MUC)

	TV31-V (r/h, T-tropic)			TV68-V (r/h, M-tropic)			HIV-1 MN (r/h, TCLA)			
	+ +	+	(+)	+ +	+	(+)	+ +	+	(+)	– *
MUC-P – total	13/36	17/36	32/36	29/36	29/36	32/36	17/36	18/36	30/36	
pregnant	10/28	12/28	25/28	23/28	23/28	26/28	13/28	14/28	23/28	
MIC-P – total	4/8	5/8	7/8	6/8	7/8	7/8	2/8	3/8	8/8	
pregnant	1/4	1/4	2/4	3/3	3/3	3/3	0/3	0/3	3/3	
TV31-P	+ +					(+)				–
TV68-P	+ +			+ +			+ +			

* = < 50% neutralization, 1 : 10 diluted plasma; (+) = ≥ 50% neutralization, 1 : 10 diluted plasma; + = ≥ 50% neutralization, 1 : 50 diluted plasma; + + = ≥ 90% neutralization, 1 : 50 diluted plasma.

no statistical comparison could be made for higher titer neutralization as no plasma from pregnant mothers that later transmitted HIV-1 to their infants were able to neutralize the MN isolate at levels above 75% at 1 : 50 dilutions, indicating an important difference at these levels of neutralization (Table 1).

Heterologous neutralization of primary HIV-1 isolates. Heterologous neutralization assays were carried out before the results for the diagnosis of the children's HIV-1 infection were available, mostly with 5–10 samples collected sequentially, using fresh supernatants as viral stocks, thereby not permitting a checker-board analysis. One experiment was carried out to compare susceptibility to heterologous neutralization by two 'rapid/high' (r/h) HIV-1 isolates, one T-tropic, isolated from a known MIC volunteer, and one M-tropic isolate, isolated from a woman who had one negative child from an earlier pregnancy, but unfortunately did not return to the Hospital after this first visit before giving birth and was therefore lost to the study. These two HIV-1 primary isolates were tested against plasma from nine MIC and 36 MUC volunteers, of which three MIC and 28 MUC were pregnant, in comparison to the HIV-1 MN neutralization (Table 2). No difference in simultaneous potency or lack of potency in neutralization of these three virus strains could be noted for the MIC or MUC groups of plasma. Although this particular experiment showed no significant differences between the MIC and MUC plasma neutralization potency, an analysis of all collected data showed that the overall neutralization at 75% level potency (1 : 10 diluted plasma) was higher for MUC than MIC plasma (81 of 89 assays versus 41 of 59 assays, $P = 0.0016$). This difference derived from the observation that while plasma from MIC showed a similar frequency in neutralizing both MIC and MUC HIV-1 isolates, MUC plasma were more frequently able to neutralize heterologous MIC at high titers than MUC HIV-1 isolates (Fig. 2).

Segregating results obtained with plasma from pregnant and nonpregnant women, no difference in neutralization potency of plasma from pregnant MIC or MUC against primary HIV-1 isolates could be noted, as the difference indicated above resulted from the greater neutralization potency observed with plasma from the whole group of nontransmitters ($P = 0.0117$ at 90% and $P = 0.0234$ at 75% neutralization levels for 1 : 50 diluted plasma).

Potency of MIC and MUC plasma neutralization against isolates with different phenotype. Although the phenotype of only 20 of the isolates was available, it could be noted that MUC plasma were more potent than MIC plasma against macrophage-tropic (M-tropic) heterologous HIV at high levels ($P = 0.0330$, Fig. 3), while no significant difference could be observed in the neutralization of T-tropic HIV-1 isolates. When isolates were segregated according to their replication velocity and titer in

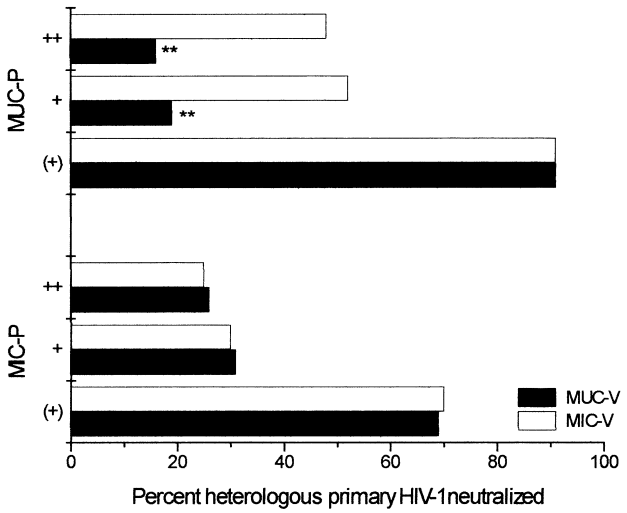

Fig. 2. Frequency of heterologous neutralization of primary HIV-1 isolates derived from transmitting (MIC-V) or non-transmitting mothers (MUC-V) by plasma MUC-P or MIC-P. (+) = ≥ 50% neutralization, 1 : diluted plasma; + = ≥ 75% neutralization, 1 : 50 diluted plasma; ++ = ≥ 90% neutralization, 1 : 50 diluted plasma; $^{**}P<0.05$ (Mann–Whitney).

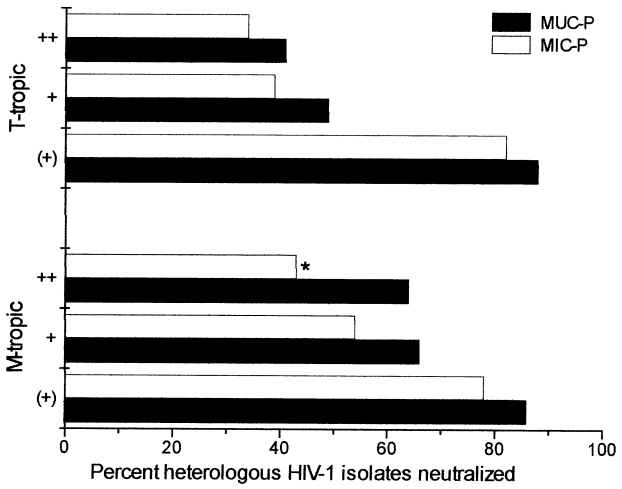

Fig. 3. Neutralization (%) of primary HIV-1 isolates grouped according to their capacity to infect MT2 cells (T-tropic isolates) or not (M-tropic isolates) by plasma from or MIC-P. (+) = ≥50% neutralization, 1 : 10 diluted plasma; + = ≥75% neutralization, 1 : 50 diluted plasma; ++ = ≥90% neutralization, 1 : 50 diluted plasma; *P=0.0330 (Mann–Whitney).

cocultures into 'rapid/high' and 'slow/low' isolates, MUC plasma were slightly more frequently effective at high titers against 'rapid/high' isolates (63 of 96 assays, 55%) than observed with MIC plasma (26 of 53 assays, 49%, $P = 0.09$). Comparison of efficacy in neutralizing 'slow/low' isolates at high titers was not possible as only two assays were carried out using 1 : 50 diluted plasma.

DISCUSSION

Mother-to-child transmission accounts for more than 80–90% of HIV-infected children [6, 24]. There are approximately 13 000 pregnant HIV-1 infected women in Brazil, with 4630 cases of vertical transmission and 40% accumulated deaths (august 1999).

Since 1997, the Zidovudine administration according to the ACTG 076 protocol [22] has been offered for free by the Brazilian Ministry of Health to all pregnant HIV-1-infected women. Before 1997, vertical transmission in Brazil was between 30 and 40%, agreeing with the transmission rates observed elsewhere, ranging from 13 to 48% [11]. The ACTG 076 protocol led to a decline of 68% of vertical HIV-1 transmission in the USA [33]. It is known that the Zidovudine administration leads to diminishing viral loads, but in HIV-1-infected pregnant women this decrease is not as dramatical as the decrease in the vertical HIV-1 transmission observed [13, 23]. In the present study, the HIV vertical transmission decreased significantly from 1995 to 1997 when the antiretroviral treatment became freely available. Despite the higher frequency of antiretroviral treatment in the latter blood samples, HIV-1 isolation was achieved in similar percentages as before, both from MUC and MIC.

Although the immunodeficiency tended to be more advanced in MIC, no significant association of the disease progression and the HIV-1 vertical transmission was observed. However, a significantly higher frequency of HIV-1 isolation was achieved from MIC in comparison to MUC PBMC, as reported before [14]. The possibility of preferential vertical transmission of different HIV-1 genetic subtypes cannot be analyzed based on the data obtained in this study, as the number of typed isolates was too low. As to phenotype, a slightly higher percentage of M-tropic and 'slow/low' isolates was obtained from MUC than from MIC, but no statistical significance was observed, agreeing with an earlier report [7].

Antibody specificity of transmitters and nontransmitters was compared by determining the binding of antibodies to synthetic peptides corresponding to immunologically important epitopes of different HIV-1 subtypes, but no difference between specificity or range of antibody binding could be observed, confirming previous studies [10, and others].

Neutralizing antibodies should be expected to be an important factor in protecting the fetus/infant from infection by the mother's HIV-1, as they can pass the placental barrier and would be present during delivery. However, it seems important that these antibodies be able to neutralize the HIV-1 strain circulating in the mothers blood during pregnancy/delivery, i.e. to be effective against the autologous viral strain. High titers of highly effective autologous neutralizing antibodies were detected in approximately 50% of the plasma tested, irrespective of the HIV-1 transmitting or nontransmitting status of the mothers. These data indicate that autologous neutralizing antibodies, even at high titers and neutralization levels, are not able to prevent vertical HIV-1 transmission on their own. However, several aspects probably converge to define transmission or nontransmission of HIV-1, and a high concentration of infectious HIV-1, as indicated by the high isolation rates and (in other studies) by high viral loads, may surpass the antibodies' capacity to neutralize all infectious HIV-1.

Neutralization of T-cell line adapted HIV-1 isolates such as the MN or LAI strains has been reported to be higher in nontransmitting mothers [6, and others], as also reported for slow- or nonprogressing patients [34], although some reports disagree with this finding [8, 35, and others]. In the present study, the efficacy of high titer HIV-1 MN neutralization was frequent for plasma from nontransmitters, even during pregnancy, a fact not observed for samples collected from pregnant HIV-1 transmitters. The 'trend to higher titer cross-neutralization of a heterologous T-cell line adapted HIV-1 isolate' [6, and as described before] was thus confirmed in this study.

The capacity to effectively neutralize several heterologous primary HIV-1 isolates was higher for the plasma from nontransmitting mothers, confirming a trend also observed by others [3]. However, a lower susceptibility for neutralization of primary isolates from transmitting mothers, reported by Lathey *et al*. [7], has not been observed in this study.

Indeed, isolates from HIV-1 transmitting mothers, although not very susceptible to neutralization by plasma from other transmitters, was significantly more so to plasma from

nontransmitting mothers. However, plasma from transmitters were equally efficient in neutralizing viral isolates obtained both from transmitting and nontransmitting mothers. This leads to the supposition that there may be a lack of some specific neutralizing immune response in transmitters, compared to nontransmitters, while the virus susceptibility to neutralization may be similar for transmitters and nontransmitters, i.e. indicating that vertical HIV-1 transmission may not be a difference between viruses but in the effectiveness of the immune response raised idiosyncratically in the infected women.

This may be more important in association with the zidovudine treatment, owing to as yet not identified effects of the zidovudine treatment apart from the observed reduction of the viral load [13, 23]. One possible explanation could be that, by reducing the activation susceptibility of the CD4 lymphocytes [36], the zidovudine treatment may indirectly lead to higher levels of cell-free virus, susceptible to neutralizing antibodies. The possibility that a more frequent neutralization could result from a direct effect of plasma zidovudine in the assay can be ignored as the effect was noted only in higher dilutions of plasma, at levels above those reported for this interference [35].

One important observation of this study was the difference in neutralization potency of plasma from pregnant and nonpregnant nontransmitters, not observed for HIV-1 transmitters. This may indicate that, although immunosuppression is known to occur during pregnancy [37], nontransmitters retain sufficient specific antibodies to contain transmission of HIV-1 to their fetus/child.

The more frequent high level neutralization of macrophage-tropic primary HIV-1 isolates by plasma from nontransmitters may be an important indication of the importance of the specificity of HIV-1 neutralizing antibodies for the prevention of vertical HIV-1 transmission, as high replication in monocyte-derived macrophages appears to be an important characteristic of HIV-1 transmitted vertically [7, 18].

In conclusion, this study indicates that, as observed in another context in the study of Moore & Burton [4], only specific high titer neutralizing antibodies appear to be associated with protection, whether for the protection of monkeys from SHIV challenge or for the protection against vertically transmitted HIV-1.

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