Co-receptor usage of HIV-1 primary isolates, viral burden, and CCR5 genotype in mother-to-child HIV-1 transmission

Lucia Ometto[a], Marisa Zanchetta[a], Monica Mainardi[a], Gian Luca De Salvo[b], Marie Cruz Garcia-Rodriguez[c], Linsay Gray[d], Marie Louise Newell[d], Luigi Chieco-Bianchi[a] and Anita De Rossi[a]

Objective: To investigate the relationship between CC chemokine receptor 5 (CCR5) genotype, viral load and co-receptor usage of maternal HIV-1 isolates in perinatal HIV-1 transmission.

Patients and methods: A total of 181 mothers and infants were studied at the time of delivery. Wild-type (wt) and Δ32 CCR5 alleles were determined by means of polymerase chain reaction (PCR). The viral load in maternal plasma samples was determined by a quantitative reverse transcriptase–PCR assay; co-receptor usage of maternal isolates was determined by viral infection in cells stably expressing CCR5 or CXC chemokine receptor 4 (CXCR4) co-receptors.

Results: HIV-1 transmission rates in wt/wt and wt/Δ32 mothers (14.7 versus 15.8%), and in wt/wt and wt/Δ32 infants (14.6 versus 14.3%) were similar. Mothers transmitting infection to wt/Δ32 infants had significantly higher HIV-1-RNA levels than those who transmitted infection to wt/wt infants (5.4 versus 4.1 $\log_{10}$ copies/ml, $P = 0.03$). In wt/wt children there was a positive relationship between transmission rate and maternal viral load over the entire range of HIV-1 values, whereas in wt/Δ32 children transmission occurred only at viral loads greater than 4.0 $\log_{10}$ copies/ml. Logistic regression analysis confirmed that the relationship between viral load and transmission varied according to the child's CCR5 genotype ($P = 0.035$; adjusted for zidovudine prophylaxis and mode of delivery, $P = 0.090$). Moreover, the majority of wt/wt transmitting mothers had R5-type isolates, whereas none of the wt/Δ32 mothers with an R5-type virus transmitted HIV-1 to their wt/Δ32 infants.

Conclusion: Taken together, these findings suggest that CCR5 Δ32 heterozygosity exerts a protective effect against perinatal transmission in children exposed to a low maternal viral burden of an R5-type isolate.

AIDS 2000, **14**:1721–1729

Keywords: CCR5 genotype, HIV-1 co-receptors, HIV-1 load, perinatal transmission

From the [a]Department of Oncology and Surgical Sciences, Oncology Section, AIDS Reference Center, University of Padua, Italy; [b]Clinical Epidemiology Section, Azienda Ospedaliera, Padua, Italy; [c]Unit of Immunology, Hospital La Paz, Madrid, Spain; and [d]Department of Epidemiology and Public Health, Institute of Child Health, London, UK.

Spondorship: This study was supported by Istituto Superiore di Sanita, grant nos. 9402-07 and 30A.0.17.

Corresponding author: Anita De Rossi, Department of Oncology and Surgical Sciences, Oncology Section, University of Padua, Via Gattamelata 64, 35128 Padua, Italy.

Tel: +39 049 8215822; fax: +39 049 8072854; e-mail: derossia@ux1.unipd.it

Received: 20 March 2000; accepted: 31 March 2000.

Introduction

HIV-1 enters target cells using the CD4 cell receptor in conjunction with co-receptor molecules. Among the various chemokine receptors that may support virus entry, the CC chemokine receptor 5 (CCR5) and the CXC chemokine receptor 4 (CXCR4) have been identified as the major co-receptors of macrophage-tropic non-syncytium inducing (NSI) and T cell tropic syncytium-inducing (SI) primary isolates, respectively [1]. It was further demonstrated that the majority of NSI isolates utilize only CCR5, whereas most SI isolates, besides CXCR4, also use additional co-receptors to enter target cells [2,3].

Homozygosity for a 32 basepair deletion (Δ32) within the CCR5 gene is associated with a substantial resistance to HIV-1 infection *in vivo* [4,5]; in-vitro studies have confirmed that peripheral blood lymphocytes and monocytes from Δ32 homozygotes do not express CCR5 protein on the cell membrane, and are resistant to infection by NSI-type strains [6,7]. On the other hand, the role of Δ32 heterozygosity in transmission and disease progression is still controversial. Compared with peripheral blood mononuclear cells (PBMC) from wild-type (wt) homozygous individuals, PBMC from Δ32 heterozygous individuals express lower levels of CCR5 molecules on their surface, show a lower infectability *in vitro* by NSI isolates [8,9], and require a significantly higher inoculum of NSI virus to become infected [10]. Although a body of evidence suggests that Δ32 heterozygosity does not prevent infection in adults [11–15], it was recently suggested that it might protect against heterosexual transmission [16]. Moreover, several investigations [12,13] showed that Δ32 heterozygosity correlated with a lower level of HIV-1 RNA in plasma; however, its protective role against disease progression was suggested by some studies [11,14,15], but was not fully confirmed in others [12,17]. The finding that a survival advantage occurred in Δ32 heterozygous patients harbouring NSI but not SI isolates [18] supports the notion that the role of Δ32 heterozygosity might depend on the co-receptor usage of the infecting strains.

A protective effect of Δ32 heterozygosity in perinatal transmission has been advanced in some studies [19,20], but has not been confirmed in others [21–23]; none of these investigations, however, addressed the role of the infant's CCR5 genotype in relation to the load and co-receptor usage of the maternal viral isolate. As perinatal transmission mostly occurs around the time of delivery [24,25], studies on mother/child pairs offer a rare opportunity to study this issue. We investigated the mother's and the child's CCR5 genotype, the maternal viral load, and the co-receptor usage of the maternal viral isolate in a large cohort of HIV-1-infected mothers and their infants.

Patients and methods

A total of 181 HIV-1-seropositive mothers and their 186 infants were studied. Five mothers delivered twins; three pairs of twins were HIV-1 negative, and in each of the remaining two pairs only the first-born was HIV-1 infected. Because of the small number of twins, dependence between twin pairs was ignored, and each infant was treated as an independent outcome. Our study population included two cohorts. One consisted of 117 infants born between June 1991 and June 1997, whose virological analyses were conducted at the AIDS Reference Center, Padua University, and for whom maternal samples (n = 113) collected within 15 days of delivery were available; these mothers and their infants attended seven hospitals in north Italy. The other cohort consisted of 68 mothers and their infants (n = 69), from whom samples were collected from July 1991 to July 1994 at the Hospital Infantil La Paz, Madrid, within the framework of the European Collaborative Study [26]. The infectious status of the infants followed in Padua was defined by virus isolation and polymerase chain reaction (PCR) assays, performed as previously reported [27]; infection in the children followed in Madrid was defined by the persistence of HIV-1-specific antibodies after 18 months of age.

CCR5 genotyping

To detect wt and Δ32 alleles, PCR was performed using 5′–CCTGGCTGTCGTCCATGCTG–3′ and 5′–CTGATCTAGAGCCATGTGCACAACTCT–3′ primers [28], which flank the 32 basepair (bp) deletion within the CCR5 gene. PBMC were lysed as described [24], and 5 μl cell lysate (corresponding to 2×10^4 cells) were mixed in 50 μl final volume of PCR buffer (10 mM Tris HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$) containing 50 pmol of each primer, 200 μM of each deoxynucleoside triphosphate, 1 μCi α-P^{33}-dATP, and 2.5 U AmpliTaqGold DNA polymerase (Perkin Elmer, Roche Molecular Systems, Inc., Branchburg, NJ, USA). A pre-PCR heating step of 95°C for 10 min to activate AmpliTaqGold DNA polymerase, and 30 amplification cycles, each consisting of 94°C for 1 min 30 s, 60°C for 1 min, and 72°C for 1 min, were carried out in an automated DNA thermal cycler (Gene Amp PCR system 2400, Perkin Elmer, Norwalk, CT, USA). Amplification products were digested with 10 U ECO RI restriction enzyme for 60 min at 37°C, electrophoresed on an 8% polyacrylamide gel, and exposed to X-ray film. After digestion, the wt amplification product was cleaved into 332 and 403 bp fragments, whereas the Δ32 product was cleaved into 332 and 371 bp fragments.

To detect the CCR5m303 point mutation, PCR was performed using 5'–GGTGGAACAACATGGATTAT CAAGTGT–3' and 5'–AAACTAAGCCATGTGCA CAACTCTGAC–3' primers. Amplification products

were digested with 10 U *Hinc*II restriction enzyme for 60 min at 37°C, electrophoresed on a 4% polyacrylamide gel, and then exposed to X-ray film. After *Hinc*II digestion, the wt amplified fragment was cleaved into 315 and 809 p fragments, whereas the mutated fragment was not cleaved because of the loss of the *Hinc*II restriction site.

HIV-1-RNA quantification

HIV-1-RNA levels in maternal plasma samples were determined by reverse transcriptase–PCR (Amplicor, Roche Molecular Systems, Branchburg, NJ, USA). The lower limit of detection for this assay was 200 copies/ml; samples in which HIV-1 RNA could not be detected were assigned the value of 100 copies/ml in order to include viral load as a continuous variable in the statistical analyses. Similar results were obtained when statistical analyses were performed by substituting the value of 100 with a random number between 1 and 200. HIV-1-RNA values were expressed on a logarithmic scale (base 10).

Viral phenotype analyses

Primary isolates were obtained by culturing PBMC from each subject with an equal amount of phytohemagglutinin (PHA)-stimulated PBMC from healthy donors, as previously described [29]. Viral isolation was successful in 21 out of 25 (84%), and in 95 out of 144 (66%) available PBMC samples from transmitting and non-transmitting mothers, respectively. Primary isolates were propagated by a single short-term passage (7 days) in PHA-stimulated donor PBMC, as reported [29]. Co-receptor usage was determined by viral infection in U87.CD4 cells stably expressing CCR5 or CXCR4 co-receptors [30], as previously reported [31]. U87.CD4 cells were used as controls. Briefly, cells were resuspended in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum, and plated in 24 well tissue culture plates (0.2×10^5 cells/well); after 24 h, the cells were rinsed with DMEM, and exposed to a 10 ng p24 equivalent dose of HIV-1 isolate in a 1 ml final volume. After 24 h the plates were washed, and 1 ml complete medium was added to each well. On days 4 and 8, supernatants were collected and tested for p24 antigen using a commercially available assay (HIV-1 p24 Core Profile enzyme-linked immunosorbent assay, Dupont Medical Products, Boston, MA, USA). As control, U87.CD4 cells were exposed to the same amount of each isolate and cultured under the same conditions; p24 values obtained in these control cultures were always below 100 pg/ml; a culture was considered positive when the p24 value was over 100 pg/ml. In agreement with the recently proposed classification [1], the viruses were typed R5, X4, or R5X4 according to their co-receptor usage.

Statistical analysis

Univariate comparisons of maternal or child's characteristics by transmission status were tested for significance using the χ^2 test for categorized variables, and the non-parametric Kruskall–Wallis test for continuous variables. Multivariate logistic regression analyses were performed to model the relationship between the child's infection status and maternal viral load, taking into account zidovudine treatment, mode of delivery and CCR5 genotypes. In all statistical analyses, the viral load was assessed as a continuous variable. For HIV-1-RNA data, statistical analyses were performed using log-transformed values. Analyses were carried out using SAS statistical software (SAS Institute, Cary, NC, USA).

Results

Our analyses were performed on a total of 186 mother/child pairs. Of the 186 infants, 27 acquired HIV-1 infection, including two (both first-born) from the five pairs of twins. The overall rate of transmission was 14.5%, which is within the confidence interval of the European Collaborative Study [26]. It should be pointed out that in this cohort only 23 mothers (20 non-transmitting and three transmitting) received zidovudine during their pregnancy, and of these 15 (14 non-transmitting and one transmitting) were administered the drug as prophylactic treatment according to the ACTG 076 protocol [32]. The HIV-1 transmission rate was higher in mothers having vaginal or emergency caesarean delivery (20.6%) than in mothers having elective caesarean delivery (4.2%), but the difference was not significant in this cohort ($P = 0.09$) (Table 1). The transmission rate among wt/wt and wt/Δ32 mothers was similar (14.7 and 15.8%, respectively), and the frequency of HIV-1 infection in wt/wt and wt/Δ32 children was the same (14.6 and 14.3%, respectively) (Table 1). Not one Δ32 homozygote was identified; the m303 allele was detected in only one uninfected infant who did not carry the Δ32 deletion [33], and in none of the mothers. The only variable that significantly correlated with an increased risk of viral transmission was the HIV-1-RNA burden at the time of delivery; indeed, the odds ratio (OR) value for one unit increase in $\log_{10}$ HIV-1-RNA copies was 1.9 (confidence interval: 1.26–2.88, $P = 0.002$) (Table 1). There was no evidence of non-linearity in the relationship between HIV-1 vertical transmission and maternal viral load on the logit scale. Treatment with zidovudine, mode of delivery, mother's and child's CCR5 genotype, and RNA viral load were included in a multivariate logistic regression analysis, with infection status as the dependent variable (multivariate analysis 1, Table 1). As the mother's and child's CCR5 genotypes were highly correlated ($r = 0.51$), only the child's genotype was used in the subsequent multivariate analysis, which included effects of viral load, zidovudine and mode of delivery (multivariate analysis 2,

Table 1. Vertical transmission of HIV-1 by selected variables

Variable	No. mother/child pairs	No. infected children (%)	Univariate analysis		Multivariate analysis 1		Multivariate analysis 2	
			Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value
Zidovudine treated								
No	107	20 (18.7)	1.53 (0.41–5.66)	0.52	0.62 (0.12–3.10)	0.56	0.85 (0.19–3.77)	0.83
Yes	23	3 (13.0)	1		1		1	
Mode of delivery								
Vaginal/emergency cesarean	107	22 (20.6)	5.95 (0.76–46.50)	0.09	6.36 (0.61–66.48)	0.12	6.37 (0.70–57.61)	0.10
Elective cesarean	24	1 (4.2)	1		1		1	
Mother's CCR5 genotype								
wt/wt	150	22 (14.7)	0.91 (0.24–3.38)	0.89	1.77 (0.23–13.62)	0.58		
wt/Δ32	19	3 (15.8)	1		1		–	
Child's CCR5 genotype								
wt/wt	158	23 (14.6)	1.02 (0.32–3.22)	0.97	0.64 (0.13–3.12)	0.58	0.85 (0.23–3.17)	0.81
wt/Δ32	28	4 (14.3)	1		1		1	
1 unit increase in log_{10} HIV-1-RNA copies/ml			1.90 (1.26–2.88)	0.002	1.61 (1.01–2.58)	0.04	1.64 (1.04–2.59)	0.03

CI, Confidence interval.

Table 1). Results of both multivariate analyses showed that only the maternal viral burden was positively correlated with transmission.

Viral burden and CCR5 genotype

The median value of HIV-1-RNA copies/ml plasma was significantly higher in transmitting mothers [4.2 $\log_{10}$, interquartile range (IR) 3.6–5.0] than in non-transmitting mothers (3.6 $\log_{10}$, IR 2.0–4.2, $P = 0.004$) (Fig. 1a). Of interest was the fact that when the viral load was analysed in relation to the mother's CCR5 genotype, there was less difference in viral load between wt/wt transmitting and non-transmitting mothers (4.1 $\log_{10}$ versus 3.6 $\log_{10}$, $P = 0.06$) than between wt/Δ32 transmitting and non-transmitting mothers (5.9 $\log_{10}$ versus 3.3 $\log_{10}$, $P = 0.02$) (Fig. 1b). Similar findings were obtained when the maternal viral load was analysed in relation to the infant's CCR5 genotype; the difference in viral load between transmitting and non-transmitting mothers having wt/wt infants (4.1 $\log_{10}$ versus 3.5 $\log_{10}$, $P = 0.03$) was lower than that between transmitters and non-transmitters having wt/Δ32 infants (5.4 $\log_{10}$ versus 3.7 $\log_{10}$, $P = 0.01$) (Fig. 1c). Moreover, mothers who transmitted the infection to wt/Δ32 infants had a significantly higher HIV-1-RNA level than those who transmitted the infection to wt/wt infants (5.4 $\log_{10}$ versus 4.1 $\log_{10}$, $P = 0.03$). When four categories of maternal viral burden were considered ($\leqslant 3.0 \log_{10}$, $> 3.0 - \leqslant 4.0 \log_{10}$, $> 4.0 - \leqslant 5.0 \log_{10}$, $> 5.0 \log_{10}$), the respective transmission rates were 9% (5/56), 13% (6/47), 19% (8/43), 33% (4/12) in wt/wt infants, and 0% (0/5), 0% (0/8), 22% (2/9), 33%(2/6) in wt/Δ32 infants; similarly, the transmission rates were 11% (5/47), 13% (6/47), 17% (7/42), 29% (4/14) in wt/wt mothers, and 0% (0/7), 0% (0/4), 25% (1/4), 50% (2/4) in wt/Δ32 mothers. These data suggested that the relationship between viral load and transmission differed by both the child's and the mother's CCR5 genotype.

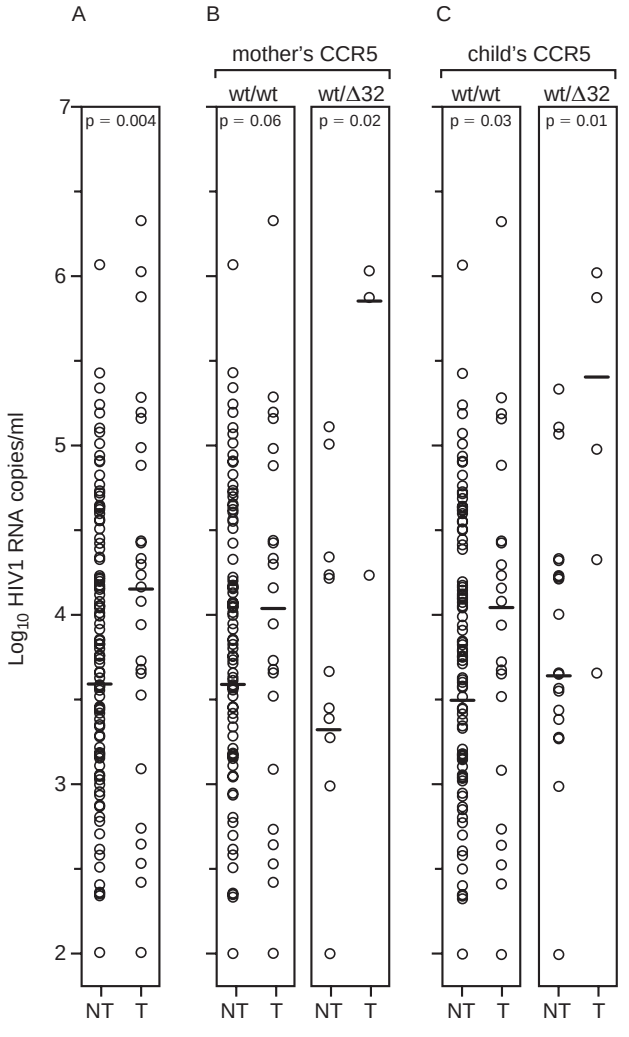

Fig. 1. (a) HIV-1 viral burden in transmitting and non-transmitting mothers was analysed according to mother's (b) and child's (c) CCR5 genotype. In each group, the horizontal bar represents the median value; *P* values were calculated by the Kruskal–Wallis test. NT, Non-transmitting; T, transmitting.

The relationship between maternal viral load and HIV-1 transmission was then modelled accounting for confounding and interaction effects of the child's or mother's genotype, zidovudine treatment, and mode of delivery. Considering first the singular effects of each of the variables, there was evidence of an interaction of maternal viral load with the child's genotype ($\chi^2 = 4.44$, $P = 0.035$) and with the maternal genotype ($\chi^2 = 3.78$, $P = 0.052$), and zidovudine and the mode of delivery were found to confound the relationship between maternal viral load and HIV-1 transmission. Fig. 2 shows clearly the distinction in the relationship between viral load and vertical transmission for wt/wt and wt/Δ32 children, based on the model including the interaction between the child' s CCR5 genotype and viral load; the apparent non-linear nature of this relationship is due to the linearity on the logit scale, resulting in the curvature of the relationship on the actual scale. This evidence led to fitting the model with zidovudine, mode of delivery and interaction of viral load with the child's CCR5 genotype for comparison with the multivariate analysis without the interaction term (multivariate analysis 2, Table 1). The resultant final model with borderline significant interaction term ($\chi^2 = 2.87$, p= 0.090) provided the estimates for the OR of 1 unit increase in log viral load for wt/wt and wt/Δ32 children shown in Table 2. The OR of 1.38 (0.84–2.25) for 1 unit increase in log viral load for wt/wt children adjusted for zidovudine treatment and mode of delivery did not differ greatly from that in the multivariate 2 analysis, but was not significant ($P = 0.204$). For a 1 unit increase in log viral load for wt/Δ32 children, there was a nearly sixfold increase in the risk of vertical transmission (OR 5.99, 0.72–49.97)

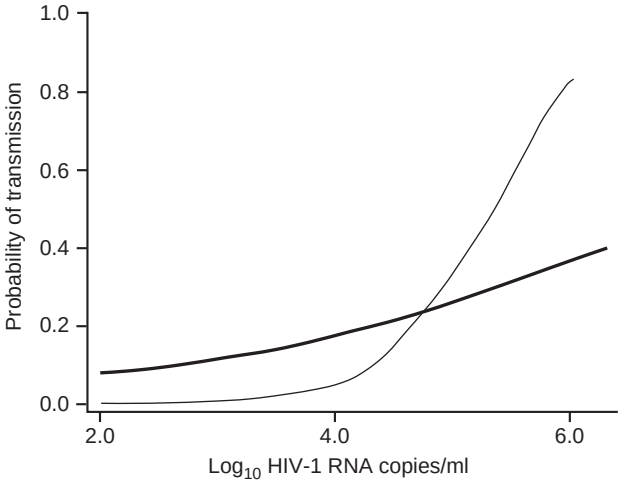

Fig. 2. Influence of maternal viral load on the probability of vertical transmission allowing for interaction between child's CCR5 genotype and viral load. Thick and fine lines represent estimated risk for wt/wt and wt/Δ32 children, respectively.

Table 2. Odds ratios of vertical transmission for a 1 unit increase in $\log_{10}$ viral load for each child CCR5 genotype in the model including interaction term, adjusted for zidovudine treatment and mode of delivery

	Odds ratio (95% CI)	*P* value
1 unit increase in $\log_{10}$ HIV-1 RNA copies/ml		
wt/wt CCR5 children	1.38 (0.84–2.25)	0.204
wt/Δ32 CCR5 children	5.99 (0.72–49.97)	0.098

CI, Confidence interval.

with borderline significance ($P = 0.098$). With adjustment by AZT treatment and mode of delivery, the individual ORs for 1 unit increase in log viral load by child's genotype from the model including interaction are less significant than the single OR ($P = 0.033$) from the model with no interaction. However, findings must be interpreted bearing in mind the limited data. These findings support the theory that the relationship between maternal viral load and transmission differed by the child's CCR5 genotype. In particular, in wt/Δ32 children, but not in wt/wt children, when maternal viral load is low, the risk of vertical transmission is lower in the former. The significance of the interaction term of maternal viral load and maternal CCR5 genotype, adjusted for zidovudine treatment and mode of delivery, was not further investigated because CCR5 typing data were not available for all the mothers; the subsequent reduction in sample size precluded reliable estimates.

Viral phenotype

It was recently suggested that CCR5 Δ32 heterozygosity constitutes a selective pressure for infecting viruses using an alternative co-receptor [34]. It has also been demonstrated that Δ32 heterozygosity is associated with a lower viral burden in individuals infected with an R5-type isolate [12,13]. In the light of these data and our findings, we hypothesized that highly viraemic wt/Δ32 transmitting mothers might harbour a primary isolate with an expanded co-receptor usage. To investigate this possibility, 116 available maternal primary viral isolates were analysed for their co-receptor usage. Positive results in indicator cells expressing the CCR5 or CXCR4 co-receptor were obtained in 104 cases; 83 isolates used only the CCR5 co-receptor (R5-type isolate), whereas 21 used the CXCR4 molecule; in line with previous observations [2,3], all but two of the latter isolates also showed a CCR5 co-receptor usage (R5X4-type isolates).

R5-type isolates were detected in 73 wt/wt, and in 10 wt/Δ32 mothers; the median value of HIV-1 RNA was lower in wt/Δ32 than in wt/wt mothers (3.1 $\log_{10}$ versus 3.7 $\log_{10}$ copies/ml). Twelve wt/wt mothers transmitted the infection to their infants, all of whom but one had a wt/wt genotype (Fig. 3a); only one wt/Δ32 mother transmitted the infection (Fig. 3b). Of interest is the fact that this mother delivered twins; the first-born was infected and had a wt/wt genotype, whereas the second-born was uninfected and had a wt/Δ32 genotype.

R5X4-type isolates were detected in 14 wt/wt and five wt/Δ32 mothers, median RNA values were 4.7 $\log_{10}$ and 5.1 $\log_{10}$ copies/ml, respectively; five wt/wt and two wt/Δ32 mothers transmitted the infection to their infants whose CCR5 genotype was concordant with that of the mother in every case. None of the two mothers with a wt/wt genotype, and an X4-type isolate transmitted the infection.

Discussion

This study addressed the role of the mother's and infant's CCR5 genotype in relation to the maternal viral burden, and the co-receptor usage of the maternal viral isolate.

We found that the frequency of the wt/Δ32 genotype was similar in infected and uninfected children, as well in transmitting and non-transmitting mothers, thus indicating that the heterozygous genotype in itself does not protect against mother-to-child HIV-1 transmission. Of interest is the fact that in wt/wt children there was a positive relationship between maternal viral load and transmission over the entire range of HIV-1 values, whereas in wt/Δ32 children transmission occurred only at viral loads greater than 4.0 $\log_{10}$ copies/ml. Similar observations were found when the mother's CCR5

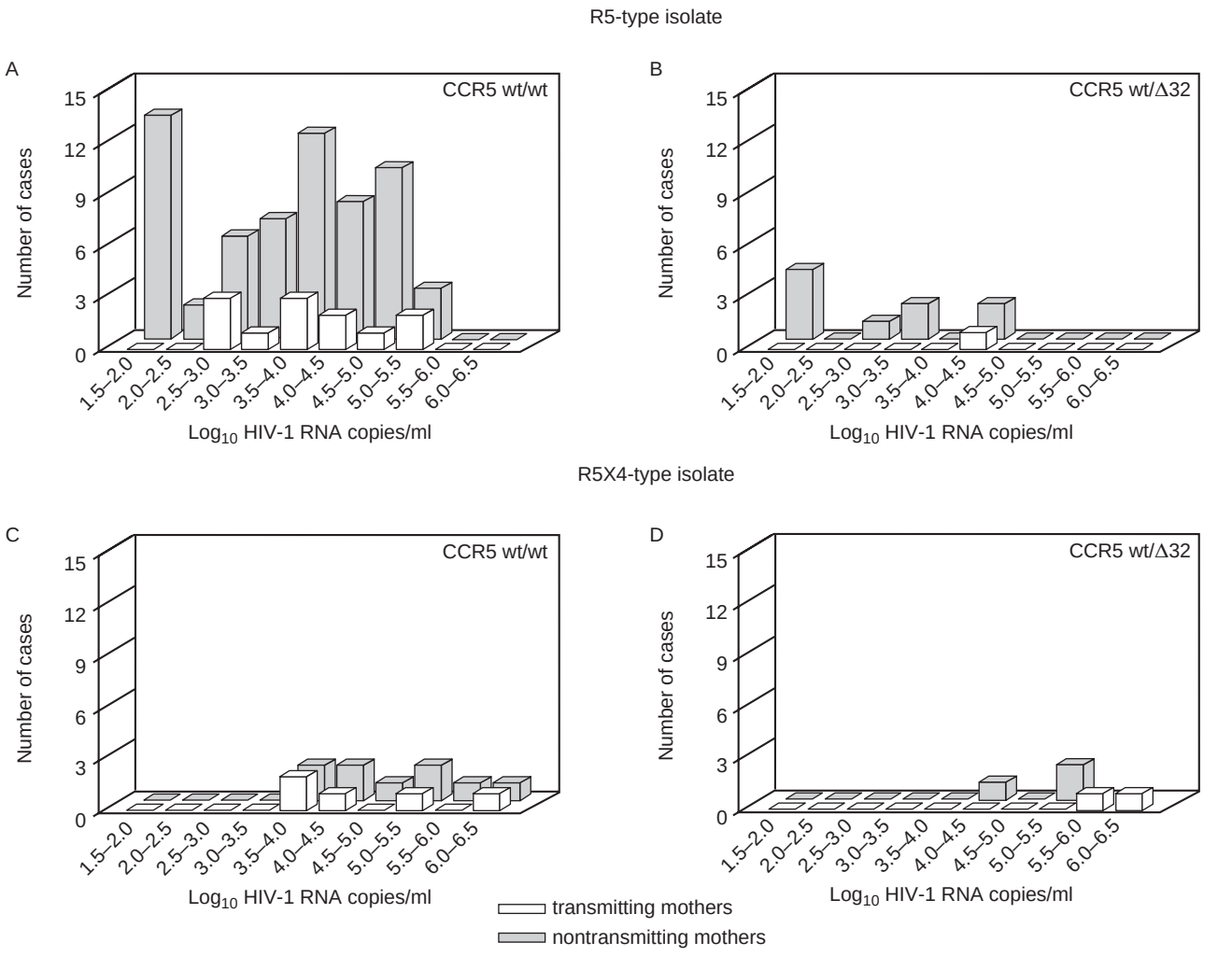

Fig. 3. Mothers were stratified according to viral burden (half $\log_{10}$ intervals from 1.5 to 6.5), CCR5 genotype, and phenotype of viral isolates. The number of transmitting and non-transmitting mothers in the different $\log_{10}$ categories is reported for (a) wt/wt and (b) wt/Δ32 mothers having R5-type isolates and for (c) wt/wt and (d) wt/Δ32 mothers having R5X4-type isolates.

genotype was considered. Logistic regression analysis disclosed that, depending on the child's CCR5 genotype, the relationship between maternal viral load and transmission differed significantly ($P = 0.035$; adjusted for zidovudine treatment and mode of delivery, $P = 0.090$). In addition, viral phenotypic analysis revealed that the majority of the wt/wt mothers transmitting infection to wt/wt infants had an R5-type isolate, whereas none of the wt/Δ32 mothers with this isolate transmitted the infection to their heterozygous infants; moreover, in mothers carrying an R5X4-type isolate, the risk of transmission was not dependent on their CCR5 genotype.

It was shown that Δ32 heterozygous cells express lower levels of CCR5 molecules on their surface than wt/wt cells [8]; moreover, PBMC from heterozygous individuals showed a lower infectability by R5-type strains than PBMC from wt/wt individuals [8,9]. As most of the infants, including all the heterozygotes, born to low viraemic mothers were exposed to an R5-type isolate, it is likely that the protective effect of the wt/Δ32 genotype in the child might be associated with a reduced expression of the receptors on the cell surface, leading to a decreased efficiency of R5-type virus entry in lymphocytes and macrophages. Moreover, in agreement with previous observations [12,13], it was found that among mothers infected with an R5-type isolate, the heterozygotes had a lower viral burden than those with the wt/wt genotype; this finding, combined with the fact that the majority of the heterozygous mothers delivered heterozygous infants, might explain why the wt/Δ32 mothers with an R5-type isolate or a low viral load had a lower risk of transmitting infection than the wt/wt mothers.

It was recently reported that PBMC from heterozygous individuals require a significantly higher viral inoculum to become infected by an R5-type isolate than PBMC from wt homozygous individuals [10], and that R5X4-type isolates were able to infect target cells regardless of

the CCR5 genotype [10,35,36]. In line with these in-vitro observations, our results indicated that the protective effect of the heterozygous genotype disappears when the child is exposed to a high maternal viral load, or to a virus with expanded co-receptor usage. In this regard, it should be pointed out that highly viraemic, transmitting and non-transmitting, wt/wt mothers had an R5- or an R5X4-type isolate, whereas highly viraemic wt/Δ32 mothers consistently carried an R5X4-type isolate. Despite the small number of cases studied, this finding is consistent with the recent suggestion that Δ32 heterozygosity constitutes a selective pressure for infecting strains with expanded co-receptor usage [34].

Studies by Mandl *et al.* [19] and Shearer *et al.* [20] suggest a protective effect of Δ32 heterozygosity in perinatal HIV-1 transmission, whereas those by Eldestein *et al.* [21], Rousseau *et al.* [22], Misrahi *et al.* [23], and Mangano *et al.* [37] argue against such an effect. Our findings suggest that the role of the CCR5 genotype may differ depending on the type and level of viral exposure, and thus strengthen the need to investigate the role of host factors, such as the co-receptor's genotype, in the context of relevant viral properties, such as the viral inoculum and virus co-receptor usage.

Acknowledgements

The authors would like to thank D. Truscia (Department of Gynecology and Obstetrics, University of Padua), C. Giaquinto (Department of Pediatrics, University of Padua), M. De Martino and L. Galli (Department of Pediatrics, University of Florence), A. Maccabruni (Departement of Infectious Diseases, University of Pavia), D. Caselli (Department of Pediatrics, University of Pavia), A. Mazza (Department of Pediatrics, General Hospital of Trento), L. Battisti (Department of Pediatrics, General Hospital of Bolzano), L. Gambaretto (Department of Pediatrics, General Hospital of Mantua), M. Franzetti (Department of Infectious Diseases, General Hospital of Vicenza), I. Bates. I. de José, and F. Hawkins (Hospital Infantil La Paz, Madrid) for providing the patient samples and clinical information. The authors would also like to thank Dan R. Littman for providing U87.CD4-CCR5 and U87.CD4-CXCR4 cell lines, Pierantonio Gallo for artwork, and Patricia Segato for help in preparing the manuscript.

References

1. Berger EA, Doms RW, Fenjo EM, *et al.* **A new classification for HIV-1.** *Nature* 1998, **391**:240.
2. Simmons G, Wilkinson D, Reeves J, *et al.* **Primary, syncytium-inducing human immunodeficiency virus type 1 isolates are dual-tropic and most can use either Lestr or CCR5 as coreceptor for virus entry.** *J Virol* 1996, **70**:8355–8360.
3. Zhang Y-J, Dragic T, Cao Y, *et al.* **Use of coreceptors other than CCR5 by non-syncytium-inducing adult and pediatric isolates of human immunodeficiency virus type 1 is rare *in vitro*.** *J Virol* 1998; **72**:9337–9344.
4. Liu R, Paxton WA, Choe S, *et al.* **Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection.** *Cell* 1996, **86**:367–377.
5. Paxton WA, Martin SR, Tse D, *et al.* **Relative resistance to HIV-1 infection of CD4 lymphocytes from persons who remain uninfected despite multiple high-risk sexual exposures.** *Nat Med* 1996, **2**:412–417.
6. Connor RI, Paxton WA, Sheridan KE, Koup RA. **Macrophages and CD4+ T lymphocytes from two multiply exposed, uninfected individuals resist infection with primary non-syncytium-inducing isolates of human immunodeficiency virus type 1.** *J Virol* 1996, **70**:8758–8764.
7. Rana S, Besson G, Cook DG, *et al.* **Role of CCR5 in infection of primary macrophages and lymphocytes by macrophage-tropic strains of human immunodeficiency virus: resistance to patient-derived and prototype isolates resulting from the Δccr5 mutation.** *J Virol* 1997, **71**:3219–3227.
8. Wu L, Paxton WA, Kassam N, *et al.* **CCR5 levels and expression pattern correlate with infectability by macrophage-tropic HIV-1, *in vitro*.** *J Exp Med* 1997, **185**:1681–1691.
9. Blaak H, van't Wout AB, Brouwer M, *et al.* **Infectious cellular load in human immunodeficiency virus type 1 (HIV-1)-infected individuals and susceptibility of peripheral blood mononuclear cells from their exposed partners to non-syncytium-inducing HIV-1 as major determinants for HIV-1 transmission in homosexual couples.** *J Virol* 1998, **72**:218–224.
10. Kim A, Pettoello-Mantovani M, Goldstein H. **Decreased susceptibility of peripheral blood mononuclear cells from individuals heterozygous for a mutant CCR5 allele to HIV infection.** *J Acquir Immune Defic Syndr Hum Retrovirol* 1998, **19**:145–119.
11. Dean M, Carrington M, Winkler C, *et al.* **Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene.** *Science* 1996, **273**:1856–1862.
12. Huang Y, Paxton WA, Wolinsky SM, *et al.* **The role of a mutant CCR5 allele in HIV-1 transmission and disease progression.** *Nat Med* 1996, **2**:1240–1243.
13. Meyer L, Magierowska M, Hubert JB, *et al.* **Early protective effect of CCR-5Δ32 heterozygosity on HIV-1 disease progression: relationship with viral load.** *AIDS* 1997, **11**:F73–F78.
14. Eugen-Olsen J, Iversen AKN, Garred P, *et al.* **Heterozygosity for a deletion in the CKR-5 gene leads to prolonged AIDS-free survival and slower CD4 T-cell decline in a cohort of HIV-seropositive individuals.** *AIDS* 1997, **11**:305–310.
15. O'Brien TR, Padian NS, Hodge T, Goedert JJ, O'Brien SJ, Carrington M. **CCR-5 genotype and sexual transmission of HIV-1.** *AIDS* 1998, **12**: 444–445.
16. Hoffman TL, MacGregor RR, Burger H, Mick R, Doms RW, Collman RG. **CCR5 genotypes in sexually active couples discordant for human immunodeficiency virus type 1 infection status.** *J Infect Dis* 1998, **176**:1093–1096.
17. Smith MW, Dean M, Carrington M, Huttley GA, O'Brien SJ. **CCR5-Δ32 gene deletion in HIV-1 infected patients.** *Lancet* 1997, **350**:741; discussion 742.
18. Michael NL, Chang G, Louie LG, *et al.* **The role of viral phenotype and CCR5 gene defects in HIV-1 transmission and disease progression.** *Nat Med* 1997, **3**:338–340.
19. Mandl CW, Aberle SW, Henkel JH, Puchhammer-Stockl E, Heinz FX. **Possible influence of the mutant CCR5 allele on vertical transmission of HIV-1.** *J Med Virol* 1998, **55**:51–55.
20. Shearer WT, Kalish LA, Zimmerman PA, for the Women and Infants Transmission Study Group. **CCR5 HIV-1 vertical transmission.** *J Acquir Immune Defic Syndr Hum Retrovirol* 1998, **17**:180–181.
21. Eldestein RE, Manns Arcuino LA, Hughes JP, *et al.* **Risk of mother-to-infant transmission of HIV-1 is not reduced in CCR5/Δ32ccr5 heterozygotes.** *J Acquir Immune Defic Syndr Hum Retrovirol* 1997, **16**:243–246.
22. Rousseau CM, Jurst JJ, Abrams EJ, Casabona J, Stein Z, King MC. **CCR5del32 in perinatal infection.** *J Acquir Immune Defic Syndr Hum Retrovirol* 1997, **16**:239–242.

23. Misrahi M, Teglas JP, N'Go N, for the French Pediatric HIV Infection Study Group. **CCR5 chemokine receptor variant in HIV-1 mother-to-child transmission and disease progression in children.** *JAMA* 1998, **279**:277–280.
24. De Rossi A, Masiero S, Giaquinto C, *et al*. **Dynamics of viral replication in infants with vertically acquired human immunodeficiency virus type 1 infection.** *J Clin Invest* 1996, **97**: 323–330.
25. Newell ML, Gray G, Bryson YJ. **Prevention of mother-to-child transmission of HIV-1 infection.** *AIDS* 1997, **11 (Suppl. A)**: S165–S172.
26. European Collaborative Study. **Vertical transmission of HIV-1:maternal immune status and obstetric factors.** *AIDS* 1996, **10**: 1675–1681.
27. De Rossi A, Ometto L, Mammano F, Zanotto C, Giaquinto C, Chieco-Bianchi L. **Vertical transmission of HIV-1: lack of detectable virus in peripheral blood cells of infected children at birth.** *AIDS* 1992, **6**:1117–1120.
28. Samson M, Libert F, Doranz BJ, *et al*. **Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene.** *Nature* 1996, **382**:722–725.
29. Ometto L, Zanotto C, Maccabruni A, *et al*. **Viral phenotype and host–cell susceptibility to HIV-1 infection as risk factors for mother-to-child HIV-1 transmission.** *AIDS* 1995, **9**:427–434.
30. Hill CM, Deng H, Unutmaz D, *et al*. **Envelope glycoproteins from human immunodeficiency virus types 1 and 2 and simian immunodeficiency virus can use human CCR5 as a coreceptor for viral entry and make CD4-dependent interactions with this chemokine receptors.** *J Virol* 1997: **71**:6296–6304.
31. Salvatori F, De Martino M, Galli L, Vierucci A, Chieco-Bianchi L, De Rossi A. **Horizontal transmission of human immunodeficiency virus type 1 from father to child.** *AIDS Res Hum Retroviruses* 1998, **14**:1679–1685.
32. Connor EM, Sperling RS, Gelber R, *et al*. **Reduction of maternal–infant transmission of human immunodeficiency virus type 1 with zidovudine treatment.** *N Engl J Med* 1994, **331**:1173–1180.
33. Ometto L, Bertorelle R, Mainardi M, Giurisato M, Chieco-Bianchi L, De Rossi A. **Analysis of the CC chemkine receptor 5 m303 mutation in infants born to HIV-1 seropositive mothers.** *AIDS* 1999, **13**:871–872.
34. D'Aquila RT, Sutton L, Savara A, Hughes MD, Johnson VA, for the NIAID AIDS Clinical Trials Group Protocol 241 Virology Team. **CCR5/Δccr5 heterozygosity: a selective pressure for the syncytium-inducing human immunodeficiency virus type 1 phenotype.** *J Infect Dis* 1998, **177**:1549–1553.
35. Zhang L, Huang Y, He T, Cao Y, Ho DD. **HIV-1 subtype and second receptor usage.** *Nature* 1996, **383**:768.
36. Aarons E, Fernandez M, Rees A, McClure M, Weber J. **CC chemokine receptor 5 genotypes and in vitro susceptibility to HIV-1 of a cohort of British HIV-exposed uninfected homosexuals.** *AIDS* 1997, **11**:688–689.
37. Mangano A, Prada A, Roldan A, Picchio G, Bologna R, Sen L. **Distribution of CCR-5 Δ32 allele in Argentinian children at risk of HIV infection: its role in vertical transmission.** *AIDS* 1998, **12**:109–110.
38. Sperling RS, Shapiro DE, Coombs RW, *et al*. **Maternal viral load, zidovudine treatment, and the risk of transmission of human immunodeficiency virus type 1 from mother to infant.** *N Engl J Med* 1996, **335**:1621–1629.
39. Burns DN, Landesman S, Wright DJ, *et al*. **Influence of other maternal variables on the relationship between maternal virus load and mother-to-infant transmission of human immunodeficiency virus type 1.** *J Infect Dis* 1997, **175**:1206–1210.
40. Cao Y, Krogstad P, Korber BT, *et al*. **Maternal HIV-1 viral load and vertical transmission of infection: the Ariel project for the prevention of HIV transmission from mother to infant.** *Nature Med* 1997, **3**:549–552.
41. Thea DM, Steketee RW, Plimer V, *et al*. **The effect of maternal viral load on the risk of perinatal transmission of HIV-1.** *AIDS* 1997, **11**:437–444.
42. The European Collaborative Study. **Maternal viral load and vertical transmission of HIV-1: an important factor but not the only one.** *AIDS* 1999, **13**:1377–1385.