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Mother-to-child Transmission of HIV-1: Advances and Controversies of the Twentieth Centuries

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Abstract

Mother-to-child transmission (MTCT) is the overwhelming source of HIV-1 infection in young children. According to the World Health Organization (WHO), during the year 2003, despite effective antiretroviral (ARV) therapy, there were approximately 700,000 new infections in children worldwide, the majority of whom were from resource-limited countries. Alternative protocols to the long-course and complex regimens of ARV drugs, which in high-income countries have almost eradicated HIV MTCT, have been shown to reduce early transmission rates by 38-50%. However, the accumulation of drug resistance and the long-term toxicities of ARVs mean that alternative approaches need to be developed. Furthermore, transmission via breastfeeding, which accounts for one third of all transmission events, can reduce the benefits of short-course therapies given to women for the prevention of MTCT.

The complex mechanisms and determinants of HIV-1 MTCT and its prevention in the different routes of transmission are still not completely understood. Despite the large contribution that many international agencies have made during the past 10-15 years in support of observational and intervention trials, as well as basic scientific research, HIV-1 MTCT intervention trials and basic research often are not integrated, leading to the generation of a fragmented picture. Maternal RNA levels, CD4+ T-cell counts, mode of delivery and gestational age were shown to be independent factors associated with transmission. However, these markers are only partial surrogates and cannot be used as absolute predictors of MTCT of HIV-1. Studies on the role of viral characteristics, immune response and host genomic polymorphisms did not always achieve conclusive results. Although CCR5-using viruses are preferentially carried by HIV-1 infected women as well as transmitted to their infants, the 32-basepair deletion of the CCR5 gene was not shown to influence perinatal MTCT. X4 viruses are apparently hampered in MTCT, although transmission of syncytium-inducing (SI) viruses, which use CXCR4, can occur when the mother carries such virus. Recently, there has been evidence of multiple virus variant transmission during peripartum MTCT. If viral escape from cytotoxic T-lymphocyte (CTL) recognition was repeatedly detected in transmitting mothers, no conclusive results were obtained on the role of the humoral immune response. The hypothesis on the mechanisms of selection during MTCT are still an open question, and include possibly that the transmitted variant is derived from a variant in the mother that escaped immune response, or that transmission is a stochastic event with the random transmission of a limited number of viral variants, or otherwise that selection occurs in the infant through a replication advantage of some transmitted viral variants.

Although global access to ARV therapy certainly remains the primary goal to achieve the immediate reduction of MTCT of HIV-1, it is also evident that new and additional innovative strategies are needed.
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Key words

HIV. Transmission. Mother-child.

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Introduction

Over the last decade of the HIV-1 epidemic the estimated number of new infections of children per day has doubled: every day during the year 2003 as many as 2,000 children acquired the infection worldwide[1]. The Joint United Nations Programme on HIV/AIDS (UNAIDS) / World Health Organization (WHO) estimated that 2.5 million of the 40 million of people living with HIV/AIDS are children under the age of 15, who mainly acquired the infection through mother-to-child transmission (MTCT) of HIV-1.

HIV-1 MTCT rates without drug access differ from 15-25% in Europe and the USA to 25-40% in some African and Asian studies[2,3]. Three different routes of HIV-1 MTCT (i.e. during pregnancy, delivery and breastfeeding) have been identified, which contribute to different degrees to the rate of transmission according also to the local setting[4]. Most of the transmission events occur during delivery and pregnancy. Approximately one third of the overall transmissions occurs postnatally through breastfeeding[5].

The availability of antiretroviral (ARV) drugs used for prevention and therapy has drastically reduced the rates of MTCT of HIV-1 and improved life expectancy in adults and children living in industrialized countries. While less than a hundred cases of child deaths were registered last year in each of these countries, half a million children died of HIV-related causes in Sub-Saharan Africa. Analogously, MTCT of HIV-1 accounts for less than a hundred cases of newly infected children only in those settings where voluntary HIV testing of pregnant women and prophylaxis with antiretroviral drugs from early on in pregnancy is associated with elective Caesarian section and avoidance of breastfeeding[6]. Thus, the vast number of new infections occurs primarily in resource-limited countries, where this long-course and expensive regimen is not appropriate for widespread or long-term use. The main obstacles to the implementation of voluntary counseling and testing as well as interventions for the prevention of MTCT (PMTCT) are the lack of adequate infrastructures and access to ARVs, as reviewed during the first international workshop on "Mother-to-child transmission of HIV: developing integration of healthcare programs with clinical, social and basic research studies" (Botswana, January 2003)[7]. Although programmed initiation of interventions for PMTCT is steadily rising in many countries, the effectiveness of the intervention approach is seldom assessed, nor is a PMTCT surveillance developed. Furthermore, the pathogenic mechanisms and determinants of MTCT of HIV-1 by the three different routes of transmission (i.e. *in utero*, peripartum and through breastfeeding) need to be more fully understood if they are to contribute to the layout of new approaches to prevention. In this regard, most of the acquired data refer to studies performed in Europe or the USA and not in those areas of the world where the epidemic is clearly increasing in intensity and significance.

Antiretroviral treatment for prevention of MTCT of HIV

Although global access to ARV therapy certainly remains the primary goal to achieve the immediate reduction of MTCT of HIV, it is also evident that alternatives to the long-course and complex regimens of ARV drugs, which in high-income countries have almost eradicated HIV MTCT, are urgently needed.

Several protocols with a series of ARV drugs have been shown to reduce MTCT to different degrees: a 38-50% reduction in early transmission rates has been observed using short courses of maternal ARV prophylactic treatments applied during the last weeks of pregnancy, labor and/or delivery (revised in[8]). The use of nevirapine (NVP), which reduces MTCT rates by 50%, given once to the mother during labor and to the newborn at birth (HIVNET 012 protocol)[9], was immediately adopted by many public health operators and authorities in resource-limited settings due to the low cost and the shortness of such a protocol.

Long-course ARV regimens are certainly more effective in reducing *in utero* and intrapartum HIV-1 MTCT. A clinical trial recently concluded in Thailand (PHPT-1) has demonstrated that MTCT rates were substantially lower when zidovudine (ZDV) was given to the women beginning at 28 weeks as compared to 35 weeks of gestation (1.6 vs. 5.1%, respectively)[10]. These results confirm that HIV-1 MTCT mainly occurs in the third trimester of gestation[11] and show evidence of the effect of treatment length on *in utero* transmission of HIV-1.

Promising results are achieved with the association of several ARV drugs. The combination of ZDV plus lamivudine (3TC) from 36 weeks of gestation, during labor, and one week postpartum to the mother and the infant, has shown to be more effective than ZDV alone given with a similar regimen but initiated from 28 weeks of gestation[12]. A recent clinical trial demonstrated the considerable efficacy of a single dose of NVP in the women added to the short-course ZDV regi-

men given from week 28 of gestation, reducing by 80% the MTCT rate compared to ZDV alone[13]. Due to its remarkable efficacy as well as its simplicity and safety, this regimen has been recommended for pregnant HIV-infected women who do not require ARV treatment, during a recent expert consultation at the WHO (Geneva, 5-6 February 2004)[14].

There are a series of unsolved questions that may hinder the success of the ARV approach. It has been shown that the reduction in MTCT rates was lower among pregnant women with more advanced immune deficiency[15]. Indeed, cumulative postnatal transmission risk of HIV-1 at two years of age was shown to be higher among ZDV-treated women with CD4+ T-cell counts below 500 cells/ml than among those with CD4+ T-cell counts above 500 cells/ml; the rates were 22 and 2%, respectively. Analogously, in another study on the efficacy of ZDV prophylaxis given from week 36 of gestation and every three hours intrapartum, the risk of MTCT of HIV at 24 months of age was significantly reduced only in the women with a plasma viral load below 50,000 copies/ml at enrollment[16]. Further studies are needed to continue and expand PMTCT efforts while preserving treatment efficacy.

ARV prophylaxis induces viral resistance, which in the long-term may threaten even the modest gains now evident in early studies. It is well documented that single-dose NVP prophylaxis induces viral resistance, which can persist for the first year after delivery[17]. Depending upon the timing and methodology, 15-60% of women and HIV-1 infected children treated with single-dose NVP have one-or-more detectable non-nucleoside reverse transcriptase inhibitor (NNRTI) mutations[18,19]. A recent study suggested that the emergence of NNRTI mutations may be virus-subtype dependent, as treated pregnant women carrying subtype-D viruses had a higher rate of resistance mutations than those carrying subtype-A viruses[20]. The emergence of viral resistance in the different subtypes or recombinant forms induced by NVP prophylaxis remains to be studied.

The potential impact of these mutations on the efficacy of future NNRTI-based treatment and MTCT prophylaxis regimens is of great concern[21]. A recent report showed that women exposed to single-dose NVP for PMTCT experienced a lower virological success rate of subsequent NNRTI-based therapy compared to non-exposed women[22]. However, the timing of treatment initiation postpartum in relation to NVP exposure affected this response; the longer the time the better the response.

Analogously, little is known about the risks associated to other prophylactic antiretroviral regimens used in pregnancy. The French Perinatal Cohort Study Group first described the risk of a neurological syndrome associated with persistent mitochondrial dysfunction in children exposed to nucleoside analogues during the perinatal period[23,24], although this dysfunction was not detected in an analogous retrospective US study[25]. Recently, the European Collaborative Study pointed to the possibility of unexpected adverse effects, such as severe prematurity and neonatal mortality, associated with highly active antiretroviral therapy (HAART) given to pregnant women[26]. Both the accumulation of drug resistance and the long-term toxicities of ARVs mean that alternative approaches need to be developed.

Several studies have demonstrated that postnatal transmission by breast milk could drastically reduce the benefits of short-course therapy given to the women for PMTCT[12,27,28]. Early breastfeeding transmission occurring within 6-8 weeks postpartum accounts for approximately 5-6% of additional events[29,30]. Thereafter, late postnatal transmission appears to be substantial (23-42% additional MTCT risk) and persistent for the whole period of breastfeeding (reviewed in[31]). While formula feeding is an obvious alternative in high-income countries, concern has been raised about the need for breastfeeding for the health of infants living in areas with endemic diarrheal diseases due to poor sanitary standards. Feeding practices have been suggested to contribute differently to transmission: exclusive breastfeeding could be associated with lower rates of breastfeeding transmission than mixed feeding of both breast milk and other milk or feeds[32]. Breastfeeding by HIV-infected women has been reported to be associated with an excess maternal mortality in a clinical trial performed in Nairobi, Kenya[33]. Subsequent studies testing the feasibility of, and the risks associated to, exclusive feeding practices indicate that exclusive breast- or formula feeding are both acceptable and feasible options for HIV+ women (reviewed in[34]). Personal motivation and support from health-care structure/staff were the determinant factors for success.

Findings from several trials in breastfeeding settings support the efficacy of short (intrapartum and one week postpartum) courses of infant ARV prophylaxis using three different regimens (NVP, ZDV alone, or ZDV/3TC) on early breastfeeding MTCT[9,25,27,29]. During the next 2-3 years a series of ongoing or planned clinical trials with different ARV regimens given to the breastfed baby and/or to the lactating mother, from

Table 1. Compilation of studies on the neutralizing activity of maternal sera against autologous primary isolates

Study*	Neutralizing capacity of the serum of		Statistic†
	Transmitting mothers	Non-transmitting mothers	p value
1	2/10	5/10	.0339
2	2/6	9/12	.0003
3	1/8	11/20	.0181
4	2/4	9/13	.0124

*Study[54]; Study[255]; Study[358], Study[457], †Chi-square test was used for statistical analysis.

delivery on for varying periods (i.e. one week, six weeks or six months, accompanied or not by early weaning) will also address the prevention of late breastfeeding transmission (reviewed in[35]). The potential side effects and toxicities of such therapies remain to be elucidated.

Indeed, several different intervention protocols have recently been or will be started to prevent MTCT of HIV-1 during pregnancy, delivery and breastfeeding. An exhaustive list of concluded, ongoing and planned clinical trials to prevent MTCT of HIV-1 is available on the website www.motherchildhiv.org.

Correlates of MTCT of HIV as a means to predict transmission

Maternal RNA levels, CD4+ T-cell counts, mode of delivery and gestational age are independent factors associated with transmission. Since the early reports performed in the USA and Europe in the mid-nineties, which showed that a high plasma virus load and a low CD4+ T-cell count of the pregnant women were independent risk factors for the transmission of HIV-1 from an untreated mother to her infant[36,37], many other studies have confirmed this correlation in several geographical settings[16,38-40]. Furthermore, maternal HIV-1 plasma viral load above the median in the study performed in Thailand was a strong risk factor for both *in utero* and intrapartum transmission[41]. The viral load of body secretions, as for example breast milk or cervicovaginal fluids, contributes to the transmission risk according to the different routes, i.e. post or peripartum transmission[38,40,42-45]. However, these markers are only partial surrogates and cannot be used as absolute predictors. Comparison of different assays for the determination of plasma and PBMC viral load showed that transmission or non-transmission occurred over the entire range of values, and no threshold value of viral load was identified which discriminated between transmitters and non-transmitters[46]. Furthermore, Contopoulos-Ioannidis showed in a meta-analysis study that maternal HIV-1 plasma load is a modest predictor of peripartum transmission for individual mothers[47].

An HIV-specific immune response of the mother has been the target for a possible prognostic marker of MTCT of HIV. Maternal antibody titers to peptides corresponding to the V3 region of gp120 and the immunodominant domain of gp41 were repeatedly evaluated and showed contradicting results with respect to perinatal transmission of HIV[48-53]. Analogously, the role of the maternal neutralizing antibody response in protection from perinatal HIV-1 transmission has not been clarified yet due to contradicting results[54-59]. In some studies a lower risk of MTCT was associated with the neutralization of the autologous virus by the maternal serum (Table 1), and in others with the neutralization of heterologous or laboratory adapted viruses[54,55,57,58,60]. Possible reasons for these inconsistent results may be ascribed to the limited cohort size of all these studies as well as to the differences in assays and reagents used. Indeed, antibody responses were mostly tested against different virus isolates (i.e. primary vs. laboratory adapted, autologous vs. heterologous, X4 vs. R5) or against peptides covering different regions of the V3 env of different viruses.

Only a few studies have investigated the role of the antibody response in breast milk. In a large cohort of lactating HIV-1 infected women in Rwanda, the presence and persistence of an anti-HIV milk secretory immunoglobulin (Ig) A and/or IgM response during the 18 month breastfeeding period was associated with a lower risk of MTCT of HIV[61]. Recently, milk anti-HIV-1 antibodies, particularly those against the gp41 ELDKWA epitope, were shown to block transcytosis *in vitro* in a monolayer of enterocytic cells[62,63].

Clear evidence of the relevance of the humoral response in preventing MTCT, however, comes from recent monkey studies. The passive administration of

a triple or quadruple combination of neutralizing human monoclonal antibodies (2G12, IgG1b12, 2F5 and 4E10) directed against conserved epitopes of HIV envelope glycoproteins achieved complete protection of the majority of infant macaques challenged orally with lentiviruses[64-67]. These promising results have encouraged the Authors to initiate a clinical trail to prevent postpartum MTCT with these same neutralizing antibodies. Furthermore, these results suggest that the epitopes recognized by these neutralizing monoclonal antibodies represent important targets against which to develop antibody response-based vaccines against HIV-1.

The data available today with regard to the relevance of the viral genotype or phenotype in perinatal MTCT are not conclusive. A heterogeneous virus population, as detected through sequencing of different regions of the viral genome, was more often described in mothers who did not transmit the virus to their babies[68,69]. A specific sequence pattern, which correlates to transmission, was however never clearly identified in any of the viral regions analyzed.

No large, controlled, prospective cohort studies have analyzed the role of the viral phenotype in MTCT of HIV-1. If all published studies are pooled, less than 300 isolates of untreated HIV-infected mothers were tested for viral phenotype with the MT-2 cell assay[55,56,58,70-76]. The single studies never found a significant correlation between viral phenotype and HIV MTCT. We and a few others have analyzed the chemokine receptor usage of viral isolates obtained from pregnant women, and showed that most maternal isolates, independently from transmission, used the CCR5 alone or in association with CXCR4 or other chemokine receptors when tested (Table 2)[73,75,77,78], thus indicating that CCR5 usage is not a predictive factor of transmission.

If these studies are analyzed together, it appears that transmitting mothers more often carry a virus capable of using CXCR4 than non-transmitting mothers: 30 and 16%, respectively (Table 2). Isolates of non-B subtypes (i.e. A, C, and G, analyzed in studies 3 and 4) more frequently used receptors (CXCR6 and CCR1) other than CXCR4[73,78]. Virus isolates, which use CXCR4 only, were extremely rare, and belonged to B or D subtypes. This is in agreement with previous reports showing that subtype-A viruses of Central Africa often use CCR5 in combination with CXCR6, whereas subtype-B and -D viruses are constituted also of a certain amount of X4 or R5X4 viruses and subtype-C viruses, which are rapidly spreading in many Southern African and Indian countries, and are to the great majority R5 viruses[79]. If the subtype affects the cell tropism of the virus, in turn it could affect the infectivity through the different routes of transmission. Studies in this regard have still to be planned.

Recently, Karlsson, et al. showed that R5 viruses can be further characterized according to their ability to use CCR5/CXCR4 chimeric receptors[80]. R5 viruses were able to use either CCR5 only, or had a more flexible use of the chimeric receptors, and were thus classified as broad CCR5 users of three types. Further studies are needed to show if this broad usage of the chemokine receptor may have consequences on cell signaling or on the sensitivity to neutralizing antibodies or chemokines, and thus also affect transmission or disease progression.

Other markers investigated as possible predictors of transmission are mutations of the host genome. The 32-basepair deletion of the CCR5 gene, which affects the expression of the chemokine receptor on the cell surface, was not shown to influence perinatal MTCT[81], as instead was the case for sexual transmission[82]. The same mutation carried by the children seems, however, to exert a protective effect against perinatal transmission in those children exposed to a low maternal viral burden of an R5 isolate[75]. Analogously, the mutation of the CCR2b gene did not show any protective effect on perinatal transmission[83]. The mutated SDF-1 gene instead has been shown to have some protective effect only in postpartum transmission in a study conducted in Africa[84].

Thus, it appears that there is the need to concentrate efforts to develop affordable, easy and standardized techniques to definitively identify markers of transmission of the virus from the mother to the infant that would be directly applicable in resource-limited settings. The assessment of the absolute risk of MTCT of HIV would have an enormous direct impact on health and financial issues: 1) by reducing the use of drugs in pregnant women; 2) by reducing the selection and amplification of drug-resistant virus variants, and 3) by reducing the possible long-term effects of such drugs on the uninfected children, and changing the implications of future drug regimens in women and children.

Portals of entry

A better understanding of the mechanisms used by the virus to establish infection in the infant may direct the development of protective and therapeutic strategies. A portal of entry for HIV in MTCT is certainly the mucosal surface. Indeed, the fetus first and the baby later swallow large amounts of contaminated fluids

Table 2. Results of published studies on the chemokine receptor usage of viral isolates of transmitting and non-transmitting mothers

Study*	Mother (number isolates tested)	Chemokine receptor usage of viral isolate					
		R5	R5X6	R5R1	R5X4	R5X4R3	X4
1	Transmitter (15)	10	0	0	3	2	0
	Non-transmitter (18)	16	0	0	1	1	0
2	Transmitter (20)	13	nt	nt	7	nt	0
	Non-transmitter (84)	70	nt	nt	12	nt	2
3	Transmitter (4)	2	2	0	0	0	0
	Non-transmitter (21)	16	2	1	1	0	1
4	Transmitter (11)	9	0	0	0	0	2
Total	Transmitter (50)	34	2	0	10	2	2
	Non-transmitter (123)	102	2	1	14	1	3

*Study 1[77]: isolates were obtained from Italian women carrying subtype B. Study 2[75]: women living in Italy; only cells expressing CCR5 and CXCR4 were used to test the chemokine receptor usage. nt = not tested. Study 3[73]: Cameroonian cohort; all isolates were subtype A except the X4 isolate, which was subtype B. Study 4[78]: women living in Sweden: 7 from Africa, 3 from Sweden and 1 from Asia. The R5 isolates were 3 subtype A, 3 subtype B, 2 subtype C, 1 subtype G; the X4 isolates were one subtype D and one CRF01.

such as amniotic fluid, vaginal secretions, blood and milk. Support for the oral transmission route comes from the association of the presence of HIV-infected cells in the infant's oropharyngeal cavity with intrapartum and early postpartum MTCT of HIV-1[85]. The portal of entry of the ingested virus is still hypothetical and may include the lymphoepithelial tissue of tonsils in the mouth, or the intestinal mucosa. Interestingly, human milk leukocytes, enterally administered to baboons with a single breastfeed, adhere for at least 60 hours to the intestinal mucosa, and cross to reach the liver and spleen[86]. Thus, HIV infected cells, prevalently macrophages and activated T-cells, ingested with milk may easily attach and cross the intestinal tract to infect underlying cells. Now, intestinal enterocytes and M-cells have been shown to selectively transcytose virus through binding with galactosyl ceramide (GalCer)[87,88]. If enterocytes appear to favor *in vitro* the transport of R5 viral variants, M-cells instead favor X4 viruses. Recently, however, Rescigno, et al. has demonstrated *in vitro* and *in vivo* that dendritic cells (DCs) can penetrate through tight junctions of the intestinal enterocytes and favor transport of enterobacteria, invasive or not, from the intestinal lumen[89]. Intestinal DCs express DC-SIGN, which can be used by HIV as receptor[89]. Furthermore, DC-SIGN has been identified as having a negative regulatory function on the ability of DCs to activate T-cells. Therefore, the binding of HIV to this receptor may also alter the functions of DCs so as to evade immunological surveillance and, thus, possibly explain the impaired virus control in the infected newborn compared to the adult[91].

The other portal of entry specific for *in utero* MTCT of HIV-1 is possibly the placenta. Throughout pregnancy, there is a tight contact between infected maternal cells and the placenta, which continuously exchanges material with the fetal blood compartment. The possible routes of *in utero* HIV-1 transmission include the transannexial or the transplacental passage. As far as the latter is concerned, two ways of infection can be considered: 1) transmission of maternal cells (monocytes and lymphocytes) through placental lesions, or 2) direct infection of placental cells. *In vivo* infection of placental cells, the trophoblastic[92-94] and the villous Hofbauer cells[95-98], has been shown as early as eight weeks of gestation, unless the pregnant women were treated with ZDV for PMTCT from early on in pregnancy[99]. However, infection of the trophoblastic cells, possibly the primary target for viral infection in the placenta, did not always result in infection of the fetus[92,100,101]. Interestingly, we demonstrated that the viral population present in these cells was shown to be a selected variant of those present in the mother's peripheral blood mononuclear cells (PBMCs)[100]. HIV entry into trophoblastic cells has still to be elucidated, as primary trophoblastic cells express CXCR4 and CCR5 but not always CD4 on the surface[102]. DC-SIGN and ICAM-1 have recently been implied to play a role in the passage of HIV between placental cells and Hofbauer cells or T-lymphocytes[103,104]. *In vitro,* HIV-1 can transcytoze across a trophoblastic barrier constituted of BeWo cells or, alternatively, infected monocytes and lymphocytes can rapidly fuse with trophoblastic cells independently from the viral chemokine-receptor

usage[105]. Interestingly, if the transcytozed virus usually retained the chemokine receptor usage of the infecting virus isolate, the virus obtained after fusion preferentially used CXCR4. These data are in strong favor of a selective process occurring at trophoblastic level. It can be envisaged that factors of the placental microenvironment, such as cytokines, chemokines, hormones or antibodies, may be involved in the selection process. A perturbation of this environment can possibly be inferred by ARV drugs, vaccines or coinfections. An example is the infection by *Plasmodium falciparum*, which was shown to be more frequent in HIV-1 infected pregnant women than in uninfected ones[106-108]. The presence of malaria parasites was associated with a higher HIV-1 load in the plasma and placental tissue[108,109]. Furthermore, placental malaria infection was shown to increase CCR5 expression in maternal macrophages and fetal Hofbauer cells, possibly increasing susceptibility to HIV infection[110]. Recent studies, however, showed discordant results with regard to increases of MTCT of HIV in association with malaria infection[107,111]. Studies on the role of coinfection with common pathogens in the developing countries should be in focus, as it is clear that such coinfections have an influence on HIV dynamics and MTCT.

How does selection occur during transmission?

The comparison of the HIV-1 sequences harbored in the peripheral blood of the mother with those of the infected infant described in the first reports at the beginning of the nineties showed that only a limited number of viral variants are transmitted[112,113]. In some cases the transmitted variant represented a minor maternal viral variant. This has corroborated the idea that the transmitted variant is derived from a variant in the mother that escaped immune response. Other hypotheses are that the minor viral variant in the peripheral blood may represent a major variant in local compartments, such as the placenta and birth canal, involved in MTCT, or that transmission is a stochastic event with the random transmission of a limited number of viral variants, or otherwise that selection occurs in the infant through a replication advantage of some transmitted viral variants. The following are some considerations on studies to unravel this dilemma.

NSI viruses (i.e. R5 viruses) are preferentially isolated from newborns[56,70,73,74,76,78]. However, the determination of a preferential transmission can be evaluated only if both the mother's and the infant's viral population are analyzed (Fig. 1). R5 viruses are in general more represented in HIV-1 infected mothers and therefore possibly more frequently transmitted to newborns. The characterization of R5 viruses according to the CCR5/CXCR4 chimeric receptor use may eventually highlight a selection process occurring for these types of viruses. It remains unclear why X4 viruses are apparently hampered in MTCT. As we and others showed, transmission of SI viruses, which use CXCR4, can occur when the mother carries such virus[56,70,76,77]. The few reports on chemokine receptor usage of transmitted viruses showed that infants always carry a virus capable of using CCR5 alone or in association with other chemokine receptors. Interestingly, both mothers who carried a R5X6 virus population transmitted a virus with the same coreceptor usage to their child. Unfortunately, the assays used for the determination of the chemokine receptor usage do not allow to distinguish between a mixed viral population of viruses which use singularly the different coreceptors, or a true multitropic virus. This would allow a better understanding of the possible selection processes. Transmission of viruses with a phenotype other than R5 may also be related to timing of transmission, i.e. *in utero* vs. intrapartum.

Only a few studies have been conducted to determine the effect of a HIV-specific cytotoxic T-lymphocyte (CTL) response on MTCT of HIV. The first study suggested that escape from CTL recognition is frequently detected in transmitting mothers, although the transmitted viral variant was not always represented by an escape variant[114]. Recently, Goulder, et al. have provided repeated evidence of transmission of HIV-1 CTL epitopes in which escape had occurred in the mother[115,116]. The different escape mutations localized in Gag were restricted by the HLA molecules B57/5801 or B27. Interestingly, the B57/5801 restricted mutation reverted to wild-type when transmitted to a B57/5801 negative child, whereas the B27 restricted mutation rarely did. These results suggest a selection process occurring already in the mother, but indicate also that the transmitted escape mutant which reverts will be a useful epitope for CTL-based vaccine development.

During the last decade, however, the evidence of multiple virus variant transmission during *in utero* or peripartum MTCT came from several reports, although the frequency of this event was not clear[117-121]. Recently, two studies detected heterogeneous viral populations in infants close to birth, independent of transmission route, i.e. *in utero* or peripartum transmission. The frequency of these events varied from 50% of the 13 in-

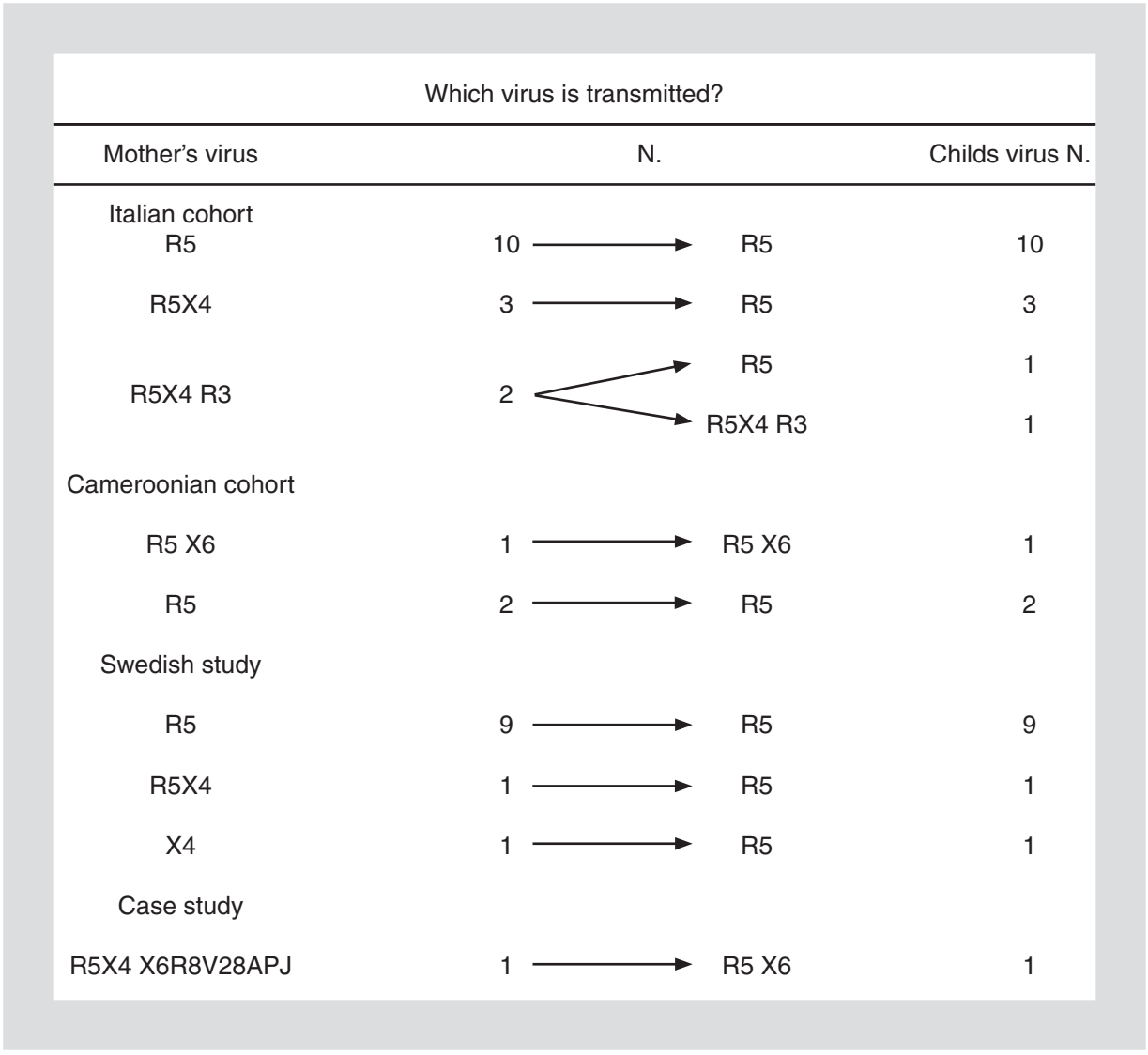

Figure 1. *Compilation of published studies on the chemokine receptor usage of viral isolates obtained close to delivery from mother-child pairs. Italian cohort[77]; Cameroonian cohort[73]; Swedish study[78]; case study[70]. N. is the number of individuals tested.*

fants to 21% of the 53 infants analyzed in the two studies, respectively[118,120]. In a geographical area where different subtypes coexist, a mixed virus subtype transmission was described in three out of nine African mother-child pairs[122]. These results would argue against selection processes as the main forces driving MTCT of HIV-1. Studies of this type are not easy as several factors, like sampling time of the infant, different transmission routes, technical approaches (heteroduplex mobility assay vs. sequencing), or viral gene studied, may bias the outcome of a viral genotypic analysis. Interestingly, similar results were recently described for African women. Multiple virus variant infection, detected in 89 of the 156 women tested, correlated with higher viral load and faster disease progression[123], and was associated with genital tract infections or hormonal contraceptive use at the time of transmission[124]. Our preliminary data suggest that multiple virus variant transmission may have an influence on the outcome of disease progression in children as two of four fast progressors, but none of five slow progressors, displayed a heterogeneous viral population during the first months of age (personal communication).

Conclusions

It is evident that considerable efforts are still needed in clinical and basic research to define innovative approaches to predict and reduce MTCT of HIV-1. There

is the need to concentrate the efforts to develop affordable, easy and standardized techniques to determine why transmission occurs and, more importantly, why most infants are not infected, despite considerable exposure through birth and breastfeeding. The assessment of correlates of protection against MTCT of HIV may direct the development of protective and therapeutic vaccine strategies. HIV vaccines and prevention methods applied for MTCT of HIV will have an enormous direct impact on public health, child survival and innovative approaches to the prevention of infectious disease.

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