VIROLOGICA SINICA, Jun 2011, 26 (3):147-155
DOI 10.1007/s12250-011-3180-2

The Role of HIV Replicative Fitness in Perinatal Transmission of HIV*

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Abstract: Perinatal transmission of Human immunodeficiency virus (HIV), also called mother-to-child transmission (MTCT), accounts for 90% of infections in infants worldwide and occurs in 30%-45% of children born to untreated HIV-1 infected mothers. Among HIV-1 infected mothers, some viruses are transmitted from mothers to their infants while others are not. The relationship between virologic properties and the pathogenesis caused by HIV-1 remains unclear. Previous studies have demonstrated that one obvious source of selective pressure in the perinatal transmission of HIV-1 is maternal neutralizing antibodies. Recent studies have shown that viruses which are successfully transmitted to the child have growth advantages over those not transmitted, when those two viruses are grown together. Furthermore, the higher fitness is determined by the gp120 protein of the virus envelope. This suggests that the selective transmission of viruses with higher fitness occurred exclusively, regardless of transmission routes. There are many factors contributing to the selective transmission and HIV replicative fitness is an important one that should not be neglected. This review summarizes current knowledge of the role of HIV replicative fitness in HIV MTCT transmission and the determinants of viral fitness upon MTCT.

Key words: Human immunodeficiency virus (HIV); Acquired immune deficiency syndrome (AIDS); Mother-to-child transmission (MTCT); Replicative fitness; Gp120

INTRODUCTION

Since the first case was found in 1981, Acquired immune deficiency syndrome (AIDS) has become a very important public health and social problem around the world. It has evoked the close attention of the World Health Organization and of national governments. The first case of an infant infected with Human immunodeficiency virus (HIV) through MTCT was reported by CDC in the US early in 1982[16]. UNAIDS reported that there were 33.3 million people estimated to be living with HIV in 2008, and about 430 000 children were newly infected with HIV, the vast majority of them infected through MTCT. There are about 700 000 HIV infected people in China in 2008. Nationally, the infection rate is 0.05% and the proportion of MTCT among the

Received: 2011-01-11, Accepted: 2011-03-31

* Foundation items: The grants of National Science Foundation of China (30970162); Program of International Collaboration of Tianjin Municipal Science and Technology Commission (08ZCGHHZ01800).

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infected population is 1.6%. It has been shown that the transmission rate was 33.3% among 461 HIV infected mothers from 15 counties of Xinjiang, Yunnan, Henan and Guangxi, where HIV infection is epidemic and antiviral treatment was not available [42].

The replication of HIV in children is faster, the virus load is higher than in adults and the progression of disease more rapid. So the prophylaxis of HIV MTCT is very important. In ideal conditions, the advanced diagnostic technique combined with the provision of antiretroviral prophylaxis and replacement feeding can reduce transmission from an estimated 30%–35% to 1%–2%. So the research on viral biological characteristics of transmitted viruses and transmission mechanism is crucial to prophylaxis and treatment strategies.

Not all the variants in mother will transmit to the infant, only a minor viral population within the chronically infected mothers is transmitted. It does not mean the maternal viruses' transmission to an infant is stochastic. In contrast, HIV MTCT might be a kind of selective transmission similar to HIV heterosexual transmission [61]. Some kinds of selective mechanisms ensure a subset of virus withstand the selection pressures imposed by perinatal transmission. There are a number of possible explanations accounting for this phenomenon [2,14,15,54,55]. On the one hand, the maternal innate immune system, including neutralizing antibody and T-cell responses plays an important role in determining which viruses could be transmitted to the infant. On the other hand, CCR-5 tropic, infant innate humoral and cellular immunity are also involved in transmission, although there are some reports on the biological characteristics of transmitted viruses such as CCR5 tropic and neutralizing antibody resistant viruses and on lack of correlation between viral genotype and phenotype. This review emphasizes HIV replicative fitness and discusses its role in HIV MTCT. It is important to fully understand the characteristics of transmitted variants and the mechanisms of HIV MTCT in order to enhance current prevention strategies.

HIV MOTHER TO CHILD TRANSMISSION

HIV MTCT is one of the HIV vertical modes of transmission. It can be acquired in utero, during delivery (intrapartum), or via breastfeeding. Although HIV MTCT has been reduced to less than 1% in the US and Europe [13,50], transmission rates in many developing countries with limited resources fluctuate between 10% and 40% [20,26]. About 5% HIV infected infants died from HIV infection or disease complications in 15 months in the US and Europe [26], but in Kinshasa, Brazzaville and Kigali in Congo and Rwanda, the death rate is from 12% to 39% [9,44,46].

Without any treatment, around 15%-30% of infants born to HIV infected women will become infected during pregnancy and delivery. A further 5%-20% will become infected through breastfeeding [39].

In utero transmission almost always happens in the last three months of the gestational period, but it can also occur earlier [41]. HIV viral particles can be detected in placentas, supporting the theory that in utero transmission occurs primarily through mother's placenta [57]. Viral particles cannot pass through the placenta trophoblast cells via cell-free transmission. Therefore they need to rely on the transcytosis of cell to cell transmission [38]. High maternal plasma HIV RNA load is associated with high transmission rate in utero transmission [22,30]. Infection by bacteria in the

maternal placenta, chorion and amnion will increase the transmission [10,47,59]. Bacterial infection may help the virus move into the maternal amniotic cavity [25,48]. Studies have also demonstrated association between infant gender and MTCT during pregnancy [12,28,56].

Approximately two-thirds of HIV exposed infants acquired HIV via intrapartum transmission [20]. When passing through the birth canal, neonates are easily exposed to maternal blood and genital secretions. The genital viral load, genital ulcer disease and breaks in the placental mucosal barrier have a close association with intrapartum transmission [17,24]. Extrinsic factors, such as prolonged duration of membrane ruptures and placental micro-transfusions were found to be associated with increased intrapartum transmission [3,37].

Transmission of HIV through breast milk is another HIV MTCT factor, and more important is that this route can be cut off entirely by an effective blocking method. Without treatment, the rate of HIV MTCT in developed countries is 15%-25%; the higher rate of 25%-35% in developing countries is due to the different custom and methods of feeding. In developing countries, breastfeeding is more prevalent and of longer duration due to a lack of access to safe water and the unaffordable cost of alternative feeding [34,49]. The viral level in breast milk is an important factor in HIV breastfeeding transmission. In general, ten-fold HIV viral load increase in breast milk will cause a two-fold increase in risk of transmission [52].

SELECTIVE TRANSMISSION IN HIV MTCT

When HIV MTCT occurs, not all strains of maternal viruses will transmit to the infant. HIV selective transmission was first found in HIV heterosexual transmission by J Koopman in 1988[36]. The concept of HIV vertical transmission was reported early in 1994 by A B van't Wout [58]. The mechanism of HIV selective transmission is called "bottle-neck theory": a lot of viruses are in the bottle, but only a fraction of the strains are transmitted from the donor to the recipient through the narrow bottle-neck. Viruses are transmitted to the recipient selectively due to some limitation either in donor or recipient. The presence of neutralizing antibodies within the donor is one of the important factors in determining which viruses are transmitted. In some cases, the transmitted virus appears to be less sensitive to neutralization [27]. The genotypic changes of *env* gene are the main determinants involved in selection of less neutralization sensitive transmitted viruses. Many factors may contribute to the changes of the *env* gene in selective transmission. Firstly, the number of potential N-linked glycosylation sites (PNGS), which anchor a "glycan shield" of carbohydrates to the envelope. Secondly, the length and genetic variation of variable loops of *env* that bind antibodies may be useful to help transmitted viruses to escape from neutralizing antibodies and determine the co-receptor usage. All the above variables are associated with survival and replication of the virus and might be related to immune escape and replication rate within the host.

In HIV mother to child transmission, the phylogenetic analyses of *env* V1-V5 amino acids showed that the infant viruses were more homogeneous than those in the maternal viruses, with the infant viruses appearing as a branch of the mother viruses on the phylogenetic tree [35]. Studies have also shown CCR5 tropic or neutralizing antibody resistant viruses are transmitted selectively [21,29,45] in MTCT. Recent researches indicate

that replicative fitness is also an important factor during selective transmission [51].

CONCEPT OF HIV REPLICATIVE FITNESS

HIV initially infects $CD4^+$T cells and the primary infection starts from a single strain infection. Due to the high mutation rate (about 300 000 frequency per site per generation) [43], capability of rapid replication (10^8-10^9 viruses per day) [32] and huge numbers of infected cells (10^7-10^8cells) [18] of HIV, a host of strains is produced. These strains are known as a quasispecies. They are dissimilar, but correlated on the level of genetic evolution. If the effective size of a HIV quasispecies is big enough, the dominant colony will be selected under the selective pressure. This typifies the enormous potential for evolution and replication.

The extent of viral adaptation to the host environment for survival and replication can be measured by replicative fitness. This concept was derived from replicative capacity which was proposed by B Asjö in 1986 [7]. The concept of HIV replicative fitness was first proposed by Domingo in 1997: according to population genetics, HIV replicative fitness means that HIV evolves to fit the host environment, surviving and replicating to generate progeny viruses [23]. This can be observed in the relative replication capability or the viral yield. HIV replicative fitness is a result of many agents' interaction. Major studies show that HIV replicative fitness is related to virulence, pathogenicity, disease progression, and drug-resistant mutations. Moreover, different co-receptor tropisms contribute to the variation in replicative fitness. Compared with CCR5-using viruses, CXCR4-using variants were observed to be transferred more efficiently from dendritic cells to $CD4^+$ T cells [6]. Recent studies of human and simian immunodeficiency virus (HIV and SIV) have demonstrated that the capacity to replicate efficiently in $CD4^+$ T cells is important for fitness [11]. The HIV-1 subtypes' different fitness was also found to correlate with their prevalence in the human population [5]. In India, subtype C HIV is the predominant subtype. From a dual infection growth competition assay in PBMC, primary subtype C isolates are shown to have higher relative fitness than subtype A primary isolate [51]. This result has also been found among HIV-1 group M with HIV-1 group O and HIV-2 isolates. Reduced replicative fitness may be contributing to the low prevalence of HIV-1 group O and HIV-2 all over the world. High replicative fitness strains have selective advantages over low replicative fitness strains. The pathogenicity of low replicative fitness strains is weaker. The comparison of historical and recent samples shows HIV replicative fitness is highest at the start of a pandemic. This may be the consequence of selective transmission and adaptation to the human host [4]. So the replicative fitness of HIV appears to be an important piece of the puzzle in understanding the prevalent and progression in human beings.

REPLICATIVE FITNESS RESEARCH SYSTEM

Heteroduplex tracking assay (HTA) and Real-time PCR are classical detection methods commonly used in HIV genetic assays [19,40]. In 2005, Abraha proposed that HTA is utilized to measure viral fitness in a growth competition assay [1]. HTA is used to evaluate the production of competing HIV variants which can be used ex vivo. Real-time PCR is a high output assay

to analyze different strains of competing HIV simultaneously. Despite the success of these methods in evaluating viral fitness, there are increasing problems with radioisotope examination: it is expensive and time-consuming and inhomogeneity between-lots and low reproducibility in Real-time PCR limits its application in viral fitness. Accordingly, new types of assay are needed which are more sensitive and which avoid the above disadvantages.

The fluorescence recombinant viral competition test proposed by Weber in 2006 [60] is a novel assay developed for evaluation of the viral replicative fitness. It is attractive for three reasons: *i*) it was developed to measure HIV replicative fitness based on recombinant viruses consisting of subtype B type strain-NL4-3 and the enhanced green fluorescent (EGFP) or the Discosoma sp.red (DsRed2) gene. Distinct from previous methods, this system allows generation of competing viruses in an intact viral genetic back-ground. It is mainly used to evaluate the contribution of drug-resistance mutations to overall viral replicative fitness via direct competition experiments. Weber focuses on the presence or absence of antiretroviral drugs' influence on HIV replicative fitness. *ii*) In the original system, subtype B strain-NL4-3 with EGFP/ DsRed2 was used as the backbone. The maternal and infantile Envelop V1-V5 region was inserted into the backbone through homologous recombination to obtain the fluorescent recombinant chimeric virus [35,60]. The fluorescent tag could tell the difference between two competing viruses when they infect target cells in mono/dual infection through flow cytometry. *iii*) The novel system could be used to evaluate any interesting target gene, except for *env,* by deletion of the different structural gene of HIV in the NL4.3 backbone.

How to choose the target cell in HIV replicative fitness research? PBMC is the natural host of HIV and it is the optimal candidate in HIV research. Because of donor selection of PBMC, cell lines expressing CD4 and co-receptor CXCR4/CCR5 could be another choice. The previous study showed that U87-CD4-CXCR4/CCR5 cells express CD4 and CXCR4/CCR5 co-receptors and could be used as the HIV target cell for infection [31]. The fitness result from U87-CD4-CXCR4/CCR5 is consistent with that from PBMC [35]. In the study of HIV chronically infected MIPs（mother and infant pairs）using the fluorescence recombinant viral competition test, the infant recombinant viruses are fitter than the maternal viruses.

DETERMINANT OF HIV REPLICATIVE FITNESS IN MTCT

HIV replicative fitness is the adaptation of the virus to host environment and is a result of the interaction of many viral and host factors. In theory, each biological process of the HIV lifecycle may be involved in determining viral replicative fitness. A previous study demonstrated that the *env* gene played a major role in determining replicative fitness in comparison with other genes such as *gag* and *pol* [8]. The current study illustrates that the *env* gene also plays a major role in the competitive test of the virus in MTCT. Transmitted HIV exhibits a phenotype of high replicative fitness in MTCT. Gp120 confers higher fitness, which means transmitted viruses have higher replicative fitness than non-transmitted viruses in chronically infected MIPs. Moreover, the higher replicative fitness of transmitted viruses is lineage specific, which only shows within the same MIPs, related mothers and infants [35].

In a well-established competitive system, the maternal and infant recombinant viruses are identical except for the *env* V1-V5 region, which is derived from mother and infant, respectively, which demonstrates the higher fitness possibly conferred by this region. It has been known that the HIV envelope gene is involved in HIV entry, co-receptor usage, the number of potential N-linked glycosylation sites (PNGS) and neutralizing antibody epitopes. It is also a major target site of the host immune system. This implies that viral binding and entry, the early events, primarily determine fitness, but whether the steps of post-entry are involved in determining fitness is still unclear. It is intriguing to investigate which domain of *env* gene correlates with fitness.

The *env* gene is responsible for the variation in HIV replicative fitness. However the role of other viral genes such as *gag* and *pol* may also play a role in viral fitness, while their function in viral replicative fitness is weaker than *env*, it should be investigated in future study [33,53]. *Gag* and *pol* are much more highly conserved than *env*, while both of them have important functions during the viral life cycle. Thus the site mutation of *gag* and *pol* will also affect viral replicative fitness as well as the cytotoxic T-lymphocyte (CTL) response of the host environment. Unlike *gag* and *pol* gene, the large variation in the *env* gene either in conserved regions (C regions) or variable loops (V regions) could cause considerable variation in fitness in individuals and it plays a pivotal role compared with other genes in determining replicative fitness.

CONCLUSION

Selective transmission occurs due to the "bottle-neck" effect upon perinatal transmission of HIV. This review highlights the role of HIV replicative fitness in perinatal transmission. HIV replicative fitness can be affected by many factors, the genetic difference being important. But until now, which domain in HIV genome is important during transmission has not been established. Nevertheless, HIV replicative fitness is an important feature during HIV MTCT. In addition, perinatal transmission of HIV is influenced by the strain of HIV and the transmission routes. Further study to clarify the effect of changes of HIV fitness upon perinatal transmission by different routes and by diverse subtypes is necessary. Transmitted viruses manifest higher replicative fitness, and the replicative fitness correlates with changes in the HIV gp120 V1-V5 domain. This implies that HIV gp120 V1-V5 is an attractive target for the development of both antiviral agents and an effective vaccine. Since *env* is involved in the entry process, a majority of the maternal viruses may be less efficient in entry, either in binding or fusion kinetics or both, whereas the post-entry step may possibly be involved in the determination of HIV fitness. There is a need for further study to investigate the relationship between HIV fitness and viral lifecycle.

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