Human Immunodeficiency Virus-1 subtypes: Could genetic diversity translate to differential pathogenesis?*

U DAYKUMAR R ANGA
Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur
Bangalore 560 064, India.
email: uday@jncasr.ac.in, Phone: 91-80-8462750 Extn. 2241, Fax: 91-80-8462766

Abstract

Human Immunodeficiency Virus (HIV) exhibits extremely high degree of variation at genetic level. This extensive genetic variation is a consequence of two factors. Firstly, there have been multiple introductions of genetically diverse simian retroviruses into the human populations. Secondly, following introduction, the viruses diversify rapidly with time, generating heterogeneous viral strains. Based on the genetic relatedness, HIV-1 and HIV-2 are classified into several distinct subtypes. Distribution of the viral subtypes across the globe is nonuniform. Additionally, epidemic outbreaks due to recombinant forms of the viruses are also becoming a serious problem in several geographical regions. Whether the various genetic subtypes and recombinant forms of HIV-1 have biological differences, for instance with respect to transmissibility and the course of disease progression, is controversial. The extent of genetic divergence among subtypes is probably more than sufficient to cause such differences. However, adequately controlled data from in vivo studies are yet to emerge. This article presents an overview of what is known on the genetic variation of the viral subtypes and its practical implication for viral pathogenesis and efficient engineering of intervention strategies.

Keywords: HIV-1, AIDS, genetic diversity, subtypes, differential pathogenesis.

1. Introduction

A little over 20 years ago, a small article appeared in a journal called Morbidity and Mortality Weekly Report published by the Center for Disease Control and Prevention (CDC), Atlanta, USA. The article was a research report on a community of gay men in Los Angeles dying of a lung infection caused by Pneumocystis carinii pneumonia [1]. Some months later there appeared another small report in the same journal on a group of 26 young gay men in New York and California diagnosed with Kaposi’s sarcoma, a rare form of skin cancer primarily seen in elderly men of Jewish and Mediterranean origin [2]. What started as a trickle eventually turned out to be a disaster during the following two decades that is today known as HIV/AIDS.

Since the beginning of the epidemic to the end of 2001, a total of 22 million people perished of AIDS (Acquired Immunodeficiency Syndrome) and a total of 40 million are presently living with HIV (Report on the global HIV/AIDS epidemic 2002, UNAIDS). In 1999, AIDS killed more people globally than both tuberculosis and malaria combined (World Health Report, 2000). In 2000, HIV surpassed other pathogens to become the world’s leading infectious agent to kill adults. Nearly 90% of the infections occur in poorer countries of Africa, Asia and Latin America, where access to knowledge is limited and resources to control the pandemic are inadequate. African countries like Burnica Faso and Botswana are devastated where infection incidence has soared up to

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30–40% levels. The virus is wreaking havoc, spreading like wild fire across the globe and killing millions every year with no signs of abatement. In spite of over two decades of intense medical research, HIV/AIDS not only continues to be a serious problem but also is attaining gigantic proportions unprecedented by any other infection in the human history. Since the bubonic plague of the 14th century, HIV is the most devastating communicable cause of adult death.

In India, the first case of HIV infection was detected in 1985 [3]. An exponential spread of the virus across all sections of the community has been reported since then [4]. In the near future India is expected to host the largest number of infected people in the world [5]. India also shares the unique distinction of having considerable number of infections caused by HIV-2 [6]. If the African continent has borne the brunt of the devastation hitherto, it appears that India is gradually slipping into that position. The vastness of the country combined with the fact that the genetically heterogeneous populations are poor and unorganized suggests that the HIV/AIDS is likely to have a serious impact on the economy and health of the people.

2. Subtypes of HIV-1

HIV being a virus exploits the host’s biosynthetic machinery for its own protein synthesis. The viral genetic material is copied by a viral polymerase called reverse transcriptase (RT) that lacks the proofreading activity. In other words, unlike in other organisms, the mistakes occurred during the process of copying the genome are not detected and corrected, rendering the process of viral genetic replication highly error prone. Gradual and cumulative accumulation of the genetic errors eventually leads to the evolution of viruses that differ considerably from one another at genetic level such that they fall into well-defined clusters that are technically referred to as viral subtypes.

Viral subtypes are akin to human races. As various human races differ from one another in minor details, still all belong to the same species, Homo sapiens, various HIV strains differ from one another at genetic or molecular levels, yet retain their viral identity. Depending on the genetic relatedness, viral strains are divided into groups and subtypes. There are three main groups, M, O and N within HIV-1. Most of the HIV-1 strains fall within group M, which consists of at least 10 to 12 genetically distinct subtypes of HIV-1, designated A through J [7]. In addition, Group O contains another distinct group of viruses identified mainly in Cameroon [8]. While the genetic subtypes of HIV-1 in group M are broadly equidistant to one another, those of group O are highly heterogeneous. A small number of strains genetically distinct from the above two groups has been mainly confined to Cameroon and Gabon. These viruses are placed in group N [9], [10]. However, it is important to note that divergence of the viral subtypes is a dynamic ongoing process and more and more viral subtypes are still evolving.

3. Subtype distribution

The subtypes of HIV-1 are unevenly distributed in the world [11], [12]. For instance, subtype-B is mostly found in the Americas, Japan, Australia, the Caribbean and Europe; subtypes A and D predominate in sub-Saharan Africa; subtype-C in South Africa, India, Brazil and China; subtype-E in Central African Republic, Thailand, North-eastern regions of India and other countries of southeast Asia. Subtypes F (Brazil and Romania), G and H (Russia and Central Africa), I (Cyprus), and group O (Cameroon) are of very low prevalence. More and more subtypes are being discov-
ered on a regular basis and several strains remain unclassified as they fail to segregate with any known group. Most of the subtypes are found on the African subcontinent, although subtype-B is less prevalent. HIV-2 strains are mostly confined to the sub-Saharan and Western Africa. There are approximately five subtypes identified within HIV-2.

In India, both HIV-1 and HIV-2 infections have been detected [6], [13]–[20]. In mainland India, several subtypes of HIV-1 have been detected, A, B, C and D [21]–[26], including recombinant strains [27]. Subtype-B, the Thai variety, is mostly seen in the north-east where the borders are common with Myanmar [28], [29]. Subtype-C strains are the most prevalent viruses seen in India causing over 85% of the total number of infections [23], [30]–[33]. Globally, subtype-C strains cause half the total infections.

4. Genetic diversity confers survival advantage on the virus

In order to adapt to the changing environments, all the DNA-based organisms, including human beings, introduce variations into their genetic content. This will ensure to provide enough diversity within the populations such that a fraction of the individuals of the population are readily equipped to cope up with the changing conditions. However, the frequency of such spontaneous genetic variation is usually low. Additionally, the doubling time of many organisms is large, hence, accumulation of too large a frequency of mutations could adversely influence the population survival. These organisms thus strive to strike a fine balance by introducing enough genetic variation into the population gene pool to offer survival advantage against adverse conditions but not to the extent that it would be deleterious for the fitness of the individual.

In contrast, RNA viruses like HIV, HCV, polio, measles and others employ high mutation rates rather as a weapon to ensure their own survival. Indeed, HIV accumulates mutations so rapidly that populations of these viruses exist not as collections of discrete and stable subtypes, as is the case with bacteria, but as a swarm of related but genetically diverse individuals, technically called quasispecies. In addition to being hugely diverse, the swarms are also highly dynamic in that with each round of replication still more mutations occur in these viruses, the result being that the entire swarm changes its overall genetic diversity on an ongoing basis.

By existing as quasispecies, RNA viruses like HIV and HCV are able to adapt to changing environments almost instantaneously. Within a swarm of quasispecies, typically numbering in the billions, there will be substantial numbers of individual viruses bearing mutations that will provide them with a selective advantage and enable them to thrive in the face of any environmental change. For instance, on initiating chemotherapy, a vast majority of the viruses in an infected individual might perish, but not without leaving behind a small population of naturally mutant viruses that are resistant to the drug and grow out in time.

However, high mutation frequency also comes at a great price as excessive accumulation of mutations renders large numbers of viruses inactive, especially if the mutations arise in vital genes. Although how many such defective viruses are produced within a quasispecies with each round of replication cannot be determined with precision. It is estimated that up to 90–95% of the viruses are replication-defective. Despite such huge losses, the small numbers of replication competent viruses are sufficient enough to keep the population proliferate as the viral population always consists of billions of viral particles.
5. Genetic diversity of HIV and the implications for pathogenesis

Classification of the viral strains into different subtypes is helpful to understand their differential geographical distribution. This also helps monitor the rapidity of viral spread in a community and the magnitude of genetic diversity generated within the population as a factor of time. More importantly, understanding subtype distribution may have a bearing on vaccine design and development. At present it is not clear whether vaccine design should be subtype specific. The scientific community is divided on the possibility of generating cross protection against different subtypes using a single vaccine.

Additionally, subtype classification of the viral strains is essential to analyze if the subtypes differ from one another in biological properties. Viral subtypes differ from one another as much as 20–25% at the genetic level. A genetic variation to this extent could also reflect in their biological properties in a way that one subtype is more infectious than others in a given clinical context. Several published reports suggested that the HIV-1 subtypes might differ in certain biological properties such as the rate of transmission from infected mothers to infants, the range of coreceptor use and others.

6. Biological differences between HIV-1 and HIV-2

The notion that subtypes of HIV-1 might demonstrate variable pathogenic properties is a logical extension of the well-documented differences between HIV-1 and HIV-2. Although HIV-1 and HIV-2 differ from each other at molecular level in the genetic structure, there are striking similarities in important properties in that they both infect only human beings and both cause AIDS after prolonged period of infection. In spite of the similarities between these viruses, good amount of evidence exists that HIV-2 is far less infectious and less pathogenic than HIV-1 [34]. Prospective studies involving mother–infant pairs have shown that the incidence of vertical transmission of HIV-2 is significantly lower than that of HIV-1 [35], [36]. Infant death rate was found to be significantly higher in HIV-1 infection than in HIV-2 [37]. Plasma viral load in HIV-2 infection was 30 times lower compared to HIV-1 infection irrespective of the length of infection suggesting that HIV-2 is possibly less pathogenic [38]. In a prospective study using a female cohort in Senegal, the rates of transmission of HIV-2 infection remained stable over the period of study while that of HIV-1 increased at a rapid rate, although both the infection rates were similar at the outset [39]. In a different study involving a cohort of infected women, disease progression from infection to AIDS was found much slower in HIV-2 infection than in HIV-1 [40]. Among HIV seropositive subjects, rate of decline in CD4+ve cells in disease progression was much slower in HIV-2-infected subjects than in HIV-1 cases. Additionally, the rate of cell decline was highly variable among HIV-2-infected subjects suggesting fundamental differences between the two infections, with HIV-1 being more pathogenic resulting in a faster and more homogeneous rate of decline than HIV-2 [41]. The difference in the rate of decline of CD4+ve cells between the viral infections was more pronounced in the later stages of infection. More importantly, a significant increase in the levels of CD8+ve cells, more so in the memory subset, was observed in the same cohort in both infections, with disease progression. In HIV-2-infected subjects, in addition to lesser degree of CD4 cell depletion, better expansion of CD8 cells was noted [42]. In a rural community in Guinea-Bissau, the adult death rate in infected subjects was found to be significantly less in HIV-2 infection as compared to HIV-1 [43] and similar observation was made by others [44]. In a recent
prospective study from Senegal, involving 472 HIV-1-and 114 HIV-2-infected subjects, annual rate of CD4 decline was found to be four times less in HIV-2 infection. However, when the rate of cell decline was corrected for baseline viral RNA load, the correlation between viral load and the rate of decline appears to be similar among all HIV-infected individuals, regardless of whether they harbor HIV-1 or HIV-2 [45]. Based on the epidemiological information that HIV-2 infections are less pathogenic, it has been proposed that a prior exposure to HIV-2 could confer natural resistance to a more virulent infection by HIV-1 [46]. However, a few recent reports contradict this hypothesis [47], [48].

The differences in the biological properties of HIV-1 and HIV-2 such as heterosexual transmission, vertical transmission, disease progression, etc. are well established. Whether similar differences also exist between various subtypes of HIV-1 is highly controversial and the information available is too inadequate. A great number of published reports have demonstrated differences among various subtypes of HIV-1 at the molecular level. Several molecular patterns that are strongly and specifically associated with one or a few of the subtypes have been identified. However, it is less understood whether such molecular differences would also mean differences in the biological properties of the subtypes. Various subtypes of HIV-1 are more or less equidistantly related to one another, exhibiting up to 20% and 20–35% difference, respectively, in the env region at the intra- and inter-subtype levels. It is reasonable to deduct that a genetic variation to this extent is likely to confer differential biological properties on the viral subtypes. In comparison, HIV-1 and HIV-2 diverge from each other up to 40% at the nucleotide sequence level in addition to having differences in the genetic structure.

7. Viral molecular determinants

Sequence variations unique for different subtypes of HIV-1 have been identified in the viral regulatory elements, including the LTR, [49]–[54], TATA box [55], TAR [55], [56], PBS [57], splice junctions [58], RRE and various other cryptic elements [59]. Additionally, viral regulatory [54], structural [49] and accessory proteins [60], also demonstrate subtype-specific sequence motifs and post-translation modifications [61], [62]. Host–virus interaction is regulated at multiple stages, from the time of the initial contact of the virus with the receptor–coreceptor–adhesion molecule complex on the surface of a target cell to the time the virus is released from the infected cell. Sequence variation in viral regulatory elements, and viral proteins therefore, could influence the overall fitness and propagation of a subtype by means of influencing virus–host interaction at multiple levels. In a broader perspective, sequence variation among subtypes and strains could influence the viral fitness at two different levels, inside and outside the target cell. In functional terms, genetic variation may influence the kinetics of viral replication inside the target cell and endow on the viral subtype to overcome adverse conditions, such as the host immune surveillance, outside the cell. A cumulative effect of multiple events at these two levels is likely to define the overall fitness for propagation of a particular viral subtype more suitable for a defined environmental condition.

This possibly could also explain the skewed nature of subtype distribution observed globally. Often in a given geographical region or a population, one of the subtypes is a predominant virus despite the fact that other subtypes are also detected in the same niche. The dominant subtype could be more suitable for these conditions, hence may enjoy survival advantage over
others. The sustenance of the other subtypes that did not ‘catch up’ was probably not supported by the host factors of the population. Host factors and environmental parameters play critical role in sustenance, spread and various other properties of pathogens. Nevertheless, it appears that some of the biological properties of HIV-1 are solely governed by viral determinants rather than the host factors such as the coreceptor use and coreceptor switch.

8. Host range and tropism

Subtype-C viruses of HIV-1 that cause over half the infections globally exclusively use CCR5 as coreceptor for viral entry. Preference for CCR5 by subtype-C over CXCR4 or other coreceptors is explicit at the time of initial infection as well as during disease progression. Unlike other subtypes, especially subtype-B strains where the coreceptor use is altered to CXCR4 during the later stages of the infection in approximately half the strains, all the subtype-C strains continue to use CCR5 even in advanced stages of the disease. The preferential use of CCR5 is highly conserved among all subtype-C strains irrespective of the geographical origin thus eliminating the possible influence of host genetics or host factors on this event.

Subtype-C strains of HIV-1 in general fail to infect target cells in the absence of CCR5. None of the primary isolates obtained from nine Ethiopian patients could grow on several T-cell lines nor could they use any of the coreceptors, including CXCR4, other than CCR5 for infection [63]. All these isolates exhibited nonsyncytium-inducing (NSI) phenotype on MT-2 cells. Complete lack of CXCR4 use of the subtype-C strains, with the exception of a small number of strains, is the characteristic feature of these viruses setting them apart from all other subtypes. While the pathogenic significance of coreceptor switch in the course of disease progression observed with nonsubtype-C strains is not understood, a complete absence of coreceptor switch in subtype-C infection is even more enigmatic. In a study using 81 primary isolates of eight different subtypes, the coreceptor use was analyzed in vitro. Majority of the subtype-C strains used only CCR5, while dual tropism (use of either CCR5 or CXCR4) was not observed in subtype-D strains [64]. This study also identified that subtype-specific coreceptor use was not linked to clinical status, CD4 count or treatment but only to the nature of the virus.

V3 loop of the env is implicated in determining critical biological properties of the virus such as the host range, cell tropism, coreceptor use, rate of growth, syncytium induction (SI), etc. [65]. Approximately 20 amino acid residues located in the V3 loop of the envelope determine cell tropism of the macrophage tropic HIV-1 strains [66]. The net charge of the V3 loop of the rapid growing SI strains is significantly positively charged as compared to the slow-growing NSI strains. The differences in the loop charge are attributed to the amino acid residues immediately flanking the loop, central GPG motif and a few other critical residues [67]. The overall charge of the V3 loop in subtype-C strains was found to be consistently 5 or below [63].

9. Transmission, infectivity and disease progression

Certain subtypes are known to be predominantly associated with specific modes of transmission. For instance, subtype-B propagates predominantly via homosexual contact and intravenous drug use, whereas subtypes-E and C, through heterosexual transmission [68]. Subtypes-C and E strains infect and replicate more efficiently in Langerhans cells (LC) which are present in the vaginal mucosa, cervix and the foreskin of the penis [69], suggesting that this property of these
subtypes allows them high potential of transmission through heterosexual contact as compared to subtype-B strains [70]. LC are a subset of specialized antigen-presenting cells of the skin dendritic cells. LC are likely the first subset of cells in the mucosa to be infected by the virus following a sexual exposure [71], [72]. After the initial exposure to the virus, LC are either directly infected themselves [71] or retain the virus on the cell surface for extended periods through virus attachment to DC-specific C-type lectin receptor DC-SIGN or a homologue DC-SIGNR [73]. DC-SIGN is expressed at high levels on LC colonizing the mucosal surfaces and specifically interacts with gp120 of HIV-1. DC-SIGN is not involved in viral entry but probably facilitates the survival of the attached viral particles for extended periods. LC with attached viral particles are believed to migrate to the secondary lymphoid structures that are rich in CD4\(^\text{+}\) T-cells thereby facilitating the spread of the viral infection [74]–[76]. The reported success of the subtype-E strains over subtype-B in establishing a rapidly spreading epidemic in Thailand is attributed to the higher magnitude of infection of Langerhans cells by these strains \textit{in vitro} [30], [70], [77].

Both subtype-E and B were introduced into Thailand independently about the same time [70]. Subtype-B is preferentially but not exclusively restricted to the intravenous drug-user communities, whereas subtype-E is spreading in the general population through heterosexual contact and eventually outnumbered the subtype-B infections that remained more or less stable. Although it has been suggested that subtype-E strains have higher potential of transmission as compared to subtype-B [78], it is important to critically evaluate the contribution made by other epidemiological factors. Compartmentalization of the subtype-B strains to IVDU could have restricted the spread of these strains to general population [79].

Additionally, the hypothesis that certain subtypes may have higher potential for transmission through heterosexual contact was not corroborated in several laboratory studies. In a study using several primary isolates of HIV-1 spanning over subtypes A through F, majority of the isolates did not display specific tropism for mature dendritic cells (DC) over PBMC. Six out of 26 isolates that showed higher infectivity for DC were distributed between both subtype-B and E strains [80]. However, the infection properties of the viral isolates could be different for DC that are highly purified and matured \textit{in vitro} as compared to the \textit{in vivo} context. The DC in the above study were obtained from the epidermal sheets of human skin, matured in a medium supplemented with GM-CSF and positively enriched using anti-CD83-specific antibody and the Minimacs system. The cell preparation obtained this way contained less than 2\% CD3\(^\text{+}\)ve cells. Importantly, coculture of DC with T-cells is essential for productive infection of the DC with HIV-1 possibly because DC being nonreplicating cells restrict viral growth [81], [82]. The above study also did not find differences in tropism between subtype-B isolates obtained from patients involved in homo- or heterosexual contact. A similar study using pools of DC and T-cells also failed to detect preferential propagation of subtype-E viruses on DC as compared to subtype-B [83].

In India where over 85\% of the infections are caused by subtype-C strains, the pattern of viral transmission is akin to the situation in Thailand. The predominant mode of transmission is through the heterosexual route where 84.72\% of the transmission is through heterosexual contact, while only 3.23\% and 3.17\% are through intravenous drug abuse and blood/blood products, respectively [84], [85] (NACO, 2002). Although no studies have been reported on the relation between the pattern of viral transmission and subtype characterization, it is believed that predominant fraction of the infections transmitted by heterosexual mode is due to subtype-C strains. Although other
subtypes, including A, B and D have also been reported from the same geographical locations [22]–[26], [31], it is not clear why these subtypes do not efficiently spread and establish parallel epidemics despite being identified as early as 1992–1994 [21], [86]. Given that the number of publications reporting on the genetic diversity of the HIV-1 strains in India is small and that the information available on the source, origin and risk factors of the infections is inadequate, the epidemiological relation between subtypes and variable infectivity, transmission and disease progression is difficult to determine. Caution must be exercised before concluding that the biological properties of subtype-C strains render them inherently superior in heterosexual transmission in India. It is critical to evaluate the impact of other socio-epidemiological factors like the population founder effect, social behavior of the populations possibly, if at all, restricting the spread of the other subtypes.

10. Population studies

Several studies evaluated the question whether subtypes differed in the rates of infection transmission and disease progression using population cohorts infected with two or more different subtypes. The number of such studies is small, and the inference drawn from these studies is conflicting and inconclusive. In a study using 49 each ethnic Ethiopians and Swedes, no significant differences in the rate of CD4 cell decline or clinical disease progression were identified over a period of two years [87]. Extending the same study, the authors also failed to see any significant difference in the rate of CD4 cell decline, clinical progression or plasma HIV-1 RNA levels among 129 individuals infected with subtypes A, B, C or D over a mean observation period of 44 months. A comparative analysis of 75 Israelis and 102 emigrant Ethiopians infected with subtype-B and C respectively, living in the same environment, resulted in similar activation profile at all stages of the infection. Surprisingly, a parallel comparison between the two uninfected ethnic groups hailing from the same population yielded contrasting immune profiles suggesting that HIV subtype is probably not a major determinant for the infection outcome [88]. Studies conducted in Thailand also failed to detect differences in pathogenicity between the two prominent subtypes circulating in that country, B and E [89], [90]. Although the latter study identified higher viral loads in the cohort infected with subtype-E during the initial months following seroconversion, at later time points identical viral load was detected in both groups. In striking contrast to the above studies, a few reports from the African continent identified that subtypes of HIV are linked to differential clinical manifestation. In Africa, several subtypes of HIV-1 are in circulation and the distribution of the subtypes is not associated with any particularly risk behavior possibly making it suitable for comparative studies. In Kenya, women infected with subtype-C underwent rapid disease progression and immunosuppression than those infected with subtypes A or D [91], [92]. In a similar study reported from Senegal, in women infected with subtypes A, C, D or G, the AIDS-free survival curves differed by subtype. Importantly, women infected with nonsubtype-A were 8 times more likely to develop AIDS [93].

Although subtype-B strains in the USA and Europe are preferentially transmitted through intravenous drug abuse, homosexual contact and blood transfusion, at other geographical regions like the Caribbean and South America they are shown to spread efficiently via heterosexual route [94]. This observation refutes the hypothesis that subtype-B strains are not efficient in transmission through heterosexual mode. Similarly, an explosive epidemic caused by a subtype-C strain was reported from Nepal that transmitted efficiently by injection drug abuse [95].
11. Vertical transmission

Differential transmission potential of HIV-1 subtypes was also analyzed using mother-to-child model. The advantage of such a study model is the feasibility to minimize the interference of various other social and epidemiological factors on the study outcome. In one of such studies from Japan, a comparison between the transmission rates from mothers infected with either subtype-E or B to their babies suggested that subtype-E has marginally higher potential (8/13 infants) of vertical transmission than subtype-B (5/13 infants) [96]. In a couple of studies from Tanzania, the rates of transmission from 51 HIV-1 seropositive mothers infected with subtypes A, C, D or recombinant strains to their infants were compared. The risk of vertical transmission was found to be significantly higher with subtype-C (6.1 times), recombinant strains (4.8 times) and subtype-D (3.1 times) than subtype-A [91], [97]. However, the sample size in all the studies above is too small to offer statistically conclusive evidence. It is important to extend these studies to larger populations. Additionally, it is also necessary to decipher the underlying mechanisms contributing to the differential transmission rates of HIV subtypes. For instance, low viral load of HIV-2 as compared to HIV-1 (nearly 37 fold less) was postulated to be responsible for the observed low mother-to-child transmission incidence of HIV-2 in Gambia [36].

12. Influential role of the viral factors

While studies conducted at the population level to characterize the relation between the genetic variation of the HIV-1 subtypes and the differences in the transmission efficiencies were often contradictory and conflicting, similar attempts to understand links between viral subtypes and pathogenesis were equally disagreeing. Following initial infection, the rate of disease progression to AIDS is highly variable. In majority of the cases it takes about 10 years on the average to develop clinical symptoms, while in several others the rate of deterioration is very rapid, progressing to AIDS within months [98]. Factors influencing the variable clinical outcome are not completely understood but are likely to be several host-related, viral and environmental factors that are interdependent. Host factors that are implicated in influencing the rate of disease progression among others are the HLA [99], [100] and polymorphism in the coreceptors especially in CCR5 [101], [102]. Influence of the viral characteristics on the rate of disease progression has been demonstrated, in addition to the comparative analyses to HIV-1 and HIV-2 infections, in a limited number of cohort studies where blood transfusions were performed from infected individuals to a few recipients inadvertently. In the case of the Sidney Blood Bank cohort, seven individuals received blood transfusion from a single infected donor [103], [104]. Phylogenetic analyses of the viruses isolated from the donor and the recipients showed that all the viruses carry multiple deletions in the Nef/LTR region [105]. Several members of the cohort remained symptom-free for periods extending over 14–18 years implicating that the defective nature of the virus was the principal factor for the delayed progression of the disease [106]. The role of the viral factors was also prominent in a study of a long-term nonprogressor that underwent a rapid transition to progressor state. Comparison of the full length virus sequences between the two clinical stages suggested that a 22 bp deletion identified within the LTR, involving the loss of one Sp1 site, of the virus possibly was responsible for the delayed disease progression in this individual [107].

Influential role of the viral factors on disease progression is further supported by the observation that infections caused by SI strains progress more rapidly to AIDS than those with NSI
viruses [108], [109]. The emergence of CXCR4 strains in the later stages of the infection has been proposed to be associated with the development of AIDS [110]. Although the significance of the coreceptor switch during the disease progression is not completely understood, CXCR4 strains are believed to be more pathogenic than CCR5 strains as their emergence is correlated with the onset of the disease. Several studies have also shown that CXCR4 strains of HIV-1 are more pathogenic in cell culture, lymphoid tissue culture, SCID-hu mice, hu-PBL-SCID mice or in the characterization of the sequential viral isolates collected from infected subjects over a period of time [110]–[114]. Most compelling evidence comes from the experiments using isogenic viral constructs that are genetically identical but differ from each other in only one or a few amino acid residues in the V3 loop. X4 virus of the isogenic pair alone caused massive depletion of human CD4 cells from PBMC and lymphoid tissue on infection [115]. Enhanced cell depletion was also reported with HIV-2 and SIV strains that use coreceptors differentially [116]. In infections with subtype-B R5 strains, biological clones isolated during the early phases of the infection were slow replicating while those from the later stages were rapid growing. Evolution of the slow-replicating isolates into rapid growing ones was also correlated with CD4 cell levels and disease progression [117]. In a parallel analysis, three biological clones of R5 phenotype isolated from three rapid progressors were shown to be less pathogenic than a standard X4 strain NL4-3 in SCID-hu Thy/Liv mice [118]. All the three subjects harbored no detectable X4 strains but exhibited rapid rates of disease progression akin to X4 infection. This study suggested that although R5 strains may not be as cytopathic as X4 viruses in vitro or ex vivo, they might be equally pathogenic in a real context of a natural infection.

In an attempt to understand why R5 strains of HIV-1 do not cause vigorous depletion of T-cells, it has been demonstrated that R5 strains are highly cytopathic for T-cells that are positive for both CD4 and CCR5 [119]. Given that expression of CCR5 is restricted only to the activated CD4 cells and that such cells constitute only a small fraction in vivo, the overall effect of R5 strains on cell depletion is marginal. However, this hypothesis ignores the fact that nearly half the infections with subtype-B and almost all the infections with subtype-C strains never undergo the process of coreceptor switch nevertheless cause the entire spectrum of clinical manifestations and finally trigger AIDS in all the infected subjects. Given that all the strains of HIV are efficient in developing AIDS, irrespective of the nature of the coreceptor use and the ability to alter coreceptor use, the pathological significance of the observations made in the in vivo, ex vivo models above is questionable.

Interestingly, the vast majority of the subtype-C strains use exclusively only CCR5 for cell entry throughout the course of the infection [63], [120], [121]. This observation compounded by the fact that CCR5 use and NSI phenotype of the strains are directly correlated [122], [123] suggests that subtype-C strains may intrinsically be less pathogenic. However, in a Scid-hu mouse model, isolates of CCR5 clones derived only from the symptomatic but not from the nonsymptomatic stage, caused severe depletion of infused human thymocytes, despite the fact that none of these isolates used CXCR4 or other coreceptor [124]. This observation suggests that despite the lack of coreceptor switch to CXCR4, CCR5 strains are intrinsically pathogenic and could contribute for the development of AIDS efficiently. The association between differential coreceptor use of the HIV strains (SI vs NSI) and the disease outcome has been studied mainly using subtype-B strains. It is of interest to extend such studies to other subtypes especially subtypes-C and E as these strains cause maximum number of infections globally. In striking
contrast to subtype-C strains that are mainly NSI, subtype-E strains are predominantly SI although both these strains share certain biological properties such as preferential transmission by heterosexual contact [125].

13. Conclusion

Since the time of introduction into the host populations, HIV-1 has been evolving rapidly. Genetically more diverse viral strains are emerging through a dynamic evolutionary process. Recombination events between two or more genetically distinct subtypes is leading to the emergence of mosaic viruses that might be endowed with biological properties more advantageous to the virus. Rapid emergence of genetic variants of the virus is posing serious challenge in developing effective control measures. Experimental evidence available is contradictory and insufficient to evaluate whether the differences observed among various subtypes at molecular level could be extrapolated to differential biological properties. More studies are urgently warranted to give essential insight into this important question. The need for such analysis is much more pronounced for countries like India where the interplay between host genetic and immune factors and subtype-C strains is least explored. Subtype-C strains have been the most successful viruses in establishing infection in most populous nations. The relative role of various epidemiological and socioeconomic factors contributing for the differential distribution of the subtypes as opposed to the viral biological properties needs evaluation.

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