

# Implications of HIV variability for transmission: scientific and policy issues

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AIDS 1997, 11:UNAIDS1–UNAIDS15

**Keywords:** HIV genetic and biological variability, cofactors of transmission and pathogenicity, molecular epidemiology

### Introduction

Current patterns of population migration and of national and international travel promote the emergence and the rapid spread of infectious agents. One of the most important pandemics of this century, fuelled by the increasingly global mixing habits of the world's population, is that of HIV/AIDS. According to the estimates of the Joint United Nations Programme on HIV/AIDS (UNAIDS), since the start of the global epidemic until 1 July 1996, approximately 28 million people have been infected with HIV. The majority of all infections in adults (approximately 70%) have been transmitted as a result of unprotected heterosexual intercourse. The contribution of other modes of transmission to the global epidemic is more limited: 8–10% cases are by mother-to-child transmission, 5–10% infections transmitted via homosexual (male-to-male) intercourse, 5–10% via sharing HIV-infected injection equipment by drug users, and 3–5% as a result of transfusion of HIV-infected blood and blood products [1].

The HIV pandemic is highly heterogeneous and dynamic in nature. It is comprised of numerous subepidemics in distinct geographical locations and population groups, each with its own specific epidemiological characteristics, modes of transmission, temporal trends, and changing patterns of incidence rates in different population groups [1–9]. The driving forces behind this epidemic are very complex and are not yet well understood. Multiple determinants of transmission are under investigation, which in general terms, could be attributed to the virus, the host and various external factors. The implications of HIV-1 genetic variability in terms of the transmissibility of the virus and possible differen-

tial pathology and the consequences these might have for public health and prevention strategies, have recently emerged as subjects of an international scientific debate [10,11]. In response to some concerns about the potential for enhanced heterosexual transmission of some genetic variants of HIV-1, UNAIDS in collaboration with Robert Koch Federal Institute of Infectious and Non-Communicable Diseases (Berlin, Germany), organized an international meeting (Berlin, 27–29 March, 1996) to discuss public health and scientific issues in relation to HIV genetic variability [12]. The UNAIDS expert group of 25 researchers in epidemiology and laboratory science, reviewed available information and made recommendations, which are summarized in this report.

### The genetic characterization of HIV-1

AIDS is caused by two types of HIV, HIV-1 and HIV-2. The more widely spread type, HIV-1, is among the most genetically variable human pathogens. A multinational effort is under way aiming at (1) comprehensively describing HIV-1 genetic variability world-wide, (2) organizing genetic data in a rational framework for establishing correlation between HIV-1 genetic variability, biological and immunological properties of the virus, and the potential correlates of vaccine-induced protection, and (3) determining the significance of HIV-1 genetic variation for the epidemiology and the pathology of HIV infection and AIDS.

Two significant milestones have been reached during the 10-year course of this effort. By 1992, the work of

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several groups had collectively established that globally collected HIV-1 strains could be grouped into several distinct genetic subtypes or clades [13,14]. These genetic subtypes are essentially equidistant and without identifiable intermediate forms. The analyses of both viral envelope (*env*) gene sequences and those encoding the matrix and core proteins (*gag*) provided phylogenetic trees of similar topology. These efforts facilitated the classification of HIV-1 into at least 10 genetic subtypes, designated A through J; all of these subtypes are classified into a major group (group M) of HIV-1 [13–22]. A number of strains remain unclassified, since they do not cluster with any of the recognized subtypes, but at the same time do not meet the criteria to be grouped into a ‘new’ subtype. In addition, a group of highly divergent HIV-1 strains was recently identified, which are referred to as ‘outliers’ or group O of HIV-1 [13,23,24]. HIV-2 isolates can also be classified into at least five genetic subtypes [13,15].

In 1995, a second milestone was reached when the contribution of recombinant strains to the global pandemic was fully recognized [25–29]. A retrospective analysis has established that a significant fraction, perhaps more than 10%, of reported HIV-1 strains represented mosaic or recombinant forms, bearing interspersed segments of genetic information from two different genetic subtypes. Recombination breakpoints have been mapped in most structural and regulatory genes and long terminal repeats (LTR) of the virus.

Traditional phylogenetic methods are often insufficient to distinguish recombinant from non-recombinant strains, and recombinant strains with similar breakpoints are prone to cluster together and be misinterpreted as ‘new’ subtypes. In fact, genetic analyses of full-length and partial genomes have shown that subtype E viruses from south-east Asia do not represent a real subtype but are mosaic viruses with genetic material from subtype A and from a yet unidentified parental subtype E virus. The subtype E viruses from Africa and Asia possess similar recombination breakpoints with most of their genome being derived from subtype A, but gp120 with an external portion of gp41, and a segment of the LTR being derived from subtype E. Subtype E viruses apparently represent recombinant forms diverged from a common ancestor and are already spreading globally without a significant alteration of the mosaic structure [27,28]. Similarly, the first analyses of HIV-1 subtype G revealed a variety of G/A recombinant forms but no parental subtype G virus, which are also widely dispersed geographically [26]. In view of these new data, genetic classification based on fragmentary genomic sequences may not be completely satisfactory. The genetic characterization effort would be facilitated by more complete sequencing of multiple full-length genomes representative of all known subtypes.

The existence of mosaic forms implies that dual infection in humans with HIV-1 strains of different subtypes may occur and may result in generation of viable and infectious progeny virions. Recombinant strains may contribute to the dynamic and unstable nature of the global epidemic, which calls for an ongoing and informed surveillance effort to monitor changing proportions and geographical shifts among subtypes.

## **Geographical distribution of HIV subtypes/descriptive epidemiology**

Although the amount of HIV-1 and HIV-2 genetic sequence information has increased rapidly in recent years, most of it has been gathered via convenience samples and outside the framework of epidemiologically based systematic attempts to characterize HIV isolates from subpopulations in a given geographical region or cohort studies. A number of collaborative efforts are under way to systematically collect and characterize larger numbers of isolates from well-defined populations. For example, the World Health Organization (WHO) Network for HIV Isolation and Characterization was established in 1991 [30] to collect HIV isolates from a number of countries world-wide and to monitor genetic and antigenic variation in potential field sites for HIV vaccine trials. The WHO Network has received further development within UNAIDS with a greater emphasis on molecular epidemiology of HIV.

The regional summaries in the following sections illustrate the diversity of the global pandemic and some of the complexities that are inherent in studies aimed at defining the relationship of HIV genetic variability to epidemiology, transmission and pathogenesis.

### **Africa**

Based on current data, the greatest diversity of HIV strains has been found in sub-Saharan Africa, which also has been the region most severely affected by the HIV/AIDS epidemic. More than 13 million adults and adolescents in Africa are estimated to be infected with HIV [1]. Serosurveillance studies indicate that HIV prevalence varies and, in general, is lower in West Africa than in eastern and southern Africa [31–37].

The major modes of transmission in Africa are through unprotected heterosexual intercourse and from mother to child. The rapid spread in some areas has not been fully explained, but appears to be linked to high numbers of sexual partners, frequency of sexual contacts with prostitutes, lack of circumcision and the high prevalence of other sexually transmitted diseases (STD) [1,6,7,38–41].

## Implications of HIV variability for transmission

The results of multiple descriptive molecular epidemiology studies in Africa indicate that all of the known HIV-1 subtypes, and HIV-2 are present on this continent. HIV-2 appears largely restricted to West Africa, and HIV-1 group O has been recovered most often from West Central Africa. In most areas where both HIV-2 and HIV-1 are present, the prevalence of the former does not appear to be changing over time, whereas in contrast the prevalence of HIV-1 has been steadily increasing, often concomitant with a reduced rate of spread of HIV-2 [42–45].

Genetic heterogeneity of HIV-1 strains from Africa shows a variation up to 35% in gp120, which reflects the presence of multiple genetic subtypes and the longer duration of the African epidemic by comparison with other regions of the world. Most of the countries in sub-Saharan Africa harbour multiple genetic variants of HIV. In some countries up to eight different subtypes of HIV can be found on small sample sizes. Such close intermixing of subtypes may explain, in part, why many of the known recombinant HIV strains have been found in this region. Certain genetic subtypes are more frequently found in specific geographical locations and are associated with epidemics with varying prevalence and incidence rates. For example, subtypes A and D are prevalent in a broad east–west belt across sub-Saharan Africa; the subtype C viruses appear to be most prevalent from north to south along Africa's eastern flank. The distribution of other genetic subtypes (e.g., B, E, F, G, H) may be broad but they do not appear to be prevalent subtypes in any African region [13–18,46–48].

With this complex picture, it is clear that few predictions can be made with respect to the mixture of subtypes that will be found in any cohort or geographical region or in the context of the likely future evolution of the virus. The ability to differentiate all HIV-1 subtypes and to identify recombinant strains is a crucial element of studies in Africa that are designed to relate HIV genetic diversity to epidemiology, transmission, pathogenesis, and potential vaccine efficacy.

### The Americas

Since the first recognition of AIDS, HIV-1 spread rapidly in this region primarily through unprotected homosexual intercourse and injecting drug use. The proportion of heterosexual transmission, which on average is at a considerably lower level than in Africa, has shown a slow but steady increase over the last 5 years [1,2,49–51]. The number of persons living with HIV/AIDS by July 1996 was estimated 780 000 in North America and more than 1.5 million in Central and Latin America. Although, in general, the rates of HIV spread have been slower in this region than in Africa and Asia, there is a wide variation in the level and speed of the epidemic among different populations,

subregions and countries. In North America, the epidemic has slowed in recent years and is approaching a stable prevalence in some regions, largely due to the decline in sexual transmission between homosexual men. However, during the last 5 years the increase in HIV/AIDS incidence has been greatest for women compared with men, as well as for African Americans and Hispanics compared with Caucasians. The epidemiological evidence in Latin America and the Caribbean indicates that the epidemic is progressively shifting to heterosexual populations, to non-urban areas, to a younger population and those with lower socioeconomic status [51–54].

A number of surveillance and monitoring programmes have been launched in the Americas. In the United States, the Centers for Disease Control and Prevention has conducted retrospective serosurveillance studies of large collections of blood samples from persons in the United States to document the prevalent HIV subtypes. Similarly, the National Institute of Allergy and Infectious Diseases is sponsoring a number of studies to monitor HIV genetic variability at domestic and international sites. The US Department of Defense has monitored the HIV epidemic by periodic evaluation of active duty, reserve and dependent populations since 1985. Addition of HIV-1 subtyping to such routine surveillance efforts will help to document the relative proportion of HIV subtypes being transmitted.

A Canadian HIV/AIDS Strain Surveillance Programme has been launched recently with an objective of ensuring systematic surveillance of HIV subtypes in Canada and generating information related to the areas of concern to public health authorities, such as HIV diagnostics, molecular epidemiology, HIV vaccine development and monitoring the trends of HIV drug resistance. The first cases of heterosexual transmission of HIV-1 subtype A in Ontario have already been documented by this programme.

In Brazil, an active HIV Characterization Network has been established which conducts molecular epidemiological studies throughout the country. As a result of these studies the presence of multiple HIV-1 subtypes, including subtypes B, B', F and C, as well as B/F recombinant viruses have been found in Brazil [30,55,56].

Overall, in the majority of cases the HIV strains in the Americas belong to subtype B which is transmitted by all known routes, including heterosexual intercourse. Recent reports have started documenting the introduction of different HIV genetic variants, including subtypes A, D, and E from group M, as well as group O isolates of HIV-1 [9,56–64], but, by and large, these subtypes appear to constitute a relatively low proportion at the present time. The epidemiological situation

in the Americas with only HIV-1 subtype B being predominant is still relatively simple compared with other geographical regions. Nevertheless, the presence of subtypes F, C and E in South America, and the introduction of subtypes A, D, E, and group O in North America calls for a concerted effort to monitor shifts in the proportions of HIV types and subtypes in the future.

### Europe

Approximately 500 000 individuals are thought to be infected in Western and Eastern Europe. The HIV transmission patterns differ significantly between individual countries and regions. In Western Europe, the epidemic started in the early 1980s among homosexual men, which peaked in the mid-1980s. Over the past 2–3 years, HIV prevalence appears to have stabilized in parts of north-western Europe, but there is no indication of its levelling in south-western Europe (Spain, Italy), where the major route of transmission has been injecting drug use. The proportion of heterosexual transmission in the western European countries is higher than that in North America, and there has been a steady increase in the proportion of female cases, which rose from 11% in 1986 to 20% in 1995 [1,2].

The eastern European countries were spared from a wide spread of HIV until the 1990s with a limited number of HIV/AIDS cases mostly among homosexual men and some sporadic cases of nosocomial outbreaks of HIV infections among hospitalized or institutionalized children in Romania and Russia [19,65,66]. However, recent reports about rapid increase of HIV incidence rates among injecting drug users (IDU) in Poland, Federal Republic of Yugoslavia, and more recently in Ukraine and Russia [67–69], coupled with high prevalence rates of STD and the presence of multiple socio-economic factors increasing the vulnerability of certain population groups, indicate that the epidemic in Eastern Europe is entering a phase of increased growth.

As in the Americas, the majority of HIV isolates reported from Europe have been of subtype B, which is widely spread among homosexual men and IDU. However, transmission patterns vary widely within this region. Several western European countries, including Belgium, France, the United Kingdom, Sweden and Portugal, have appreciable numbers of HIV infections with HIV-2, HIV-1 group O, and with HIV-1 subtypes other than B. The majority of non-subtype B infections were acquired in Africa. [13,21,70–72], which thus far have not appeared to spread widely beyond the imported cases and their direct contacts. In Belgium, HIV-1 subtype surveillance has shown that non-B HIV-1 subtypes have been present for some time in the country without causing substantial secondary heterosexual or other chains of transmission [73].

In Eastern Europe, the presence of nearly all known genetic subtypes of HIV-1 has been reported, including subtypes of extremely low prevalence in other parts of the world, such as subtypes F, G, H and I [19,21,74–77]. The present HIV subtype distribution on the background of low prevalence of HIV/AIDS in Eastern European countries mostly reflects multiple introductions of HIV in the region and largely relates to the specific population migration patterns and routes of drug trafficking. Prospective molecular epidemiological studies of HIV in this region, if conducted in sufficient detail, may provide a unique opportunity to better understand the role of HIV variability in transmission.

### Asia and the Pacific

Although the extensive spread of HIV-1 in this region with about 60% of the world's population, did not begin until the late 1980s the number of HIV infections has increased rapidly and is estimated to be over 3 million [1,2,78]. The HIV epidemiology in Asia is extremely diverse, ranging from countries with low prevalence (Mongolia, Democratic People's Republic of Korea) to countries with high prevalence (Thailand, Cambodia, Myanmar). The experience with Thailand illustrates the potential for the rapid HIV-1 transmission in Asia. Due to a well-developed public health programme and sentinel surveillance systems, the Thai epidemic is relatively well documented. In 1988, an explosive HIV-1 transmission occurred among a large population of IDU in Bangkok [79,80]. This was followed by a larger epidemic of heterosexual HIV-1 transmission, largely related to the common practice of male patronage of female sex workers [79,81–83]. Molecular epidemiological studies indicated that these were two independent introductions causing separated epidemics with two different genetic subtypes of HIV-1 [84,85]. It has been proposed that increased infectiousness occurring in the primary phase of HIV infection with high viraemia, and the large number of clients served by commercial sex workers, compounded by high prevalence of STD, contributed greatly to the rapid spread of the epidemic in Thailand [6,83].

Molecular epidemiological studies in Thailand have helped to characterize patterns of distribution and segregation of certain HIV subtypes by modes of transmission. Extensive HIV-1 transmission among IDU in Thailand was largely due to subtype B strains. Some early strains were closely related to North American subtype B strains while another genetic variant of subtype B did emerge in Thailand, known as Thai subtype B (or B', also in earlier publications referred to as 'genotype A'). The Thai B variant has a 'GPGQ' motif at the crown of the V3 loop, which makes this variant serologically distinct from the typical North American subtype B strains [86]. The second wave of the heterosexual epidemic was caused by subtype E virus. More

recent studies have documented that subtype E viruses have also started spreading in increasing proportions among IDU in Bangkok [87]. In addition, the molecular data reveal an extremely narrow range of intra-subtype genetic heterogeneity of HIV strains harboured in this region, not exceeding 2–5%, which is comparable to the variability within a single infected individual [28,30,84,85,87]. These results are supportive of other epidemiological observations indicating the staged/independent introductions of HIV-1 with rapid clonal expansion of the virus in different population groups which are separated by relatively independent sexual networks or other behavioural risk factors [6,22,84,85].

Two important foci for the future global epidemic are represented by China and India, the two countries with the world's largest populations. The reports from India suggest an extensive spread of HIV in many parts of the country which is fuelled by low use of condoms, wide spread of STD and injecting drug use. The epidemic in China is starting to gain speed with an extensive spread of HIV among IDU in Yunnan Province and heterosexual transmission in southern China [1,2,85,88–90]. Another example of a rapid spread of the epidemic could be seen in Vietnam, where the HIV seroprevalence among IDU has reached 32% during 1992–1995, with a parallel rapid heterosexual spread of HIV among young men and women in the south of the country [1,2,91].

Similar to the situation in Bangkok, the heterosexual epidemic in China is related to subtype E strains [88,89]. However, the epidemic among IDU in Yunnan province, which was started with Thai variant of subtype B strains, is now also being caused by subtype C HIV-1 [89,90], which probably reflects the drug trafficking links between China, Thailand and India. The epidemics in the north of Thailand and Vietnam from the very beginning have been quite different because subtype E viruses have always dominated both IDU and heterosexual routes of transmission [84,85,91].

The molecular epidemiology data from India indicate the presence of both major types of HIV, HIV-1 and HIV-2. Among the HIV-1 subtypes, subtype C strains, which are genetically close to the viruses from South Africa, have been dominating the heterosexual epidemic in the country, although subtypes A, B and D have also been reported [92–94]. These results demonstrate that there are no geographical limits to the spread of HIV-1 subtypes between widely separated continents and countries, which most probably is related to population migration patterns.

## Correlation between genetic subtypes and biological variability of HIV-1 *in vitro* and *in vivo*

### Biological variability of HIV-1

The relationship of HIV-1 genetic subtypes to biological and immunological variation is an important area of enquiry, and has received increasing attention in recent years. The *in vitro* characterization of HIV-1 has resulted in definition of distinct biological phenotypes of HIV-1 based on growth kinetics (slow/low versus rapid/high), target cell tropism (lymphocytes, monocytes/macrophages, and established cell lines), cytopathic effects [syncytia-inducing (SI), SI versus non-SI (NSI)], susceptibility to neutralization and susceptibility to antiretroviral drugs. Most rapid/high viruses are also highly cytopathic (i.e., SI) and possess a broader range of target cell tropism, while the slow/low viruses are of the NSI phenotype with preferential tropism to monocytes/macrophages and to a lesser extent to CD4+ lymphocytes [95–97].

The biological properties of HIV-1 are largely determined by genetic variation in the *env* gene, in particular in the region encompassing the V3 loop. Point mutations, which lead to substitutions by positively charged amino acids at defined positions in the V3 loop and result in an overall increase of its positive charge, strongly correlate with SI properties of the virus [95]. In addition, other regions in the *env* gene, including V1, V2, C4 and the gp41 transmembrane region have been suggested to play a role in determining the biological properties of the virus [98].

Most of the knowledge about the biological variability of HIV-1 was built up through the studies of subtype B viruses. More recent studies on a large panel of internationally collected HIV-1 isolates, belonging to six major subtypes (A–F), have led to the important conclusion that, despite substantial genetic variation between different subtypes, the genetic determinants of biological phenotypes are largely conserved and exhibit similar relationships as in the case with subtype B viruses [99].

Neutralization by monoclonal and polyclonal human antisera has been demonstrated for a variety of HIV isolates. This biological property of the virus may guide the development of effective vaccines. In HIV-1-infected human individuals, neutralizing activity can be detected in approximately 50% of cases. It may be specific either to the infecting strain or may have broader activity. The initial hypothesis about possible correlation between the known genetic subtypes and neutralization serotypes has been difficult to verify experimentally. In a number of independent checkerboard cross-neutralization studies, it has been shown that genetic subtypes do not represent the classical neu-

tralization serotypes [100,101]. Unlike HIV-1 infection, serum neutralizing activity is regularly present in HIV-2 infection [102], which may suggest the importance of an effective neutralizing antibody response in delaying disease progression in HIV-2-infected individuals. Further research in reagents, methodology and large-scale cross-neutralization analyses coupled with a detailed genetic characterization will be required to determine the existence of neutralization serotypes of HIV-1. Such work is likely to have important implications for HIV vaccine development.

The cellular arm of the human immune system is also important in combating HIV-1 infection. A recent compilation provides an overview of what is known about the CTL epitopes in HIV and reveals a general lack of information on important CTL targets in *env*, *gag*, *pol*, *nef*, for subtypes other than B. The available data suggest the existence of at least partial cross-reactivity in many CTL epitopes across subtypes. However, such information has to be considered with caution due to the possible interference of additional variables, in particular the heterogeneity in human leukocyte antigen (HLA) restriction patterns between infected individuals [103,104].

Another layer of complexity in understanding the relationships between viral and host genetic variability and immune protection is brought to light by recent research in a variety of factors, including the existence of multiple cytotoxic T-lymphocyte (CTL) epitopes encoded by structural and regulatory genes, HLA heterogeneity, the role of secondary receptors and chemokines in HIV binding and fusion and host genetic factors influencing receptor specificity [104–110].

### The relationship of HIV variability to pathogenesis

The significance of the biological variability of HIV *in vivo* has been the subject of many natural history studies. The pathogenesis of the two major genetic types of HIV has been shown to differ significantly, with HIV-2 being both less transmissible and less pathogenic than HIV-1 [42–45,111,112].

The role of HIV-1 biological variability in pathogenesis has been investigated mostly in the cases of HIV infection with subtype B viruses in health-care settings typical of the developed world. It has been demonstrated that SI and rapid/high biological phenotypes are typically associated with late stages of immunodeficiency, whereas viruses lacking this property are more commonly associated with asymptomatic individuals or patients with mild symptoms [95–97].

With respect to HIV-1 subtype B virus infection, viral load is closely associated with pathogenesis or the rate of progression to AIDS. The prognostic value of the

viral load measurement has been generally acknowledged recently as a prognostic laboratory marker of disease progression in most natural history studies and clinical trials of antiretroviral drugs [105,106]. Careful evaluation of the viral load dynamics in longitudinal studies in patients infected with subtypes other than B will provide important data regarding relative pathogenicity of different genetic subtypes of HIV-1. Some additional tools will be required to collect this data, including quantitative viral load assays standardized across different genetic subtypes.

Several other viral biological characteristics and corresponding host immune responses have been suggested as correlates with the slow rates of disease progression, such as infection with naturally occurring attenuated viruses, polyclonal expansion and sustained levels of CD8+ CTL and the persistence of high levels of broadly cross-neutralizing antibodies, which have frequently been found in long-term non-progressors [103–107].

On the other hand, similar to the mechanisms of immune protection, the role of multiple genetically determined host factors could affect HIV pathogenesis, including the range and the quality of both humoral and cellular arms of immune responses, and the activity of the immunoregulatory lymphokine networks [104]. The most recent research findings have provided evidence about the role of host genetics in resistance to HIV primary infection and the rate of progression to AIDS. It has been shown that HIV attachment to and fusion with the target cells is determined not only by its binding with CD4 molecule, but also by at least two other secondary binding sites, represented by CCR5 and CXCR4 receptors [108,109]. It has been found that individuals who are homozygous for a 32-base-pair deletion in the CCR5 gene are less frequently infected with HIV, whereas the individuals who are heterozygous for the same mutation become infected but still can be protected against rapid progression to disease compared with the individuals homozygous for the normal CCR5 gene. In addition, it was demonstrated that utilization of these secondary receptors is determined by the biological phenotype (NSI and SI) of viral strains and not by their genetic subtype. The NSI variants, regardless of their subtype, could only use the CCR5 receptor as a secondary binding site, whereas SI variants could use either CCR5 or CXCR4 receptors [110]. These results further strengthen prior observations indicating that the major viral properties such as infectivity, transmissibility and pathogenicity are more likely determined by viral biological characteristics rather than by association to a certain genetic subtype.

Examination of all of these variables in longitudinally evaluated cohorts harbouring multiple HIV-1 subtypes will be required to better understand the host–virus

interplay in the outcome of HIV infection. However, such studies will be costly to implement and will require interdisciplinary teams of researchers, including virologists, immunologists and epidemiologists.

### The relationship of HIV variability to transmission

Two observations from HIV molecular epidemiological studies have coalesced to generate hypotheses concerning the relative efficiency of transmission of HIV subtypes by different modes of transmission. The highly variable geographical distribution of HIV subtypes, with subtype B predominating in Europe and in North America but all other subtypes predominating in Africa, coupled with the data indicating that the major mode of transmission is heterosexual in Africa but largely via homosexual exposure or injecting drug use in Europe and North America, fuelled speculation that mode of transmission has played a significant role in determining which HIV genetic (and/or biological) subtypes become established and spread in susceptible populations. However, the interpretation of the current data linking transmissibility and viral subtypes is complex.

The only convincing evidence of differential transmission rates of HIV has been shown between HIV-1 and HIV-2. It was documented that sexual and mother-to-child transmission efficiency of HIV-2 strains is much lower than that of HIV-1 [42–45,111,112].

With regard to differences in transmissibility of different subtypes of HIV-1, there are relatively few studies completed to date. In a cross-sectional study of HIV-1 index men and their sex partners, Kunanusont *et al.* [113] found higher seroconcordance rates among couples infected with subtype E, compared with those infected with subtype B. However, in this study most men infected with subtype B were also IDU, whereas most men infected with subtype E were probably infected sexually. Studies that avoid this potential confounding effect will be required in the future to fully examine virus-specific differences in transmissibility.

Per-sex-act transmission probabilities, although difficult to estimate, do provide a more accurate measure of transmission risk. In general, as with other sexually transmitted infections, there appears to be a higher probability of male-to-female HIV-1 transmission than that for female-to-male transmission. Studies of heterosexual discordant couples in which one partner is at risk of HIV infection only from their partner have estimated very similar per-sex-act transmission probabilities for subtype B in Europe and North America ( $P = 0.001$ ) and for subtype E ( $P = 0.002$ ) in northern Thailand [6,114].

In mother-to-child transmission, approximately one-quarter to one-third (depending on the study) of infants

born to HIV-1-infected women became infected in the absence of interventions; no consistent relationship with subtype has been demonstrated, although there are insufficient data available. In contrast, mother-to-child transmission of HIV-2 occurs at a rate of about 1% [111,112].

Laboratory investigations of the cell tropism, viral load in blood and other body fluids, and of the replicative potential of biological and genetic variants of HIV-1, provide an independent and important approach in determining whether HIV-1 genetic subtypes and biological phenotypes differ in ways that may influence transmission. The role of the virus cell tropism, specifically to Langerhans' cells, has been the subject of recently reported studies. The interest in Langerhans' cells is explained by differential localization of these cells in specific anatomical sites, such as vagina, cervix and penile foreskin, and the absence of these cells in rectal or colon mucosa, which may shed light on mechanisms of heterosexual transmission of HIV.

Langerhans' cells belong to the class of CD4+ dendritic antigen-presenting cells, which after the uptake of antigen migrate to regional lymph nodes, where they undergo a profound functional and phenotypic switch and mature into potent immunostimulatory cells. In earlier studies, it was shown that laboratory subtype B strains of HIV-1 (LAI and SF162) could productively infect purified cultures of Langerhans' cells. The coculture experiments with HIV-1-infected Langerhans' cells and uninfected CD4+ T cells showed that HIV-1-infected Langerhans' cells readily formed clusters and syncytia with T cells, resulting in efficient cell-to-cell transmission. It was also demonstrated that during the cell-to-cell interaction, Langerhans' cells not only effectively infect T cells, but also activate them resulting in enhanced virus replication [115,116].

Soto-Ramirez *et al.* [117] evaluated the replication efficiency of selected HIV-1 strains belonging to subtypes B, E and C in peripheral blood mononuclear cells (PBMC) and purified Langerhans' cells. They demonstrated that although all HIV strains grew efficiently in human PBMC, the replication of subtype E and C viruses in Langerhans' cells was significantly more efficient than subtype B strains. These results together with other published epidemiological data convinced the authors to put forward a hypothesis about the role of differential Langerhans' cell tropism of different subtypes of HIV-1 in heterosexual transmission.

A slightly different mechanism of mucosal transmission was suggested by Spira *et al.* [118], using the SIV/rhesus macaque model. It was documented that in the case of SIV, a retrovirus which is closely related to HIV, the primary target cells during mucosal transmission were the dendritic cells located in the lamina propria of the

vaginal mucosa. The investigators could not confirm the presence of infected Langerhans' cells in the epithelium following exposure to the virus, and suggested that at the primary stages of infection the virus could bind to Langerhans' cells without infecting them, and these virus-bound cells would serve as a vehicle to transport the virus to the lamina propria where they pass the infection on to dendritic cells. On the other hand, in chronically infected animals SIV-infected Langerhans' cells are frequently found in the epithelium, which is explained by the investigators as a result of secondary viral infection. Another important observation in this study is that the thickness of epithelium could be an important barrier for the virus, whereby passage of the virus through a single layer of epithelial cells (as in endocervix) is much easier than passage through multiple layers in the vagina. This suggestion is further strengthened by another experiment with the SIV/macaque model, where it was shown that injections of progesterone, which regulates the cyclic changes of the multilayer structure and desquamation in vaginal mucosa during menstrual periods, may significantly modify the susceptibility of the animals to infection via vaginal mucosa [119].

It is also important to bear in mind the role of viral load in semen and vaginal secretions in heterosexual transmission. D. Cain (personal communication, 1996), compared the levels of the vaginal viral load in HIV-infected women from the United Kingdom and South Africa. All of the South African women were infected with subtypes A and C, whereas the majority of the UK samples were from women infected with subtype B. The results of this study showed a significantly higher (up to 10-fold) level of vaginal viral load in South African women than in women from the United Kingdom. But it should be noted that in this study the contribution of other cofactors, such as other STD or time since seroconversion, was not appropriately controlled, which limits the generalizability of these results.

When analysing the role of HIV genetic variability in transmission, it is very important to keep in mind that sexual transmission is influenced by multiple cofactors, which may positively or negatively interact with each other, such as specific sexual practices, use of condoms and contraceptives, presence of sexually transmitted infections, the stage of HIV infection, and other viral and host factors. The transmission probability, effective rate of partner exchange and duration of infectiousness, all determine the basic reproductive rate of the virus (the rate at which a given strain generates secondary cases of infection). Due to the potential role of these multiple factors that control transmission, which in turn are also highly variable between individuals and over time, research in this area needs to take into account sociobehavioural (e.g., sexual practices), anatomic (e.g.,

circumcision), immunological, clinical and other established factors, for example STD and host genetics.

In summary, much research has begun transmissibility, but it does not yet provide a clear picture of the most important determinants. A systematic exploration of HIV-1 subtypes and biological variants, both *in vitro* and in the settings of longitudinal cohorts, well-characterized with respect to sociobehavioural, anatomic, and clinical factors, where transmission is monitored concomitantly with viral and host factors, will be required to conclusively establish links between viral subtypes and the efficiency of transmission via different routes.

### Laboratory methods for HIV molecular epidemiology

The high genetic variation of HIV has significant implications for the sensitivity and specificity of diagnostic and screening methods. The isolation of HIV-1 in 1983 [120,121] and the development of a serological test soon thereafter were major breakthroughs. However, the subsequent identification of HIV-2 required significant modification of diagnostic tests. The identification of the highly divergent genetically group O strains of HIV-1 required another modification of some diagnostic tests, particularly those utilizing synthetic peptides and recombinant proteins as antigens. At the same time, the techniques based on whole virus lysates still are capable of reliably detecting all HIV-1 variants. The newer generation of laboratory techniques for HIV detection have been modified to include the group O specific antigens and thus improving the sensitivity and specificity of currently available HIV screening and diagnostic techniques [23,24,63].

This experience with group O HIV-1 has heightened awareness of the possible problems that may arise in the case of emergence of new highly divergent genetic variants, which may render the existing diagnostic and screening tools less efficient and undermine their value for public health. It is therefore important to monitor the sensitivity and specificity of all current and future diagnostic kits.

With regard to laboratory assays for molecular epidemiological studies, each of the available techniques has its own limitations for large-scale screening, which may be related either to low levels of sensitivity and specificity for different HIV-1 genetic subtypes or to the technical complexities of the test making it not ideal for systematic analysis of large numbers of biological samples.

One of the most attractive and widely used techniques for molecular epidemiological studies have been serological assays. Routine serological screening tests, even



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if only partially effective in detecting variant HIV strains, remain an essential first step in surveillance. The traditional HIV antibody screening tests combined with confirmatory assays and HIV antigen detecting assays are able in most cases to detect HIV infection, as well as to distinguish between HIV-1 and HIV-2. However, these tests do not distinguish between different subtypes and further characterization in subtyping is required.

Subtype-specific serological techniques, such as synthetic peptide-based enzyme immunoassays, can be useful for preliminary subtyping in certain situations. The third hypervariable loop (V3 loop) of the external envelope glycoprotein, gp120, has been widely used because the majority of HIV-positive individuals are seroreactive to it and because of its extensive variability between subtypes. In Thailand, for example, this approach has been effective since only two antigenically distinct subtypes, B and E, appear to predominate. However, application of serological subtyping has been shown to be more complicated in Africa, where highly heterogeneous genetic variants of HIV-1 are present. Additionally, the occurrence of inter-subtype recombinant HIV-1, with its implication of occasional infection of humans with more than one HIV-1 subtype, further complicates test development by giving rise to unresolvable interpretations of cases of seroreactivity to multiple V3 peptides. Nevertheless, the relative ease of subtype-specific antibody detection makes it a useful complement to other techniques, especially in large-scale studies of HIV-1 subtype distribution [122–124].

The application of molecular techniques based on the polymerase chain reaction has dramatically improved the detection and genetic characterization of infectious pathogens, such as HIV. The major challenge to the large scale genetic screening of HIV subtypes has been the development of more efficient and feasible molecular techniques [37,47,125,126]. However, as discussed above, the problems related to the frequent occurrence of genomic recombination in HIV may have a significant impact on the reliability of results obtained by genetic screening techniques, which typically rely on a small segment of the viral genome for subtyping. Genetic screening methods can be strengthened by inclusion of multiple, short genomic regions, rather than dependence on a single region. The development of efficient screening methods, such as heteroduplex mobility assay (HMA) for multiple genome regions would be a logical step at this time.

When DNA sequencing is the primary approach, each of the regions sequenced should contain sufficient genetic diversity to allow reliable estimation of phylogenetic relationships. Any discordance of subtype assignment between different genome regions, or any segment that behaves as a genetic outlier to established subtypes, should be investigated by full-length *gag* and

*env* sequencing or, preferably, by full-genome sequencing.

In order to meet the needs of large-scale monitoring of HIV subtypes and to ensure higher sensitivity and specificity of laboratory methods used for these purposes, there have been several algorithms suggested, which combine different techniques, such as V3-peptide serology, HMA and sequencing [91,124]. These algorithms take into account the relative value and limitations of each method and attempt to rationalize the HIV-1 subtype determination. It was shown that prescreening of samples by peptide serology, and further confirmation of samples with indeterminate results by HMA or sequencing facilitates an increased efficiency of HIV-1 subtyping efforts [87]. In addition, sequencing of the region encompassing V3 region provides important information that could be used for further fine-tuning of the V3-peptide serological tests and their adaptation to the specific geographical locations.

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## Methodology considerations related to molecular epidemiology of HIV

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As understanding of the significance of HIV subtypes' genetic and biological variability increases, knowledge of their frequency and distribution will play an increasingly important role in determining their relationship to transmission and pathogenesis. At present, most data regarding HIV subtypes are derived from studies conducted for purposes other than surveillance. It is important to establish world-wide surveillance networks, which would allow the collection of reliable information on distribution and the rate of spread of different subtypes in different populations with various transmission risk factors. Nevertheless, in the absence of such networks to date, the ongoing convenience sampling and increased retrospective and cross-sectional testing still may provide useful information, which could be useful in planning for more definitive molecular epidemiological studies, better defining the objectives, selecting appropriate populations and sites, and identifying the role of potential cofactors that could influence an in-depth analysis.

An important strategy, which is both resource effective and most likely to detect shifts in genetic constitution of HIV populations, involves identifying and screening accessible subpopulations who have higher risks of HIV infection. As mentioned previously, efficient laboratory testing algorithms are also needed for large-scale screening of different populations. This is important both in areas where the HIV epidemic has been present the longest and in which extensive genetic heterogeneity of HIV has had time to develop, and in countries where the epidemic is more recent and separate intro-

ductions of HIV variants may be detected. Efforts should be made to sample on a longitudinal, cross-sectional and long-term basis, since only consistent monitoring of defined populations over extended period of time can reliably detect changes in the presence and frequency of various strains (in other words to detect the direction of evolution).

In addition to sampling at the population level, sampling at the individual level, including different body compartments, should be considered, and appropriate methods for specimen collection should be selected and standardized.

Finally, in order to be able to comprehensively interpret virological findings, studies should attempt to control for various factors, such as demographic, exposure risk, immunological and clinical parameters, which may influence HIV transmission.

Timely reporting and dissemination of these data should allow other investigators to confirm previous findings and prioritize new areas for surveillance and research. There may be significant value in compiling, organizing and distributing molecular epidemiological data concerning HIV-1 transmission and pathogenesis with comprehensive listing of ongoing and proposed cohort studies around the world. The Los Alamos National Laboratory Human Retroviruses and AIDS Database, which provides a compendium of HIV sequence information, is an excellent example of the value of such efforts, and could serve as model for an HIV-1 epidemiology database resource.

## Major findings and conclusions

Human immunodeficiency viruses (HIV-1 and HIV-2) are characterized by extensive genetic variability. There are at least 10 distinct genetic subtypes (designated from A to J) in the major group (group M) of HIV-1, in addition to a group of highly heterogeneous strains, which are designated as group O ('outliers'). At present, specific subtypes are found more frequently in certain countries or regions of the world; however, given the mixing of people within and between countries, it is likely that multiple subtypes of HIV-1 will appear in most countries.

The implications of genetic differences between HIV-1 subtypes, in terms of ease with which they transmit in different populations at risk of infection and the possible differential pathology they may induce, are of considerable public health and scientific interest.

Recent research findings suggest that some of the types or subtypes of HIV may be more easily transmitted

than others. To date, the clearest evidence for differences in transmissibility and virulence has come from comparative epidemiological studies of HIV-1 and HIV-2 in communities where both viral types are present. HIV-1 is both more transmissible by all routes and more pathogenic (in terms of shorter incubation period in the human host). However, current evidence of biological differences between different HIV-1 subtypes is inconclusive.

Overall, the available data can be summarized as follows below.

Indirect evidence in favour of differences in transmissibility of HIV-1 subtypes is based on the following:

- (1) *In vitro* experiments showing higher replication of selected isolates of subtype E and C, compared with subtype B viruses, in Langerhans' cells, which have been suggested as a primary target during heterosexual transmission.
- (2) Ecological observations of variation in prevalence and segregation of different subtypes in different vulnerable communities, where the major transmission route differs.
- (3) A cross-sectional study of heterosexual couples in Thailand, which suggests a higher risk of heterosexual transmission of subtype E than that of subtype B.

Evidence against major biological differences of different HIV-1 subtypes includes the following:

- (1) The ongoing epidemics caused by subtype B viruses in heterosexuals in specific countries.
- (2) The presence of many subtypes other than B in Europe without a major spread in heterosexuals.
- (3) Studies of maternal-infant transmission of HIV-1 in different parts of the world that suggest similar rates of vertical transmission between different subtypes of HIV-1.
- (4) Very similar per-sex-act transmission probabilities among couples with subtype B in North America and couples with subtype E in northern Thailand.

Laboratory and epidemiological data remain inconclusive and are insufficient to reject the null hypothesis that key ecological and biological properties do not differ significantly between HIV subtypes. The major reason for this uncertainty is related to the lack of studies that integrate basic virology, epidemiology and social behavioural approaches.

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In terms of future projections, it is argued that although the virus is evolving rapidly, both within and between affected communities, the question of whether evolution will select for increased pathogenicity concomitant with increased transmissibility, or indeed whether multiple subtypes of HIV-1 will co-exist within the same communities when the endemic (as opposed to epidemic) state is reached in defined populations, will not be resolved quickly. Evolution in this context will occur over many decades, which highlights the need for expanded surveillance of HIV genetic variability and the direction of evolution. Evolution may move in different directions in different locations depending on behavioural and host genetic factors in defined populations.

Finally, the group unanimously agreed that 'genetic variation in HIV-1 and the rapid evolution of the virus world-wide in no way alters current public health policy on measures required to slow the spread of infection either within vulnerable groups in particular countries or worldwide.' HIV/AIDS remains a life threatening disease, and the best public health strategies should continue to include the important approaches that have shown to be effective, such as promotion of safer sex practices, including the use of condoms, the reduction of the number of sex partners, and the control of other sexually transmitted infections. These three key strategies will achieve the best results in a supportive environment which makes safer choices socially acceptable and removes social and economic barriers to individuals' abilities to protect themselves against HIV/AIDS.

### Recommendations for further research

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In interpretation of the observed patterns of spread of different HIV-1 subtypes, many confounding factors, such as prevalence of cofactor STD, host genetic factors, heterogeneity in sexual behaviour and mixing patterns between risk groups, can significantly influence the accuracy with which comparisons between subtypes can be made. It was therefore recognized that there is a clear need for more detailed epidemiological and behavioural studies within the same risk group where more than one subtype is present and where multidisciplinary research teams, including molecular virologists, epidemiologists and social scientists, would attempt to tease apart the influences of genetic variation and other factors. With regard to the further research needs, the expert group made the following recommendations in specific areas.

### Virology and basic science

Recent research reveals that genetic recombination of different HIV-1 subtypes arising from dual infection is much more common than previously realized. The implications of this finding for classification of HIV-1 viral diversity and its measurement, relevance for the rate of virus evolution, its transmission and pathogenicity are in urgent need of investigation.

Frequent recombination between different HIV-1 subtypes indicates the importance of whole genome sequencing of viral isolates, particularly from regions where many subtypes are circulating in the same community and those HIV-1 subtypes for which whole genome sequences are not available or available for only a small number of isolates. This recommendation implies a much greater scientific effort internationally to generate sufficient and representative information on HIV-1 genetic diversity, perhaps via establishment of additional research centres or facilities for whole genome sequencing of HIV. The implementation and the analysis of these projects should be conducted through international collaboration efforts, including the existing UNAIDS HIV Characterization and Molecular Epidemiology Network.

In view of the possible relationship between genetic variability and major biological properties of the virus, such as target cell tropism, transmissibility and pathogenicity, it is recommended that investigation in this area be expanded to include all recognized HIV-1 subtypes, using well-characterized samples, collected as part of specially designed epidemiological and natural history studies. In addition, these studies should address questions related to the mechanisms of dual infections with different subtypes, and the role of resulting recombinant viral forms in pathogenesis and transmission.

Although the group mainly concentrated on viral evolution and genetic diversity, it did not fully recognize the need for combining both the virological and epidemiological studies of viral evolution with investigation of the roles of host genetic background, host genetic diversity, cross-immunity between subtypes and the dynamics of the viral load during different stages of infection in determining viral evolution, pathogenicity and transmissibility.

### Epidemiology

The complexities of the biology and epidemiology of HIV-1, in particular the rapid evolution of the virus, the long and highly variable incubation period between infection and the development of AIDS, the sexual route of transmission with much heterogeneity in behaviours within and between communities, make epidemiological study of different HIV-1 subtypes difficult. Therefore, there is a need for more standardization and rigour in the

design of cross-sectional and longitudinal studies, where greater emphasis should be placed in the latter by following cohorts of people over time, concordant with detailed behavioural, clinical and virological studies to determine pathogenicity (rates of disease progression) and transmissibility of different subtypes.

In order to better understand the global molecular epidemiology of HIV, it will be necessary to expand surveillance of different HIV subtypes using samples of adequate size, accompanied by precise and detailed epidemiological information. Ongoing sentinel serosurveillance and serial cross-sectional epidemiological studies with subtyping can be effective in monitoring trends over time. Not only is this important for better characterizing the transmission patterns, but is crucial for diagnostics, blood safety, monitoring for drug resistance and HIV vaccine development.

UNAIDS should establish a World-Wide Web site for the compilation of a registry of the major ongoing cohort-based epidemiological studies, to promote interdisciplinary research and enable molecular virologists to obtain isolates from well-characterized populations.

In addition to expansion of surveillance of HIV subtypes and more complete genomic characterization in all parts of the world, regions with more than one predominant HIV subtype may provide important opportunities to investigate the potential of genetic and phenotypic differences in HIV to affect transmission probability, pathogenesis and responses to therapies and vaccines.

### Methodology and study design

Transmission probability, effective rate of partner exchange, duration and level of infectiousness all influence the basic reproductive rate of HIV and hence its ability to spread and persist in different populations. Research in this area should incorporate sociobehavioural (e.g., sexual practices), anatomic (e.g., circumcision), immunological, clinical, STD and host genetic cofactors alongside viral parameters, such as viral load, genetic subtype, and biological phenotype/tropism, and host genetic factors into studies of HIV molecular epidemiology.

Studies of transmission, both sexual and perinatal, may be most cost-effectively incorporated into ongoing cohort studies. Prospective cohort studies and cross-sectional partner studies are the most appropriate approaches to reliably describing the incidence, natural history and transmissibility (both sexual and perinatal) of different HIV subtypes. To be more practical and cost-effective, these should be incorporated, whenever possible, into ongoing studies.

Current technology does not provide all the research tools required to address the question of relative patho-

genicity and the transmissibility of HIV-1 subtypes. The development of and relevant reference reagents (with full genomic sequencing of the isolates used) for serotyping, genotyping and measuring the viral load that are standardized across all known HIV-1 subtypes were identified as attainable goals.

### Acknowledgements

The meeting was organized by the Joint United Nations Programme on HIV/AIDS (UNAIDS) and co-sponsored by the Federal Ministry of Health of Germany. UNAIDS is grateful for the assistance provided by the Robert-Koch Institute of Infectious and Non-Communicable Diseases, Berlin, Germany and its staff, in particular B. Schwartländer and S. Kissling, in the organization and conduct of the meeting. Technical assistance and secretarial support was provided by C. Brenner, UNAIDS. UNAIDS expresses its gratitude to all participants of the meeting for their valuable contribution to the successful outcome of the meeting. In particular, UNAIDS extends its thanks to R. Anderson for chairing the meeting and F. McCutchan and D. Hu for serving as rapporteurs and for preparation of the meeting report.

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## Appendix

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