EDITORIAL REVIEW

The puzzle of HIV-1 subtypes in Africa

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Introduction

The genetic variation of microorganisms and their evolution in time have important implications for the control of infectious diseases. Genetic variation may be reflected in differences in biological characteristics that may determine transmissibility, pathogenesis, and immunogenicity. Genetic variability of microorganisms needs to be taken into account when developing or adapting diagnostic tests and vaccines, and when making projections of the burden of morbidity and mortality. Identification of strains or subtypes has also proved to be an invaluable tool in studying the spread of infectious pathogens. For instance, according to several reports in the recent scientific literature about the use of restriction fragment length polymorphism (RFLP) analysis in investigating outbreaks of tuberculosis, classical epidemiological investigations were found to be too crude or too insensitive.

HIV displays important genetic variability. Differences between HIV-1 and HIV-2 are fairly well documented in terms of transmissibility, pathogenesis, and pattern of spread [1,2]. Our knowledge about the biological characteristics and the epidemic spread of the different HIV-1 strains, however, is still patchy. So far it has been very difficult to conduct large-scale comparative studies of these subtypes because of the lack of a simple, rapid, and cheap test for the identification of HIV-1 subtypes. Of the two types, HIV-1 and HIV-2, the former type is by far the most widely distributed and also the most studied virus. This review will focus on our current state of knowledge about HIV-1 subtypes in sub-Saharan Africa. By way of an introduction we will first give a brief overview of the basis for HIV-1 subtyping. This will be followed by a review of the distribution patterns of HIV-1 subtypes in sub-Saharan Africa and a discussion of the implications of this for the development of diagnostic tests and vaccines, and for surveillance.

Classification of HIV-1 strains in subtypes

Basis for classifying HIV-1 subtypes

Before 1992, HIV-1 strains were classified on the basis of their geographic origin into two subgroups, North American and African variants [3]. Since 1992, the *env* coding sequence has been used to classify globally prevalent viruses. The first five sequence subtypes — A, B, C, D and E [4] — thus identified differed from each other by approximately 30% in their *env* coding sequences and 14% in their *gag* coding sequences. Thereafter, five more subtypes were described and labelled F through J [5–8], of which I and J viruses have been reported only once. Moreover, several sequences still await classification. These 10 subtypes constitute the group M (major) HIV-1 viruses. They differ from one another by an average nucleotide percentage distance of 27% (range, 21–31%) [9]. Again, within each subtype, a plethora of HIV-1 variants are expressions of minor intrasubtype genomic diversity; their average nucleotide distances are approximately 11%.

The following criteria were laid down for the current classification system into subtypes: (i) subtypes are approximately equidistant from one another in *env*; (ii) the *env* phylogenetic tree is for the most part congruent

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with the *gag* phylogenetic tree; and (iii) two or more samples are required to define a new sequence subtype. Envelope coding sequences were used as the basis for classifying the 1992 compendium, but as the database of gene sequences has grown this basis is increasingly challenged. Subtype E viruses, for instance, are equidistant from subtypes A–D viruses in their *env* coding sequences, but they appear to be similar to subtype A viruses in their *gag* coding sequences [10–12]. Subtype E for *gag* coding sequences has so far not been documented.

In 1994, HIV-1 viruses isolated from Cameroonian patients were described that were highly aberrant and could not be classified in any of the known group M subtypes [13,14]. These viruses share approximately 50% homology in their *env* genes with group M viruses and are almost genetically as equidistant from each other as are the members of different HIV-1 group M subtypes. They were classified into a separate group of viruses, group O (outliers) [15]. There is as yet no set of criteria for assigning viruses to group O subtypes.

Phylogenetically, group M and group O viruses form a double-star-like configuration, which suggests that each group has evolved from a single ancestral virus. The double-star phylogeny (i.e., two centres of evolutionary radiation) would suggest that HIV-1 arose from at least two separate zoonotic transmissions [16].

Mechanisms of HIV-1 variability

In order to interpret the distribution of HIV-1 subtypes and to foresee the emergence of new subtypes, a clear understanding is needed of the mechanisms underlying the variability of HIV-1. For many years it was believed that errors in reverse transcriptase and the presence of virion RNA as a dimer were the driving force behind the genetic variation of HIV [17]. Results from *in vivo* studies suggested that HIV-1 infection is characterized by an extremely high turnover of virus, in the range of 10^9 virions per day [18]. On the basis of these data, it was estimated that the HIV-1 sequences (*env* gene) in an infected person and between infected persons in a population change by approximately 1% per year [19]. If this estimate is accurate, two different HIV-1 subtypes are at least 30 years apart from each other. This would mean that the distribution patterns of HIV-1 subtypes that we now see in the world are the result of virus exchanges between populations (viral migration), rather than of mutations or diversification within different human populations [16].

Since 1995, however, HIV-1 intersubtype recombination and dual infection of persons by two different HIV-1 subtypes have been described [20]. Analysis of the 1993 HIV database revealed that 10% of the reported sequences are in fact recombinants [21], which suggests that recombination plays a more important role in the genetic diversification of HIV-1 than was previously thought. What are the implications of these new insights into the mechanisms of genetic diversification?

First, there are implications for the classification of subtypes. The third variable region of the envelope is the best documented region of the genome, and although sequences encoding C2–V3 contain a suboptimal length for phylogenetic analysis, they have all along been considered a reliable basis for subtype determination [22]. However, now that HIV-1 intersubtype recombination seems to occur frequently, subtype naming should refer to the region analysed [23]. For instance, the analysis of full genome sequences for subtypes A, B, C, D, E and G revealed that subtypes E and G actually consist of recombinant viruses. Full-length genome sequences are not available for subtypes F, H, I and J, but it is likely that some of these subtypes are also recombinants [24]. The following changes have already been proposed in the criteria for subtype classification: (i) at least two epidemiologically unrelated isolates cluster together and are separated from established genotypes; (ii) contiguous sequences of at least 1.5 kb have to be described; and (iii) no subsegment can join established genotypes [25]. Obviously these new criteria will have a bearing on the method used for subtyping.

Secondly, the stated value of 1% single base changes per year may be regarded as a minimum rate [19], and the subtypes that we see now may not be as far apart from each other in time as previously thought. Finally, it stands to reason that the frequency of intersubtype recombinants depends on the prevalence rates of different HIV-1 subtypes circulating in a certain population and the probability that certain groups in this population acquire multiple infections. As a consequence, the emergence of new intersubtype recombinant HIV-1 variants is to be expected mainly in those populations where there is a larger variety of HIV-1 subtypes and where there is a high probability of people acquiring multiple infections.

Methods of subtyping

Although sequencing remains the most accurate approach to characterizing virus genomes, several less cumbersome and less expensive techniques have been based on polymerase chain reaction (PCR), for example heteroduplex mobility assay (HMA) [26], RFLP analysis [27,28], and oligonucleotide probe hybridization [29]. The last two methods have so far proven their usefulness only in studies where only a few subtypes need to be differentiated. Subtype-specific probe hybridization has been used successfully to determine *env* subtypes B and E in Thailand [29], and to distinguish *env* subtypes A and D in Uganda [30]. The V3 peptide enzyme-linked immunosorbent assay

(ELISA) [31] has proved useful in areas where the number of subtypes is limited [32], but it does not perform well on sera from Central Africa where a large variety of subtypes are circulating [33]. This was clearly shown in a multicentre study which assessed the performance of V3 peptide ELISA on a panel of genetically characterized samples of HIV-1 subtypes A, B, C, D, F, G and H (unpublished data). More research is needed to improve the specificity and sensitivity of serologic subtyping, which is the only method that can be used on a large scale at an affordable cost, not only in resource-constrained settings but also in industrialized countries.

Screening and confirmation of group O infections are mainly performed by use of serologic methods [34]. As the first representatives of HIV-1 group O (ANT-70 and MVP5180) were discovered, a strategy was developed whereby the presence of HIV-1 group O antibodies to the synthetic ANT-70/MVP5180 V3-loop peptides was monitored by ELISA. Sera reactive in ELISA were retested in a Line Immuno Assay (LIA; Research Product, Innogenetics, Ghent, Belgium), in which different biotinylated HIV-1 group M and group O V3 peptides were applied as streptavidin complex in parallel lines on nylon strips. A positive reaction with only the HIV-1 group O V3 peptides in LIA is indicative of the presence of HIV-1 group O infection [35]. As the number of newly documented HIV-1 group O infections increased, it was observed that not all HIV-1 group O antibodies react in ANT-70 and MVP5180 V3 peptide ELISA. In addition, some sera from HIV-1 group M-infected individuals do cross-react in ANT-70/MVP5180 V3 peptide ELISA. Preliminary results of a collaborative study, whereby 33 HIV-1 group O sera were analysed for their antibody reactivity to 22 synthetic V3 peptides derived from various HIV-1 group O strains, indicated that combinations of group O V3 peptides gave 100% sensitivity (unpublished data). Alternatively, group O infections can be detected by genetic methods, including PCR using group O and group M-specific primers [36] and restriction analysis of a *pol* fragment [37].

Distribution of HIV-1 subtypes in the different regions of sub-Saharan Africa

Overview of the HIV-1 distribution patterns

Numerous studies have been conducted on the distribution of different HIV from sub-Saharan Africa. Table 1 summarizes the distribution of HIV-1 group M subtypes in regions of sub-Saharan Africa. The studies in Table 1 were selected on the basis of two criteria: first there were at least 10 isolates, unless data from two similar populations could be pooled; and secondly, subtyping was performed by sequencing, HMA, RFLP analysis or oligonucleotide probe hybridization. Subtyping based on serology was not considered reliable in the African context because of the large variety of HIV-1 subtypes, as well as the lack of subtype specific-peptides used in V3 peptide ELISA [33].

None of the studies in Table 1 was conducted on a truly random sample of HIV-infected persons from the general population. With the exception of the studies from Kenya and Benin, most studies were not even performed on homogenous groups of HIV-seropositive persons. Study subjects could have been in different stages of disease and thus would have been infected in different years. Second, study subjects were not always similar with regard to their region of residence or origin. Third, the year blood was taken was not always indicated. Last but not least, samples came from study subjects who belonged to different risk categories, making comparisons difficult. It can for instance be questioned to what extent commercial sex workers (CSW) in Cotonou are comparable to pregnant women in Abidjan with regard to distribution of subtypes.

The distribution patterns (Table 1) may therefore not correctly reflect the distribution patterns of HIV-1 subtypes in the general population in different regions in sub-Saharan Africa. However, there are a few striking features from which some conclusions can be drawn. The epidemics in all parts of sub-Saharan Africa, except Southern Africa (South Africa and Malawi), seem to be dominated by subtype A. There is no doubt that the largest variety of HIV-1 subtypes are found in Central Africa (Cameroon, Gabon, Central African Republic, Zaïre), where even in relatively limited studies as many as seven different subtypes have been found. The variety in subtypes is much less in the other regions. In West Africa the most prevalent subtype by far is subtype A. The epidemics in Eastern Africa seem to involve two main subtypes, A and D, which were found in Uganda in nearly equal proportions. Subtype C has so far been found to play an important role only in the epidemics in Southern Africa.

Subtype B, the most prevalent subtype in Europe and the United States, is not prevalent in sub-Saharan Africa with the exception of South Africa. In South Africa, subtype B is mainly found among homosexuals and there is evidence to suggest that it was introduced through contacts with gay communities in the United States and in Europe [59,60]. Subtype E, which is associated with an explosive epidemic among heterosexuals in Thailand, is also not commonly found in Africa, except in the Central African Republic [61]. The link between subtype E in the Central African Republic and Thailand remains elusive.

Group O viruses have been mainly found in Central Africa, with Cameroon as the epicentre, where 5%

Table 1. Distribution of HIV-1 subtypes in sub-Saharan Africa.

Country	Ref	Total	Subtypes										Populations sampled
			A	B	C	D	E	F	G	H	O	?	
West Africa													
Côte d'Ivoire	[38]	13	11	1		1							HIV+ patients in Abidjan
	[28]	106	106										89 TB patients and 20 pregnant women in Abidjan
Bénin	[39]	21	19						2				CSW at Cotonou
Ghana	[40]	19	16			2			1				Different areas mostly East
Central Africa													
Cameroon	[41]	18	11	1			1	3		1	1		HIV+ patients in Yaounde and Douala
	[42]	47	32	2	2	2	1	4			4		HIV+ patients
Gabon	[43]	17	7		2	2		1	4		1		Asymptomatic patients and AIDS patients from Libreville and Franceville
CAR	[12,44]	27	12		1	1	8		1	1		3	AIDS patients in Bangui
Zaïre	[45]	14	10						4				Women in Kinshasa
Eastern Africa													
Uganda	[46]	12				12							AIDS patients in Kampala
	[47]	22	11			11							AIDS patients in Kampala
	[30]	67	38			29							Leftover blood from hospitals and clinics in five districts
	[48]	10	8			2							Asymptomatic seropositive subjects in Northern Uganda
	[49]	16	13			3							HIV+ individuals
Rwanda	[49]	13	13										HIV+ individuals
Kenya	[50]	23	19			3						1	Pregnant women in Nairobi
	[51]	8	6		1	1							Pregnant women in Nairobi
Tanzania	[52]	14	4			10							Patients in clinic in Turani
	[53]	10	2			8							AIDS patients in Dar-es-Salaam
Malawi	[54]	14			14								HIV+ individuals from Blantyre
Southern Africa													
South Africa	[55]	84		42	35	6	1						Patients in local clinics in Cape Town
	[56]	13		1	11	1							Malawians working in the gold mines and patients in hospital
	[57]	46			46								Mining cohort from gold fields in Gauteng
	[58]	53		20	28	5							Patients in local clinic in Cape Town

CAR, Central African Republic; TB, tuberculosis; CSW, commercial sex workers; HIV+, HIV-seropositive.

[62–64] of the HIV-1 infections are with group O viruses. HIV-1 group O infections have also been documented in Gabon [62], Equatorial Guinea [65], and less frequently in Benin [66], Senegal, Togo, Niger and Chad [67], Nigeria [35,67] and Kenya [67,68] (Table 2). Dual infections by group O and group M viruses have been described in Benin [66], Kenya [68], and Cameroon [64] (Table 2).

Interpreting these variations in distribution patterns in the regions of sub-Saharan Africa is very difficult. At least 15 years have elapsed since the start of the HIV/AIDS epidemic in Africa and the distribution patterns we see now are the result of a long historical evolution which is impossible to reconstruct as the prevalence of different HIV-1 subtypes in a certain population is determined by a complex interplay of several factors. As outlined earlier, the emergence of subtypes is intrinsically a biological phenomenon. On the other hand, it is reasonable to postulate that the coexistence of different subtypes in a population is determined by the biological characteristics of subtypes, mainly transmissibility and virulence, as well as by interactions and cross-infections between different risk groups in the population. The best documented example is from Thailand. Two HIV-1 subtypes, E and B, were almost simultaneously introduced in two risk groups in Thailand, injecting drug users, and CSW and their clients, respectively [61]. As the epidemic progresses there are shifts in the distribution of these subtypes, and it is still uncertain how far these changes are the result of interactions between the different risk groups or due to differences in biological characteristics [69]. In sub-Saharan Africa the most notable example is from South Africa, where HIV first spread among the homosexual population after introduction of the virus, presumably through contacts with gay communities in the United States and in Europe [59,60]. The predominant subtype associated with homosexual transmission is subtype B, the same as in the United States and Europe [57]. The spread within the heterosexual population of South Africa occurred at a later date, and here the virus, mainly subtype C, was probably introduced via contacts with migrant labourers from countries

Table 2. Prevalence of HIV-1 group O in Africa.

Country	n/total	Reference
West Africa		
Burkina Faso	0/398	[67]
Burundi	0/97	[67]
Mali	0/816	[67]
Niger	5/1459	[67]
Senegal	1/1283	[67]
Nigeria	2/183	[35]
Togo	1/670	[67]
Bénin	1/142	[66]
Central Africa		
Chad	2/619	[67]
Cameroon	9/187	[62]
	16/240	[63]
	8/332	[67]
Equatorial Guinea	4/41	[65]
Congo	0/288	[67]
Gabon	5/203	[62]
	2/213	[67]
Zambia	1/720	[67]
East Africa		
Kenya	1/250	[68]

The numbers of HIV-1 group O samples identified (n) per number of HIV-1-positive samples analysed (total) for each country that was subjected to HIV-1 group O prevalence studies are shown.

north of South Africa [57]. Each of the risk categories for HIV infection has its own subtype and because heterosexual transmission of HIV is gaining in importance it can be expected that non-B subtypes (i.e., C and D) will gain in importance, assuming that the following two conditions are met: there is no extensive cross infection between the gay community and the heterosexual population, and subtypes B, C and D are comparable in transmissibility and virulence.

HIV-1 subtypes and the course of the HIV epidemic: are subtypes motors or markers of the HIV-1 epidemic?

The rate of spread of HIV is not uniform across sub-Saharan Africa. HIV infection has spread most rapidly and most extensively in East Africa. Surveillance data on HIV infection among pregnant women suggest that in Central Africa the rate of spread has been relatively slower and more stable [70]. For instance, in Kinshasa (Zaïre) the prevalence of HIV infection in pregnant women was 6.3% in 1986 and 3.1% in 1991, whereas in Blantyre (Malawi) the corresponding prevalence rates were 2–4.2 and 25.9%, respectively. Considering these data and the distribution patterns of HIV-1 subtypes, the relatively slow epidemics seem to be associated with a higher variety of subtypes, whereas in areas with an explosive epidemic only one (or at most three) subtype is prevalent.

We postulate the following explanation for this finding. In populations where there is a large variety of subtypes, groups of people infected with different subtypes belong to different sexual networks with few links (and thus few opportunities for the exchange of virus strains) with other sexual networks. Such a pattern of sexual networking (i.e., the existence of a multitude of smaller networks) would be consistent with a relatively slow spread of the virus. This is in contrast to the pattern of very extensive concurrent partnerships which is believed to be associated with a very rapid spread of the virus [71]. In populations with this latter sexual behaviour pattern the introduction of one or a few viruses of different subtypes will rapidly lead to an explosive epidemic that is dominated by only one or a few subtypes. In other words, we hypothesize that the large variety of HIV-1 subtypes in Central Africa has been made possible by the relatively slow spread of HIV in the populations of this part of Africa.

Practical implications of the variability of HIV-1 in sub-Saharan Africa

Diagnostic tests, HIV vaccines, and antiretroviral drugs are mainly designed and developed in the industrialized countries of Europe and North America. In these parts of the world, the B subtype is by far the most common HIV-1 subtype. Tests, vaccines, and drugs based on research on subtype B viruses might perform suboptimally in populations where non-B subtypes are circulating, which is true in sub-Saharan Africa. What is the evidence so far?

Diagnostic tests

Because HIV infection is diagnosed with serological tests of antigen/antibody reactions, even subtle changes in antigenic structure may affect the sensitivity of these tests. In particular, several serological antibody assays have been reported to lack sensitivity to antibodies to some group O viruses, which also frequently produce indeterminate Western blot results [72,73]. Therefore, there is a need to identify and further characterize these aberrant viruses in order to incorporate their antigens to improve the sensitivity of current ELISA. So far, the ELISA systems do not seem to lack sensitivity to any subtype within the group M viruses. However, there is an urgent need to evaluate third-generation assays, which are increasingly being used to screen blood for transfusion, for immunoglobulin M detection. These assays incorporate subtype B antigen and perform well in narrowing the seroconversion window phase of HIV infection in industrialized countries, but their accuracy in detecting early seroconversion of non-subtype B infection is unknown.

Perhaps the most underdeveloped field in HIV diagnosis in Africa is that of nucleic acid-based assays, such as commercial polymerase chain reaction (PCR) assays and multiprobe branched signal amplification. Although extensive evaluation on HIV-1 non-B subtypes has not been performed, preliminary data suggest that most commercial PCR kits fail to detect several

non-B HIV-1 subtypes [74,75]. In addition, no commercial assay exists for HIV-2 amplifications. Increasingly, viral load quantification will be required in Africa to better assess the outcome of clinical trials, such as the efficacy of zidovudine in preventing perinatal transmission. We therefore need to consider non-B subtypes in the design of molecular biology assays.

Vaccine development

Several studies have attempted to demonstrate cross-neutralization with human serum or plasma in HIV-1 subtype checkerboard experiments. Specific neutralization serotypes corresponding to genetic subtypes have not been demonstrated, regardless of the type of neutralization assay protocol used [9,76,77]. Instead, extensive intra- and intergenetic subtype cross-neutralization by human sera has been observed [77,78]. The main conclusion from these studies is that the large variety of HIV-1 subtypes do not correspond to neutralization serotypes. This suggests that the immune system responds to a conformational structure, not to the linear DNA sequences used for phylogenetic analysis that code for these structures. Together, these observations argue for the presence of conserved neutralizing antibody epitopes across clades. On the basis of these studies, one obvious focus of vaccine research seems to be the mapping of these epitopes, which could then be used in vaccines to elicit a broad enough immune response to protect against most subtypes. Whether this is realistic can be assessed by novel approaches, including computer-based modelling, to analyse neutralization data. One such approach investigates the correlation between genetic subtypes and cross-clade neutralizing capacity, with spectral map analysis [79]. This analysis identified three key HIV-1 isolates, which when neutralized by any serum were highly predictive of the presence of broadly reactive neutralizing antibodies in the specimen. A critical analysis of such epitopes involved in broad neutralization should make genetic variation surmountable in the design of broadly reactive vaccine or immune therapy. However, it remains to be determined whether a strong humoral cross-clade neutralization response in a vaccinated naive person will be an equally good prognostic marker for protection against infection or progression to disease, as was reported previously for some other successful vaccines such as measles, polio, influenza, and hepatitis B.

Response to antiviral drugs

We currently lack *in vivo* studies of the response to therapy of different HIV-1 subtypes. For other RNA virus infections, such as hepatitis C, the response to therapy is genotype-dependent [80]. One *in vitro* study has shown that HIV-1 group O viruses compared with group M viruses are resistant to TIBO [8-chloro-tetrahydro-imidazo (4, 5, 1-jk) (1, 4) – benzodiazepin-2 (1H)thione] non-nucleoside products [81]. Resistance of non-B subtypes to antiretrovirals may pose problems for the application of these therapies in populations where the predominant HIV-1 subtypes are non-B subtypes. Although HIV antiretroviral drugs are not widely used in Africa at present, this may change in the foreseeable future and it may eventually become necessary to conduct surveillance for drug-resistant non-B HIV-1 strains.

Conclusions

There are many questions on the biological characteristics of different HIV-1 subtypes that remain to be answered. Differences in biological characteristics could have consequences for the spread of HIV-1, the detection of infection, the treatment of infected persons and the development of a vaccine. More work needs to be done on documenting and monitoring distribution patterns of HIV-1 subtypes [82]. There are several good reasons for choosing Central Africa as a priority site for setting up a surveillance system for HIV-1 subtypes.

At present, surveillance is hampered by the lack of a simple, rapid and cheap test for subtyping. Even if such a test becomes available, surveillance for subtyping will not be a simple undertaking, neither will it be cheap. For reasons of efficiency, it makes sense to set up surveillance where one finds the largest variety of subtypes and where one is most likely to see the emergence of new subtypes. According to our current knowledge of HIV-1 subtypes, the largest variety of subtypes in the world are found in Central Africa.

Secondly, it would be naive to assume that what is happening in Central Africa in terms of HIV-1 subtype variability does not affect the rest of the world. Humankind has seen pandemics of syphilis and plague in a time, many centuries ago, when intercontinental travel was a laborious and extremely dangerous undertaking. Recently, there have been reports of the introduction of non-B subtypes into the northern hemisphere [83–85]. Surprisingly these reports caused some alarm. 'Surprisingly' because in our time of extensive and rapid movement of people between continents it is all one can expect, as has been shown in several European countries [86,87]. Research teams working on vaccines, diagnostic tests, and antiretroviral drugs will need to take into account the non-B subtypes found in sub-Saharan Africa.

References

1. De Cock KM, Adjorlolo G, Ekpini E, *et al.*: **Epidemiology and transmission of HIV-2: why there is no HIV-2 pandemic.** *JAMA*

1993, **270**:2083–2086.
2. Kanki PJ, De Cock KM: **Epidemiology and natural history of HIV-2.** *AIDS* 1994, **8** (suppl 1):S85–S93.
3. Myers G, Rabson RB, Berzofsky JA, Smith RF, Wong-Staal F (Eds): *Human Retroviruses and AIDS 1990.* Los Alamos: Los Alamos National Laboratory; 1990.
4. Myers G, Korber B, Berzofsky JA, Smith RF, Pavlakis GN (Eds): *Human Retroviruses and AIDS 1992.* Los Alamos: Los Alamos National Laboratory; 1992.
5. Myers G, Korber B, Wain-Hobson S, Jeang K-T, Henderson L, Pavlakis GN (Eds): *Human Retroviruses and AIDS 1994.* Los Alamos: Los Alamos National Laboratory; 1994.
6. Janssens W, Heyndrickx L, Fransen K, *et al.*: **Genetic and phylogenetic analysis of *env* subtypes G and H in Central Africa.** *AIDS Res Hum Retrovirus* 1994, **10**:277–279.
7. Kostrikis LG, Bagdades E, Cao Y, Zhang L, Dimitriou D, Ho DD: **Genetic analysis of human immunodeficiency virus type 1 strains from patients in Cyprus: identification of a new subtype designated subtype I.** *J Virol* 1995, **69**:6122–6130.
8. Leitner T, Alaeus A, Marquina S, Lilja E, Lidman K, Albert J: **Yet another subtype of HIV type 1?** *AIDS Res Hum Retrovirus* 1995, **11**:995–997.
9. Moore JP, Cao Y, Leu J, Qin L, Korber B, Ho D: **Inter- and intraclade neutralization of human immunodeficiency virus type 1: genetic clades do not correspond to neutralization serotypes but partially correspond to gp120 antigenic serotypes.** *J Virol* 1996, **70**:427–444.
10. McCutchan FE, Hegerich PA, Brennan TP, *et al.*: **Genetic variants of HIV-1 in Thailand.** *AIDS Res Hum Retrovirus* 1992, **8**:1887–1895.
11. Louwagie J, McCutchan FE, Peeters M, *et al.*: **Phylogenetic analysis of *gag* genes from 70 international HIV-1 isolates provides evidence for multiple genotypes.** *AIDS* 1993, **7**:769–780.
12. Murphy E, Korber B, Georges-Courbot MC, *et al.*: **Diversity of V3 region sequences of human immunodeficiency viruses type-1 from the Central African Republic.** *AIDS Res Hum Retrovirus* 1993, **9**:997–1006.
13. vanden Haesevelde M, Decourt J-L, De Leys RJ, *et al.*: **Genomic cloning and complete sequence analysis of a highly divergent African human immunodeficiency virus isolate.** *J Virol* 1994, **68**:1586–1596.
14. Gürtler LG, Hauser PH, Eberle J, *et al.*: **A new subtype of human immunodeficiency virus type 1 (MVP-5180) from Cameroon.** *J Virology* 1994, **68**:1581–1585.
15. Charneau P, Borman AM, Quillant C, *et al.*: **Isolation and envelope sequence of a highly divergent HIV-1 isolate: definition of a new HIV-1 group.** *Virology* 1994, **205**:247–253.
16. Myers G: **Tenth anniversary perspectives on AIDS. HIV: between past and future.** *AIDS Res Hum Retrovirus* 1994, **10**:1317–1324.
17. Temin HM: **Retrovirus variation and reverse transcription: abnormal strand transfer result in retrovirus genetic variation.** *Proc Natl Acad Sci USA* 1993, **90**:6900–6903.
18. Ho DD, Neuman AU, Perelson AS, *et al.*: **Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection.** *Nature* 1995, **373**:123–126.
19. Myers G, MacInnes K, Myers L: **Phylogenetic moments in the AIDS epidemic.** In *Emerging Viruses.* Edited by Morse SS. New York: Oxford University Press; 1993:120–135.
20. Artenstein AW, Van Cott TC, Mascola JR, *et al.*: **Dual infection with human immunodeficiency virus type 1 of distinct envelope subtypes in humans.** *J Infect Dis* 1995, **171**:805–810.
21. Robertson DL, Sharp PM, McCutchan FE, Hahn BH: **Recombination in HIV-1.** *Nature* 1995, **374**:124–126.
22. Korber BTM, Allen EE, Farmer AD, Myers GL: **Heterogeneity of HIV-1 and HIV-2.** *AIDS* 1995, **9** (suppl A):S5–S18.
23. Myers G, Korber B, Wain-Hobson S, Smith R, Pavlakis G: *Human Retroviruses and AIDS 1993.* Los Alamos: Los Alamos National Laboratory; 1993.
24. McCutchan FE, Salminen MO, Carr JK, Burke DS: **HIV genetic diversity.** *AIDS* 1996, **10** (suppl 3): S513–S520.
25. Salminen MO, Carr JK, Burke DS, McCutchan FE: **Genotyping of HIV-1.** In *Human Retroviruses and AIDS 1995. Part III.* Edited by Myers G, Korber B, Hahn BH, *et al.* Los Alamos: Los Alamos National Laboratory; 1995.
26. Delwart EL, Shpaer EG, Louwagie J, *et al.*: **Genetic relationships determined by a DNA heteroduplex mobility assay: analysis of HIV-1 *env* genes.** *Science* 1993, **262**:257–261.
27. Pieniazek D, Janini LM, Ramos A, *et al.*: **HIV-1 patients may harbor viruses of different phylogenetic subtypes: implications for the evolution of the HIV/AIDS pandemic.** *Emerg Infect Dis* 1995, **1**:86–88.
28. Ellenberger D, Luo C-C, Pieniazek D, *et al.*: **Analysis of genetic diversity of HIV-1 in Côte d'Ivoire using restriction fragment length polymorphism (RFLP) analyses.** *XI International Conference on AIDS.* Vancouver, July 1996 [abstract TuA2065].
29. Subbarao S, Luo C-C, Limpakarnjanarat K, *et al.*: **Evaluation of oligonucleotide probes for the determination of the two major HIV-1 *env* subtypes in Thailand [letter].** *AIDS* 1996, **10**:350–351.
30. Luo C-C, Downing R, Dela Torre N, *et al.*: **The evaluation of probe hybridization method used for the large scale screen of the distribution of HIV in Uganda.** *XI International Conference on AIDS.* Vancouver, July 1996 [abstract WeC344].
31. Cheingsong-Popov R, Lister S, Callow D, *et al.*: **Serotyping HIV type 1 by antibody binding to the V3 loop: relationship to viral genotype.** *AIDS Res Hum Retrovirus* 1994, **10**:1379–1386.
32. Pau C-P, Lee-Thomas S, Auwanit W, *et al.*: **Highly specific V3 peptide enzyme immunoassay for serotyping HIV-1 specimens from Thailand.** *AIDS* 1993, **7**:337–340.
33. Massangan M, Ndoyo J, Hu DJ, *et al.*: **A highly heterogenous HIV-1 epidemic in the Central African Republic.** *Emerg Infect Dis* 1996, **2**:222–224.
34. Peeters M, Nkengasong J, Willems B, *et al.*: **Antibodies to V3 loop peptides derived from chimpanzee lentiviruses and the divergent HIV-1_{ANT-70} isolate in human sera from different geographic regions.** *AIDS* 1994, **8**:1657–1661.
35. Mulanga Kabeya C, Esu-Williams E, Eni E, Peeters M, Saman E, Delaporte E: **Evidence for HIV-1 group O infection in Nigeria [letter].** *Lancet* 1995, **346**:308.
36. Janssens W, Fransen K, Loussert-Ajaka I, *et al.*: **Diagnosis of HIV-1 group O infection by polymerase chain reaction.** *Lancet* 1995, **346**:451–452.
37. Heyndrickx L, Janssens W, Fransen K, *et al.*: **Specific diagnosis of HIV-1 group O by restriction analysis of the *pol* gene fragment.** *XI International Conference on AIDS.* Vancouver, July 1996 [abstract TuA2086].
38. Janssens W, Heyndrickx L, Van de Peer Y, *et al.*: **Molecular phylogeny of part of the *env* gene of HIV-1 strains isolated in Côte d'Ivoire.** *AIDS* 1994, **8**:21–26.
39. Heyndrickx L, Janssens W, Alary M, *et al.*: **Genetic variability of HIV type 1 in Bénin.** *AIDS Res Hum Retrovirus* 1996, **12**:1495–1497.
40. Ishikawa K, Janssens W, Brandful J, *et al.*: **Genetic and phylogenetic analysis of HIV-1 *env* subtypes in Ghana, West Africa.** *AIDS Res Hum Retrovirus* 1996, **12**:1575–1578.
41. Nkengasong JN, Janssens W, Heyndrickx L, *et al.*: **Genotypic subtypes of HIV-1 in Cameroon.** *AIDS* 1994, **8**:1405–1412.
42. Takehisa J, Zekeng L, Miura T, *et al.*: **Phylogenetic analysis of HIV-1/2 in Cameroon based on the *pol* and *env* regions: evidence of mixed infection.** *XI International Conference on AIDS.* Vancouver, July 1996 [abstract TuA372].
43. Delaporte E, Janssens W, Peeters M, *et al.*: **Epidemiological and molecular characteristics of HIV infection in Gabon, 1986–1994.** *AIDS* 1996, **10**:903–910.
44. Janssens W, Heyndrickx L, Fransen K, *et al.*: **Genetic and phylogenetic analysis of *env* subtypes G and H in Central Africa.** *AIDS Res Hum Retrovirus* 1994, **10**:877–879.
45. Potts KE, Kalish ML, Bandea CI, *et al.*: **Genetic diversity of human immunodeficiency virus type 1 strains in Kinshasa, Zaire.** *AIDS Res Hum Retrovirus* 1993, **9**:613–618.
46. Oram JD, Downing RG, Roff M, *et al.*: **Sequence analysis of the V3 loop regions of the *env* genes of Ugandan human immunodeficiency proviruses.** *AIDS Res Hum Retrovirus* 1991, **7**:605–614.
47. Albert J, Franzen L, Jansson M, *et al.*: **Ugandan HIV-1 V3 loop sequences closely related to the US/European consensus.** *Virology* 1992, **190**:674–681.
48. Buonaguro L, Del Gaudio E, Monaco M, *et al.*: **Heteroduplex mobility assay and phylogenetic analysis of V3 region sequences of human immunodeficiency virus type 1 isolates from Gulu, Northern Uganda.** *J Virol* 1995, **69**:7971–7981.
49. WHO Network for HIV Isolation and Characterization: **HIV type-1 variation in World Health Organization-sponsored vaccine evaluation sites: genetic screening, sequence analysis, and preliminary biological characterization of selected viral strains.** *AIDS Res Hum Retrovirus* 1994, **10**:1327–1343.
50. Janssens W, Heyndrickx L, Fransen K, *et al.*: **Genetic variability**

of HIV type 1 in Kenya. *AIDS Res Hum Retrovirus* 1994, **10**:1577–1579.
51. Zachar V, Goustin AS, Zacharova V, *et al.*: **Genetic polymorphism of envelope V3 region of HIV type 1 subtypes A, C, and D from Nairobi, Kenya.** *AIDS Res Hum Retrovirus* 1996, **12**:75–78.
52. Zwart G, Wolfs TFW, Bookelman R, *et al.*: **Greater diversity of the HIV-1 V3 neutralization domain in Tanzania compared with The Netherlands: serological and genetic analysis.** *AIDS* 1993, **7**:467–474.
53. Wieslaw S, Schwinn A, Baczko K, *et al.*: ***vpu* and *env* sequence variability of HIV-1 isolates from Tanzania.** *AIDS Res Hum Retrovirus* 1994, **10**:1753–1754.
54. Orloff GM, Kalish ML, Chiphangwi J, *et al.*: **V3 loops of HIV-1 specimens from pregnant women in Malawi uniformly lack a potential N-linked glycosylation site.** *AIDS Res Hum Retrovirus* 1993, **9**:705–706.
55. Williamson C, Engelbrecht S, van Harmelen J, van Rensburg EJ, Bredell W, Wood R: **Distinct HIV-1 subtypes associated with different risk groups in South Africa.** *XI International Conference on AIDS.* Vancouver, July 1996 [abstract TuA101].
56. Becker MLB, de Jager G, Becker WB: **Analysis of partial *gag* and *env* gene sequences of HIV type 1 strains from Southern Africa.** *AIDS Res Hum Retrovirus* 1995, **11**:1265–1267.
57. Williamson C, Engelbrecht S, Lambrick M, *et al.*: **HIV-1 subtypes in different risk groups in South Africa.** *Lancet* 1995, **346**:782.
58. MRC AIDS Virus Research Unit: **Genetic subtyping of HIV-1 in Gauteng using a heteroduplex mobility assay.** In *National Institute for Virology. Annual Report 1995.* Johannesburg: Department of Health, National Institute for Virology; 1995:28.
59. Ras GJ, Simson IW, Anderson W, Prozesky OW, Hamerma T: **Acquired immunodeficiency syndrome. A report of 2 South African cases.** *S Afr Med J* 1983, **64**:140–142.
60. Sher R: **HIV infection in South Africa, 1982–1988: a review.** *S Afr Med J* 1989, **76**:314–318.
61. Ou C-Y, Takebe Y, Weniger BG, *et al.*: **Independent introduction of two major HIV-1 genotypes into distinct high-risk populations in Thailand.** *Lancet* 1993, **341**:1171–1174. [Erratum in *Lancet* 1993, **342**:250].
62. Nkengasong JN, Peeters M, vanden Haesevelde M, *et al.*: **Antigenic evidence of the presence of the aberrant HIV-1_{ANT70} virus in Cameroon and Gabon [letter].** *AIDS* 1993, **7**:1536–1538.
63. Zekeng L, Gürtler L, Afane Ze E, *et al.*: **Prevalence of HIV-1 subtype O infection in Cameroon: preliminary results [letter].** *AIDS* 1994, **8**:1626–1628.
64. Zekeng L, Tsague JM, Afane Ze E, *et al.*: **HIV-1 group O infection in Cameroon.** *XI International Conference on AIDS.* Vancouver, July 1996 [abstract MoC1475].
65. Hunt JC, Golden AM, Vallari A, *et al.*: **Molecular and serologic characterization of four HIV-1 group O sera from Equatorial Guinea [abstract].** *AIDS Res Hum Retrovirus* 1995, **11** (suppl 1):S144.
66. Heyndrickx L, Alary M, Janssens W, Davo N, van der Groen G: **HIV-1 group O and group M dual infection in Bénin.** *Lancet* 1996, **347**:902–903.
67. Peeters M, Mboup S, Gueye A, *et al.*: **Geographical distribution of HIV-1 group O viruses in Africa.** *AIDS* 1997, **11**:493–498.
68. Songok EM, Libondo DK, Rotich MC, Oogo SA, Tukei PM: **Surveillance for HIV-1 subtypes O and M in Kenya [letter].** *Lancet* 1996, **347**:1700.
69. Mastro T, Ungchusak K, Vanichseni S, *et al.*: **The evolution of HIV-1 subtypes B and E in heterosexuals and injecting drug users (IDUs) in Thailand 1992-1995.** *XI International Conference on AIDS.* Vancouver, July 1996 [abstract WeC342].
70. Buvé A, Caraël M, Hayes R, Robinson NJ: **Variation in HIV prevalence between urban areas in sub-Saharan Africa: do we understand them?** *AIDS* 1995, **9** (suppl 1):S103–S109.
71. Morris M, Sewankambo N, Wawer M, Serwadda D, Lainjo B: **Concurrent partnership and HIV transmission in Rakai District, Uganda.** *IX International Conference on AIDS and STD in Africa.* Kampala, December 1995 [abstract TuC105].
72. Loussert-Ajaka I, Ly TD, Chaix ML, *et al.*: **HIV-1/HIV-2 seronegativity in HIV-1 subtype O infected patients.** *Lancet* 1994, **343**:1393–1394.
73. Schable C, Zekeng L, Pau CP, *et al.*: **Sensitivity of United States HIV antibody tests for detection of HIV-1 group O infections.** *Lancet* 1994, **344**:1333–1334.
74. Loussert Ajaka I, Descamps D, Simon F, *et al.*: **Genetic diversity and HIV detection by polymerase chain reaction.** *Lancet* 1995, **346**:912–913.
75. Arnold C, Barlow KL, Kaye S, Loveday C, Balfe P, Clewley JP: **HIV type 1 sequence subtype G transmission from mother to infant: failure of variant sequence species to amplify in the Roche Amplicor test.** *AIDS Res Hum Retrovirus* 1995, **11**:999–1001.
76. Kostrikis LG, Cao Y, Ngai H, Moore JP, Ho D: **Quantitative analysis of serum neutralization of human immunodeficiency virus type 1 from subtypes A, B, C, D, E, F, and I: lack of direct correlation between neutralization serotypes and genetic subtypes and evidence for prevalent serum-dependent infectivity enhancement.** *J Virol* 1996, **70**:445–458.
77. Nyambi P, Nkengasong J, Peeters M, *et al.*: **Reduced capacity of antibodies from patients infected with human immunodeficiency virus type 1 (HIV-1) group O to neutralize primary isolates of HIV-1 group M viruses.** *J Infect Dis* 1995, **172**:1228–1237.
78. Nkengasong JN, Peeters M, Ndumbe P, *et al.*: **Cross-neutralizing antibodies to HIV-1_{ANT70} and HIV-1_{IIIB} in sera of African and Belgian HIV-1-infected individuals.** *AIDS* 1994, **8**:1089–1096.
79. Nyambi P, Nkengasong J, Lewi P, *et al.*: **Multivariate analysis of human immunodeficiency virus type 1 neutralization data.** *J Virol* 1996, **70**:6235–6243.
80. Kanai K, Kako M, Okamoto H: **HCV genotypes in chronic hepatitis C virus and response to interferon [letter].** *Lancet* 1992, **339**:1543.
81. Descamps D, Collin G, Loussert-Ajaka I, Saragosti S, Simon F, Brun-Vézinet F: **HIV-1 group O sensitivity to antiretroviral drugs [letter].** *AIDS* 1995, **9**:977–978.
82. Hu DJ, Dondero TJ, Rayfield MA, *et al.*: **The emerging genetic diversity of HIV. The importance of global surveillance for diagnostics, research, and prevention.** *JAMA* 1996, **275**:210–216.
83. Rowe PM: **HIV-1 group O infection identified in USA [letter].** *Lancet* 1996, **348**:116.
84. Artenstein AW, Coppola J, Brown AE, *et al.*: **Multiple introductions of HIV-1 subtype E into the Western hemisphere.** *Lancet* 1995, **346**:1197–1198.
85. Brodine SK, Mascola JR, Weiss PJ, *et al.*: **Detection of diverse HIV-1 genetic subtypes in the USA.** *Lancet* 1995, **346**:1198–1199.
86. Fransen K, Buvé A, Nkengasong JN, Laga M, van der Groen G: **Longstanding presence in Belgians of multiple non-B HIV-1 subtypes [letter]**. *Lancet* 1996, **347**:1403.
87. Clewley JP, Arnold C, Barlow KL, Grant PR, Parry JV: **Diverse HIV-1 genetic subtypes in UK [letter]**. *Lancet* 1996, **347**:1487.