Genetic diversity of HIV-1: the moving target

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Introduction

One of the major characteristics of HIV is the extremely high genetic variability. This extensive heterogeneity is the result of two factors. First, there have been multiple introductions of genetically diverse simian viruses into humans [1]. Numerous African primates have now been found to be infected with SIV, and the two major viral types infecting humans, HIV-1 and HIV-2, represent zoonotic transmissions from two different sources, namely chimpanzees (*Pan troglodytes*) [2] and sooty mangabeys (*Cercocebus atys*) [3,4], respectively (Fig. 1). Second, since these simian viruses entered the human population, they have rapidly accumulated more genetic diversity because of the high error rate of reverse transcriptase [5] and the fast turnover of virions in HIV-infected individuals [6,7]. On top of this, reverse transcriptase is known to be highly recombinogenic [8], so that radically different genomic combinations may be generated in individuals infected by genetically diverse viruses.

The initial epicentres of HIV-1 and HIV-2 infection appear to have been Central Africa and West Africa, respectively, reflecting the natural habitats of chimpanzees and sooty mangabeys. From these roots, different lineages of viruses have spread to very different extents around the world. For example, the original survey describing HIV-2 infection in commercial sex workers in West Africa in 1985 revealed no HIV-1 infection in the same population [9]. HIV-2 is still primarily found in West Africa, and HIV-2 prevalences are currently stable or even decreasing [10]. In contrast, HIV-1 has spread throughout Africa, including West Africa, and some lineages of HIV-1 have dispersed around the world, so that HIV-1 is predominant globally.

This review will focus on the current state of knowledge of HIV-1 diversity and its geographical distribution, as well as the implications of this genetic variability for diagnosis, treatment, and the development of vaccines.

Classification of HIV-1 strains

HIV-1 groups

Phylogenetic analyses of numerous strains of HIV-1, isolated from diverse geographic origins, have revealed three distinct clades of viruses, which have been termed groups M (main), N (new, or non-M, non-O) and O (outlier). Since SIVcpz lineages are interspersed among these three HIV-1 lineages (Fig. 1; see also [1,2,11]), each of the groups must have arisen from a separate cross-species transmission event [2]. All of the HIV-1 strains described in the 1980s, and still the vast majority of strains found worldwide and responsible for the pandemic, belong to just one of these lineages, group M. Within group M, there is further phylogenetic structure, which has allowed the classification of strains into numerous subtypes (see later).

Group O isolates were first described around 1990 [12]. These viruses are highly divergent from group M (Fig. 1), exhibiting only about 50% amino acid sequence identity to group M in the *env* gene [13,14]. Group O seems to be endemic to Cameroon and neighbouring countries in West Central Africa, but

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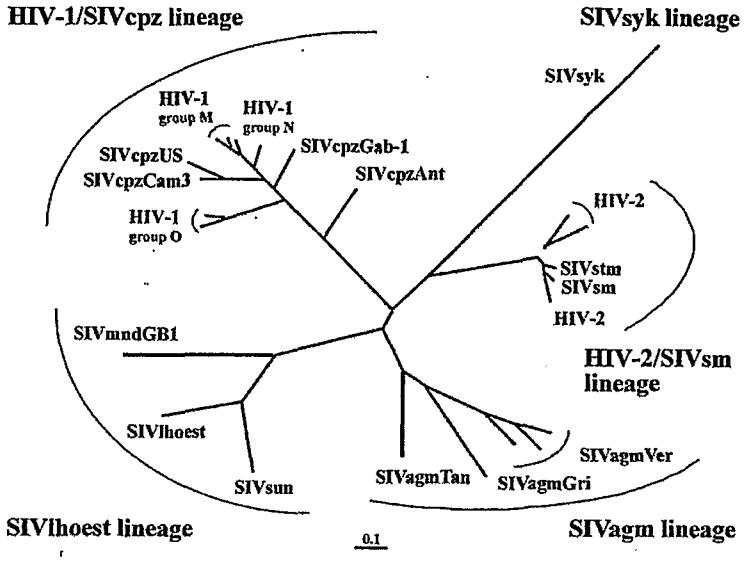

Fig. 1. Evolutionary relationships among the primate lentiviruses HIV-1, HIV-2 and SIV from various species of simians. Five major clades are evident: the HIV-1/SIVcpz lineage, which includes three groups of HIV-1 plus examples of SIVcpz isolated from chimpanzees; the HIV-2/SIVsm lineage, including several subtypes of HIV-2 plus SIVsm isolated from sooty mangabeys and SIVstm from a captive macaque; the SIVagm lineage, which includes isolates from four species of African green monkeys (viruses from tantalus, grivet and vervet monkeys are shown); the SIVlhoest lineage, including isolates from L'Hoest monkeys, sun-tailed monkeys, and mandrills; and the SIVsyk lineage from Sykes' monkeys. This unrooted phylogenetic tree was derived by maximum likelihood analysis of Pol protein sequences. (Data from Hahn *et al.* [1].)

even there, these viruses represent a minority of HIV-1 strains: their highest prevalence is 2–5% of HIV-1-positive samples [15,16]. Phylogenetic analyses of group O strains have not revealed the same substructure as found within the evolutionary tree of group M, and so this group has not been classified into subtypes. The lack of such a phylogenetic structure within group O may be the result of the relatively slow and limited spread of these viruses. Nevertheless, several groups have recently proposed that there are distinct clades within group O [17,18], and sequence data from more isolates are needed to clarify this issue.

Group N has only been identified recently, and is so far represented by only two isolates from Cameroonian patients [19]. The phylogenetic position of YBF-30, the only group N representative for which a full genome sequence is so far available, depends on the gene studied. Using sequences from the 5′ half of the genome, YBF-30 forms an independent lineage most closely related to, but still distant from, group M (as in Fig. 1). In contrast, with sequences from the 3′ half of the genome, YBF-30 clusters more closely with a chimpanzee virus (SIVcpzUS) [2,11]. These differences are symptomatic of a mosaic virus generated by recombination (see later).

HIV-1 group M subtypes

Since 1992, phylogenetic analyses of *env* and *gag* sequences have been used to classify the prevalent viruses observed in the global AIDS epidemic [20,21]. Subtypes were proposed because most sequences were found to fall into a limited number of discrete clades. Initially, five subtypes were identified, but more extensive global sampling has revealed additional subtypes: at the latest count, there are 11, designated A–K [22]. The subtypes are approximately equidistantly related (Fig. 2), exhibiting 25–35% amino acid sequence difference in their Env proteins, and up to 20% difference within subtypes [23]. This 'starburst' radiation at the root of the group M evolutionary tree is consistent with an exponentially growing population size [24]; in this case, meaning an exponential growth of the number of infected individuals at the start of the M group radiation.

It is important to distinguish between pure subtypes and recombinant viruses (see later). To be considered as a subtype, isolates should resemble each other, and no other existing subtype, across the entire genome. In this light, there are only nine subtypes of HIV-1 group M, since the prototypic viruses of subtypes E and I have been found to be recombinants, in which substantial fractions of the genome were derived from

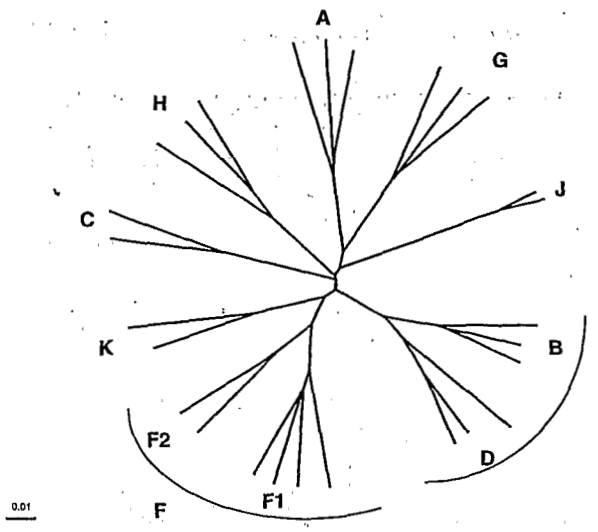

Fig. 2. Evolutionary relationships among non-recombinant HIV-1 group M subtypes, based on neighbour-joining phylogenetic analysis of near-full-length genome sequences. Each of the internal branches defining a subtype or sub-subtype is supported by 100% of bootstraps.

other subtypes. In addition, in the case of subtype G, there is some ambiguity about the origins of the accessory gene region, which resemble subtype A [25,26]; however, most of the subtype G genome is phylogenetically distinct.

A number of HIV-1 isolates remain unclassified, because they neither fall within any of the designated subtypes nor are recognizable inter-subtype recombinants, but have not been assigned to a new subtype because they are unique. Since full-length genome sequencing has become technically easier, and with the development of a variety of tools to detect the existence of mosaic (i.e. recombinant) genomes, the criteria to propose a novel subtype have been revised recently [27]. Three near-full-length genomic sequences, preferably, or two complete genomes in conjunction with partial sequences, are needed to define a new subtype. These strains must be identified in at least three individuals with no direct epidemiological linkage.

Within some subtypes, further phylogenetic structure can be identified, leading to a classification into subclades. Subtype F is subdivided into two subclades, F1 and F2. F1 viruses have been found in Brazil, Romania and Central Africa, whereas the F2 subclade consists exclusively of viruses from Cameroon [22]. Interestingly, the extent of divergence between subclades F1 and F2, while hardly any greater than the diversity seen within some other subtypes, is similar to that seen between subtypes B and D. With the benefit of hindsight, it is clear that for consistency, subtypes B and D would be better considered as subclades of a single subtype but, for historical reasons, it is difficult to change these designations. Subtype B gained its subtype status because it is the common form of HIV-1 in North America and Europe, and was very heavily represented among the first strains characterized.

It seems likely that the various subtypes, and subclades within subtypes, have been generated by founder effects. Thus, subtype B arose from a single strain that emerged from Africa. The phylogenetic structure at the root of subtype B is again a starburst, mirroring the initial radiation of group M subtypes, and again most likely due to exponential growth in the number of infected individuals at the start of the epidemic in Central and North America, and Europe.

Recombinant HIV-1 viruses

Following the designation of group M subtypes, it was realized that certain isolates seem to belong to different subtypes, depending on the region of the genome used for phylogenetic analysis [28]. Such discordant phylogenetic positions most likely indicate that the virus is an inter-subtype recombinant. One of the first African HIV-1 isolates to be characterized, MAL from Zaire [29], had been identified as a probable recombinant [30], but it was not until 1994 that the first multiply-infected individual was identified [31]. Subsequent reports have identified many more mosaic viruses, and confirmed their frequency at about 10–20% of newly characterized strains [28,32,33]. That these mosaic viruses are indeed recombinants is supported by the fact that discrete breakpoints can be identified between the genomic regions with differing phylogenetic affinities [25,26], and is further reinforced by the observation that most of these strains can be traced to geographical areas where the relevant subtypes co-circulate [25,34]. For example, numerous inter-subtype mosaic combinations have been reported from Africa [35–37], where all subtypes are found, while B/F recombinants have been found in Brazil, where subtypes B and F are both common [34].

Some of these mosaic HIV-1 genomes are unique, or restricted to small transmission clusters. However, others have been identified in several, apparently unlinked, individuals and play a major role in the global AIDS epidemic: these are now designated as 'circulating recombinant forms' (CRF) [38]. Members of a CRF should resemble each other over the entire genome, with similar breakpoints reflecting common ancestry from the same recombination event(s). There are currently several CRF of HIV-1: under new nomenclature proposals, each will be designated by an identifying number, with letters

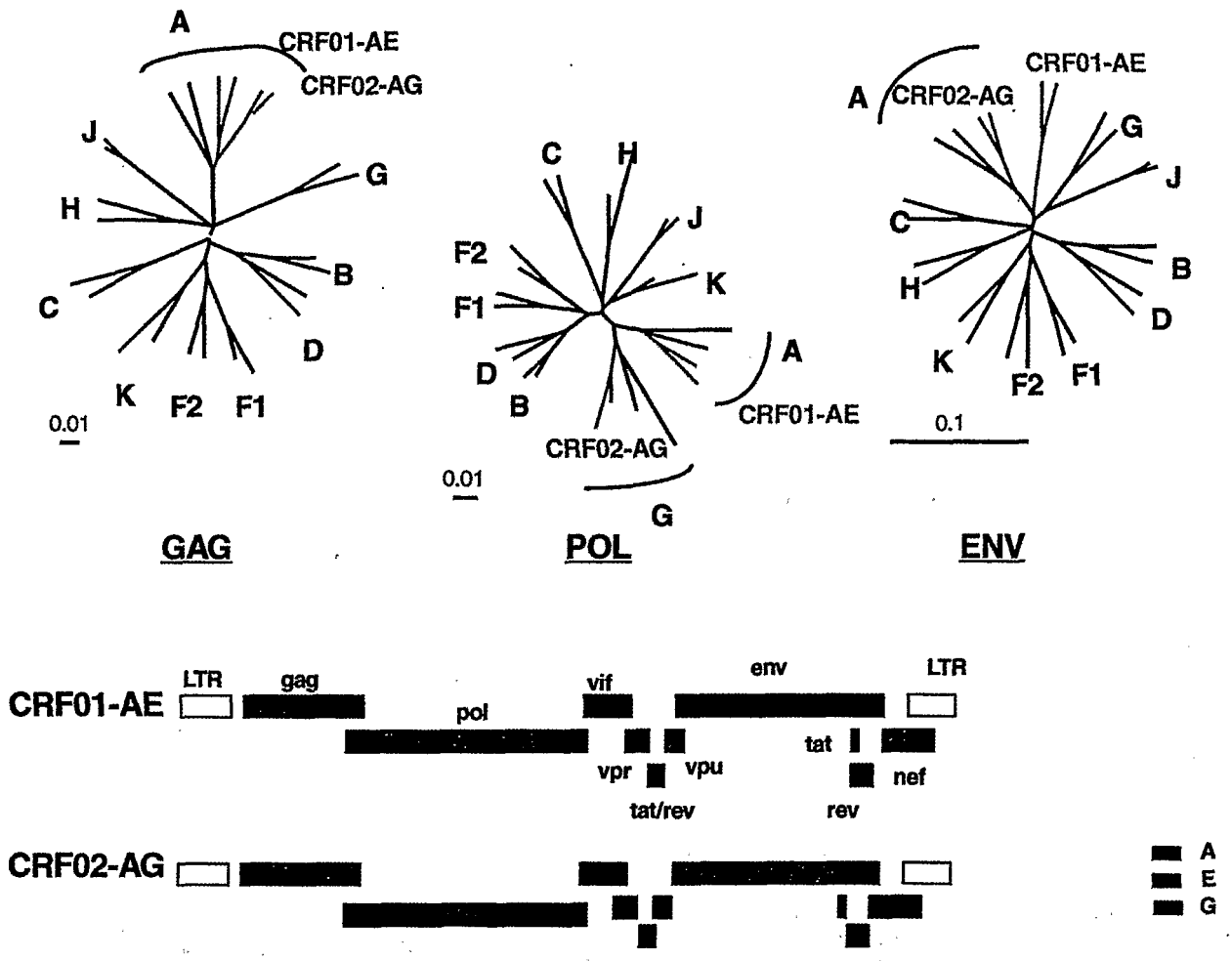

Fig. 3. Evolutionary relationships of the circulating recombinant forms, CRF01-AE and CRF02-AG, in different regions of the genome, based on neighbour-joining phylogenetic trees of the full-length *gag* sequences, 3′ end sequences of the *pol* gene (covering the protease and the 3′ end of the reverse transcriptase gene), and the gp120 *env* sequences. The mosaic structure of the genomes of CRF01-AE and CRF02-AG are shown.

indicating the subtypes involved [27]. If the genome contains sequences originating from more than three subtypes, the letters will be replaced by 'cpx', denoting 'complex'.

All known representatives of what was initially described as subtype E appear, in fact, to be recombinants of subtypes A and E [39,40], and are now designated CRF01_AE [27]. The only non-subtype A sequences are found within (most of) the *env* gene, parts of *vif*, *vpr* and *nef*, and the *LTR* (Fig. 3). Subtype E was first designated on the basis of the distinct phylogenetic position of these viruses in *env* trees. However, no virus has yet been described that represents a full-length non-recombinant, subtype E sequence, perhaps simply because that lineage has become extinct. This absence of one of the 'parental' lineages leads to some technical difficulties in formally proving the recombinant nature of these viruses.

The IbNg strain from Ibadan, Nigeria, was initially described as a divergent lineage within subtype A, based on partial gp120 sequences [41]. However, after the determination of a full-length sequence, IbNg was recognized as a complex mosaic of alternating subtype A and subtype G sequences [26]. Since a number of similar viruses have been reported from countries in both West and East Africa, this clade is now designated CRF02_AG (Fig. 3).

An epidemic among intravenous drug users (IVDU) in Kaliningrad, Russia, involves viruses that are mosaics of subtypes A and B [42,43], and which are therefore termed CRF03_AB.

Isolate 94CY032 from Cyprus was designated as the prototype of subtype I based on gp120 sequences [44]. However, full genome sequencing revealed this virus to be a complex mosaic with multiple break-

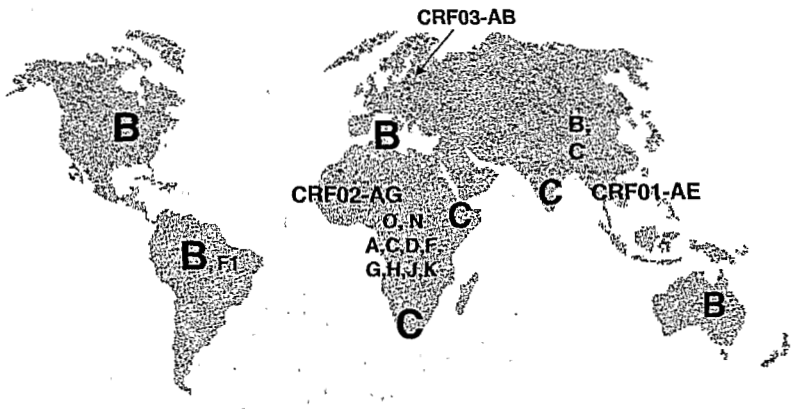

Fig. 4. Distribution of HIV-1 groups, subtypes and circulating recombinant forms (CRF) in different parts of the world. Only the predominant forms in each region are shown. Due to international travel and intermixing of populations, many subtypes other than those indicated are reported in each region.

points between regions of several distinct subtypes, including A and G [45]. Two similar viruses have been described from epidemiologically unlinked individuals from Greece [46]. As in the case of subtype E, no full-length non-recombinant subtype I virus has yet been identified. These viruses are now designated CRF04-cpx [27].

Recently, two near-full-length genomes of similar complex mosaic viruses, containing fragments of (at least) subtypes A, G and J, have been described in patients from Burkina Faso (BFP90) and Mali (95ML84), and partial sequences of additional viruses from Nigeria and Senegal indicate that these may share a common ancestry [47,48]. However, as with the definition of a novel subtype, a third full-length genome sequence or partial sequences, confirming the breakpoints, from a third epidemiologically unlinked individual will be required to designate these new CRFs [27].

Worldwide distribution of HIV-1 variants

Subtype designations have been powerful molecular epidemiological markers to track the course of the HIV-1 pandemic. Extensive efforts have been made to collect and characterize HIV-1 isolates from around the world, and a broad picture of the distribution of subtypes and CRFs have emerged (Fig. 4). Globally, the predominant viral forms are subtypes A and C, followed by subtype B, and the recombinants CRF01_AE and CRF02_AG [33,49].

The greatest genetic diversity of HIV-1 has been found in Africa. Group M subtypes A and C are most common, but all groups and subtypes are found, consistent with this continent being the source of the epidemic. As expected, given the presence of numerous co-circulating subtypes, a high frequency and a wide variety of recombinants have also been reported in Africa [36,37]. In South and East Africa, subtype C predominates. In West and Central Africa, as judged by *env* sequences, subtype A-like viruses are most common [50]. Full-length genome sequences of viruses resembling IbNg, representing CRF02_AG, have recently been reported from different locales in Senegal, Cote d'Ivoire, and Cameroon, and it seems likely that the majority of viruses with subtype A *gag* and/or *env* sequences in West Africa and West Central Africa belong to this circulating recombinant form [33,51]. In contrast, in East Africa, the subtype A viruses are predominantly non-recombinant [52]. Subtype D is present at frequencies of 5–40% in Central and East Africa [50,53], while subtype G has been documented in many West and Central African countries. Subtypes F, H, J and K, as well as CRF01_AE, are mainly seen in Central Africa [22,36,37,50]. We have already noted that HIV-1 group O is only common in West Central Africa, and group N has only been reported in the same region.

Specific distributions of the various subtypes are seen in other continents, although sporadic cases of many different variants of HIV-1 have now appeared in most parts of the world. In North America, Europe and Australia, subtype B is by far the most common. However, various other group M subtypes, and even group O viruses, have been reported in the United States [54–56] and several European countries [57–60]. For example, a study of blood donors in France showed a 16% increase between 1984 and 1995 in the prevalence of individuals infected by non-subtype B viruses [61], and other varieties are also on the increase in Sweden and Germany [62,63].

In South America, subtype B predominates, but subtypes F and C are also found. Several instances of B/F recombinants have been reported from Brazil and Argentina [49,64,65].

Across Asia, different forms of HIV-1 have taken hold in different regions. In India, subtype C predomi-

nates, but subtypes A and B co-circulate, and A/C recombinants are also found [66,67]. In contrast, in Thailand, first reports indicated two separate epidemics, of subtype B and 'subtype E' (i.e. CRF01_AE) viruses, spreading in different sections of the population: IVDU were infected with subtype B viruses, whereas those infected heterosexually predominantly carried subtype E (now CRF01_AE) viruses. More recent data show that the proportion of CRF01_AE has increased in almost all population groups, so that 35% of IVDU in Bangkok are now infected with this variant, and it is also the predominant form among younger individuals [68–70]. CRF01_AE is also increasing in frequency in other South-East Asian countries [71]. This has led to speculation that CRF01_AE is more transmissible than subtype B, but it is difficult to discount that the differential spread merely reflects epidemiological factors (see later).

The example of Thailand, in particular, clearly illustrates that the global distribution of different forms of HIV-1 is a dynamic process. As more HIV-1 variants inevitably intermix in different parts of the world, so the likelihood of generating new recombinant viruses will increase. Indeed, the pattern of mosaicism will become even more complex, since recombination involving viruses that are already recombinant will occur. Mosaics involving CFR02_AG have already been observed in various African countries [33,72]. Continued monitoring is necessary to determine the future role of non-subtype B viruses in North America and Europe, and to chart the emergence of new predominant subtypes and CRF around the world.

Methods of classification

Sequence determination is the most accurate approach to characterize virus genomes, but there is a clear need for continued development of techniques for typing that are less cumbersome, and less expensive. Serological methods, as well as polymerase chain reaction (PCR)-based techniques, are continually being refined. These approaches can usually define the subtype affinity of a particular region of the viral genome, but they will not detect whether other regions are mosaic or belong to a different subtype.

Serological methods for subtyping

Serological assays are greatly preferable, because they are easy to use and large numbers of samples can be tested. Enzyme-linked immunosorbent assays detecting antibodies against the V3 loop of the envelope protein have been used successfully to discriminate among HIV-1 group M, N and O infections [15,19,73]. However, V3 serotyping is less useful in determining group M subtypes. These enzyme-linked immunosorbent assays are only useful in areas where a limited number of major variants co-circulate, as with subtype B and CRF01_AE in Thailand, or subtypes B and C in South Africa [68,69,74]. The technique has also proved useful in France, to discriminate between subtype B and non-subtype B infections [58]. However, in regions where many subtypes have been co-circulating for a long period, serotyping can lead to misidentification of genotypes [75,76].

PCR-Based approaches

The heteroduplex mobility assay (HMA) is a PCR-based method that allows rapid subtyping using a set of reference reagents representing different subtypes [77]. Less sophisticated equipment is required for this technique than for sequencing, and HMA has in fact been widely used in developing countries to identify *env* gene subtypes. One limitation of HMA is that, as HIV-1 sequences continue to diverge and as new geographic regions are sampled, the primers used are failing more often, and a greater number of samples remain indeterminate. Another is that *env* HMA cannot discriminate between subtype A and CRF02_AG viruses, which are also subtype A in the *env* region analyzed. More recently, HMA has been developed to characterize *gag* genotypes, which can distinguish between subtype A and CRF02_AG. Simultaneous use of *gag* and *env* HMA can give a preliminary estimation of the frequency of recombinant viruses [78].

Other PCR-based methods have been developed, including a restriction fragment length polymorphism technique to determine protease region genotypes [79], and oligonucleotide assays that have been used to distinguish between *env* sequences of subtypes B and E in Thailand [80]. Subtype A-specific PCR of the *env* gene has been developed for areas where that clade is highly prevalent [81].

Sequence determination

Nevertheless, sequencing remains the most accurate approach to identify HIV-1 variants. Even only partial *gag* and/or *env* sequences give more precise information than serotyping or HMA with regard to the presence of subclades or recombinant viruses. Also, partial genome characterization allows a preliminary selection of important strains for full sequencing. Full-length genome sequencing is needed for the continued surveillance of HIV-1 global variation. A full-length sequence is also necessary to exclude the possibility that a new isolate is recombinant, or to determine the pattern of mosaicism within an isolate that is recombinant.

A variety of complementary approaches have been developed to identify sequences that are recombinants,

and to map the positions of breakpoints within mosaic sequences. General methods to identify recombinant sequences examine whether the evolutionary relationships, or more simply relative distances between sequences, vary among different windows along a sequence alignment [82–84]. Breakpoints between sequence regions with different evolutionary histories can be identified by mapping the linear distribution of divergent and/or phylogenetically incompatible sites [85,86]. In the specific case of HIV-1 sequences, because the different subtypes of the M group have been well defined, potential intersubtype recombinants can be analyzed in a more-or-less automated fashion. Using moving window analysis, diversity plots [25,67] can be used to display the extent of similarity of a new sequence to representatives of other subtypes; the recombinant identification program [87] uses distance measures to assign subtypes to regions within the new sequence, and the strength of bootstrap support for the phylogenetic placement of the new sequence with any subtype representative can be assessed [88]. Fine-scale mapping of recombination breakpoints has been performed using informative site analysis [25,89]. Much of the software used to perform these analyses is freely available and/or can be accessed online.

Studies of HIV-1 variants in Nigeria, the most populous African country, illustrate the value of rapid screening assays that can be performed locally, and also the need for more sophisticated analysis of a subset of samples in order to completely understand the situation. In Nigeria, two *env* subtypes, A and G, were found to be predominant by HMA. However, partial *gag* sequencing revealed that more than 30% of samples had discordant *gag* and *env* subtype designations. Phylogenetic tree analysis of *gag* and *env* sequences showed several subclusters within subtypes A and G, and numerous A/G recombinants with different genomic structures. Thus, despite the limited number of common HIV-1 subtypes in Nigeria, there is a complex mix of viruses circulating in that country [90,91].

Implications of HIV-1 variability

Whether the various groups, subtypes and recombinant forms of HIV-1 have biological differences, for example with respect to transmissibility and the course of disease progression, is not yet well known. Clearly, the extent of genetic divergence among subtypes (let alone among groups) is more than sufficient to cause such differences, but adequately controlled data from *in vivo* studies may take a long time to emerge. Importantly, diagnostic tests, antiretroviral drugs, and HIV-1 vaccines have so far mainly been developed only for subtype B viruses.

Serological and molecular diagnostic tests

Examples of HIV-1 group O were initially identified because they were not detected by certain commercial serological screening assays, and group O sera can give indeterminate results in confirmatory tests such as Western blots [92,93]. The majority of commercial assays are now capable of detecting group O antibodies, either through the use of broadly cross-reactive group M antigens, or by addition of specific group O antigens. However, continued surveillance is necessary, since even subtle changes in antigenic structure of some HIV-1 variants may affect the sensitivity of these tests. During seroconversion, a significantly lower sensitivity was observed for the detection of non-subtype B infections using screening assays with subtype B-derived antigens [94]. Several studies have reported that commercial viral load assays were either not able to detect or incorrectly quantified viral RNA in plasma from patients infected with non-subtype B strains of HIV-1 [95]. These assays have been re-evaluated, and other assays are under development, but as yet no assay is commercially available that can detect and quantify HIV-1 group O or HIV-2 strains. As even greater variability of HIV-1 emerges, these tests will need to be continuously monitored to evaluate the efficiency of the primers or probes used.

Antiretroviral treatment

Antiretroviral drugs are not yet widely used in developing countries, where non-subtype B strains of HIV-1 predominate. However, this situation could change in the coming years, as several governments and international organizations have recently made antiretroviral treatments available to certain developing countries, and antiretroviral drugs are being introduced to prevent perinatal transmission. *In vitro* data have indicated that HIV-1 group O viruses, as well as HIV-2, are naturally resistant to non-nucleoside reverse transcriptase inhibitors [96,97]. There are also indications of variation in drug susceptibility within group M. Thus, some subtype F samples are less susceptible to 8-chloro-tetrahydro-imidazo(4,5,1-*jk*)(1,4)-benzodiazepin-2(1*H*)thione, a non-nucleoside reverse transcriptase inhibitor [98], while some subtype G strains are less susceptible to protease inhibitors [99]. Many amino acid mutations associated with secondary resistance to protease inhibitors have been reported as natural variants in treatment-naïve patients infected with non-subtype B HIV-1 strains, but the biological consequences of this remain to be studied [100–103].

Biological differences, transmission and disease progression

Differences between HIV-1 and HIV-2 with respect to transmissibility and disease progression have been clearly documented: HIV-2 exhibits a lower rate of

heterosexual transmission, a near absence of vertical transmission, and a longer incubation period before development of AIDS [104–106]. The question remains whether similar differences exist among the various forms of HIV-1.

There have been some indications that there may be biological differences among HIV-1 groups and subtypes, but also some apparently contradictory results. A prospective cohort study among female sex workers in Senegal showed that subtype A-infected women were eight times less likely to develop AIDS [107]. The CXCR4-positive phenotype, previously known as rapid/high or syncytium inducting, is rare among subtype C-infected patients [108–110]. With subtype B, the presence of CXCR4-positive viruses is associated with a more rapid progression to AIDS, but it remains to be determined whether subtype C viruses are less virulent or are associated with different clinical outcomes. No difference in disease progression was found between patients infected with subtypes B and C in Israel [111], or among patients infected with subtypes A, B, C and D in Sweden [112]. However, in a cohort of pregnant women in Kenya, higher viral loads and lower CD4 cell counts were seen in subtype C patients than in those infected with subtypes A or D [113].

The rate of heterosexual transmission for CRF01_AE in Thailand is fivefold higher than for subtype B in the United States [114]. More prospective epidemiological studies from different regions are needed to determine whether this is really due to intrinsic properties of the virus rather than population-related factors. No difference was found between CRF01_AE and subtype B in rate of disease progression during a 3-year follow-up study in Thailand [115].

The rate of disease progression varies from person to person and is influenced by multiple virological and host factors. The evaluation of associations between subtypes and phenotypes will be very difficult, and it will not be easy to determine whether any association is causal or due to bias or chance. Nevertheless, it would be premature to conclude that there are no subtype-specific differences in virus biology, transmission or disease development.

Vaccines

Most candidate HIV-1 vaccines currently in development or in clinical trials are based on subtype B antigens, and the degree to which these will elicit cross-protection against other subtypes is poorly understood. In one study, in Thailand, neutralizing antibodies appeared to be subtype specific [116]. However, several other studies have suggested that there is no correlation between HIV-1 subtypes and neutralizing serotypes: some sera appear to be broadly cross-reactive, and some isolates highly susceptible to neutralization by many sera, but other isolates seem neutralization resistant [117].

Similarly, while one study reported a subtype-specific cytotoxic T-lymphocyte response [118], several others have shown cross-clade cytotoxic T-lymphocyte reactivities in individuals infected with non-B subtypes, as well as in recipients of a subtype B vaccine [119,120]. These data suggest that some cytotoxic T-lymphocyte epitopes may be conserved among different subtypes, and emphasize the importance of including some of the more conserved proteins in vaccine constructs.

Implications of recombination

Retroviral recombination requires the simultaneous infection of a cell with two different proviruses, allowing the encapsidation of one RNA transcript from each provirus into a heterozygous virion. The discovery of large numbers of recombinant viruses clearly implies that co-infection with divergent HIV-1 strains is not as rare as once thought. Indeed, dual infections with different subtypes have been reported in regions where multiple variants co-circulate [31,65,72,121]. In any geographic region, the proportion of recombinant viruses will depend on a number of factors, including the prevalence rates of different subtypes, the probability that certain population groups acquire multiple infections and transmit their viruses further, and the fitness of any mosaic viruses generated. However, the frequency of recombinant viruses is almost certain to increase; recombination, once it has occurred, cannot be undone, and so the frequency of 'pure' subtype strains is likely to decrease.

It remains to be determined whether there are constraints, during the course of HIV infection, on when superinfection can occur. In macaques, it has recently been shown that superinfection with divergent strains of HIV-2 is possible only during certain periods when antibodies are not yet efficiently expressed [122]. In contrast, in a chimpanzee, superinfection with a CRF01_AE virus 32 weeks after experimental infection with a subtype B strain led to a dual infection with the rapid appearance of recombinant viruses [123]. The latter result suggests that superinfection is not restricted to the early phase of infection, and implies that the humoral and cellular antibody response are not efficient against divergent strains.

It was initially suspected that homologous recombination between group M and group O viruses may not be possible because of their high degree of divergence. However, two recent reports have documented intergroup recombinants in two different patients from Cameroon [72,124]. One study showed

that these M/O mosaic viruses replicated well *in vivo* and *in vitro*, and indeed became the predominant variants within the patient's viral population [72]. Recombination between such divergent strains could contribute substantially to the emergence of new HIV-1 variants, and would have important implications both for diagnosis by serological and molecular tests, and for treatment. This observation also suggests the possibility that HIV could recombine with SIV, for example in individuals who are exposed to SIV by cross-species transmission, and so by this means additional SIV sequences could spread into the human population.

While dual infections with HIV-1 and HIV-2 have frequently been reported in regions where both viruses circulate [37], no recombinants between them have as yet been described. In this case, the level of genetic divergence may be too high for successful recombination, although its possibility cannot be entirely excluded.

Clearly, superinfection and consequent recombination can affect the biological properties, and pathogenesis, of HIV strains. Several studies have found that, under the selective pressure imposed by antiretroviral drugs, recombination between strains with different drug sensitivities occurred, resulting in new HIV-1 variants with dual drug resistance [125]. *In vitro* experiments with feline and murine retroviruses have demonstrated that, under appropriate selection pressures, mixed infections can generate recombinant viruses with altered tissue tropism, pathogeneicity, and host range, or with changes in antigenic epitopes [126,127]. Finally, recombination also has important implications for vaccine strategies based on live-attentuated viruses, since these could recombine with infecting strains, even if the two are quite divergent.

Conclusions and perspectives

It is increasingly evident that the distribution of HIV-1 genetic subtypes is a dynamic and unpredictable process. The geographical distribution of subtypes is evolving, and intermixing of HIV-1 variants is inevitable. Recombinant viruses are already contributing substantially to the global pandemic, and the frequency and complexity of recombinant viruses will increase as the various subtypes spread to all continents, and viruses that are already mosaic are involved in further recombination events.

If there are biological differences among the various subtypes and CRFs, it is important that these should be clearly defined, and it remains important to track the molecular epidemiology of HIV-1. In future studies, both 'pure' subtypes and CRFs will need to be monitored.

More studies are needed to understand the role and the implications of recombinant viruses in the global HIV evolution. It is important to study in more detail the impact of viral recombination on viral properties, since recombination may introduce genetic and biological consequences that are far greater than those resulting from the steady accumulation of single mutations. In order to develop an efficient vaccine, it remains to be determined when superinfection can occur during the course of HIV infection, and to what extent humoral and cellular antibody response are efficient against divergent strains.

References

1. Hahn BH, Shaw GM, De Cock KM, Sharp PM. **AIDS as a zoonosis: scientific and public health implications.** *Science* 2000, **287**:607–614.
2. Gao F, Bailes E, Robertson DL, *et al.* **Origin of HIV-1 in *Pan troglodytes troglodytes*.** *Nature* 1999, **397**:436–441.
3. Hirsch V, Olmsted R, Murphey-Corb M, Purcell, Johnson P. **An African primate lentivirus (SIVsm) closely related to HIV-2.** *Nature* 1989, **339**:389–392.
4. Gao F, Yue L, White AT, *et al.* **Human infection by genetically-diverse SIVsm-related HIV-2 in West Africa.** *Nature* 1992, **358**:495–499.
5. Preston BD, Poiesz BJ, Loeb LA. **Fidelity of HIV-1 reverse transcriptase.** *Science* 1988, **242**:1168–1171
6. Wei X, Ghosh SK, Taylor ME, *et al.* **Viral dynamics in human immunodeficiency virus type 1 infection.** *Nature* 1995, **373**:117–122
7. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. **Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection.** *Nature* 1995, **373**:123–126.
8. Hu WS, Temin HM. **Retroviral recombination and reverse transcription.** *Science* 1990, **250**:1227–1233.
9. Barin F, Mboup S Denis F, *et al.* **Serological evidence for virus related to Simian T-Lymphotropic retrovirus III in residents of West Africa.** *Lancet* 1985, **ii**:1387–1389.
10. Van der Loeff M, Aaby P. **Towards a better understanding of the epidemiology of HIV-2.** *AIDS* 1999, **13(suppl A)**:S69–S84.
11. Corbet S, Muller-Trutwin M, Versmisse P, *et al.* **Env sequences of simian immunodeficiency viruses from chimpanzees in Cameroon are strongly related to those of human immunodeficiency virus group N from the same geographic area.** *J Virol* 2000, **74**:529–534.
12. Deleys R, Vanderborght B, Vanden Haesevelde M, *et al.* **Isolation and partial characterization of an unusual human immunodeficiency retrovirus from two persons of west-central African origin.** *J Virol* 1990, **64**:1207–1216.
13. Vanden Haesevelde M, Decourt J, De Leys R, *et al.* **Genomic cloning and complete sequence analysis of a highly divergent African human immunodeficiency virus isolate.** *J Virol* 1994, **68**:1586–1596.
14. Gurtler LG, Hauser P, Eberle J, *et al.* **A new subtype of human immunodeficiency virus type 1 (MVP-5180) from Cameroon.** *J Virol* 1994, **68**:1581–1585.
15. Peeters M, Gueye A, Mboup S, *et al.* **Geographical distribution of HIV-1 group O viruses in Africa.** *AIDS* 1997, **11**:493–498.
16. Zekeng L, Gurtler L, Afane E, *et al.* **Prevalence of HIV-1 subtype O infection in Cameroon: preliminary results.** *AIDS* 1994, **8**:1626–1628.
17. Janssens W, Heyndrickx L, Van der Auwera G, *et al.* **Interpatient variability of HIV-1 group O.** *AIDS* 1999, **13**:41–48.
18. Mas A, Quinones-Mateu ME, Domingo E, Soriano V. **Phylogeny of HIV type 1 group O isolates based on *env* gene sequences.**

AIDS Res Hum Retroviruses 1999, **15**:769–773.

19. Simon F, Mauclere P, Roques P, *et al.* **Identification of a new human immunodeficiency virus type 1 distinct from group M and group O.** *Nat Med* 1998, **4**:1032–1037.
20. 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.
21. Louwagie J, Janssens W, Mascola J, *et al.* **Genetic diversity of the envelope glycoprotein from human immunodeficiency virus type 1 (HIV-1) isolates from African origin.** *J Virol* 1995, **69**:263–271.
22. Triques K, Bourgeois A, Vidal N, *et al.* **Near-full length genome sequencing of divergent African HIV-1 subtype F viruses leads to the identification of a new HIV-1 subtype designated K.** *AIDS Res Hum Retroviruses* 2000, **16**:139–151.
23. Sharp PM, Robertson DL, Gao F, Hahn BH. **Origins and diversity of human immunodeficiency viruses.** *AIDS* 1994, **8**:S27–S42.
24. Holmes EC, Nee S, Rambaut A, Garnett GP, Harvey PH. **Revealing the history of infectious disease epidemics through phylogenetic trees.** *Philos Trans R Soc London B* 1995, **439**:33–40.
25. Gao F, Robertson DL, Carruthers CD, *et al.* **A comprehensive panel of near-full-length clones and reference sequences for non-subtype B isolates of human immunodeficiency virus type 1.** *J Virol* 1998, **72**:5680–5698.
26. Carr JK, Salminen MO, Albert J, *et al.* **Full genome sequences of human immunodeficiency virus type 1 subtypes G and A/G recombinants.** *Virology* 1998, **247**:22–31.
27. Robertson DL, Anderson J, Bradac J, *et al.* **HIV-1 subtype And recombinant nomenclature proposal.** *Science* 2000, **288**:55–57.
28. Robertson DL, Sharp PM, McCutchan FE, Hahn BH. **Recombination in HIV-1.** *Nature* 1999, **374**:124–126.
29. Alizon M, Wain-Hobson S, Montagnier L, Sonigo P. **Genetic variability of the AIDS virus: nucleotide sequence analysis of two isolates from African patients.** *Cell* 1986, **46**:63–74.
30. Li W-H, Tanimura M, Sharp PM. **Rates and dates of divergence between AIDS nucleotide sequences.** *Mol Biol Evol* 1988, **5**:313–330.
31. Artenstein AW, VanCott 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.
32. Cornelissen M, van den Burg R, Zorgdrager F, Lukashov V, Goudsmit J. **Pol gene diversity of five human immunodeficiency virus type 1 subtypes: evidence for naturally occurring mutations that contribute to drug resistance, limited recombination patterns, and common ancestry for subtypes B and D.** *J Virol* 1997, **71**:6348–6358.
33. Montavon C, Toure-Kane C, Liegeois F, *et al.* **Most *env* and *gag* subtype A HIV-1 viruses circulating in West and West Central Africa are similar to the prototype AG recombinant virus IBNG.** *J Acquir Immune Defic Syndr* 2000, **23**:363–374.
34. Sabino EC, Sphaer EG, Morgado MG, *et al.* **Identification of HIV-1 envelope genes recombinant between subtypes B and F in two epidemiologically-linked individuals from Brazil.** *J Virol* 1994, **68**:6340–6346.
35. Renjifo B, Chaplin B, Mwakagile D, *et al.* **Epidemic expansion of HIV type 1 subtype C and recombinant genotypes in Tanzania.** *AIDS Res Hum Retroviruses* 1998, **14**:635–638.
36. Janssens W, Buve A, Nkengasong JN. **The puzzle of HIV-1 subtypes in Africa.** *AIDS* 1997, **11**:705–712.
37. Kanki PJ, Peeters M, Gueye-Ndiaye A. **Virology of HIV-1 and HIV-2: implications for Africa.** *AIDS* 1997, **11(suppl B)**:S33–S42.
38. Carr JK, Foley B, Leitner T, Salminen M, Korber BT, McCutchan FE. **Reference sequences representing the principal genetic diversity of HIV-1 in the pandemic.** In *Human Retrovirus and AIDS.* Part III. Los Alamos, NM: Los Alamos National Laboratory; 1998.
39. Gao F, Robertson DL, Morrison S, *et al.* **The heterosexual human immunodeficiency virus type 1 epidemic in Thailand is caused by an intersubtype (A/E) recombinant of African origin.** *J Virol* 1996, **70**:7013–7029.
40. Carr JK, Salminen MO, Koch C, *et al.* **Full-length sequence and mosaic structure of a human immunodeficiency virus type 1 from Thailand.** *J Virol* 1996, **70**:5935–5943.
41. Howard TM, Rasheed S. **Genomic structure and nucleotide sequence analysis of a new HIV type 1 subtype A strain from Nigeria.** *AIDS Res Hum Retroviruses* 1996, **12**:1413–1425.
42. Bobkov A, Kazennova E, Selimova L, *et al.* **A sudden epidemic of HIV type 1 among injecting drug users in the former Soviet Union: identification of subtype A, subtype B, and novel gagA/envB recombinants.** *AIDS Res Hum Retroviruses* 1998, **14**:669–676.
43. Liitsola K, Tashkinova I, Laukkanen T, *et al.* **HIV-1 genetic subtype A/B recombinant strain causing an explosive epidemic in injecting drug users in Kaliningrad.** *AIDS* 1998, **12**:1907–1919.
44. Kostrikis LG, Bagdades E, Cao YZ, Zhang LQ, 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.
45. Gao F, Robertson DL, Carruthers CD, *et al.* **An isolate of human immunodeficiency virus type 1 originally classified as subtype I represents a complex mosaic comprising three different group M subtypes (A, G and I).** *J Virol* 1998, **72**:10234–10241.
46. Nasioulas G, Paraskevis D, Magiorkinis E, Theodoridou M, Hatzakis A. **Molecular analysis of the full-length genome of HIV-1 subtype I: evidence of A/G/I recombination.** *AIDS Res Hum Retroviruses* 1999, **15**:745–758.
47. Oelrichs RB, Workman C, Laukkanen T, McCutchan FE, Deacon NJ. **A novel subtype recombinant full-length HIV type 1 genome from Burkina Faso.** *AIDS Res Hum Retroviruses* 1998, **14**:1495–1500
48. Montavon C, Bibollet-Ruche F, Robertson DL, *et al.* **The identification of a complex A/G/I/J recombinant HIV-1 virus in different West African countries.** *AIDS Res Hum Retroviruses* 1999, **15**:1707–1712.
49. Workshop report from the European Commission and the Joint United Nations Programme on HIV/AIDS. **HIV-1 subtypes: implications for epidemiology, pathogenicity, vaccines and diagnostics.** *AIDS* 1997, **11**:UNAIDS17–UNAIDS36.
50. Peeters M, Esu-Williams E, Nzilambi N, *et al.* **Molecular epidemiology of HIV-1 genetic subtypes in West and Central Africa.** *XII International Conference on AIDS.* Geneva, June 1998 [abstract 11163].
51. Andersson S, Norrgren H, Dias F, Biberfeld G, Albert J. **Molecular characterization of human immunodeficiency virus (HIV)-1 and –2 in individuals from Guinea-Bissau with single or dual infections: predominance of a distinct HIV-1 subtype A/G recombinant in West Africa.** *Virology* 1999, **262**:312–320.
52. Carr JK, Laukkanen T, Salminen MO, *et al.* **Characterization of subtype A HIV-1 from Africa by full genome sequencing.** *AIDS* 1999, **13**:1819–1826.
53. Rayfield MA, Downing RG, Baggs J, *et al.* **A molecular epidemiologic survey of HIV in Uganda.** *AIDS* 1998, **12**:521–527.
54. Brodine S, Mascola J, Weiss P, *et al.* **Detection of diverse HIV-1 genetic subtypes in the United States.** *Lancet* 1995, **346**:1198–1199.
55. Brodine SK, Shaffer R, Starkey M, *et al.* **Drug resistance patterns, genetic subtypes, clinical features, and risk factors in military personnel with HIV-1 seroconversion.** *Ann Intern Med* 1999, **131**:502–506.
56. Rayfield M, Sullivan P, Bandea C, *et al.* **HIV-1 group O virus identified for the first time in the United States.** *Emerg Infect Dis* 1996, **2**:209–212.
57. Heyndrickx L, Janssens W, Coppens S, *et al.* **HIV type 1 C2V3 env diversity among Belgian individuals.** *AIDS Res Hum Retroviruses* 1998, **14**:1291–1296.
58. Simon F, Loussert-Ajaka I, Damond F, Saragosti S, Barin F, Brun-Vézinet F. **HIV type 1 diversity in northern Paris, France.** *AIDS Res Hum Retroviruses* 1996, **12**:1427–1233.
59. Lasky M, Perret JL, Peeters M, *et al.* **Presence of multiple non-B subtypes and divergent subtypes B strains of HIV-1 in individuals infected after overseas deployment.** *AIDS* 1997, **11**:43–51.
60. Alaeus A, Leitner T, Lidman K, Albert J. **Most HIV-1 genetic subtypes have entered Sweden.** *AIDS* 1997, **11**:199–202.
61. Barin F, Courouce AM, Pillonel J, Buzelay L. **The retrovirus study group of the French society of blood transfusion: increasing diversity of HIV-1M serotypes in French blood donors over a 10-year period (1985–1995).** AIDS 1997, **11**:1503–1508.
62. Sonnerborg A, Durdevic S, Giesecke J, Sallberg M. **Dynamics of the HIV-1 subtype distribution in the Swedish HIV-1 epidemic**

during the period 1980–1993. *AIDS Res Hum Retroviruses* 1997, **13**:343–345.
63. Dietrich U, Ruppach H, Gehring S, *et al.* **Large proportion of non-B HIV-1 subtypes and presence of zidovudine resistance mutations among German seroconvertors.** *AIDS* 1997, **11**:1532–1533.
64. Marquina S, Leitner T, Rabinovich RD, Benetucci J, Libonatti O, Albert J. **Coexistence of subtypes B, F, and a B/F env recombinant of HIV type 1 in Buenos Aires, Argentina.** *AIDS Res Hum Retroviruses* 1996; **12**:1651–1654.
65. Janini L, Tanuri A, Schechter M, *et al.* **Horizontal and vertical transmission of human immunodeficiency virus type 1 dual infections caused by viruses of subtypes B and C.** *J Infect Dis* 1998, **177**:227–231.
66. Jameel S, Zafrullah M, Ahmad M, Kapoor GS, Sehgal SA. **Genetic analysis of HIV-1 from Punjab, India reveals the presence of multiple variants.** *AIDS* 1995, **9**:685–690.
67. Lole K, Bollinger R, Paranjape R, *et al.* **Full-length human immunodeficiency virus type 1 genomes from subtype C infected seroconverters in India, with evidence of intersubtype recombination.** *J Virol* 1999, **73**:142–160.
68. Wasi C, Hering B, Ratkam S, *et al.* **Determination of HIV-1 subtypes in injecting drug users in Bangkok, Thailand, using peptide-binding enzyme immunoassay and heteroduplex mobility assay: evidence of increasing infection with HIV-1 subtype E.** *AIDS* 1995, **9**:843–849.
69. Kalish ML, Baldwin A, Raktham S, *et al.* **The evolving molecular epidemiology of HIV-1 envelope subtypes in injecting drug users in Bangkok, Thailand: implications for HIV vaccine trials.** *AIDS* 1995, **9**:851–857.
70. Kitayaporn D, Vanichseni S, Mastro T, *et al.* **Infection with HIV-1 subtypes B and E in injecting drug users screened for enrollment into a prospective cohort in Bangkok, Thailand.** *J Acquir Immune Defic Syndr Hum Retrovirol* 1998, **19**:289–295.
71. Chen YM, Lee CM, Lin RY, Chang HJ. **Molecular epidemiology and trends of HIV-1 subtypes in Taiwan.** *J Acquir Immune Defic Syndr Hum Retrovirol* 1998, **19**:393–402.
72. Peeters M, Liegeois F, Torimiro N, *et al.* **Characterization of a highly replicative intergroup M/O recombinant HIV-1 virus isolated from a Cameroonian patient.** *J Virol* 1999, **73**:7368–7375
73. Mauclere P, Damond F, Apetrei C, *et al.* **Synthetic peptide ELISAs for detection of and discrimination between group M and group O HIV type 1 infection.** *AIDS Res Hum Retroviruses* 1997, **13**:987–993.
74. Cheingsong-Popov R, Williamson C, Lister S, *et al.* **Usefulness of HIV-1 V3-loop serotyping in studying the HIV-1 epidemic in South Africa.** *AIDS* 1998, **12**:949–950.
75. Hoelscher M, Hanker S, Barin F, *et al.* **HIV-1 V3 serotyping in Tanzanian samples: possible reasons for mismatching with genetic subtyping.** *AIDS Res Hum Retroviruses* 1998, **14**:139–149.
76. Nkengasong J, Willems B, Janssens W, *et al.* **Lack of correlation between V3-loop peptide enzyme immunoassay serologic subtyping and genetic sequencing.** *AIDS* 1998, **12**:1405–1412.
77. Delwart E, Shpaer E, Louwagie J, *et al.* **Genetic relationships determined by a DNA heteroduplex mobility assay: analysis of HIV-1 *env* genes.** *Science* 1993, **262**:1257–1261.
78. Heyndrickx L, Janssens W, Zekeng L, *et al.* **Simplified strategy for detection of recombinant human immunodeficiency virus type 1 group M isolates by *gag/env* heteroduplex mobility assay.** *J Virol* 2000, **74**:363–370.
79. Ellenberger D, Pienazek D, Nkengasong J, *et al.* **Genetic analysis of human immunodeficiency virus in Abidjan, Ivory Coast, reveals the predominance of HIV type 1 subtype A and introduction of subtype G.** *AIDS Res Hum Retroviruses* 1999, **1**:3–9.
80. Subbarao S, Luo C, Limpakarnjanarat K, *et al.* **Evaluation of oligonucleotide probes for the determination of the two major HIV-1 *env* subtypes in Thailand.** *AIDS* 1996, **10**:350–351.
81. Peeters M, Liegeois F, Bibollet-Ruche F, *et al.* **Subtype-specific polymerase chain reaction for the identification of HIV-1 genetic subtypes circulating in Africa.** *AIDS* 1998, **12**:671–686.
82. Weiller GF. **Phylogenetic profiles: a graphical method for detecting genetic recombinations in homologous sequences.** *Mol Biol Evol* 1998, **15**:326–335.
83. McGuire G, Wright F, Prentice MJ. **A graphical method for detecting recombination in phylogenetic data sets.** *Mol Biol Evol* 1997, **14**:1125–1131.
84. Grassly NC, Holmes EC. **A likelihood method for the detection of selection and recombination using nucleotide sequences.** *Mol Biol Evol* 1997, **14**:239–247.
85. Jakobsen IB, Easteal S. **A program for calculating and displaying compatibility matrices as an aid in determining reticulate evolution in molecular sequences.** *CABIOS* 1996, **12**:291–295.
86. Maynard Smith J. **Analysing the mosaic structure of genes.** *J Mol Evol* 1992, **34**:126–129.
87. Siepel AC, Halpern AL, Macken C, Korber BT. **A computer program designed to screen rapidly for HIV type 1 intersubtype recombinant sequences.** *AIDS Res Hum Retroviruses* 1995, **11**:1413–1416.
88. Salminen MO, Carr JK, Burke DS, McCutchan FE. **Identification of breakpoints in intergenotypic recombinants of HIV type 1 by bootscanning.** *AIDS Res Hum Retroviruses* 1995, **11**:1423–1425.
89. Robertson DL, Hahn BH, Sharp PM. **Recombination in AIDS viruses.** *J Mol Evol* 1995, **40**:249–259.
90. Peeters M, Esu-Williams E, Vergne L, *et al.* **Predominance of subtype A and G HIV type 1 in Nigeria, with geographical differences in their distribution.** *AIDS Res Hum Retroviruses* 2000, **16**:315–325.
91. McCutchan FE, Carr JK, Bajani M, *et al.* **Subtype G and multiple forms of A/G intersubtype recombinant human immunodeficiency virus type 1 in Nigeria.** *Virology* 1999, **254**:226–234.
92. Loussert-Ajaka I, Ly T, Chaix M, *et al.* **HIV-1/HIV-2 seronegativity in HIV-1 subtype O infected patients.** *Lancet* 1994, **343**:1393–1394.
93. Schable C, Zekeng L, Pau C, *et al.* **Sensitivity of United States HIV antibody tests for detection of HIV-1 group O infections.** *Lancet* 1994, **344**:1333–1334.
94. Apetrei C, Loussert-Ajaka I, Descamps D, *et al.* **Lack of screening test sensitivity during HIV-1 non-subtype B seroconversions.** *AIDS* 1996, **10**:F57–F60.
95. Parekh B, Philipps S, Granade T, Baggs J, Hu D, Respess R. **Impact of HIV type 1 subtype variation on viral RNA quantitation.** *AIDS Res Hum Retroviruses* 1999, **15**:133–142.
96. Descamps D, Collin G, Letourneur F, *et al.* **Susceptibility of human immunodeficiency virus type 1 group O isolates to antiretroviral agents: in vitro phenotypic and genotypic analyses.** *J Virol* 1997, **71**:8893–8898.
97. Quinones-Mateu M, Albright J, Mas A, Soriano V, Arts E. **Analysis of pol heterogeneity, viral quasispecies, and drug resistance in individuals infected with group O strains of human immunodeficiency virus type 1.** *J Virol* 1998, **72**:9002–9015.
98. Apetrei C, Descamps D, Collin G, *et al.* **Human immunodeficiency virus type 1 subtype F reverse transcriptase sequence and drug susceptibility.** *J Virol* 1998, **72**:3534–3538.
99. Descamps D, Apetrei C, Collin G, Damond F, Simon F, Brun-Vezinet F. **Naturally occurring decreased susceptibility of HIV-1 subtype G to protease inhibitors.** *AIDS* 1998, **12**:1109–11011.
100. Tanuri A, Vicente A, Otsuki K, *et al.* **Genetic variation and susceptibilities to protease inhibitors among subtype B and F isolates in Brazil.** *Antimicrob Agents Chemother* 1999, **43**:253–258.
101. Pieniazek D, Rayfield M, Ramos A, *et al.* **Molecular diversity of HIV-1 group M protease gene sequences worldwide: evidence for naturally occurring drug-resistant mutations in drug-naive individuals.** *Sixth Conference on Retroviruses and Opportunistic Infections.* Chicago, January–February 1999 [abstract 278].
102. Vergne L, Peeters M, Reynes J, *et al.* **Molecular diversity of HIV-1 group M protease and RT gene sequences in Africa: no evidence for major drug resistant mutations in drug naive individuals.** *Seventh European Conference on Clinical Aspects and Treatment of HIV infection.* Lisbon, October 1999 [abstract 385].
103. Shafer RW, Chuang TK, Hsu P, White CD, Katzenstein DA. **Sequence and drug susceptibility of subtype C protease from human immunodeficiency virus type 1 seroconverters in Zimbabwe.** *AIDS Res Hum Retroviruses* 1999, **15**:65–69.
104. Kanki P, Travers K, Mboup S, *et al.* **Slower heterosexual spread of HIV-2 than HIV-1.** *Lancet* 1994, **343**:943–946.
105. De Cock K, Adjorlo G, Ekpini E, *et al.* **Epidemiology and transmission of HIV-2. Why there is no HIV-2 pandemic.** *JAMA* 1993, **270**:2083–2086.
106. Marlink R, Kanki P, Thior I, *et al.* **Reduced rates of disease**

devolpment after HIV-2 infection as compared to HIV-1. *Science* 1994, **265**:1587–1590.

107. Kanki P, Hamel D, Sankale JL, *et al.* **Human immunodeficiency virus type 1 subtypes differ in disease progression.** *J Infect Diseases* 1999, **179**:68–73.
108. Tscherning C, Alaeus A, Frederiksson R, *et al.* **Differences in chemokines coreceptor usage between genetic subtypes of HIV-1.** *Virology* 1998, **241**:181–188.
109. Bjorndal A, Sonnerborg A, Tscherning C, Albert J, Fenyo E. **Phenotypic characteristics of human immunodeficiency virus type 1 subtype C isolates of Ethiopian AIDS patients.** *AIDS Res Hum Retroviruses* 1999, **15**:647–653.
110. Peeters M, Vincent R, Perret JL, *et al.* **Evidence for differences in MT2 cell tropism according to genetic subtypes of HIV-1: the switch from non-syncytium to syncytium inducing variants seems rare in subtype C HIV-1 viruses.** *J Acquir Immune Defic Syndr Hum Retrovirol* 1999, **20**:115–121.
111. Weisman Z, Kalinkovich A, Borkow G, Stein M, Greenberg Z, Bentwich Z. **Infection by different HIV-1 subtypes (B and C) results in a similar immune activatin profile despite distinct immune backgrounds.** *J Acquir Immune Defic Syndr* 1999, **21**:157–163.
112. Aleus A, Lidman K, Bjorkman A, Giesecke J, Albert J. **Similar rate of disease progression among individuals infected with HIV-1 genetic subtypes A–D.** *AIDS* 1999, **13**:901–907.
113. Neilson J, John G, Carr J, *et al.* **Subtypes of human immunodeficiency virus type 1 and disease stage among women in Nairobi, Kenya.** *J Virol* 1999, **73**:4393–4403.
114. Nelson K, Rungruengthanakit K, Margolick J, *et al.* **High rates of transmission of subtype E human immunodeficeincy virus type 1 among heterosexual couples in Northern Thailand: role of sexually transmitted diseases and immune compromise.** *J Infect Dis* 1999, **180**:337–343.
115. Amornkul PN, Tansuphasawadikul S, Limpakarnjanarat K, *et al.* **Clinical disease associated with HIV-1 subtype B and E infection among 2104 patients in Thailand.** *AIDS* 1999, **13**:1963–1969.
116. Mascola J, Louwagie J, McCutchan F, *et al.* **Two antigenically distinct subtypes of human immunodeficiency virus type 1: viral genotype predicts neutralization serotype.** *J Infect Dis* 1993, **169**:48–54.
117. Van der Groen G, Nyambi P, Beirnaert E, *et al.* **Genetic variation of HIV type 1: relevance of interclade variation to vaccine development.** *AIDS Res Hum Retroviruses* 1998, **14**:S211–S221.
118. Dorrel L, Dong D, Ogg G, *et al.* **Distinct recognition of non clade B human immunodeficiency virus type 1 epitopes by cytotoxic T lymphocytes generated from donors infected in Africa.** *J Virol* 1999, **73**:1708–1714.
119. Cao H, Kanki P, Sankale JL, *et al.* **Cytotoxic T-lymphocyte cross-reactivity among different human immunodeficiency virus type 1 clades: implications for vaccine development.** *J Virol* 1997, **71**:8615–8623.
120. Bertoletti A, Cham F, McAdam S, *et al.* **Cytotoxic T cells from human immunodeficiency virus type 2-infected patients frequently cross-react with different human immunodeficiency virus type 1 clades.** *J Virol* 1998, **72**:2439–2448.
121. Quinones-Mateu M, Arts EJ. **Recombination in HIV-1: update and implications.** *AIDS Rev* 1999, **1**:89–100.
122. Otten RA, Ellenberger DL, Adams DR, *et al.* **Identification of a window period for susceptibility to dual infection with two distinct Human Immunodeficiency virus type 2 isolates in a Macaca nemestrina (Pig-tailed Macaque) model.** *J Infect Dis* 1999, **180**:673–684.
123. Fultz PN, Yue L, Wei Q, Girard M. **Human immunodeficiency virus type 1 intersubtype (B/E) recombination in a superinfected chimpanzee.** *J Virol* 1997, **71**:7990–7995.
124. Takehisha J, Zekeng L, Ido J, *et al.* **Human immunodeficiency virus type 1 intergroup (M/O) recombination in Cameroon.** *J Virol* 1999, **73**:6810–6820.
125. Moutouh L, Corbeil J, Richman D. **Recombination leads to the rapid emergence of HIV-1 dually resistant mutants under selective drug pressure.** *Proc Natl Acad Sci USA* 1996, **93**:6106–6111.
126. Golovkina T, Jaffe A, Ross S. **Coexpression of exogenous and endogenous mouse mammary tumor viruses RNA in vivo results in viral recombination and broadens the virus host range.** *J Virol* 1994, **68**:5019–5026.
127. Tumas K, Poszgay J, Avidan N, *et al.* **Loss of antigenic epitopes as the result of *env* gene recombination in retrovirus-induced leukemia in immunocompetent mice.** *Virology* 1993, **192**:587–595.

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