Molecular Epidemiology of HIV-1 Variants in the Global Aids Pandemic: an Update

Michael M. Thomson and Rafael Nájera
Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda (Madrid), Spain

Abstract

The picture of HIV-1 genetic diversity in the global pandemic continues to evolve. Identification of new variants, including circulating and unique recombinant forms, recognition of new outbreaks and of changes in established epidemics, and characterization of growing numbers of full-length genomes provide a view of high dynamism and increasing complexity. The pervasive role of recombination as a major driving force in the generation of diversity in the HIV-1 pandemic is becoming evident, and is particularly visible in areas in which different genetic forms meet, referred to as "geographic recombination hotspots". The importance of superinfection and its impact on HIV-1 diversification and propagation is surfacing, although restrictions to superinfection are also apparent. Genetic diversity within subtypes is increasing over time and new geographically localized lineages deriving from point introductions are being recognized. Characterization of such variants may be of relevance to vaccine development and may allow the detection of intrasubtype recombination and superinfection. Recent studies supporting the correlation of HIV-1 clades to immune responses and to drug resistance-associated mutations lend increasing relevance to the role of molecular epidemiology as an essential tool in combating the AIDS pandemic. However, knowledge on the global HIV-1 genetic diversity and its implications is still far from adequate and a major scaling up of efforts is needed. **(AIDS Reviews 2005;7:210-24)**

Key words

HIV subtypes. Molecular epidemiology. Recombinant viruses. Genetic diversity.

Introduction

Molecular epidemiology refers to the use of molecular tools for epidemiologic studies. When applied to the HIV-1 pandemic, it commonly refers to studies on the propagation and distribution of HIV-1 molecular forms in different geographic areas (Fig. 1) and groups with different epidemiologic features, such as risk practices, including the analysis of temporal trends in transmission[1,2]. Such studies have been of prime importance for the recognition of recombination as a major mechanism of HIV-1 evolution in the pandemic. More recently, molecular epidemiology has also been applied to studies on prevalences of HIV-1 isolates resistant to antiretroviral drugs. Various molecular tools have been used for studies on the epidemiology of HIV-1 genetic variants, but phylogenetic sequence analysis is the most reliable method and the one providing more information on global and regional patterns of propagation of HIV-1 infection. The application of phylogenetic methods to epidemiologic studies on HIV-1 and other RNA viruses is possible thanks to their rapid evolution, allowing accurate reconstruction of transmission events even within closely related clusters[3]. In the initial studies on molecular epidemiology of HIV-1 clades, *env* and *gag* segments were the regions of the genome most commonly analyzed, although more recently, sequencing of *pol* segments has become more common, mainly due to its usefulness for detecting drug resistance-associated

Correspondence to:
Michael M. Thomson
Centro Nacional de Microbiología
Instituto de Salud Carlos III
28220 Majadahonda
Madrid, Spain
E-mail: mthomson@isciii.es

Figure 1. *Global geographic distribution of HIV-1 genetic forms. Letters and numbers represent subtypes and CRF, respectively. Those with larger sizes represent predominant genetic forms in each area. Genetic forms circulating in particular countries are shown followed by the countries' names in parentheses.*

mutations. Given the extraordinarily high recombination potential of HIV-1, characterization of full-length genomes, of which more than 1000 are currently available in public sequence databases[4], may provide much epidemiologically relevant phylogenetic information that could be missed in the analyses of short genome segments. In all these studies, appropriate sampling is of the utmost importance in order to examine sufficiently representative numbers of samples of each geographic area and groups with different risk behaviors, including recently acquired infections.

A review of the literature reveals the growing proportion of studies related to HIV-1 genetic variants. The relevance of HIV-1 molecular epidemiology is reinforced by recently published studies supporting the correlation of HIV-1 genetic forms to susceptibility to immune responses, including neutralizing antibodies[5] and cell-mediated immunity[6,7], and to development of drug-resistance mutations[8], with obvious implications for the development of effective vaccines able to cope with the global HIV-1 diversity, and for the introduction of antiretroviral therapies in developing nations. Recent studies have also provided new data on the role of recombination in the generation of HIV-1 diversity, diversification within subtypes, frequency of superinfection, new outbreaks, and changes within established epidemics.

Newly identified recombinant forms

HIV-1 recombinant forms can be classified from an epidemiologic perspective as unique recombinant forms (URF), detected in a single individual or a single epidemiologically linked cluster, which constitute substantial proportions of infections in areas with heterogeneous circulating clades, and circulating recombinant forms (CRF), which have propagated to some epidemiologic extent. Identification of a new CRF requires characterization of at least three epidemiologically unlinked viruses with identical mosaic structures, at least two of them characterized in near full-length genomes (> 8 kb)[9]. Twenty CRF have been reported to date. CRF that have spread widely are:

- CRF01_AE, of Central African origin, which is the predominant genetic form in Southeast Asia;
- CRF02_AG, prevalent in West and parts of West-Central Africa;
- CRF07_BC and CRF08_BC, the major variants circulating among injecting drug users (IDU) in China;
- CRF12_BF which circulates widely in Argentina and Uruguay, and, with a lower prevalence, in Chile (unpublished data).

The list of HIV-1 CRF is growing incessantly, both as a consequence of the emergence of new recombinants in areas where different clades co-circulate and of the new characterization of old, previously unrecognized

Table 1. Geographic variants of HIV-1 subtypes and CRF

Subtype/CRF	Country/area of introduction of variant	Countries/areas of further propagation	Names given	CRF derived from variants
A1	Ukraine	FSU*	IDU-A, FSU-A	03_AB
	West Africa	West Africa	A3	
	East Africa	East Africa		
B	Thailand	SE Asia, China, India	B',Thai B	07_BC 08_BC 15_01B
	Ukraine	Russia	IDU-B	03_AB
	Korea			
	Trinidad and Tobago			
	Cuba			
C	South Africa	South, SE Asia	C'	
	India	Myanmar, China, Nepal	C_{IN}	07_BC 08_BC
	Brazil	Argentina, Uruguay		
	Ethiopia		C", Eth2220 cluster	
	Cuba			
D	West-Central Africa	South Africa		05_DF
	East Africa			10_CD
F1	Brazil			12_BF
	Romania			
G	Portugal	Spain	G^P, G_{ES}	14_BG
	Cuba			20_BG
	Nigeria			
01_AE	Central African Republic			
	Thailand	SE Asia, China, Finland		15_01B
02_AG	Uzbekistan			
06_cpx	Estonia			

*FSU: countries of the Former Soviet Union, including Russia, Belarus, Moldova, Latvia, Estonia, Kazakhstan, and Uzbekistan.

CRF. Six have been added to the list in the last two years: CRF09_cpx, CRF16_A2D, CRF17_BF, CRF18_cpx, CRF19_cpx, and CRF20_BG.

CRF09_cpx is a complex recombinant with segments of subtypes A, F, G, K and one or more unclassified subtypes[10,11]. It is related in part to CRF02_AG and to Z321, a URF from the Democratic Republic of Congo (DRC) that was isolated in 1976. CRF09_cpx is circulating as a minor variant in West and Central Africa, and has been identified in Senegal, Ghana, Ivory Coast, and Cameroon[10-13]. Recombinants between CRF09_cpx and CRF02_AG or CRF06_cpx have been detected in the last three countries[11,13,14].

CRF16_A2D has been identified only in four persons, one in Kenya, one in Korea, and two in Argentina[15].

CRF17_BF has been detected in seven individuals from several South American countries (Argentina, Paraguay, Bolivia, and Peru)[16]. Sequences are still unavailable and its recombinant structure is unpublished.

The last three CRF have been identified in Cuba, a country with a low HIV-1 prevalence, but with an unusually high HIV-1 genetic diversity, derived from the introduction of multiple African variants[17,18] (Fig. 2). CRF18_cpx and CRF19_cpx were originally identified in partial *pol* and *env* sequences (7 and 21%, respectively) of individuals from Cuba[17]. CRF18_cpx is a complex AFGHKU intersubtype recombinant which also circulates in Cameroon and probably in other central African countries (DRC, Republic of Congo, Gabon, and Angola)[19]. CRF19_cpx is an ADG intersubtype recombinant[17,20]. Viruses representative of its probable parental strains, an AG recombinant from Cameroon and a subtype D virus from Gabon, have been identified[20]. CRF20_BG and phylogenetically related BG recombinants, including two other, still unnamed, CRF, have recently expanded in Cuba, mostly among men who have sex with men (MSM) in Havana City[18,21,22]. They derive from subtype B and G strains circulating in Cuba. Infections with these BG recombinants have increased in recent years, representing 31% of HIV-1 diagnoses in 2003 among MSM in Havana City[18].

A fact that is becoming apparent with the analysis of several CRF is that some of them are part of larger families of related recombinant forms, including other CRF and URF. Thus, BF recombinants of Argentina and other South American countries (Uruguay, Bolivia, Chile, and Venezuela – but not Brazil), including CRF12_BF and a high diversity of URF, appear to derive from a common recombinant ancestor, an idea

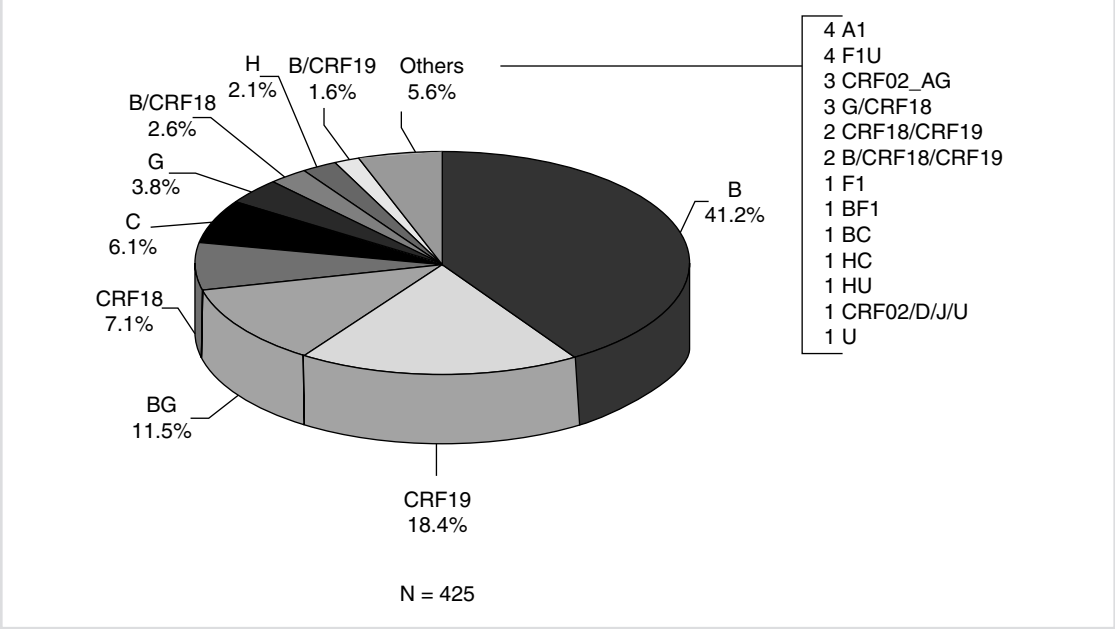

Figure 2. *Distribution of genetic forms in Cuba in samples collected in 2003, based on* pol *sequences*[18].

inferred from coincidence in multiple recombination breakpoints and clustering in phylogenetic trees of partial sequences[23,24]. Some of these recombinants appear to be secondary recombinants of CRF12_BF, but others, with simpler structures or separate phylogenetic clustering, seem to derive from lineages which are ancestral to CRF12_BF[23]. Similarly, the three BG intersubtype CRF and one URF from Cuba (characterized by us in full-length genomes) have multiple coincident breakpoints and cluster in trees of segments of subtypes B and G, suggesting a common ancestry[22]. BC recombinants circulating in China (CRF07_BC and CRF08_BC) are also partially homologous in their mosaic structures and derive from common parental strains[25-27], and URF related to them have been identified in Myanmar and China[28-30], some of them apparently secondary recombinants, but others probably derived from an ancestor of the CRF. CRF05_DF might also be part of a larger family of closely related DF recombinants of Central Africa[31]. Related recombinants might derive from recombination between the parental strains in a single dually or multiply infected individual, in which different recombinants can predominate along the course of the infection[32] and be transmitted to different individuals, or they may be generated by successive rounds of recombination in different individuals within a transmission chain. CRF thus would be the result of a selection process from a larger pool of diverse related recombinants, one or more of which become circulating thanks to their biologic properties and to chance introductions in the appropriate transmission networks.

Geographic variants within subtypes and CRF

All HIV-1 subtypes (except probably B) have originated in Central Africa, and derive from point introductions and expansion of Central African viruses in the incipient pandemic, probably in the DRC[33]. Subtype B probably derives from the point introduction of a subtype D-related virus in Haiti or the USA. Dates of origin of subtypes B and C have been estimated by molecular clock analyses; for subtype B they range from the late 1950s to the early 1970s[34-38], and for subtype C from the 1950s to the 1960s[38,39].

The earliest subtype B infections were documented in homosexual men in the USA in 1978[40]. In the following years, subtype B propagated among homosexual men and IDU in Western Europe and in the Americas. The subtype B epidemics in Western Europe[41] and Latin America appear to derive from multiple introductions from the USA, but in some countries local variants of subtype B deriving from point introductions have

been reported. One of these, introduced in the late 1980s in Thailand[42], has spread to other countries in the area. Other subtype B variants of monophyletic origin with more limited spread have been reported in Ukraine and Russia[43,44], Trinidad and Tobago[45], Korea[46,47], and Cuba[17,18]. The Southeast Asian variant, usually named B' (but also Thai B) was introduced initially among IDU in Thailand in 1988[42,48]. It has spread to neighboring countries, mainly among IDU, including Myanmar (here also transmitted via heterosexual contact), China, Manipur state (northeast India), Malaysia, and Singapore[49]. A homogeneous B' subvariant of monophyletic origin has been identified in paid blood donors in China, infected in large numbers through unsafe collection procedures, mostly in the central provinces of Henan and Hubei[50,51]. Full-length genomes of viruses of this sublineage from Henan, as well as of other B' viruses from China, Thailand, and Myanmar, have been characterized[52-55]. B' is parental of CRF07 and CRF08_BC[26,27], which are circulating widely in China, and of CRF15_01B[56], a minor recombinant of Thailand.

The Ukrainian subtype B variant (named IDU-B)[57], was introduced among IDU in the seaport city of Nikolayev in 1994[44]. It has had a limited spread in Ukraine and Russia, but it has recombined with the subtype A variant circulating among IDU in the Former Soviet Union (FSU) to generate CRF03_AB[43], which caused an explosive epidemic among IDU in the Russian Baltic city of Kaliningrad and has been occasionally detected in several countries of the FSU.

Local subtype B variants of monophyletic origin have been reported in Trinidad and Tobago[45], where it causes a heterosexually transmitted epidemic, in Korea[46,47], transmitted mainly among homosexual men, and Cuba[17], where a local variant is responsible for about half of HIV-1 infections among homosexual men in Havana City. Full-length genome sequences of the Korean and Cuban variants have been obtained. The existence of a subtype B variant specific of IDU in Northwestern Europe has been claimed on the basis of several nucleotide polymorphisms which are absent from the homosexual population[58]. In Brazil, a local subtype B variant (B_{br}) has been identified on the basis of a characteristic Env V3 loop tetrapeptide[59]. In both cases, however, phylogenetic characterization of full-length genomes supporting the existence of such variants has not been published.

The epidemic among IDU in countries of the FSU is mainly caused by a subsubtype A1 variant (most commonly designated IDU-A, but also FSU-A)[57,60,61] derived from a single introduction, first detected in the seaport city of Odessa in southern Ukraine in 1995[44]. Since 1996 it has spread widely among IDU in a vast geographic area comprising most FSU countries of eastern Europe and central Asia, including Russia[57,60-62], Belarus[63], Moldova[64], the Baltic States of Latvia[65] and Estonia[66], and the central Asian republics of Kazakhstan[67] and Uzbekistan[68,69]. Heterosexual transmission of this variant has also been reported in Russia and Belarus[60,63]. In spite of its wide geographic dispersion and of the aging of the epidemic, it is still characterized by a surprisingly low genetic diversity[60]. A subvariant of IDU-A with characteristic polymorphisms in protease, including V77I mutation, is responsible for many of the more recent outbreaks in Russia, Belarus, Kazakhstan, and Uzbekistan[62,69,70]. As mentioned previously, IDU-A is, with IDU-B, parental of CRF03_AB[43].

Recently, the existence of a new A sub-subtype, designated A3, circulating in West Africa has been proposed[71]. A3 viruses were detected in Ivory Coast, Nigeria, Niger, Guinea-Bissau, Benin, and Equatorial Guinea[72]. In Dakar, Senegal, 9.4% of infections in female prostitutes were attributed to A3. The designation of this variant as sub-subtype was based on genetic distances with A1 and A2 viruses within the range defined for inter-sub-subtype distances. However, in phylogenetic trees of full-length genomes, A3 variants cluster with 100% bootstrap support with A1 references and interdigitate with the Eastern European A1 cluster and a large cluster comprising all other A1 references from East Africa (Kenya, Uganda, and Tanzania) (Fig. 3). This tree topology suggests that A3 viruses, rather than a new sub-subtype, may represent a West African variant of sub-subtype A1.

Subtype C variants of monophyletic origin have been reported in India, Ethiopia, and Brazil. The Indian subtype C variant (C_{IN})[73,74] has spread widely in that country mainly via heterosexual contact, but also parenterally among IDU in the northeastern state of Manipur[75,76]. Subtype C viruses of Indian origin have propagated in Myanmar[53,77,78], in Yunnan province of China[28,79], and in Nepal[80]. Viruses from Myanmar and China are closely related to each other[77], and may represent a subvariant within the C_{IN} strain, which is parental of two CRF (CRF07_BC and CRF08_BC)[26,27].

The existence of two subtype C Ethiopian variants, named C' and C", have been proposed by analysis of some partial genome segments[81,82], in which 20% of the viruses analyzed in *pol* and the *env* V3 region were found to be recombinant between both lineages. However, in analyses of full-length genomes, a single Ethiopian

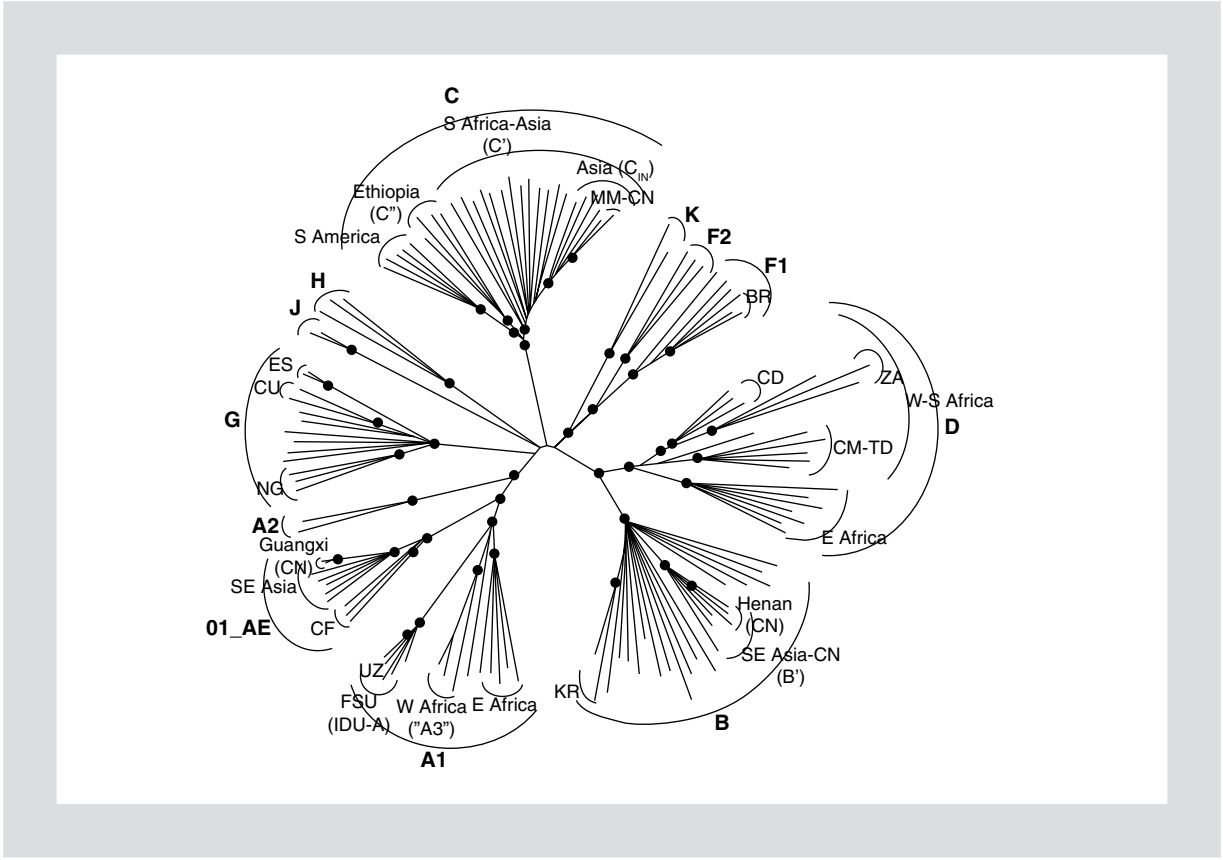

Figure 3. *Neighbor-joining tree of full-length genomes showing geographic clusters within HIV-1 genetic forms. Nodes signaled with a dot are supported by ≥ 90% bootstrap values. BR: Brazil; CD: Democratic Republic of Congo; CF: Central African Republic; CN: China; CM: Cameroon; CU: Cuba; ES: Spain; KR: Korea; MM: Myanmar; NG: Nigeria; TD: Chad; UZ: Uzbekistan; ZA: South Africa. FSU: countries of the Former Soviet Union.*

subcluster was found, which was not sustained in several subgenomic regions[83]. It is possible that extensive recombination between both variants has blurred the distinction between them, altering the tree structures of full genomes and of some subgenomic segments. In Cuba, a subtype C variant related to the Ethiopian cluster is circulating in a minority of homosexual men, of which we have recently characterized two full-length genomes (unpublished data).

In Brazil, a subtype C variant of monophyletic origin has rapidly expanded in recent years in the Southern states of the country, where prevalences of 30-45% of infections have been reported, reaching 58% in recent infections in the city of Rio Grande[84-88]; this variant is not associated with any particular risk factor. Sporadic cases of infection with the Brazilian C variant have been reported in Argentina, Uruguay, and Paraguay[15,89]. Coalescent methods, by which the genealogy and changes in population size and growth rates are inferred from randomly sampled sequences, indicate that the Brazilian subtype C variant has a single origin in the 1990s and that its growth rate is about twice that of subtype B or of African subtype C[90].

Subtype C viruses circulating in Southern Africa have been reported to derive from multiple introductions[90,91], although in another study[81] they formed a monophyletic cluster with viruses from India and of the Ethiopian C' variant in some subgenomic regions. The monophyletic origin of most viruses from South Africa and India is also supported in trees of full-length genomes, after excluding several Ethiopian and South African viruses (some of them probable intrasubtype recombinants) (Fig. 3). Some authors have applied the designation C', originally used for an Ethiopian strain, to all viruses of the South African/Indian cluster[92]. By use of coalescent methods, it has been inferred that subtype C in sub-Saharan Africa is growing exponentially, with a current doubling time of 2.4 years[38].

Two phylogenetic clusters are distinguished in subtype D: the East African cluster, circulating in Uganda and Kenya, and the West African cluster, comprising isolates from the DRC, Cameroon, Chad, and strains

from the early epidemic in South Africa[93-95]. The West African cluster is subdivided in two subclusters, one including most viruses from the DRC and those from South Africa and the other including viruses from Cameroon and Chad[95]. CRF05_DF (31) and CRF10_CD[96] derive from West and East African subtype D parental strains, respectively.

Two distinct sub-subtype F1 variants have been reported in Brazil and Romania, forming separate clusters in phylogenetic trees[97]. In Brazil, subtype F viruses represent 10-15% of HIV-1 infections. Extensive recombination with subtype B has generated frequent URF and has rendered pure subtype F uncommon in Brazil[98]. A BF recombinant of Brazilian origin is the ancestor of CRF12_BF and related recombinants[24], which are widely circulating in Argentina and have been sporadically detected in other countries of South America (except Brazil). A distinct subtype F variant is predominant in Romania both among adults and children (the latter being horizontally infected through contaminated injection equipment and blood transfusions)[99]. Greater distances between the isolates from adults indicate an initial introduction in this population[99]. Only analyses of partial sequences have been published, but we have recently obtained a near complete genome sequence of a Romanian F subtype virus (unpublished data), which is closely related to the MP411 isolate, collected from a French man but probably acquired in central Africa.

A subtype G variant of monophyletic origin was identified among IDU in Galicia (northwestern Spain)[100,101], although more recently it has been shown to be circulating more widely in Portugal, where it is transmitted both via heterosexual contact and among IDU[102,103]. In a recent survey, subtype G viruses, most of them of the local variant, represented 18% of infections in Portuguese subjects[102]. Among IDU in Lisbon, the proportion of infections with viruses of subtype G was 49.5% in *gag* and 24% in *env*, with 29 of 30 G^{env} viruses belonging to the local strain[103]. A mean interisolate distance of 11% in *env* suggests that subtype G was introduced in Portugal more than a decade ago[103]. Phylogenetic analyses show a close relationship of the Portuguese/Galician strain with viruses from Cameroon and Gabon[104]. This variant is parental of CRF14_BG[100] and of diverse URF detected in Portugal and Galicia[100,105]. In Cuba, a local subtype G variant, characterized in full-length genomes, is found in 5% of individuals, mainly transmitted heterosexually[17]. It is parental of CRF20_BG and of two other related BG CRF, which have spread among homosexual men in Havana City[21,22,100]. Subtype G variants in Nigeria have also been characterized in partial sequences[106], forming three clusters in *env* and two in *gag*. Characterization of full-length genomes has not been published, but four of five available subtype G sequences from Nigeria group in a monophyletic cluster (Fig. 3).

Among CRF01_AE isolates, two main clusters are distinguished, corresponding to the Central African and Southeast Asian variants[107,108]. In Southeast Asia, CRF01_AE was introduced among female prostitutes and their male patrons in Thailand in 1989[42,48,109]. Since the mid-1990s it has replaced subtype B' (introduced one year earlier) as the main genetic form transmitted among IDU[110]. CRF01_AE has propagated widely to all countries in the region, including Myanmar, Cambodia, Laos, Vietnam, China, Malaysia, Singapore, Indonesia, Philippines, and Taiwan[49]. It is transmitted mainly via heterosexual contact, but in some countries (Thailand, Myanmar, Vietnam, China, and Malaysia) it also spreads among IDU. In China, it is circulating mainly among IDU in the southeastern province of Guangxi, close to the Vietnamese border. Viruses from Guangxi and northern Vietnam form a highly homogeneous subvariant which has been characterized in full-length genomes[26,111,112]. CRF01_AE is parental of CRF15_01B, detected in 1.7% of samples in Thailand, both among IDU and heterosexuals[56]. In Malaysia, 22% of newly diagnosed infections were CRF01/B' recombinants, mainly found among IDU, but also among homosexuals and heterosexuals; 19 shared common breakpoints in protease-reverse transcriptase, and might represent a new CRF[113]. A limited outbreak of a CRF01_AE strain of Southeast Asian origin has been reported among IDU in Finland[114].

Variants of CRF02_AG and CRF06_cpx of monophyletic origin have caused recent outbreaks among IDU in Uzbekistan[69] and Estonia[115], respectively, where they have generated URF by recombination with IDU-A viruses. A CRF11_cpx variant has been reported to circulate among a minority of IDU in Switzerland[116], although full-length genomes are not available. In Cuba, a variant of CRF18_cpx, of Central African origin, is transmitted both via heterosexual and homosexual contact, and has recombined locally with CRF19_cpx, and subtypes B and G[17-19,104].

In summary, several subtypes and CRF have diversified through point introductions in different geographic areas, generating local variants and subvariants sharing a common ancestry, many of which have been characterized in full-length genomes. Some of these variants have spread extensively, such as IDU-A in

countries from the FSU, C' in South Africa , and a subvariant, C_{IN}, in India, Myanmar and China, variants of subtypes A and D in East Africa, B' and CRF01_AE in Southeast Asia and China, and, in a more localized scale, subtypes C and F in Brazil, and subtype G in Portugal and Spain. Some of these variants have recombined with subtype B strains circulating locally, generating diverse recombinant forms, including CRF: CRF12_BF, circulating in Argentina and Uruguay, CRF14_BG in Portugal and Spain, CRF03_AB in countries of the FSU, CRF07_BC and CRF08_BC in China, CRF15_01B in Thailand, and CRF20_BG and related CRF in Cuba, all of which (except the Cuban BG recombinants) appear to have originated among IDU. The recognition of these variants may be relevant to the characterization of intrasubtype or intra-CRF recombinants and superinfections in areas in which two or more variants are circulating, and may be also relevant for studies on virus biology, susceptibility to immune responses, response and development of resistance to antiretroviral drugs, pathogenicity, and transmissibility. It is possible that there are intrasubtype differences on these points, as has been reported between subtypes. It must be considered that the age of subtypes is currently similar or even greater than that of HIV-1 group M when subtypes originated, which would be reflected in intrasubtype distances which are similar or larger, and increasing over time, than intersubtype distances at the birth of subtypes. Therefore, it may be necessary to adopt uniform definition criteria and a nomenclature system, and to establish reference isolates for these variants, similarly to subtypes, sub-subtypes, and CRF.

Geographic recombination hotspots

In areas in which different HIV-1 genetic forms co-circulate in the same population, high frequencies of unique mosaic viruses derived from recombination between the co-circulating clades are usually detected, a fact which attests to the high frequency of dual or multiple infections with different variants. These areas have been referred to as "geographic recombination hotspots", a denomination first applied to Central Myanmar[53], but which is similarly applicable to many other areas. This doesn't necessarily imply that HIV-1 recombination is common only in those areas. It may also be frequent in areas in which a single clade circulates or is highly predominant, although intraclade recombinants may remain undetected by usual phylogenetic analyses.

Table 2. Geographic recombination hotspots

Country	Subtypes/CRF involved in recombination
Central Africa	
DRC	A1,A2,C,D,F1,G,H,J,K,01,02,U
Cameroon	A,B,D,F2,G,H,J,01,02,09,11, 13,18,U,O
Gabon	A1,C,D,G,H,02,U
R. of Congo	A1,D,F1,G,H,J,U
Angola	A1,A2,B,C,G,H,U,05
West Africa	
Ghana	02,06,09,A1,D,G
Ivory Coast	02,06,09
Niger	02,06
Nigeria	A1,G
East Africa	
Kenya	A1,C,D
Tanzania	A1,C,D
Uganda	A1,C,D
Ethiopia	C',C"
Europe	
Portugal	B,G,14
Estonia	06,A1
Southeast Asia	
Myanmar	B,C,01
Yunnan (China)	B,C,07,08
Thailand	B,01
Manipur (India)	B,C
South America	
Brazil	B,F1,C
Argentina	B,12
Caribbean	
Cuba	B,G,18,19

High prevalences of URF have been reported in central Africa (DRC, Cameroon, Gabon, Republic of Congo, and Angola), West Africa (Ivory Coast, Ghana, Niger, and Nigeria), East Africa (Uganda, Kenya, Tanzania, and Ethiopia), Asia (central Myanmar, Thailand, Yunnan province of China, and Manipur state of India), Europe (Portugal and Estonia), and the Americas (Argentina, Brazil, and Cuba) (Table 2). Some of these geographic recombination hotspots have been identified recently. In Ghana, 64 (25.7%) of 249 viruses analyzed in *gag, pol,* and *env* were URF, the majority having CRF02_AG as parental, with other recombining clades being subtypes A, D, and G and CRF06_cpx[117]. In another study in Ghana, 24% were URF in protease-reverse transcriptase, involving recombination between CRF 02, 06, and 09, and subtypes A, D, and G[13]. In Gabon, six of 41 (14.6%) samples analyzed in *pol* and *env* were URF[118]. In Angola, 37 and 17% prevalences of URF, respectively, involving multiple subtypes, were reported in two studies[119,120]. In Ethiopia, 20% of samples analyzed in *gag-pol* and the *env* V3 region were

intrasubtype recombinants between different variants circulating in the country[81]. Outside of Africa, most recombination hotspots are found in areas in which IDU represent a major proportion of infections (except Cuba, where most infections are transmitted sexually). In Portugal, 25% of viruses from IDU in Lisbon were BG recombinants, most of which did not belong to CRF14_BG, and therefore were potential URF[103]. In Estonia, 16% of samples, most of them from IDU, were CRF06/A URF[115]. In Northern Thailand, five of 38 seroconverting IDU harbored CRF01_AE/B URFs, which represented four of seven (57.1%) new infections in 2002[121]. In Manipur state in Northeastern India, B'C recombinants were detected in three of 14 and three of 28 samples, respectively, in two studies among IDU or their sexual partners[75,76]. It should be noted that in most studies only short genome segments have been analyzed, resulting in an underestimation of the real prevalence of URF.

Geographic recombination hotspots are the birthplace of most CRF, some of which have propagated widely outside of their place of origin. CRF represent only a few recombinants among the thousands generated in these areas, which have become selected for epidemic expansion due to their biologic properties and to chance introduction in appropriate transmission networks. Thus, CRF01_AE, CRF05_DF, and all the complex CRF (04, 06, 09, 11, 13, 18 and 19) have most likely originated in Central Africa; CRF02_AG probably originated in Central or West Africa, where subtypes A and G co-circulate; CRF10_AD in East Africa; CRF14_BG in Portugal; CRF07 and 08_BC in Myanmar or South China; CRF12_BF in Brazil; and CRF20_BG and two other related CRF in Cuba.

Changes in established epidemics

The persistent predominance of one or more HIV-1 variants in a geographic area has been attributed to a founder effect[122], whereby the first genetic form successfully introduced in a population gains an initial advantage over other genetic forms arriving later, and remains predominant due to similar transmission efficiencies and to restrictions to superinfection, particularly during the chronic phase of infection[123]. However, there are multiple examples, some of them reported recently, which show that the distribution of HIV-1 variants in an epidemic is not static but can evolve relatively rapidly, even within groups sharing a transmission route.

The case of IDU in Thailand has been known for several years: subtype B' was first introduced among IDU in 1988, but CRF01_AE, introduced initially among promiscuous heterosexuals one year later, has become the predominant genetic form transmitted in IDU since the mid-1990s[110]. Infection with CRF01_AE in IDU was not associated with sexual risk behaviors[124,125]. One study found higher transmission rates for CRF01_AE compared to subtype B among IDU in Bangkok, although it was difficult to differentiate the contributions of biologic from epidemiologic factors[126]. Similarly, in IDU of the Yunnan province in south China, a shift in predominance from B' (introduced since 1989) to C^{env} viruses was noticed in the mid-1990s[127]. More recently in Brazil, subtype C has expanded in the southern states of the country[85,87,88]. Coalescent analyses indicate faster expansion of the Brazilian subtype C variant, compared to subtype B or to the South African subtype C[90]. In Cuba, three BG intersubtype recombinant forms have expanded recently among homosexuals from Havana City[18,22]. No infections with these recombinants were detected in individuals diagnosed in 1998 or earlier, but they have increased gradually since 1999, reaching 31.4% of infections in 2003 diagnoses, with a concomitant decrease of subtype B from 88% in 1997 to 40% in 2003[18] (Fig. 4). In Uzbekistan, only subtype A and CRF03_AB viruses were detected in samples collected in 1999-2000, but 9.2% of infections with a CRF02_AG variant of monophyletic origin were detected in 2002-2003[69]. In Kinshasa, DRC, subtype C infections among female prostitutes rose from 0% in 1997 to 18.9% in 2002[128].

In several countries of Western Europe, an increase in non-subtype B infections has been noticed in recent years, particularly among nonimmigrant individuals, although evidence for the local circulation of non-subtype B genetic forms is lacking, except for subtype G and CRF14_BG in Portugal and Northwestern Spain[100-103] and CRF11_cpx among IDU in West Switzerland[116]. In Spain, 7.6% of seroconversions from 1997 through 2004 were with non-subtype B viruses, all recorded in the last three years[129]. In a recent study in clinics of sexually-transmitted infections of England and Wales, 71% of infections among heterosexuals were with non-subtype B viruses, with proportions increasing in newly diagnosed infections[130]. In France, non-subtype B infections increased from 10.6 to 19% in acute infections between 1996-1998 and 1999-2000. Among chronically infected individuals of European descent, non-subtype B infections increased from 5.9 to 14% in this period[131]. The majority of these individuals did not report a partner from sub-Saharan Africa. Similar trends have been observed in Italy[132] and Belgium[133].

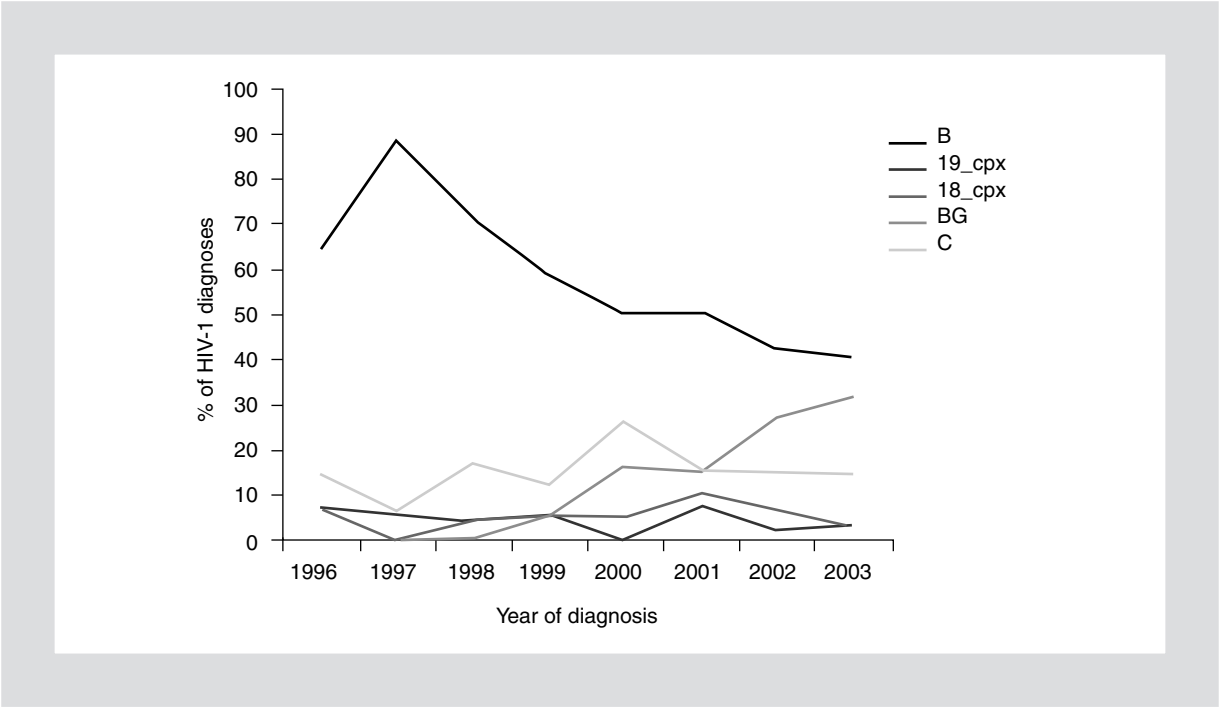

Figure 4. *Changes in relative prevalences of main genetic forms of Cuba among homosexual men from Havana City according to year of diagnosis[18].*

Rapid changes in the distribution of HIV-1 genetic forms in established epidemics, either by the introduction of new strains, by the expansion of minor variants, or by the local generation and expansion of new recombinant forms, support the need for continued surveillance to monitor the genetic composition of the epidemic and the variants transmitted at each moment, which may have implications for vaccine development, response to antiretroviral therapies, or validity of tests used in clinical practice.

The role of superinfection in the HIV-1 pandemic

Superinfections can lead to the generation of new recombinant forms, or to the replacement of the initially infecting strain with the reinfecting virus as the predominant form in an individual. In some areas they may contribute to the high frequencies of URF, some of which exhibit complex mosaic structures apparently derived from multiple successive rounds of recombination[134], suggesting that dual infections, including superinfection, are relatively common in those populations. Reports of superinfection have multiplied since the first cases were published in 2002[32,135-139]. However, several studies also point to the existence of restrictions to superinfection, particularly during chronic infection[140-143]. Of the 27 reported cases of superinfection, 18 were intersubtype, one intergroup, and only eight intrasubtype. These figures may not necessarily reflect their relative frequencies, since they may be biased by the use of methods detecting only intersubtype superinfection. In at least three of the reported cases, superinfection has resulted in recombination between the initial and the secondary strains, two of them intersubtype and one intrasubtype[32,144,145]. The majority of superinfection have occurred in the early stages of infection: only two of seven intrasubtype and six of 18 intersubtype superinfection were detected more than one year after detection of the initial infection. Of the two intrasubtype superinfections detected after one year post-seroconversion, one occurred in a patient treated with antiretroviral drugs during the acute infection[146]. Several population-based studies have reported the rarity of superinfection during chronic infection. In the USA, where only subtype B is circulating, no superinfections were detected during 1072 person-years of observation in one study[140], or among IDU after 215 person-years of follow-up in another one[141]. In the latter study, the relative risk of superinfection was significantly lower than initial infection in the study population. In a population of IDU in Switzerland among whom subtype B and CRF11_cpx are circulating, only two cases of superinfection after 346 person-years of

follow-up were detected[116]. No case of superinfection was detected among 14 treated seroconcordant couples harboring divergent HIV-1 strains followed up during one to four years[142], nor among nine individuals with established untreated infection with high-risk exposure (four transfused with HIV-1-infected blood and five IDU consistently sharing injection equipment with other IDU)[143]. In studies of early infection, results are discordant: some suggest that superinfection is relatively common[139,147] and others that it is rare[136,148,149]. Discrepant results may derive from multiple factors that may affect the frequency and detection of superinfection: whether a single clade or multiple clades circulate in the study population, type and frequency of risk exposure, frequency of antiretroviral drug treatment, duration of follow up, and sensitivity of the method in detecting minor strains. We have found that detection of dual infection may also depend on the clinical material used for study: one case of dual infection with two CRF (CRF12_BF and CRF14_BG), a very probable superinfection with each CRF acquired in Argentina and Spain, respectively, was readily detected by direct sequencing, clonal sequencing, and by clade-specific PCR in DNA from PBMC, but not in plasma RNA where only CRF12_BF was detected[150].

The reported rarity of superinfection during chronic HIV-1 infection in humans is concordant with studies in nonhuman primates, reporting a window period after initial infection beyond which superinfection would be restricted[151,152]. This may reflect the time required for the immune responses to mature[153]. If this is so, it would suggest that immune mechanisms could protect against superinfection, which may offer hope for an effective vaccine against HIV-1, although results from one published case appear to indicate that even strong $CD8^+$ T-cell-mediated responses against the initial strain may not be sufficient for protection against reinfection[146]. Also of potential relevance for vaccine development would be to determine whether the degree of genetic relatedness between viruses correlates to protection from superinfection. Studies aimed at examining such hypothesis may be feasible in areas in which different subtypes or CRF and variants within them co-circulate in the same population (e.g. Cuba[17,18]).

Concluding remarks

Recent studies supporting correlations of HIV-1 genetic clades with susceptibility to neutralization by antibodies[5] and to cell-mediated immune responses[6,7], and with development of specific drug resistance-associated mutations[8] reinforce the role of molecular epidemiology as an essential tool for guiding interventions aimed at the control of the HIV-1 pandemic. However, more studies are needed, in which main variants within subtypes and CRF should be included. The high frequency and complexity of URF detected in many areas indicate that recombination is a major mechanism for the generation of HIV-1 diversity in the pandemic. Many of the recombination events could result from superinfections, which are more common than previously thought, although there also appear to exist restrictions to their occurrence, particularly during chronic infection. They constitute natural experiments which may allow examining the correlation between HIV-1 genetic variants and protective immune responses.

In spite of rapidly accumulating data on global HIV-1 diversity, much more remains to be known, and a major scaling-up of efforts is still needed, especially in developing countries with high HIV-1 prevalences. In particular, it is important to carry out studies with epidemiologically representative sampling of the population, including the collection of relevant epidemiologic data. In 1996, Hu, et al.[154] proposed to establish "worldwide surveillance networks to monitor the molecular epidemiology of HIV by systematically collecting isolates of representative HIV strains from different populations with various transmission risk factors, to help understand the degree of genetic diversity within subtypes and what subtypes predominate in these populations" and added that "efforts should be made to sample on a continual basis […] sampling should also attempt to capture recent infections so as to reflect the strains currently transmitted". These recommendations are still far from being fulfilled. At the time this was written, the importance of recombination and superinfection for generation of diversity in the pandemic was still not fully recognized. Today, we should add to the aforementioned proposal the need for characterization of full-length genomes of representative samples, particularly in areas in which multiple genetic forms are circulating, and for carrying out well-designed population-based studies on superinfection, including examination of its frequency, timing during infection, and correlation with immune responses and genetic clades.

References

1. Thomson M, Pérez-Álvarez L, Nájera R. Molecular epidemiology of HIV-1 genetic forms and its significance for vaccine development and therapy. Lancet Infect Dis 2002;2:461-71.

2. Nájera R, Delgado E, Pérez-Álvarez L, Thomson MM. Genetic recombination and its role in the development of the HIV-1 pandemic. AIDS 2002;16 (Suppl 4):S3-16.
3. Leitner T, Escanilla D, Franzen C, Uhlen M, Albert J. Accurate reconstruction of a known HIV-1 transmission history by phylogenetic tree analysis. Proc Natl Acad Sci USA 1996;93:10864-9.
4. HIV Sequence Database. Theoretical Biology and Biophysics Group. Los Alamos National Laboratory.Los Alamos, NM. 2005.
5. Binley J, Wrin T, Korber B, et al. Comprehensive cross-clade neutralization analysis of a panel of anti-HIV-1 monoclonal antibodies. J Virol 2004;78:13232-52.
6. Coplan P, Gupta S, Dubey S, et al. Cross-reactivity of anti-HIV-1 T-cell immune responses among the major HIV-1 clades in HIV-1-positive individuals from 4 continents. J Infect Dis 2005; 191:1427-34.
7. McKinnon L, Ball T, Kimani J, et al. Cross-clade CD8+ T-cell responses with a preference for the predominant circulating clade. J Acquir Immune Defic Syndr 2005;40:245-9.
8. Wainberg M. HIV-1 subtype distribution and the problem of drug resistance. AIDS 2004;18 (Suppl 3):S63-S68.
9. Robertson D, Anderson J, Bradac J, et al. HIV-1 nomenclature proposal. Science 2000;288:55-6.
10. McCutchan F, Sankale J, M'Boup S, et al. HIV-1 circulating recombinant form CRF09_cpx from west Africa combines subtypes A, F, G, and may share ancestors with CRF02_AG and Z321. AIDS Res Hum Retroviruses 2004;20:819-26.
11. Toni T, Adje-Toure C, Vidal N, et al. Presence of CRF09_cpx and complex CRF02_AG/CRF09_cpx recombinant HIV-1 strains in Cote d'Ivoire, West Africa. AIDS Res Hum Retroviruses 2005;21:667-72.
12. Burda S, Konings F, Williams C, Anyangwe C, Nyambi P. HIV-1 CRF09_cpx circulates in the North West Province of Cameroon where CRF02_AG infections predominate and recombinant strains are common. AIDS Res Hum Retroviruses 2004;20:1358-63.
13. Delgado E, Ampofo W, Torpey K, et al. High prevalence of HIV-1 unique recombinant forms in Ghana involving CRF02_AG, CRF06_cpx and CRF09_cpx. 3rd IAS Conference on HIV Pathogenesis and Treatment. Abstract MoPe14.1B06. Rio de Janeiro, Brazil. July 2005.
14. Ndembi N, Takehisa J, Zekeng L, et al. Genetic diversity of HIV-1 in rural eastern Cameroon. J Acquir Immune Defic Syndr 2004;37:1641-50.
15. Gómez-Carrillo M, Quarleri J, Rubio AE, et al. Drug resistance testing provides evidence of the globalization of HIV-1: a new circulating recombinant form. AIDS Res Hum Retroviruses 2004;20:885-8.
16. Carr J, Montano S, Laguna-Torres V, et al. BF intersubtype recombinants are concentrated in the Southern Cone of South America. XV International AIDS Conference. Abstract ThOrC1365. Bangkok, Thailand. July 2004.
17. Cuevas M, Ruibal I, Villahermosa M, et al. High HIV-1 genetic diversity in Cuba. AIDS 2002;16:1643-53.
18. Pérez L, Thomson M, Aragonés C, et al. HIV-1 molecular epidemiology in Cuba: high diversity of genetic forms, frequent recombination, and recent expansion of BG intersubtype recombinant viruses. 3rd IAS Conference on HIV Pathogenesis and Treatment. Rio de Janeiro, Brazil. July 2005.
19. Thomson M, Casado G, Posada D, Sierra M, Nájera R. Identification of a novel HIV-1 complex circulating recombinant form (CRF18_cpx) of Central African origin in Cuba. AIDS 2005;19:1155-63.
20. Casado G, Thomson M, Sierra M, Nájera R. Identification of a novel HIV-1 circulating ADG intersubtype recombinant form (CRF19_cpx) in Cuba. J Acquir Immune Defic Syndr 2005; 40:532-7.
21. Sierra M, Thomson M, Casado G, Nájera R. Near full-length genome characterization of phylogenetically related HIV-1 BG intersubtype recombinant viruses and their parental subtype B and G strains from Cuba. XV International AIDS Conference. Abstract MoPeC3433. Bangkok, Thailand. July 2004.
16. Sierra M, Pérez L, Thomson M, Delgado E, Casado G, Nájera R. Identification of diverse phylogenetically related HIV-1 BG intersubtype recombinant viruses in Cuba, including a novel circulating recombinant form (CRF20_BG). 12th Conference on Retroviruses and Opportunistic Infections. Abstract 359. Boston, USA. February 2005.
23. Sierra M, Thomson M, Ríos M, et al. The analysis of near full-length genome sequences of HIV-1 BF intersubtype recombinant viruses from Chile, Venezuela and Spain reveals their relationship to diverse lineages of recombinant viruses related to CRF12_BF. Infect Genet Evol 2005;5:209-17.
24. Thomson M, Delgado E, Herrero I, et al. Diversity of mosaic structures and common ancestry of HIV-1 BF intersubtype recombinant viruses from Argentina revealed by analysis of near full-length genome sequences. J Gen Virol 2002;83:107-19.
25. McClutchan F, Carr J, Murphy D, et al. Precise mapping of recombination breakpoints suggests a common parent of two BC recombinant HIV-1 strains circulating in China. AIDS Res Hum Retroviruses 2002;18:1135-40.
26. Piyasirisilp S, McCutchan F, Carr J, et al. A recent outbreak of HIV-1 infection in southern China was initiated by two highly homogeneous, geographically separated strains, circulating recombinant form AE and a novel BC recombinant. J Virol 2000;74:11286-95.
27. Su L, Graf M, Zhang Y, et al. Characterization of a virtually full-length HIV-1 genome of a prevalent intersubtype (C/B') recombinant strain in China. J Virol 2000;74:11367-76.
28. Yang R, Xia X, Kusagawa S, et al. On-going generation of multiple forms of HIV-1 intersubtype recombinants in the Yunnan Province of China. AIDS 2002;16:1401-7.
29. Takebe Y, Ma Y, Yang C, et al. Geographical hotspots of extensive intersubtype recombination in Asia: «melting pot» that generates diverse forms of HIV-1 unique recombinant forms. 2004. XV International AIDS Conference. Abstract ThOrA1364. Bangkok, Thailand. July 2004.
30. Yang R, Kusagawa S, Zhang C, et al. Identification and characterization of a new class of HIV-1 recombinants comprised of two circulating recombinant forms, CRF07_BC and CRF08_BC, in China. J Virol 2003;77:685-95.
31. Casado G, Thomson M, Delgado E, et al. Near full-length genome characterization of an HIV-1 CRF05_DF virus from Spain. AIDS Res Hum Retroviruses 2003;19:719-25.
32. McCutchan F, Hoelscher M, Tovanabutra S, et al. In-depth analysis of a heterosexually acquired HIV-1 superinfection: evolution, temporal fluctuation, and intercompartment dynamics from the seronegative window period through 30 months postinfection. J Virol 2005;79:11693-704.
33. Rambaut A, Robertson DL, Pybus O, Peeters M, Holmes E. HIV. Phylogeny and the origin of HIV-1. Nature 2001;410:1047-8.
34. Korber B, Muldoon M, Theiler J, et al. Timing the ancestor of the HIV-1 pandemic strains. Science 2000;288:1789-96.
35. Salemi M, Strimmer K, Hall W, et al. Dating the common ancestor of SIVcpz and HIV-1 group M and the origin of HIV-1 subtypes using a new method to uncover clock-like molecular evolution. FASEB J 2001;15:276-8.
36. De Oliveira T, Salemi M, Gordon M, et al. Mapping sites of positive selection and amino acid diversification in the HIV genome: an alternative approach to vaccine design? Genetics 2004;167:1047-58.
37. Robbins K, Lemey P, Pybus O, et al. U.S. HIV-1 epidemic: date of origin, population history, and characterization of early strains. J Virol 2003;77:6359-66.
38. Walker P, Pybus O, Rambaut A, Holmes E. Comparative population dynamics of HIV-1 subtypes B and C: subtype-specific differences in patterns of epidemic growth. Infect Genet Evol 2005;5:199-208.
39. Travers S, Clewley J, Glynn J, et al. Timing and reconstruction of the most recent common ancestor of the subtype C clade of HIV-1. J Virol 2004;78:10501-6.
40. Foley B, Pan H, Buchbinder S, Delwart E. Apparent founder effect during the early years of the San Francisco HIV type 1 epidemic (1978-1979). AIDS Res Hum Retroviruses 2000;16:1463-9.
41. Hue S, Pillay D, Clewley J, Pybus O. Genetic analysis reveals the complex structure of HIV-1 transmission within defined risk groups. Proc Natl Acad Sci USA 2005;102:4425-9.

42. Ou C, Takebe Y, Luo C, et al. Wide distribution of two subtypes of HIV-1 in Thailand. AIDS Res Hum Retroviruses 1992;8:1471-2.
43. Liitsola K, Holm K, Bobkov A, et al. An AB recombinant and its parental HIV type 1 strains in the area of the former Soviet Union: low requirements for sequence identity in recombination. UNAIDS Virus Isolation Network. AIDS Res Hum Retroviruses 2000;16:1047-53.
44. Nabatov A, Kravchenko O, Lyulchuk M, Shcherbinskaya A, Lukashov V. Simultaneous introduction of HIV type 1 subtype A and B viruses into injecting drug users in southern Ukraine at the beginning of the epidemic in the former Soviet Union. AIDS Res Hum Retroviruses 2002;18:891-5.
45. Cleghorn F, Jack N, Carr J, et al. A distinctive clade B HIV type 1 is heterosexually transmitted in Trinidad and Tobago. Proc Natl Acad Sci USA 2000;97:10532-7.
46. Kang M, Cho Y, Chun J, et al. Phylogenetic analysis of the nef gene reveals a distinctive monophyletic clade in Korean HIV-1 cases. J Acquir Immune Defic Syndr Hum Retrovirol 1998;17:58-68.
47. Kim Y, Cho Y. Monophyletic clade of HIV-1 subtype B in Korea: evolutionary pressure or single introduction? AIDS Res Hum Retroviruses 2003;19:619-23.
48. Ou C, Takebe Y, Weniger B, et al. Independent introduction of two major HIV-1 genotypes into distinct high-risk populations in Thailand. Lancet 1993;341:1171-4.
49. Oelrichs R, Crowe S. The molecular epidemiology of HIV-1 in South and East Asia. Curr HIV Res 2003;1:239-48.
50. Su B, Liu L, Wang F, et al. HIV-1 subtype B' dictates the AIDS epidemic among paid blood donors in the Henan and Hubei provinces of China. AIDS 2003;17:2515-20.
51. Zhang L, Chen Z, Cao Y, et al. Molecular characterization of HIV-1 and HCV in paid blood donors and injection drug users in china. J Virol 2004;78:13591-9.
52. Graf M, Shao Y, Zhao Q, et al. Cloning and characterization of a virtually full-length HIV type 1 genome from a subtype B'-Thai strain representing the most prevalent B-clade isolate in China. AIDS Res Hum Retroviruses 1998;14:285-8.
53. Takebe Y, Motomura K, Tatsumi M, et al. High prevalence of diverse forms of HIV-1 intersubtype recombinants in Central Myanmar: geographical hot spot of extensive recombination. AIDS 2003;17:2077-87.
54. Brown B, Darden J, Tovanabutra S, et al. Biologic and genetic characterization of a panel of 60 HIV-1 isolates, representing clades A, B, C, D, CRF01_AE, and CRF02_AG, for the development and assessment of candidate vaccines. J Virol 2005;79:6089-101.
55. Liu L, Su B, Zhuang K, et al. Genetic characterization of full-length HIV type 1 genomes from 3 infected paid blood donors in Henan, China. J Acquir Immune Defic Syndr 2005;40:501-3.
56. Tovanabutra S, Watanaveeradej V, Viputtikul K, et al. A new circulating recombinant form, CRF15_01B, reinforces the linkage between IDU and heterosexual epidemics in Thailand. AIDS Res Hum Retroviruses 2003;19:561-7.
57. 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;14:669-76.
58. Kuiken C, Thakallapalli R, Esklid A, de Ronde A. Genetic analysis reveals epidemiologic patterns in the spread of HIV. Am J Epidemiol 2000;152:814-22.
59. Morgado M, Sabino E, Shpaer E, et al. V3 region polymorphisms in HIV-1 from Brazil: prevalence of subtype B strains divergent from North American/European prototype and detection of subtype F. AIDS Res Hum Retroviruses 1994;10:569-76.
60. Bobkov A, Kazennova E, Selimova L, et al. Temporal trends in the HIV-1 epidemic in Russia: predominance of subtype A. J Med Virol 2004;74:191-6.
61. Bobkov A, Cheingsong-Popov R, Selimova L, et al. An HIV-1 epidemic among injecting drug users in the former Soviet Union caused by a homogeneous subtype A strain. AIDS Res Hum Retroviruses 1997;13:1195-201.
62. Vázquez de Parga, Rakhmanova A, Pérez-Álvarez L, et al. Analysis of drug resistance-associated mutations in treatment-naive individuals infected with different genetic forms of HIV-1 circulating in countries of the former Soviet Union. J Med Virol 2005;77:337-44.
63. Lazouskaya N, Eremin V, Adema K, et al. The HIV type 1 epidemic in Belarus: predominance of Eastern European subtype A strains and circulation of subtype B viruses. AIDS Res Hum Retroviruses 2005;21:830-3.
64. Pandrea I, Descamps D, Collin G, et al. HIV type 1 genetic diversity and genotypic drug susceptibility in the Republic of Moldova. AIDS Res Hum Retroviruses 2001;17:1297-304.
65. Balode D, Ferdats A, Dievberna I, et al. Rapid epidemic spread of HIV type 1 subtype A1 among intravenous drug users in Latvia and slower spread of subtype B among other risk groups. AIDS Res Hum Retroviruses 2004;20:245-9.
66. Ustina V, Zilmer K, Tammai L, et al. Epidemiology of HIV in Estonia. AIDS Res Hum Retroviruses 2001;17:81-5.
67. Bobkov A, Kazennova E, Sukhanova A, et al. An HIV-1 subtype A outbreak among injecting drug users in Kazakhstan. AIDS Res Hum Retroviruses 2004;20:1134-6.
68. Kurbanov F, Kondo M, Tanaka Y, et al. HIV in Uzbekistan: epidemiological and genetic analyses. AIDS Res Hum Retroviruses 2003;19:731-8.
69. Carr J, Nadai Y, Eyzaguirre L, et al. Outbreak of a West African recombinant of HIV-1 in Tashkent, Uzbekistan. J Acquir Immune Defic Syndr 2005;39:570-5.
70. Roudinskii N, Sukhanova A, Kazennova E, et al. Diversity of HIV-1 subtype A and CRF03_AB protease in Eastern Europe: selection of the V77I variant and its rapid spread in injecting drug user populations. J Virol 2004;78:11276-87.
71. Meloni S, Kim B, Sankale J, et al. Distinct HIV-1 subtype A virus circulating in West Africa: sub-subtype A3. J Virol 2004;78:12438-45.
72. Meloni S, Sankale J, Hamel D, et al. Molecular epidemiology of HIV-1 sub-subtype A3 in Senegal from 1988 to 2001. J Virol 2004;78:12455-61.
73. Lole K, Bollinger R, Paranjape R, et al. Full-length HIV-1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. J Virol 1999;73:152-60.
74. Shankarappa R, Chatterjee R, Learn G, et al. HIV-1 env sequences from Calcutta in eastern India: identification of features that distinguish subtype C sequences in India from other subtype C sequences. J Virol 2001;75:10479-87.
75. Bhanja P, Sengupta S, Singh N, et al. Determination of Gag and Env subtypes of HIV-1 detected among injecting drug users (IDUs) in Manipur, India: Evidence for intersubtype recombination. Virus Res 2005;114:149-53.
76. Tripathy S, Kulkarni S, Jadhav S, et al. Subtype B and subtype C HIV-1 recombinants in the northeastern state of Manipur, India. AIDS Res Hum Retroviruses 2005;21:152-7.
77. Motomura K, Kusagawa S, Kato K, et al. Emergence of new forms of HIV-1 intersubtype recombinants in central Myanmar. AIDS Res Hum Retroviruses 2000;16:1831-43.
78. Motomura K, Kusagawa S, Lwin H, et al. Different subtype distributions in two cities in Myanmar: evidence for independent clusters of HIV-1 transmission. AIDS 2003;17:633-6.
79. Yu X, Wang X, Mao P, et al. Characterization of HIV-1 heterosexual transmission in Yunnan, China. AIDS Res Hum Retroviruses 2003;19:1051-5.
80. Oelrichs R, Shrestha I, Anderson D, Deacon N. The explosive HIV-1 epidemic among injecting drug users of Katmandu, Nepal, is caused by a subtype C virus of restricted genetic diversity. J Virol 2000;74:1149-57.
81. Pollakis G, Abebe A, Kliphuis A, et al. Recombination of HIV type 1C (C'/C») in Ethiopia: possible link of EthHIV-1C' to subtype C sequences from the high-prevalence epidemics in India and southern Africa. AIDS Res Hum Retroviruses 2003;19:999-1008.
82. De Baar M, Abebe A, Kliphuis A, et al. HIV-1 C and C' subclusters based on long terminal repeat sequences in the Ethiopian type 1 subtype C epidemic. AIDS Res Hum Retroviruses 2003;19:917-22.
83. Harris M, Maayan S, Kim B, et al. A cluster of HIV-1 subtype C sequences from Ethiopia, observed in full genome analysis, is not

sustained in subgenomic regions. AIDS Res Hum Retroviruses 2003;19:1125-33.
84. Guimaraes M, Moreira A, Morgado M. Polymorphism of HIV-1 in Brazil: genetic characterization of the nef gene and implications for vaccine design. Mem Inst Oswaldo Cruz 2002;97:523-6.
85. Soares E, Santos R, Pellegrini J, et al. Epidemiologic and molecular characterization of HIV-1 in southern Brazil. J Acquir Immune Defic Syndr 2003;34:520-2.
86. Brindeiro R, Diaz R, Sabino E, et al. Brazilian Network for HIV Drug Resistance Surveillance (HIV-BResNet): a survey of chronically infected individuals. AIDS 2003;17:1063-9.
87. Soares M, de Oliveira T, Brindeiro R, et al. A specific subtype C of HIV-1 circulates in Brazil. AIDS 2003;17:11-21.
88. Soares E, Martinez A, Souza T, et al. HIV-1 subtype C dissemination in southern Brazil. AIDS 2005;19 (Suppl 4):S81-S86.
89. Carrión G, Eyzaguirre L, Montano S, et al. Documentation of subtype C HIV-1 strains in Argentina, Paraguay, and Uruguay. AIDS Res Hum Retroviruses 2004;20:1022-5.
90. Salemi M, de Oliveira T, Soares M, et al. Different epidemic potentials of the HIV-1B and C subtypes. J Mol Evol 2005;60:598-605.
91. Gordon M, de Oliveira T, Bishop K, et al. Molecular characteristics of HIV-1 subtype C viruses from KwaZulu-Natal, South Africa: implications for vaccine and antiretroviral control strategies. J Virol 2003;77:2587-99.
92. Iversen A, Learn G, Skinhoj P, et al. Preferential detection of HIV subtype C' over subtype A in cervical cells from a dually infected woman. AIDS 2005;19:990-3.
93. Hierholzer M, Graham R, El Khidir I, et al. HIV-1 strains from East and West Africa are intermixed in Sudan. AIDS Res Hum Retroviruses 2002;18:1163-6.
94. Vidal N, Koyalta D, Richard V, et al. High genetic diversity of HIV-1 strains in Chad, West Central Africa. J Acquir Immune Defic Syndr 2003;33:239-46.
95. Loxton A, Treurnicht F, Laten A, van Rensburg EJ, Engelbrecht S. Sequence analysis of near full-length HIV-1 subtype D primary strains isolated in Cape Town, South Africa, from 1984-1986. AIDS Res Hum Retroviruses 2005;21:410-3.
96. Koulinska I, Ndung'u T, Mwakagile D, et al. A new HIV-1 circulating recombinant form from Tanzania. AIDS Res Hum Retroviruses 2001;17:423-31.
97. Bandea C, Ramos A, Pieniazek D, et al. Epidemiologic and evolutionary relationships between Romanian and Brazilian HIV-subtype F strains. Emerg Infect Dis 1995;1:91-3.
98. Thomson M, Sierra M, Tanuri A, et al. Analysis of near full-length genome sequences of HIV-1 BF intersubtype recombinant viruses from Brazil reveals their independent origins and their lack of relationship to CRF12_BF. AIDS Res Hum Retroviruses 2004; 20:1126-33.
99. Op de Coul E, van den Burg R, Asjo B, et al. Genetic evidence of multiple transmissions of HIV-1 subtype F within Romania from adult blood donors to children. AIDS Res Hum Retroviruses 2000;16:327-36.
100. Delgado E, Thomson M, Villahermosa M, et al. Identification of a newly characterized HIV-1 BG intersubtype circulating recombinant form in Galicia, Spain, which exhibits a pseudotype-like virion structure. J Acquir Immune Defic Syndr 2002;29:536-43.
101. Thomson M, Delgado E, Manjón N, et al. HIV-1 genetic diversity in Galicia Spain: BG intersubtype recombinant viruses circulating among injecting drug users. AIDS 2001;15:509-16.
102. Esteves A, Parreira R, Venenno T, et al. Molecular epidemiology of HIV-1 infection in Portugal: high prevalence of non-B subtypes. AIDS Res Hum Retroviruses 2002;18:313-25.
103. Esteves A, Parreira R, Piedade J, et al. Spreading of HIV-1 subtype G and envB/gagG recombinant strains among injecting drug users in Lisbon, Portugal. AIDS Res Hum Retroviruses 2003;19:511-7.
104. Casado G, Delgado E, Sierra M, et al. HIV-1 subtype G viruses circulating in northwestern Spain and Portugal have a common ancestry and are related to viruses from Central Africa and Cuba. 3rd IAS Conference on HIV Pathogenesis and Treatment. Abstract MoPe14.1B03. Rio de Janeiro, Brazil. July 2005.
105. Delgado E, Casado G, Thomson M, et al. Near full-length genome characterization of HIV-1 CRF14-BG and unique BG recombinants from Galicia (Spain). 2nd Conference on HIV Pathogenesis and Treatment. Abstract 255. Paris, France. July 2003.
106. Peeters M, Esu-Williams E, Vergne L, et al. Predominance of subtype A and G HIV-1 in Nigeria, with geographic differences in their distribution. AIDS Res Hum Retroviruses 2000;16:315-25.
107. Murphy E, Korber B, Georges-Courbot M, et al. Diversity of V3 region sequences of HIV-1 from the Central African Republic. AIDS Res Hum Retroviruses 1993;9:997-1006.
108. Gao F, Robertson D, Morrison S, et al. The heterosexual HIV-1 epidemic in Thailand is caused by an intersubtype (A/E) recombinant of African origin. J Virol 1996;70:7013-29.
109. McCutchan F, Hegerich P, Brennan T, et al. Genetic variants of HIV-1 in Thailand. AIDS Res Hum Retroviruses 1992;8:1887-95.
110. Kalish M, 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;9:851-7.
111. Kato K, Shiino T, Kusagawa S, et al. Genetic similarity of HIV-1 subtype E in a recent outbreak among injecting drug users in northern Vietnam to strains in Guangxi Province of southern China. AIDS Res Hum Retroviruses 1999;15:1157-68.
112. Yu X, Chen J, Shao Y, et al. Emerging HIV infections with distinct subtypes of HIV-1 infection among injection drug users from geographically separate locations in Guangxi Province, China. J Acquir Immune Defic Syndr 1999;22:180-8.
113. Tee K, Pon C, Kamarulzaman A, Ng K. Emergence of HIV-1 CRF01_AE/B unique recombinant forms in Kuala Lumpur, Malaysia. AIDS 2005;19:119-26.
114. Liitsola K, Ristola M, Holmstrom P, et al. An outbreak of the circulating recombinant form AECM240 HIV-1 in the Finnish injection drug user population. AIDS 2000;14:2613-15.
115. Adojaan M, Kivisild T, Mannik A, et al. Predominance of a rare type of HIV-1 in Estonia. J Acquir Immune Defic Syndr 2005;39:598-605.
116. Yerly S, Jost S, Monnat M, et al. HIV-1 co/super-infection in intravenous drug users. AIDS 2004;18:1413-21.
117. Fischetti L, Opare-Sem O, Candotti D, et al. Molecular epidemiology of HIV in Ghana: dominance of CRF02_AG. J Med Virol 2004;73:158-166.
118. Pandrea I, Robertson D, Onanga R, et al. Analysis of partial pol and env sequences indicates a high prevalence of HIV-1 recombinant strains circulating in Gabon. AIDS Res Hum Retroviruses 2002;18:1103-16.
119. Bartolo I, Epalanga M, Bartolomeu J, et al. High genetic diversity of HIV-1 in Angola. AIDS Res Hum Retroviruses 2005;21:306-10.
120. Abecasis A, Paraskevis D, Epalanga M, et al. HIV-1 genetic variants circulation in the north of Angola. Infect Genet Evol 2005;5:231-7.
121. Tovanabutra S, Beyrer C, Sakkhachornphop S, et al. The changing molecular epidemiology of HIV-1 among northern Thai drug users, 1999 to 2002. AIDS Res Hum Retroviruses 2004;20:465-75.
122. Hu D, Buve A, Baggs J, van der G, Dondero T. What role does HIV-1 subtype play in transmission and pathogenesis? An epidemiologic perspective. AIDS 1999;13:873-81.
123. Gross K, Porco T, Grant R. HIV-1 superinfection and viral diversity. AIDS 2004;18:1513-20.
124. 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;19:289-95.
125. Vanichseni S, Kitayaporn D, Mastro T, et al. Continued high HIV-1 incidence in a vaccine trial preparatory cohort of injection drug users in Bangkok, Thailand. AIDS 2001;15:397-405.
126. Hudgens M, Longini I, Jr., Vanichseni S, et al. Subtype-specific transmission probabilities for HIV-1 among injecting drug users in Bangkok, Thailand. Am J Epidemiol 2002;155:159-68.
127. Luo C, Tian C, Hu D, et al. HIV-1 subtype C in China. Lancet 1995;345:1051-2.
128. Vidal N, Mulanga C, Bazepeo S, et al. Distribution of HIV-1 variants in the Democratic Republic of Congo suggests increase of subtype

C in Kinshasa between 1997 and 2002. J Acquir Immune Defic Syndr 2005;40:456-62.
129. De Mendoza C, Rodriguez C, Colomina J, et al. Resistance to NNRTI and prevalence of HIV-1 non-B subtypes are increasing among persons with recent infection in Spain. Clin Infect Dis 2005;41:1350-4.
130. Tatt I, Barlow K, Clewley J, Gill O, Parry J. Surveillance of HIV-1 subtypes among heterosexuals in England and Wales, 1997-2000. J Acquir Immune Defic Syndr 2004;36:1092-9.
131. Chaix M, Descamps D, Harzic M, et al. Stable prevalence of genotypic drug resistance mutations but increase in non-B virus among patients with primary HIV-1 infection in France. AIDS 2003;17:2635-43.
132. Monno L, Brindicci G, Lo C, et al. HIV-1 subtypes and circulating recombinant forms (CRFs) from HIV-infected patients residing in two regions of central and southern Italy. J Med Virol 2005;75:483-90.
133. Snoeck J, Van Laethem K, Hermans P, et al. Rising prevalence of HIV-1 non-B subtypes in Belgium: 1983-2001. J Acquir Immune Defic Syndr 2004;35:279-85.
134. Casado G, Thomson M, Delgado E, et al. Full genome characterization of HIV-1 unique and circulating recombinant viruses of African origin in Galicia (northwestern Spain). 2nd IAS Conference on HIV Pathogenesis and Treatment. Abstract 241. Paris, France. July 2003.
135. Van der Kuyl A, Kozaczynska K, van den B, et al. Triple HIV-1 infection. N Engl J Med 2005;352:2557-9.
136. Hu D, Subbarao S, Vanichseni S, et al. Frequency of HIV-1 dual subtype infections, including intersubtype superinfections, among injection drug users in Bangkok, Thailand. AIDS 2005; 19:303-8.
137. Smith D, Richman D, Little S. HIV superinfection. J Infect Dis 2005;192:438-44.
138. Plantier J, Lemee V, Dorval I, et al. HIV-1 group M superinfection in an HIV-1 group O-infected patient. AIDS 2004;18:2444-6.
139. Chohan B, Lavreys L, Rainwater S, Overbaugh J. Evidence for frequent reinfection with HIV-1 of a different subtype. J Virol 2005;79:10701-8.
140. Gonzales M, Delwart E, Rhee S, et al. Lack of detectable HIV-1 superinfection during 1072 person-years of observation. J Infect Dis 2003;188:397-405.
141. Tsui R, Herring B, Barbour J, et al. HIV-1 superinfection was not detected following 215 years of injection drug user exposure. J Virol 2004;78:94-103.
142. Chakraborty B, Valer L, de Mendoza C, Soriano V, Quinones-Mateu M. Failure to detect HIV-1 superinfection in 28 HIV-seroconcordant individuals with high risk of re-exposure to the virus. AIDS Res Hum Retroviruses 2004;20:1026-31.
143. Diaz R, Pardini R, Catroxo M, et al. HIV-1 superinfection is not a common event. J Clin Virol 2005;33:328-30.
144. Fang G, Weiser B, Kuiken C, et al. Recombination following superinfection by HIV-1. AIDS 2004;18:153-9.
145. Yang O, Daar E, Jamieson B, et al. HIV-1 clade B superinfection: evidence for differential immune containment of distinct clade B strains. J Virol 2005;79:860-8.
146. Altfeld M, Allen T, Yu X, et al. HIV-1 superinfection despite broad CD8+ T-cell responses containing replication of the primary virus. Nature 2002;420:434-9.
147. Smith D, Wong J, Hightower G, et al. Incidence of HIV superinfection following primary infection. JAMA 2004;292:1177-8.
148. Gottlieb G, Nickle D, Jensen M, et al. Dual HIV-1 infection associated with rapid disease progression. Lancet 2004;363:619-22.
149. Grobler J, Gray C, Rademeyer C, et al. Incidence of HIV-1 dual infection and its association with increased viral load set point in a cohort of HIV-1 subtype C-infected female sex workers. J Infect Dis 2004;190:1355-9.
150. Sierra M, Thomson M, Casado G, et al. Dual infection with two HIV-1 circulating recombinant forms (CRF12_BF and CRF14_BG) detected in peripheral blood mononuclear cells' DNA but not in plasma RNA. XV International AIDS Conference. Abstract MoPeC3435. Bangkok, Thailand. July 2004.
151. Otten R, Ellenberger D, Adams D, et al. Identification of a window period for susceptibility to dual infection with two distinct HIV type 2 isolates in a Macaca nemestrina (pig-tailed macaque) model. J Infect Dis 1999;180:673-84.
152. Cole K, Rowles J, Jagerski B, et al. Evolution of envelope-specific antibody responses in monkeys experimentally infected or immunized with SIV and its association with the development of protective immunity. J Virol 1997;71:5069-79.
153. Cole K, Rowles J, Jagerski B, et al. Evolution of envelope-specific antibody responses in monkeys experimentally infected or immunized with SIV and its association with the development of protective immunity. J Virol 1997;71:5069-79.
154. Hu D, Dondero T, Rayfield M, et al. The emerging genetic diversity of HIV. The importance of global surveillance for diagnostics, research, and prevention. JAMA 1996;275:210-6.