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Worldwide molecular epidemiology of HIV

Epidemiologia molecular do HIV no mundo

ABSTRACT

Human immunodeficiency virus (HIV) is the worldwide disseminated causative agent of acquired immunodeficiency syndrome (AIDS). HIV is a member of the *Lentivirus* genus of Retroviridae family and is grouped in two types named HIV-1 and HIV-2. These viruses have a notable ability to mutate and adapt to the new conditions of human environment. A large incidence of errors at the transcriptional level results in changes on the genetic bases during the reproductive cycle. The elevated genomic variability of HIV has carried important implications for the diagnosis, treatment and prevention as well as epidemiologic investigations. The present review describes important definitions and geographical distribution of subtypes, circulating recombinant forms and other genomic variations of HIV. The present study aimed at leading students of Biomedical Sciences and public health laboratory staff guidance to general and specific knowledge about the genomic variability of the HIV.

KEYWORDS: HIV, genetics. HIV infections, epidemiology. Acquired immunodeficiency syndrome, epidemiology. HIV subtypes. Circulating recombinant forms.

RESUMO

O vírus da imunodeficiência humana (HIV), disseminado em todo o mundo, é o agente responsável pela síndrome da imunodeficiência adquirida (Aids). O HIV é um membro do gênero *Lentivirus* da família Retroviridae e compreende os tipos HIV-1 e HIV-2. Esses vírus possuem notável capacidade de mutar e se adaptar às novas condições do ambiente humano. Uma grande incidência de erros ao nível transcricional do genoma resulta em alterações nas bases genéticas durante o ciclo reprodutivo. A elevada variabilidade genômica do HIV apresenta importantes implicações para o diagnóstico, tratamento e prevenção, bem como nas investigações epidemiológicas. A elaboração desta revisão traz importantes conceitos sobre definições e distribuição geográfica de subtipos, formas recombinantes circulantes e outras variações genômicas do HIV. O estudo pretendeu direcionar os estudantes de ciências biomédicas e os profissionais de laboratórios de saúde pública aos conhecimentos gerais e específicos acerca da variabilidade genômica do HIV.

DESCRITORES: HIV, genética. Infecções por HIV, epidemiologia. Síndrome de imunodeficiência adquirida, epidemiologia. Subtipos de HIV. Formas recombinantes circulantes.

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INTRODUCTION

The causative agent of acquired immunodeficiency syndrome (AIDS) is the worldwide disseminated human immunodeficiency virus (HIV). About 85% of the HIV isolates from humans are grouped into two types, HIV-1 and HIV-2. The worldwide main agent of AIDS is HIV-1, while the occurrence of HIV-2 is restricted to some regions of Western and Central Africa.

HIV and simian immunodeficiency virus (SIV) are genetically related members of *Lentivirus* genus of the Retroviridae family. Lentiviruses include complex exogenous viruses responsible for a variety of neurological and immunological diseases, but not directly implicated in any malignancies. Genomes of these viruses are characterized by structural genes *gag*, *pol*, *env,* and a complex combination of other additional genes. The retrovirus genome is unique among viruses in several aspects, including its physical organization, its mode of synthesis, and its functions in replication. The diploid virus genome is composed of two identical copies of single-strand ribonucleic acid (RNA) and is synthesized and processed by the host cell messenger RNA (mRNA) handling machinery.[43,80]

Distinct lentiviruses have been isolated from several nonhuman primate species including African green monkeys (SIV_{AGM}), sooty mangabeys (SIV_{SMM}), mandrills (SIV_{MND}), sykes (SIV_{SYK}), and chimpanzees (SIV_{CPZ}). Several SIV have also been isolated from Asian macaque species including SIV from Rhesus macaques (SIV_{MAC}), nemestrine macaques (SIV_{MNE}), and stump-tailed macaques (SIV_{STM}). In these primates, the SIVs cause endemic AIDS-like disease. Despite extensive genomic diversity, a unifying feature of human and nonhuman primate lentiviruses is that the cell receptor is the CD4 antigen, a differentiation marker on the surface of T-helper lymphocytes.[43,44,53,58,97,107]

At present, the origins of HIV-1 and HIV-2 infections remain an enigma, although some investigations have indicated that both viruses have risen from zoonotic transmissions between nonhuman primates and humans.[97] The analysis of genetic sequencing has revealed that the genomes of SIV_{SMM} and HIV-2 exhibit a high degree of homology although SIV_{CPZ} is most closely related to HIV-1 strain.[41,45,52,53,59] HIV-2 has a genetic structure very similar to HIV-1 but a nucleotide homology[46] of only 60%.

Several authors have admitted that humans are not the natural host of either HIV-1 or HIV-2. Instead, these viruses have entered the human population as a result of zoonotic or cross-species transmission. African primates represent an extremely large reservoir of lentiviruses with the potential for infecting other species, including humans, in their natural habitats. Probably, there was a certain moment in which two different SIV strains infected and replicated in a chimpanzee, originating thus the recombinant virus now named HIV. That proves their biological fitness by becoming major circulating forms of epidemiological significance, capable of infecting humans and causing AIDS epidemic. Many of the SIVs known to infect mainly chimpanzees exhibit biological properties that render them at least candidates for natural transmission to humans, such as the ability to replicate efficiently in primary human lymphocytes.[7,47,53]

When did HIV-1 enter humans, and when did the current phylogeny of HIV-1 genotypes arise have been extremely controversial questions. Studies using linear distance-time correlations estimate that HIV-1 first diverged from HIV-2 around 1930 and then diverged to its current phylogeny over the past 40 years. In the evolutionary origin of HIV and SIV it is estimated that the HIV-1/HIV-2 divergence node occurred in the early 1,800s and, the oldest node linking all human and simian viruses date back 600 to 1,200 years, while the HIV-1 strain causing AIDS were estimated to be 50 to 100 years old.[36,44,45,77-79,97]

Studying HIV genetic variation at the global level is needed, not only to learn the origins and understand the epidemiology of HIV-1, but also the emergence of subtypes, Circulating Recombinant Forms (CRFs), and intra-subtypes that may be more readily transmitted or have altered virulence, and to ensure that vaccine antigens are directed against strains of the virus that are currently circulating within specific populations.

GENOMIC VARIABILITY

HIV presents a remarkable ability to mutate and adapt to the new conditions of the human environment. A large incidence of errors at transcriptional level results in changes on the genetic bases during the reproductive cycle of HIV. Reverse transcriptase plays a major role in generation of diversity of retroviruses. Several error mechanisms have been ascribed to polymerases in general, and a high frequency of mutations occurs including genetic substitutions, deletions, recombinations, repetitions, and insertions. Each of these events may involve one or more nucleotides. By reason of these various error mechanisms, in addition to the recombinant events, reverse transcriptase plays a distinguished role for producing HIV sequence diversity in infected individuals.

The elevated genomic variability of HIV gives raise to important implications for laboratory diagnosis, treatment, prevention, and for epidemiological investigation as well.[80,109]

Replication of HIV, like all RNA viruses that lack enzymes for editing the freshly replicated nucleotides strands, is liable to error prone.[113] HIV-1 generates, on average, one error per 10^4 nucleotides, which is also the size of its genome. Potentially, each provirus is a new mutant strain, unique at least in one base site. Mutations accumulate over successive replication cycles, leading to a myriad of closely related but non-identical viruses in every infected individual. Blood and lymphoid tissue from a HIV-infected human adult individual contains 10^{11} CD4-positive lymphocytes, of which between 10^9 and 10^{10} can be showed to harbor viral DNA. On account of 10^7 HIV-infected patients worldwide, there may be as many as 10^{17} HIV genetically unique strain variants in circulation. This vast reservoir of genetic variants may increase the potential for successful adaptation of the HIV-1 strains.[64,74,79,92] Antiviral immune responses and other factors, such as cell tropism and cytopathicity, provide selective pressure for accumulation of viral variants, designated *quasi-species*, in the host. The *quasi-species* is the realization of the distribution of forms within the sequence space. The term *quasi-species* has come to be used more loosely in the HIV literature to simply refer to the set of viruses found in an infected individual. Under circumstances of selective pressure, such as therapy or immune pressure, the frequency of forms in the viral population can be shifted.[34,65,111,112]

SUBTYPES

Human immunodeficiency virus type-1 (HIV-1) is classified into three distinct groups: M (major), O (outlier) and N (non M/non O, new). Groups M, N and O viruses are members of primate *Lentivirus* lineage that includes also SIV_{CPZ} strains. In *Lentivirus* lineage, groups M and N, and SIV_{CPZ}, are approximately equidistant from each other, whereas group O is the most distantly related to the other strains.[41] HIV-1 group M has spread worldwide, causing the global AIDS pandemic. Group O infections are less common, and they have been endemic in West Central Africa mainly in Cameroon, Gabon, Nigeria, and Equatorial Guinea. This group has early been identified in Africa and spread in Europe and the United States, however, most of infections due to group O might be directly linked to persons who had had any connection to West Central Africa. Group O has been accounted for less than 10% of HIV-1 infections worldwide.[5,23,24,41,60,125,151-153]

The earliest case of HIV-1 group M infection was identified in human blood specimen collected in 1959 in Kinshasa, Democratic Republic of Congo[157,158] while the most ancient case of group O infection was found in a Norwegian patient that would be infected in the early 1960s.[60,138] Group N infections have been identified in West Central Africa, only in some people from Cameroon.[5,23,125]

Since the introduction into humans, group M has been described as a major phylogenetic entity of HIV-1. Group M is composed of 11 subtypes or clades named A through K, besides more than 15 CRFs which circulate among varying extents in populations around the globe. In relation to the group O, there is no analogous classification of subtypes, however it has been proposed a classification into five phylogenetic clusters designated from I to V. HIV-2 also presents variants that are classified into five distinct and equidistant subtypes named from A to E.[151,152]

Subtype designations have been powerful molecular epidemiological markers to track the course of the HIV-1 epidemic. The subtypes of group M are genotypic variants defined mainly in function of sequence variation in *env* gene, which encodes the glycoprotein gp120 in virion membrane. Regions of *pol*, *gag* and *vif* genes are also sequenced to characterize the subtypes.[88,89,138] The subtypes of group M are equidistant from each other in a radial phylogenetic distribution from a common ancestor.[117,151,152]

The next conditions are proposed for establishing the subtypes of a virus isolate: (a) at least two isolates should be sequenced in their entireties, (b) they should resemble each other but not to other existing subtypes throughout the genome, and (c) they should have to be found in at least three epidemiologically unlinked individuals.[117]

In order to make easier the vaccine design and evaluation, several programs for typing the HIV-1 isolates have been implemented mainly for countries suitable as sites for conducting the phase III vaccine efficacy trials.[35] For these proposals, the employed techniques include serotyping,[137] heteroduplex mobility assay (HMA),[14,30,31] RT-polymerase chain reaction (RT-PCR)-gag fingerprinting[50,73,85,118,145] and lately DNA microarray assays have been introduced for this purpose.[124]

The HIV subtyping has been an important molecular tool for monitoring the geographic changes in worldwide AIDS epidemic. Existence of significant differences in HIV subtypes-associated pathogenesis or transmissibility has still to be determined. The de-

gree to which the vaccines based on one subtype will elicit cross-protection against other subtypes is still poorly understood. However, there is well-established evidence that differences related to diagnostic assays performance and to antiretroviral drugs efficiency do exist among the several HIV-1 variants. Therefore, it remains important to track the HIV-1 molecular epidemiology and the genetic characterization of prevalent HIV-1 strains.[30,31]

According to genetic sequencing study of group M, the subtypes A to K have been regionally dispersed. The subtype A has been responsible for 80% of the HIV-infections in Western Africa, and for 30% in Eastern Africa.[54,89] In Eastern Europe the subtype A has been disseminating since 1995 in the countries from the Former Soviet Union, mainly Russia and Ukraine.[12,83,98]

HIV subtype B has been the main epidemic component in the Western Europe (60%), together with subtype A (11%), C (5%) and other subtypes (11%).[32,50,51,130,134] Subtype B is also predominant in Americas,[78,79,144] and in the Australian continent,[48] and also in some Asian countries as Korea,[102] India and Singapure.[122] In Japan,[66,146] where the subtype B has been predominant (74%), other subtypes such as C (3.5%), A (2.0%), F (1.0%) are also circulating together with subtype E (20%).

Subtype C represents 60% of HIV-infections worldwide, predominantly in East Africa and South Asia.[20,69,77,83] Subtype C has been reported in Malaysia and Southwest China, which may reflect the virus spreading via links with India where this subtype predominate.[20,25,110,136] In Tanzania (Africa)[3] subtype C shows 50% prevalence followed by subtypes A and B. Recent epicenter of HIV subtype C has been verified in Southern Africa involving Botswana, Zimbabwe, Malawi, Zambia, Namibia, South Africa and it has spread to India, Nepal and China. In Europe subtype C has also increased in Scotland since 2000, mainly due to transmission from individuals with evident exposure outside the United Kingdom such as African and Asian countries.[153]

Subtype D has been responsible for 5 to 40% in countries of East and Central Africa where it has been circulating together with subtype A.[74,109] Subtype E (renamed as CRF01_AE) has been common in Vietnam and neighbor countries, in the majority of infected intravenous drug users (IDUs).[22,71] Subtype E has been predominant in Thailand (>80%), with small proportion of subtype B, also in majority of IDUs.[22,129]

Subtype F has been the most common virus in Eastern Europe, mainly in Romenia,[104] and is also constituent part of Southern American subtypes.[114,116] Subtype I was first identified in Cyprus and Greece in the early 1990s and from there it has spread to the Mediterranean Region.[71,105]

The subtypes A, E, G, H, J and K have been described to be prevalent in Burkina Faso, Mali, Nigeria, Ivory Coast, Gabon, and Democratic Republic of Congo, from where they have spread to South Europe, and Asia.[6,16,55,89,106,122]

In South America, HIV-1 subtype B has been predominant in Brazil, followed by subtypes F, and C, with small proportion of subtype D.[13,15,28,30,39,94,114,116,127] In Argentina, Bolivia, Peru, Paraguay, Uruguay and Venezuela[21] and also in Caribbean Islands[140] these same subtypes have been found since the mid-1990s.

The main cells targeted by HIV *in vivo* are T-lymphocytes, macrophages and probably dendritic cells. This tropism is predominantly determined by the cell surface receptors required for HIV to attach to and gain entry into cells. Usually, entry to target cells requires both CD4 and one of the chemokine coreceptors. Chemokine CCR5 is the coreceptor predominantly used *in vivo*, however, variants that use another coreceptor CXCR4 evolve during disease in some AIDS patients.[26] Several reports indicate that HIV-1 subtypes present different immunobiological properties. Subtypes A and D differ in ratio for coreceptor usage. Most subtype A isolates use the CCR5 receptor tropism for attaching to T-cells. In contrast, most subtype D isolates use the CXCR4 coreceptor. However the dual CCR5/CXCR4 tropism has not been observed among them yet.[88]

Recent observation in Senegal showed that the time for disease progression in HIV-1 subtype A-infected has been slower than in non-A subtype-infected individuals.[62] In contrast, subtype A is associated with a higher risk for vertical transmission than subtype D.[115] Results obtained from Sub-Saharan Africa have suggested that HIV-1 subtypes A, C, D, and E are well adapted for heterosexual transmission while subtype B is less efficiently transmitted by this route. On the other hand, in North America, Western Europe, South Asia and India, subtype B is efficiently transmitted by intravenous drug users, and among homosexual individuals, in whom the infection by HIV-1 subtypes A, C, D and E does not occur.[1,49,59,68]

CIRCULATING RECOMBINANT FORMS

When two distinct HIV-1 strains are circulating, and these strains are initially introduced into different

social networks but these barriers eventually can collapse, the two virus strains become highly intermixed in an individual, establishing conditions for intersubtypes recombination.[4,117] If a individual is infected by two different HIV-1 subtypes, and if the resultant genetic combination is satisfactorily established in the environment, then this is designated CRF. Currently, about 20 CRFs have been described and numbered according to the order in which they have adequately described. As example, the designation CRF01_AB indicates the first occurrence of CRF in a person, a recombinant strain composed of two subtypes, A and B, which have been genetically recombined.[19,69,116]

On the basis of the newly proposed nomenclature, the formal requirement for assigning a new CRF is the existence of at least three epidemiologically independent complete genomic sequences that share the same recombinant structure, and form a monophyletic cluster in all regions of the genome. Or it should produce two full-length genome sequences plus partial sequences of a third strain that cluster with full-length genome sequences, and share identical breakpoints. Monophyletic groups (or clades) are defined as groups containing species which are more closely related to each other than to any outside of the group.[116,117]

CRF occurrence varies worldwide and sometimes differs according to the local or regional predominant subtypes. In a geographic region, the proportion of recombinant virus emergence depends on a series of factors, including (a) the prevalence index of the different virus subtypes, (b) the probability in which certain population groups acquire multiple infections, and the chance of these viruses being further transmitted, and (c) the occurrence of generation of genomic recombinations. Once these factors occur recombination can still be broken and then the frequency of pure subtype is likely to increase. Recombination may introduce genetic and biological consequences that are better than those resulting from firm accumulation of single mutations. From this, it is conceivable to presume what kind of impact the diversity caused by CRF structures may have over the development of highly efficacious HIV/AIDS vaccines. HIV-1 subtypes and CRF dissemination have been a dynamic and unpredictable process, and the geographical spreading of these ongoing virus variants has been unpreventable.[35,131,148]

The generation of intersubtype recombinant HIV-1 may occur in a setting of intermixing of subtypes and their recombinants in populations, with many cycles of co-infection and back-crossing before extrinsic factors such as geographic dispersal, or entry into a different social network, lead to "fixation" of particular recombinant forms.[117] In Kaliningrad, Russia, in mid-1990s, an epidemic among IDUs involved recombinant viruses that are mosaics of disseminated subtypes A and B from the Former Soviet Union. This virus, which was designated CRF03_AB, has spread all over East Europe. Subtype A and B strains from Ukrainian IDUs were showed to be the probable parental viruses of the Kaliningrad AB recombinant strain.[76,99]

A complex mosaic of alternating subtype A and subtype G sequences, with origin in Ibadan, Nigeria, was recognized as CRF02_AG. This virus emerged among several African countries and became epidemic in the African continent, predominantly in West and West Central Africa, where it represents between 50 to 70% of the circulating strains.[2,24,38,83,91,109,143] Thus, from Africa CRF02_AG they were introduced in Europe and its epidemic has lately been rapidly spreading in France,[137] Belgium,[126] Italy,[90] and United Kingdom.[134] African and European immigrants are responsible by a large panel of CRFs in United Kingdom, such as CRF01_AE, CRF14_BG, CRF03_AB, CRF05_DF, CRF06_cpx, and CRF11_cpx, together with CRF02_AG and several URFs.[2,43,82,143]

In China where circulates an Indian subtype C in addition to a Chinese subtype B variant (B_{Ch}), mainly among IDUs, the recombination of both viruses generated two new B/C recombinants, CRF07_BC and CRF08_BC. The CRF07_BC appears to contain two small subtype C segments interspersed with subtype B (a C/B/C configuration). CRF08_BC seem to have a B/C/B configuration with a long subtype C segment interrupted by small, closely spaced subtype B segments. Probably, in a certain time, a "parental" BC recombinant should have arise and widely dispersed. Then, different individuals with such a BC strain could have become co-infected with subtype C, generating recombinants of different but related structure in two separate backcrosses (the cross of F1 hybrid to one of the parental types). The shared breakpoints of CRF07_BC and CRF08_BC offsprings may be those that were retained from the "parental" BC recombinant. As the two CRFs became dispersed geographically, moving away from the main concentrations of both subtype C and subtype B, further backcrossing may have been curtailed, fixing these strains in the population.[85,132,145,155] In Thailand, the frequency of subtype B increased in early-1990s mainly among IDUs and, by the end of that decade, its decrease was observed just when the prevalence of subtype C began to increase and, at the same time, the emergence of Indian subtypes E and F was recog-

nized, thereof the new recombinants BC, BE and BF became predominant.[83]

In Portugal, there has been a potential spreading of multiple CRFs owing to the return of native Portuguese people from former Portuguese colonies in Africa, where they lived or worked. In this way, European HIV-1 subtype B may recombinate with African subtypes A, C, D, G, H, and J from Angola, Mozambique and Guinea Bissau.[37] In Spain, as occurred in Portugal, European subtype B might also be recombinated with African subtypes A and J brought from the former Spanish colony of Equatorial Guinea.[18, 54]

In Argentina, Uruguay, and Brazil the CRF12_BF became prevalent in heterosexual population, and in vertically infected children.[17,36,51,114,116,120,121] In Brazil, the distribution of HIV-1 subtypes assumes diversified patterns according to the geographic regions. In the Brazilian Southern and Southeastern States the rate of subtype B arise to 50%, and subtypes C and F represent 28% and 7%, respectively,[127] while in Northeastern region occurs a high prevalence of B (>80%) followed by a low frequency (<3%) of subtype F and BC recombinant.[39] Other recombinant forms such as B/C, C/B, B/F, F/D, and the triplet B/C/F are consistent with the three main circulating subtypes.[74,94,116,127]

The genetic arrangement caused by the subtype recombinations has made it difficult the understanding of CRF genomic composition, and then its ensuing definition. All representing subtype E strains initially described in Southeast Asia seem to be recombinant forms of subtypes A and E, and thus they have been designated CRF01_AE. Genetic sequencing of this strain is divergent from a subtype A in the *env* gene, parts of *vif*, *vpr*, and *nef* genes and the LTR. However, a full-length non-recombinant subtype E sequence has not been described yet, and the absence of one of the "parental" lineages makes it difficult to formally proving the recombinant nature of these viruses. By this sense, it has not been considered as subtype E for A-K classification. In spite of CRF01_AE being an incorrect name because the putative "parental" non-recombinant E strain has not been found, as the "E" designation for *env* region of these strains is very common used, renaming it would lead to confusion. Thus, the "E" designation will be retained for the viruses CRF01_AE.[2,117]

CRF01_AE viruses have been documented at low frequencies in several Central African countries, like Central African Republic, Cameroon and the Democratic Republic of Congo.[100,102,141] However they are responsible for the explosive epidemic in Southeast Asia, especially in Thailand from where these viruses have further spread to surrounding countries like Vietnam,[64,87] Cambodia,[88] Myanmar,[95] China,[146] and Taiwan.[25]

Genomes in mosaic forms, containing sequences originated from more than two subtypes have been named by replacing "cpx," denoting complex. Subtype I, from Greece and Cyprus[71,105] has not still been recognized as a full-length genome. This virus resembles a mosaic of subtypes A, G, H and K, and a putative new subtype I. Thus, subtype I was removed from the genetic classification of subtypes A-K and it is now called CRF04_cpx.[40,101,117] Due to the renaming of subtype E as CRF01_AE and the subtype I as CRF04_cpx, in contrast to 11 subtypes A-K, several authors have admitted only nine subtypes of HIV-1 in the group M, then designated A-D, F-H, J and K.[79,82,96,106,117]

Others important "cpx" include CRF-06_cpx from Burkina Faso and Mali that is composed of successive fragments of subtypes A, G, J and K. CRF09_cpx described in Senegal is composed of circulating subtypes A, C and D. CRF11_cpx comprising subtypes A, G, J, and fragments of CRF01_AE was observed in Cameroon and the Central African Republic. CRF13_cpx was recently also found in Cameroon and is a mosaic of genomic regions also characterized as subtypes A, G, J and CRF01_AE, with the subtype J substantially different from that one circulating in Democratic Republic of Congo.[91,146,147] Cuban CRF18_cpx and CRF19_cpx that exhibit multiple segments of African subtypes A, D, G and H, were recently described.[19]

Recombinations where virus are discordant in gene regions but they do not resemble any previously known CRFs are defined as Unique Recombinant Forms (URFs). Most URFs are detected in regions where multiple subtypes and CRFs co-circulate.[83,133] There are URFs involving more than two subtypes or also including CRF genomic fragments. The first CRF01_AE/B recombinant identified in Thailand was isolated from an individual with both heterosexual and IDU exposure and the full-length genome showed the strain to be mostly subtype B with the gp120 of envelope from CRF01_AE. It is likely that a similar process occurred to generate the recombinant CRF01_AE/B in Georgia, USA, composed also of HIV-1 subtype B and CRF_AE.[149] CRF01_AE/B has been disseminated also in Malaysia.[150] In Cameroon, where recombinant CRF02_AG accounts for 60% of prevalence, followed by subtype A with 13%, a new complex recombinant CRF02_AG/A is the newest variant originated.[142,149,152] A novel recombinant between Ukrainian subtype A and African CRF06_cpx origi-

nated in early 2000s the mosaic CRF06_cpx/A that is disseminating in Estonia.[156] In Switzerland, a new intermixed CRF11_cpx/B has also disseminated.[153] And in Cuba, a intermixed recombinant named CRF18_cpx/CRF19_cpx was recently recovered.[19]

Epidemiological studies have showed that HIV-1 subtypes and CRFs are segregated among people with different risk behaviors.[30,31] A multicenter survey in Taiwan from 1988 to 1998 showed that among heterosexual and homosexual men the subtype B infection corresponded to 52% and 78% respectively, and CRF01_AE infections, 44% and 21% respectively. In this same study, a comparison between subtype B and CRF01_AE infections in females and males showed significant differences, 68% in men versus 14% in women for the subtype B, and 30% in men versus 70% in women for the CRF01_AE. In regard to IDU AIDS-patients, the same authors found the proportion of subtype B and CRF01_AE equal to 67% and 33%, respectively.[24] This interesting Taiwanese study provides parameters to understand differences in the distribution of HIV-1 subtypes and recombinant forms according to regional epidemics and human behaviors.

Several authors have acknowledged that in the early 1980s when the HIV-1 epidemic started, pure subtypes were prevailing, while CRFs have increased in the 1990s. However, when HIV subtypes were initially genetically characterized in the early 1990s, the first identified viruses were assumed to represent pure subtypes. The viruses found afterward were then compared to these prototypic strains. Recent study including serum samples collected in the mid-1980s in Kinshasa (Democratic Republic of Congo) showed that substantial intersubtype recombination was already high in 1980s, when HIV-1 viruses were initially classified. Thus, at least some of the recombinant viruses mainly in Central Africa were likely classified as pure subtypes after being exported from Africa and establishing regional epidemics in other parts of the world.[62]

INTRA-SUBTYPES

The genomic region *env* gp120 of HIV-1 has a pattern structure of five variable regions named from V1 to V5 in loop form, interspersed with five conserved regions C1-C5. Into this complex structure the V3 loop is a region which has been designated the principal neutralizing determinant. Virus neutralizing antibodies are elicited by amino acid sequences encompassing the V3 loop. In infected individuals, B-lymphocytes as well as T-lymphocytes responses are directed to the V3 loop. Thus, V3 loop is presumed to play an important role in the early stages of infection, perhaps by interfering with the interaction of gp120 and CD4 and/or a distinct coreceptor.[150]

The V3 region of gp120 contains determinants for coreceptor affinity and cell tropism as well as immune evasion, and is associated with a preferential use of the CCR5 coreceptor, which is also characteristic of most vertically transmitted HIV-1 viruses. The amino acid sequence of V3 loop is a variable domain in the gp120 subunit of HIV-1, containing 35 amino acids arranged in a disulfide loop involving residual Cys301 and Cys336 interval. This domain plays an important role in regulating several biological properties of the virus such as cell tropism, cytopathicity, syncytium formation and fusogenicity. Deletions in the V3 loop abrogate viral infectivity. The variable domain V3 loop possesses relative conserved subdomains as well as variable subdomains located at the top or crown of the loop. Genetic studies have showed that the conserved amino acids in the V3 loop influence several properties of HIV-1 that are controlled by the *env* gene. However, mutations as substitutions and small deletions in V3 do not affect the ability of gp120 to interact with CD4 receptors. Genetic variability into a determined subtype may introduce diversified motifs characterized by V3 loop amino acids and alterations in determined sequence of amino acids in the crown of V3 loop serves as indicative of the geographic origin of a certain mutant HIV-1. Using C2V3C3 *env* region nucleotide sequencing, several amino acid motifs from the V3 loop crown have been determined, showing wide spectrum of phenotypes into a same subtype and a same geographical territory.[57,66]

The subtype B strains of Spain[18] revealed a prevalent octamer HIGPGRAF (30%) followed by PIGPGRAF (9%) and NIGPGRAF (6%). In Switzerland, several motifs were found into the subtype B, characterized by the predominant motif IGPGRAF (84%) and other tetrameric sequences of amino acids GPGR and GWGR.[132]

Korean subtype B strains are characterized by the tetrameric motifs GPGR (55%), GPGS (20%), and GPGQ, GPGG, GQGR and APGS (one case each, 5%).[103] Current subtypes B in Thailand have predominantly octameric motifs HIGPGKAF, HIGPGRAF and PIGPGAFF at the top of V3 loop. Other Thai subtype named B_B is identified by HLGPGQAW and PLGPGQAW octameric motifs also present in Myanmar and China.[20]

In Brazil subtype B strains (or B_{Br}) present predominantly GPGGAF motif that circulate also in other South American countries including Argentina, Uruguay and

Venezuela.[13] Another subtype B with V3 loop motif GPGRTW was recently described in Venezuela.[21] Brazilian subtype C (or C_{Br}) has as a characteristic tetramer GPGQ,[13] also found in Portugal,[36] Japan,[99] and India.[80] In relation to the subtype F, Brazilian F_{Br} possesses GPGR motif[13] that has a small difference compared to the Romanian subtype F_R with predominant GPGQ motif, occasionally also observed in Brazil.[7]

The different motifs found in subtypes have immediate implications also in the originated CRFs. A new B/F recombinant diverse of the South American CRF12_BF was recently identified into Venezuelan strains, and involves motifs GPGRVV from subsubtype F1 and GPGRTW and GPGGAF from different subtypes B. The presence of mosaic genomes highlights the need to improve subtyping protocols with the molecular analysis of at least two distinct viral regions to identify the circulant recombinants forms and dual infection with different subtypes, and a HMA *env*/*gag* protocol has been recently proposed for this confirmatory study.[21]

The genetic variability of HIV poses special problems for HIV diagnosis, treatment and HIV vaccine development. It is therefore important to monitor the distribution and dynamics of HIV subtypes at a global level, and this is an objective of the WHO-UNAIDS – sponsored "Network for HIV Isolation and Characterization".[35,65]

RECOMBINATION BETWEEN HIV GROUPS

Due to the high degree of divergence between HIV groups M and O it had been suspected that homologous recombination between HIV groups may not be possible. However, recent reports have described recombinant intergroup M/O in three different patients from Cameroon. These M/O mosaic viruses were proved to replicate well *in vivo* and *in vitro*, and can even become the predominant variant within the patient's viral population.[108,133]

Dual infections with HIV-1 group M and group O have been recently identified in Cameroon[152] among AIDS patients. The amplification of both group M and O genomic sequences from all four genome regions (*gag*, *pol*, *env* gp120-V3 and *env* gp 41-IDR) from two distinct specimens had suggested the presence of at least two viruses, with both group M- and O-derived sequences across the full-genome. One specimen contained a group M CRF02_AG virus and a group O clade V virus, and other specimen contained a group M recombinant of U (unclassified), CRF02_AG and subtype A, and a group O clade Ib virus. A third and more complex specimen contained a group O clade IV virus together with a group M/O recombinant of M CRF02_AG and O clade IV.

Other types of dual infections including HIV-1 and HIV-2 have also frequently been reported in Central Africa regions where both viruses co-circulate, however, until now, no recombinants between these two viruses have been described. Probably the level of genetic divergence between HIV-1 and HIV-2 strains may be too high for successful recombination, although this possibility cannot be entirely excluded. There is no protective effect of HIV-2 against subsequent HIV-1 infection. Instead, subjects who have HIV-2 infection are at a substantially higher risk of acquiring HIV-1 infection, whether for behavioral or biological reasons.[62,107,139,152]

Dual infections may represent either: (a) concurrent infection of two or more viral strains occurring at or near the same time; or (b) sequential superinfection of a second viral strain after primary seroconversion to the initial strains. A superinfection is defined as the re-infection of an individual after a primary HIV-1 infection, with a heterologous strain belonging to the same or different subtype as the primary strain. Dual infections and recombinants involving different strains or clades of HIV-1 have been reported over the past decades, then it was not clear how frequently these events occur. Dual infection and superinfection have been reported in a handful of HIV-1 infected individuals. Characterization of new CRFs and/or URFs in increasing numbers over recent years has suggested that co-infection and superinfection are occurring relatively frequently.[56,70,153]

Recombination between highly divergent groups within lentiviruses has also been discussed. Group N forms are an independent lineage most closely related to but still distant from group M. Genomic analyses have showed that group N viruses cluster more closely with a chimpanzee virus (SIV_{cpz}). Thus, some authors have suggested that group N viruses are probably the result of a recombination event between an SIV_{cpz} like and an HIV-1 like viruses, which would corroborate with a substantial zoonotic transmission from chimpanzee to human. Group M and O strains as divergent recombination could contribute substantially to the emergence of new HIV-1 variants, and would have important implications both for diagnosis by serological and molecular testing, treatment with antiretroviral drugs and also for vaccines production.[27,41,62,108,109]

CONCLUSIONS

Worldwide distribution of HIV subtypes and other intermixed variants is a dynamic and unpredictable

process and contributes substantially to the global AIDS pandemic. Prevalence rates of different subtypes implicate in the proportion of new recombinant viruses. The probability of certain population groups acquiring multiple infections and transmitting their viruses further, and the fitness of any mosaic viruses generated also contribute with the proportion of recombinant viruses. Genomic recombination of HIV strains may introduce genetic and biological consequences that are far greater than those resulting from the steady accumulation of single mutations.

A consistent genetic classification of subtypes, CRFs and other recombinant genomes bears important implications for monitoring the pandemic, vaccine designs, and detection of genetic determinants related to a particular HIV. Studying HIV genetic variation at the global level is needed, not only to learn the origins and understand the epidemiology of HIV-1, but also to identify the emergence of subtypes, CRFs, and intra-subtypes that may be more readily transmitted or have altered virulence. Thus, it ensures that vaccine antigens are directed against strains of the viruses that are currently circulating within specific populations. Several countries have been selected as World Health Organization field site for HIV-1 vaccine trials programs, and priority has been given to molecular investigation of the prevalence and genetic diversity of HIV-1 strains circulating in the countries. Genomic variation studies are important for developing AIDS vaccines and also predicting the global evolution of HIV.

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