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HIV Diversity, Recombination and Disease Progression: How Does Fitness "Fit" Into the Puzzle?

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Abstract

HIV appears to have diverged into several lineages upon multiple zoonotic introductions from the nonhuman primates. The HIV-2 and HIV-1 groups M, N, and O likely represent different cross-species transmission events. The radial evolution of group M in multiple clades or subtypes is likely due to adaptation and expansions in the human hosts. It is not well understood why HIV strains such as HIV-1 subtype C in particular or group M in general have spread disproportionately as compared to other subtypes, groups, or types, which often remained geographically constrained to local epidemics. Host genetic effects, transmission bottlenecks, social/behavioral and environmental limitations, founder effect and other viral factors could have contributed to variable spread through the human population. Even after transmission, viruses evolve at different rates during disease progression. Recent studies have explored phenotypic differences between HIV types, groups, and subtypes in attempts to explain or understand this radial evolution and expansion. This review explores some of the important aspects relating to fitness during disease progression, during global distribution of different HIV subtypes, and related to circulation of recombinant forms in the epidemic. **(AIDS Reviews 2007;9:75-87)**

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Key words

HIV subtype. Recombination. Disease progression. Viral fitness.

Introduction

The extreme heterogeneity of HIV is a result of rapid viral turnover (~ 2.6 days/replication cycle), a high virus burden (10^{10} viral particles/day) and the error-prone nature of the reverse transcriptase (RT) enzyme which lacks proofreading activity (3×10^{-5} mutation per base pair per cycle)[1-4]. HIV can also recombine, giving rise to viruses leading to major antigenic shifts and, if stable, alterations in fitness or virulence[5-8]. HIV-1 survives in a host as a swarm of genotypically related clones termed quasispecies[9]. A strong genetic bottleneck is evident during host-to-host transmission such that a relatively homogenous set of virions establishes a new infection. During disease progression, this population rapidly evolves into a genotypic and phenotypic mixture of different variants[10]. Persistence of different variants in a given environment is highly linked to the replicative adaptability of individual viruses, known simply as fitness. Genetic variability is of relevance since it can affect disease progression through adaptation to immune response and antiretroviral therapy. This review focuses on the possible impact of replicative fitness on (i) transmission and disease progression, (ii) HIV global diversity/expansion in the epidemic, and finally, on (iii) the emergence of circulating recombinant forms.

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HIV origin and classification

Simian immunodeficiency virus (SIV) appears to have crossed the species barrier (simians to humans) multiple

times over several decades, leading to the various types, groups and possibly clades of HIV. Phylogenetic relationship indicates that HIV-1 and -2 jumped from the closely related SIV-infected nonhuman primates: chimpanzees (*Pan troglodytes troglodytes*) and sooty mangabeys (*Cercocebus atys*), respectively[11-13]. The origin of HIV-1 group M can be linked to chimpanzees inhabiting the eastern equatorial forests of Cameroon[14]. Interestingly, a close follow-up study showed that distinct HIV-1 group O viruses are prevalent among gorillas (*Gorilla gorilla*) in this region of Cameroon[15]. Even though studies were limited to Cameroon, it is likely that similar cross-species transmission events could have occurred in the equatorial rain forest of Central Africa such as Gabon and the Central African Republic. While the origin of HIV-1 in humans dates back to the 1920-1950s[16,17], it is predicted that HIV-2 crossed to the human population before 1930-1955 in West Africa, while the epidemic took off in Guinea Bissau[11,16,18].

'Pure' subtypes or non-recombinants

HIV is divided into types 1 and 2. HIV-1 is further classified into three groups: Major (M), Outlier (O), and non-M/non-O (N). While HIV-1 and -2 nucleotide sequence variation might reach 50%, the differences between groups are greater than 30%. Group M dominates the epidemic and is composed of nine subtypes; A-D, F-H, J, and K. Subtypes A and F have been further subdivided into sub-subtypes A1-A3 and F1-F2, respectively. Sequence similarity between subtypes is between 70-90% (Fig. 1), with the greatest genetic differences observed in the *env* gene (up to 30% nucleotide diversity) followed by *gag* (20%) and *pol* (15%)[19]. During the early phase of the epidemic, genotypic analysis of the envelope gp120 coding region was used for the classification of HIV-1 subtypes. However, as the epidemic progressed it became clear that sequence analysis of several regions of the genome was imperative if HIV diversity was to be better understood. Analysis of multiple gene fragments or the entire genome suggested that certain individuals either carried recombinant viruses or were infected with multiple subtypes[20-22], particularly in regions of Central Africa and East Asia[23].

HIV group O viruses were first described in 1994, about 12 years after discovery of HIV. However, the earliest known infection by group O was reported in a Norwegian sailor and his family circa 1960, but only identified from stored frozen samples in 1997[24,25]. Group O viruses were named "outlier" because of their distinct clustering from other members of the group M viruses (Fig. 1)[25]. Several attempts have been made using partial *gag*, *pol,* and *env* sequences to classify Group O viruses into subtypes[26-30]. Results from these studies indicate group O viruses exhibit a high diversity, but do not show the same subtype radiation like group M viruses[26,31].

The group N viruses have only been identified in five patients and within the past five years. Phylogenetically, they form an independent clade related to group M, whereas sequences from the 3' end cluster more closely with the SIVcpzUS chimpanzee virus (Fig.1)[13] suggesting a possible ancient recombination event within humans or prior to cross-species transmission[32]. The epidemiology and molecular phylogeny of HIV-2 is more understood than that of the rare HIV-1 groups O and N. HIV-2 can be subdivided into subtypes A-G, but only A and B clusters comprise of more than three fully sequenced isolates. The nucleotide and amino acid sequence diversity within HIV-2 cluster is greater than that of HIV-1 group M. The HIV-2 subtypes are therefore analogous to HIV-1 groups M, N, O in sequence diversity[18,33-35].

Recombination and the HIV epidemic

For HIV recombination to occur, it is necessary that a cell is first infected by two different viruses to generate a heterozygous or heterodiploid virion, i.e. containing a genome from a different virus. *De novo* infection by this heterodiploid virus can result in a recombined retroviral DNA sequence due to the switching of reverse transcriptase between RNA genomes during (–) strand DNA synthesis[3,4,7,8]. Although retroviral recombination was largely a laboratory phenomenon discovered by Howard Temin, *in vivo* recombination was quite evident with the discovery of intersubtype HIV-1 recombinants in the mid-1990s. It is now known that "intersubtype recombination" is an integral player in increasing the genetic variability of HIV[36-39]. Viruses from different lineages within group M have recombined to form various recombinant viruses with epidemic proportions known as circulating recombinant forms (CRF). Complex CRF (CRFcpx) are recombinants which are comprised of more than two different subtypes. To define any new CRF, at least two full length viruses and a third viral sequence must have identical recombination breakpoints and be isolated from epidemiologically unlinked individuals. Unique recombinant forms (URF) have heterogeneous recombination breakpoints and have limited transmission in the human population[40,41]. Circulating recombinant forms account for about 10-20% of all new infections while URF are responsible for over 30% of the infections where several HIV-1 subtypes co-cir-

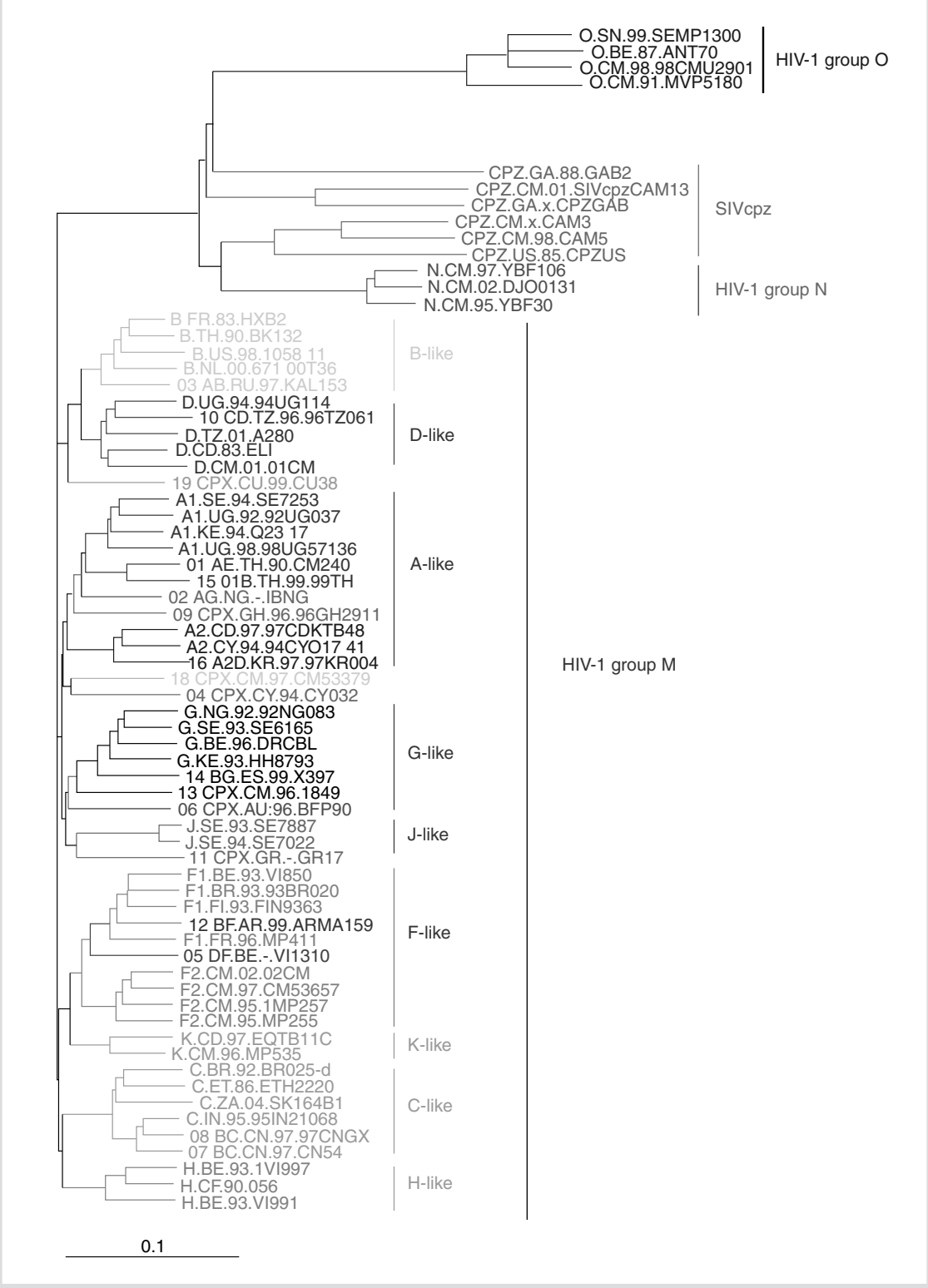

Figure 1. *Phylogenetic tree representing the genetic diversity in HIV-1 groups M, N, O and SIVcpz. Full genome sequences were aligned and the tree constructed using Clustal-X version 1.83 and schematically represented with the Tree View Program. HIV-2 was used as the out group. Subtypes and CRFs are represented in black and different shades of grey. Within HIV-1 group M the term "x-like" is used to denote clusters which are similar to the different group M subtypes.*

culate (e.g. Uganda and Kenya)[38,40,42]. Of the 34 CRF described, the most dominant forms in the epidemic are the CRF01_AE, CRF02_AG, CRF07,08_BC, and CRF_BF-like viruses found primarily in Asia, West Africa, China and South America, respectively (Table 1). Some of these CRF have recombined with other pure subtypes or CRF to form "second generation" recombinants which are also spreading in certain regions of the world (Table 1). Despite initial assumptions that HIV-1 group M and O might be too diverse to recombine, several studies have reported M/O intergroup recombinants in West Africa[43-46]. Even though a million individuals appear to be dually infected with HIV-1 and -2, intertype recombinants have not been identified. However, the recombination event potentially involved in generation of HIV-1 group N leaves open the possibility that HIV-1 and -2 could still recombine[46,47]. Much attention is placed on the possible emergence of human H5N1 influenza A strain due to genetic rearrangements between bird H5N1 and another influenza strain. However, another human lentiviral outbreak could arise from a recombination between HIV-1 and -2, which could also have a devastating impact on the human population. Current vaccine efforts and drug design would have to be reconsidered due to poor efficacy of our current strategies.

Distribution of HIV throughout the world

The expansion of different HIV strains throughout the globe has been largely uneven. Founder effects, human genetic (host restrictive factors) as well as social/behavioral factors can all contribute to this differential spread. For example, specific HIV subtypes tend to circulate in particular geographic regions, strongly supporting the founder effect theory (single introduction followed by a rapid spread) rather than the host restrictive factor theory (host factor preventing viral infection). However, the impact of initial founder event is now minimized due to multiple introduction of different subtypes and co-circulation within the same transmission groups. Diversity and prevalence of HIV is highest in sub-Saharan Africa due in part to the origin of this lentivirus[48].

The highest prevalence of HIV-1 is found in Southern Africa (Botswana, Lesotho, Namibia, South Africa, Zimbabwe, Malawi, Mozambique, Zambia, and Swaziland), which also has the lowest HIV-1 diversity due to the dominance of subtype C. In fact subtype C viruses comprise about 52% of the HIV-1 infections worldwide[49]. In East Africa subtypes A, D as well as C are predominant. However, recombinants between these three subtypes are responsible for over 30% of the infections in Uganda, Kenya, and Tanzania. Aside from CRF10_CD which is prevalent in Tanzania, these intersubtype recombinants have been classified as URF, suggesting a high frequency of dual infections (Table 1)[50,51]. West and Central Africa, described as an "HIV diversity hotspot" carries a mix of nearly all HIV strains. For example, the Democratic Republic of Congo (DRC) and Cameroon have likely "seeded" the entire HIV-1 global epidemic and nearly every HIV type, group, subtype, and recombinant form can still be identified in the infected population[52,53]. Unlike HIV-1 group M and aside from some sporadic infections, HIV-1 groups N and O never established epidemics outside of West Africa[54-57]. Unlike the dominance of subtype C in much of the world, subtypes A and G viruses are more common in West African countries including Nigeria, Ghana, Côte d'Ivoire and Cameroon. In most of these countries, however, CRF02_AG is responsible for the majority of the HIV-1 infections[22,58,59]. CRF09, 11 and 13 occur rarely and sporadically in West and Central Africa (Table 1)[60-63]. CRF06_cpx likely emerged in Burkina Faso and has resulted in regional epidemics within Burkina Faso, Niger, Mali, Nigeria, Côte d'Ivoire, and in the intravenous drug users in Kalingrad, a geographically separate region of Russia on the Baltic Sea[42,64]. Recent studies in different African countries have shown high rates of intermixing of different subtypes and recombinant forms giving rise to more complex recombinant strains. For example, CRF30_0206 is a recombinant between CRF02_AG and CRF06_cpx circulating in Niger and Burkina Faso[63,65]. HIV-2 is rare in central Africa, but has been reported more frequently in West Africa (Senegal and Guinea Bissau) where subtype A predominates and Côte d'Ivoire where subtypes B and G have been reported[18,35,47]. Subtypes C, D and E have been reported in Sierra Leone and Liberia[35,47].

The most dynamic HIV epidemics are occurring in Asia and Eastern Europe. Unlike Africa, all of the subtypes that co-circulate in Asia are due to multiple founder events[41]. Although subtype B was the first to be introduced into many Asian countries including India, Thailand, and China in the mid-1980s, its expansion has not been at the pace of other subtypes and mainly subtype C. In China, subtype C and B are highly prevalent and have recombined to form the dominant CRF07_BC and CRF08_BC. In Thailand, CRF01_AE (formerly classified as subtype E) has spread in the general population overtaking the founder Thai subtype B, initially more prevalent among drug users[23]. Molecular epidemiologic studies in India indicate that subtype C is the most common subtype[66,67], followed by the Thai B

Table 1. Characteristics and geographical distribution of Circulating Recombinant Forms (CRF) described in the global HIV pandemic

Name	Reference strain	Subtypes	Distribution	Fitness	Identified sequences*	Nº. of infections†	Epidemic impact
CRF01_AE	CM240	A, E	Thailand, Central Africa	group M-like	6332	1,303,689	5+
CRF02_AG	IbNG	A, G	West and Central Africa	AG > A = G	3769	2,601,251	6+
CRF03_AB	Kal153	A, B	Eastern Europe	unknown	149	6000	2+
CRF04_cpx	94CY032	A, G, H, K, U	Cyprus, Greece	unknown	22	7500	2+
CRF05_DF	VI1310	D, F	Belgium	unknown	29	5920	2+
CRF06_cpx	BFP90	A, G, J, K	West Africa	unknown	845	100,332	3+
CRF07_BC	CN54	B', C	China, Taiwan	unknown	136	108,000	3+
CRF08_BC	GX-6F	B', C	China	unknown	187	126,900	3+
CRF09_cpx	96GH2911	CRF02, A, U	West and Central Africa	unknown	39	14,100	2+
CRF10_CD	TZBF061	C, D	East Africa	unknown	202	17,640	2+
CRF11_cpx	GR17	A, CRF01, G, J	Central Africa	unknown	529	46,030	3+
CRF12_BF	ARMA159	B, F	South America	F > B = BF‡	348	51,932§	3+
CRF13_cpx	96CM-1849	A, CRF01, G, J, U	Central Africa	unknown	78	27,260	2+
CRF14_BG	X397	B, G	Europe, Asia	unknown	90	5550	2+
CRF15_01B	99TH. MU2079	CRF01, B	Thailand	unknown	18	9860	1+
CRF16_A2D	KISII5009	A2, D	Kenya, Korea, Argentina	unknown	6	NA	1+
CRF17_BF	ARMA038	B, F	South America	F > B = BF‡	7	51,932¶	3+
CRF18_cpx	CU76	A1, F, G, H, K, U	Cuba, Central Africa	unknown	36	<1000	1+
CRF19_cpx	CU7	A1, D, G	Cuba	unknown	10	<1000	1+
CRF20_BG	CB228	B, G	Cuba	unknown	5	NA	1+
CRF21_A2D	99KE_ KER2003	A2, D	Kenya	unknown	2	NA	1+
CRF22_ 01A1	CM53122	CRF01, A1	Cameroon	unknown	3	NA	none
CRF23_BG	CB118	B, G	Cuba	unknown	2	NA	none
CRF24_BG	CB378	B, G	Cuba	unknown	3	NA	none
CRF25_cpx	02CM_ 1918LE	A, G, U	Cameroon	unknown	2	NA	none

Continue

Table 1. Characteristics and geographical distribution of Circulating Recombinant Forms (CRF) described in the global HIV pandemic *(continued)*

Name	Reference strain	Subtypes	Distribution	Fitness	Identified sequences*	Nº. of infections†	Epidemic impact
CRF26_AU	Pending	A, U	Pending	unknown	NA	NA	none
CRF27	Pending	Pending	Pending	unknown	NA	NA	none
CRF28_BF	BREPM12609	B, F	South America	F > B = BF‡	2	51,932§	3+
CRF29_BF	BREPM16704	B, F	South America	F > B = BF‡	3	51,932§	3+
CRF30_ 0206	Pending	Pending	Niger	unknown	1	NA	none
CRF31_BC	Pending	B, C	Brazil	unknown	2	NA	none
CRF32_ 06A1	EE0369	CRF06, A1	Eastern Europe (Estonia)	unknown	23	NA	none
CRF33_01B	Pending	CRF01, B	Asia (Malaysia)	unknown	3	3631	1+
CRF34_01B	Pending	CRF01, B	Pending	unknown	NA	NA	none

*based on information from the HIV database and published data.
†global estimate based on the number of infected individuals/country multiplied by the prevalence of a subtype or CRF in each country where data is available.
‡preliminary results, analysis in progress.
§all BF-like viruses combined; NA: data not available; none: only reference strains or > 3 sequences but all from the same country or epidemiological significance of "pending" sequences is still to be determined; 1+ to 6+ = based on estimated global prevalence (6+: > 2 million; 5+: 1-2 million; 4+: 200,000-1 million; 3+: 25-200,000; 2+: 5-25,000; 1+: < 5000 infections, respectively).

strains. New recombinant forms involving B/C as well as A/C have now been reported in some parts of India[68,69].

In Eastern Europe, subtype A1 dominates, but subtype B as well as CRF03_AB co-circulate in the epidemic. The increase in HIV-1 prevalence coincided with the dissolution of the former Soviet Union. Intravenous drug users as well as men who have sex with men are a main source of transmission[70,71]. In North America, Western Europe, and Australia, subtype B is the most prevalent form of HIV-1. However, there has been a rapid increase in non-B subtypes and CRF in Western Europe, first due to immigration but now also among Europeans[72-76]. Similarly, HIV-2 subtypes A and B, which are both epidemic in West Africa, circulate in some European countries, particularly those with historical ties to this part of Africa (e.g. Portugal)[77]. South America, mainly Brazil and Argentina have HIV epidemics with considerable subtype and CRF diversity including subtypes B, C, and F. The BF recombinants (CRF12_BF, CRF17_BF, CRF28_BF, CRF29_BF) are responsible for about 80% of the infections in Argentina[78-80]. In Brazil, subtype C dominates the southern province, Rio do Sul, due to the rapid increase in subtype C prevalence from 35% in 1996 to 52% in 2002 among the HIV-infected population[81]. In Cuba, some new CRF (CFR_20, 23 and 24) have been identified and involve recombination between subtypes B and G[82].

HIV evolution and fitness during disease progression

The concept of "survival of the fittest" drives evolution in a complex population. Recent studies suggest an association between HIV fitness, diversity, recombination, rate of transmission, and disease progression[83-86]. Evolution of HIV is a continuous process involving migrating through a host, overcoming transmission barriers, escaping from different immune pressures, and resisting antiretroviral therapies. The most fit virus in an *ex vivo* culture suggests an increased virulence in a host. However, rapid disease progression is also related to faster extinction of this viral isolate in the human population. The very fit viruses therefore have to adapt to a given environment in order to survive. Fitness, therefore, is a complex term related to replicative adaptation of an organism to a given environment. For HIV, this environment is multi-layered due to complex

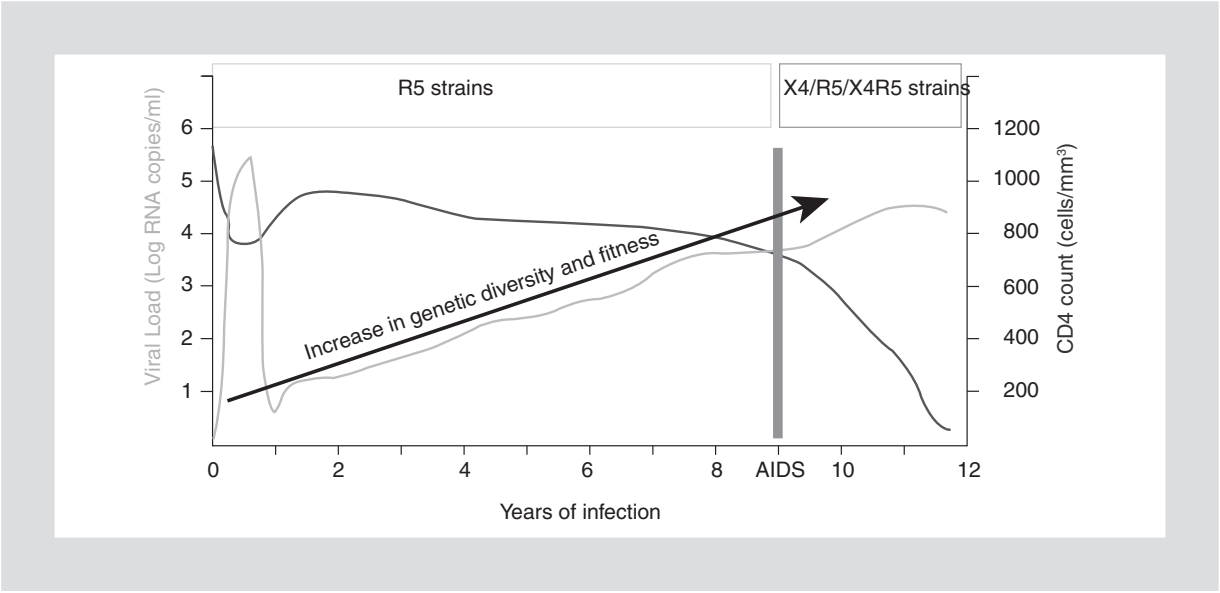

Figure 2. *Schematic representation of the evolution of HIV tropism, genetic diversity and fitness during disease progression. During HIV transmission, only CCR5-using strains are transmitted and persist during the asymptomatic stage. After several years marked by increased viral load and decrease in CD4 cell counts, CXCR4-using strains emerge in about 50% of infected subjects. Both R5 and dual tropic R5X4 strains are also present during late disease. Genetic diversity and fitness increase as disease progresses and both correlate very closely with viral load.*

interplay between host and pathogen[9]. Sequence variation in viral proteins and viral regulatory elements could affect the overall fitness of an isolate by influencing virus-host interactions or the kinetics of viral replication. The observation that individuals infected with a virus lacking the open reading frame of *nef* progress more slowly to AIDS, provided the first hint of *nef's* role in disease progression and possible impact of impaired fitness on virulence[87,88]. In tissue culture, *nef* deleted or mutated viruses are significantly impaired in replicative fitness[89].

Within a given quasispecies, each clone has a fitness representative of those viral properties, undergoing selection in that particular environment. During viral replication, different genomes encode virus that replicate at high rates, continually mutating but remain under selective pressures. Positive selection implies that one or more members of the quasispecies are better suited to a given environment, while negative selection eliminates unfit variants.

A significant genetic bottleneck is apparent during transmission of HIV-1 by any route of infection. However, individuals may be exposed to varying amounts of virus depending on the mode of transmission. The virus population recovered from primary infections is more homogenous with a narrow genetic variation of quasispecies than found in the donor (Fig. 2)[10]. The transmitted variants have less adaptive potential than the donor variants due to the genetic bottleneck. Factors that impose the genetic bottleneck during transmission are diverse and include: the density of target cells at the site of infection, number of transmitted virions, structure of the quasispecies as well as the host's innate immune response and genetic polymorphism[9,90]. During initial infection, the viral properties selected for efficient transmission may differ from those actually required for efficient dissemination and rapid turnover during acute infection. Rapid selection of fit virus in such circumstances may only occur in exposures with a significant load of diverse HIV-1 quasispecies. Environmental differences such as pH, target cells and mucosal composition at the site of exposure may affect the efficiency of transmission, fitness of the infecting isolates, and subsequent disease progression[90-92]. For example, phenotypic and genotypic differences between HIV-1 variants infecting men and women following heterosexual contact were related to the high diversity seen among women, due to the fact that women were typically infected by multiple viruses from their partners whereas most men were productively infected by only a single genotype[92,93].

During initial infection there is a clear occurrence of phenotypic selection in which mostly slowly replicating NSI/R5 strains are transmitted[94]. These R5 viruses continue to dominate the viral quasispecies throughout the asymptomatic phase characterized by a "steady state" in which there is a continual and rapid viral replication

but virus dissemination is partly controlled by the host immune response[95]. The fast-replicating SI/X4 HIV-1 isolates generally emerge during AIDS or in late disease in about 50% of patients, coincident with a rapid decline in CD4 cells, a burst in viral load, and the onset of AIDS (Fig. 2). This observation, first related to subtype B infections, has been confirmed among different HIV-1 subtypes with the exception of subtype C where X4 variants rarely occur even at late stages of disease[22,59,96-98]. The hypothesis that SI/X4 viruses are more fit than their NSI/R5 counterparts has been challenged using peripheral blood mononuclear cell (PBMC) *ex-vivo* cell culture[83]. This study demonstrated NSI/R5 HIV isolates from rapid progressors could actually outcompete SI/X4 HIV-1 isolates from long-term nonprogressors (LTNP) in dual competitions performed in PBMC[83]. However, in the vast majority of competitions between viruses exclusively utilizing different coreceptors, the SI/X4 viruses win related to increased replication kinetics and higher CXCR4 than CCR5 expression in PBMC. The relative fitness difference in relation to disease progression was more pronounced when isolates of the same phenotype were compared (X4 vs. X4 or R5 vs. R5). Typically, individuals with slower disease progression are infected with less fit HIV-1 isolates as determined by *ex vivo* competitive fitness assays. When relative fitness was compared to different markers of disease progression, there was a significant positive correlation between viral load and relative fitness. Therefore, fitness and viral load were significant predictors of slow versus rapid progression to AIDS. Subsequent studies revealed that replicative fitness increases from the founder or infecting HIV-1 during disease and does so as a correlate of increasing viral loads and decreasing CD4 cell counts[84]. Although RNA viral load remains a widely accepted parameter to monitor therapy[99], a recent study indicated that the decline in CD4+ T-cell counts and not the increase in viral load is a better predictor of disease progression[100]. However, it is quite possible that HIV-1 replicative fitness may be the best predictor of disease rates[84].

Ex vivo fitness of primary HIV isolates typically maps to the *env* gene and is largely controlled by the efficiency of host cell entry[101-103]. On average, sequence changes occur at a rate of 1% per year in *env*. Even though the relationship between disease progression and HIV *env* genetic diversity has been controversial, *env* diversity from founding or infecting viruses has been shown to increase continuously and peaks at the onset of AIDS (Fig. 2)[10]. This prolonged increase in *env* divergence is ultimately due to mutations, but is shaped by immune selection, changes in cellular tropism, and fitness of the encoded virus[104-107].

HIV genotypes, fitness and their implication in the HIV pandemic

Transmission and pathogenic fitness: the case of HIV-2, HIV-1 subtype C and HIV-1 group O

Two reasons could be responsible for the rapid spread of a particular virus strain: efficient transmissibility also known as *ex vivo* "transmission fitness" and rapid replication known as *ex vivo* "pathogenic fitness". Understanding the *ex vivo* fitness and transmissibility of different HIV strains requires a robust culture system able to mimic *in vivo* transmission and pathogenesis. HIV pathogenic fitness in PBMC correlates with disease progression within a host, while transmission fitness is best monitored in a monocyte derived dendritic cell (MO-DC) culture system[85,108] or using explant cervical or vaginal tissue.

Until very recently, the role of fitness in the geographic distribution of HIV strains during the global pandemic had not been studied. HIV-1 group M has been efficiently spread across the world, unlike HIV-2 which is limited to local or regional epidemics. Although HIV-2 and HIV-1 group M appeared to expand exponentially in the 1980s, HIV-2 is responsible for less than 1 million infections as compared to 28 million HIV-1 group M cases[48,109]. Progression to AIDS was slower in HIV-2 as compared to HIV-1 infected patients in West Africa. However, poor transmissibility as compared to HIV-1 in individuals with similar opportunities for sexual contacts is more likely to contribute to the lower HIV-2 prevalence[110-113]. Within HIV-1, group N and O have not spread beyond local epidemics, with Cameroon as the epicenter[114,115].

Recent *ex vivo* studies on replicative fitness suggest that HIV-1 group M viruses are more fit than HIV-2, which in turn are more fit than group O viruses[108] (Fig. 3). This order in replicative fitness is nearly a perfect match to their prevalence in the epidemic. Low HIV-2 fitness in PBMC may reflect reduced virulence and pathogenicity *in vivo*. Unfortunately, little is known about the rates of disease progression in group O infected individuals. Based on the close relationship between *ex vivo* fitness in PBMC with virulence in humans, one could speculate that disease progression is very slow in group O infected individuals. To go beyond this speculation, it would be interesting to determine if infection by HIV-1 group O could even be

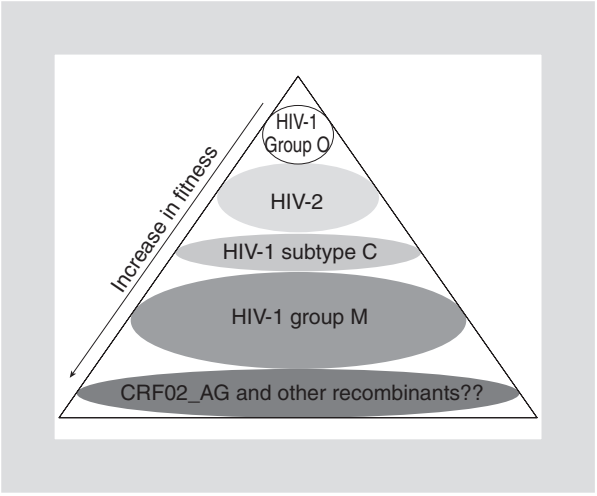

Figure 3. *A "Fitness pyramid" showing the pathogenic fitness order of HIV types, groups and subtypes. HIV-1 group O is the least fit while group M is the most fit. Subtype C is the least fit among group M, but is, however, more fit than HIV-2, which in turn is more fit than HIV-1 group O. Two studies have shown that CRF02_AG is more fit than its parental isolates HIV-1 subtypes A and G. There are, however, no other published studies comparing CRF with their parental isolates. This position of CRF02_AG at the base of the pyramid with respect to other group M isolates still has to be confirmed. Fitness data for other CRF and HIV-1 group N are completely lacking.*

cleared by the host immune response. Follow-up studies of two group O patients (ANT 70 and her spouse) suggest disease progression in this group is similar to those of group M[28,116]. Increased prevalence of HIV-1 group M over HIV-2 and group O in West Africa may be related more to transmission efficiency rather than pathogenesis in the host. After all, group O viruses have been reported to circulate in Central Africa as early as the 1960s[24]. Models of sexual transmission suggest that dendritic cells (DC) may be the initial targets of primary infection[91,117-120]. Accordingly, competition experiments using DC (a model for transmission fitness) showed extremely low fitness of group O and HIV-2 as compared to HIV-1 group M strains[108].

Predominance of HIV-1 group M over HIV-1 group O, N, or HIV-2 does not imply an equal subtype distribution throughout the world or even in a distinct geographic region. For example, subtype C has rapidly expanded in the epidemic due to multiple founder events throughout the world. However, given the higher prevalence of subtypes A, B, D, G and some CRF in Africa in the late 1980s, it is somewhat surprising that only subtype C has lead to rapid expansion in the heterosexual transmission group. Full-length subtype C sequences from India were found to cluster distinctively from other subtype C isolates, suggesting a single introduction followed by rapid spread[66,121,122]. In recent years, increase of subtype C prevalence or its derivative recombinant forms is quite evident in several regions such as Rio do Sul, Brazil (from 35% in 1996 to 52% in 2002)[81], in Kinshasa and Mbuji-Mayi, DRC (from 2.1 and 16.3% in 1997 to 9.7 and 25% in 2002, respectively)[123], and in Yunnan, China (from 5.1% in 1992 to 90% in 2002). However, there are also studies reporting no change in subtype prevalence in Kenya[124]. Several studies have suggested that rapid spread of subtype C might be related to a "better" replicative capacity compared to other HIV subtypes. Most subtype C isolates appear to have an extra or third NFκB element in the LTR, which may augment transcription in the presence or absence of the HIV Tat protein[125,126]. In a recent study, Centlivre, et al. compared the transmission efficiency of isogenic SHIV carrying subtypes B, C and E minimal core-promoter/enhancer elements by coinfecting rhesus macaques or tissue culture containing rhesus PBMC, thymus histocultures, and gut-associated lymphoid tissue (GALT, an IL-2 rich microenvironment)[127,128]. They found that the SHIV carrying the subtype C promoter was dominant in primary infection, but was eventually replaced by the SHIV containing the subtype B promoter. The subtype C viruses dominated when thymic histocultures or GALT were used suggesting enhanced transmission. However, when cultured in *rhesus* PBMC, the subtype C viruses were more fit than E but less fit than B[127,128].

In a recent study competing primary subtype B and C HIV-1 isolates in PBMC cultures, all subtype C isolates were out competed by subtype B, even though no differences were found in intra subtype B or C competitions[101]. These experiments only involved NSI/R5 subtype C and B viruses, considering that subtype C isolates rarely switch to SI/X4 even late in disease[129]. When employing rare SI/X4 subtype C isolates, their fitness was still 100-fold less than SI/X4 group M isolates. To date, over 2000 competitions have been performed with over 40 primary subtype A, B, C, D, and CRF01 HIV-1 isolates. HIV-1 subtype C is unique from all other subtypes in terms of lower replicative fitness in PBMC. In contrast, competition experiments in Langerhan cells embedded in human skin blisters showed equal "transmission" fitness between subtypes B and C, a result recently confirmed with a larger panel of subtype C viruses[130]. This maintenance of high "transmission" fitness in DC but low "pathogenic" fitness in PBMC is again unique to subtype C, considering that group O and HIV-2 isolates had much lower "pathogenic" and "transmission" fitness. Poor relative fitness of subtype C isolates could be linked to a model where decreased

fitness is associated with slower disease progression, which could result in longer transmission times and opportunities[83].

Are recombinant viruses always more fit than their parental isolates?

It is conceivable that following recombination or other evolutionary bottlenecks, only the fittest viruses in a population would survive. It would therefore be expected that after recombination, the new recombinants would eventually outgrow the parental isolates. However, the fact that some CRF seem largely predominant over other circulating HIV strains in certain areas may have several explanations. First, the recombinant form may have some biological advantage over the parental strains, including a possibly higher replicative fitness and/or transmission capacity. Second, the recombinant strain could have been introduced first into a particular region and as result established an epidemic before other subtypes entered, commonly known as the founder-effect. CRF02_AG dominates the HIV epidemic in West and Central Africa. The founder effect hypothesis does not seem applicable in the DRC where ancient subtype A and G subtypes have been reported[131]. Two independent groups recently compared the *ex vivo* fitness of parental subtypes A and G viruses against its putative CRF02_AG strain[85,86]. Konings, et al. followed the replication kinetics of four NSI subtype A and G viruses each, as well as five CRF02_AG in a monoinfected PBMC culture. On day 11 postinfection, the CRF02_AG infection supported slightly higher levels of virus production (1.4 to 1.9-fold) than the parental subtypes A and G infections. Njai, et al. on the other hand carried out a more detailed study which involved dual competition assays of five subtypes A, five subtype Gs and 10 CRF02_AG, all originating from Cameroon[85]. These viruses were competed in parallel in stimulated PBMC (pathogenic fitness) and MO-DC (transmission fitness). The CRF02_AG isolates were significantly more fit than the parental A and G viruses in PBMC. However, this advantage was again minimal and not significant in MO-DC, but still suggesting that the wide distribution of CRF02_AG viruses might be due to its pathogenic fitness.

The HIV epidemic in Argentina is characterized by CRF12_BF and many related forms[41,78,79]. An initial rapid phase of exponential growth of CRF12_BF infections appeared to be due to mother-to-child transmission considering a recent reduction in Argentina[132]. To investigate the reason why CRF12_BF had spread so rapidly in Argentina, Turk, et al. studied the role of LTR and tat activity in viral replication and demonstrated a higher activity for the $LTR^{BF(ARMA159)}$ /$Tat^{BF(ARMA159)}$ complex when compared to its B subtype counterpart[133]. They also showed that the LTR from subtype B and Tat proteins from CRF12_BF or vice versa could not efficiently complement each other and mediate transcription. Considering that CRF12_BF contains a "subtype B" LTR and "subtype F" Tat, it was not surprising to find several substitutions in the *tat* gene and in the LTR sequence from CRF12_BF samples which may have appeared due to adaptive evolution[133]. This study concluded that the observed transcriptional improvement may be a consequence of the recombination process between two different HIV-1 subtypes and selection forces favoring the spreading of these recombinant forms. Regardless of the interaction between the LTR and Tat, transcriptional regulation of HIV-1 is only one step in the retroviral lifecycle that can affect replicative fitness. It is important to note that several studies have indicated that efficiency of host cell entry controls replicative fitness and overrides the minor increases or decreases in the rates of completing other steps in the retroviral lifecycle. In ongoing PBMC competitions using primary isolates of subtypes B, F and CRF12_BF primary isolates from Argentina, it appears that subtype F isolate is more fit than B or CRF12_BF isolates. These results are preliminary, but still suggest that the rapid expansion of CFR12_BF is not directly related to "pathogenic" fitness. Of course, higher transmission fitness may still play an important role for CFR12_BF expansion. Since there are four different BF recombinant viruses circulating in Argentina, it would be interesting to compare the prevalence and replicative fitness of each.

Conclusion

The global spread of HIV subtypes and different recombinant forms have been extensively studied in the past. However, phenotypic differences between HIV-1 isolates have not been the subject of intense investigation until recently. Competition assays have now been performed in HIV isolates of the same or different types, group, or subtypes to determine a replicative fitness order and to correlate this fitness to disease progression. HIV-1 subtypes, mainly C, continue to expand in the human epidemic and they are also recombining to form multiple CRF. The replicative fitness of some widely spread CRF such as CRF07,08_BC, CRF06cpx and BG-like viruses (Table 1)

are still unknown, but some data is now available on the fitness of CRF02_AG and CFR_12_BF. It is conceivable that with continual HIV-1 evolution coupled with the increase in global migration, new forms of HIV will arise, possibly spread disproportionately, and even displace existing subtypes through rapid expansion in a local population. Continuous monitoring is necessary to determine if these new viruses can adapt and survive the immune response or administration of antiretroviral drugs. There is no doubt that HIV genotype and fitness will continue to play a major role in the spread of HIV.

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