PERSPECTIVES

Is HIV-1 evolving to a less virulent form in humans?

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Abstract | During the rapid spread of HIV-1 in humans, the main (M) group of HIV-1 has evolved into ten distinct subtypes, undergone countless recombination events and diversified extensively. The impact of this extreme genetic diversity on the phenotype of HIV-1 has only recently become a research focus, but early findings indicate that the dominance of HIV-1 subtype C in the current epidemic might be related to the lower virulence of this subtype compared with other subtypes. Here, we explore whether HIV-1 has reached peak virulence or has already started the slow path to attenuation.

There are four classified retroviruses that infect humans and cause symptomatic disease. Human T-cell lymphotropic virus (HTLV) types 1 and 2 are members of the *Deltaretrovirus* genus in the subfamily *Orthoretrovirinae*, show a low rate of evolution in the human population, and probably originated from a non-human primate millions of years ago[1–4]. The high frequency of HTLV-1 infections was localized in specific endemic areas (for example, southern Japan) until recent times, indicating a relatively slow spread in the human population compared with another human retrovirus, HIV-1.

HIV-1 was introduced into the human population just 60–80 years ago[5] but an estimated 40 million individuals are currently infected with the virus[6]. The spread and expansion of HIV-1 across Africa and throughout the world has been accompanied by one of the most rapid evolutionary rates described for a human pathogen[6], aside from hepatitis C virus. HIV-1 remains one of the most lethal pathogens (100% mortality) that currently infects humans, whereas infection by other human viruses that are often more feared, such as Ebola, severe acute respiratory syndrome (SARS), influenza H5NI and Lassa Fever, can have a mortality rate of <50% (REFS 7–9).

Whether or not a lethal pathogen evolves to become a relatively benign parasite or even a commensal organism has been a subject of great debate. The survival of Euroasian populations during the European bubonic plague (1347–1352) and the 1918 influenza epidemic is often perceived as evidence for pathogen attenuation. However, neither *Yersinia pestis* nor the H1N1 influenza virus seem to have attenuated during these relatively short human epidemics. Surviving the *Y. pestis* epidemic is thought to be more related to fractionation of the human population, the resistance of rodent ectoparasites and, possibly, the selection of resistant human hosts[10,11].

Nonetheless, there are many examples of microparasite evolution and host selection leading to attenuation or resistance to disease. The best documented and controlled example is the rapid outgrowth of an attenuated myxoma virus following its introduction into Australia in 1950 to control the expanding rabbit population[12–14]. This virus might have attenuated as a result of altered immunomodulatory properties in addition to a reduction in the replication rate[13,14]. More recent analyses of archived reports from the fifteenth century suggest that the dramatic decrease in the severity of syphilis symptoms that took place over a period of less than 10 years was related to a reduction in virulence rather than the selection of resistant hosts[15]. Rapid (decades to centuries) versus slow (thousand to millions of years) attenuation might be related to the severity of disease, the rate of lethality and the transmission efficiency or transmission routes. The suggestion that the simian lentiviruses have attenuated in their non-human primate hosts during millions of years of co-evolution[16] has been challenged by recent phylogenetic analyses that indicate the introduction of simian immunodeficiency virus (SIV) into African primates might be a relatively recent event that unfolded in the past hundreds or thousands of years, therefore indicating that primate lentiviruses might frequently jump between primate species and then rapidly adapt to the new host[17,18].

Studies that have modelled the expansion and contraction of various epidemics have indicated that the inter-relationships between lethal pathogens and their hosts are complex and frequently not comparable[19–22]. These questions can only be answered by understanding pathogen evolution, dynamics and spread within the population at large and within infected hosts. The propensity for HIV-1 to evolve rapidly in response to various immune and other host pressures indicates that this virus is more likely to become attenuated through a reduction of virulence rather than selection of resistant hosts.

Defining pathogen fitness and virulence

The phylogenetic approaches that are commonly used to describe the evolution of pathogens do not examine how these genetic differences actually impact on the physical attributes, often termed the fitness, of the pathogen. This is understandable, given the complexities of measuring fitness, which can be defined as the adaptability or reproductive success of an organism in a specific environment. In the case of HIV-1 research, fitness is frequently misrepresented or over-interpreted[23,24]. For example, HIV-1 virions in which the reverse transcriptase (RT) harbours mutations that confer resistance to antiretroviral drugs (ARVs) such as 3TC (Lamivudine or Epivir) seem less fit than virions carrying the wild-type RT[25,26]. However, this reduced fitness in a reconstituted *in vitro* assay does not necessarily imply that the drug-resistant virus will be less fit in a human host receiving ARVs. Fitness in each

environment can be inter-related but is not necessarily consequential. For the purposes of this Opinion article, HIV-1 fitness will be defined in a specific environment and only significant correlations between environments will be highlighted as being potentially consequential.

Fitness is not always synonymous with virulence, which is typically defined as the rate of host mortality as a consequence of infection[27] but which can be further refined to include the reproduction rate and pathogenic potential of the pathogen[28]. These parameters also define the fitness of a pathogen within a host. Typically, virulence is difficult to measure for viruses such as HIV-1, given the long asymptomatic period after infection. Therefore, intermittent measures of HIV-1 replicative fitness during disease can often provide insights into the rates of disease progression[29–32]. Confusion between these principles of virulence and fitness is introduced when examining fitness within the host compared with examining fitness within a population[24,33]. Maynard, Ewald, Anderson, May, Novak and others[22,33–36] suggest that pathogen strain 'A', with slow replicative fitness but high transmission efficiency, will be favoured in a host population over strain 'B', with increased replicative fitness. However, when the strains compete together in a single host, it is clear that strain 'B' is more virulent and would out-compete and, consequently, be fitter than, strain 'A'. In terms of a pathogen spreading through a host population, high pathogen fitness is then defined by a reduction in reproduction rate and virulence without a loss in transmission. This article will address the possible direct relationship between HIV-1 virulence and replicative fitness but it is important to note that attenuation of virulence is not always synonymous with lower replicative capacity. For example, the attenuation of myxoma virulence in the Australian rabbit population might be more related to changes in virus-mediated immunomodulation than to lower replicative fitness in rabbits[13,14].

Expansion of HIV-1 in humans

HIV-1 was first introduced into the human population from the chimpanzee subspecies *Pan troglodytes troglodytes*[37–39]. Most recently, Keele *et al.* have discovered a region in southern Cameroon where chimpanzees carry SIV strains (SIV$_{cpzptt}$) that are closely related to two distinct lineages of HIV-1, the main (M) and new (N) groups[37] (FIG. 1). The origin of the outlier (O) group might be related to a jump from *Gorilla gorilla*[40]. SIV$_{cpzptt}$ was introduced into the human population multiple
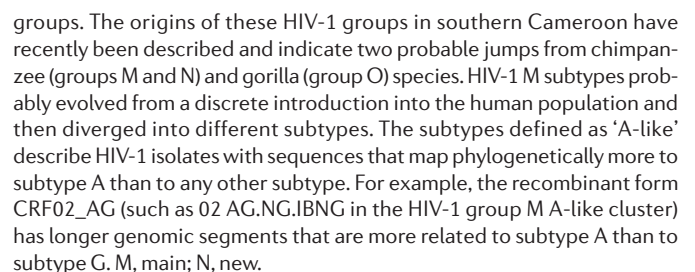
times, over decades to possibly centuries, but these transfer events probably started to increase in the 1920s to the 1950s as a result of human migration into this dense tropical region[5,37–39]. Similar to the pending introduction of influenza H5N1 from the avian to the human host[41], a distinct but possibly rare SIV$_{cpzptt}$ strain might have had the capacity for efficient replication and sexual transmission in humans[17,37]. Following this founder event, human population density and contact had to be sufficiently high for subsequent transmission and spread. It is possible that a single transmission of HIV-1 group M spawned the >60–80 million infections that have taken place since the beginning of the epidemic[37]. By contrast, HIV-2 originated in humans from a cross-species transmission from sooty mangabeys in west Africa (near or in Guinea-Bissau) around 1930–1955 (REFS 42,43). In the 1980s, the incidence of HIV-1 group M in central Africa and HIV-2 in west Africa expanded exponentially but clearly at different rates, as the prevalence of HIV-1 M infections (28 million) far exceeds that of HIV-2 infections (<1 million)[6,44,45]. In stark contrast, HIV-1 groups O and N are responsible for <25,000 infections, most of which are in Cameroon and Gabon[6,46–49]. The expansion and divergent evolution of HIV-1 group M into diverse subtypes (FIG. 1) has been dated to 1956–1976 and might have coincided with human emigration and the seeding of new regional epidemics in central Africa[5,50,51].
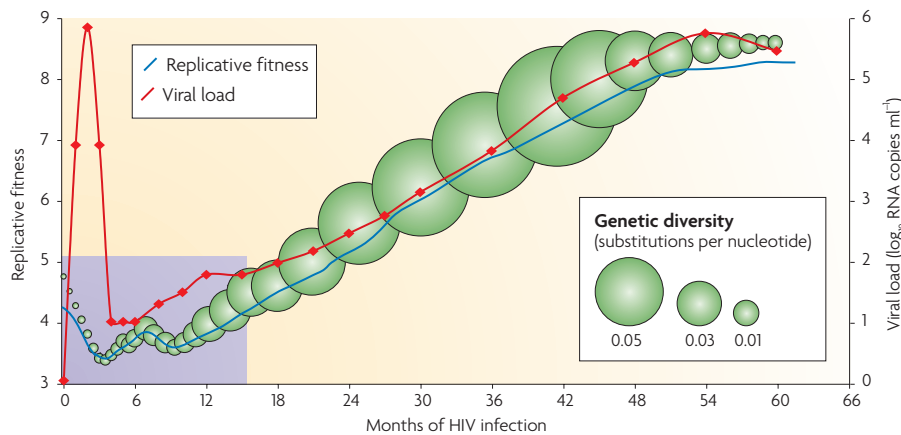
HIV-1 subtypes share 70–90% sequence identity, groups share <70%, and HIV-1 and HIV-2 can differ as much as 50% at the nucleotide level (FIG. 1). Given this extreme genetic diversity, is it reasonable to assume that not all HIV types, groups, subtypes and even isolates have evolved to maintain similar virulence? Although many research articles have described genotypic differences between subtypes, there are only a handful of studies that have examined potential phenotypic differences among the human lentiviruses[52–56]. No studies have modelled how phenotypic or 'fitness' differences among these viruses might affect disease progression in infected individuals or the general spread of the virus in the human population. This article can only provide predictions of temporal HIV-1 attenuation based on published studies and significant trends during the human epidemic. To understand how attenuation of virulence can occur, it is important to understand the population dynamics and fitness of HIV-1 in each infected host and during host-to-host transmission.

How HIV-1 infects, and evolves in, a host

In relation to other sexually transmitted pathogens, HIV-1 is transmitted with moderate efficiency by sexual contact and apparently greater efficiency by direct blood-to-blood contact (for example, needle sharing by intravenous drug users)[57,58] or vertical mother-to-child perinatal transmission[59]. There are many cases of discordant couples in which the HIV-positive individual fails to transmit the virus to the uninfected partner despite frequent opportunity[60]. A schematic of transmission events and disease progression is provided in FIG. 2 and BOX 1. An increased viral load in the donor and consequently in the inoculating dose increases the transmission efficiency[61,62]. An increased viral load in the donor also corresponds to greater genetic diversity of the inoculating HIV-1 population (termed the virus isolate)[63] (FIG. 2), and yet few HIV-1 clones will establish an infection in the recipient[64]. This dramatic bottleneck might be related to accessing the initial target cells (cells of the dendritic lineage)[65,66] and establishing a productive, systemic infection. Based on extensive studies on RNA viruses, it is clear that restrictive genetic bottlenecks invoked by various selection pressures will reduce replicative fitness[67–69]. Unfortunately, nearly all of these viral fitness studies have been done in tissue-culture infections and not in animal models or in defined donor–recipient transmission pairs. Greater HIV-1 genetic diversity during acute or early infection has been associated with more rapid disease progression[70]. In addition, the genetic diversity of HIV-1 seems to correlate directly with replicative fitness[29].

HIV is the obvious aetiological agent of AIDS, yet it is commonly assumed that the phenotypic characteristics and replication efficiency (the *ex vivo* fitness) of the infecting, wild-type HIV isolates have little impact on the rate of disease progression. Much attention has been focused on host correlates of disease progression, such as the strength of HIV-specific immune responses[71–77] and host genetic polymorphisms that might affect HIV-1 replication[78–82]. RNA load remains the best predictor of HIV disease progression[83] and the dramatic reductions in this load achieved by ARV treatment can delay disease progression[84]. It is important to note that a high viral load is not an absolute in relation to the severity of the disease. In non-human hosts such as sooty mangabeys, a high SIV load is not associated with clinical symptoms[85]. Recent studies indicate that the decline in CD4+ T-cell counts, and not the increase in viral load, is a better predictor of disease progression[86].

Figure 1 | **Phylogenetic tree of human and simian lentiviruses.** The genetic similarity between different HIV and simian immunodeficiency virus (SIV) strains was compared by aligning the full genome sequences of 87 human and simian lentiviruses using ClustalX v.1.83 (the accession numbers are available on request). Phylogenetic trees based on nucleotide distance were constructed by neighbour-joining methods as implemented in ClustalX with 1,000 bootstrap resamplings (not presented) and schematically represented with the TreeView program. HIV-2 and HIV-1 share only 50–60% sequence identity and cluster at distinct locations on the phylogenetic tree whereas SIV$_{cpz}$ branches out from the root of the HIV-1

groups. The origins of these HIV-1 groups in southern Cameroon have recently been described and indicate two probable jumps from chimpanzee (groups M and N) and gorilla (group O) species. HIV-1 M subtypes probably evolved from a discrete introduction into the human population and then diverged into different subtypes. The subtypes defined as 'A-like' describe HIV-1 isolates with sequences that map phylogenetically more to subtype A than to any other subtype. For example, the recombinant form CRF02_AG (such as 02 AG.NG.IBNG in the HIV-1 group M A-like cluster) has longer genomic segments that are more related to subtype A than to subtype G. M, main; N, new.

Figure 2 | **A hypothetical example of changes in replicative fitness and viral load during HIV-1 disease progression.** An individual is typically infected by a few HIV-1 clones (depicted by a small green circle), which dramatically increase in copy number but not in genetic diversity during the first 1–2 months of infection. Following this acute infection period, viral load is reduced partly as a result of strong HIV-specific cell-mediated immunity. The virus population is thought to oscillate between expansion of HIV-1 populations owing to immune escape from existing HIV-specific cytotoxic T-lymphocyte (CTL) clones and contraction caused by new genetic bottlenecks that are induced by newly emerging CTL clones. The replicative fitness and genetic diversity of the HIV-1 population seem to track closely together and, following early disease (purple box), both increase at a relatively linear rate with the length of infection. This increase in replicative fitness correlates with increases in viral loads (right axis) and decreases in CD4+ T-cell counts (not shown). The scale for replicative fitness is arbitrary but is derived from the relative HIV-1 fitness values, that is, the ability of one HIV-1 isolate to out-compete another in *ex vivo* dual-virus competition experiments.

The first evidence that specific viral genetic traits might affect virulence was the identification of long-term non-progressing individuals harbouring a defective HIV-1 isolate lacking a *nef* open reading frame[87,88]. Mutations or deletions in this accessory-protein-coding gene can significantly impair fitness in tissue-culture infections[89]. Even with this discovery 10 years ago, the effect of relative HIV-1 replicative fitness on disease progression has not been the subject of intense study owing to difficulties in accurately measuring HIV-1 fitness.

There have been only limited studies examining how virus fitness changes during natural infections[31,32,90,91]. The replication capacities of different HIV-1 isolates are commonly compared by measuring *ex vivo* growth kinetics in single virus infections[31,32]. The viruses used in many studies are not isolated from infected patients but rather are chimeric recombinant viruses containing gene(s) from the patient's virus within the genome of a laboratory HIV-1 strain[32,92]. Small environmental differences in separate single virus infections and the inherent variability of detection assays can prevent accurate estimates of viral fitness by these methods. By contrast, dual virus infections or competition assays involve multiple rounds of virus replication, are less affected by environmental changes and, as a result, can quantify small but significant

differences in fitness[29,93,94]. In HIV research, multiple and single-cycle single virus infections, and competition experiments, have been used in studies that described impaired HIV-1 replicative fitness conferred by drug-resistance mutations[95,96]. Nevertheless, the impact of these drug resistance mutations in primary HIV-1 isolates (as opposed to chimeric viruses) has not been fully assessed.

Initial reports using HIV-1 competition assays in peripheral blood mononuclear cell (PBMC) cultures to measure fitness indicated that many long-term survivors harbour HIV-1 with an impaired replication capacity, whereas rapid progressors can be infected with more aggressive HIV-1 isolates[30]. Subsequent studies indicated that the genetic diversity and fitness of the intra-patient HIV-1 population continues to increase during disease and to diverge from the founder HIV-1 clones[29,63,70] (FIG. 2). This diversity reaches an inflection point just before the inception of AIDS symptoms. During the asymptomatic period, the steady increase in HIV-1 diversity occurs concomitantly with increasing viral load and, more significantly, with increasing *ex vivo* replicative fitness of the infecting HIV-1 isolate[29] (FIG. 2). Red Queen dynamics (in which the interaction between a parasite and its host leads to a constant evolutionary process of adaptation and counter-adaptation) predict

that increasing replicative fitness must be associated with a continual expansion of the genetic breadth and size of a population[97,98]. Any strong selective pressure can result in the contraction of population size, the appearance of deleterious mutations and decreasing fitness[99–101]. Interestingly, the introduction of ARVs as a strong selective pressure during asymptomatic disease resulted in a dramatic decrease in HIV-1 load, genetic diversity and replicative fitness, even in the absence of drug resistance mutations[29].

Following transmission, it is possible that the replicative fitness and genetic diversity of newly infecting HIV-1 isolates is lower than that of the inoculating virus from the donor (BOX 1). Such a continual increase in HIV-1 diversity and fitness is in direct conflict with the idea that the HIV-specific acquired immune response can counter this viral expansion[102]. Discrepancies in these data might simply be due to the time period of sampling and analyses. HIV-1 genetic diversity and fitness only increases after the first one to two years of infection[29] (FIG. 2). Immediately following acute infection, both host genetics and the HIV-specific immune response might have crucial roles in establishing a genetic diversity and fitness set point, which might also correspond with the well-documented viral-load set point[83,103] (FIG. 2). Cell-mediated immunity could be the most dominant factor in specifically reducing viral load and, as a consequence, fitness and diversity[104–107].

How can HIV-1 virulence be attenuated?
The genes at the human leukocyte antigen (HLA) loci are extremely variable. In humans in most geographical regions, it is typically rare to find a perfect match in the HLA A–G alleles that encode major histocompatibility complex (MHC) class I and in the six HLA D genes that encode MHC class II. Even in regions of specific ethnicities, there is considerable population diversity in HLA alleles, which encompass at least 21 major A, 35 B and 15 C alleles (see the dbMHC web site). Compared with Eurasian populations, HLA diversity is much greater in the main ethnic groups of sub-Saharan Africa; a study of >4,000 individuals characterized at the A, B and C loci found no preference for a single allele[108]. The least HLA diversity is often observed in more homogeneous Caucasian populations, most notably, the Caucasian Australian population (see the dbMHC web site)[109].

How might a human population that is diverse in terms of HLA type or other host polymorphisms influence HIV-1 evolution?

Quantitative aspects of the HIV-specific immune response (for example, high levels of HIV-specific cytotoxic T lymphocytes (CTLs) versus low levels) are self-limiting because this immunity is not transferred between individuals. However, a qualitative difference in terms of what the immune system 'sees' in the infecting HIV-1 strain could have a significant impact on virulence. A mismatch of HLA genes between the donor and recipient of a transmission pair would result in the presentation of a different set of HIV-1 peptide epitopes to CD8+ T cells in the recipient that were not recognized in the donor[110,111]. With each new transmission, the infecting virus must then evolve to escape this pressure, which in turn seems to reduce replicative fitness[112,113]. In human populations with a low HLA diversity (for example, in Australia), CTL escape mutations might become fixed along with compensatory mutations that restore or maintain HIV-1 virulence[109] (BOX 1). By contrast, the constant passage of HIV-1 through humans of different HLA types (for example, in Africa) might prevent the accumulation of compensatory mutations, which could stabilize CTL escape mutations (BOX 1). However, a model involving only HLA types is obviously too simplistic, considering the effects of other host factors. For example, polymorphisms in other host restriction factors, such as ABOBEC3G/3F and TRIM5α, could also alter disease progression[114,115]. Furthermore, host polymorphisms in the HIV-1 co-receptor CCR5 and its ligands (for example, CCL3L1) alter co-receptor or ligand expression levels and, as such, affect the ability of HIV-1 to use the co-receptor[78–82]. If these polymorphisms exist at high frequencies in the human population, they too could result in an oscillation between HIV-1 sequences that, in this example, reduce or increase co-receptor avidity, a correlate of replicative fitness.

In general, HIV-1 virulence might attenuate if, on average, the loss in fitness that occurs following transmission is greater than the gain in replicative fitness that occurs during asymptomatic disease progression (BOX 1). Loss of fitness is probably due to the genetic bottleneck during transmission and/or escape from host restrictions or the host immune response during early disease. The continual introduction of new selective pressures and genetic bottlenecks would lead to the accumulation of deleterious mutations, which in turn would reduce replicative fitness or virulence. However, these hypotheses have not been empirically tested using HIV-1 transmission pairs.

Box 1 | A model for HIV-1 attenuation

The figure shows a model for HIV-1 attenuation in a human population with diverse and limited genetic polymorphisms that are associated with HIV-1 disease progression. In this illustration, patient A is the founder who will infect patient B approximately 4 years into the infection of A. Before transmission from patient A to patient B, the replicative (or pathogenic) fitness of the virus has increased from 4 to 7 (arbitrary units, see FIG. 2 legend). Although the replicative fitness increases in patient A, the transmission from patient A to patient B results in a genetic bottleneck that resets the fitness to a lower baseline or set level (dotted line). As patients A and B are not an HLA match, the cell-mediated immune response (or CTL response) of patient B recognizes a different set of HIV-1 epitopes than in patient A. In patient B, mutations in this new set of epitopes are necessary for CTL escape and for the virus to expand in the face of this anti-viral response. However, this 'escape' from the CTL response comes at a fitness cost and reduces the replicative fitness in the first 1–2 years of infection. Following this escape, the infecting HIV-1 isolates seem to continually increase in number (that is, the viral load increases)[83] and gain replicative fitness[29] (FIG. 2). This process is repeated with each transmission event in patients with diverse HLA alleles.

Patient B transmits to patient C and patient C transmits to patient D during acute infection. Increased transmission efficiency is associated with higher viral loads, which are highest during acute infection. However, the short time interval of acute infection provides limited opportunity for transmission. As the disease progresses and viral load increases, the efficiency of transmission will increase even though opportunities for transmission can remain constant. Consequently, more transmission events might result from prolonged, compared with rapid, disease progression (for example, transmission from patient D to E) even when accounting for the high transmission efficiencies during acute infection[149,150]. In this model, owing to the fitness bottlenecks at transmission and the subsequent loss in fitness caused by CTL escape[111–113], the circulating HIV-1 isolate will start to lose virulence. This loss in virulence would decrease pathogenesis, prolong the time to AIDS or even result in a non-symptomatic infection. The complete clearance of HIV-1 is unlikely because of the stable integration of the provirus into memory T cells with long half lives and possible re-activation of virus replication in these cells[160,161].

In patients with limited genetic diversity and possible HLA matches, loss of fitness might be associated with the genetic bottleneck following transmission but the infecting HIV-1 isolate could be more 'resistant' to the breadth of possible CTL responses. For example, patient A transmits HIV-1 to patient X 1.5 years into the infection. After 1.5 years, the HIV-1 isolate in patient A has mutated the dominant HIV-1 epitopes and escaped CTL activity. When patient A infects patient X (who is a match at some or all of the HLA alleles), the virus from patient A cannot be efficiently recognized by the CTL response of patient X and so could result in a more rapid disease progression. An HIV-1 isolate passing through patients of limited HLA diversity might actually increase in virulence. CTL, cytotoxic T lymphocyte; HLA, human leukocyte antigen.
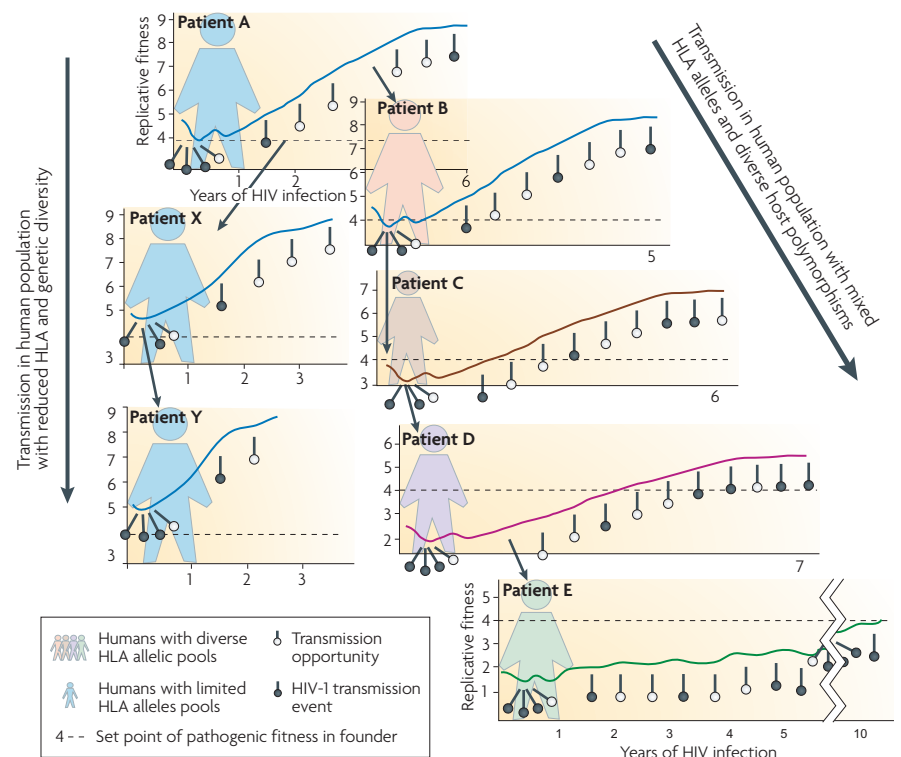
Is there evidence for attenuation of HIV-1?

With the exceptions of anecdotes, a few case reports and one published study on a large cohort, there is no evidence that HIV-1 virulence has either increased or decreased during the past 20–30 years of the pandemic[116–121]. One of the main obstacles in comparing disease progression in the mid-1980s with the current day epidemic in the developed world is the significant medical advancements that have been made. In the developing world, until recently, few if any cohorts have been analysed for disease progression. Finally, owing to increased awareness and education about AIDS, patients are now diagnosed earlier, sometimes during acute infection, whereas in the 1980s many patients did not present to a clinic until the onset of AIDS. As a result, there is often the impression that HIV-1 infections are less aggressive now than they were at the beginning of the pandemic.

In a study published last year, >100 primary HIV-1 isolates were obtained from patient samples in Antwerp, Belgium, but only twelve could be matched from the periods 1985–1987 and 2002–2003 (REF. 121). All 24 viruses from both time periods were competed against each other in PBMC from HIV-negative donors. Based on >500 head-to-head virus competition experiments, the recent HIV-1 isolates were significantly less fit than the historical isolates[121]. These findings indicate that HIV-1 might have attenuated in replicative fitness over the past 15–18 years. If the fitness of the infecting HIV-1 strain is a predictor of disease progression[29–32], reduced *ex vivo* fitness in PBMC might be a strong correlate of decreased virulence. However, these results should be interpreted with caution owing to the limited sample size involved. Furthermore, the cross-sectional design of this study results in sampling of HIV-1 isolates at a single time point during disease progression, which was

controlled for disease stage based on CD4+ T-cell count and viral load. Nonetheless, the increased fitness of the 1980 viruses versus the 2000 viruses was significant and greater than any increase in HIV-1 fitness observed during disease progression[29,121]. Future studies on large cohorts that span the epidemic temporally in various geographical regions are warranted but, as described below, these studies will again be limited to analyses of HIV-1 virulence (that is, replicative fitness) as rates of disease progression will not be available (see below).

Rates of disease progression have now been examined in a European HIV-infected cohort spanning the years 1986–2002 (REF. 119). The slopes of CD4+ T-cell decline were calculated in antiretroviral-treatment-naive patients with confirmed dates of infection, at no fewer than nine time points over periods >1 year. Following this early period of the AIDS epidemic, there seems to be some evidence for slowing rates of disease
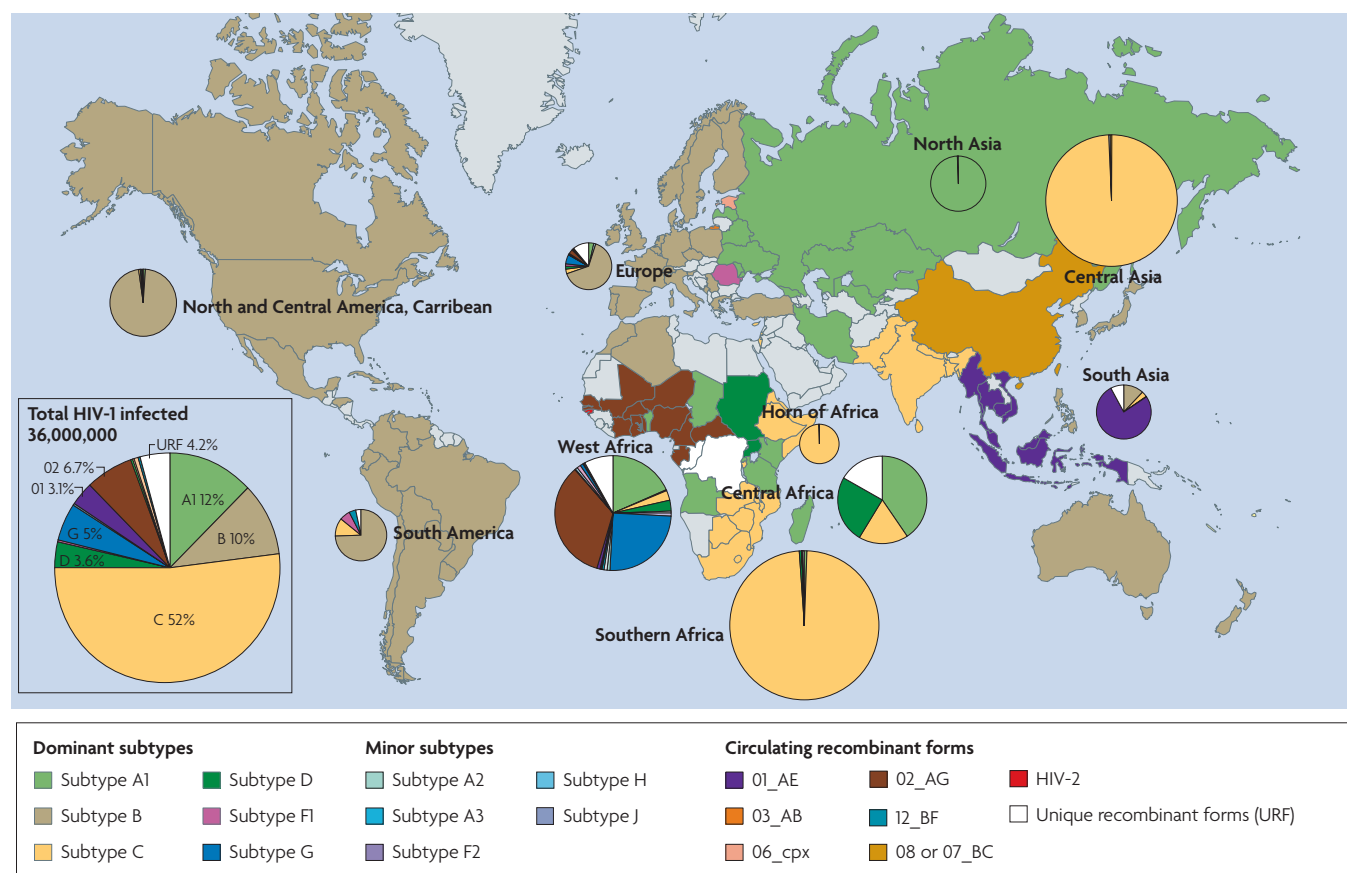
Figure 3 | HIV-1 diversity in the worldwide epidemic. The frequency of each HIV-1 subtype and recombinant form was estimated in each country based on published findings. A complete breakdown of subtype prevalence per country and the countries present in each region are listed in the Supplementary information S1 (table). The countries are colour-coded based on the dominant HIV-1 group main (M) subtype. The countries coloured grey have a low level of HIV-1 prevalence or were not represented in the scientific literature related to HIV-1 subtype prevalence. The pie charts depict the proportion of each subtype or recombinant form in each geographical region. The size of the pies is proportional to the number of HIV-1 infected individuals in that particular region.

progression from 1992–1998. Interestingly, the period following 1992 also corresponds to increasing use of combination ARV treatment, which could only be indirectly related to this attenuation as none of the patients received ARVs during the study period. It is possible that drug resistance mutations might have appeared at a higher frequency during sub-optimal treatment from 1992–1998, that is, before the widespread use of highly active antiretroviral therapy (HAART). These mutations have been associated with reduced fitness[95,96] but their presence following a new transmission and in the absence of ARV treatment is low (<5–20%)[122,123] and would not fully explain why the rates of disease progression might have decreased between 1992–1998.

The detection of fluctuations in the rate of CD4^{+} T-cell decline at the start of the HIV-1 epidemic in Europe is surprising given the preliminary data suggesting that replicative fitness might have been higher in the 1980s compared with the 2000s (REF. 121). Slopes of CD4^{+} T-cell decline can be heavily influenced by the length of longitudinal follow-up, the follow-up period post-infection, and the number of data points[124]. For example, most patients in the 1980s presented to a clinic with advanced disease when the slopes of CD4^{+} T-cell decline are commonly lower than in early disease (especially once the level falls below 200 CD4^{+} T cells per μl)[124,125]. Therefore, 'slow' disease progression during 1986–1988 might be associated with the accrual of patients with more advanced disease. In general, natural history cohorts starting at early or acute infection and ending with AIDS-defining illnesses or morbidity (typically 3–10 years without ARV treatment) provide the best estimates of CD4^{+} T-cell decline and disease progression rates[125]. In the developed world, cohorts from the 1980s are not readily available owing to late diagnosis, whereas the current cohorts are difficult to assess for the natural history of disease due to ARV intervention. Finally, given the reduced HLA diversity and greater ethnic homogeneity in the Caucasian population, the attenuation rate of subtype B HIV-1 virulence might be less dramatic than the attenuation possible with non-B subtypes in sub-Saharan Africa.

In vivo and *ex vivo* fitness differences

Early observations on the rates of disease progression in west Africa showed that HIV-2 infections were less aggressive than HIV-1 infections[126,127]. HIV-2 is also transmitted less frequently than HIV-1 in human populations of similar demographics and similar opportunities for sexual contacts[128,129]. Whereas HIV-1 has spread across the globe, founder events of HIV-2 in areas other than west Africa did not result in regional epidemics (FIG. 3). Even HIV-1 infections are not evenly distributed among the different groups and subtypes: group N infections have only been characterized in five Cameroonians, whereas ~30,000 group O infections are estimated in Cameroon and Gabon[6,46].

HIV-1 group M subtype C has spread more rapidly than any other M subtype, partly as a result of recent subtype C pandemics in southern Africa, south America and Asia[130–133] (FIGS 3,4a; Supplementary information S1 (table)). 'Pure' HIV-1 subtype C or HIV-1 recombinant forms containing at least the envelope gene of subtype C are now responsible for >50% of the HIV-1 infections worldwide[130]. A founder event of subtype C or a C-containing recombinant form in many regions has also resulted in an apparent displacement of existing HIV-1 subtypes (such as subtypes B and CRF01_AE in south China[131,132], many subtypes in Kinshasa, Democratic Republic of Congo[134] and subtype B in southern Brazil[133]) (FIGS 3,4a). This displacement could be due to the introduction of subtype C into a population by different transmission routes (heterosexual transmission versus intravenous drug use) or increased sexual activity (for example, commercial sex workers)[135]. A subtype might initially predominate in a specific transmission group but there is little supporting evidence to suggest that subtypes A, B, C, D and CRF01_AE (also referred to as E) are any more or less transmissible by a specific route, in a specific ethnic group or in specific cell types[53,136–141]. In addition, such initial predominance is generally lost during an epidemic. For example, in Thailand HIV-1 subtypes B and CRF01_AE were segregated to intravenous drug users and heterosexuals, respectively, in the late 1980s but are now more uniformly mixed in the Thai population[142].

Recent studies have attempted to establish the *ex vivo* 'pathogenic' and 'transmission' fitness of different HIV types, groups and subtypes[52]. *Ex vivo* 'pathogenic fitness' has been previously described as the relative replication capacity of HIV-1 in primary blood cells (CD4^{+} T cells and PBMCs), which in turn, might be related to HIV-1 virulence[30,53]. By contrast, *ex vivo* 'transmission fitness' is measured by competing primary HIV-1 isolates in cells of the dendritic lineage, which are derived from blood monocytes or human skin[53,143]. Future dual HIV-1 competition experiments using vaginal or cervical biopsy explants might be the best model for transmission fitness[65,66].

To first establish the 'pathogenic fitness' (or virulence) of HIV, pair-wise competitions were done with eight HIV-2, six HIV-1 group O and 15 HIV-1 group M isolates (2 subtype A, 5 subtype B, 4 subtype C, 2 subtype D and 2 subtype CRF01_AE) in PBMCs[144] (FIG. 4b). These viruses were classified as using either the CCR5 (R5) or the CXCR4 (X4) co-receptor for entry. When HIV isolates of the same phenotype (X4 or R5) were competed against each other, the following fitness order (based on >2,000 competitions) was observed (FIG. 4b): HIV-1 group M (subtype A, B, D and CRF01_AE) had a greater fitness than subtype C, which in turn was greater than HIV-2, and HIV-1 group O showed the lowest fitness.

With the exception of subtype C, this order reflects the prevalence of these human lentiviruses in the human population and the proposed rates of transmission efficiency[128,129]. The R5 HIV-1 subtype C isolates were at least 100-fold less fit than HIV-1 isolates of any other group M subtype[53]. Aside from subtype C, no other HIV-1 subtype has shown a significant fitness difference in group M[53,144]. Subtype C HIV-1 isolates are preferentially CCR5-tropic and non-syncytium-inducing (NSI) throughout disease, whereas infections with HIV-1 isolates of other subtypes result in a switch from CCR5 to CXCR4-tropic virus in approximately 50% of late disease cases. A few rare X4 subtype C isolates have been obtained from infected Zimbabweans late in disease[145] but even the switch from R5 to X4 did not restore fitness, compared with other group M isolates (Nankya, I. *et al*, unpublished data).

What causes differential spread?

Differential spread of human lentiviruses in the host population is controlled by both transmission efficiency and opportunity, which is collectively referred to as R_0 (REF. 22). Several models of microparasite expansion in humans have been proposed[20,22,33,146,147] but most assume that during the early stages of the epidemic, the rate of infection (dY/dt) simply increases as a function of the number of infected individuals (Y) during an infectious period (v = rate of moving into a non-infectious state), the probability of infecting a new host (β) and the frequency of sexual contact (c) (REF. 22).

$$dY/dt \sim (\beta c - v)Y \qquad (1)$$

The opportunities for HIV-1 transmission relate to the number of sexual contacts, which are more frequent during acute infection and in the asymptomatic period of disease than during AIDS. An infected individual (without
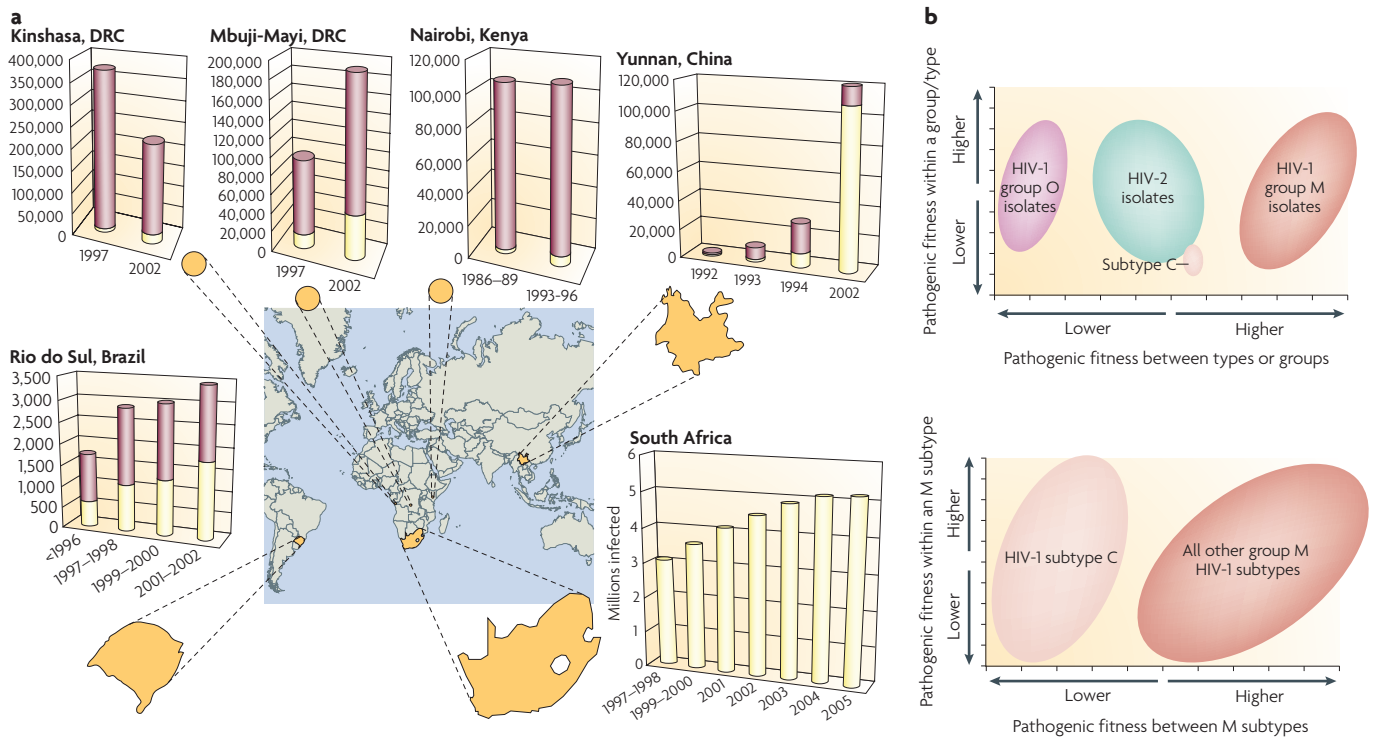
Figure 4 | **Relationship between the increasing prevalence of HIV-1 subtype C and its low pathogenic fitness.** The prevalence of HIV-1 subtype C or subtype C-containing recombinant forms has increased in proportion to other HIV-1 subtypes in Rio do Sul, Brazil (from 35% in 1996 to 52% 2002)[133], in Kinshasa and Mbuji-Mayi, Democratic Republic of Congo (DRC) (from 2.1% and 16.3% in 1997 to 9.7% and 25% in 2002, respectively)[134] and in Yunnan, China (from 5.1% in 1992 to 90% in 2002)[162,163]. By contrast, subtype C is dominant in South Africa[164] and did not increase in proportion to subtypes A and D or recombinant forms in Kenya[165]. The number of HIV-1 cases increased in all of these regional epidemics (except in Kinshasa)[6].

a | The number of HIV-1 infections in specific years is subdivided into subtype C (yellow) and non-subtype-C (maroon) HIV-1 infections. **b** | A schematic representation of the pathogenic fitness of human lentiviruses, derived from >3,000 pair-wise dual HIV-1 competition experiments[26,27,49,50,108,120,123,125,165]. The coloured ovals plot the fitness of primary HIV-1 isolates competed against isolates of the same type, group or subtype (y axis; mean fitness of 1 or equal fitness) compared with the fitness of primary HIV-1 isolates competed against isolates of different types, groups or subtypes. Each oval encompasses the fitness (x and y fitness values) of at least 10 to 20 primary HIV-1 isolates. M, main; O, other.

general knowledge of the transmission routes or infection status) could engage in several sexual encounters during the acute or early phase (approximately 3 months)[148], the period of highest HIV-1 load[61,62]. Therefore, the exponential growth of HIV-1 infections in the early 1980s could have been primarily the result of transmission from acutely infected men having sex with men or from intravenous drug users[148–150] (BOX 1). However, in heterosexual populations with a reduced frequency of sexual contacts, a donor might transmit HIV-1 to more recipients during a long asymptomatic period (2–5 years) of infection, with moderate viral load, than during the acute or early infection stage (1–3 months), with high viral load. Low HIV-1 virulence, which could lead to longer asymptomatic periods, could also increase the opportunity for transmission (BOX 1).

Similarly, poor fitness of HIV-1 group O and HIV-2 compared with HIV-1 group M isolates was found when competitions were carried out in both PBMCs (*ex vivo*

pathogenic fitness) and in dendritic–T-cell cultures (*ex vivo* transmission fitness)[144]. In relation to this *ex vivo* model, poor transmission efficiency has been well documented for HIV-2 compared with HIV-1 in the human populations in which both circulate[128,129]. By contrast, subtype C HIV-1 isolates had similar *ex vivo* transmission fitness[53] compared with group M subtype B isolates in Langerhans cells even though subtype C isolates are at least 100-fold less fit in PBMC cultures[53]. *In vivo* observations indicate that subtype C HIV-1 is transmitted as efficiently as other HIV-1 group M isolates in human cohorts[135,137] and in rhesus macaques, through the use of SIV–HIV *env* chimeric virus (SHIV-MJ4)[151]. These *ex vivo* and *in vivo* observations imply that HIV-1 subtype C is efficiently transmitted but is less virulent than other HIV-1 group M isolates.

HIV-2 and HIV-1 groups O and N might have had limited expansion in the human population due to poor host adaptation and transmission efficiencies. By contrast, HIV-1

group M seems to be more virulent as well as more transmissible. Therefore, the progenitor of the HIV-1 group M lineage might have been more 'fit' for human infection, promoting further adaptation through rapid evolution and passage through the human population. New founder events in segregated human populations in central Africa might have led to subsequent divergence into subtypes. HIV-1 subtypes probably evolved and diverged under similar selection pressures, unless infecting a human population with distinct genetic polymorphisms that had an effect on virulence. Adaptation to the human population followed by attenuation of lethal virulence might, however, be occurring at different rates for divergent HIV-1 clades. If diversity in HLA types has a significant role in HIV-1 evolution, subtypes predominating in Africa, such as subtype C, might attenuate virulence at a faster rate than HIV-1 subtype B, which infects the Caucasian population of lower HLA diversity in the developed world.

Prevalence and reduced virulence

HIV-1 subtype C might be in a more advanced stage of attenuation than other HIV-1 subtypes[53,96,144]. If it is less virulent than other subtypes, subtype C infections might result in slower disease progression, longer periods of asymptomatic infection and more opportunities for transmission; that is, it would be associated with an increase in R_0 (see equation [1]; BOX 1). To support a model of disproportionate expansion of HIV-1 subtype C in the human population (FIGS 3,4a), the transmission efficiency of subtype C must be greater than, or comparable to, other HIV-1 group M strains. Preliminary data suggest that subtype C is transmitted as efficiently as other HIV-1 group M subtypes[53,135,137,151]. Therefore, decreased virulence could have a key role in the spread of subtype C by increasing the opportunity for transmission[33]. So far, comparisons of cohorts infected with subtype C and other subtypes show similar or higher viral loads in the subtype C-infected individuals[152,153]. These initial observations would argue against the possible subtype C attenuation and would support the more popular assumption, that subtype C is more virulent.

However, there are few studies on the natural history and progression of patients infected by any HIV-1 subtype other than subtype B[154–157]. Two studies have described faster disease progression in individuals infected with subtype D compared with subtype A- or subtype C-infected individuals in Uganda and Tanzania[154–156]. The possibility of greater subtype D virulence has been attributed to a higher propensity to switch from the NSI/R5 phenotype to the more aggressive syncytium-inducing (SI)/X4 phenotype[158]. By contrast, subtype C rarely switches from the NSI/R5 to the SI/X4 phenotype. At a recent presentation at the sixteenth International AIDS Conference in Toronto, Canada[159], interim analyses of disease progression were described in a cohort of 256 subtype A-, C- and D-infected women, recruited at acute or early infection and followed for a mean of 36 months. The mean slope of CD4+ T-cell decline in the subtype C-infected women from Zimbabwe was 2.3-fold slower than in subtype A- and D-infected women from Uganda ($p<0.008$). However, there were no differences in the mean viral loads at 3 and 12 months between the countries or between different subtypes. Slower disease progression, as described by slower declines in CD4+ T-cell counts, was not attributable to any other factors (such as opportunistic infections, sexual activity, diet, age and weight) apart from being infected

with subtype C versus subtype A or D. In the Bantu corridor extending from Zimbabwe to Uganda, the frequency of HLA-A, -B and -C alleles and other host polymorphisms (for example, CCR5 deletions or promoter polymorphisms) are similar and could rule out obvious human genetic differences skewing HIV-1 evolution[108].

Summary

The attenuation of infectious pathogens remains contentious and many doubt that the spread of HIV-1 through the human population will lead to decreased virulence as HIV-1 isolates actually gain replicative (or pathogenic) fitness during infection of human hosts. However, this gain might not be sufficient to reverse the loss in pathogenic fitness that occurs during transmission and early disease. The extreme genetic bottleneck following transmission reduces the HIV-1 population size and diversity by at least 10,000-fold. *In vitro* studies indicate that this genetic reduction correlates with a significant fitness loss. The few HIV-1 clones establishing infection must evade the immune system, resulting in yet another series of genetic bottlenecks reducing fitness. Therefore, attenuation of virulence might occur if the net fitness loss during transmission or early disease is greater than the increase in pathogenic fitness during disease progression and between transmission events. Greater diversity in HLA alleles in African populations compared with Caucasian populations might result in stronger immune pressure in the human population and possibly faster rates of HIV-1 attenuation. Is the global dominance of HIV-1 subtype C related to an attenuation of virulence? Does subtype C infection lead to slower disease progression and more opportunities for new transmission events? These questions might hold the key to the future of the HIV-1 epidemic and could provide a new focus for ARV treatment and vaccine design.

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Acknowledgements

Competing interests statement

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