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Review

Impact of human immunodeficiency virus (HIV) subtypes on HIV-associated neurological disease

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Among the many variables affecting transmission and pathogenesis of the human immunodeficiency virus type 1(HIV-1), the effects of HIV subtypes, or clades, on disease progression remain unclear. Although debated, some studies have found that the variable *env* and *pol* sequences of different subtypes of HIV-1 may endow some subtypes with greater degrees of cell tropism, virulence, and drug resistance, which may lead to differences in overall disease progression. HIV-associated dementia (HAD) appears to be associated with viral diversity and markers of immune activation. Africa has the highest prevalence of HIV, largest viral diversity, and is where clade recombination occurs most frequently. All of these factors would suggest that HAD would pose the largest threat in this region of the world. Although investigations into the effects of different subtypes on overall disease progression are well documented, few have looked into the effects of subtypes on neurological disease progression. This review highlights the need for more international research involving the neurological effects and especially the clinical presentation of dementia for the entire range of the group M HIV-1 subtypes. *Journal of NeuroVirology* (2007) **13,** 291–304.

Keywords: dementia; HIV; subtype

Introduction

Among the myriad of variables influencing the transmissibility and pathogenicity of human immunodeficiency virus type 1 (HIV-1), the effects of HIV subtypes, or clades, on disease progression are still unclear (Hu *et al*, 1999). Studies have found inconsistent correlations between HIV-1 subtypes and indicators of disease progression and drug resistance. It is known that HIV enters the nervous system early in the course of the disease, and causes substantial neurological disease in some (Davis *et al*, 1992). However, the relationship of subtype specific differences in neurological disease progression are less clear and the paucity of data in this regard substantiates the need for further research.

The extensive genetic diversity that characterizes the human immunodeficiency virus (HIV) poses deep concerns for differences in disease progression, effective antiretroviral therapy (ART), and the outlook of the constantly evolving pandemic (Geretti, 2006). The sources of this diversity range from basic differences in the modes of transmission (Soto-Ramirez *et al*, 1996) to the complex functioning of genetic mechanisms (Roberts *et al*, 1988; Spira *et al*, 2003; Yang *et al*, 2005). Differences between the two distinct members of the lentivirus family of retroviruses, HIV-1 and HIV-2, have been well documented (Kanki *et al*, 1999). Although both have been shown to exhibit cross-species transmission, the crossovers originated from different primate species. Evidence supports that HIV-2 was originally transmitted from sooty mangabey (Clavel *et al*, 1986; Reeves and Doms, 2002), whereas HIV-1, the major source of the acquired immunodeficiency syndrome (AIDS) pandemic, appears to originate from chimpanzees (Gao *et al*, 1999; Sharp *et al*, 1995). Although HIV-2 seems to be less pathogenic than HIV-1 (Kanki *et al*, 1994; Marlink *et al*, 1994), the inherent resistance of HIV-2 to the entire class of non-nucleoside reverse transcriptase inhibitors, as well as other differences in drug susceptibility create treatment problems (Maniar *et al*, 2006; Ren *et al*, 2002; Yang *et al*, 1996).

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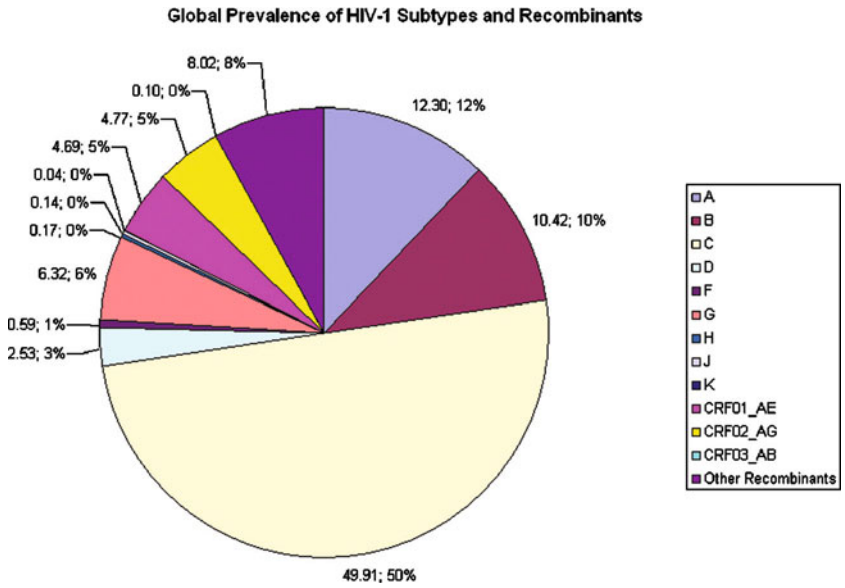

Figure 1 Global distribution of HIV-1 subtypes and recombinants, 2004. Created from data in the WHO/UNAIDS report titled "Global and regional distribution of HIV-1 genetic subtypes and recombinants in 2004" by Hemelaar et al. (2006).

The three classes of HIV-1, group M (major), group O (outlier), and group N (new, non-M, non-O), are believed to result from three separate species crossovers from chimpanzees (Gao *et al*, 1999; Hahn *et al*, 2000). Group M (Figure 1) is responsible for greater than 90% of reported HIV/AIDS cases worldwide and is represented by nine major clades or subtypes (A–D, F–H, J, and K). The virus also recombines frequently and intersubtype recombinants are believed to originate from individuals with multiple coexisting infections (Kijak and McCutchan, 2005). The resulting recombinant forms are divided into circulating recombinant forms (CRFs), which are common and defined as fully sequenced viruses identified in at least three epidemiologically unlinked individuals, and unique recombinant forms (URFs), which are only found in individuals. Studies have shown that an HIV-infected individual has "swarms" of HIV that are related, but nonidentical viral genomes, called quasispecies. Those with greater exposures also tend to have greater unique variation (McCutchan, 2006). The impact of CRFs on the future of the HIV pandemic cannot be overlooked. Although not genetically considered as major HIV-1 subtypes, CRF01_AE (formerly termed subtype E) and CRF02_AG represented around 9.5% of the global HIV-1 distribution in 2004 (Figure 1) (2006; Gao *et al*, 2001; Hemelaar *et al*, 2006; Robertson *et al*, 2000; Spira *et al*, 2003). Groups O and N were identified more recently and are responsible for a small minority of HIV-1 strains. Groups O and N are primarily endemic to Cameroon and its neighbors in West Central Africa, the region marked by the greatest diversity of HIV strains (Jaffe and Schochetman, 1998; Simon *et al*, 1998; Zekeng *et al*, 1994). Differences in disease transmission and drug susceptibility between the broad classes of HIV-1 and the separate subtypes of group M are a source of on-going debate and research. Some studies have even found differences in disease progression of HIV-1 depending on whether it was transmitted via homosexual intercourse and intravenous (IV) drugs as is common in developed nations, or through heterosexual and mother-to-child transmission characteristic of the developing countries (Hu *et al*, 1999; Soto-Ramirez *et al*, 1996).

Geographic distributions

In West Central Africa, where the original cross-species transmissions are believed to have occurred (Gao *et al*, 1999), virtually every subtype of HIV-1 group M, as well as strains of group N, group O, and HIV-2 are represented. However, in other parts of Africa and the various regions of the world, certain subtypes predominate over others, providing a background where differences in disease progression could be observed (Figure 2).

Within group M, subtype A and A/G recombinant account for around 17% of worldwide infections, the second leading source of the pandemic, and mainly preside in West and Central Africa. Subtype B, responsible for around 10% of infections, predominates in Europe, Australia, and the Americas, but is also observed in Africa, the Middle East, and Asia. Accounting for over 49% of worldwide infection, subtype C is mainly observed in Southern and Eastern Africa, India, Nepal, and China. Subtype D is mainly observed in East and Central Africa, but has also been found in West and Southern Africa, Eastern Europe, and Central Asia. Formerly referred to as subtype E, the A/E mosaic or CRF01_AE appears

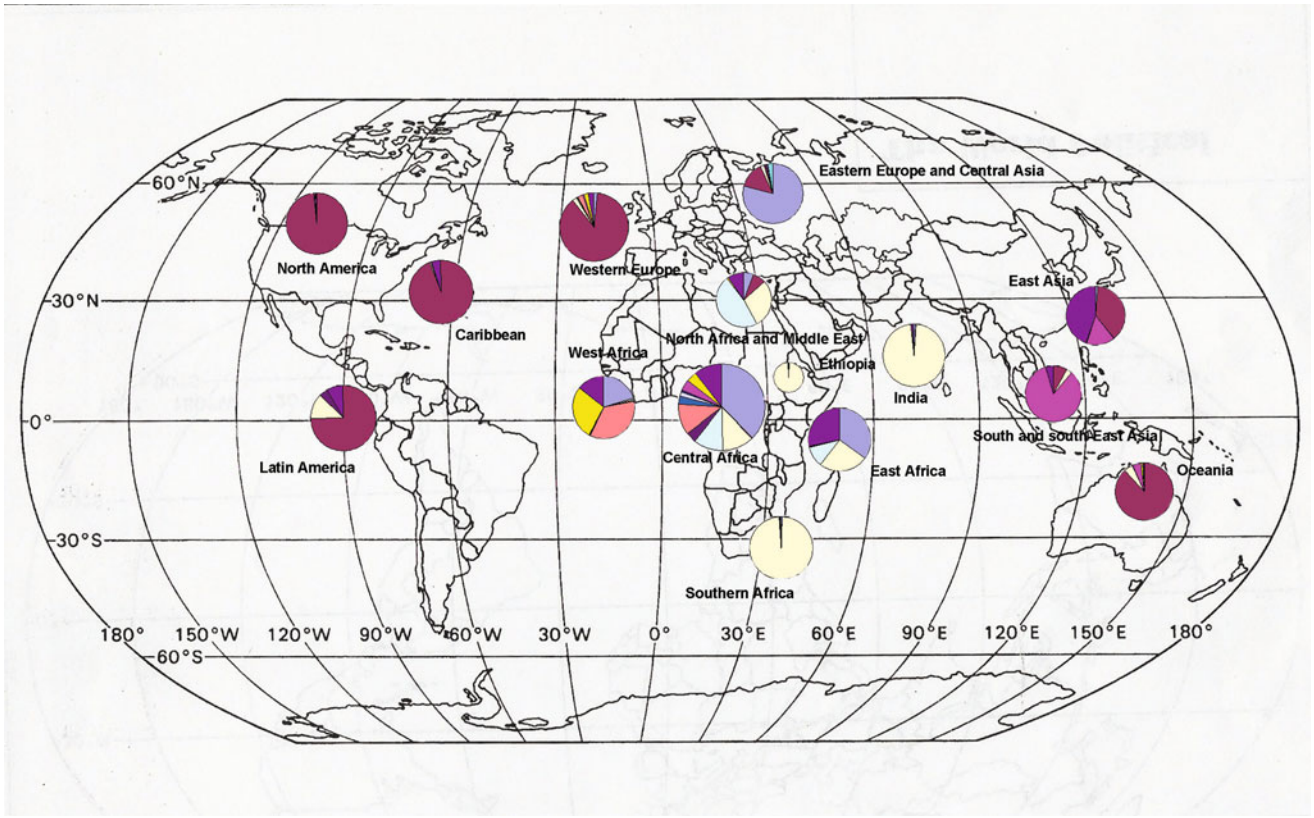

Figure 2 Regional distribution of HIV-1 subtypes and recombinants, 2004. Created from data in the WHO/UNAIDS report titled "Global and regional distribution of HIV-1 genetic subtypes and recombinants in 2004" by Hemelaar et al. (2006).

in Southeast Asia and Central Africa. Subtype F has been found in Central Africa, Latin America, and Eastern Europe, whereas subtype G occurs in West and Central Africa, the Middle East, Eastern Europe, Taiwan, and Korea. Subtypes H and K are largely limited to Central Africa, the Demographic Republic of Congo, and Cameroon, whereas subtype J is primarily observed in Central and South America. CRFs and other recombinants (URFs) now account for nearly 18% of global infections (Hemelaar *et al*, 2006).

Groups N and O have only been observed in Central Africa, possibly due to two more recent cross-species transmissions in Cameroon and the neighboring countries (Hahn *et al*, 2000; Jaffe and Schochetman, 1998; Simon *et al*, 1998; Zekeng *et al*, 1994). The eight main subtypes of HIV-2 are all found in West Africa, but have also been reported in France, Portugal, and India (Brennan *et al*, 1997; Damond *et al*, 2001; Kandathil *et al*, 2005; Pieniazek *et al*, 1999; Soriano *et al*, 2000).

Genetic mutation driving diversity

The genetic variation of HIV strains involves multiple factors. As reverse transcriptase (RT) transcribes DNA from viral RNA, it makes many errors matching the correct nucleotides in sequence. To make things worse, RT does not possess the exonuclease proofreading capacity that occurs with normal DNA transcriptase to correct these mistakes. The high mismatch error rate during transcription combined with the high replication rate and propensity for recombination leads to substantial genetic variation and frequent mutation. Acting on this backdrop of variation are a wide range of host, environmental, and therapeutic selection pressures, driving the overall evolution of the virus (Liesch and DeStefano, 2003; Preston *et al*, 1988; Roberts *et al*, 1988; Simon and Ho, 2003).

Genetic distinction of different groups and subtypes of HIV-1 is largely based on differences in the envelope protein (Env protein) nucleotide sequence (Figure 3). Env proteins of groups M and O can differ by as much as 30% to 50%, whereas those of group N appear "phylogenetically equidistant" from groups M and O (Spira *et al*, 2003; Thomson *et al*, 2002). Within group M, interclade variations within the *env* gene of 20% to 30%, and intraclade variations of 10% to 15%, have been observed (Gao *et al*, 1996; Gao *et al*, 1998). Variations in the *env* gene may play an important role in viral entry and neuropathogenicity, both directly and indirectly. Variations in the *pol* gene, although less divergent, are particularly important to drug susceptibility. The *pol* gene encodes reverse transcriptase (RT) and protease, two enzymes vital to viral replication and fitness. If this gene is

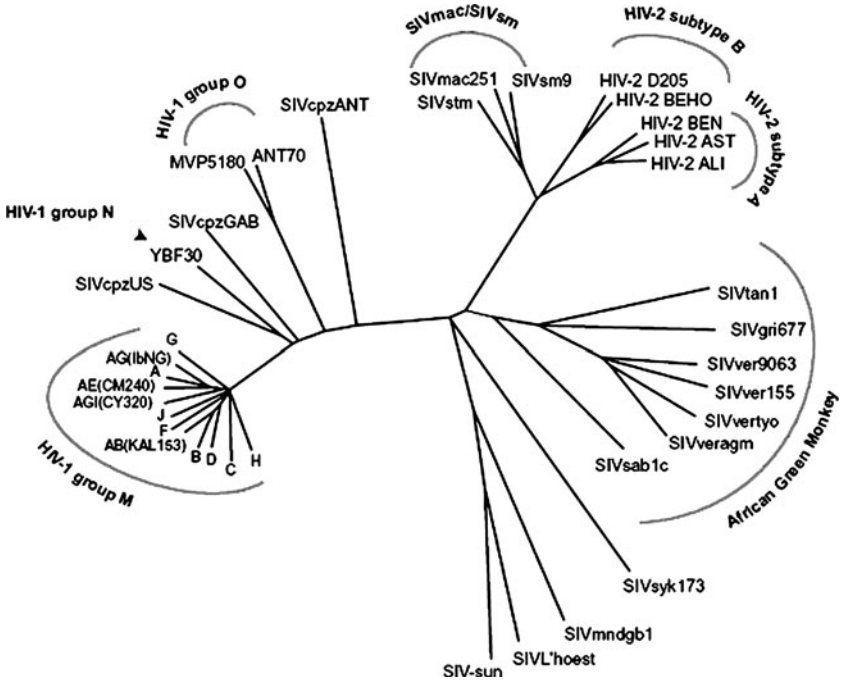

Figure 3 Neighbor-joining phylogenetic tree constructed by Los Alamos National Laboratory using the HIV Sequence Database and the PHYLIP DNADIST and WEIGHBOR programs. This diagram shows the relationships between HIV-1 and HIV-2, HIV-1 groups M, N, and O, as well as the various clades within HIV-1 group M (http://www.hiv.lanl.gov/content/hiv-db/COMPENDUM/1992/2/intro.pdf).

excessively mutated, it could render these critical enzymes and therefore the next generation of virus non-functional. Less drastic variations in this gene are specifically significant to treatment because many antiretroviral drugs target RT (nucleoside reverse transcriptase inhibitors [NRTIs], non-nucleoside reverse transcriptase inhibitors [NNRTIs]) and protease (protease inhibitors [PIs]) (Spira *et al*, 2003).

HIV-1 drug resistance

Given that mutations in the *env* and *pol* genes of subtype B viruses can confer genetic resistance to antiretroviral therapy (ART), interclade differences in these genes could also confer resistance. Development of drug resistance to ART may be both a cause and consequence of insufficient viral suppression and treatment failure (Kantor *et al*, 2005). Recently, transmissions of drug resistant virus in up to 20% of new HIV-1 subtype B infections in the United States and Europe have been recorded (Boden *et al*, 1999; Brodine *et al*, 1999; Little *et al*, 2002; Simon *et al*, 2002; Yerly *et al*, 1999). These drug-resistant mutations found in untreated subtype B–infected individuals were formerly only associated with failed drug therapy, but may in fact result from either transmission from a treated individual or from naturally drug resistant variants differing from the wild-type (Kantor *et al*, 2005). Numerous observational studies maintain that existing RT inhibitors and protease inhibitors are equally effective against both subtype B and non-B subtype viruses (Alexander *et al*, 2002; Bocket *et al*, 2005; Pillay *et al*, 2002). Several have shown that a high baseline viral load and use of only NRTIs prolong time to undetectable viral load (Bocket *et al*, 2005; De Wit *et al*, 2004). These results, however, are not ubiquitous.

Non-B subtypes predominate in developing countries where access to effective combination therapy is limited or nonexistent. Major mutations developing during the course of suboptimal drug treatment and resulting virologic failure may be the underlying cause of differences in HIV-1 disease progression, and several studies have indeed found differences in drug susceptibility and HIV-1 pathogenicity. Kantor and Katzenstein (2005) analyzed 157 non-B–infected persons and 1400 subtype B–infected persons receiving ART within the Stanford HIV RT and Protease Sequence Database. Some amino acid substitutions in non-B viruses occurred at high rates at positions linked with resistance in subtype B RT and protease sequences. Mutations were observed at 22/31 (71%) of known subtype B RT-resistant positions, 15/18 NRTI and 7/13 NNRTI positions, as well as 13/21 PI-resistant positions. Higher percentages of resistance mutations to NRTIs and PIs and less mutations with NNRTIs are possibly due to the more recent introduction of NNRTIs into treatment regimens (Kantor *et al*, 2005).

Given the characteristically rapid evolution of HIV in general, differentiating between wild-type strains of specific clades, natural variants, and "mutant" strains may be difficult. Deviations from the established "wild-type" for each clade may occur

during transcription/replication, leading to altered messenger RNA (mRNA) for production of proteins or an altered genome for the next generation of virus. Deviations may also occur during translation of mRNA due to natural *ribosomal frameshifting* prominent in retroviruses, where the ribosome may slip forward or backward one or several nucleotides on the same mRNA, resulting in different proteins with different survival benefits (Mendez, 2003). As described briefly above, the term mutation used in this paper is based on deviations from known HIV-1 subtype B RT and protease sequences that do respond to ART (Kantor *et al*, 2005). Simply stated, deviations from these "ideal" subtype B sequences may in fact be natural to other subtypes, but are referred to as mutations since they are associated with drug resistance.

Although variability in the *env* gene may change the effectiveness of drugs that target viral entry (as with fusion inhibitors) (Geretti, 2006), variability of the *pol* gene is particularly important as previously noted. Interclade variability in the *pol* gene of 10% to 12% at the nucleotide level and 5% to 6% and the amino acid level has been reported (Kantor *et al*, 2005). Although there are usually no major resistance mutations found in non-B–infected, drug-naïve patients, minor changes are common (Vergne *et al*, 2000). Though these minor mutations do not directly confer resistance to treatment-naïve patients, they may facilitate the more rapid development of resistance during the course of suboptimal therapy once started. Looking at several studies on drug sensitivities, subtype D appears to have diminished sensitivity possibly due to rapid growth kinetics, whereas subtypes A, B, C, and A/E had comparable sensitivities (Palmer *et al*, 1998; Spira *et al*, 2003). In a study of pregnant women in Uganda, subtype D also appeared more resistant than subtype A to nevirapine (NVP) (Eshleman *et al*, 2001). Subtype C seems to have greater resistance to NNRTIs over subtype B (Spira *et al*, 2003). Although they may not directly confer resistance, the mutations V106M, A98S, and G190A have been linked to the ability of clade C viruses to select for resistance to NNRTIs more rapidly than clade B (Grossman *et al*, 2004a; Grossman *et al*, 2004b; Loemba *et al*, 2002). These studies also determined that the V106M and G190A mutations correlated with resistance to all NNRTIs. Another study found the V106M and G190A mutations occurring at a higher rate in subtype CRF01_AE RT over subtype B (Hsu *et al*, 2005). One study revealed that during therapy with the protease inhibitor nelfinavir the L90M mutation occurred in subtypes C, G, and CRF01_AE, whereas the D30N mutation occurred in subtype B. L90M seems to confer a greater degree of cross-resistance to several protease inhibitors whereas D30N only conferred resistance to nelfinavir (Grossman *et al*, 2004b). The Y181C and Y181I mutations endow resistance to all drugs within the entire NNRTI class to group O and HIV-2, respectively (Descamps *et al*, 1997; Tantillo *et al*, 1994; Vergne *et al*, 2000).

Research on disparities in drug resistance among the different HIV-1 subtypes is ongoing and often contradictory. Some studies claim that despite minor mutations, no substantial differences in drug sensitivity and disease pathogenesis exist (Alexander *et al*, 2002; Bocket *et al*, 2005; Pillay *et al*, 2002). Some present mutations that appear to confer resistance to entire drug classes (Descamps *et al*, 1997; Descamps *et al*, 1995; Grossman *et al*, 2004a; Tantillo *et al*, 1994), whereas others suggest that minor mutations just predispose certain subtypes to select for resistant strains once therapy begins (Kantor *et al*, 2005; Vergne *et al*, 2000). Although fewer mutations have been reported at known subtype B NNRTI-resistant positions compared to NRTI- and PI-resistant mutations (Kantor *et al*, 2005), the effects of these NNRTI mutations represent a large portion of discussions on HIV-1 subtype–dependent drug susceptibility.

As a protected compartment, HIV in the central nervous system (CNS) behind the blood-brain barrier (BBB) is likely to play a unique role in development of resistance (Smit *et al*, 2004). Penetration of antiretrovirals into the CNS is limited by the BBB, allowing virus to be exposed to subclinical drug exposure levels and increasing potential for resistance. Virus within the CNS has been shown to genetically vary from systemic compartments (Harrington *et al*, 2005; Ritola *et al*, 2005). It has also been shown in models that virus in CSF is efficient at reseeding the systemic compartment (Liu *et al*, 2006). It remains to be shown if certain subtypes of HIV are more efficient at crossing into the CNS and establishing infection. Presumably, such subtypes would be more efficient in developing antiretroviral resistance in addition to potentially increased neuropathogenesis.

Subtype deviations in disease progression

The research to date on HIV-1 subtype–specific differences in disease progression is marked by contrast. Some studies have concluded that among the many variables affecting the pathogenicity of HIV-1, it is highly unlikely that a single factor such as subtype could explain significant differences in disease transmission and progression (Hu *et al*, 1999; Kandathil *et al*, 2005). However, these results are not universally accepted and some studies have discerned disparities in transmission and virulence among the different HIV-1 subtypes.

In Thailand where two subtypes, B and the A/E recombinant (formerly referred to as subtype E), were introduced at approximately the same period, the ratio of new infections due to subtype A/E has increased in almost all population groups (Hu *et al*, 1999). Hu *et al* (1999) conclude that in spite of the disproportionate increase in subtype A/E infections, the apparent difference in transmissibility may be

the chance results of a founder effect, where subtype A/E was introduced first and enjoyed an adequate time window to become established before subtype B was introduced. In a study describing rates and correlates of disease progression and survival among 194 female sex workers in Northern Thailand, Kilmarx *et al* (2000) found disease progression strongly correlated with viral load, but not significantly associated with any other factors. Their results suggest that disease progression with HIV-1 subtype A/E infection in Southeast Asia was not notably different from subtype B infections observed in other studies (Kilmarx *et al*, 2000). A study by Mehendale *et al* (2002) found similar viral set points in Indian seroconverters where clade C predominates and untreated HIV seroconverters in the United States where clade B predominates, but the median trajectory of increasing viral load was greater in the Indian seroconverters. Their data suggest that early virological and host events following primary HIV infection may be responsible for the more rapid disease progression described in resource-poor settings, but does not clearly indicate any inherent differences based on subtype alone (Mehendale *et al*, 2002).

Recognized differences in transmission and virulence of HIV-2 compared to HIV-1 suggest that HIV viruses may possess different pathogenic potentials. Observations found that HIV-2 was less transmissible and slower in disease progression than HIV-1. In addition, biological properties that vary with distinct HIV-1 subtypes may lead to differences in pathogenesis (Donnelly *et al*, 1993; Kanki *et al*, 1994; Marlink *et al*, 1994). For example, contrary to the studies in Thailand described above, Costello *et al* (2005) observed a shorter time period from infection to death among a heterosexual Thai population than among subtype B–infected populations in the pre-HAART (highly active antiretroviral therapy) era. They also acknowledge that comparisons of HIV subtype B and subtype A/E rates of progression are confounded by almost entirely different modes of transmission, intra-venous drug use versus heterosexual intercourse (Costello *et al*, 2005).

Several studies have found differences in the effects of HIV-1 subtypes A, C, and D on disease progression. In a prospective study of registered female sex workers in Senegal, Kanki *et al* (1999) found that HIV-1 subtype-specific AIDS incidence rates per 100 person-years varied widely, from 3.45 for subtype A to 15.95 for subtype C and 14.45 for subtype D. Within each subtype, 87.0% of women infected with subtype A were AIDS-free compared with 30.0% of subtype C– and 60.0% of subtype D–infected women. Women infected with subtype A had a longer AIDS-free survival period than those infected with non-A subtypes (Kanki *et al*, 1999). Kaleebu *et al* (2002) investigated the effect of HIV-1 envelope subtypes A and D on disease progression in 1045 adults in Uganda. Subtype D corresponded with significantly lower average CD4 cell count during follow-up period, as well as a significant difference in mortality compared to subtype A (Kaleebu *et al*, 2002).

In Tanzania, where the predominant subtypes are A, C, and D, Vasan *et al* (2006) determined that subtype D had the strongest association with risk of death relative to the risk associated with subtype A, followed by risks associated with subtype C and recombinant viruses. Subtype D again showed a strong association with disease progression, relative to the disease progression associated with subtype A. Women infected with subtype D progressed to death or the World Health Organization (WHO) stage 4 illness more than twice as fast as subjects infected with subtype A, whereas no significant differences were found between women infected with subtype A and those infected with subtype C, or recombinant viruses (Vasan *et al*, 2006). Interestingly, the authors note that differential use of coreceptors for viral entry into the cell by each subtype may have some effect. HIV-1 subtype A and especially subtype C appear to favor the CCR5 receptor for viral entry throughout the duration of the disease (Peeters *et al*, 1999). Subtype D, however, displays a tropism for the syncytium-inducing CXCR4 receptors primarily observed in T cells (Tscherning *et al*, 1998; Zhang *et al*, 1996) and possibly a dual tropism for both coreceptors (Kaleebu *et al*, 2007; Laeyendecker, 2006; Vasan *et al*, 2006) throughout the course of infection. The use of CCR5 receptors observed in subtypes A and C correlates with a non-syncytium inducing macrophage-tropic version of HIV-1 associated with slower viral growth and replication (Abebe *et al*, 1999; Peeters *et al*, 1999; Tscherning *et al*, 1998). The more rapid viral growth and replication associated with CXCR4 T-cell-tropic strains could theoretically allow subtype D to infect more cells per unit of time than other subtypes, explaining the greater propensity for drug resistance and more rapid disease progression (Vasan *et al*, 2006). The effects of coreceptor usage are described in more detail below, as they are particularly relevant to HIV-1 neuropathogenesis. We would hypothesize that subtype D would be more neuropathogenic, given the propensity for rapid systemic disease progression and immunocompromise often associated with neurologic disease in HIV, in addition to other factors noted below.

In a study examining cognitive difficulties present among individuals infected with HIV-1 clade C virus in India, Yepthomi *et al* (2006) reported as many as 56% of patients with advanced HIV meeting the benchmark for impairment in two cognitive domains. This study highlights the need for additional research to determine if HIV-1 subtype C is more or less prone than subtype B to cause neurological deficits (Yepthomi *et al*, 2006). The impact of antiretroviral therapy on neurocognitive dysfunction in clade C and other non-B subtypes has to be fully determined as well.

Subtype-related neurological divergence

All lentiviruses infect the brain and eventually lead to chronic neurological disease in their unfortunate hosts (Bangham, 1993; Power *et al*, 2004). The significant loss of neurons occurring with AIDS clearly correlates with neurocognitive impairment (Masliah *et al*, 1992; Masliah *et al*, 1997; Mattson *et al*, 2005). Cells primarily infected by HIV within the brain are blood-derived macrophages, resident microglia, and perhaps astrocytes, but most studies suggest that neurons do not appear to be directly infected (Epstein and Gendelman, 1993; Kure *et al*, 1990; Takahashi *et al*, 1996; Trillo-Pazos *et al*, 2003). However, neural damage is likely caused directly by shed viral proteins such as gp120 (Dreyer *et al*, 1990; Lannuzel *et al*, 1995) and Tat (Behnisch *et al*, 2004; Jones *et al*, 1998; Maragos *et al*, 2003; Nath *et al*, 1999) or indirectly through the elevated production of neurotoxic molecules released by infected astrocytes (Levi *et al*, 1993; Merrill *et al*, 1992; Mollace *et al*, 1993; Nath *et al*, 1999; Patton *et al*, 2000), macrophages (Gendelman *et al*, 1994; Levi *et al*, 1993; Merrill *et al*, 1992; Nath *et al*, 1999; Philippon *et al*, 1994), and microglia (Gendelman *et al*, 1994; Jones *et al*, 1998; Kramer-Hammerle *et al*, 2005; Levi *et al*, 1993; Mattson *et al*, 2005; Nath *et al*, 1999).

Coreceptors, cell tropism, and syncytium inducibility

The hypervariable V3 loop of the envelope glycoprotein gp120 is involved in HIV-1 entry into the CD4 cell and is an important source of neuropathogenesis regardless of HIV subtype (Hwang *et al*, 1991; Zhang *et al*, 2001). However, clade D strains have been identified with a more variable pattern of V3 loop amino acids compared to other group M subtypes and clade C on the other hand displays less variation than the other subtypes (De Wolf *et al*, 1994; Ping *et al*, 1999; Zhong *et al*, 1995). Thus, differences in the V3 loop between subtypes may affect host cell tropism and neurovirulence (De Jong *et al*, 1992; Zhong *et al*, 1995). Meanwhile, differences in coreceptor usage and syncytium inducibility may also affect disease progression (Peeters *et al*, 1999; Tscherning *et al*, 1998). Syncytium-inducing (SI) strains characteristically infect T cells using the β chemokine receptor CXCR4 and replicate quickly. Non–syncytium-inducing (NSI) strains tend to infect macrophages using the β chemokine receptor CCR5 and grow more slowly. A large portion of most HIV clades change coreceptor usage as the disease progresses, usually appearing as the CCR5/NSI macrophage infecting strain during the early disease stage and then transitioning to the CXCR4/SI phenotype or a dual tropism for CXCR4 and CCR5 during the rapidly progressing end stage of the disease (Bjorndal *et al*, 1997; Ida *et al*, 1997; Kaleebu *et al*, 2007; Keet *et al*, 1993; Kozak *et al*, 1997; Nielsen *et al*, 1993; Oberlin *et al*, 1996; Tersmette *et al*, 1988; Tscherning *et al*, 1998). Clades A, C, and D in particular do not fit this pattern well. As previously mentioned, clade A tends to favor CCR5 throughout infection which could account for the slower progression to AIDS recorded in several studies. Clade C extremely favors CCR5 and rarely becomes CXCR4/SI tropic. Clade D, however, shows preferential tropism for CXCR4, or dual tropism for CXCR4 and CCR5, throughout the course of disease, possibly accounting for the more rapid disease progression recorded in clade D–infected individuals compared to those infected with clades A and C (Abebe *et al*, 1999; Kaleebu *et al*, 2002; Kanki *et al*, 1999; Peeters *et al*, 1999; Tscherning *et al*, 1998; Vasan *et al*, 2006). There is some disagreement in the literature over the switch to a CXCR4-tropic or a dual-tropic phenotype for clade D. Some sources claim that the more rapid disease progression associated with clade D infections is due to a simultaneous dual tropism throughout infection (Kaleebu *et al*, 2007; Laeyendecker, 2006). For example, in a subgroup of patients in Rakai, Uganda, 5/31 seroconverters had dual tropic infections. All 5 were subtype D and 4/5 died within 3 years of infection. Other sources have not observed dual tropism in subtype D SI phenotypes. In one study, the ability to use the CXCR4 coreceptor correlated with all of the 26 rapidly progressing SI isolates from all HIV subtypes; however, none of the 55 slow progressing NSI isolates could use CXCR4 and instead used CCR5. Thirteen of the 26 rapid/SI phenotypes could use CXCR4 and CCR5 simultaneously, none of which were clade D isolates (Tscherning *et al*, 1998). Although the presence of the CXCR4/SI phenotype is a strong predictor for greater CD4+ T-cell depletion and progression to AIDS, approximately half of AIDS patients will not have detectable CXCR4-tropic virus (Koot *et al*, 1993).

HIV-1 envelope–mediated neuronal death

HIV infection of the brain is characterized by viral molecular diversity, but restricted viral protein abundance (Clements *et al*, 2002; Morris *et al*, 1999; Power *et al*, 1994; Power *et al*, 1998; Power *et al*, 2004; Teo *et al*, 1997). Viral envelope diversity in several other retroviruses has been shown to influence the progression of neurological disease, so similar effects from HIV Env could occur (Johnston *et al*, 2000; Lynch *et al*, 1991; Mankowski *et al*, 1997). HIV-1 strains from patients with dementia differ from those without dementia. The dementia-associated strains mainly differ in the V1 and V3 region of the gp120 envelope protein, regions that also account for some diversity in the different HIV-1 subtypes (Mankowski *et al*, 1997; Power *et al*, 1994; Ritola *et al*, 2005; Zink and Clements, 2002).

A large fraction of HIV-1 species within an individual are replication incompetent, but still express viral proteins (for example gp120) (Englund *et al*, 1995; Stevenson *et al*, 1990; Teo *et al*, 1997; Wiskerchen

and Muesing, 1995). Despite their limited abundance, viral proteins may be directly toxic to neurons and therefore may be sufficient for neuronal death (Ohagen *et al*, 1999; Teo *et al*, 1997; Toggas *et al*, 1994). The V3 region of the envelope is known to influence the release of neurotoxins from infected macrophages, and may be directly toxic itself (Kaul and Lipton, 1999; Kong *et al*, 1996; Power *et al*, 1998; Yeung *et al*, 1995; Zhao *et al*, 2001).

In one of the few studies on interclade differences in neurological effects, Zhang *et al* (2003) found that neurotoxicity was independent of viral replication for both direct and indirect neuronal death. Although neurons express both CCR5 and CXCR4 chemokine receptors, HIV-1 entry and replication are not permitted. However, these receptors may still interact with the HIV Env protein, incurring neurotoxic effects. LAN-2 neurons (for direct effects) and macrophages (for indirect effects) were pretreated with anti-CCR5 and anti-CXCR4 antibodies or G-coupled-protein inhibitory PTX. Antibodies and pertussis toxin (PTX) reduced direct neurotoxicity from 20% to 80% for total neural death and 15% to 33% for neural apoptosis. So chemokine receptors appear to be integral components in HIV-direct neuronal death, but indirect toxicity caused by macrophages may have different mechanisms. Indirect toxicity results from a cascade of macrophage-microglial cell signaling events leading to the production of numerous neurotoxins and inflammatory molecules. In macrophages infected with different viral strains, HIV induced a 2.4- to 5.9-fold increase in tumor necrosis factor (TNF)-α, interleukin (IL)-β, and inducible nitric oxide synthase (iNOS) mRNA levels. Viral protein expression alone was able to induce the release of these neurotoxins from macrophages. Overall, levels of neurotoxicity did not differ significantly among strains from different clades, but all clades investigated (A, B, and D) displayed some neurotoxicity (Zhang *et al*, 2003).

Conclusions

Viral replication and cell tropism are key factors in HIV-related systemic disease progression (Cao *et al*, 1995; Levy, 1991; Littman, 1998; Rodes *et al*, 2004). However, HIV-associated dementia is not definitively correlated with viral load in the brain or cerebrospinal fluid (CSF) during the HAART treatment era (Glass *et al*, 1995; Johnson *et al*, 1996; Lazarini *et al*, 1997; McArthur *et al*, 2004; Sevigny *et al*, 2004). Instead, HIV-associated dementia (HAD) appears to be associated with viral diversity and markers of immune activation (Glass *et al*, 1995; Sevigny *et al*, 2004; Zhang *et al*, 2003). High replication rates, lack of proofreading capacity for mismatch errors, the propensity for recombination, as well as a number of other host and environmental factors foster this genetic diversity (Liesch and DeStefano, 2003; Preston *et al*, 1988; Roberts *et al*, 1988; Simon and Ho, 2003). Although highly debatable, it has been demonstrated by some that the variable *env* and *pol* sequences of the different subtypes of HIV-1 may endow some subtypes with greater degrees of cell tropism, virulence, and drug resistance, which may lead to differences in overall disease progression (Gao *et al*, 1996; Gao *et al*, 1998; Geretti, 2006; Kantor *et al*, 2005; Spira *et al*, 2003; Vergne *et al*, 2000). It cannot be reiterated enough that insufficient treatment, such as the cheaper and simpler two-drug combinations currently used in the developing world, create an environment that harbors more frequent mutations and inevitably drug resistance. In sub-Saharan Africa, where HIV viral diversity is the greatest and clade recombination occurs most frequently (Hemelaar *et al*, 2006; Vidal *et al*, 2000), HAD poses the largest threat, potentially becoming an epidemic within a pandemic. Although investigations into the effects of different subtypes on overall disease progression are well documented (regardless of contrasting findings), few have looked into the effects of subtypes on neurological disease progression. In light of the correlations of viral diversity and viral envelope proteins to neurotoxicity (Ohagen *et al*, 1999; Stevenson *et al*, 1990; Teo *et al*, 1997; Wiskerchen and Muesing, 1995; Zhang *et al*, 2003), more research involving the neurological effects and especially the clinical presentation of dementia for the whole gamut of the group M HIV-1 subtypes is of paramount importance.

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