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Journal of Antimicrobial Chemotherapy (2003) **51**, 229–240
DOI: 10.1093/jac/dkg079
Advance Access publication 14 January 2003

Reviews

Impact of clade diversity on HIV-1 virulence, antiretroviral drug sensitivity and drug resistance

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HIV-1 infection is characterized by genetic diversity wherein distinct viral subtypes (clades A, B, C, D, E, F, G, K and O) are expanding in different geographical regions. This article deals with the topic of HIV-1 subtype diversity in the context of sensitivity to antiretroviral drugs, drug resistance and viral fitness. Increasing evidence suggests that all clades of HIV probably display similar sensitivity to antiviral drugs. However, viruses from some subtypes and/or geographical regions may have a greater propensity to develop resistance against certain drugs than do other viral variants. In addition, differences in regard to replication capacity or fitness may exist among various HIV subtypes and differences in this regard may potentially become magnified under conditions of drug resistance. Immunological pressures may also play an important role in the evolution of viral subtypes that may impact on ultimate drug resistance profiles.

Keywords: HIV, clade, resistance

Introduction

Contrary to predictions made at the inception of the AIDS epidemic two decades ago, HIV-1 has evolved to assume multiple guises, which differ from place to place across the globe. The predominant subtype that is found in the developed Western World, clade B, differs considerably from those subtypes and recombinants that exist in Africa and Asia, where the vast majority of HIV-infected persons reside. Thus, serious discrepancies may exist between the subtype B retrovirus that medical practitioners encounter in North America and Europe and those viral subtypes that plague humanity on a global scale. To bridge this gap, the following exposition of HIV clade diversity and its clinical consequences is in order.

The large genomic diversity of viral subtypes in different geographical regions is the consequence of the astonishingly high mismatch error rate of the HIV reverse transcriptase (RT) enzyme coupled with the absence of an exonuclease proof-reading activity. Other factors that contribute to the rapid pace of genetic diversification include the replicative rate of each viral subtype, the number of mutations arising in each replicative cycle, the viral propensity for genomic recombination and viral fitness. In addition, high rates of genomic evolution may result from host, environment and/or therapeutic selection pressures.[1–6]

Three classes of HIV-1 have developed across the globe: M (major), O (outlying) and N (new).[2,3] Among the M group, which accounts for >90% of reported HIV/AIDS cases, viral envelopes have diversified so greatly that this group has been subclassified into nine major clades including A–D, F–H, J and K, as well as several circulating recombinant forms.[1–7] Viral diversity appears to radiate out of sub-Saharan Africa, where over 28 million of the total 40 million infected persons live.[6–10]

Geographical spread

As depicted in Figure 1, the demographic distribution of patients infected with particular clades, or subtypes, is heterogeneous, with predominant clades in a given region, as follows:

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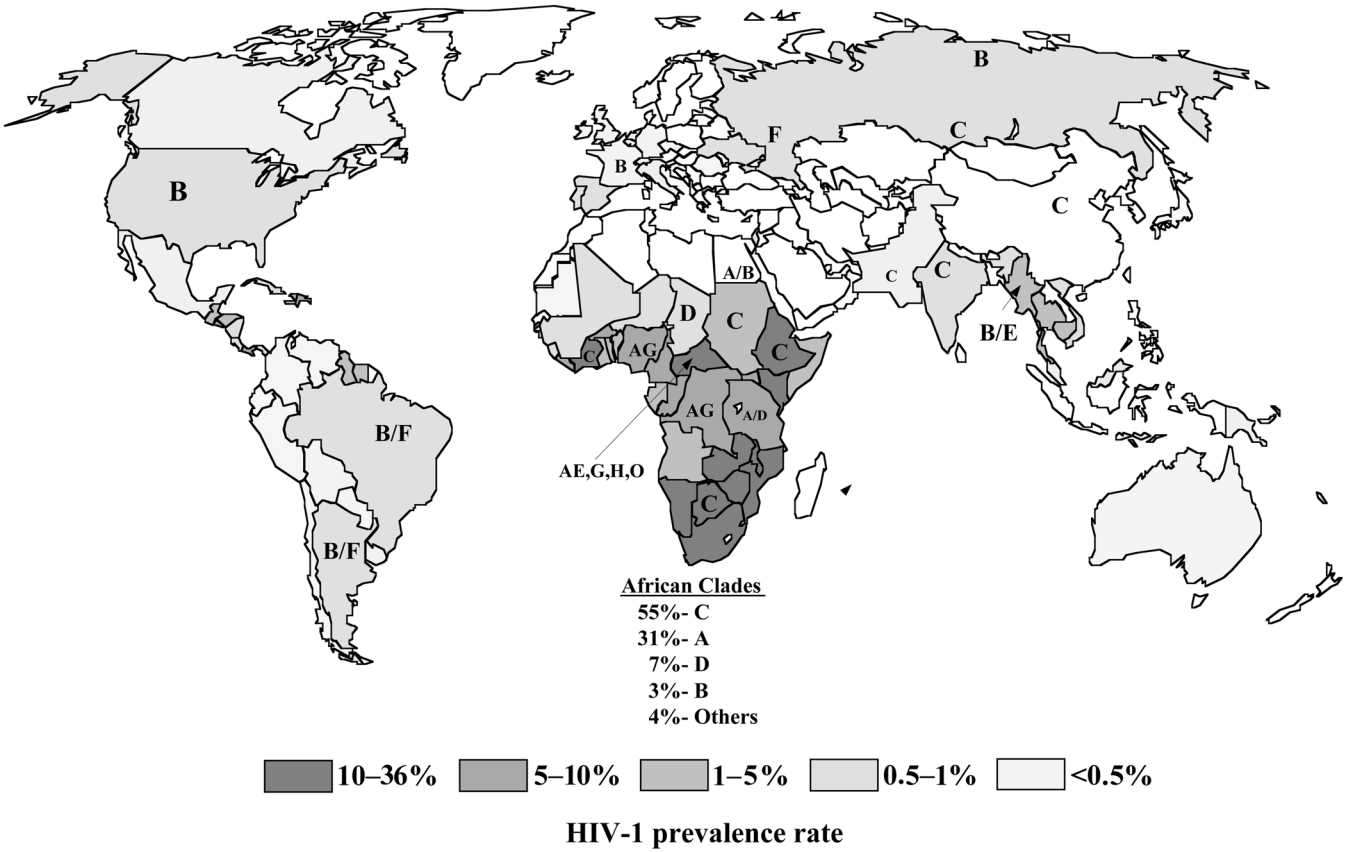

Figure 1. Subtype diversity of HIV-1 infections prevalent worldwide.

A and A/G recombinant variants predominate in West and Central Africa.[6]

B has been the predominant species in Europe and the Americas. However, with increasing immigration and globalization, >40% of new infections in Europe are presently non-B African and Asian variants.

C is largely predominant in Southern and Eastern Africa, India and Nepal. Indeed, clade C has created the recent epicentres of the HIV pandemic by its uncontrolled spread throughout Botswana, Zimbabwe, Malawi, Zambia, Namibia, Lesotho, South Africa, India, Nepal and China.[3,4,6,7,11–15]

D is generally limited to East and Central Africa, with sporadic cases observed in Southern and Western Africa.[6,7,9,10,16,17]

E has never materialized alone, but rather appears as an A/E mosaic detected in Thailand, the Philippines, China and Central Africa.[4,6,7,18–20]

F has been reported in Central Africa, South America and Eastern Europe.

G and A/G recombinant viruses have been observed in Western and Eastern Africa as well as in central Europe.[6]

H has only been detected in Central Africa.[4–10,21]

J has been reported exclusively in Central America.[10,16]

K has recently been identified in the Democratic Republic of Congo and Cameroon.[5]

This list is not exhaustive, for more subtypes are constantly being discovered, and migrating populations are shaping new patterns of infection.[22]

Of particular concern are HIV-1 clades C and A, as well as the A/G and A/E recombinant forms, which represent the predominant subtypes in Africa and Asia where HIV disease is dangerously out of control.

In sharp contrast, the other genre of retrovirus, HIV-2, has not spread much beyond West Africa where it is presently endemic. Some sporadic cases have been observed elsewhere in Africa but the virus appears to be significantly less pathogenic than HIV-1.[6,7,16,17]

Genomic diversity of clades

HIV-1 clades are phylogenetically classified on the basis of the 20–50% differences in envelope (*env*) nucleotide sequences. The Env proteins of groups M and O may differ by as much as 30–50%. The N subtype, in turn, appears to be phylogenetically equidistant from M and O.[1,2,23,24] Within M subgroups, inter-clade *env* variations differ by 20–30% whereas intra-clade variation of 10–15% is observed.[24–27]

The *pol* region of HIV-1 is two to three times less divergent than *env* because this region encodes two critically important

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enzymes, RT and protease, which, if excessively mutated, render the virus inoperative. *gag* sequences are even further intolerant of mutations, seeing as they encode for relatively inflexible core protein sequences.

Inter- and intra-clade variations within *pol* sequences are particularly relevant insofar as this region encodes RT and protease proteins, against which many antiviral drugs are directed. Variations in these regions may therefore affect drug susceptibility and development of drug resistance. Ethiopian clade C isolates differ (with respect to RT) from clade B by 6.8–10%, and intra-clade differences of 3.5–5.8% have been reported for strains from Africa, India and South America.[28–30]

The fact that any given percentage variation in nucleotide sequence translates into lower amino acid sequence variation is notable because many genetic mutations are silent. For instance, the 10% nucleotide divergence between RT sequences in clades E and B yields only a 7% divergence in amino acid residues.

Not only do *env* genes vary substantially from clade to clade, but so do the long terminal repeat (LTR) sequences, which contain transcriptional promoters of HIV replication.[31–33] Each clade has its own LTR copy number as well as an exact nucleotide sequence of enhancer and promoter structures, despite the uniformity in other LTR features, i.e. Sp1 sites, TATA box and TAT-responsive element.[31,32,34] Moreover, diversity is seen in numbers of transcriptional promoters. These include the NF-κB binding sites (three to four in C, two in B and just one in E), as well as in sequences upstream of NF-κB sites, such as the *nef*-overlapping USF gene, which is incident only in clade B,[32] and the AP-1 transcriptional factor binding site (which exists as one site in subtypes C, E and G, two in A and F, and none in B or D).[32] The –170 region of U3, containing a specific motif for the NF-IL6 transcriptional factor (C/EBP-B), is harboured by clade B but not by A, C, D or O.[33] This factor transactivates the HIV-1 LTR in cells of monocytic origin.[33,35] Additionally, subtype discrepancies arise between the negative regulatory element (NRE) seen in clades C, D and E versus that detected in clade B.[34]

Given these genetic distinctions between HIV-1 promoters, it is not surprising to find that clades respond differentially to various transcriptional factors. The NF-κB binding factor, Rel-p65, NF-κB and nuclear HeLa cell extract all stimulate HIV-1 clade C to a far greater extent than clade B or E.[31,34] Likewise, tumour necrosis factor (TNF)-α activates the LTRs of clade C more impressively than those of clades A, B, D, F and G, with the lowest stimulation seen in clade E.[32]

One might inquire as to whether clade diversity bears any impact on HIV-1 gene expression and replication kinetics, pursuant to these structural and functional differences, coupled with the fact that the LTR is believed to play a pivotal role in cellular tropism.[36] The matter requires further elucidation by studies of HIV-1 transcription and replication kinetics, which scrutinize subtypes and cell types.

Recent experiments indicate that the sequence of the viral regulatory protein, Nef, also differs between HIV-1 clades, ranging in variation from 14.4% to 23.8%, with the closest Nef configurations being those of B and D.[37] The clinical implications of Nef sequence diversity are currently unknown but potentially great, given the recent observation that Nef sequences may change in clade B-infected patients as a function of disease progression.[38,39]

Lastly, there is evidence that other regulatory and accessory HIV-1 genes may play an important role in subtype diversity. This relates partly to the fact that clade C contains a uniquely truncated Rev protein and an enlarged Vpu product, as well as the finding that clade D expresses a Tat protein with a C-terminus deletion.[27]

Subtype diversity in co-receptor usage, cell tropism and syncytium inducibility

Clades may show differences in co-receptor usage and syncytia-inducing capacity that may impact on disease progression. The cytopathic property of B strains can be either syncytium inducing (SI) or non-SI (NSI), the former being consistent with a virus that infects T cells and replicates swiftly, whereas the latter is characteristic of a virus that infects macrophages and grows more slowly.[40–42] The chief co-receptor of SI virus is the β chemokine receptor CXCR4, whereas that of NSI virus is the β chemokine receptor CCR5.[43–46] Most HIV clades cause disease by assuming the CCR5+/NSI phenotype during early disease and the CXCR4+/SI phenotype during the end stages of disease.[47–49] However, this correlation does not hold true for clade A, C or D. Clade A viruses tend to favour CCR5 even at later stages, a pattern that is seen to a more extreme extent in C strains, which rarely become CXCR4+/SI even in moribund patients. Subtype D displays simultaneous tropism for CCR5 and CXCR4 throughout the course of disease.[15,50–53]

Some researchers postulate the reason for the absence of the CXCR4 phenotype among clade C HIV to be that African patients may experience persistent immune activation by co-existent infections, which constantly trigger CCR5 over-expression.[51,54–56] However, in light of the fact that C strains are actually commonplace in countries with starkly contrasting immunological backgrounds, this notion may be inaccurate. Equally unclear is the contention arising from preliminary studies to the effect that subtypes vary in their ability to infect Langerhans cells, with such tropism linked to vaginal/cervical transmission.[11,57–59] Further experimental analysis is required.

Any discussion of HIV-1 host cell tropism as a function of subtype would not be complete without reference to the V3 loop of the envelope glycoprotein gp120 involved in HIV-1 entry into CD4 cells.[60–62] Particular amino acid substitutions are believed to be essential for co-receptor usage, infectivity

and cell tropism in clade E.[60] Clade D strains demonstrate a highly variable pattern of V3 loop amino acids compared with other group M subtypes. At the opposite extreme, clade C displays less variation in the V3 loop than all the others, and also lacks the highly conserved N-linked glycosylation site traditionally associated with other subtypes.[53,63–65]

The relationship between HIV-1 subtype diversity and disease transmissibility and progression is poorly understood. Moreover, inter-subtype studies may be complicated by host, societal and virological factors that are difficult to control.[6] Subtype diversity may impact on modes of HIV transmission.[6,7,11,12,57,58,66,67] Homosexual and intravenous drug abuse are the primary modes of transmission observed for clade B strains in Europe and the Americas. In contrast, clades A, C, D and E predominate in Africa and Asia where heterosexual transmission predominates. Further analysis of this topic is warranted, as are studies among different clades.[12,68,69]

Several studies suggest that AIDS progression differs as a function of infecting subtype.[6,57,68–70] In one study, patients hosting clade A or G would appear to live symptom-free for the longest, whereas those infected by clade D live an intermediate span and those hosting clade C experience rapid disease progression.[70] In a recent large cohort study, subtype D was associated with lower CD4 cell count and faster disease progression and death compared with subtype A.[69] Likewise, a cross-sectional study found clade C patients to suffer the highest rates of viraemia coupled with lowest CD4 counts, with progression to AIDS before their A- or D-infected counterparts.[68]

To better appreciate the virological characteristics of each subtype, additional longitudinal studies with various HIV subtypes are needed. These may help researchers to innovate superior strategies for disease control.

Subtype diversity in the face of antiretroviral drugs

In developed countries, clade B HIV infections have been managed with highly active antiretroviral therapy (HAART) using nucleoside- and non-nucleoside RT inhibitors (NRTIs and NNRTIs) as well as protease inhibitors (PIs). Such therapy has sharply reduced HIV transmission, morbidity and mortality, but has also created the long-term spectre of drug resistance. Advances in genotypic analysis have identified the changes in sequence that can confer resistance to each antiretroviral drug and even against entire classes of NRTIs, NNRTIs and PIs.[71–74] However, very little data are available as to how subtype diversity may affect drug susceptibility and resistance. Despite the fact that antiretroviral therapy has been scientifically fine-tuned to target the *pol* gene products (RT and protease), nucleotide divergence within this sequence as a function of HIV subtype is only now coming to light.[75–79]

It has been shown that the Y181C and Y181I mutations render group O and HIV-2 resistant to all drugs within the entire NNRTI class, respectively.[80–83] In a less absolute manner, clade F shows some measure of resistance to the non-commercialized NNRTI, the TIBO compound, while remaining sensitive to other NNRTIs, such as nevirapine and delaviridine (DLV), as well as NRTIs and PIs.[84] Clade C isolates from treatment-naive Zimbabweans appear to be as drug sensitive as clade B isolates.[85,86] In contrast, our results indicate that some clade C isolates may show inherent resistance against NNRTIs due to the presence of a G190A mutation.[28–30] A different study found clade D viruses to function with diminished drug sensitivity owing to rapid growth kinetics, whereas subtypes A, B, C and E demonstrated comparable sensitivity.[87] Preliminary results from the paediatric PENTA 5 study show that resistance rates are higher for non-B versus B clades.[88]

Our group has recently evaluated the effects of clade genotypic diversity on drug susceptibility and drug resistance patterns.[28–30] Clade C isolates were obtained from treatment-naive immigrants to Canada, Ethiopians living in Israel and subjects from Botswana and India.[28–30] There was low phylogenetic divergence (3.8–5%) among clade C strains from Ethiopia, Botswana and India, indicating that these variants are more closely related than those previously observed for other clades.[89–91] This is similar to other studies showing less diversity with clade C viruses than other African strains.[2]

As observed in Africa, we show that clade A can be subdivided into clade A, A/E and A/G subclusters. Recombination has been reported to be a common feature among retroviruses and, as well, between HIV-1 strains, particularly clade A.[2,4,92] Ominously, mutation and recombination may both contribute to rescuing high-fitness HIV-1 variants. Recent identification of individuals infected with HIV-1 isolates from two subtypes and inter-subtype species suggests that this effect may frequently materialize among viruses co-circulating in specific geographical regions.[4,8,93–95]

Genotypic analyses of viruses of different clades show many nucleotide changes (silent mutations), polymorphisms and secondary mutations within RT and protease regions implicated in the emergence of resistance to NRTIs, NNRTIs and PIs used in HIV-1 treatment. Whereas, in treatment-naive patients, many of these changes do not confer resistance to drugs *per se* among different clades, they may facilitate the development of resistance. A recent study in Ivory Coast showed how almost all HIV-1 patients were infected with non-B subtypes, predominantly by A/G recombinants.[96] In addition, a high prevalence of 57.4% genotypic and phenotypic HIV-1 drug-resistant strains was reported among 68 patients who were treated with NNRTIs, NRTIs and PIs between 1998 and 1999.[96] Indeed, a comparably high 30–50% proportion of viral variants harbouring drug resistance mutations had previously been reported in some developing

countries among HIV individuals receiving antiretroviral therapy.[97,98] These findings underscore how the introduction of suboptimal therapy and poor patient adherence may lead to a more rapid appearance of resistant variants in African or Asian strains than that reported for clade B infections in developed countries where HAART is available. This last point is particularly worrisome in the light of the fact that suboptimal antiretroviral therapy is becoming increasingly prevalent in developing countries.[7,76,82,99]

Our laboratory carried out *in vitro* drug selection studies on 15 clade C isolates to contrast the emergence of mutations that confer drug resistance in HIV-1 clade C strains compared with that observed for clade B.[28–30] Ethiopian and Botswanan clade isolates carried a clade C-specific KVEQ cluster of silent mutations (amino acids 65, 106, 138, 161, respectively), which allowed for RT-based phylogenetic characterization. There were numerous baseline polymorphisms and silent mutations in treatment-naive subjects within RT at sites linked to resistance to NNRTIs and NRTIs [azidothymidine (AZT), multi-NRTI resistance], as well as protease polymorphisms that facilitate resistance to PIs.[28–30] These include R211K and L214F (NRTI), A98S and E138K (NNRTI) and M36I (PI) secondary or accessory mutations that were not shown to confer phenotypic resistance on their own. Nevertheless, evaluation of baseline drug susceptibility demonstrated similar sensitivities among most clade C viruses from treatment-naive persons, confirming observations reported for clade C strains from Zimbabwe.[28,30,85] Intrinsic resistance to nevirapine and efavirenz was seen in one of the 15 individuals where G190A expression led to innate NNRTI resistance mutation. In this isolate, resistance to nevirapine and efavirenz was observed with no DLV resistance. This result corroborates data on the resistance impact of the G190A substitution in clade B isolates.[100]

Our laboratory carried out studies to ascertain the dosage and time to development of resistance to NNRTIs in clade C isolates compared with clade B viruses.[28,30] The final drug concentration required for the development of resistance mutations conferring NNRTI resistance was significantly lower for clade C than clade B viruses for each of nevirapine (2 versus 10 μM), efavirenz (0.01 versus 1 μM) and DLV (1 versus 10 μM), respectively.[28,30] Moreover, resistant variants were fully selected more rapidly with the clade C isolates (8 or 9 weeks with nevirapine or DLV and 13 weeks with efavirenz) than with the clade B control (at least 15 weeks with nevirapine or DLV and 30 weeks with efavirenz).[28] In general, at the middle interval of the selection period, the subtype B viruses harboured a mixture of wild-type and mutated forms, whereas clade C isolates were mutated forms in regard to all the NNRTIs (nevirapine, DLV or efavirenz). These findings suggest that clade C viruses can more rapidly select for resistance to NNRTIs. Recently, it was reported that non-subtype B HIV-1 strains were likely to be less susceptible to HAART.[101] In addition, non-B sequences were statistically associated with rapid progression to resistance after antiretroviral therapy, and had different mutational patterns to B isolates.[102] Another recent study showed some evidence of HIV-1 subtype impact on the development of NNRTI resistance mutations; there was an increased prevalence of specific mutations and polymorphisms among non-clade B viruses that may have predisposed their hosts to NNRTI treatment failure.[102] These *in vivo* reports correlate with observations in cell culture; discrepancies seen in the development of NNRTI-resistant mutations were not previously noted *in vitro* for subtype C RT.

Our studies also indicate that novel resistance mutations can develop in clade C isolates.[28–30] Two of five Ethiopian clade C isolates initially harboured the A98S secondary mutation associated with resistance to nevirapine.[28] After cell culture selection with nevirapine, a new S98I mutation arose conferring primary phenotypic resistance. In subtype B HIV-1 strains, the mutation at this position has been reported to be A98G and has been observed *in vivo*.[103] This was the first report on the presence of the S98I mutation in RT *in vitro* selected by nevirapine. In clade C-resistant variants selected with nevirapine, several other amino acid changes were also generated, including A98I, A98S, K103N, V106M, V108I and Y181C.[28] The baseline polymorphism at codon 106 in clade C viruses facilitated development of a novel V106M mutation, conferring efavirenz resistance.[28–30]

Apart from S98I and V106M, the codon changes at positions 103, 106, 108 and 181 observed with clade B isolates were also noted in subtype C infections and patients failing NNRTI therapy.[28–30,71,104] Yet the emergence of some NNRTI resistance mutations may be more accelerated in certain HIV-1 non-B subtypes and facilitated by pre-existing genetic polymorphisms. In a recent clinical trial conducted in Uganda for prevention of mother-to-child transmission of HIV-1 with nevirapine, the K103N mutation was detected in 20% of treated women by 6 weeks after receiving a single dose of nevirapine at the onset of labour.[105] This leads to a concern for the postpartum transmission of resistance through breastfeeding.[105]

After selection with DLV, a silent mutation, A62A, initially observed in one Ethiopian isolate, became A62V, a mutation associated with multi-drug resistance against NRTIs.[28,30,71] This shows that silent mutations at sites related to drug resistance in clade C RT have potential impact in facilitating codon changes for emergence of resistance. Another secondary mutation at a site associated with cross-resistance among multiple NRTIs, i.e. substitution V75E, was generated in one of five Ethiopian clade C isolates, grown under conditions of DLV pressure.[28,30,71] Once again, this suggests that clade C RT may have specific patterns of drug resistance that need to be considered. In addition, these findings demonstrate that clade C viruses may progress rapidly to resistance after treatment

with NNRTIs.[28] Additional prospective studies need to be conducted in order to assess the incidence of drug resistance-related mutations in populations infected with subtype C strains and undergoing drug therapy.

It has been established that HIV-2 RT, showing 60% sequence homology with HIV-1 RT, is not inhibited by any of the known NNRTIs.[83] This has been mechanistically linked to differences in the NNRTI binding pocket of HIV-2 harbouring the natural Y181I polymorphism. In other studies, 10 Cameroonian group O viral isolates were shown to be naturally resistant to NNRTIs (nevirapine, DLV, R82913), while showing sensitivity to NRTIs [AZT, didanosine, zalcitibine, lamivudine (3TC)] and PIs (saquinavir, ritonavir).[81,82] Group O viruses carry the natural Y181C polymorphism similar to the Y181I divergence seen in HIV-2. Four Romanian clade F isolates, showing 7.4% genotypic variation from clade B isolates, were also shown to have reduced sensitivity to the TIBO compound, while demonstrating phenotypic susceptibility to other NNRTIs such as nevirapine and DLV, as well as to NRTIs and PIs.[84] In contrast, the phenotypic sensitivity of clade C isolates from five drug-naive infected Zimbabweans to NRTIs and NNRTIs was reported to be similar to that of clade B isolates.[85,86]

In our study, the presence of certain secondary mutations associated with resistance to NNRTIs and to zidovudine (ZDV) did not significantly decrease the susceptibility of Ethiopian or Botswanan clade C strains to RT inhibitors, except for one virus that harboured G190A, a nevirapine resistance primary mutation.[28,30] However, resistance to efavirenz and nevirapine developed more rapidly. This has serious clinical ramifications since these two drugs are inexpensive and accessible to resource-poor nations.

Immunodominant epitopes

Several immunodominant regions have been characterized in HIV-1 clade B RT. Therefore, the peptides harbouring drug-selected mutations that appear in these epitopes may be of interest in therapeutic immunization protocols to restrict emergence of escape mutations and antiviral drug resistance. However, little is known about potential divergence among RTs of different HIV-1 subtypes. Diversity in the *pol* regions of HIV-1 clade C, corresponding to known cytotoxic T lymphocyte (CTL) and T-helper epitopes within clade B RT, could be important and confound immunotherapeutic strategies that target RT immunogenic regions.

A total of 14 clade C antiviral treatment-naive isolates were included in our studies, comprising five previously characterized clade C isolates from Ethiopia and nine other HIV-1 isolates obtained from nine drug-naive individuals originating from Botswana.[30] Screening for the diversity of immunodominant regions of HIV-1 subtype C RT was carried out to identify amino acid substitutions that may affect recognition of these epitopes by cellular immune response effectors.

A polymorphism was identified that clustered within certain CTL epitopes of clade C isolates from Ethiopia and Botswana.[30] Such clustering has been reported in Gag-specific CTL epitopes in HIV-1-infected individuals.[106] These clustered mutations in Gag may be required for HIV-1 escape from HLA-B27-restricted CTL responses.[107] To date, there has been no previous report on the genotypic divergence of CTL epitope sequences in clade C RTs from different regions. Such inter- and intra-clade C variations may affect RT immunogenicity and CTL cross-reactivity for different strains of HIV-1, allowing viral escape from immune control.

In addition, analysis of T-helper epitopes in clade C RT of Ethiopian and Botswanan isolates has also shown that clustered polymorphisms were present in certain CD4+ T-cell epitopes, mainly in the N-terminal part of the RT fingers and the C-terminal region of the RT palm subdomain. T-helper epitope diversity observed in clade C RT is another factor that may potentially contribute to a divergent immune cross-reactivity of RT regions. The role of CD4+ T cells in priming immune responses against HIV has been widely documented. T-helper lymphocytes have been reported to be critical for the induction of CTL responses, as well as for maintaining CD8+ T-cell memory and for the maturation of CD8+ T-cell function.[108,109] The polymorphism found in clade C RT sequences, corresponding to known clade B regions that trigger immunodominant T-helper responses, emphasizes the need for global screening for distinct immunogenetic patterns among HIV-1 subtypes. This may reveal immune correlates for a broadly cross-reactive immune therapeutic approach to prevent the destruction of CD4+ T cells by HIV.

Despite the natural polymorphisms among different HIV-1 subtypes, the recognition of RT epitopes by CTLs and T-helper cells has been reported to be affected by antiviral drug resistance mutations.[110–112] A series of mutations selected by NNRTIs and NRTIs can lead to viral drug resistance.[104,113] Mutations generated during antiviral therapy could also decrease immune responsiveness to RT, in the case of amino acid substitutions within epitopes that are normally recognized by CTLs and T-helper cells.

To identify drug resistance mutations that might be generated within immunogenic motifs of clade C RT, our group used increasing concentrations of different NNRTIs (nevirapine, DLV and efavirenz) as well as NRTIs (3TC and ZDV).[28–30] Experiments carried out with Ethiopian and Botswanan subtype C strains revealed a panel of common mutations at sites associated with drug resistance as well as odd amino acid changes, A62V, V75E, L210M associated with secondary resistance to NRTIs, as well as S98I, K103E, V106M conferring NNRTI resistance. In the case of RT CTL recognition motifs, mutations at drug resistance sites were mainly noted within two relatively conserved immunogenic

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regions of RT overlapping CTL epitopes 103–118, 108–123, 175–184 and 180–190.[30] In the case of RT T-helper epitopes, half of the drug resistance mutations arose in relatively conserved regions 48–73, 62–78 and 88–100, and half arose in highly polymorphic epitopes, i.e. 171–191, 195–210 and 196–216.[30] Many other mutations, unrelated to drug resistance sites, were also noted within regions overlapping the CTL and T-helper epitopes of HIV-1 clade C RT. The impact of these residues, including the drug resistance mutations and amino acid substitutions due to clade C polymorphism, on the specific immunogenicity of clade C RT is unknown. Some antiviral drug mutations in clade B RT have been reported to increase the immunogenicity of previously poor immunogenic regions of RT.[112]

We have mainly investigated the fingers and palm subdomain of RT, as the majority of drug resistance mutations are generated within these areas of RT.[71,83,104] We have noted the existence of an important polymorphism in the CTL and T-helper epitopes in clade C RT. An average sequence homology of 88.4–91.5% was found among Ethiopian and/or Botswanan clade C isolates and other HIV-1 group M subtype prototype strains. These observations suggest that large immunological differences may exist among RTs.

The immunogenic properties of HIV-1 RT have been documented in several studies.[112,114–117] Thus, HIV-1 subtype natural polymorphisms which exist at critical amino acids may anchor positions within epitopes that may alter mechanisms of HIV-1 RT fragment processing and presentation, allowing immune escape of HIV-1 subtype variants.[118–120] There is ample evidence to demonstrate that the immune system may control and significantly suppress viral replication during early stages of HIV-1 infection.[109,121] Genotypic divergence noted in clade C RT suggests that a global characterization of CTL and T-helper anchor motifs and predicted drug selected mutations in HIV-1 non-B subtype RT is warranted. Knowledge of frequently arising baseline polymorphisms and drug-related mutations, in the context of immune responsiveness to RT, may boost our understanding of the immunotherapeutic control of HIV infection.

Conclusions

These considerations of genetic diversity and its potential consequence on drug resistance are of paramount significance in treating non-B HIV. Antiviral drug regimens presently in use have been designed against clade B, and so might not be equally effective in Africa or Asia. Indeed, as noted above, non-B infections are both less susceptible to HAART and statistically associated with a more rapid post-HAART progression of mutational patterns than B isolates.[101,102] Moreover, quite apart from genetic considerations, the fact that developing countries can scarcely afford multiple drug regimes for each patient, and must hence resort to suboptimal therapy, e.g. bitherapy (AZT/3TC), favours the accelerated development of drug resistance in these regions. For instance, 7–29% of pregnant women administered AZT monotherapy developed AZT-resistant strains, as did 5–21% of their infected offspring.[122] Developed countries have a vested interest in screening the phenotypes and genotypes of non-B subtypes and in ensuring the availability of antiretroviral drugs for the treatment of HIV disease throughout the planet.

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