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Drug resistance in non-subtype B HIV-1

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Abstract

Treatment of HIV-1 with antiretroviral therapy may select mutations in the *pol* gene associated with resistance to reverse transcriptase inhibitors and protease inhibitors. To provide durable clinical benefit, emergence of drug resistance is countered by prescription of alternative drug regimens. Data on sequential treatments that are effective after virologic failure and the selection of drug resistance is largely confined to HIV-1 subtype B, the clade that has circulated in North America and Europe. However, HIV-1 subtype B currently accounts for only 12% of the estimated 40 million HIV infected individuals worldwide. The global HIV-1 epidemic includes infection with nine identified HIV-1 group M subtypes (A–K), as well as distinct sub-subtypes and numerous chimerical or recombinant forms. Increasing access to treatment of HIV-1 in the developing world and increasing non-subtype B infection through travel and migration pose new questions about the susceptibility and response of these diverse HIV-1 viruses to antiretroviral drugs. Here we review HIV diversity and the published literature on drug resistance, comparing the known resistance mutations in individuals infected with subtype B to the growing experience in the treatment of non-subtype B HIV-1 worldwide.

Keywords: HIV-1 subtypes; Non-B; Drug resistance

1. HIV-1 diversity

HIV is characterized by a wide range of viral genetic diversity among distinct types, groups and clades (Hu et al., 1996; McCutchan, 2000; Peeters, 2001; Peeters and Sharp, 2000). The viruses that cause AIDS are the only known members of the lentivirus family of retroviruses, which infect humans, after undergoing cross species transmission events from non-human primates (Fauci and Desrosiers, 1997). There are two distinct types of human retroviruses, HIV-1 and HIV-2, and at least 18 distinct simian immunodeficiency viruses that naturally infect different African primates. These viruses are distinguished by their genome organizations and phylogenetic relationships (Hahn et al., 2000).

Differences between HIV-1 and HIV-2 are well documented in terms of transmissibility, pathogenesis and pattern of spread (De Cock et al., 1993; Kanki and De Cock, 1994). HIV-2 was the first human lentiviral infection for which there was sufficient evidence to substantiate its zoonotic origins, from sooty mangabey monkeys (Clavel et al., 1986; Reeves and Doms, 2002). Similarly, it appears that HIV-1 arose as a consequence of transmission from chimpanzees to humans (Hahn et al., 2000). Notably, there are very important differences between HIV-1 and HIV-2 isolates with respect to drug susceptibility. Specifically, non-nucleoside reverse transcriptase inhibitors (NNRTIs) are active only against HIV-1 reverse transcriptase (RT), and demonstrate little activity against HIV-2 RT (Hizi et al., 1993; Ren et al., 2002; Yang et al., 1996).

HIV-1 is the major pathogen responsible for the AIDS pandemic. Further analyses of different strains of HIV-1 from diverse geographical origins, show that isolates can be subdivided into groups, subtypes, sub-subtypes and circulating recombinant forms (CRFs), based on phylogenetic sequence differences.

Groups refer to distinctive HIV-1 lineages M (for Major), O (for Outlier), and N (for New, or Non-M, Non-O). All of

Abbreviations: AIDS, acquired immunodeficiency syndrome; CRF, circulating recombinant form; FDA, Food and Drug Administration; HIV, human immunodeficiency virus; HAART, highly active antiretroviral therapy; IAS, international AIDS society; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; RT, reverse transcriptase

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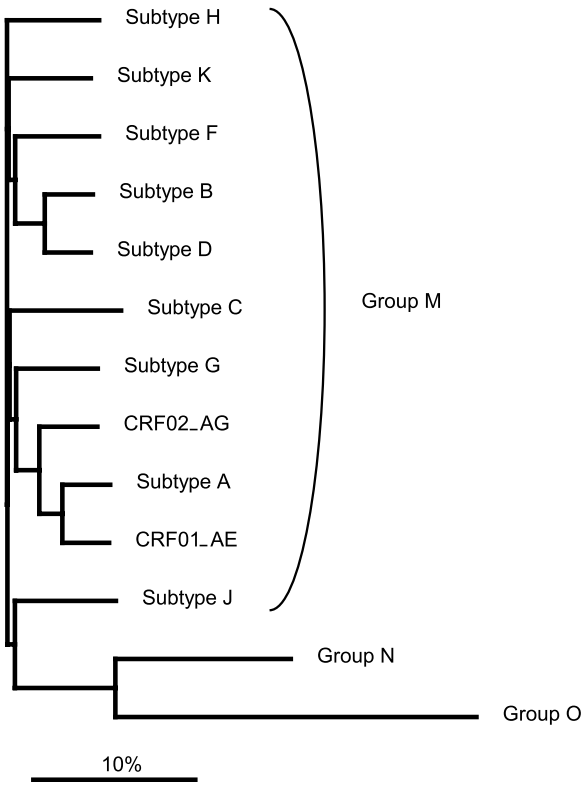

Fig. 1. HIV-1 diversity. Neighbor-joining tree constructed from *pol* sequences of 13 reference isolates (Subtype A isolate U455, CRF01_AE isolate U54771, CRF02_AG isolate L39106, Subtype B isolate HXB2, Subtype C isolate C2220, Subtype D isolate NDK, Subtype F isolate 93BR020, Subtype G isolate SE6165, Subtype H isolate 90CR056, Subtype J isolate SE9173c, Subtype K isolate 97EQTB11C, Group N isolate YBF30, Group O isolate AZT70C). The scale bar represents a 10% nucleotide difference. The tree was constructed using the PHYLIP phylogenetic package (Felsenstein, 1993).

the HIV-1 strains described in the 1980s, and still the vast majority of strains found worldwide, belong to one of these lineages, group M. Group O seems to be endemic to and largely confined to Cameroon and neighboring countries in West Central Africa, where these viruses represent a small minority of HIV-1 strains (Gurtler et al., 1994; Jaffe and Schochetman, 1998; Janssens et al., 1999; Nkengasong et al., 1998; Vanden et al., 1996). Group N has only recently been identified, and is so far represented by a limited number of isolates from Cameroonian persons (Fonjungo et al., 2000; Simon et al., 1998).

Within group M, there is further phylogenetic distinction, which was initially noted in the most variable part of the virus—the envelope glycoproteins—allowing the classification of a limited number of discrete clades or subtypes even within the relatively conserved *pol* gene (Fig. 1). The subtypes are approximately equidistantly related, and are distinct from one another across the entire genome (Korber et al., 2000; Robertson et al., 2000a,b). There are currently nine accepted subtypes of HIV-1 group M (A–D, F–H, J and K). Within some subtypes further phylogenetic structure can be identified, leading to a classification into subclades

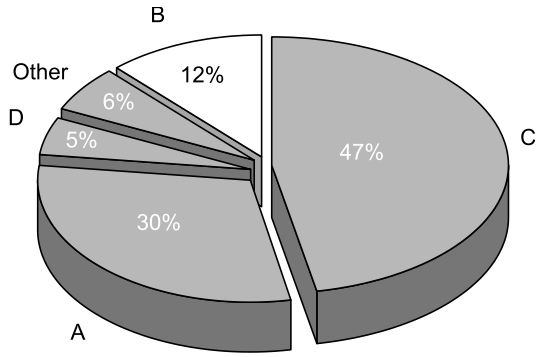

Fig. 2. A pie chart representing global distribution of HIV-1 subtypes in new infections in the year 2000 (Osmanov et al., 2002). Gray pie slices represent non-B subtypes, accounting for 88% of infections. The white pie slice represents subtype B, accounting for 12% of infections. (A) subtype A and CRF01_AE and CRF02_AG; (B) subtype B; (C) subtype C; (D) subtype D; Other- other subtypes and CRFs.

(A1 and A2, F1 and F2) (Gao et al., 2001; Robertson et al., 2000a,b; Triques et al., 2000).

By 1995 phylogenetic analyses of the HIV-1 full-length genome sequences demonstrated that certain isolates clustered with two or more subtypes, and recombination was identified as an additional source of HIV-1 variation (Robertson et al., 1995a,b; Salminen et al., 1995). Accumulating global data show that a significant percentage of strains are mosaics of two or more genetic subtypes, often mixtures of the prevalent subtypes circulating in the region. Thus, in addition to distinct subtypes, CRFs are continuously being identified (Kuiken et al., 2000). The two most common CRFs are CRF01_AE and CRF02_AG, dominant in Southeast Asia and West Africa, respectively.

According to a recent report by the WHO–UNAIDS Network for HIV Isolation and Characterization (Osmanov et al., 2002), in the year 2000, most new infections were due to HIV-1 subtype C, which caused 47.2% of all new HIV-1 global infections. Subtype C HIV-1 predominates in Southern Africa, Ethiopia and India. The second most common clade was subtype A, which caused 30% of all new infections, (including CRF01_AE and CRF02_AG). Overall, more than 18% of new infections were attributed to HIV-1 recombinants. Subtype B was responsible for only 12.3% of global infections. It is the predominant subtype in the Western world, although there are increasing numbers of non-subtype B HIV-1 infection in North America and in Western Europe (Fig. 2) (Balotta et al., 2001; Boni et al., 1999; Brodine et al., 1999; Couturier et al., 2000; Deroo et al., 2002; Holguin et al., 2001; Irwin et al., 1997; Thomson and Najera, 2001).

2. HIV-1 drug resistance

Current FDA approved antiretroviral medications include 16 drugs targeted against two *pol* gene enzymes, the RT and protease: seven nucleoside RT inhibitors (NRTIs) (abacavir,

didanosine, lamivudine, stavudine, tenofovir, zalcitabine and zidovudine), three NNRTIs (delavirdine, efavirenz, and nevirapine) and six protease inhibitors (PIs) (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir and saquinavir).

Antiretroviral drug resistance is the major obstacle to durable treatment of HIV-1. Our understanding of drug resistance is largely limited to HIV-1 from those areas of the world where subtype B viruses predominate and antiretroviral therapy has been accessible.

The development of drug resistance to the NRTIs, NNRTIs and PIs, is both a cause and a result of virologic treatment failure with incomplete virus suppression. The selection of drug resistance poses ongoing challenges to the success of future treatment regimens. An increasing number of studies in subtype B HIV-1 show that the presence of drug resistance before starting a new drug regimen is an independent predictor of virologic response to that regimen (DeGruttola et al., 2000; Hanna and D'Aquila, 2001; Haubrich and Demeter, 2001). In randomized clinical trials, subjects whose physicians have access to drug resistance data, particularly genotypic resistance data, respond better to therapy than control subjects, whose new regimens are crafted without access to the results of these assays (Baxter et al., 2000; Cingolani et al., 2002; Cohen et al., 2002; Durant et al., 1999; Haubrich et al., 2001; Meynard et al., 2002; Tural et al., 2002).

A standardized RT and protease numbering system for HIV-1 drug resistance mutations has been developed, which is used for genotypic interpretations. All sequences are currently compared with a consensus B sequence (http://hiv-web.lanl.gov). Mutations are represented by a letter indicating the consensus B wild-type amino acid followed by the amino acid residue number, followed by a letter indicating the mutation (e.g. L90M). It is common to label some mutations either primary (or less commonly 'major') or secondary (or 'minor'; Hirsch et al., 2000). Primary mutations reduce drug susceptibility by themselves whereas secondary mutations reduce drug susceptibility or improve the replicative fitness of isolates with a primary mutation (Shafer, 2002).

Several web-accessible sites which review antiretroviral drug resistance include the International AIDS society (IAS) USA (http://www.iasusa.org); Los Alamos Database (http://hiv-web.lanl.gov); Stanford HIV Drug Resistance Database (http://hivdb.stanford.edu); and HIV Resistance Web (http://www.hivresistanceweb.com). Reviews of HIV-1 RT and protease drug resistance critically examine the accumulating evidence for genotypic resistance of subtype B HIV-1 (Deeks, 2001; Erickson et al., 1999; Hirsch et al., 2000; Loveday, 2001; Miller, 2001; Shafer, 2002; Shafer et al., 2000; Soriano and de Mendoza, 2002a,b).

3. HIV-1 drug resistance in untreated individuals

Detection of RT and protease mutations associated with drug resistance in subtype B HIV-1 in individuals who have not been exposed to antiretroviral therapy is thought to result from either transmission from a treated individual, or drug resistant variants that differ from the wild type consensus sequence.

In the last decade transmission of drug resistant virus has been recognized among HIV-1 subtype B newly infected individuals in the US and Europe (Boden et al., 1999; Brodine et al., 1999; Erice et al., 1993; Little et al., 1999, 2002; Yerly et al., 1999). Thus mutations, usually associated only with drug therapy, have been observed in up to 20% of cases of acute and early HIV-1 infection. Moreover, some unique genotypes with intermediate amino acid residues associated with reversion (back-mutation) of resistance mutations to a wild type genotype (e.g. RT T215D/N/S/C/E) in the context of recent transmission are increasingly observed (Garcia-Lerma et al., 2001; Goudsmit et al., 1997; Rubio et al., 1997; Yerly et al., 1998). The escalating rates of transmission of drug resistant virus observed in the past few years, coupled with the poorer response to treatment in persons with drug resistant virus, are being considered as a basis for a recommendation that resistance tests should be performed routinely for persons with new HIV infection (Little et al., 2002).

In the absence of any drug exposure, RT and protease sequences from B and non-B HIV-1 are polymorphic among about 40% of the first 240 RT amino acids and 30% of the 99 protease amino acids. Some of these amino acid substitutions occur at high rates in non-subtype B viruses at positions associated with drug resistance in subtype B. These include protease positions 10, 20, 36, 63, 71, 77 and 93, and RT positions 69, 75, 98, 106, 118 and 179 (Kantor and Katzenstein, 2003 Holguin and Soriano, 2002).

Thus mutations at polymorphic and conserved sites that are associated with drug resistance in subtype B HIV-1 are found at high rates in untreated persons infected with non-subtype B HIV-1. As an increasing number of individuals with non-subtype B infection access antiretroviral therapy, these genotypic differences in non-B consensus (wild-type) sequences may result in new drug resistant forms as well as differences in the long-term outcomes of antiretroviral therapy.

4. HIV-1 drug resistance in treated individuals

The prevalence of drug resistance mutations in subtype B infected individuals in the United States and Canada may be as high as 78% among treated individuals with detectable levels of HIV-1 RNA (Richman et al., 2001). There is a growing and extensive literature on sequence data from untreated and treated persons infected with subtype B virus. This has led to increasingly accurate, albeit complex, interpretations of subtype B drug resistance (http://www.aidsinfo.nih.gov) (Hirsch et al., 2000). Patterns of mutations arising during virologic failure with specific drugs have become increasingly recognizable making it possible to recommend alternative drugs to treat persons with resistance.

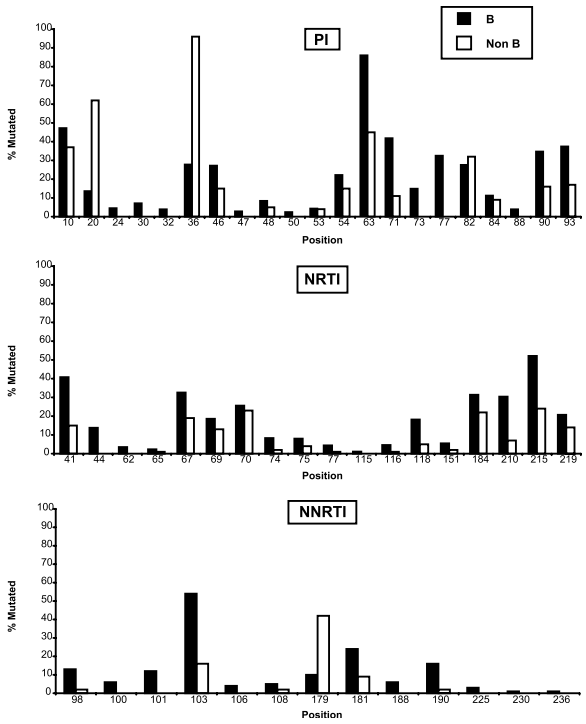

Fig. 3. Frequency of mutations at RT and protease positions associated with subtype B drug resistance in isolates from treated persons infected with B and non-subtype B HIV-1 isolates. PI denotes protease inhibitors, NRTI denotes nucleoside RT inhibitors, NNRTI denotes non-nucleoside RT inhibitors. Non-B isolates include 6 and 9 RT and protease subtype A isolates, 9 and 6 subtype C, 30 and 10 subtype D, 15 and 5 subtype F, 5 and 3 subtype G, 3 and 1 subtype H, 2 and 0 subtype J, 7 and 1 subtype K, 30 and 2 CRF01_AE, 19 and 16 CRF02_AG isolates, respectively.

However, such data are generally not available for non-B subtypes. Frequency and patterns of specific mutations and response to antiretroviral drug therapies have not been characterized. RT and protease sequences from recent publications including 157 non-B infected persons, have been compiled in the Stanford HIV RT and Protease Sequence Database (Kantor et al., 2001). Fig. 3 shows the frequency of NRTI, NNRTI and PI related drug resistance mutations in these 157 non-B infected persons (including subtypes A, C, D, F, G, H, J, K, CRF01_AE and CRF02_AG) and in 1400 subtype B infected persons, who received antiretroviral therapy. These sequences and the individually associated treatment histories are available on the Stanford Database website (http://hivdb.stanford.edu). Within each subtype, frequencies of observed changes in amino acids are distinguished from the expected residues at each position of the subtype B consensus sequence (http://hiv-web.lanl.gov).

Higher percentages of drug resistance mutations to NRTIs and PIs and less evidence of resistance to NNRTIs probably reflect the more recent introduction of NNRTIs into clinical care, particularly in developing countries. Some mutations that are associated with drug resistance in subtype B (protease positions 20, 36, 82 and RT position 179) are more common in non-B subtypes. However, even in this relatively small sample of isolates from drug treated non-B infected individuals, mutations are observed at 22/31 (71%) known subtype B RT drug resistance positions (15/18 NRTI and 7/13 NNRTI related positions), and 13/21 (62%) known subtype B protease drug resistance positions.

A summary of the recently published studies of persons infected with non-B HIV-1 receiving antiretroviral therapy demonstrates the geographic diversity and the relatively small numbers of persons with non-subtype B viruses who have been followed in treatment protocols (Table 1). For most of these studies sequences and/or treatment history data are not available and thus they are not included in the compilation in Fig. 3.

5. Studies in Africa

C. Adje et al. described genotypic and phenotypic data on RT and protease sequences from 68 non-B infected persons from Cote D'Ivoire (Adje et al., 2001). In this study 57% of persons had at least one RT or protease primary resistance

Table 1
Published studies on drug resistance in persons infected with HIV-1 non-B isolates

Region	Country	Reference	Main subtypes	Number of persons
Africa	Cote d'Ivoire	Adje et al., 2001	A, AG, D, G, H	68
	Uganda	Weidle et al., 2002	A, C, D	94
	Gabon	Vergne et al., 2002	A, AG, AE, D, G, H, J	22
	Zimbabwe	Kantor et al., 2002	C	21
Europe	United Kingdom	Barlow et al., 2001	A, C, D, F, G, H	25
		Cane et al., 2001	C	43
		Pillay et al., 2002	A, AG, AE, C, D, F, G, H	67
		Frater et al., 2001	A, C, D	18
	Spain	Perez-Alvarez et al., 2001a,b	G, BG	31
Latin America	Brazil	Caride et al., 2000, 2001	F, A	5
	Brazil	Brindeiro et al., 2002	A, C, F	17
	Cuba	Ruibal-Brunet et al., 2001	A, C	16
Middle East	Israel	Grossman et al., 2001	C	73

AE denotes CRF01_AE and AG denotes CRF02_AG.

mutation. Possible explanations given for the high prevalence of resistance included suboptimal treatment regimens, interruption in supply of antiretroviral drugs, and poor adherence leading to inadequate suppression and selection of resistance.

L. Vergne et al. reported on drug resistance in Gabon, in 22 treated and 13 drug naïve persons infected with non-B HIV-1 (Vergne et al., 2002). In this study 58% of persons treated for a mean of 17.7 months of antiretroviral therapy, had major drug resistance mutations (mostly to NRTIs). The high percentage, similar to the findings in Cote d'Ivoire, was thought to be due to poor access to drugs and limited capacity to deliver highly active antiretroviral therapy (HAART).

P. Weidle et al. described a pilot program in Uganda, assessing clinical and laboratory information from 94 persons receiving antiretroviral therapy, infected with subtypes A, C and D (Weidle et al., 2002). The authors tested phenotypic drug resistance in a subset of specimens. Resistance to at least one drug was found in 65% of persons in whom a result was available. Lower resistance rates were seen for persons who have received HAART versus non-HAART regimens.

We analyzed RT and protease sequences from 21 Zimbabwean persons infected with subtype C who were failing antiretroviral drug therapy (Kantor et al., 2002). Of the 21 persons, 81% had known drug-resistance mutations. We compared these sequences to RT and protease sequences from untreated persons infected with subtype C, and to RT protease subtype B sequences from treated and untreated persons and identified subtype C specific polymorphisms and candidate drug resistance mutations.

6. Studies in Europe

K. Barlow et al. described 25 subjects infected with HIV-1 CRFs, among 118 isolates from non-B infected individuals in the United Kingdom (Barlow et al., 2001). Overall, there appeared to be no difference in the frequency or distribution of key resistance mutations between persons infected with single subtypes and those with recombinant viruses. Furthermore, there were no obvious subtype-associated differences in the key resistance mutations. Evidence for differences between subtypes was seen in mutations such as protease M36I, which is considered a secondary resistance mutation in subtype B, but is virtually universal among subtype C viruses.

P. Cane et al. reported on drug resistance in the protease genes of 55 subtype C persons (43 treated and 12 drug naïve) in the United Kingdom (Cane et al., 2001). Among the 20 persons taking PIs at the time of sampling, 65% had primary protease mutations. The authors noted a high frequency of L90M (a primary protease mutation related to saquinavir, nelfinavir, indinavir and ritonavir), secondary protease mutations at 36 and 93 and a low frequency of D30N (a primary protease mutation related to nelfinavir).

D. Pillay et al. recently reported on the association between virologic response and HIV-1 subtype in 113 non-B infected children receiving antiretroviral therapy (Pillay et al., 2002). No differences were observed in the frequency of development of primary resistance mutations (protease positions 30, 90; RT position 184) in B and non-B viruses, however, differences were observed between subtypes in the prevalence of secondary PI-resistance mutations (positions 20, 36, 77).

A. Frater et al. followed 79 African antiretroviral drug-naïve persons infected with subtypes A, C and D, who were prescribed HAART (Frater et al., 2001). Of these, 76% had undetectable viral load after 12 months. Analysis of the baseline sequences before antiretroviral therapy and comparison of non-B to B sequences revealed several inter-subtype differences at RT and protease positions, which were not, however, associated with a lack of response to therapy. Sequences from 18 persons who failed antiretroviral therapy were further analyzed. Several known subtype B drug resistance mutations were seen, as well as several new mutations, not known to be related to drug resistance in subtype B.

L. Perez-Alvarez et al. from Spain published two reports on 31 persons infected with subtype G and with BG recombinants (Perez-Alvarez et al., 2001a,b). Some of the persons were naïve to drug therapy, and some were treated. In general, a similar frequency of primary RT and protease mutations was observed in B and non-B subtypes. Different frequencies of secondary mutations were seen in some positions (protease positions 36, 46 and RT positions 98, 211).

7. Studies in South America and Cuba

E. Caride et al. published two papers from Brazil, evaluating the RT and protease mutations profile for five persons, four of who were infected with subtype F (Caride et al., 2000, 2001). In the RT, similar genotypic and phenotypic resistance patterns were found. In the protease, different mutation patterns were seen, despite similar drug usage. Primary protease mutations at positions 84 and 90 were not seen in non-B infected persons, and the resistance pathways included mutations at positions 48 and 82. The authors noted several genotypic differences from subtype B, which did not change in vitro drug susceptibility but were thought to lead to the different resistant pathways seen.

An additional recent Brazilian study by Brindeiro et al. compared genotypic and phenotypic resistance in non-B versus B HIV-1 isolates from children failing antiretroviral therapy (Brindeiro et al., 2002). They found significant differences among subtypes in the prevalence of secondary protease mutations, reflecting the prevalence of polymorphisms in non-B isolates.

I. Ruibal-Brunet et al. determined the prevalence of drug resistance and analyzed non-B subtypes in Cuba (Ruibal-Brunet et al., 2001). In a sample of 103 persons,

76 received drug therapy. Of the treated individuals, 21% were infected with subtypes A and C. Similar profiles of resistance mutations were seen in isolates from B and non-B infected persons.

8. Studies in Israel

Z. Grossman et al. compared sequence data from 87 mostly subtype C infected immigrants from Ethiopia, (14 drug naïve) to 78 subtype B infected persons (20 drug naïve) from Israel (Grossman et al., 2001). Different rates of mutations were found between subtypes both in drug-naïve and in treatment-experienced persons. The protease D30N mutation, which is associated with nelfinavir therapy, and the AZT related RT mutations were noted to occur at lower rates in subtype C infected persons who received these drugs.

9. Summary and conclusions

Although HIV-1 subtypes other than B are responsible for most new HIV infections worldwide, virus sequence data is described from a limited number of persons infected with HIV-1 non-B subtypes. Review of publications which examined the response among non-B infected individuals to antiretroviral drugs demonstrates that about 60% of non-B infected persons who fail antiretroviral therapy have genotypic evidence of drug resistance. This figure is comparable with the findings in the US and Canada (Richman et al., 2001).

Similar primary drug resistance mutations appear to evolve in the various subtypes. RT and protease polymorphisms in non-B infected untreated individuals, some of which are at drug resistance positions, may result in differences in response to therapy and the selection of drug resistance. Emerging differences in antiretroviral drug resistance to different drug classes in some non-B isolates exist, as summarized below.

9.1. NRTIs

Although several studies found lower rates of some NRTI resistance mutations in non-B isolates, rates of primary drug resistance mutations appear similar to subtype B. Different mutation patterns may be due to the use of dual nucleoside therapies, which are more widely reported from Southern Africa and other resource limited settings where non-B subtypes predominate. Some of the described subtype specific polymorphisms (RT positions 39, 43, 60, 207, 211) are adjacent to sites related to drug resistance, and may have an effect on drug efficacy and drug resistance evolution.

9.2. NNRTIs

These drugs have not been widely available in most developing countries until 1999–2000, and are only recently becoming a preferred treatment due to price reduction and ease of administration. There are accruing reports of K103N and additional major NNRTI-related drug resistance mutations in untreated persons (Clevenbergh et al., 2002). In addition, mutations at RT positions 98 and 179, which are considered secondary NNRTI associated mutations in subtype B, and specific adjacent polymorphisms (RT positions 177 and 178), are frequently identified among drug naïve non-B infected persons. The significance of these mutations needs to be defined.

9.3. PIs

There is emerging evidence for the existence of different pathways of drug resistance based on subtype, for example the predominance of L90M rather than D30N among persons with non-B infection exposed to nelfinavir. In addition, isoleucine instead of the wild-type valine in the protease 82 position (a site of primary resistance mutation for several PIs) is more prevalent in some non-B isolates and may decrease susceptibility to PIs (Descamps et al., 1998; Kaplan et al., 1994; Maguire et al., 2002). Mutations at secondary subtype B PI-related positions such as 10, 20, 36, 71, 77 and 93 occur at high frequency in non-B viruses. It is not clear whether this may reduce the activity of some PIs, or if this is a basis for the evolution of different primary resistance mutations and cross-resistance.

In summary, the use of antiretroviral therapy in persons infected with non-B HIV-1 leads to the selection of drug resistance mutations, which are generally similar to those described in subtype B. Use of specific drug regimens in different regions and populations and development of treatment strategies depend on the investigation and understanding of drug resistance in the context of diverse viral subtypes. Many sequences with data on drug doses, duration, adherence and clinical outcomes are needed to analyze these complex patterns of drug resistance in non-B infected individuals. Worldwide collaboration is essential to identify similarities and differences between B and non-B HIV-1, and to optimally interpret non-B sequence data in clinical settings.

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