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FOK I POLYMORPHISM AS A GUN TO DISCRIMINATE BRAZILIAN HIV-1 SUBTYPE B SAMPLES

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Three HIV-1 subtypes B,F and C have been found in Brazil so far, in addition to recombinant B/F genomes, with a clear predominance of isolates belonging to the B subtype (88.5%). Those studies have also shown that many Brazilian subtype B isolates present a typical amino acid composition (GWGR) at the conserved crown of the gp120 V3 loop, suggesting that subtype B isolates could be split in two main groups, one corresponding to the USA/Europe consensus B sequence and a second one, called B', typically found in Brazil. Recently, a very sensitive technique to define HIV-1 subtypes was developed (Heteroduplex mobility assay) which facilitate molecular epidemiological studies in large scale. However, in Brazil in addition to the identification of the HIV-1 subtype, it is also important to discriminate its B and B' variants. DNA sequencing has been used for such discrimination, however, it is too expensive and time consuming to be useful for epidemiological studies. In order to overcome this problem, we tried to discriminate B and B' variants of Brazilian HIV-1 samples by restriction fragment length polymorphism using Fok I restriction enzyme , identified as discriminative for both variants based on the corresponding restriction maps of the V3 region. DNA samples were PCR amplified using a nested protocol with outer primers ED3/ED14 and inner primers ED31/ED33 followed by HIV-1 subtyping by heteroduplex mobility assay as described (Delwart et al,1993,Science 262:1257-61). After determination as B subtype, the sample PCR amplified were digested with Fok I restriction enzyme and eletrophoresed thorough 2% agarose gels for 1h in 1XTBE. In order to confirm the specificity of this digestion we have determined the DNA sequence of the V3 region for a sample subset. Based on the analysis of 127 samples from Rio de Janeiro City typed as B subtype, at least 5 different restriction fragment patterns could be detected after Fok I digestion. For the 50 samples (39,4%) typed as the B' variant, two patterns corresponding to 410bp and 230bp (96,0%) or 410bp,120bp and 80bp (4,0%) were identified, as confirm by DNA sequencing. For the 77 (60,6%) conventional B subtype samples, 74 (96,1%) were undigested with this restriction enzyme, whereas 2 (2,6%) gave a 500bp and 120bp pattern, and (1,3%) a 350bp and 275bp pattern. In conclusion, the 410bp Fok I fragment seems to be discriminative of the B' Brazilian HIV-1 samples. Attention has to be made on the eletrophoresis molecular weight determination as even conventional B subtype can be digested with this restriction enzyme giving, although distinct, closer patterns.

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