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IS THE HUMAN LEUCOCYTE ANTIGEN B*5701 PREDICTED BY SEQUENCE VARIATIONS OF HIV-1 SUBTYPE F REVERSE TRANSCRIPTASE?

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Abstract. Objective: to evaluate the predictive value of mutations in position 245 of HIV reverse-transcriptase as a marker of the presence of HLA-B*5701 in HIV-1 subtype F1 infected patients. **Methods**: HIV drug resistance testing was performed by using the ViroSeq Genotyping System. Sequence-based HLA typing was used for detection of HLA-B*5701. **Results**: A high sequence conservation in HIV reverse-transcriptase codon 245 was found both in treatment-naïve and in treatment-experienced patients. Wild-type sequence was present in all 4 patients with B*5701, but also in 109 (98.1%) of 111 subjects without B*5701. There was no significant association between HLA-B*5701 and mutations in HIV reverse-transcripase codon 245. **Conclusions**: Sequence variation in HIV reverse transcriptase 245 position is not a useful marker for predicting HLA-B*5701 in subtype F infected patients.
Keywords: abacavir hypersensitivity, HLA-B*5701

Introduction

Adverse drug effects are among the most common reasons for low adherence to antiretroviral (ARV) therapy and represent a major limitation to a long-term successful treatment of the human immunodeficiency virus (HIV) type 1 infection. The risk of adverse effects is, therefore, an important factor in the selection of the ARV regimens [1].

Abacavir (ABC) is a nucleoside reverse-transcriptase inhibitor with effective antiviral activity against HIV-1, few drug interactions and a favourable long-term toxicity profile. The most important treatment-limiting adverse effect of abacavir is a hypersensitivity reaction (HSR), which occurs in approximately 5% of the treated patients. Symptoms of HSR usually appear within the first six weeks of starting abacavir and include any two or more of the following: fever, rash, gastrointestinal / respiratory symptoms, malaise, fatigue [2]. These symptoms increase in intensity with continued ABC use, but resolve within several days after discontinuation of ABC. Therefore, abacavir or abacavir-containing drugs should be discontinued immediately when hypersensitivity is suspected, and rechallenge should be strictly avoided because more severe and even life-threatening systemic manifestations can occur rapidly.

These symptoms are nonspecific and consequently ABC HSR can be difficult to distinguish from concomitant infection, inflammatory disease or reaction to other drugs (rash induced by efavirenz, hypersensitivity reaction to nevirapine, gastrointestinal symptoms associated with protease inhibitors). This can lead to false positive clinical diagnoses in a quarter of cases: in double-blind, comparative studies, HSR was diagnosed clinically

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in 7%–8% of patients who were receiving abacavir, but also in 2 to 3% of persons not taking abacavir [3,4,5].

The use of skin patch testing decreases the problem of false positive clinical diagnosis by identifying patients with an immunologically mediated hypersensitivity reaction to abacavir. However, cutaneous patch testing cannot be used as a predictive screening tool, because previous ingestion of the drug is necessary for the immunologic priming. Although patch testing has been successfully used to improve ABC HSR diagnostic precision in recent clinical trials, this test remains a research tool and not a validated diagnostic test [1,6].

A large body of evidence has demonstrated a strong association between the presence of the HLA B*5701 allele and ABC HSR. The sensitivity of HLA-B*5701 test for clinically diagnosed ABC HSR is between 45 and 50% in white subjects and 14% in black subjects. When clinical diagnosis is improved by skin patch testing, the sensitivity of HLA-B*5701 as marker for ABC HSR increases to 100% for white and black patients, with a specificity of 96–98% for both groups [7,8]. The screening of HLA-B*5701 prior to abacavir administration eliminates immunologically confirmed ABC HSR and significantly reduces the rate of diagnosis of clinical HSR [7,9].

Although the HLA testing for HLA-B*5701 screening is cost-effective [1,10,11], its routine use in the clinical practice could be limited by the complexity and the costs of the high resolution HLA typing. Thereby, current development of alternative methods is expected to increase the accessibility of the HLA-B*5701 screening for HIV infected patients.

Cytotoxic T lymphocytes (CTLs) recognizing human leukocyte antigen (HLA) class I– restricted viral epitopes play a major role in the immune control of HIV-1 infection. Multiple evidences indicate that HLA class I – restricted CTLs select escape mutations reproducible among individuals with the same HLA alleles [12,13]. On the basis of these data, some authors hypothesized that the presence of a specific escape mutation selected in the HLA-B*5701–specific ISW9 epitope in HIV reverse-transcriptase may serve as an indirect marker of B*5701 expression [14]. The detection of this escape mutation in the codon 245 of reverse transcriptase (RT) by using the standard HIV drug-resistance testing may provide prescreening informations with no additional costs [14]. Since this method has a high sensitivity and a very high negative predictive value, the presence of the clade B wild-type 245V amino acid sequence could be useful to identify individuals who may be safely treated with abacavir. Some studies have shown that this method has a high sensitivity also in small cohorts of patients infected with non-B subtypes [14,15], but this observation needs further investigation in larger cohorts of patients infected with distinct non-B subtypes.

The objective of our study was to evaluate if mutations in position 245 of HIV RT could be used as a marker of the presence of HLA-B*5701 in HIV-1 subtype F1 infected patients. For this issue we have used sequence-based typing (SBT), which is the gold standard technique for identifying specific HLA alleles. This method is very precise and highly reproducible, giving results with less ambiguities compared to other techniques, such as sequence specific oligonucleotide probe (SSOP). Sequencing of the HLA-B locus provides high resolution HLA typing, needed to identify the HLA-B*5701 allele and to differentiate it from closely related alleles, such as HLA-B*5702, HLA-B*5703, and HLA-B*5801/5802, which do not appear to be associated with ABC HSR [10,16].

Methods

Patient and data collection

The National Institute for Infectious Diseases "Prof Dr Matei Balş" monitors a large cohort of HIV infected patients. Adult patients with HIV-1 subtype F1 infection from this cohort were invited to participate in the study. At least one HIV drug resistance test was available for each of the participants in the study. Informed consent was obtained from all participants.

HIV sequencing

HIV genotype analysis was performed on virus from plasma samples by using a commercially available kit (ViroSeq HIV-1 genotyping system, Celera Diagnostics) according to the manufacturer's recommendation. Briefly, the HIV RNA was extracted, reverse-transcribed and then amplified by PCR. The RT-PCR product was sequenced bidirectionally on an ABI Prism 3100 Avant genetic analyser (Applied Biosystems) by using six primers. The sequences were analysed with the Sequencing Analysis Software v 3.7 and then assembled with ViroSeq 2.5/2.7 HIV-1 genotyping system software. The operator

validated the correctness of each electropherogram interpretation and then saved the sequences in FASTA format. The HIVSeq Program, available at the Stanford database (http://hivdb.stanford.edu), was then used for sequence interpretation. For subtyping purposes we used the publicly available algorithm from REGA HIV-1&2 Automated subtyping tool version 2.0 (http://jose.med.kuleuven.be/genotypetool/html/subtypinghiv.html).

HLA sequencing

Human Leucocyte Antigen typing was performed using the AlleleSEQR HLA Sequencing kit (Applied Biosystems), according to the manufacturer's recommendation. The human DNA extracted from frozen blood samples was purified, then amplified by a PCR protocol. The PCR product was purified with ExoSAP-IT, then used for the sequencing PCR, together with BigDye Terminator ddNTP and primers specific for the exons 2, 3 and 4 of the HLA-B gene. The DNA sequences were then detected by capillary electrophoresis on an Avant 3100 Genetic Analyzer (Applied Biosystems) and the data were processed with *Assign SBT* (Conexio Genomics), a dedicated allelic typing software available at www.conexio.iinet.net.au.

Statistical analysis

The association between the presence of specific mutations at HIV RT codon 245 and the presence of a HLA-B allele observed in our cohort were determined using Fisher's exact test.

Results

Prevalence of mutations at codon 245 of HIV-1 reverse transcriptase

In order to evaluate the prevalence of mutations in codon 245 of RT, we analysed HIV-1 sequences from 228 patients, with equal proportions of ARV-naïve and treatment-experienced. The sequences from our cohort were compared with BCI-RO7, the reference strain for Romanian subtype F1 (Genebank accession number AF447853) and with HXB2, the reference strain for subtype B (Genebank accession number AF033819) (fig 1). The most common (wild-type) amino acid in position 245 in the pretherapy sequences was glutamine (Q). This represents a subtype F polymorphism compared with the subtype B consensus sequence, where valine (V) is in the RT 245 position. In ARV treated patients, glutamine was also the most frequent amino acid in position 245, being present in 112 (98%) patients. Another amino acid detected in this position, both in ARV-naïve and ARV-experienced patients was lysine (K). Moreover, none of the 65 subjects with multiple resistance tests presented sequence variation in RT 245 codon during ARV treatment. The high sequence conservation in HIV RT codon 245 (99% in treatment-naïve and 98% in treatment-experienced patients) suggests that the association, if any, between the amino acid in 245 position and B*5701 might be a useful marker, because it is not impaired by mutations selected by antiretroviral therapy.

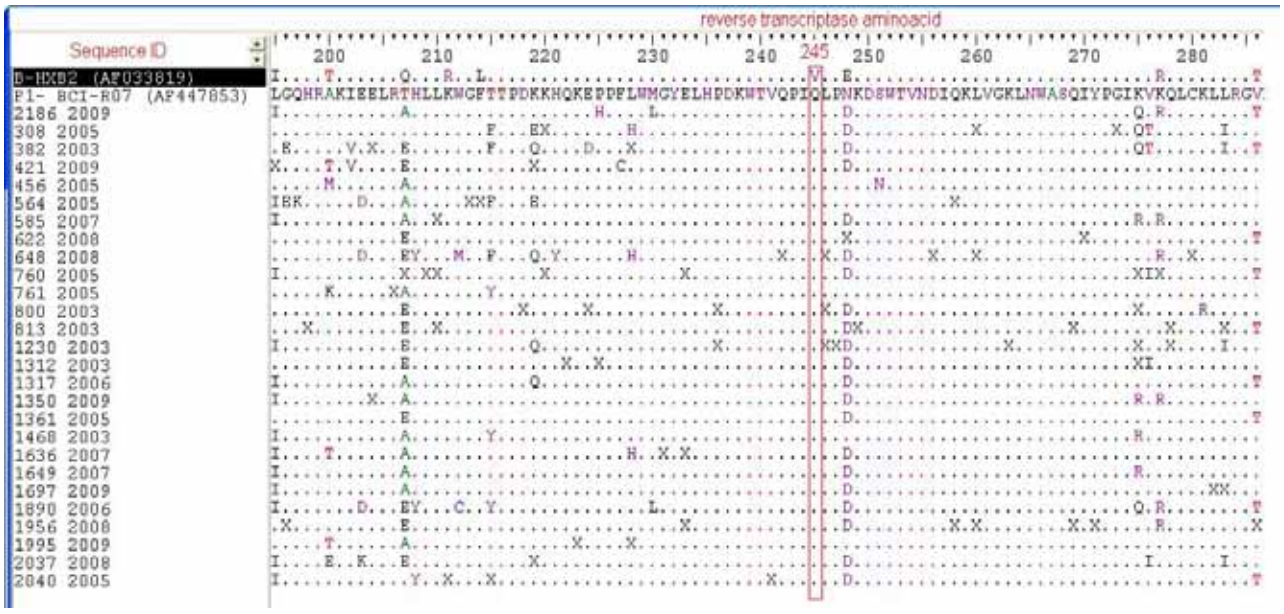

Figure 1. Comparative analysis of F1 reverse transcriptase sequences along with B and Romanian F1 reference strains
The figure represents a part of the total alignment of reference and patient sequences. The amino acid in position 245 of reverse transcriptase for subtype B reference strain B-HXB2, subtype F1 Romanian reference strain F1-BCI-RO07 and patients with subtype F1 is distinctly marked in red.

Prevalence of HLA-B*5701

For 115 patients, most of them (95.6%) on treatment, high resolution HLA-B typing was also performed. The HLA-B*5701 allele was present in 4 (3.4%) of these subjects. This result is comparable with the 2.8% prevalence of HLA-B*5701 described in a larger cohort of Romanian HIV-infected patients (unpublished data).

Association between HLA-B*5701 and polymorphisms at RT codon 245

We analysed the association between HLA-B*5701 and polymorphisms at RT codon 245: glutamine, the subtype F wild-type amino acid in position 245, was present in all 4 patients with B*5701, but also in 109 (98.1%) of 111 subjects without B*5701. The presence of non-wild type amino acid lysine at RT codon 245 was observed in two patients, one with HLA-B*1502, B*3501 and one with HLA-B*5103, B*5501. The sensitivity and specificity of the presence of glutamine at RT codon 245 for predicting B*5701 were 100% and 1.8%, respectively; the negative and positive predictive values of this test were 100% and 3.53%, respectively. There was no significant association between 245 Q and HLA-B*5701, or between 245K and other HLA-B alleles.

Discussions

Recent prospective [7] and retrospective [8] studies have shown that the sensitivity and the specificity of HLA-B*5701 detection as a marker for immunologicaly confirmed ABC HSR is 100% and 96–99%, respectively. The positive and negative predictive values of HLA-B*5701 testing are influenced by the prevalence of HLA-B*5701 in the population.

Assuming the sensitivity and specificity of HLA-B*5701 testing described in these studies and the approximately 3% carriage frequency of HLA-B*5701 observed in our cohort of Romanian HIV positive patients, the estimated positive predictive value of screening would be 52%, and the negative predictive value would be 100%. Thus, in a population of 100 patients this screening would identify 97 HLA-B*5701 negative and 3 HLA-B*5701 positive subjects. Avoidance of ABC therapy for the 3 HLA-B*5701–positive patients would prevent 2 cases of ABC HSR and would deny ABC therapy to 1 patient who would have tolerated the drug [8].

The HLA-B screening would also provide the opportunity of a more accurate diagnosis of symptoms during the first few weeks of ARV therapy and maintenance of an abacavir-containing regimen in HLA-B*5701 negative patients [9]. However, the routine use of a pharmacogenetic test in clinical practice would be favourised by the availability of a simple, inexpensive, robust, allele specific test [17]. The sequence-based HLA typing, the gold standard for identifying specific HLA alleles, is relatively expensive, time consuming and requires extensive expertise and equipment. For this reason, if the association between HLA-B*5701 and sequence variation in position 245 of RT is significant, the use of routine HIV drug resistance testing might become an alternative for the HLA-B locus typing. One study showed that for HIV subtype B infected patients the detection of mutations in RT codon 245 as a marker for HLA-B*5701 has a sensitivity of >96% and a negative predictive value of 99.6% [14]. Consequently, the detection of wild-type RT 245V (present in more than 70% of subtype B-infected individuals) could be used as an indirect indicator of the absence of B*5701, thereby identifying individuals with a substantially reduced risk for ABC HSR and reducing the total number of persons requiring HLA-B typing by 70% [14].

In our cohort of subtype F infected patients we found a high level of sequence conservation in RT codon 245: glutamine is present in more than 98% cases, both in treatment-naïve and in treatment-experienced patients. One possible reason for this situation would be that this amino acid represents an escape mutation selected in the HLA-B*5701–specific ISW9 epitope and thus, offers a selective advantage for the viral population. This epitope is the second most frequently targeted epitope in acute or early infection in the entire HIV genome among B*57-expressing individuals [18]. This could also explain why wild-type sequence was present in all B*5701-expressing patients from our cohort. Although our results indicate that the presence of the wild-type RT sequence has a very high negative predictive value for HLA-B*5701, mutations in the codon 245 were detected only in 2% of individuals and therefore are not useful for reducing the number of persons requiring HLA-B typing.

We conclude that in subtype F infected patients, at least from our cohort, sequence variation in HIV reverse transcriptase position 245 is not a useful marker for predicting HLA-B*5701.

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