A Simple Screening Approach to Reduce B*5701-Associated Abacavir Hypersensitivity on the Basis of Sequence Variation in HIV Reverse Transcriptase

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Background. Abacavir hypersensitivity is strongly associated with the human leukocyte antigen (HLA)–B*5701 allele; however, the cost of routine high-resolution HLA typing before initiation of therapy remains prohibitive. We propose a simple approach to reduce B*5701-associated abacavir hypersensitivity based on the screening of human immunodeficiency virus (HIV) reverse transcriptase (RT) for a signature B*5701-associated cytotoxic T lymphocyte escape mutation at RT codon 245.

Methods. The correlation between HLA-B*5701 and RT codon 245 variation was investigated in 392 HIV-infected, antiretroviral-naive adults who were initiating highly active antiretroviral therapy. The relationship between codon 245 variation and premature abacavir discontinuation was investigated in a larger cohort of treated individuals ($n = 982$). Associations between HLA-B*5701 and codon 245 variants were determined using Fisher's exact test or the χ^2 test.

Results. A very strong association between HLA-B*5701 and RT codon 245 variation was observed. Only 1 (4.2%) of 24 subjects with B*5701 harbored virus with the clade B "wild-type" amino acid 245V, compared with 278 (75.5%) of 368 who did not have B*5701 ($P < .001$). The sensitivity and specificity of codon 245 substitutions for predicting HLA-B*5701 were 96% and 75%, respectively, and the positive and negative predictive values were 20% and 99.6%, respectively. This association remained robust even after antiretroviral treatment was administered (negative predictive value, 100%; $n = 269$). In abacavir-treated individuals ($n = 982$), codon 245 substitutions were predictive of premature abacavir discontinuation ($P = .02$).

Conclusions. As HIV RT sequence is incidentally obtained as a part of routine drug-resistance testing, the examination of sequence variation at RT codon 245 could be adopted as a simple, low-cost screening method to identify individuals who could be safely treated with abacavir and/or who could benefit from HLA characterization.

Cytotoxic T lymphocytes (CTLs) recognizing human leukocyte antigen (HLA) class I–restricted viral epitopes presented on the surface of infected cells play a major role in the immune control of HIV-1 infection [1]. However, HIV is able to evade host CTL responses through the accumulation of specific mutations in HLA-restricted CTL epitopes [1, 2]. This phenomenon, known as "CTL escape," has been documented on both an individual basis [1–4] and a population basis [5], and HLA class I–mediated selection pressure is now recognized as a major force shaping in-host—as well as populational—HIV sequence evolution [5]. Indeed, available data indicate that selection of CTL escape mutations occurs along common and reproducible mutational pathways [3, 5], suggesting that viral evolution may be largely predictable on the basis of an individual's HLA class I profile [3].

In addition to representing a major force shaping viral sequence evolution, the HLA class I locus is also strongly associated with immune-mediated hypersensitivity reactions to specific antiretroviral agents. For example, the HLA class I allele B*5701 is strongly

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associated with a >100-fold increased risk of hypersensitivity reaction to the nucleoside analogue reverse-transcriptase inhibitor (NRTI) abacavir [6–8], particularly in the context of the 57.1 ancestral haplotype containing HLA-B*5701, HLA-DR7, and HLA-DQ3 [6]. This hypersensitivity is a dramatic, idiosyncratic reaction that occurs in 4%–8% of patients who initiate abacavir therapy [9]. This reaction compromises treatment and is an absolute contraindication for future abacavir use, with potentially life-threatening consequences upon rechallenge [9]. Studies have begun to evaluate the utility of "skin-patch" testing for abacavir hypersensitivity [10, 11], and genetic screening for HLA-B*5701 [11–13] is likely to be useful in the clinical setting [14]. Unfortunately, routine high-resolution HLA typing remains relatively costly, and its accessibility varies greatly, from approaching a standard of care in some regions to being rarely available in others. These factors present barriers to widespread implementation of this pharmacogenetic approach to prescribing abacavir [15].

On the basis of independent observations that selection of CTL escape mutations are reproducible among individuals sharing HLA alleles [3, 5] and that HLA-B*5701 screening is likely to be recommended prior to initiating abacavir-containing regimens, we hypothesized that the presence of a signature HLA-B*5701–selected CTL escape mutation in HIV reverse transcriptase (RT) [16, 17], identified as a part of routine HIV drug-resistance testing, may serve as an indirect marker for the presence of HLA-B*5701 among HIV subtype B–infected individuals and, thus, as a simple and cost-effective prescreening method to identify persons who are at risk for the abacavir hypersensitivity reaction. Here we show that the presence of an HLA-B*5701–associated amino acid change at HIV RT codon 245 [3, 16, 17], which lies within a known HLA-B*5701–restricted epitope spanning codons 244–252 [16, 18], represents a highly sensitive method of identifying possible B*5701-expressing individuals. Conversely, the presence of HLA-B*5701 is exceedingly rare in individuals harboring the "wild-type" amino acid at RT codon 245; therefore, the presence of wild-type 245V (where V represents the HIV subtype B consensus) could be used to identify individuals who may be safely treated with abacavir.

METHODS

Study Subjects

The British Columbia HIV/AIDS Drug Treatment Program. In the province of British Columbia, Canada, antiretroviral drugs are distributed free-of-charge to HIV-infected individuals through a centralized HIV/AIDS drug treatment program, previously described in detail [19]. Antiretroviral drugs are prescribed according to specific guidelines set by the British Columbia Therapeutic Guidelines Committee, which are revised regularly and are in accordance with international guidelines. Sociodemographic, therapeutic, and clinical data (including CD4 cell counts and plasma viral loads determined at baseline and approximately every 3 months thereafter) from persons enrolled in the British Columbia drug treatment program are stored in a centralized database. Since its inception, ~7000 HIV-infected British Columbians have received treatment through the drug treatment program.

The HAART Observational Medical Evaluation and Research (HOMER) cohort. The HOMER cohort is an open, treatment-based cohort that includes all antiretroviral-naive adult British Columbians who have initiated HAART through the drug treatment program since August 1996 [19]. It has been the focus of a number of population-based studies and has been described in detail previously [20–23]. This study represents a nonrandom sample of HOMER subjects who initiated HAART between August 1996 and September 1999 ($n = 1188$) for whom a blood sample for HLA typing (765 of 1188 patients) and a baseline (pretherapy) HIV drug-resistance genotyping incorporating the sequence at RT codon 245 (392 of 765 patients) were available. These subjects represent individuals who attended St. Paul's Hospital, Vancouver (British Columbia), and who underwent antiretroviral-resistance testing as requested by a physician or according to research protocols. The baseline characteristics of the 392 subjects included in this study were comparable to those of the remaining 796 HOMER subjects for whom HLA and/or RT codon 245 data were not available (table 1). There were no differences observed in median baseline CD4 cell count or plasma viral load ($P = .2$) among included and excluded individuals; however, patients included in the study were slightly older (median age, 38 vs. 37 years; $P = .03$) and more likely to be male (88% vs. 83%; $P = .04$) than those who were excluded. Ethics approval for this study was obtained from the institutional ethics board of Providence Health Care and University of British Columbia.

HLA-B Sequence-Based Typing and HIV RT Genotyping

Sequence-based typing for HLA-B was performed using DNA extracted from a PBMC-enriched frozen blood sample that was obtained from each subject. The sequence-based typing protocol, a validated "in-house" procedure based on International Histocompatibility Working Group protocols, involves a nested PCR amplification of exons 2 and 3 of the HLA-B locus (primers available upon request), followed by bidirectional automated DNA sequencing using an ABI 3700 DNA sequencer (Applied Biosystems). HLA allele interpretation was performed by comparing sequence-based typing data against all alleles listed in the Immunogenetics (IMGT)–HLA database (FTP site available at http://www.ebi.ac.uk/imgt/hla/download.html) up to August 2005, yielding intermediate-to-high–level resolution

Table 1. Baseline (pretherapy) characteristics of the HAART Observational Medical Evaluation and Research (HOMER) cohort, stratified by the availability of both human leukocyte antigen–B and HIV reverse transcriptase codon 245 data.

Baseline characteristic	Total HOMER cohort (n = 1188)	Availability of HLA-B and RT codon 245 data: No RT codon 245 or HLA-B data available (n = 796)	Availability of HLA-B and RT codon 245 data: RT codon 245 and HLA-B data available (n = 392)	P
Male sex	1003 (84.4)	660 (82.9)	343 (87.5)	.04
Age, median years (IQR)	37.1 (32.0–43.7)	36.7 (31.4–43.3)	37.9 (32.6–44.2)	.03
HIV pVL, median $\log_{10}$ copies/mL (IQR)	5.08 (4.62–5.49)	5.08 (4.59–5.46)	5.11 (4.67–5.53)	.19
CD4 cell count, median cells/mm^3 (IQR)	280 (130–420)	290 (130–430)	260 (120–400)	.18
Baseline AIDS diagnosis	157 (13.2)	105 (13.2)	52 (13.3)	1.00
History of injection drug use	351 (29.6)	238 (29.9)	113 (28.8)	.70

NOTE. Data are no. (%) of patients, unless otherwise indicated. IQR, interquartile range; pVL, plasma viral load.

for most alleles. Population-based HIV RT sequences up to codon 400 were obtained from plasma HIV RNA as a part of routine HIV drug-resistance testing, as previously described [24]. The HOMER cohort consists of ~97% HIV subtype B infections [25].

Statistical Analyses

Association between a pretherapy RT codon 245 genotype and HLA-B*5701. For each subject, the latest plasma sample collected in the 180 days prior to initiation of HAART (the "baseline" sample) was genotyped for antiretroviral resistance. Associations between the presence of specific mutations at HIV RT codon 245 at baseline and possession of each HLA-B allele observed in our cohort were determined using Fisher's exact test.

Effect of exposure to HAART on the prevalence of substitutions at RT codon 245. Fisher's exact test was used to examine associations between HLA-B alleles and RT codon 245 variation in antiretroviral-resistance genotypes after initiation of HAART. For each subject with an available post-HAART genotype (n = 269), the subject's latest "on-therapy" genotype was used.

Association of RT codon 245 with early discontinuation of abacavir treatment. We performed a larger analysis of all HIV-infected individuals who were enrolled in the drug treatment program who initiated abacavir-containing therapy at any time between March 1998 and July 2004 (n = 1448) and who had HIV RT codon 245 data available (n = 982; 68%). The association between RT codon 245 substitutions in the subject's latest "on-therapy" genotype and premature discontinuation of abacavir treatment was assessed using the χ^2 test. We defined premature discontinuation as permanently stopping abacavir therapy ⩽3 months after initiation with a report of abacavir-related adverse effects or hypersensitivity or permanently stopping abacavir ⩽1 month after initiation without a documented reason.

RESULTS

Prevalence of HLA-B*5701 and substitutions at RT codon 245 at baseline. In the baseline (pretherapy) HOMER cohort sequences, HIV RT codon 245 exhibited 71% sequence conservation. Consistent with the HIV-1 subtype B consensus sequence [26], the most common (wild-type) amino acid in our cohort was 245V. The HLA-B*5701 allele was detected in 24 (6.1%) of 392 patients examined. We observed an exceedingly strong association between HLA-B*5701 and polymorphisms at RT codon 245: wild-type 245V was observed in 1 (4.2%) of 24 subjects with B*5701, compared with 278 (75.5%) of 368 subjects without B*5701 ($P < .001$). The sensitivity and specificity of the presence of non–wild-type amino acids at RT codon 245 for predicting B*5701 were 95.8% (95% CI, 77%–100%) and 75.5% (95% CI, 71%–80%), respectively; negative and positive predictive values of this assay were 99.6% (95% CI, 98%–100%) and 20.4% (95% CI, 14%–29%), respectively. For individuals with HLA-B*5701, the most common substitution at RT codon 245 was E (n = 11; 45.8%), followed by M (n = 4; 16.7%), L (n = 2; 8.3%), and others. Note that no unique characteristics were identified for the single subject (who was an exception) who had both B*5701 and wild-type 245V at baseline.

Other associations with substitutions at RT codon 245 at baseline. The relatively low specificity (75.5%) of the relationship between RT codon 245 substitutions and HLA-B*5701 may be largely explained by 2 main factors. The first is infection with non-B HIV subtypes. All 14 (3.6%) of 392 subjects infected with non-B subtypes had non-V amino acids at RT codon 245 at baseline; however, none had HLA-B*5701. The most common amino acid substitutions at RT codon 245 among non-B

subtypes in our cohort were Q ($n = 9$; 64.3%), followed by E ($n = 2$; 14.3%), and others. The second factor that explains the relatively low specificity of this assay is possession of the closely related HLA-B*58 allele, which also has a CTL epitope overlapping RT codon 245 and also selects for an escape mutant at this position [18]. Only 1 (6.7%) of 15 individuals with HLA-B*58 had the HIV subtype B wild-type 245V sequence, compared with 278 (73.7%) of 377 subjects without B*58 ($P < .001$). Similar to the observations for HLA-B*5701, the most common substitutions at RT codon 245 for individuals with B*58 were E ($n = 5$; 33.3%), followed by L ($n = 2$; 13.3%), T ($n = 2$; 13.3%), and others. No other HLA-B allele observed in our cohort was significantly associated with variation at RT codon 245. For individuals with other HLA-B alleles, the most common amino acid sequence was 245V ($n = 277$; 78.5%), followed by substitutions E ($n = 18$; 5.1%), M ($n = 13$; 3.7%), K ($n = 12$; 3.4%), and others.

Effect of exposure to antiretroviral therapy on the prevalence of substitutions at RT codon 245. To be a useful marker, the association between RT codon 245 and B*5701 should not be impaired by mutations selected by antiretroviral therapy. Posttherapy (follow-up) RT sequences were available for 269 (68.6%) of the 392 individuals who were investigated. In the posttherapy sequences, wild-type 245V was observed in 0 of 13 subjects with B*5701, compared with 195 (76.2%) of 256 subjects without B*5701 ($P < .001$), indicating that the association between B*5701 and RT codon 245 variation remains significant, regardless of antiretroviral exposure. The sensitivity and specificity of RT codon 245 substitutions for predicting HLA-B*5701 after receiving antiretroviral therapy were 100% (95% CI, 78%–100%) and 76.8% (95% CI, 71%–81%), respectively; negative predictive value and positive predictive value were 100% (95% CI, 98%–100%) and 19.7% (95% CI, 12%–30%), respectively. Consistent with pretherapy data, the most common substitution at RT codon 245 in individuals with HLA-B*5701 was E ($n = 9$; 50.0%). Six subjects experienced a change from the wild-type sequence to RT codon 245 polymorphisms (all mixtures containing V) after initiation of therapy, whereas 4 subjects changed from polymorphisms to the wild-type sequence (3 V-containing mixtures and 1 with 245M); none of these individuals had B*5701. Note that posttherapy HIV genotype data were unavailable for the single individual who had both B*5701 and 245V at baseline.

Association of RT codon 245 with early discontinuation of abacavir. As a preliminary clinical validation, we wished to examine whether sequence variation at RT codon 245 was associated with early discontinuation of abacavir treatment (defined as permanently stopping abacavir treatment ⩽3 months after initiation with a report of abacavir-related adverse effects or hypersensitivity or permanently stopping abacavir treatment after ⩽1 month without a documented reason) in a larger group of treated individuals. Of the 1448 people who initiated abacavir therapy in British Columbia between March 1998 and July 2004 (regardless of previous antiretroviral therapy), RT sequence data were available for 982 individuals (67.8%). A total of 46 (16.4%) of 280 subjects with RT codon 245 substitutions prematurely discontinued abacavir, compared with 76 (10.8%) of 702 individuals with 245V ($P = .02$). Thus, even with these relatively broad definitions, detection of substitutions at RT codon 245 was indeed linked to early abacavir discontinuation. The substitution 245E was present in 14% of individuals who prematurely discontinued abacavir therapy, compared with 8% of those who continued to receive therapy ($P = .005$). Other RT codon 245 substitutions associated with trends toward early abacavir discontinuation were 245M (8% vs. 5%) and K (7% vs. 4%).

DISCUSSION

The HLA-B*5701 allele, observed in 5%–10% of white individuals, is associated with a >100-fold increased risk for the serious and potentially fatal abacavir hypersensitivity reaction [6–8]. On the basis of the severity of this reaction, genetic testing for HLA-B*5701 as a screening method for abacavir use has been simulated and found to be potentially cost-effective [12] and clinically useful [14], and it is possible that screening for HLA-B*5701 may be recommended for all HIV-infected individuals who are initiating antiretroviral therapy. High-resolution HLA typing, however, remains a relatively time-consuming and costly process and, thus, the development of more-rapid and simple B*5701 screening procedures—including allele-specific PCR, flow cytometry–based screens, or the identification of indirect diagnostic indicators—would be advantageous [15]. The availability of these HLA screening methods varies greatly among different locations.

On the basis of previous evidence indicating that HLA class I alleles select for reproducible patterns of escape mutations in the HIV genome [3, 5], we hypothesized that specific HLA-B*5701–selected sequence changes in HIV infection may serve as an indirect marker of B*5701 expression. Here we show that the presence of previously documented HLA-B*5701–selected sequence changes at HIV RT codon 245 [3, 16, 17], representing an escape mutation selected at the first anchor residue of the HLA-B*5701–specific ISW9 epitope in HIV RT, may serve as a useful marker for HLA-B*5701 expression, whereas the presence of the clade B wild-type 245V amino acid sequence virtually excludes the possibility of HLA-B*5701 expression. This epitope is the second most frequently targeted epitope in acute or early infection in the entire HIV genome among B*57-expressing individuals [27].

Despite only a moderate specificity, the presence of non-V amino acid substitutions at HIV RT codon 245 (where V represents the HIV subtype B consensus or wild-type sequence)

predicts the presence of HLA-B*5701 with extremely high sensitivity (96%–100%). Similarly, although the positive predictive value of this test was low (subjects with B*5701 account for only 20% of all subjects with non-V substitutions at RT codon 245), the negative predictive value was >99% (meaning that the presence of wild-type V excludes the possibility of B*5701 in >99% of cases). These results suggest the utility of examining HIV sequence variation at RT codon 245 to identify those individuals who are at a substantially reduced risk for the abacavir hypersensitivity reaction. On the basis of the high sensitivity and negative predictive value of this assay, the presence of wild-type RT 245V (present in >70% of subtype B-infected individuals) could be used as an indirect indicator of the absence of B*5701, thereby identifying individuals who may safely be treated with abacavir. This screening method would reduce the total number of persons requiring HLA-B typing by >70%.

One limitation of this study is that we did not directly determine associations between RT codon 245 sequence variation and incidence of the abacavir hypersensitivity reaction. In the absence of comprehensive abacavir patch test screening data, we used an "early abacavir discontinuation" definition, which may not accurately reflect the prevalence of the hypersensitivity reaction in our cohort. Nevertheless, data suggest that RT codon 245 substitutions are specifically associated with early discontinuation of abacavir treatment and are not simply a marker of therapy discontinuation in general. For example, these substitutions were not correlated with premature discontinuation of treatment with nevirapine, an unrelated drug that has also been associated with hypersensitivity reactions in some individuals [28]. Another limitation is that our cohort represents a chronically HIV-infected population that was about to initiate therapy with relatively low median CD4 cell counts. Because the time-course of selection for HLA-associated polymorphisms over the natural course of HIV infection remains incompletely characterized, it is not clear whether the association between HLA-B*5701 and RT codon 245 would be as robust earlier in the HIV disease progression. Finally, it is important to note that the predictive value of wild-type RT 245V to identify individuals potentially at reduced risk for the abacavir hypersensitivity reaction is presently restricted to those who have HIV subtype B infection, which represents the only subtype with "V" as the consensus amino acid sequence at this position [26]. Additional research will be required to determine whether escape from the wild-type 245Q observed in non-B clades of HIV is also associated with HLA-B*5701. Despite this limitation, however, this assay is still likely to be of clinical utility, given that HIV subtype B infections predominate in North America and Western Europe, and HLA-B*5701 is most prevalent—as well as most strongly predictive of abacavir hypersensitivity reactions—in white individuals [7, 8, 12].

The greatest advantage of the RT codon 245 test is that HIV RT genotypes can be obtained through routine HIV drug-resistance testing and, therefore, could be available to patients and clinicians at no additional cost or effort. It may, therefore, serve as a rapid and economical screening method for identifying patients who are at risk for experiencing the abacavir hypersensitivity reaction. On the basis of the results of this study, as well as the potential utility of using other specific HLA class I allele–selected HIV sequence changes as indirect genetic markers in future assays, we recommend that all commercially available antiretroviral-resistance tests report all amino acid differences, from consensus to at least RT codon 245. Finally, it is important to note that no screening approach can replace the need for pharmacovigilance in the decision to discontinue abacavir treatment in patients who have clinically suspected abacavir hypersensitivity.

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