Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir

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Summary

Background The use of abacavir—a potent HIV-1 nucleoside-analogue reverse-transcriptase inhibitor—is complicated by a potentially life-threatening hypersensitivity syndrome in about 5% of cases. Genetic factors influencing the immune response to abacavir might confer susceptibility. We aimed to find associations between MHC alleles and abacavir hypersensitivity in HIV-1-positive individuals treated with abacavir.

Methods MHC region typing was done in the first 200 Western Australian HIV Cohort Study participants exposed to abacavir. Definite abacavir hypersensitivity was identified in 18 cases, and was excluded in 167 individuals with more than 6 weeks’ exposure to the drug (abacavir tolerant). 15 individuals experienced some symptoms but did not meet criteria for abacavir hypersensitivity. p values were corrected for comparisons of multiple HLA alleles (p) by multiplication of the raw p value by the estimated number of HLA alleles present within the loci examined.

Findings HLA-B*5701 was present in 14 (78%) of the 18 patients with abacavir hypersensitivity, and in four (2%) of the 167 abacavir tolerant patients (odds ratio 117 [95% CI 29–481], p<0·0001), and the HLA-DR7 and HLA-DQ3 combination was found in 13 (72%) of hypersensitive and five (3%) of tolerant patients (73 [20–268], p<0·0001). HLA-B*5701, HLA-DR7, and HLA-DQ3 were present in combination in 13 (72%) hypersensitive patients and none of the tolerant patients (822 [43–15 675], p<0·0001). Other MHC markers also present on the 57·1 ancestral haplotype to which the three markers above belong confirmed the presence of haplotype-specific linkage disequilibrium, and mapped potential susceptibility loci to a region bounded by C4A6 and HLAC. Within the entire abacavir-exposed cohort (n=200), presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 had a positive predictive value for hypersensitivity of 100%, and a negative predictive value of 97%.

Interpretation Genetic susceptibility to abacavir hypersensitivity is carried on the 57·1 ancestral haplotype. In our population, withholding abacavir in those with HLA-B*5701, HLA-DR7, and HLA-DQ3 should reduce the prevalence of hypersensitivity from 9% to 2·5% without inappropriately denying abacavir to any patient.

Lancet 2002; 359: 727–32
See Commentary page 722

Introduction

Abacavir is a commonly used nucleoside analogue with potent antiviral activity against HIV-1. About 5% (range 0–14%) of patients treated with abacavir develop a hypersensitivity reaction characterised by multisystem involvement that can be fatal in rare cases.1 Symptoms usually appear within the first 6 weeks of treatment (median time to onset 11 days) and characteristically include fever, rash, gastrointestinal symptoms (nausea, vomiting, diarrhea, or abdominal pain) and lethargy or malaise. Less common manifestations include respiratory or musculoskeletal symptoms, headache, paraesthesia, oedema, renal or hepatic failure, or anaphylaxis.1 Symptoms related to the hypersensitivity reaction worsen with continued therapy and usually improve within 24 h of abacavir discontinuation. Rechallenge with abacavir after a hypersensitivity reaction typically results in recurrence of symptoms within hours, with the potential to induce a more severe clinical syndrome with increased risk of life-threatening hypotension and death.

Several observations support the possibility that genetic susceptibility factors for this idiosyncratic hypersensitivity syndrome exist, and more specifically that involved genetic loci lie within the MHC region. First, only a subset of individuals exposed to abacavir develop hypersensitivity, typically within 6 weeks of starting therapy, and those who do not develop the syndrome within this time remain at low risk despite continued therapy. Second, a meta-analysis of 25 clinical studies involving 5248 participants showed that ethnic origin might influence abacavir hypersensitivity, with decreased risk associated with black race.1 Familial predisposition has also been reported.1 Third, there is evidence that the pathogenesis of several similar multisystem drug hypersensitivity reactions involves MHC-restricted presentation of drug or drug metabolites, with direct binding of these non-peptide antigens to MHC molecules or haptenation to endogenous proteins before T-cell presentation.2–10

Investigation of associations between MHC alleles and clinical phenotypes can be facilitated by an understanding of expected linkage disequilibrium across the MHC. Particular arrangements of alleles, referred to as ancestral haplotypes, are known to be maintained and transmitted through generations en bloc, with a low frequency of
recombination events within these genetic blocks. Ancestral haplotypes and their simple recombinants (i.e., a haplotype resulting from one historical crossover event between two ancestral haplotypes) have been suggested to account for at least 70% of the observed haplotypes in white populations. Individual alleles can be haplospecific (e.g., HLA-B*5701 and the 57-1 ancestral haplotype) or common to multiple ancestral haplotypes (e.g., HLA-DRB1*0701 represented in 57-1, 47-1 and 13-1 haplotypes).

We therefore sought associations between MHC alleles and abacavir hypersensitivity in the first 200 individuals treated with abacavir in the Western Australian HIV Cohort.

Patients and methods

Patients

581 active participants in the Western Australian HIV Cohort Study on Dec 31, 2001 were considered for this study. These patients had been HLA-typed at the HLA-A, HLA-B, HLA-C, HLA-DR, and HLA-DQ loci at enrolment into the cohort study, and had been followed up at 1–3-monthly intervals. A clinician recorded details of the antiretroviral treatment history and adverse drug reactions, especially to abacavir, at each visit. The first 200 participants of the cohort prescribed abacavir until Dec 31, 2001, were included in the study; abacavir prescription was validated in all cohort cases through the use of the Royal Perth Hospital pharmacy database. The medical records of abacavir-exposed individuals were reviewed by a single clinician (GM), who was unaware of HLA-typing results, for evidence of abacavir hypersensitivity, using standard diagnostic criteria. These criteria were onset of at least two of the following symptoms within 6 weeks of abacavir initiation: fever, rash, gastrointestinal symptoms (nausea, vomiting, diarrhoea, or abdominal pain), lethargy, malaise, arthralgia, myalgia or respiratory symptoms (dyspnoea, sore throat, or cough); resolution of symptoms within 72 h of discontinuation of abacavir; and absence of an alternative likely explanation for the symptoms.

Cases were reviewed and classified as: definite cases of abacavir hypersensitivity, in whom abacavir hypersensitivity could be excluded—i.e., for whom abacavir therapy had been continuous for at least 6 weeks (abacavir-tolerant); or cases for whom abacavir hypersensitivity could not be excluded because symptoms in the first 6 weeks of abacavir exposure did not meet diagnostic criteria (abacavir hypersensitivity not excluded). Cases from the last group were excluded from analyses of HLA allele frequency and abacavir hypersensitivity, but were included in calculations of positive and negative predictive values of the presence of HLA alleles for abacavir hypersensitivity so as to provide an estimated predictive value for an unselected population of patients.

Additional non-HIV-infected control individuals were recruited from the Western Australian bone-marrow donor registry (n=3212). As a requirement of the registry, all patients.

Results

Prevalence of abacavir hypersensitivity

In the cohort of 200 individuals, 18 definite cases of abacavir hypersensitivity were identified, and 167 HLA-B*5701 in all cases). HLA-DRB1 typing was done by DNA sequencing. HLA-DQ typing was done by either sequencing or PCR sequence-specific oligonucleotide typing in HLA-DR7-positive patients. Complement (C4, Bf) allotyping was done by immunofixation electrophoresis.

This study exploits current knowledge of the MHC and the alleles of polymorphic microsatellite and single nucleotide polymorphism (SNP) markers. Since our initial data indicated an association between abacavir hypersensitivity and HLA alleles specific for the 57-1 ancestral haplotype, we sought to define the boundaries of the abacavir hypersensitivity susceptibility region through the study of participants with recombinant 57-1 ancestral haplotypes. The 57-1 ancestral haplotype includes HLA-B*5701, C4A6, HLA-DRB1*0701 (DR7), and HLA-DQB1*0203 (DQ3). HLA-DR-DQ haplotypes show extremely high linkage disequilibrium in all populations, but DR7 can be associated with either DQ3 (on the 57-1 ancestral haplotype) or DQ2 (on other haplotypes). Therefore, the combination of HLA-DR7 plus HLA-DQ3 in individuals who did not have HLA-DQ2 was necessary for the assignment of a recombinant 57-1 ancestral haplotype.

Samples were typed for the –238G→A and –308G→A substitutions in the gene encoding tumour necrosis factor α (TNFA), and four microsatellites spanning the central MHC region (D6S1014, D6S273, MIB, and MICA-TM) were used for mapping of this region. Statistical analysis

p values were corrected for comparisons of multiple HLA alleles (p.c) by multiplication of the raw p value by the estimated number of HLA alleles present within the loci examined (35 for HLA-B, 18 for HLA-DR, and seven for HLA-DQ). Hence, all p values were multiplied by a factor of 4410. Odds ratios were calculated with Haldane’s modification, which adds 0.5 to all cells to accommodate possible zero counts; Woolf’s method was used to estimate SEs and CIs. Comparisons of demographic and related data in those with abacavir hypersensitivity and those who were abacavir tolerant were done with Fisher’s exact tests for dichotomous variables, and t tests for continuous variables.

Role of the funding source

The sponsor had no role in the design, conduct, interpretation, or writing up of the study.

Table 1: Demographic variables, antiretroviral exposure, and immunological status in abacavir-hypersensitive and abacavir-tolerant groups

<table>
<thead>
<tr>
<th>Abacavir hypersensitive (n=18)</th>
<th>Abacavir tolerant (n=167)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>16 (89%)</td>
</tr>
<tr>
<td>Female</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>Ethnic origin</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>18 (100%)</td>
</tr>
<tr>
<td>Non-Caucasoid</td>
<td>0</td>
</tr>
<tr>
<td>ART naive</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>Mean (SD) age (years)</td>
<td>44.6 (9.0)</td>
</tr>
<tr>
<td>Mean (SD) percentage CD4</td>
<td>19.8 (8.1)</td>
</tr>
<tr>
<td>Mean (SD) absolute CD4</td>
<td>444.2 (233)</td>
</tr>
</tbody>
</table>

ART=antiretroviral therapy.
individuals had had more than 6 weeks’ exposure to abacavir without developing hypersensitivity (abacavir tolerant). Among the patients defined as having definite abacavir hypersensitivity reactions, one started therapy with efavirenz and two started nevirapine (which can also cause hypersensitivity reactions) at the time abacavir was introduced. These individuals were included in the analysis because they fulfilled the diagnostic criteria. Additionally, 15 individuals had non-specific symptoms during the first 6 weeks of abacavir exposure, but did not meet the criteria for definite abacavir hypersensitivity. In this group, eight patients were excluded because their symptoms were insufficient to fulfill diagnostic criteria, including one in whom symptoms persisted after abacavir was withdrawn, and one in whom symptoms resolved spontaneously despite the fact that abacavir was not stopped. In the other seven patients, whose features met the diagnostic criteria, an alternative explanation was available. An alternative drug was implicated in three patients (one with recurrence of symptoms with efavirenz rechallenge, one with probable nevirapine hepatotoxicity, and one who had developed similar symptoms after multiple other drug exposures), and in the remaining four individuals, the likely alternative explanation for (non-observed) symptoms was anxiety; one of the four also had localised abdominal pain suggestive of cholelithiasis. On the basis of the application of these diagnostic criteria, the prevalence of abacavir hypersensitivity in our population was 9%.

Table 1 shows demographics, exposure to antiretroviral therapy, and immunological status (CD4 T-cell proportion and absolute number) in the group with definite abacavir hypersensitivity and the group who were abacavir tolerant. The proportion of caucasoid individuals in the abacavir hypersensitive group was slightly higher than in the tolerant group, but the groups were similar in terms of other variables considered. Within the entire abacavir-exposed cohort (n=200), 176 participants were classified as caucasoid; the non-caucasoid group consisted of seven Africans, 11 indigenous Australians, and six Asians.

### HLA allele frequency

HLA-B*5701 was present in a significantly higher proportion of abacavir-hypersensitive patients than abacavir-tolerant patients, as was the combination of HLA-DR7 and HLA-DQ3 (table 2). The HLA-B*5701, HLA-DR7, HLA-DQ3 haplotype was found to be more predictive of the clinical syndrome than its component alleles (table 2). Within the entire abacavir-exposed cohort (n=200), we found a higher frequency of HLA-B*5701 (odds ratio 103 [95% CI 25–423], p<0.0001), HLA-DR7 plus HLA-DQ3 (4 [17–239], p<0.0001), and the combined alleles (724 [38–13 811], p<0.0001), among patients with abacavir hypersensitivity than among the abacavir-tolerant group. The presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 haplotype was associated with positive and negative predictive values of 100% and 97%, respectively.

HLA typing revealed no alleles characteristic of the 57-1 ancestral haplotype in the two patients with a diagnosis of abacavir hypersensitivity who started nevirapine and abacavir therapy concurrently, although the TNFA-308G→A polymorphism—a TNFA promoter polymorphism implicated in susceptibility to carbamazepine hypersensitivity15—was present in both patients.

**Frequency of complete or partial 57-1 ancestral haplotype in control populations**

The frequency of the C4A6 allele, an additional specific marker of the 57-1 ancestral haplotype within the central MHC, was assessed in the abacavir-exposed groups, as well as in two control populations (381 HIV-infected patients not exposed to abacavir and 3212 HIV-negative bone-marrow donors). The distribution of the HLA-B*5701, C4A6, and HLA-DR7 plus HLA-DQ3 alleles of the 57-1 ancestral haplotype was similar in the three groups (table 3, figure 1). By contrast, alleles of the 57-1 ancestral haplotype were greatly over-represented in the abacavir hypersensitive group compared with the tolerant group (table 3, figure 1).
Mapping of putative susceptibility loci within the MHC

Central MHC markers characteristic of the 57·1 ancestral haplotype (D6S1014*137, C4A6, D6S273*135, TNFA 238A, MICA*194, MIB*344) were examined to confirm the presence of the haplotype and to map the extent of the non-recombinant haplotype in cases and controls (figure 2). Mapping of recombinant 57·1 haplotypes among abacavir hypersensitive and abacavir tolerant patients identified a candidate region of about 300 Kb telomeric to the C4A6 allele that was unique to abacavir hypersensitive patients in the cohort. The telomeric boundary of this region was marked by the D6S273*135 microsatellite present in intron 1 of megakaryocyte-enhanced gene transcript 1 (MEGT1/G6d). Among the 14 patients with abacavir hypersensitivity and the HLA-B*5701 allele, we found carriage of all markers between C4A6 and HLA-Cw6 (ie, D6S273*135, TNFA 238A, MICA*194, MIB*344, HLA-B*5701), consistent with the presence of non-recombinant 57·1 ancestral haplotype in this region. Hence, presence of 57·1 ancestral haplotype alleles in a region identified by the presence of C4A6 at the centromeric boundary, and telomeric of HLA-Cw6, provided sufficient conditions for abacavir hypersensitivity susceptibility in this group (figure 2).

Figure 1: Markers of 57·1 ancestral haplotype in the abacavir-hypersensitive group, abacavir-tolerant group, and control populations

Presence of indicated markers is shown by shading. Vertical axes have been scaled for all groups to show proportion of patients with various components of 57·1 ancestral haplotypes.
The major finding in this study of 200 consecutive abacavir-treated individuals in the Western Australian HIV Cohort is that the presence of the HLA-B*5701, HLA-DR7, HLA-DQ3 haplotype is strongly associated with susceptibility to abacavir hypersensitivity—a serious and potentially life-threatening clinical syndrome encountered in about 5% of abacavir-treated patients. The presence of this allelic combination is associated with greatly increased risk of developing the syndrome, with an odds ratio similar to that seen in the association between HLA-B27 and ankylosing spondylitis. These data might provide a plausible basis for the observed differences in abacavir hypersensitivity between ethnic groups, and for cases of familial predisposition. Although we noted ethnic differences in susceptibility to abacavir hypersensitivity, there was no evidence that this potential confounding factor accounted for our findings. Similarly, a comprehensive assessment of HIV-seronegative and HIV-infected control populations provided evidence against the possibility of a biased representation of the frequency of HLA-B*5701, or of the 57·1 ancestral haplotype, in the abacavir-exposed cohort.

Current practice guidelines for the prevention and management of abacavir hypersensitivity focus on early recognition of suggestive symptoms in the appropriate interval after initiation of abacavir therapy, and prompt cessation of abacavir after diagnosis. The positive and negative predictive values (100% and 97%, respectively) associated with the presence of the HLA-B*5701, HLA-DR7, HLA-DQ3 haplotype in this study support an important and immediately applicable clinical role for HLA typing in this setting. However, further studies in abacavir-exposed cohorts from a range of ethnic and racial populations are required to confirm these findings, so that the predictive value of genetic testing for this hypersensitivity syndrome can be examined and generalised as appropriate. Such transracial studies will also be essential to the characterisation of the polymorphic locus or loci that are common to cases of abacavir hypersensitivity.

On the basis of these data, our current practice is to withhold abacavir in patients with the HLA-B*5701, HLA-DR7, HLA-DQ3 haplotype. However, in four cases of abacavir hypersensitivity (ie, 2% of the cohort) no part of this putative susceptibility haplotype could be identified. Two of these cases had concurrent exposure to nevirapine. In this context, HLA typing at these alleles cannot be regarded a screening test, and current clinical practices should continue to underpin the management of abacavir hypersensitivity.

These findings are consistent with a direct role for MHC-specific alleles in the pathogenesis of abacavir hypersensitivity. The susceptibility locus or loci marked by the presence of HLA-B*5701, HLA-DR7, HLA-DQ3 could feasibly participate directly in abacavir-specific antigen recognition by the immune system (ie, α/β or γδ T cells). This possibility is supported by the existence of a complex intracellular pathway for the metabolism of abacavir, in which this carbocyclic guanine derivative is
converted to carbovir monophosphorylation via multiple pathways involving deamination and phosphorylation steps, before undergoing kinase-mediated activation to the active carbovir triphosphate form. The numerous metabolites thus formed might then undergo haptenation to endogenous proteins to achieve antigenicity, or bind directly to MHC molecules to elicit T-cell activation, consistent with the proposed pathogenesis of a number of idiosyncratic drug reactions.

In relation to recombinant mapping of candidate susceptibility regions, the most parsimonious region identified contains several central MHC genes, including a family of heat-shock-protein genes that might be involved in antigen chaperoning and folding as well as providing direct immunostimulatory signals in the presence of antigen. However, a larger region defined by C4A6 and HLA-Cw6 on the 57·1 haplotype provides sufficient conditions for susceptibility to abacavir hypersensitivity, raising the possibility that two or more loci on this region of the 57·1 ancestral haplotype (including HLA-B*5701, HLA-DR7, and HLA-DQ3) requires the identification of informative abacavir-hypersensitive and abacavir-tolerant cases with recombinant 57·1 haplotypes. This approach is more appropriate than multivariate analyses of individual alleles that assume linkage disequilibrium to be mainly attributable to genetic distance. In multivariate analyses, markers that are haplospecific but distant from a true susceptibility locus can display stronger associations than non-haplospecific markers that are close to it. Recognition of these effects has prompted the development of specific methods to adjust for the confounding effects of haplotype-associated linkage disequilibrium.

Abacavir is a member of the nucleoside reverse-transcriptase inhibitor (NRTI) class of antiretroviral drugs, and has a favourable profile in relation to the risk of mitochondrial toxic effects—the major duration-dependent toxic effect associated with NRTI use. The drug is commonly incorporated into triple NRTI regimens, and has the advantage of presentation in a co-formulation with zidovudine and lamivudine. In this context, a highly predictive test for abacavir hypersensitivity would be predicted to provide a significant reduction in the total burden of toxic effects associated with abacavir therapy, and allow for the safer use of this drug without inappropriate denial of access to its use. The strong association between abacavir hypersensitivity and HLA-B*5701, HLA-DR7, and HLA-DQ3 identified in this study, although requiring confirmation in further studies involving racially diverse populations, provides a plausible basis for the development of such a test, as well as for an increased understanding of the pathogenesis of this potentially life-threatening clinical syndrome.

**Conflict of interest statement**

S Mallal has spoken at meetings sponsored by GlaxoSmithKline, and has sat on the HIV advisory boards for GlaxoSmithKline and Merck Sharp and Dohme.

**Acknowledgments**

We thank Martyn French, Patricia Martinez, and Ric Chaney for providing clinical information.

**References**