Original article

Cytokine profiling in abacavir hypersensitivity patients

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Background: Abacavir hypersensitivity in genetically susceptible individuals implicates an abacavir-specific T-cell response to either the parent drug or a metabolite generated in vivo. We have analysed the cytokine profile in antigen-presenting cells and the T-lymphocytes that are involved in the pathological immune response to abacavir.

Methods: In this study, we compared abacavir-specific cytokine responses in cultured peripheral blood mononuclear cells (PBMCs) from HIV-infected abacavir hypersensitive, tolerant and naive individuals. Cells were cultured in the presence or absence of abacavir. Cytokine expression was determined by microarray analysis, enzyme-linked immunosorbent assays and flow cytometry.

Results: We demonstrated using in vitro models of immune activation that the production of interferon-γ was specifically induced by abacavir treatment in PBMCs obtained from hypersensitive patients carrying the HLA-B*5701 allele (median 123.86 compared with -30.83 for tolerant controls, P=0.001).

Conclusion: These results provide further insight into the immunological and metabolic basis of abacavir hypersensitivity syndrome. In vitro assays could assist in the identification of susceptible loci by providing a surrogate marker for the hypersensitivity reaction. Such a marker could be studied in unexposed individuals to shed further light on the immunopathogenesis of the abacavir hypersensitivity syndrome.

Introduction

Adverse drug reactions are a major health problem and are associated with increased patient morbidity, mortality and hospital care costs [1,2]. Approximately 15% of these side effects are thought to be drug-induced hypersensitivity reactions generated by the immune system in response to the drug [3]. The reactions are not strictly dose-dependent and appear to represent immunological responses that are drug-specific, but which do not relate to the pharmacological properties of the drug [1,4]. Clinical phenotypes of these adverse drug effects are diverse. These effects can include varying combinations of fever, skin rash, hepatotoxicity, gastrointestinal and/or respiratory involvement and also eosinophilia, depending on the offending drug [4]. Clinical severity also varies, although it is notable that fatal outcomes can be reported for severe drug hypersensitivity reactions, particularly for Stevens-Johnson syndrome and toxic epidermal necrolysis [1,4,5].

Most drugs are chemically inert, with a molecular weight of less than 1 kDa, and are considered to be poor or non-activators of the immune system [1,6–9]. It has therefore been hypothesized that these agents are haptenated or conjugated to endogenous proteins following bio-activation of the parent drug, leading to the recognition and presentation of the hapten–peptide complex to the immune system [1,6–9]. The resultant major histocompatibility complex (MHC) restricted T-cell-mediated immune response can be directed against the hapten itself or against the peptide and is followed by the release of pro-inflammatory cytokines and chemokines [1,3,6–9]. Accordingly, in vitro evidence has indicated that drug hypersensitivity reactions involve MHC-restricted presentation of the parent drug or its metabolites before T-cell presentation [6–10].

In this context, abacavir hypersensitivity may provide an illustrative example in which susceptibility has a strong genetic basis. Abacavir is a nucleoside reverse transcriptase inhibitor used to treat HIV-1 infection and has a generally favourable toxicity profile. However, ≈8% of HIV patients treated with
abacavir develop a multisystem hypersensitivity reaction to the drug [11–13]. This clinical syndrome usually develops within 6 weeks of initiating therapy, while those patients who do not develop the hypersensitivity reaction within this period remain at low risk despite ongoing therapy. Epidemiological data have indicated ethnic and familial associations with the abacavir hypersensitivity reaction [13,14], and subsequent genetic associations have revealed that genes located in the MHC are highly predictive of hypersensitivity reactions in abacavir-exposed populations (positive predictive value >70%). The susceptibility locus/resides within the 57.1 ancestral haplotype (AH) comprising HLA-B*5701, C4A6 and the DRB*0701, DQ3 combination [10,15]. Recombinant haplotype mapping suggests that the candidate region includes the haplo-typic heat shock protein 70 (Hsp70)-Hom M493T polymorphism within the central MHC region, which in combination with HLA-B*5701 is highly predictive of an abacavir hypersensitivity response [16].

In this study, the immunological basis of the abacavir hypersensitivity response was investigated with the aim of finding a specific diagnostic marker of this adverse reaction. The elucidation of a characteristic cytokine profile associated with abacavir-specific immune responses was undertaken to determine the role of type 1 and type 2 immune cells, classified according to the cytokines they secrete, and their role in the induction of a cell-mediated inflammatory response or antibody production [17–19]. In general, type 1 cells primarily produce interferon (IFN)-γ, tumour necrosis factor (TNF)-β and interleukin (IL)-2 that stimulate macrophages leading to the development of cell-mediated immune responses, while a number of anti-inflammatory cytokines such as IL-4, -5, -10 and -13 are secreted by type 2 cells, which primarily produce interferon (IFN)-γ and interleukin (IL)-10 that stimulate macrophages leading to the development of an antibody-mediated response or antibody production [17–19]. In general, type 1 cells primarily produce interferon (IFN)-γ, tumour necrosis factor (TNF)-β and interleukin (IL)-2 that stimulate macrophages leading to the development of cell-mediated immune responses, while a number of anti-inflammatory cytokines such as IL-4, -5, -10 and -13 are secreted by type 2 cells, which primarily produce interferon (IFN)-γ and interleukin (IL)-10 that stimulate macrophages leading to the development of an antibody-mediated response or antibody production [17–19]. In general, type 1 cells primarily produce interferon (IFN)-γ, tumour necrosis factor (TNF)-β and interleukin (IL)-2 that stimulate macrophages leading to the development of cell-mediated immune responses, while a number of anti-inflammatory cytokines such as IL-4, -5, -10 and -13 are secreted by type 2 cells, which primarily produce interferon (IFN)-γ and interleukin (IL)-10 that stimulate macrophages leading to the development of an antibody-mediated response or antibody production [17–19].

**Methods**

**Patients**

HIV-positive patients carrying markers of the 57.1 AH (n=74) were identified from the West Australian HIV Cohort. All patients who consented to participate in the study were recruited. Peripheral blood mononuclear cells (PBMCs) were collected from 11 ABC HSR individuals, 9 ABC non-HSR and 8 ABC-naive patients. Prior approval was obtained from the institutional ethics committee. Abacavir hypersensitivity was diagnosed on clinical presentation and epicutaneous patch testing as previously described [16].

**Separation of PBMCs**

PBMCs from heparinized blood were separated on a Ficoll Paque Plus (Amersham Biosciences, Uppsala, Sweden) gradient and stored in liquid nitrogen in 10% dimethylsulfoxide (VWR International Ltd, Leicestershire, UK) and 90% heat-inactivated fetal calf serum (HI-FCS) (ThermoTrace, Melbourne, VIC, Australia). The thawed PBMCs were washed once in cold RPMI 1640 (Gibco Invitrogen Corporation, Auckland, New Zealand) and resuspended at a concentration of 10^6 cells/ml in 10% HI-FCS/RPMI 1640. Cells were aliquoted into 14 ml round-bottom polypropylene tubes (Falcon, Becton-Dickinson, San Jose, CA, USA) to give a final concentration of 0.5×10^6 cells/ml.

**Measurement of cytokine RNA**

Abacavir (commercially available Ziagen® tablets or powder kindly supplied by GlaxoSmithKline, Uxbridge, Middlesex, UK) was dissolved in phosphate-buffered saline (PBS) (pH 7.4) and stored at -80°C until used. PBMCs from ABC HSR and non-HSR patients were incubated at 37°C in an atmosphere of 5% CO₂ in the presence or absence of 4 μg/ml abacavir for 0 (baseline) and 24 h.

The RNase Mini Kit (Qiagen Inc, Valencia, CA, USA) was used to extract RNA from PBMCs according to the manufacturer’s instructions. Briefly, PBMCs were pelleted (6 min, 5,000 rpm, room temperature). Culture supernatants were harvested and stored at -80°C until used. Cell pellets were lysed in 350 μl buffer RLT, filtered through a QIAshredder spin column (Qiagen Inc.) and stored at -80°C. RNA was isolated in 30 μl of RNase and DNase-free water and stored at -80°C until used [23]. RNA isolated from ABC HSR (n=9) and ABC non-HSR (n=6) patients after 0 and 24 h exposure to abacavir was pooled and re-extracted as described above. The eluted RNA concentration was determined by spectrophotometry (ND-1000 spectrophotometer, BioLab, Melbourne, VIC, Australia) at a wavelength of 260 nm. The extracted RNA was stored at -80°C.

Equal concentrations of RNA (0.5 μg) from each patient group at the two time points were converted to biotinylated cDNA by reverse transcription. The cDNA was hybridized to four GEArray Q Series Human Common Cytokine Gene arrays according to the manufacturer’s instructions (SuperArray Bioscience Corporation, Frederick, MD, USA). The nylon membranes were blocked and incubated with alkaline-phosphatase-conjugated streptavidin and detected using CDP-Star chemiluminescent substrate. Chemiluminescent signals were captured on X-ray film (Cronex 5, Agfa, Melbourne, VIC, Australia), scanned...
on a ScanJet ADF (Hewlett Packard, Melbourne, VIC, Australia) and converted into grayscale TIFF images. The images were analyzed using ScanAlyze 2.44 (http://rana.lbl.gov). The numerical data were further analyzed using GEArray Analyzer software (www.superarray.com).

Quantification of TNF-α and IFN-γ cytokine levels

PBMCs from two ABC HSR patients and one ABC non-HSR individual were exposed to a range of drug concentrations (0.4, 1, 4, and 10 μg/ml) for 3 and 6 h at 37˚C in a 5% CO2 incubator. Negative controls were set up in parallel. Brefeldin A (Sigma Aldrich Co, Sydney, NSW, Australia) was added to cell cultures after a 1 h incubation at a final concentration of 10 μg/ml.

After incubation PBMCs were placed on ice and washed once in PBS (Ca2+- and Mg2+-free) supplemented with 1% bovine serum albumin (BSA; Sigma Aldrich Co) and 0.2% sodium azide. All antibodies (unless stated otherwise) were obtained from Immunotech, Miami, FL, USA. Cells were simultaneously stained with energy-coupled dye-conjugated anti-CD45 (clone J33) and fluorescein isothiocyanate (FITC)-conjugated anti-TNF-α and anti-CD14+ (clone 93). Staining for intracellular TNF-α was carried out using Intraprep Permeabilization Reagent (Immunotech) according to the manufacturer’s instructions. TNF-α was detected with phycoerythrin (PE)-conjugated anti-TNF-α (clone 188). Positive and negative populations were differentiated by isotype controls conjugated to FITC and PE. All samples were analysed on a Coulter EPICS XL-MCL Flow Cytometer (Beckman Coulter, Melbourne, VIC, Australia). Monocyte and lymphocyte populations were separated by their CD45 and side-scatter characteristics. CD14+/TNF-α+ monocytes were gated using the isotype-matched controls as reference [23]. A total of 20,000 live events were collected for each sample. Differences between percentages of CD14+ cells expressing TNF-α in abacavir-exposed and -unexposed samples were displayed graphically.

Similarly, PBMCs from an ABC HSR HLA-B*5701-positive patient and an ABC non-HSR HLA-B*5701-negative control were exposed to the drug for 16 h for the detection of intracellular IFN-γ. Staining was carried out using the IFN-γ-secreation assay detection kit (FITC) according to the manufacturer’s instructions (Miltenyi Biotech, Bergisch Gladbach, Germany). Expression of this cytokine was analysed on CD8+ cells using anti-PE-CY5-conjugated antibodies (clone B9.11) on a FACS Calibur Flow Cytometer (Becton-Dickinson). A total of 10,000 live events were collected based on forward and side-scatter characteristics.

A time-course was initially carried out on PBMCs from a patient from each group using 4 μg/ml abacavir. Culture supernatants were harvested at 0, 3, 6, 24 and 48 h and IFN-γ (BD Pharmingen, San Jose, CA, USA) levels were quantified by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s instructions. PBMCs from ABC HSR (n=9) and non-HSR (n=6) patients were incubated for 48 h in the presence and absence of abacavir. PBMCs from ABC-naive patients (n=8) were also cultured for the same time period with 4 μg/ml of abacavir-free drug base. Culture supernatants were collected and stored at -80˚C until used. IFN-γ, IL-2 and IL-4 (R&D Systems, Minneapolis, MN, USA) levels were measured in triplicate by ELISAs according to the manufacturer’s instructions (average SD was <5 and interassay variation was <10%).

Data analysis

Intracellular TNF-α and extracellular IFN-γ and IL-2 expression were corrected by subtracting abacavir-unexposed results before analysis. Cytokine levels for each group are presented as medians. Statistical differences between the ABC HSR, non-HSR and ABC-naive groups were assessed by non-parametric Kruskal–Wallis or exact Mann–Whitney tests, as appropriate, for continuous variates or Fisher’s exact tests for categorical data. Statistical significance was defined as corresponding to P<0.05.

Results

Patient characteristics

Demographics of patients who consented to participate in the in vitro study are presented in Table 1. All three patient groups were reasonably matched for CD4+ and CD8+ T-cell counts (P=0.89, P=0.14 respectively, Kruskal–Wallis test). However, carriage of HLA-B*5701 was significantly higher amongst patients in the ABC HSR group compared with the non-HSR (P=0.0005, Fisher’s exact test) and ABC-naive (P=0.02, Fisher’s exact test) groups, while the number of Caucasians in each group was similar (P=0.17, Fisher’s exact test). All patients were on active antiretroviral therapy at the time of the study and 10/11 ABC HSR, 4/9 ABC non-HSR and 5/8 ABC-naive patients had undetectable viral loads (data not shown).

Expression of inflammatory and type 1 cytokines in ABC HSR patients

Optimal drug concentration and time-points for harvesting PBMC cultures were determined by dose-response and time-course experiments (Figures 1 and 2). From these results, a concentration of 4 μg/ml was chosen for cell culture experiments. The culture period depended on the assay and a 3 h time-point was chosen for intracellular flow cytometric analysis whilst culture supernatants were harvested after a 48 h exposure to abacavir.
Table 1. Characteristics of patients in ABC HSR, non-HSR and ABC-naive groups

<table>
<thead>
<tr>
<th></th>
<th>ABC HSR</th>
<th>ABC non-HSR</th>
<th>ABC naive</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients, n</td>
<td>11</td>
<td>9</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Mean CD8+ T-cell count/μl (SD)</td>
<td>945.6 (475.2)</td>
<td>1,073.1 (596.3)</td>
<td>1,379 (414)</td>
<td>0.89</td>
</tr>
<tr>
<td>Mean CD4+ T-cell count/μl (SD)</td>
<td>532 (302.1)</td>
<td>539.3 (316.7)</td>
<td>625.8 (431.6)</td>
<td>0.14</td>
</tr>
<tr>
<td>Caucasian count, n (%)</td>
<td>11 (100)</td>
<td>7 (77.78)§</td>
<td>8 (100)</td>
<td>0.17</td>
</tr>
<tr>
<td>HLA-B*5701 count, n (%)</td>
<td>11 (100)</td>
<td>2 (22.22)</td>
<td>4 (50)</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

*ABC HSR, abacavir hypersensitive patients. †ABC non-HSR, abacavir tolerant controls. ‡ABC naive, abacavir-unexposed individuals. § Includes two patients who were HLA-B*5701-positive. All three patient groups were reasonably matched for CD4+ and CD8+ T-cell counts (P=0.89 and P=0.14, respectively, Kruskal-Wallis test).

Carriage of HLA-B*5701 was significantly higher amongst patients in the ABC HSR group compared with the non-HSR (P=0.0005, Fisher’s exact test) and ABC-naive groups (P=0.02, Fisher’s exact test).

Figure 1. TNF-α expression in CD14+ monocytes after in vitro exposure to abacavir

Peripheral blood mononuclear cells (0.5x10^6) from two ABC HSR individuals (triangles and diamonds) and one tolerant control (squares) were cultured in the presence and absence of abacavir for (A) 3 h and (B) 6 h. Intracellular expression of tumour necrosis factor α (TNF-α) measured by flow cytometry was higher in the ABC HSR patients at both time points compared with the ABC non-HSR individual. TNF-α expression in T-cells was not significantly different between the abacavir-exposed and -unexposed samples. ABC HSR, hypersensitive to abacavir; ABC non-HSR, abacavir tolerant.

Figure 2. IFN-γ and IL-2 levels in culture supernatants from 0 to 72 h after in vitro exposure to 4 μg/ml abacavir

(A) Interferon (IFN)-γ and (B) interleukin (IL)-2 levels in culture supernatants from 0 to 72 h after in vitro exposure to 4 μg/ml abacavir. Supernatants were harvested after 48 h culture with the drug. Levels of both cytokines were higher in the ABC HSR patient (triangles) compared with the tolerant control (squares). IL-4 could not be measured as the levels of this cytokine were below the limit of detection of the assay. ABC HSR, hypersensitive to abacavir.
Elevated type 1 cytokine RNA expression in ABC HSR patients

Profiling of immune response gene expression was carried out following abacavir exposure. To identify a cytokine specific to the abacavir hypersensitivity response, RNA extracted from PBMCs obtained from ABC HSR \((n=9)\) and non-HSR \((n=6)\) patients was exposed to 4 \(\mu\)g/ml abacavir at time 0 and after 24 h and applied to microarray analysis. The RNA was pooled before converting to cDNA to reduce interindividual heterogeneity [24]. Cytokine RNA expression was normalized against D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The change in cytokine expression after abacavir stimulation in the ABC HSR and non-HSR groups was depicted graphically and an arbitrary threshold of 50 units was chosen as the cutoff. Patients in the HSR group had higher inflammatory and type 1 cytokine expression compared with ABC non-HSR individuals. In particular, IL-1\(\beta\), IL-6, IL-8, TNF-\(\alpha\), IL-12B, TNF-\(\beta\), IFN-\(\gamma\) and IL-6, IL-8, TNF-\(\beta\), IFN-WI and IL-1 were expressed at 60- to 655-fold higher levels in ABC HSR compared with ABC non-HSR individuals, whereas the expression of IL-9, IL-11, IL-19 and IFN-\(\gamma\) decreased after a 24 h abacavir exposure relative to levels in the tolerant controls (Table 2).

TNF-\(\alpha\) and IFN-\(\gamma\) were chosen as inflammatory and type 1 markers, respectively, for further investigation. We have previously shown that the fold difference in the percentage of CD14\(^+\) monocytes expressing intracellular TNF-\(\alpha\) was significantly higher in the ABC HSR group (median fold increase 13.4) compared with the ABC non-HSR patients (median fold reduction 8.9, \(P=0.008\), Mann–Whitney test) [16,25]. In this study, this group of patients had increased IFN-\(\gamma\) in culture supernatants compared with both ABC non-HSR (\(P=0.001\), exact Mann–Whitney test) and ABC-naive patients (\(P=0.002\), exact Mann–Whitney test; Figure 3). Levels of this cytokine were significantly higher in the ABC HSR group compared to non-HSR and ABC-naive patients after culturing with either Ziagen \({ }^{\text{TM}}\) or abacavir-free drug base (data not shown). Flow cytometric analysis of intracellular IFN-\(\gamma\) production showed that the difference in the expression of this cytokine between abacavir-exposed and -unexposed samples was higher in the CD8 \(^{+}\)-T-lymphocyte population in the ABC HSR HLA-B*5701-positive patient (1.96) compared with a non-HSR HLA-B*5701-negative individual (-0.11) (data not shown). IL-2 and IL-4 levels in culture supernatants were also quantified as markers of a type 1 and type 2 cytokine response but could not be detected.

**Discussion**

Most drugs are chemically inert but may undergo conjugation to endogenous proteins following metabolism to reactive metabolites, which can result in the recognition and presentation of a hapten–peptide complex to the immune system. For the majority of agents, the hapten is a chemical metabolite generated on bio-activation of the parent drug and then processed by antigen-presenting cells. The resultant T-cell-mediated immune response is restricted against the hapten and may be associated with the release of pro-inflammatory cytokines and chemokines [1–3,9,16]. For this reason, T-lymphocytes and the cytokines they secrete were investigated in order to characterize the abacavir hypersensitivity response and to thus identify a surrogate marker for this adverse reaction.

ABC HSR patients were originally identified on the basis of clinical presentation at the time of adverse reaction. However, these clinical symptoms occur in other drug hypersensitivity reactions or in unrelated illnesses such as immune restoration diseases. For this reason, epicutaneous patch tests were used to support and confirm diagnosis of abacavir hypersensitivity [16,25–27]. Once the cohorts were identified, cytokine profiling was characterized using microarrays,

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**Table 2. Pro-inflammatory and type 1 cytokine RNA expression is increased in ABC HSR patients after a 24 h in vitro exposure to 4 \(\mu\)g/ml abacavir compared with ABC non-HSR individuals**

<table>
<thead>
<tr>
<th>Increased RNA expression</th>
<th>Decreased RNA expression</th>
</tr>
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<tbody>
<tr>
<td>Pro-inflammatory [37]</td>
<td>IL-1(\beta), TNF-(\alpha), IL-6, IL-8(^*)</td>
</tr>
<tr>
<td>Anti-inflammatory [22]</td>
<td>IL-10(^*)</td>
</tr>
<tr>
<td>Type 1 promoting response [18,21,38–40]</td>
<td>TNF-(\beta), IL-12B(^+)</td>
</tr>
<tr>
<td>Type 2 promoting response [18,22,41]</td>
<td>IFN-(\gamma), IFN-W1(^<em>), FasL(^</em>)</td>
</tr>
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</table>

* Differential RNA expression >100. \(^*\) Differential RNA expression >50 but <100. \(^+\) Differential RNA expression >100 but <250. \(^\dagger\) Differential RNA expression >250. AIF, allograft inflammatory factor; CSF, colony-stimulating factor; FasL, Fas ligand; FGF, fibroblast growth factor; IFN, interferon; IL, interleukin; PDGF, platelet-derived growth factor; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor. ABC HSR, hypersensitive to abacavir; ABC non-HSR, abacavir-tolerant.
intracellular flow cytometry and ELISAs. From the results, we have demonstrated that ABC HSR patients express higher amounts of inflammatory and type 1 cytokines than abacavir-treated non-HSR individuals. This altered cytokine expression profile correlated with ABC HSR patient drug outcomes.

The increased RNA expression of type 1 cytokines, such as TNF-β and IL-12B, and protein expression of IFN-γ is consistent with the hypothesis that the abacavir hypersensitivity response is mediated by type 1-producing cells. Although IFN-γ RNA expression at 24 h was decreased, protein levels of this cytokine were elevated after a 48 h in vitro culture (Figure 2A). This difference in the kinetics between IFN-γ RNA and protein expression has been observed for other antigens [28].

Previous results from recombinant mapping, in vitro studies and epicutaneous patch testing suggested that the abacavir hypersensitivity reaction occurs via MHC class I restriction involving CD8+ T-cells [10,14]. In this study, we have demonstrated a rapid production of IFN-γ restricted to cultures of PBMCs stimulated with abacavir from individuals with previous abacavir hypersensitivity. Although it is not possible to discount the contribution of CD4+ T-cells and natural killer (NK) cells to this response, a limited intracellular flow cytometric analysis of PBMCs taken from one ABC HSR HLA-B*5701-positive patient and one ABC non-HSR individual showed a restriction of IFN-γ staining to CD8+ cells in the HSR patient sample (data not shown).

In addition, we have previously shown that plasma TNF-α levels were abrogated in a population of PBMCs depleted of CD8+ T-lymphocytes compared with the undepleted and CD4+ T-cell-depleted populations [16]. In this study, we have examined other potential cellular contributors of TNF-α to this reaction and showed that evidence of enhanced TNF-α production is present in CD14+ monocytes from two ABC HSR patients but not one tolerant control and that there was no increased expression detectable in lymphocytes. This is consistent with abacavir having a direct effect on monocytes in susceptible individuals and further studies looking at the mechanism behind this response in ABC-naive subjects are needed.

These data also support the clinical observation that carriage of HLA-B*5701 and related haplospecific genetic markers within the class I and class III regions of the MHC have strong positive predictive associations with the abacavir hypersensitivity response in the Western Australian HIV cohort, and that these gene products may be involved in antigen presentation [16]. Involvement of the extended 57.1 MHC haplotype in conferring susceptibility to abacavir hypersensitivity also suggests that polymorphic MHC-encoded immunoregulatory genes may contribute to the abacavir-specific immune response in these patients. For example, the -238A promoter polymorphism of TNF-α is haploptic to the 18.1 and 57.1 AHs. This polymorphism might play a role in the induction of the abacavir hypersensitivity response within the 57.1 haplotype [16,27] and could potentially influence an innate immune response to abacavir, as suggested by restriction in TNF-α expression to CD14+ monocytes from individuals that have a history of hypersensitivity (Figure 1) [16]. In addition, elevated cytokine production may be directly correlated with the carriage of the Hsp70-Hom variant and the abacavir hypersensitivity response [16]. HSP70 proteins assist in the presentation of exogenous antigens to CD8+ T-lymphocytes [29] and can induce the production of pro-inflammatory cytokines like TNF-α and IL-6 through the CD14 and Toll-like receptor pathway, initiating the innate immune response [17,30]. Hence, genetic regulation of TNF-α and IFN-γ may contribute to the severity of the clinical syndrome in the ABC HSR group of patients. Our current investigations are focused on elucidating differences in the innate immune response in ABC HSR and non-HSR patients after in vitro abacavir exposure and specifically what potential roles monocytes and dendritic cells play in the initiation of this reaction.

In addition to the altered RNA levels of pro- and anti-inflammatory, type 1 and type 2 cytokines, expression of other common cytokine genes changed after in vitro abacavir exposure (Table 2). For example, Fas
ligand (FasL) expression was upregulated after 24 h exposure to the drug, whereas CD40L levels were decreased. Both these molecules are members of the TNF ligand family [31–34]. FasL is an activation molecule expressed by CD4+ type 1 cells as well as by CD8+ cytotoxic T-lymphocytes [32]. The binding of FasL to a cell bearing its receptor Fas is necessary for initiation of apoptosis [34]. The binding of CD40L to CD40 can also induce apoptosis in CD4+ T-lymphocytes, as well as generating memory CD8+ T-cells [35].

The success of prospective screening for HLA-B*5701 in the Western Australian cohort has resulted in a significant decrease in the incidence of abacavir hypersensitivity, from 8% to <2% [36]. Despite the small number of ABC HSR patients in this study, we have shown that pro-inflammatory and type 1 cytokines are upregulated on exposure to abacavir in patients who had the hypersensitivity reaction. These observations are consistent with our previous results showing that the abacavir hypersensitivity reaction is MHC class I restricted and involves CD8+ T-lymphocytes. Hence, in vitro assays may assist in the identification of susceptibility loci in predisposed individuals by providing objective evidence of an abacavir-specific immune response, while also helping to clarify the immunological mechanisms involved in the pathogenesis of abacavir hypersensitivity.

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Disclosure statement

The authors declare no conflicts of interest.

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