Research Article

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Susceptibility to chronic hepatitis C virus infection is influenced by sequence differences in immunodominant CD8+ T cell epitopes

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Background & Aims: The antiviral immune response against HCV by CD8+ T cells plays a central role in viral containment. In a large HCV genotype 1b outbreak in Ireland, HLA-B*08 was identified as a risk allele for chronic infection and HLA-A*03 and HLA-B*27 were associated with higher clearance rates. Here we took advantage of a similar large common source HCV genotype 1b outbreak (East-German cohort) to determine the role of HLA class I alleles and the sequence of the infection source, in immunodominant CD8+ T cell epitopes for disease outcome.

Methods: HLA-type and *IL28B* genotype were determined in 216 patients with chronic and 95 with spontaneously resolved HCV infection. The viral sequence in immunodominant epitopes was determined in the infection source and in patients with chronic infection.

Results: In contrast to the Irish cohort, HLA-B*08, HLA-A*03 and HLA-B*27 were neutral for disease outcome even when the cohort was stratified for the *IL28B* genotype. Sequence analysis of the immunodominant epitopes revealed that pre-existing substitutions in the infection source of both cohorts influenced the impact of the corresponding HLA-allele. The immunodominant epitopes presented by the "protective" alleles HLA-A*03 and -B*27 in the Irish cohort contained substitutions in the source virus of the East-German outbreak. Importantly, the pre-existing substitutions altered subsequent selection pressure and viral evolution in the East-German cohort.

Conclusions: This study highlights that subtle sequence differences in the infection source may have profound effects on the ability to clear HCV infection in the presence of particular HLA class I alleles.

Keywords: HLA class I; Immune escape; Selection; IL28B; Anti-D.

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Abbreviations: HCV, hepatitis C virus; HLA, human leukocyte antigen; ALT, alanine amino transferase; HIV, human immunodeficiency virus; RNA, ribonucleic acid; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism.

Introduction

Upon acute infection with the hepatitis C virus, two fundamentally different clinical courses are observed. In a subgroup of patients, sustained control of viral replication is achieved whereas in the majority of patients HCV persists in the liver. Numerous studies have tried to identify prognostic factors for the different disease outcomes and to dissect the exact underlying mechanisms. There is a large body of evidence that both the innate and adaptive immune responses are important for successful clearance of HCV infection (reviewed in [1]). From a clinical perspective, spontaneous clearance of HCV infection is more frequently observed in patients with severe clinical signs of hepatitis and jaundice [2]. This has been attributed to the cytotoxic response by HCV-specific CD8+ T cells that become detectable when ALT levels start to rise in the serum. Accordingly, a strong immune response by CD8+ T cells has been linked to spontaneous control of HCV infection [1]. This has been confirmed in chimpanzee studies where depletion of CD8+ T cells was associated with absence of immune control [3].

CD8+ T cells recognize infected hepatocytes through the interaction with viral epitopes presented on the cell surface in context with HLA class I alleles. The epitope repertoire that is presented on the cell surface is therefore largely determined by the HLA class I molecules encoded by the host genome. The importance of particular HLA class I alleles is highlighted in HIV-1 infection where HLA-B*27:05, HLA-B*57:01, and HLA-B*13 are associated

with control of viral replication whereas HLA-B*35 is associated with high viral load and faster disease progression [4,5]. It has been demonstrated that CD8+ T cells reproducibly select for escape mutations in key epitopes presented by protective HLA class I alleles [6]. These escape mutations may severely impair viral replication and thereby attenuate the disease course [7,8]. Alternatively, protective HLA class I alleles may have unique properties in the composition of their binding motif, as highlighted in a recent study [9]. These unique properties may result in thymic selection of a naïve CD8+ T cell repertoire with the capacity of broad crossreactivity.

In HCV infection, the effect of particular HLA class I alleles on disease outcome is less clear [10]. For many alleles, conflicting results have been observed, however, there are a few alleles that seem to have a reproducible effect. These include HLA-B*27 and HLA-B*57, which were reported to be associated with spontaneous clearance of HCV infection in various studies [11–14]. In contrast, HLA-B*08 has been reported as a "protective" allele in one study [14], but was reported as a risk allele in others [12,13]. Some of these differences may be the consequence of the quite heterogeneous composition of the cohorts that have been studied. This problem can be overcome by analysis of single source outbreaks where sequence heterogeneity of the inoculum and most transmission factors are well controlled. There are two large single source HCV genotype 1b outbreaks that occurred, after inoculation of contaminated anti-D immunoglobulin, to women in the late '70s in Ireland and Germany. Studies of the Irish HCV outbreak were very informative for the identification of protective host HLA class I alleles [13]. Here we studied the very similar HCV outbreak that occurred in 1978/79 in the former German Democratic Republic [15]. Interestingly, despite the fact that the source of infection in both the Irish and East-German cohorts was HCV genotype 1b, there are differences in the impact of particular HLA class I alleles on disease outcome. We compared sequences in the immunodominant CD8+ T cell epitopes from the infection source of both cohorts and the consequences for immune selection pressure and viral evolution in the East-German cohort.

Patients and methods

Patients

Samples from the East-German anti-D cohort have been collected since 2008 by members of the East German HCV Study Group. For 311 patients of Central-European origin, information about the outcome after acute infection (spontaneous resolution *vs.* persistent infection) was available. The study cohort includes 95 (30.5%) spontaneous resolvers and 216 (69.5%) patients with chronic infection. There is a selection bias in sample recruitment towards patients with persistent infection. In the East-German cohort about 50% of all exposed individuals spontaneously resolved infection [15]. Written informed consent was obtained from all patients and the study was approved by the ethics committee.

Sequences of the East-German and the Irish anti-D cohorts

The genomic region covering NS3 and NS5B was amplified and sequenced from 113 chronically infected subjects from the East-German cohort. Viral RNA extraction, reverse transcription and amplification by nested PCR were performed as previously described [16]. Parts of the sequences were previously published under accession numbers HQ908282–HQ908359. Sequences from the Irish outbreak with the accession numbers AF313916 (source) and AB154177–AB154206 (patient isolates) were downloaded from GenBank. All sequences were manually aligned with Se-Al (available from http://evolve.zoo.ox.ac.uk).

HLA class I and IL28B genotyping

DNA of patients was extracted from peripheral blood lymphocytes using spin columns (Qiagen, Hilden, Germany). HLA-A and HLA-B typing at two-digits resolution-level was performed using sequence-specific primers (PCR-SSP) methodology or alternatively using sequence-specific oligonucleotides (LABType methodology, both provided by One Lambda Inc., Canoga Park, CA, USA)[17]. The *IL28B* genotype was determined using LightSNiP rs12979860 hu *IL28B* melting curve analysis (TIB MOLBIOL, Berlin, Germany) according to the manufacturer's instructions.

Impact of epitope variants on CD8+ T cell response

PBMCs from ten HLA-A*03-positive injection drug users (five with chronic and five with spontaneously resolved HCV infection) were cultured in RPMI 1640 medium containing 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 10 mM HEPES buffer, 25 U/ml recombinant interleukin-2 (IL-2), 0.1 μg/ml anti-CD28 and anti-CD49d and the prototype peptide (TVYHGAGTK) or a variant (TVFHGAGSK; 1 μg/ml per peptide). After 10 days, cells were restimulated with both peptides (10 μg/ml) in the presence of brefeldin A (100 ng/ml) for 5 h and then analysed for their IFN-γ secretion via flow cytometry.

Statistical analysis

Univariate associations between HLA class I alleles and outcomes of HCV-infection were assessed using Fisher's exact tests. Fisher's exact tests were also used for associations between expression of HLA class I alleles and viral mutations.

Results

Impact of individual HLA class I alleles on the outcome of HCV infection in the East-German cohort

The HLA-A and -B phenotype frequencies were compared in 311 individuals of the East-German anti-D cohort according to the outcome of infection. The analysis included patients who spontaneously cleared infection (n = 95) and patients who developed chronic infection (n = 216). HLA-A*31 was the only allele that was associated with differential outcome of HCV infection by univariate analysis with a significantly higher frequency of this allele in patients with spontaneous clearance (12.6% *vs.* 3.2%; RR = 3.9; uncorrected $p = 0.003$). No significant association between any other HLA class I alleles and infection outcome was observed in this cohort (Fig. 1A). This was a surprising finding because a differential impact on disease outcome was observed in the Irish cohort for HLA-A*03, HLA-B*27, and HLA-B*08 [13]. As previously described in a smaller subset of patients [18], the rs12979860 C/C genotype was strongly associated with spontaneous viral clearance in the East-German cohort (Fig. 1B). Presence of jaundice was a predictive marker for spontaneous clearance only in patients with the non-C/C genotype [18]. Assuming that jaundice is the result of the cytotoxic T-cell response, we hypothesized that the impact of HLA class I alleles on disease outcome may be more prominent in rs12979860 non-C/C patients. However, no significant association between any HLA class I allele and disease outcome was observed when only patients with the rs12979860 non-C/C genotype (204 of 311 women) were included (Fig. 1C), although this subanalysis was underpowered for low frequency HLA alleles.

Sequence differences in the infection source between the Irish and the East-German cohort in key epitopes

We hypothesized that sequence differences in the infection source in key epitopes of the CD8+ T cell response may be