Virology 386 (2009) 398–406

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Impact of viral selected mutations on T cell mediated immunity in chronically evolving and self limiting acute HCV infection

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ARTICLE INFO

Article history:
Received 6 November 2008
Returned to author for revision
23 December 2008
Accepted 15 January 2009
Available online 20 February 2009

Keywords:
HCV
Acute infection
CD8$^+$ T lymphocytes
Viral escape

ABSTRACT

The ability of HCV to mutate in response to cytotoxic T lymphocyte (CTL) pressure is increasingly recognized, but the influence of such a mechanism in viral persistence and final disease outcome has not been ascertained. In this study, we performed a detailed longitudinal analysis of cell mediated immunity and HCV evolution in two self limiting and two chronically evolving HCV acutely infected patients, one of whom transiently controlled viremia. Aminoacid mutations in immunodominant regions of viruses were observed in all patients, although they conferred viral escape from CTL responses only in chronically infected individuals. Resurgence of viremia coincided with the replacement of the original virus quasispecies with mutant viruses that had escaped recognition by primary CD8$^+$ T cell responses and infection persisted in the presence of variant viruses which were less efficiently recognized by preexisting and *de novo* induced T cell responses.

Introduction

Hepatitis C virus (HCV) is one of the major causes of chronic hepatitis worldwide (Lauer and Walker, 2001). To date, the mechanisms by which HCV establishes persistence in chronically evolving individuals are not fully understood. In chimpanzees, a strong association between viral persistence and acquisition of mutations in MHC class I restricted epitopes has been demonstrated (Erickson et al., 2001). In humans, selection of escape mutations by CD8 immune pressure was initially demonstrated by longitudinal studies in chronically evolving patients. In such analyses, escape mutations were detected in HLA-restricted epitopes of the NS3 protein, before, during and after IFN-alpha treatment (Tester et al., 2005; Timm et al., 2004). Furthermore, occurrence of escape mutations in different regions of HCV polyprotein (Core, E1, E2, NS2, NS3) was documented by prospective partial genomic sequencing and genome wide analysis of T cell responsiveness (Cox et al., 2005). Finally, the role of cell mediated immunity in shaping HCV evolution has been confirmed by population-based analysis. In such studies, accumulation of viral sequence polymorphisms was found at different sites of the NS3, NS5A, NS5B proteins in chronically infected patients sharing the same class I HLA-allele (Gaudieri et al., 2006; Timm et al., 2007). Although all the previous investigations have provided clear evidence that CD8$^+$ T cell responses represent a driving force of HCV evolution, the role of such a mechanism in viral persistence and final disease outcome is uncertain. Indeed, development of escape mutations has been documented in the absence of viral control, and some reports have demonstrated that progression to chronic infection may occur in the absence or with limited selection pressure on immunodominant cytotoxic T cell epitopes (Kuntzen et al., 2007; Urbani et al., 2005a,

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doi:10.1016/j.virol.2009.01.020

Table 1
Clinical and virological characteristics of HCV acutely infected patients

Patient	Genotype	Risk factor	Age (yr)	Sex	Outcome	ALT (IU/ml)[a]	Viral load (IU/ml)				
							T0	T1	T3	T6	T12
A18	3a	IVDU	28	M	Chronicity	803	225,000	504,000	78,900	148,000	nd
A90	1b	Iatrogenic	58	F	Chronicity	363	14,400	<600	86,000	2930	2104
A46	1b	Needle	46	F	Self-recovery	1185	223,000	139,000	<15	<15	<15
A87	3a	IVDU	22	M	Self-recovery	1967	2120	54,300	<15	<15	<15

T = months after diagnosis.
nd = not determined.
[a] Values at the moment of diagnosis.

2005b). In a previous study, by performing an in depth analysis of the early cellular immune responses and viral evolution, we provided clear evidence of the relationship between HCV mutation in an immunodominant NS3 epitope and the loss of immune control of viremia (Guglietta et al., 2005). In the present study, to further delineate the role and consequences of escape mutations for immune control, we performed a detailed longitudinal analysis of cell mediated immunity and viral evolution in two self limiting and two chronically evolving HCV acutely infected patients, one of whom transiently controlled viremia. We found selection of amino acidic mutations in immunodominant regions of the virus, but they caused escape from T cell recognition only in chronically evolving patients. In particular, in the patient who transiently controlled viremia, viral resurgence was concomitant with the selection for a virus population mutated at a single amino acid residue that escaped T cell recognition. Subsequent to viral resurgence, the patient became persistently infected by variant viruses which were less efficiently recognized by preexisting and *de novo* induced T cell responses. Altogether, these data provide further support for a causative role of escape mutation in viral persistence and provide novel insights in the interplay between virus and host immune responses during HCV infection.

Results

Characterization of the cellular immune response in HCV acutely infected individuals

The early HCV specific T cell response occurring in HCV acutely infected individuals A18, A90, A46 and A87 was evaluated. The characteristics of these individuals are summarized in Table 1. Subjects A90 and A46 were infected by HCV genotype 1b and A18 and A87 by genotype 3a. All four subjects displayed serum ALT elevation and were viremic at the moment of diagnosis (T0; Table 1). Patients A46 and A87 spontaneously cleared the virus and remained HCV RNA negative

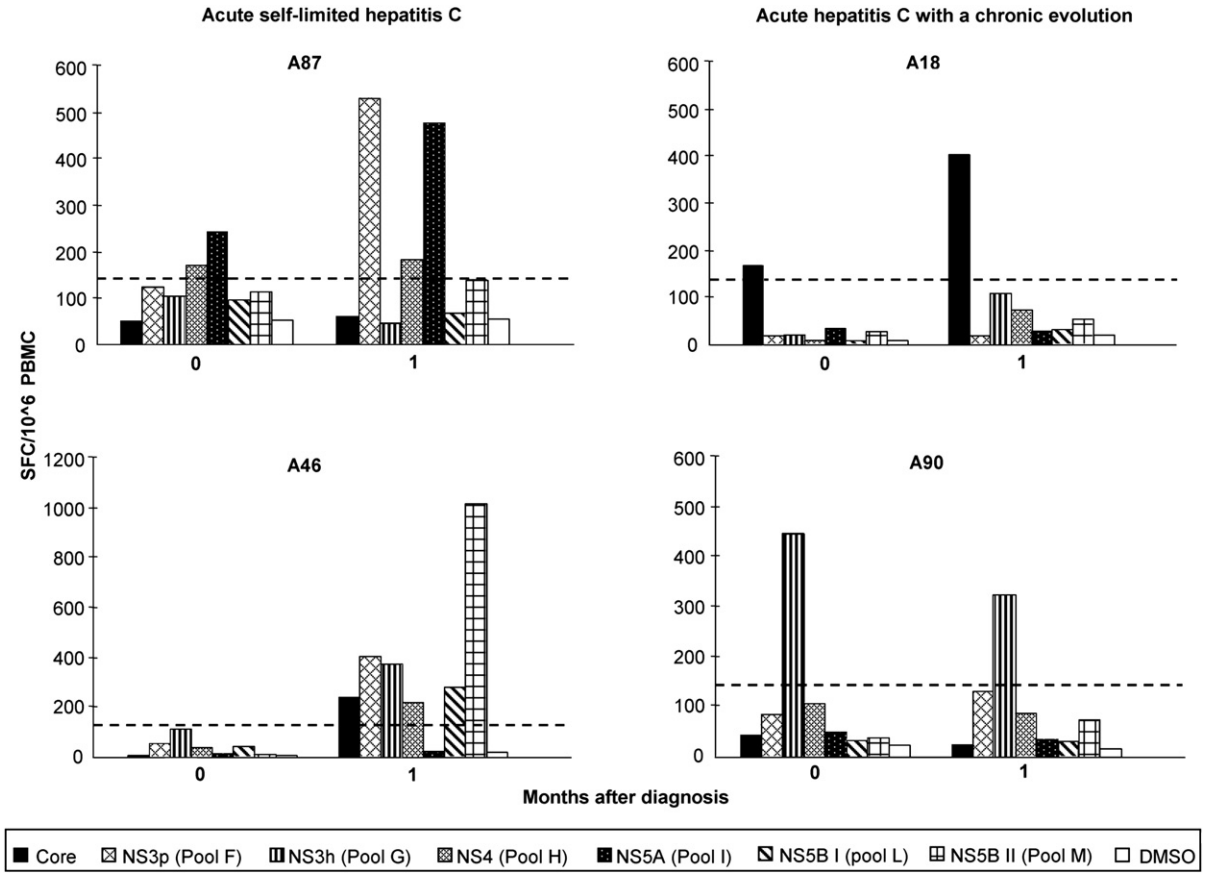

Fig. 1. Magnitude and breadth of HCV-specific T cell responses in HCV acutely infected patients. PBMC samples collected at the moment of diagnosis and one month later were tested by IFN-γ ELIspot assay against seven peptides pools corresponding to Core (pool C) and genotype matched NS3 protease (pool F), NS3 helicase (pool G), NS4 (pool H), NS5A (pool I) and NS5B (pool L and M). DMSO refers to control. Numbers represent Spot Forming Cells/10^6 PBMC. The horizontal dashed line represents the cut-off levels to define a positive response.