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Hepatitis C virus infects T cells and affects interferon-γ signaling in T cell lines

Yasuteru Kondo [a], Vicky M.H. Sung [a], Keigo Machida [a], Minyi Liu [a], Michael M.C. Lai [a,b,*]

[a] *Department of Molecular Microbiology and Immunology, University of Southern California Keck School of Medicine, 2011 Zonal Avenue, Los Angeles, CA 90033, USA*
[b] *Institute of Molecular Biology, Academia Sinica, Taipei 115, Taiwan*

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Abstract

It has been reported that hepatitis C virus (HCV) may infect and replicate in human T cells, particularly in perihepatic lymph nodes, but the extent and consequence of T-cell infection in patients is unclear. This study is conducted to characterize the parameters and functional consequences of HCV infection in T lymphocytes. By using a lymphotropic HCV strain, we showed that HCV could infect T cell lines (Molt-4 and Jurkat cells) in vitro. Both positive- and negative-strand HCV RNA were detected for several weeks after infection. Viral proteins could also be detected by immunofluorescence studies. Moreover, infectious HCV particles were produced from Molt-4 cell cultures, and could be used to infect naïve T cell lines. HCV could also infect human primary $CD4^+$ T cells, particularly naïve ($CD45RA^+CD45RO^-$) $CD4^+$ cells, in culture. The amounts of STAT-1 and phosphorylated STAT-1 proteins in the infected Molt-4 cells were significantly less than those in uninfected cultures, suggesting the possibility of defect in interferon-γ signaling. Indeed, T-bet and STAT-1 mRNA levels after interferon-γ stimulation in infected Molt-4 were suppressed. In conclusion, HCV could infect and transiently replicate in T cells and that HCV replication suppressed the IFN-γ/STAT-1/T-bet signaling due to the reduction of STAT-1 and inhibition of its activation (phosphorylation).

Keywords: CD4; CD45RA; STAT-1; T-bet; Lymphotropism; HCV

Introduction

Hepatitis C virus (HCV) infects about 170 million people worldwide and is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) (Alter et al., 1999). Cellular immune response to HCV plays an important role in the pathogenesis of chronic hepatitis, liver cirrhosis, and HCC (Chang et al., 1997).

HCV antigen-driven proliferation of $CD4^+$ T cells is weak or absent in patients who develop persistent infection (Koziel, 1997). It has been shown that depletion of $CD4^+$ T cell results in a weak $CD8^+$ T cell response, which partly controls viremia, followed by viral rebound and persistence in chimpanzee infection studies (Rice and Walker, 1995).

Pegylated interferon (IFN)-α/ribavirin combination therapy causes sustained virological response (SVR) in some patients with chronic hepatitis. However, SVR rates in genotype 1 and genotype 4 infections are still as low as 42% and 50%, respectively (Grakoui et al., 2003; McHutchison et al., 2002). IFN-based therapy may restore the HCV-specific Type 1 T cell response in the SVR patients group but not the non-responder patients group; proper Th1 response is essential to eradicate HCV (Cramp et al., 2000; Kamal et al., 2002). Several mechanisms have been proposed for the failure of HCV-specific $CD4^+$ and $CD8^+$ T cell response, including deletion, anergy, CTL exhaustion, suppression via regulatory $CD4^+$–$CD25^+$ T cell and interleukin-10 (IL-10)-secreting regulatory $CD8^+$-T cell (Accapezzato et al., 2004; Boettler et al., 2005; Semmo et al., 2005; Ulsenheimer et al., 2003). Although HCV core protein has been reported to directly suppress T cell functions via binding with gC1qR (Yao et al., 2005), the

* Corresponding author. 2011 Zonal Ave. HMR500, Los Angeles, CA 90033, USA. Fax: +1 323 442 1721.
E-mail addresses: yasuteru@ebony.plala.or.jp (Y. Kondo), mvsung@uci.edu (V.M.H. Sung), kmachida@usc.edu (K. Machida), minyiliu@usc.edu (M. Liu), michlai@usc.edu (M.M.C. Lai).

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influence of HCV replication in the T cells on their functions is not fully understood.

Recently, the role of innate immune response (type I IFN system) in the control of viral infection has been well documented in the studies using HCV-producing hepatocyte cell lines and replicon systems (Foy et al., 2003; Lin et al., 2005; Pflugheber et al., 2002). One possible mechanism for suppression of IFN-γ signaling is that HCV induces degradation of STAT-1 (Lin et al., 2005), thus interfering with the Jak-STAT signaling pathway. Since STAT-1 activation is essential for both innate immune system and acquired immune system, especially for Th1 commitment and development (Siebler et al., 2003; Takeda et al., 2003a), we are interested in knowing whether HCV could replicate in T cells, thus affecting T cell response.

Previous reports have indicated that HCV replication could be detected in B cells, T cells, fibroblasts, peripheral blood mononuclear cells and hepatocytes of HCV patients (Bare et al., 2005; Hu et al., 2003; Laporte et al., 2003; Li et al., 2005; Lindenbach et al., 2005; Pal et al., 2006; Shimizu et al., 1992; Sung et al., 2003). Existence of HCV strains with preferential lymphocyte tropism suggests a potential role of T cells as an HCV reservoir (Bare et al., 2005; Hu et al., 2003; Laporte et al., 2003; Li et al., 2005; Lindenbach et al., 2005; Pal et al., 2006; Shimizu et al., 1992; Sung et al., 2003). Furthermore, viral replication in T cells may directly affect T cell functions. We have previously established a B cell line (SB cells) that produces HCV particles, which can further infect B lymphocytes in vitro (Sung et al., 2003). The SB strain of HCV belongs to genotype 2b. The buoyant density of the HCV particles from SB culture supernatant in sucrose was 1.13 to 1.15 g/ml (Sung et al., 2003), in agreement with the reported density for HCV particles from hepatocytes (Kanto et al., 1994). The HCV quasispecies pattern in SB cells is very similar to that in the spleen but different from that in the serum of the same patient, suggesting that the origin of this virus was from splenic cells (Sung et al., 2003). Thus, the SB-HCV strain appears to be relatively lymphotropic. Here we report that this HCV strain can also infect T cells, modulating T cell functions.

Results

HCV can infect T cell lines in culture

Our group has previously reported that HCV persistently produced from a particular B cell line (SB), which was established from an HCV-positive B-cell lymphoma, can infect and replicate in established B cell lines (Raji, Daudi) and primary B lymphocytes (Machida et al., 2004a, 2004b; Sung et al., 2003). We therefore studied whether this virus could also infect T cells. Two T-cell lines (Molt-4 and Jurkat) were infected with SB culture supernatant, and HCV RNA was then detected from the culture supernatant and intracellular RNA by semiquantitative strand-specific RT-PCR at various time points after infection. The results showed that both positive- and negative-strand HCV RNA could be detected in the intracellular RNA of Molt-4 and Jurkat cell lines at several time points after infection for at least 17 days after inoculation (Fig. 1B and data not shown). The amount of positive-strand RNA was more abundant than negative-strand RNA, as expected. Negative-strand RNA could be detected on days 8–17 post-infection in Molt-4 cells, but only on day 11 in Jurkat cells (Fig. 1B–D). By comparison, HCV infection of Raji cells (B cells) resulted in an HCV RNA level at least 4 times higher (Fig. 1A, C, D). The finding that the viral RNA titer was highest at certain time points post-infection (day 8–11 in Molt-4), instead of being at the beginning of incubation, further suggests that these RNAs were not contamination from the input RNA, but reflected authentic HCV RNA replication. However, the replication level of HCV RNA in T cell lines was very low. Quantitation of intracellular HCV RNA titer was not attempted because our preliminary data showed that false positive and negative amplification of HCV RNA frequently occurred due to the very little amount of HCV-RNA as compared with the large excess of total cellular RNA, resulting in under-estimation of viral RNA copies. Underestimation of RNA copies and wide standard deviation were observed in intracellular HCV RNA quantification by conventional real time-PCR, particularly with very low amount of HCV-RNA (10^2 to 10^4 copies/ug) (data not shown).

Next, we examined HCV RNA in the culture supernatant. Real-time PCR amplification was carried out since HCV-RNA quantification in culture supernatant could be performed more reliably than intra-cellular HCV-RNA quantification because of lower background. HCV RNA was detected in the Molt-4 culture supernatant transiently; the peak titer was over 5×10^3 copies/ml (Fig. 1E). By comparison, HCV-infected Raji cells produced HCV for a longer period of time. NS5A and NS3 proteins could be detected by confocal laser microscopy in infected Molt-4 cells at several time points (Day 5, Day 11, Day 17 post-infection) (Fig. 1F and G and data not shown). The percentage of the HCV antigen-positive cells at various time points (NS5A: Day 5, 16.3%, Day 11, 29.8%, Day 17, 8.8%) (NS3: Day 5, 10.0%, Day 11, 18.3%, Day 17, 6.8%) followed the similar kinetics to those of RNA

Fig. 1. Detection of HCV replication in the cell lines. (A, B) Representative gels showing the semiquantitaive detection of positive- and negative-strand HCV RNA in Raji and Molt-4 cells. For internal control, semi-quantification of β-actin mRNA was performed by using the same RNA preparations. For negative control, extracted HCV RNA was run in every RT-PCR test without an upstream HCV primer. Cell lines infected with UV-irradiated SB-HCV were used for uninfected cell control. The data of two independent studies had similar results. (C, D) Comparison of strand-specific HCV RNA titer in the cells among the three different cell lines. Titer is expressed as the highest dilution giving a visible band of the correct size. (E) Time course of the positive-strand HCV RNA titer in the culture supernatant. The cells were infected with HCV and incubated for 5 days. Then cell number was reduced to 1×10^5 cells/ml every 3 days. The culture supernatant collected during the 3-day period was used for real time PCR. RNA titer is expressed as the copies/ml of culture supernatant. (F) Detection of the NS5A and NS3 proteins in the HCV-infected Molt-4. The cells were stained with polyclonal anti-NS5A and NS3 antibodies. For negative control, cells infected with UV-irradiated HCV and cells reacted with the detection antibody only were used. (G) Percentage of NS5A- and NS3-expressing cells. Percentage of infectivity was determined by counting numbers of the positive stained cells in 400 cells. For negative control, cells treated with UV-irradiated HCV and detection antibody alone were used.