# Initial burst of viremia related to CD8 effector memory T cells after living donor liver transplantation in hepatitis C virus-infected recipients

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**The post-transplant immune responses, viremia, and allograft histology after living donor liver transplantation were studied in 39 hepatitis C virus (HCV)-infected recipients. The recipients were classified into the following groups according to a hierarchical clustering of their preoperative CD8CD45 T-cell isoforms: group I, naive-dominant; group II, effector memory-dominant; and group III, effector-dominant. Plasma HCV-RNA rapidly increased and then peaked as an initial burst around postoperative day (POD) 25 in group I, on POD 40 in group II, and on POD 55 in group III. The initial burst of viremia was suppressed by the high expression of $CD8^{+}CD28^{-}CD27^{-}$ subsets. The progression of fibrosis ≥F2 was significantly more frequent for those patients with the highest viremia levels. Moreover, the initial T-cell immune response became less important throughout time, and new immune responses emerged after 2 months that modified the host–virus interaction. It is suggested that the interferon (IFN)-alpha/ribavirin therapy starting 2 months may be an effective option and now is undertaken. (Translational Research 2010;156:68–79)**

**Abbreviations:** ANOVA = analysis of variance; CCR7 = chemokine receptor 7; CM = central memory T cell; CMV = cytomegalovirus; CTL = cytotoxic T lymphocyte; E (effector) = effector T cells; EBV = Epstein-Barr virus; EM = effector memory T cells; HBV = hepatitis B virus; HCV = hepatitis C virus; HLA = human leukocyte antigen; HSI = highest susceptibility to infection; IFN-$\gamma$ = inferferon-gamma; FN-$\gamma$ = interferon gamma; IL = interleukin; LC = liver cirrhosis; LDLT = living donor liver transplantation; MHC = major histocompatibility complex; PCR = polymerase chain reaction; POD = postoperative day; Tac = tacrolimus; TNF-$\alpha$ = tumor necrosis factor-alpha

When compared with hepatitis B virus (HBV) and nonviral-infected recipients, hepatitis C virus (HCV) recurs in virtually all transplant recipients after an otherwise technically successful liver transplantation. Liver transplantation for HCV-related liver failure is followed invariably by acute infection of the allograft associated with vigorous contact between $CD8^{+}$ T cells and HCV-infected hepatocytes.[1] A sharp decrease in the HCV viral load occurs immediately after liver transplantation most likely from a lack of virion

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## AT A GLANCE COMMENTARY

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### Background

Thirty-nine hepatitis-C-virus-infected recipients were classified according to their preoperative CD8CD45 T-cell isoforms as follows: group I was naive-dominant, group II was effector memory (EM)-dominant, and Group III was effector-dominant. Plasma HCV-RNA peaked around post-operative day (POD) 25 in group I, on POD 40 in group II, and on POD 55 in group III. The initial burst of viremia was preceded by an increase in the $CD8^+$ EM T-cell pool and was suppressed by the high expression of $CD8^+CD28^-CD27^-$ subsets.

### Translational Significance

New immune responses emerged after 2 months. The interferon-alpha/ribavirin therapy starting 2 months may be an effective option.

production and hepatic viral clearance. HCV infection of the allograft, however, begins with extreme levels of viral replication within a few hours after liver transplantation.[2,3] Indeed, HCV infection recurs almost universally within 4 weeks of liver transplantation with a 10- to 20-fold increase in preoperative viral levels. Furthermore, persistent post-transplant HCV infection can cause severe graft damage.[4-6] From this sequence of events, because the host immune response plays a critical role in controlling HCV replication and liver damage, we became interested in how early immune responses may be predictive of the subsequent initial burst of HCV-viremia and fibrosis progression.

A strong, virus-specific cytotoxic T lymphocyte (CTL) response toward human immunodeficiency virus-1, Epstein-Barr virus (EBV), cytomegalovirus (CMV), and HCV is well documented according to viral specificity in cases of persistent viral infection in different individuals.[7,8] In regard to the phenotypic characteristics of HCV infection and the distinct stages of differentiation, the relationship between HCV-specific memory $CD8^+$ phenotype and effector capacity has been proposed in 2 hypotheses. First, the HCV-specific memory $CD8^+$ T-cell pool largely comprises preterminally differentiated $CD45RA^-$ chemokine receptor 7-negative ($CCR7^-$) T cells that have been designated as effector memory (EM) T cells during chronic infection. Second, a stepwise loss of CCR7, CD28, and CD27 associated with the progressive up-regulation of cytotoxic activity has been found to characterize $CD8^+$ T-cell differentiation.[8] Such studies of $CD8^+$ T-cell responses against infection have been facilitated by major histocompatibility complex (MHC) class I tetramer technology[9] and single cell assays for cytokine production.[10,11] However, because this assay is restricted to MHC-type and antigenic peptides, the pool of potential subjects available for study is limited. Accordingly, it is of great importance to determine how phenotypic and functional analyses of $CD4^+$ and $CD8^+$ T-cell subsets such as naive T cells, central memory (CM) T cells, EM T cells, and effector T cells can reflect cytotoxic reactivities against a relatively small number of viral epitope peptides presented in the context of host MHC class I molecules.[12-15] Other invaluable information would include how both the distribution of circulating $CD8^+$ T-cell subsets and the initial burst of plasma HCV-RNA could be influenced by the vigorous replication of HCV after living donor liver transplantation (LDLT) and which EM T cells or effector T cells are predominantly generated as CTLs during HCV reactivation. Furthermore, the appropriate generation of effective CTL responses for viral clearance often requires help from $CD4^+$ T-helper cells.[16-21]

The present study examined how viremia during the first 3 months after LDLT (before the start of antiviral therapy) is modulated by the $CD8^+$ T-cell differentiation phenotype before LDLT. We performed longitudinal quantitative and qualitative analyses of global nonspecific $CD4^+$ and $CD8^+$ T-cell responses by analyzing the expressions of the CCR7, CD45RO, and CD28/CD27 subsets, in relation to HCV viremia and histological fibrosis after LDLT in numerous heterogeneous recipients.

## PATIENTS AND METHODS

**Patients and grafts.** The subjects consisted of 102 patients (39 HCV-infected, 28 HBV-infected, and 35 nonviral-infected recipients) who had undergone standard LDLT[22] between 2002 and 2008 at Kyoto University Hospital. Written informed consent was obtained from all subjects prior to the start of the study, which was approved by the Ethics Committee of Kyoto University Hospital and was conducted in accordance with the Declaration of Helsinki (1975), as revised in 1996.

**Immunosuppression.** Methylprednisolone (initial steroid bolus; 10 mg/kg) was administered just before the start of graft reperfusion, followed by 1 mg/kg of intravenous methylprednisolone for 3 days and 0.5 mg/kg of intravenous methylprednisolone for another 3 days. Oral prednisolone (0.3 mg/kg) then was continued for 3 months.[23]

Two types of immunosuppression protocols have been performed routinely from postoperative day (POD) 2, a regular protocol using tacrolimus (Tac) and