Accelerated liver disease in aggressive hepatitis C recurrence post-liver transplantation may be due to enhanced apoptosis mediated by both virus and immunosuppressants

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Introduction
Hepatitis C (HCV)-related liver failure is now the commonest indication for liver transplantation (OLT) in Australia.

These patients are noted to have a poorer survival compared to those transplanted for other indications.

This is because HCV recurrence in the allograft commonly follows an aggressive course, with at least 20% of patients developing cirrhosis within five years of transplantation.

Induction of hepatocyte apoptosis may be one mechanism by which HCV drives liver injury.

In post-liver transplant HCV recurrence, the combination of immunosuppressants and viral replication is postulated to increase hepatocyte apoptosis and accelerate liver fibrosis.

Aims
We investigated:
1) Hepatocyte apoptosis in liver biopsies of HCV-infected patients pre- and post-liver transplant.
2) The effects of HCV and immunosuppressants on cell death in primary human hepatocytes (PHH), primary murine hepatocytes (PMoH), and human hepatoma cells (Huh7).

Materials and Methods
Human Liver Immunohistochemistry
Hepatocyte apoptosis was assessed via immunohistochemistry in liver tissue of pre- and post-transplant liver biopsies of HCV-infected and HCV-negative patients for markers of apoptosis:
- M30 CytoDEATH (M30)
- Cleaved PARP (cPARP)

Cell Experiments
Huh7 cells, PHH (from Lonza), and PMoH (from C57BL/6 mice) were infected with recombinant adenoviruses encoding
- GFP (used as control viral infection)
- HCV-CoreE1E2 (structural proteins)
- HCV-NS3-5B (non-structural proteins)

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Results
Increased Apoptosis in HCV-infected Liver

Effect of Immunosuppressants on Cell Viability

Infection with rAdHCV infection alone decreased cell viability by 1.6-fold.

Effect of Immunosuppressants on Apoptosis

Effect of rAdHCV Infection on Apoptosis

PHH harvested at 48 hours post-infection with rAdHCV.

Effect of Immunosuppressants Alone on Apoptosis

- In PHH, CyA at 1 µg/mL had no effect on cleaved PARP or cleaved caspase 3 compared to mock.
- In contrast, MMF at 5 µg/mL increased cleaved caspase 3 by 1.3-fold.

Effect of Immunosuppressants on rAdHCV-infected cells

In PMoH harvested at 48 hours post-treatment
- Effect of CyA
  - Cleaved Casp3 by 2.6-fold
  - Cleaved PARP by 4.5-fold
- Effect of MMF
  - Cleaved Casp3 by 1.5-fold
  - Cleaved PARP by 1.9-fold

Inhibition of Cell Death

Effect of Q-VD and Nec-1 on rAdHCV infection in Huh7 cells:
- Infection with rAdHCV
  - cell viability by 1.7-fold
- In the presence of Q-VD:
  - rAdHCV cell viability by only 1.2-fold (P = 0.03)
- In the presence of Nec-1:
  - rAdHCV cell viability by only 1.2-fold (P = 0.15)

In Huh7 infection sensitized with 10 ng/mL of TNF-a
- rAdHCV infection + TNF-a
  - cell viability by 2.7-fold
- This was improved significantly by the addition of both Q-VD and Nec-1

Effect of Q-VD and Nec-1 on immunosuppressant-treated rAdHCV infection
- Addition of Q-VD
  - greatly reduced clPARP by 50- to 120-fold (P < 0.006).
- Addition of Nec-1
  - had no effect on clPARP (P = 0.720).

Conclusions
- Hepatocyte apoptosis was significantly increased in HCV-infected patients pre- and post-OLT compared to HCV-negative patients.
- HCV infection reduced cell viability and increased apoptosis.
- Immunosuppressant agents CyA and MMF further promoted cell death, and may explain the acceleration of progression of liver disease in post-liver transplant HCV recurrence.
- Inhibition of apoptosis by Q-VD-Oaph partially restored cell viability and reduced cell death in rAdHCV-infected hepatocytes.
- Partial reversal of cell death by Necrostatin-1 suggests a possible alternate pathway of cell death in HCV infection (ie. necroptosis).

These results provide an insight into the mechanisms responsible for accelerated liver fibrosis seen in post-liver transplantation and possible novel therapeutic targets in this setting.

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