HIV/HCV Co-Infection Accelerated Liver Disease is Associated With Induction of M2-Like Macrophages

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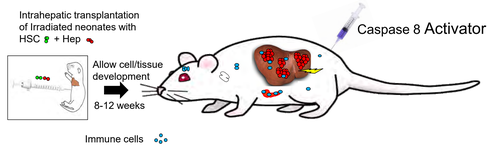
ABSTRACT

Chronic hepatitis B/C virus-induced liver diseases including fibrosis and cancer are exacerbated by human immunodeficiency virus (HIV) co-infection. Furthermore, HCV and HIV co-infection is a major cause of mortality in HIV-infected patients in western countries. The underlying pathogenic mechanisms by which chronic hepatitis viruses and HIV-1 co-infection induce and exacerbate liver diseases respectively remain unclear due to a lack of appropriate small animal models. Several studies show macrophages and other innate immune cells play a critical role in modulating host response and pathology; with M1 macrophages promoting pathogen clearance and immunity via secretion of Th1 associated cytokines, while M2-like macrophages impair Th1 immune response and promote tissue fibrosis and neoplasia via secretion of immunosuppressive cytokines. We have recently developed humanized mouse models, which carry both human immune system and human liver cells. The humanized mouse model is permissive for chronic hepatitis B/C virus and HIV infections. Importantly, the humanized mouse model is the only *in vivo* model that support anti-viral human immune responses, and develops human immune cells mediated chronic liver inflammation, fibrosis and neoplasia in chronic hepatitis virus infections. HIV infection of humanized mice results in AIDS associated pathogenesis including CD4+ T cells depletion and hyper-immune activation. Additionally, preliminary results show HIV/HCV co-infection of humanized mice results in exacerbated liver pathogenesis. Analysis of HCV-induced liver inflammation in both humanized mice and patient liver samples showed high levels of macrophages of predominately M2-like phenotypes localized to cirrhotic and neoplastic regions. Interestingly, chronic HIV infection is also associated with M2-like macrophage polarization in both humanized mice and patients. Importantly, HIV/HCV co-infection-induced accelerated liver disease was associated with increased M2-like macrophage activation, thus suggesting a critical role for macrophage polarization in HIV/HCV-induced liver pathogenesis.

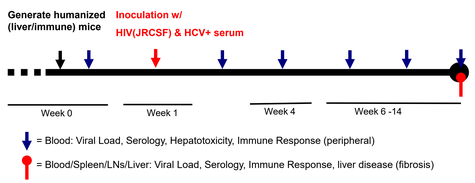
METHODS

Generation of the AFC8 Humanized Mouse Model for HCV/HIV Co-Infection

AFC8/DKO Transgenic mice were tranplanted w/ progenitor liver and hematopeoietic stem cells. Induction of apoptosis in mouse liver cells and liver regeneration by human Hep cells was induce by treatment with the AFC8 transgene activating drug. Human liver and immune reconstitution was examined and highly humanized mice was used for co-infection studies.



AFC8 humanized mice were chronically infected with HIV (JRCSF) and HCV. Uninfected and monoinfected (HCV or HIV) were used as controls to examine the effect of co-infection of HCV and HIV induced immunopathogenesis and associated liver disease.



RESULTS

Chronic HCV-induced liver inflammation and fibrosis is associated w/ human M2-like macrophages in the AFC8/DKO humanized mouse model.

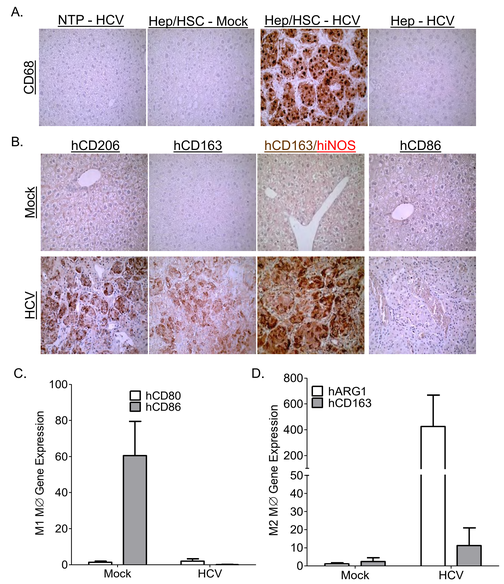


Figure 1: Elevated levels human M2-like macrophages in chronic HCV infected humanized mice. NTP-HCV (Non-transplanted inoculated with HCV), Hep/HSC TP - Mock or HCV (human hepatocytes and immune cells humanized mice inoculated with Mock or HCV+ serum), Hep TP-HCV (adult hepatocytes transplanted mice inoculated with HCV) were stain for human pan-macrophage marker - CD68 (A) and human M2-like and M1 markers (B). Additionally, Liver RNA was isolated from Hep/HSC TP - Mock or HCV and qPCR analysis was performed to examine human macrophage (C) M1 and (D) M2-like macrophages.

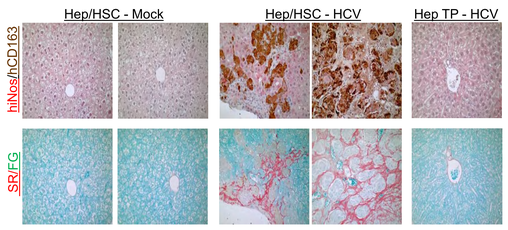


Figure 2: Human M2-like (iNOS/Red negative and CD163/Brown positive) macrophages localizes to fibrotic (Sirius Red - SR positive) regions in chronic HCV infected humanized mice. (A) Hep/HSC TP - Mock or HCV (human hepatocytes and immune cells humanized mice inoculated with Mock or HCV+ serum); Hep TP (adult hepatocytes transplanted mice inoculated with HCV) were stained for M1 and M2 macrophages and collagen.

Chronic HIV-induced immunopathogenesis is associated w/ human M2-like macrophages in the AFC8/DKO humanized mouse model.

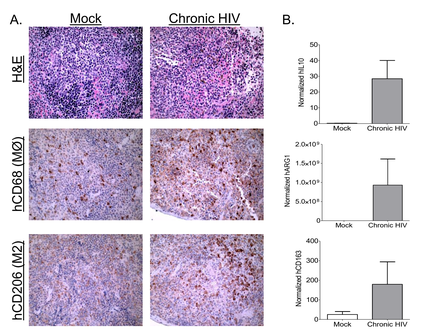


Figure 3: Chronic HIV – induced immunopathogenesis is associated with M2-like macrophage activation. Macrophage activation profile was examined in lymphoid tissue (Spleen) from chronic HIV (JR-CSF – 20 weeks post infection) infected humanized mice (n=20) and Mock control (n=4) using (A) histochemical and (B) qPCR analysis.

Chronic HCV and HIV co-infection exacerbates liver disease in the AFC8 hu-mouse model.

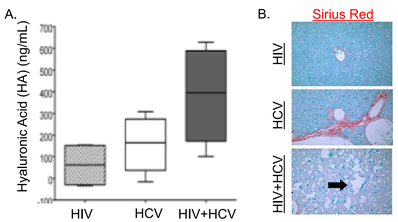


Figure 4: Chronic HIV co-infection exacerbates HCV – induced liver disease in the humanized mouse model. Liver disease was examined in AFC8 - humanized mice (n=2 per group) mono- or co-infected with HCV (genotype 1) and HIV (JR-CSF) using (A) hyaluronic acid (HA) assay and (B) histochemical (Sirius Red stain) analysis.

Chronic HCV/HIV-induced liver inflammation and fibrosis is associated with M2-like macrophage activation in the human patients.

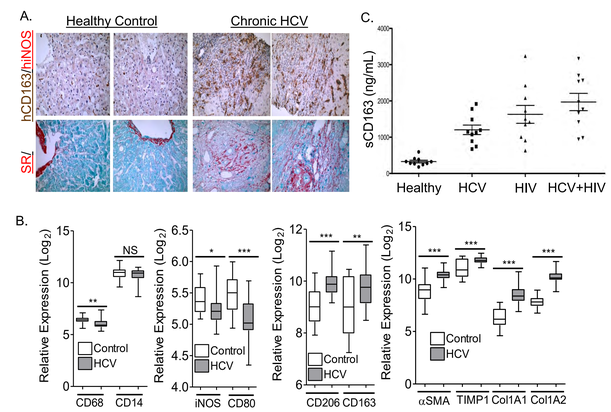


Figure 5: Chronic HCV induced – liver disease is associated with M2-like macrophage polarization in humans. (A) M2-like (iNOS negative and CD163 positive) macrophages localizes to inflammatory and fibrotic regions in chronic HCV patients. (B). M2-like macrophage gene expression profile correlates with fibrogenesis in chronic HCV infected livers. (C) Elevated levels of human M2-like macrophage activation (sCD163) in chronic HCV and chronic HIV monoinfection; chronic co-infection elevates sCD163 levels.

HCV –induce M2-like macrophages promotes hepatic stellate cell activation.

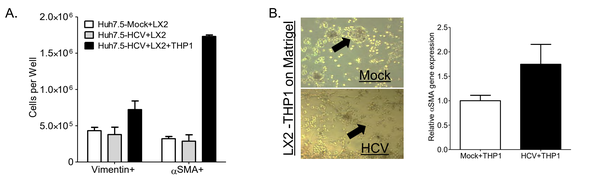


Figure 6: HCV-induced M2-like macrophage promote hepatic stellate cell activation in quiescent human hepatic stellate cells. (A) Hepatic stellate cell line (LX2 cells) cultured on matrigel coated plates were co-culture with human monocytic cell line (THP1 cells) and uninfected or HCV (JFH1 virus) infected human hepatocyte cell (Huh7.5) and LX2 activation was examined by flow cytometry analysis after 3 days. (B) LX2 cells cultured on matrigel coated plates were co-culture with THP1 cells and inoculated with Mock (H77SAAG) or HCV (JFH1 virus) and hepatic stellate cells activation was examined morphologically or using gene expression analysis after 6 days.

CONCLUSIONS

- The AFC8/DKO humanized (Immune system/Liver) mouse model supports both HIV/HCV co-infection, tissue immunopathogenesis and accelerated liver disease.
- Chronic HCV and HIV associated tissue immunopathogenesis is associated with M2-like macrophage activation in the AFC8/DKO humanized (Immune system/Liver) mouse model. Additionally, chronic HCV associated liver immunopathogenesis and fibrogenesis is also associated with M2-like macrophage activation in humans and in cell culture systems and HCV/HIV co-infection – induced exacerbated liver disease is associated with M2-like macrophage activation.

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