Systemic CD8⁺ T-cell function is associated with liver disease severity in chronic HCV infection and remains unresolved after DAA treatment

PROGRESSIVE LIVER FIBROSIS IN HCV INFECTION

The liver filters 1/3 of the body’s blood every minute!

Effect of inflammatory, fibrotic environment on circulating immune cells?
IMMUNE IMPAIRMENT IN CHRONIC HCV INFECTION

▶ Innate immunity dysfunction
- Monocytes, macrophages, dendritic cells
- Natural killer cells, neutrophils
- Myeloid-derived suppressor cells
- Kupffer cells, hepatic stellate cells

▶ Adaptive immunity dysfunction
- B-cells
- CD8⁺ T-cells
- CD4⁺ T-cells
- Tregs

IMPAIRED HCV-SPECIFIC CD8⁺ T-CELLS IN CHRONIC HCV INFECTION

HCV-specific CD8⁺ T-cells (0.05-2% of CD8⁺ T-cells)
GENERALIZED CD8+ T-CELL DYSFUNCTION IN HCV INFECTION

Bulk CD8⁺ T-Cell Dysfunction

↓ Perforin, ↓ Fas
↓ Bcl-2, ↓pSTAT1
↑PD-1


Studies conducted in HCV+ with minimal fibrosis, or fibrosis score not indicated.

BULK CD8⁺ T-CELL IMPAIRMENT IN HCV INFECTION

IL-7-induced pSTAT5 and Bcl-2 is reduced in HCV mono- and HIV-HCV co-infection

Burke Schinkel S. et al., PlosOne 2016
REDUCED BCL-2 IN HCV INFECTION WITH ADVANCED FIBROSIS

Burke Schinkel S. et al., PlosOne 2016

HCV CORE REDUCES CD8⁺ T-CELL PROLIFERATION

Khan S. et al., Immunol. 2017
HCV core reduces CD8⁺ T-cell perforin production and degranulation

**HYPOTHESIS**

Generalized CD8⁺ T-cell dysfunction in HCV infection is associated with advanced liver fibrosis and is not resolved following DAA treatment.

**STUDY OBJECTIVES**

- Evaluate bulk CD8⁺ T-cell function in chronic HCV infection in the context of liver fibrosis stage.

- Determine the effect of DAA therapy on generalized CD8⁺ T-cell function in the context of liver fibrosis stage.
CHRONIC HCV+ PATIENT CHARACTERISTICS

- HCV RNA+ > 6 months, HIV-, HBV-
- Untreated for HCV infection
- Fibrosis scoring (transient elastography)

LIVER FIBROSIS STAGE

AND

CD8+ T-CELL PHENOTYPE

Bulk
- Naïve
- Effector (E)
- Early effector memory (e-EM)
- Late-EM (I-EM)
- Central memory (CM)
IFN-$\gamma$ EXPRESSION AND ADVANCED LIVER FIBROSIS

In HVC+ advanced fibrosis:
Enhanced IFN-$\gamma$ production by naïve and early effector memory cells

HIGH PERFORIN BASELINE IN ADVANCED FIBROSIS
INCREASED PERFORIN PRODUCTION IN ADVANCED LIVER FIBROSIS

In HCV+ advanced fibrosis:
- Increased perforin production in nearly all cell types
- Greatest difference in naïve, EM and CM subsets

INDUCTION OF DEGRANULATION MARKER (CD107) ALTERED IN ADVANCED LIVER FIBROSIS

In HCV+ advanced fibrosis:
- Not all cell types show evidence of fibrosis-stage dep. degranulation
- Degranulation of naïve cells
- Late-effector memory cells less inducible to degranulate
OBJECTIVE #2: EFFECT OF DAA ON CIRCULATING CD8+ T-CELLS IN THE CONTEXT OF LIVER FIBROSIS STAGE.

DAA (Paritaprevir-Ombitasvir-Dasabuvir) +/- Ribavirin

- Non-cirrhotic, HCV 1a/b DAA +/- ribavirin, n=16
- Cirrhotic, compensated HCV 1a/b DAA + ribavirin, n=8

AFTER DAA THERAPY: WEEK 0 vs. WEEK 48
IFN-γ PRODUCTION IS NOT REDUCED IN ADVANCED FIBROSIS

In HVC+ advanced fibrosis:
IFN-γ production is not reduced after DAA tx
AFTER DAA THERAPY: **WEEK 0 vs. WEEK 48**

**PERFORIN PRODUCTION IS NOT REDUCED IN ADVANCED FIBROSIS**

In HCV+ advanced fibrosis:
Perforin production is reduced in naïve and CM cells after DAA tx
Effector cells continue to produce perforin ≈ wk0

AFTER DAA THERAPY: **WEEK 0 vs. WEEK 48**

**DEGRANULATION MARKER IS NOT REDUCED IN ADVANCED FIBROSIS**

In HVC+ advanced fibrosis:
Degranulation is not reduced after DAA tx
CD8+ T-cells are dysfunctional in HCV+ infection with advanced liver fibrosis

- Cells exhibit overactive, yet inefficient functions compared to HCV+ infection with minimal liver fibrosis or uninfected controls. This dysfunction is not reversed after DAA treatment.
MECHANISMS OF CD8+ T-CELL DYSFUNCTION?

- Murine models of chronic infection (LCMV): long-lasting effects on T-cell exhaustion (*Wherry et al.,)
  - Imprinting T-cell dysfunction in advanced liver fibrosis?
- Hyperimmune activation – polyclonal, non-specific immune response
- Other dysfunctional immune cells? E.g. monocytes/macrophages? Kupffer cells? HSC?

WHY STUDY BULK, CIRCULATING CD8+ T-CELLS?

- Important clinical implications – direct/indirect links?
  - HCV reinfection (+/- immunodominant epitopes)
  - HCC: risk after DAA, loss of tumour-specific T-cells
  - Susceptibility to important co-infections e.g. M.tb.
  - HIV-HCV: More rapid progression to AIDS and liver disease progression
  - Response to T-cell mediated vaccines (e.g. HCV vaccines in development)
  - Response to antibody-mediated vaccines for bacterial infections
ACKNOWLEDGEMENTS

Crawley lab (past/present):
*Dr. Agatha Vranjkovic, PhD (RA)
*Felicia Deonarine
Faria Ahmed (CanHepC graduate student 2016-18)
Sarwat Khan (2x CanHepC summer student)
Stephanie Burke, MSc
Andrea Ibrahim; Winston Karges (CanHepC summer student);
Julie Matte, Aditya Mohan, Faith Mottahedi

Dr. Curtis Cooper, MSc, MD, FRCP – Dir. Ottawa and Reg. Hepatitis Clinic
Ottawa and Regional Hepatitis Clinic – nursing staff

Dr. Ashok Kumar, Children’s Hospital of Eastern Ontario/uOttawa

RESEARCH FUNDING

Student funding: Ontario Graduate Scholarship;
Queen Elizabeth II-GSST, Canadian Network for Hepatitis C