Fine needle aspirates in Hepatitis B

Mala Maini
Division of Infection and Immunity
UCL, UK

HBV Forum:
Intrahepatic Panel
Liver Sampling in Hepatitis B:
A vital “telescope onto the battlefield”

Why?

When?

How?
Compartmentalised intrahepatic virology and immunology

• Histological assessment, exclusion of other pathology

• Compartmentalised viral reservoirs: cccDNA & integrated DNA

• What about immune responses?

• Can blood monitoring adequately represent the hepatic immune landscape - the site of host/pathogen interaction?

Recent advances in basic science
Liver sampling: a vital window into HBV pathogenesis on the path to functional cure

Upkar S Gill¹, Laura J Pallett², Patrick T F Kennedy¹, Mala K Maini²

Gut 2018 Jan 13
Unique immune responses are sequestered in the liver.

**Tissue-resident lymphocytes: Frontline pathogen defence**

a) Blood

- HBV+CD8+ T cell
- NK cell
- gMDSC

b) Liver

- Resident NK cell
- HBV-specific CD8+ T cell
- Resident T cell
- CD8+ T cell
- gMDSC

**CXCR6 marks a novel subset of T-bet^{1.0}Eomes^{hi} natural killer cells residing in human liver**

**IL-2^{high} tissue-resident T cells in the human liver: Sentinels for hepatotropic infection**

Laura J. Pallett, 1 Jessica Davies, 1 Emily J. Colbeck, 1 Francis Robertson, 2 Navjot Hansi, 3 Nicholas J. W. Eason, 1 Alice R. Burton, 1 Kerstin A. Stegmann, 1 Anna Schurich, 1 Leo Swadling, 1 Upkar S. Gill, 2 Victoria Male, 3 Tu Vinh Luong, 2 Amir Gander, 2 Brian R. Davidson, 2 Patrick T.F. Kennedy, 1 and Mala K. Maini 1
WHEN is liver sampling needed in CHB?

- **Basic research into HBV pathogenesis**

- **New insights into fundamental aspects of tissue-specific immunology**
  - discovery novel cell types & unique adaptations

- **Optimising HBV functional cure strategies**
  - antiviral approaches: indirect intrahepatic immune reconstitution?
  - immunomodulators: liver-targeted mechanism of action?
  - early phase studies: understanding how to tailor therapy or select optimal combos

- **Identifying peripheral biomarkers reflecting intrahepatic virological/immunological changes**
  - for larger scale drug trial immune monitoring using blood samples
HOW to approach liver sampling in Hepatitis B

• Liver biopsy remains gold standard
  – Optimise research samples when clinically indicated
  – Paired biopsies to assess some new therapies?

• Need in situ immunostaining for spatial relationships
e.g. between infected hepatocytes and immune cells
  – Make use of historical paraffin-embedded / frozen tissue blocks
  – Apply new multiplexed immunostaining

• Unbiased comprehensive analyses e.g. scRNA-seq

• Hypothesis-driven focused studies tailored to the drug target
e.g. HBV-specific immunity, cccDNA
Can fine needle aspirates provide an alternative to biopsies in HBV?

Upkar Gill
Patrick Kennedy
Barts & the London

Diagnostic liver biopsies being replaced by non-invasive fibrosis tests

Can fine needle aspirates (FNA) be used for longitudinal monitoring of novel HBV therapies?

FNA pioneered in HBV by Janssen group 2005
Used for repetitive longitudinal therapy monitoring in HCV Pembroke Gut 2014

Can multiparameter flow cytometry of FNA samples probe compartmentalised liver immunity?

Gill, Pallett et al, Gut 2018
FNA reflect immune composition of biopsies

CD8 T cells

MAIT cells

NK cells

B cells

Biopsy

FNA
FNA sample liver-resident NK cells
FNA sample liver-resident CD8 T cells

Liver-resident CD69+CD103+ T cells

pre-gated on CD3+ CD8+

blood

CD103

CD69

0.39

9.7

aspirate

CD103

CD69

7.42

43.7

biopsy

CD103

CD69

16.7

72.8

% subset

% CD8+ T<sub>RM</sub> cells in biopsy

% CD8+ T<sub>RM</sub> cells in aspirate

p = 0.0005

r = 0.54

***
FNA sample functional HBV-specific T cells

ICS for IFN-γ with OLP
FNA can dissect the specificity of intrahepatic HBV-specific T cell reconstitution on antivirals

ICS for IFN-γ with OLP
FNA for simultaneous analysis of lymphocytes and hepatocytes

Live CD45- hepatocytes

Live CD45+ lymphocytes

PD-L1+ hepatocytes
Fine needle aspirates for HBV functional cure trials

**Fine needle aspirates:**

- **sufficient cells for 3 flow cytometry panels (16-30 parameters each)**
- **simultaneous analysis of leukocytes & hepatocytes**
  e.g. PD1 T cells /PD-L1 hepatocytes; HBV-specific T cells/HBsAg+hepatocytes
- **proportional representation of all intrahepatic leukocytes including MAITs, myeloid cells, DCs**
- **sample tissue-resident T and NK cells**
- **enriched for functional HBV-specific T cells**
- **can be scored for "liver-like" sampling for standardization**
- **well-tolerated for repeated sampling**

Gill et al, Gut Apr 2018
Gill, Pallett et al, Gut Nov 2018
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