Towards a better understanding of chronic co-infections by hepatitis delta and B viruses: from eco-evolutionary aspects to the improvement of the treatment and prevention of associated liver diseases

InterLabex meeting
2016, December 13th
An emerging program on HDV research in Lyon?

- Set a “virtual HDV laboratory” in Lyon with international visibility to develop within 5 years an integrated and sustained program of research on HDV.

- Gather the expertise of 6 research and clinical teams/units working in complementary areas of research (ecology, epidemiology, virology, immunology, oncology, medical research).

- 4 main axes of research covering scopes of DevWeCan and Ecofect LabEx.

- Build-up a long-term funding strategy on a “win-win” situation based on a “boot funding” from InterLabex initiative.
Epidemiology of HDV/HBV co-infection

HBV
250 millions

HDV(HBV)
15-20 millions

Partially undiagnosed

Globally undiagnosed

HDV is a satellite virus of Hepatitis B virus (HBV)

HBV

- Circular partially double-stranded DNA virus
- Genome = 3,2 kB, coding for:
  - Polymerase
  - Surface (S, M, L- HBsAg)
  - Core (HBcAg) and Precore (HBeAg)
  - HBx

- Around 250 millions chronic carriers
- No curative treatment
- Cirrhosis : 5 to 20 % in 10 years
- ≈ 50% of HCC are due to HBV worldwide (part due to HDV?)
- HCC incidence: 0.5-2% per year

HDV

- Single-stranded negative sense RNA virus
- Genome = 1,6 kB, coding for:
  - HDAg – no enzymatic activity
- Depends on:
  - Cell RNA polymerases for replication
  - HBV envelop for spreading

- ≈ 5-10 % of HBV chronically infected patients → 15-20 millions worldwide, but under diagnosed !
- High rate of fulminant hepatitis
- No efficient txt (Peg-IFNa <10 % efficacy)
- Cirrhosis : ≈ 70 % in 10 years
- HCC incidence: >> 5 %/year
- Very poor survival after 10 years (< 50%)

Schematic overview of HBV and HDV life cycles

HBV RNA transcription

cccDNA

HBV RNA replication

HBV proteins synthesis

pgRNA encapsidation

HBV replication

HDV RNA replication

HDV proteins synthesis

NTCP

Hepatocyte

HBeAg

NTCP
Existing and investigational anti-HDV drugs

- Entry inhibitor: Myrcludex
- Egress inhibitor: Lonafarnib, Rep2139

Key processes:

- HBV RNA transcription
- cccDNA
- HBV replication
- HBV proteins synthesis
- pgRNA encapsidation

- NTCP
- HBeAg

Immune stimulation: Peg-IFNa
HDV/HBV co-infections medical research challenges

- HBV/HDV increased pathogenicity (→HCC)
- IFN-α treatment failure
- New anti-HDV therapies
HDV/HBV co-infections medical research challenges

- Better understanding of HDV biology
- HBV/HDV increased pathogenicity (→ HCC)
- IFN-α treatment failure
- New anti-HDV therapies

HDV

HBV

Hepato/liver
Four axes of research proposed in a long-term program

✓ Axis#1: Eco-infectiology of HDV-related viruses in wild animal/human contact areas (Cosset/Pontier)

✓ Axis#2: Co-evolution of HDV and HBV genomes in human population in link with pathogenic geographical particularities (Deny/Combet/Scholtès)

✓ Axis#3: Interplay between HDV/HBV and liver (innate) immunity → relevance for immune-driven pathogenesis (Lucifora/Durantel)

✓ Axis#4: Genetic, epigenetic, and molecular patterns of HDV-driven carcinogenesis (Levrero/ Zoulim)
Axis#3: Interplay between HDV/HBV and innate immunity

How and which kind of responses are induced by HDV?

How the hepatocyte response influences HDV replication?

How HDV interferes with HBV replication?

more insight into
HBV/HDV increased pathogenicity

IFN-α treatment failure
IFN-driven pathogenesis

How the hepatocyte response induced by HDV influences HBV replication?

Private funding

Academic funding

GILEAD

HBV

anRS
Agence autonome de l’Inserm

Infect-ERA
Axis#3: Main relevance of research

- As in the case of HIV or HCV, a **sustained viro-induced IFN response** has incidence on responsiveness to IFN treatment and pathogenesis.
- **Peg-IFNa is not efficient** against HDV: understanding of underlying mechanism should help improving IFN based therapy and develop novel immune therapeutic options.
- **Uncontrolled viro-induced inflammation** in liver is a main driver of pathogenesis.
- **HDV/HBV co-infections** represent the worst situation with respect to development of HCC.
- 50% of patients if left untreated and/or without care die after 10 year! **Faster than any other liver infections!**
- Few research done in this area worldwide, because **HDV infections are neglected**.

Define the role of HDV-driven sustained IFN response on pathogenesis

Restore permisiveness of HDV to Peg-IFNa in co-infected patients OR develop novel immune-stimulatory strategies.
HBV-driven carcinogenesis: genetic and epigenetic patterns

- Complex mutational landscape
- Epigenetic changes
- Insertional mutagenesis
- Viral proteins (HBx, HBc)
- Co-morbidities (aflatoxin B1)
- HBV replication correlates with HCC
- Sustained HBV viral suppression
  - no inflammation
  - « regression of cirrhosis » but HCC risk is unchanged
- HBV / HDV co-infection:
  - more rapid and disease progression
  - increase HCC risk
- L-HDAg
  - high inflammation
  - NF-kB signaling
  - increased oxidative stress
  - ER stress
- S.HDAg
  - increased expression of “clusterin”
    - Hyperacetylation
    - epigenetic mechanism

HDV / HBV co-infection:
- more rapid and disease progression
- increase HCC risk

HDV replication:
- Dysregulation of pathways
- Increased oxidative stress
- Epigenetic changes
- Transformative events

Axis #4: Genetic, epigenetic, and molecular patterns of HDV-driven HCC

Levrero & Zucmann-Rossi, J. Hepatol 2016
HDAg and HBx double role in viral replication and hepatocarcinogenesis

HDAg acts as an histone to recruit a ISWI-type chromatin remodeling complex

HDAg and HBx double role in viral replication and hepatocarcinogenesis

Axis#4: Genetic, epigenetic, and molecular patterns of HDV-driven HCC
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- Genetic landscape of HDV/HBV tumors
  [Exome sequencing, MiSeq amplicon panels]
  (Collab. with Dr Jessica Zucmann-Rossi, Paris)

- HDV/HBV tumors profiling
  [RNAseq, miRseq]
  compare with infected cells, pre-neolastic lesions
  (Collab. with Dr Francesco Negro, Geneva)

- Epigenetic profiles in HDV/HBV tumors
  [HDAg ChIPseq, histone PTMs ChIPseq]
  cross analysis with HBx and HBc available and ongoing ChIPseq

- HDAg interaction with cellular RNAs (mRNA, IncRNAs)
  [HDAg RIPseq]

- Validation and functional experiments in \textit{in vitro/in vivo} HDVHBV models
  [PHH, liver-humanized mice, transgenic mice…]

Blood, biopsies, T/PT tissues from:
- Lyon / France
- Italy
- Turkey

Prognostic and treatment allocation signatures
Axis#1: Eco-infectiologicy of HDV-related viruses

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- Define transmission and ecology of HDV-like viruses in their natural bat hosts (field studies in metropolitan France, French Guiana, Tunisia and Gabon)
  - PCR assays as well as serological longitudinal studies in caves (aim: ca. 10,000 samples – faeces, serum)
  - Identification of bat species via high-throughput sequencing (>40 bat species)
  - Identification and quantification of HDV-like viruses by sequencing/qPCR
  - Phylogeny of HDV-like viruses

- Determine intrinsic barriers and pathways to viral cross-species adaptation
  - Understand the role of the HDV-like viruses’ envelope glycoproteins found in bats in the potential crossing of the interspecies barrier
  - Characterize the cell entry molecular determinants of HDV-like viruses compared to the human HDV/HBV
  - Study HDV-like viruses in bat liver chimeric mice

- Develop an integrative approach linking infectiology and eco-evolutionary biology to emphasize constraints shaping virus circulation between species
  - Develop probabilistic models
  - Complete bat species phylogeny
  - Understand bat species ecology
Axis#2: Co-evolution of HDV/HBV genomes in human pop.

- Define several **well annotated** cohorts (n=20) of HDV/HBV co-infected patients (genotypically-paired with corresponding HBV-infected patients) from various geographical origins (France/Lyon, Italy, South America, Turkey, Eastern Europe...)

- Use **NGS approaches** to sequence full length HDV/HBV genomes in cross-sectional conditions

- Analyze co-evolution of genomes using **mathematical/bioinformatic means** in link with clinically determined pathogenesis

- Identify association of HDV/HBV genotypes/subtypes correlating with pathogenesis

- **Additional markers** to be found in same cohorts in link with axis#3 and 4

- Co-infections in **liver humanized mouse model and in vitro** to study early/late impact on hepatocyte physiology in link with pathogenesis/HCC
Axis#1 and 2: Chimeric mouse platform already set, to study cross-species strains and virus co-evolution.
Model of implementation

Axis#1: Eco-infectiology of HDV-related viruses in wild animal/human contact areas

Axis#2: Co-evolution of HDV and HBV genomes in human population

Axis#3: Interplay between HDV/HBV and innate immunity IFN-driven pathogenesis

Axis#4: Genetic, epigenetic, and molecular patterns of HDV-driven carcinogenesis

Boot funding 2017-2019?