DELTA STILL AN ORPHAN NEGLECTED DISEASE DUE TO A MYSTERIOUS VIRUS

HDV 40 th Birthday

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Immunofluorescence detection of new antigen-antibody system (δ/anti-δ) associated to hepatitis B virus in liver and in serum of HBsAg carriers

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SUMMARY A new antigen-antibody system associated with the hepatitis B virus and immunologically distinct from the HB surface, core, and *e* systems is reported. The new antigen, termed δ, was detected by direct immunofluorescence only in the liver cell nuclei of patients with HBsAg positive chronic liver disease. At present, the intrahepatic expression of HBcAg and δ antigen appears to be mutually exclusive. No ultrastructural aspect corresponding to the δ antigen could be identified under the electron microscope. δ antibody was found in the serum of chronic HBsAg carriers, with a higher prevalence in patients with liver damage. The nuclear fluorescence patterns of HBcAg and δ antigen were similar; it is only possible to discriminate between the two antigens by using the respective specific antisera.

While studying liver biopsies from patients who were seropositive for the hepatitis B surface antigen (HBsAg) in direct immunofluorescence, it was noted that an antiserum against the hepatitis B core antigen (HBcAg), as well as staining specimens in which core particles could be demonstrated by the electron microscope (EM), also reacted with additional biopsies which did not contain core particles (at electron microscopy) and were negative with other reference antisera against HBcAg.

When the EM core positive and core negative specimens were tested with several HBsAg positive sera, it soon became apparent that some sera reacted with either one or the other liver substrate; this suggested that there were two distinct nuclear antigenic specificities.

The identification of this new antigen and of its antibody as an immunological system independent of other known reactions associated with the HB virus is reported in this communication. Provisionally, we propose that it should be called δ.

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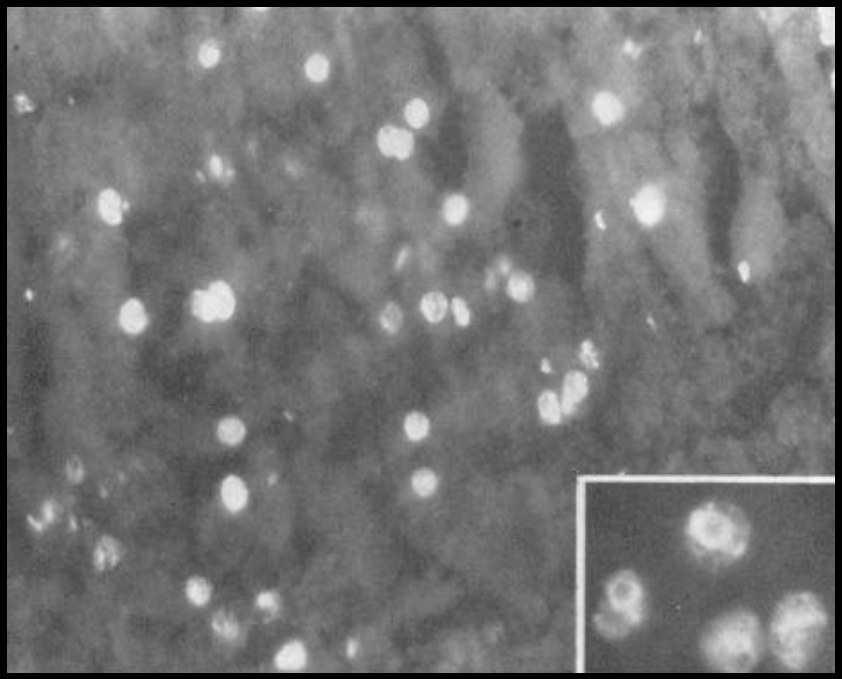
Methods

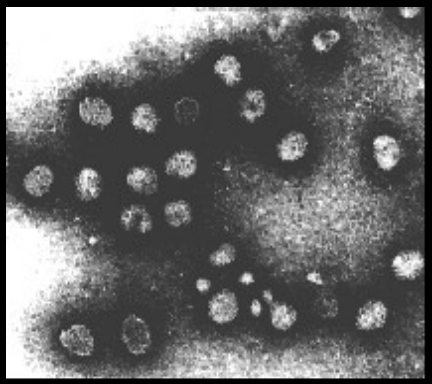
PREPARATION OF STANDARD FLUORESCENT ANTISERA AGAINST δ ANTIGEN (δ ANTISERUM), AGAINST HBcAg (HBc ANTISERUM), AGAINST HBsAg (HBs ANTISERUM), AND AGAINST *e* ANTIGENS ($e_\alpha + e_\beta$ ANTISERUM), STANDARD δ ANTIGEN (δ) AND HBcAg POSITIVE LIVER SUBSTRATES

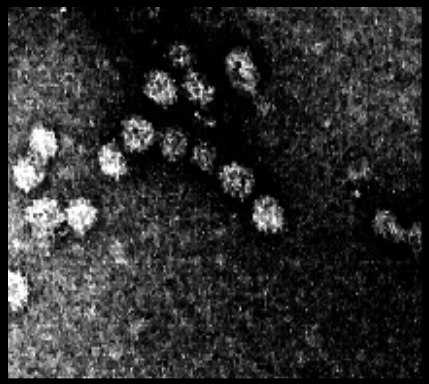
A fluorescein isothyocianate (FITC) conjugated antiserum against HBsAg was prepared from Beheringwerke rabbit precipitating serum RBBO4 (Rizzetto *et al.*, 1976b). A FITC conjugated antiserum against *e* antigens ($e_\alpha + e_\beta$) was prepared from a human serum as previously described (Trepo *et al.*, 1976).

A FITC antiserum monospecific against HBcAg and one monospecific against δ were prepared from the blood of two apparently healthy HBsAg carriers; both sera were negative when tested by the Reuma and Waaler-Rose techniques. The gamma globulin fractions, isolated after precipitation with $(NH_4)_2SO_4$, did not contain autoantibodies (in indirect Immunofluorescence (IFL)), antibodies against HBsAg, *e* antigens, or *e* antibodies.

After conjugation with FITC, the HBc antiserum







Rizzetto M et al., Gut 1977; Bonino F et al J Virol 1986; Ryu et al., J Virol

MAJOR CHARACTERISTICS

- Unique agent
- Defective virus
- Highly pathogenic
- Reemerging
- Most challenging therapy

HDV HBV

HDV INHIBITS HBV REPLICATION

Anti-transcriptional effec

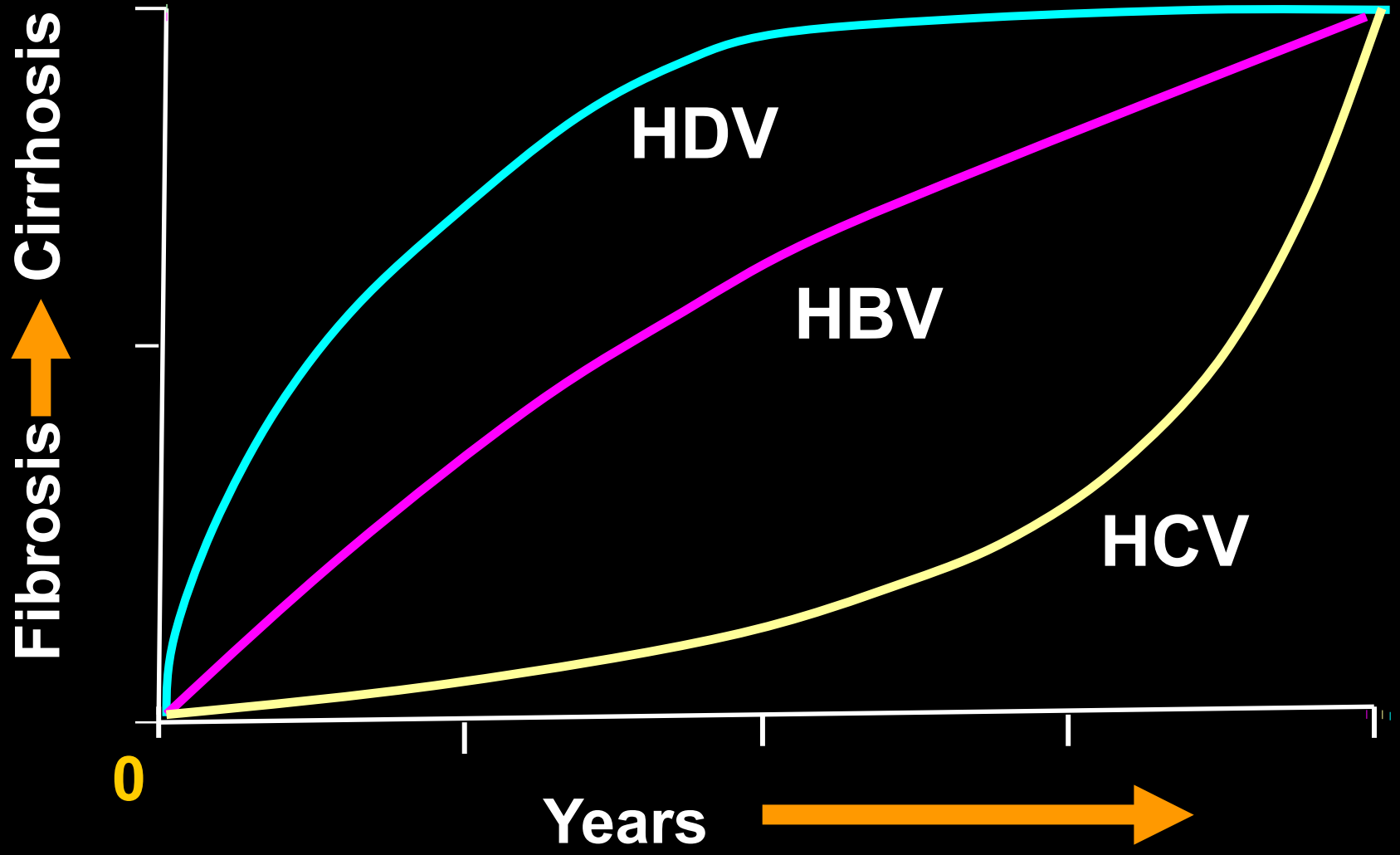
Competition envelope

Cytokines (MxA ?)

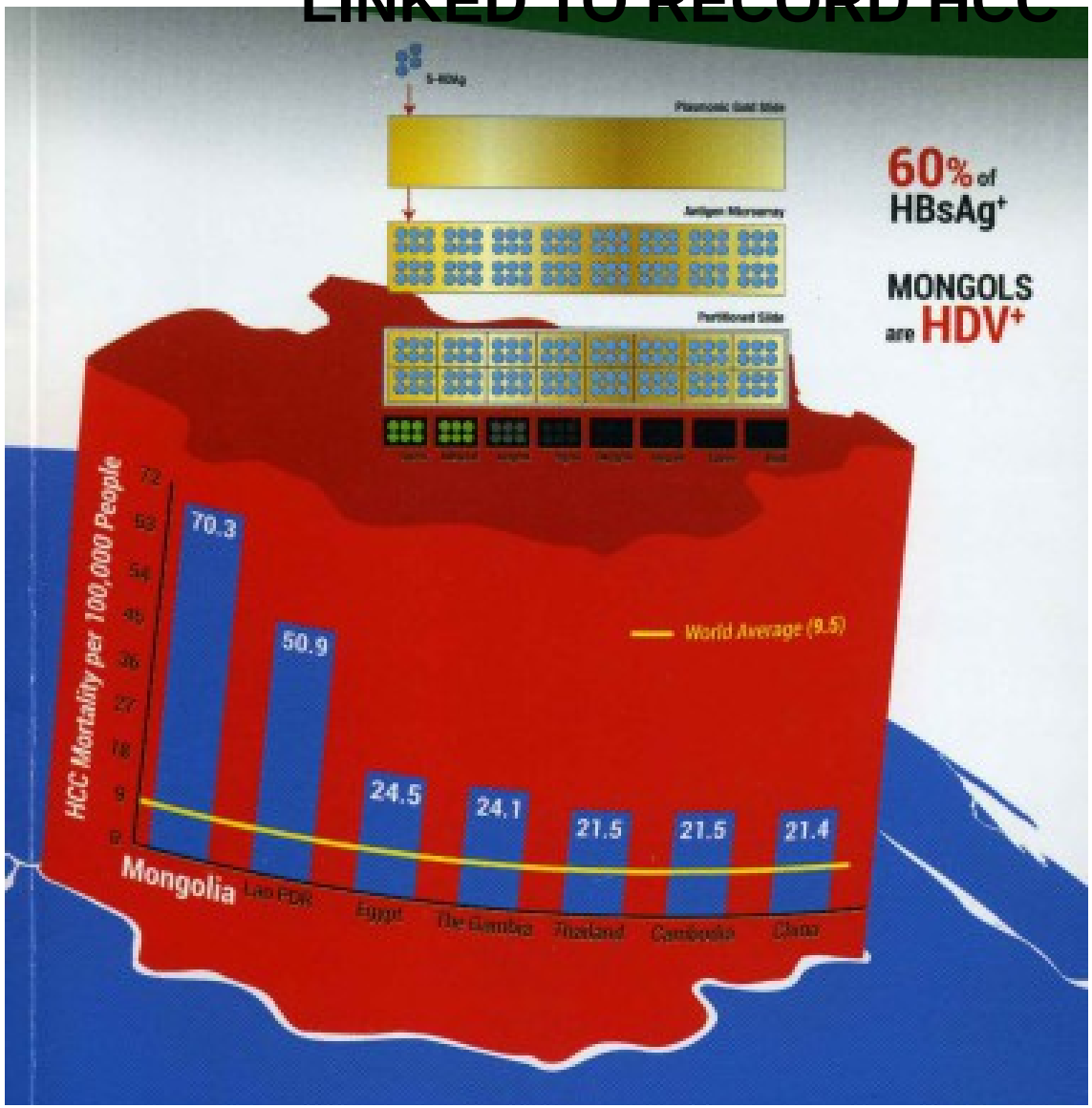
HDV
Highly Pathogenic

HDV causes the least common but most severe form of chronic viral hepatitis leading to cirrhosis in about 80% of the cases

Evolution of Hepatitis D Compared to Hepatitis B and C



HIGH HDV PREVALENCE IN HBV MONGOLIANS LINKED TO RECORD HCC



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HDV THERAPY DILEMNA

- Overlooked deadly disease
- Severity linked to HDV replication
- IFN only approved drug

BUT

Only beneficial at high dose for long time

Relapse > 50 %

Hopeless !?

Brazilian experience

interferon-alpha pegylated plus Entecavir in Treatment of patients WITH chronic hepatitis delta from Western Amazon Region, Brazil. Week 48 Interim Analysis.

Borzacov, L.M1, Vieira, D.S2, Botelho, L.F2, Santos, A.O2, Villalobos-Salcedo, J.M1

Cohoort of 36 ptes with HDV Gen III in Brazil.;
mean age 45.3 years; F3/F4 28%.
60% patients were positive HBV-DNA in 4-week including negative baseline patients.
36 patients completed 48-week of treatment and only one was HDV-RNA positive.

Conclusion: Combined treatment with PEG-IFN-a 2a plus Entecavir, resulted in high early virologic response (EVR) rates in co-infected HBV/HDV patients with genotype III, but long term follow up is required to validate this therapeutic regimen

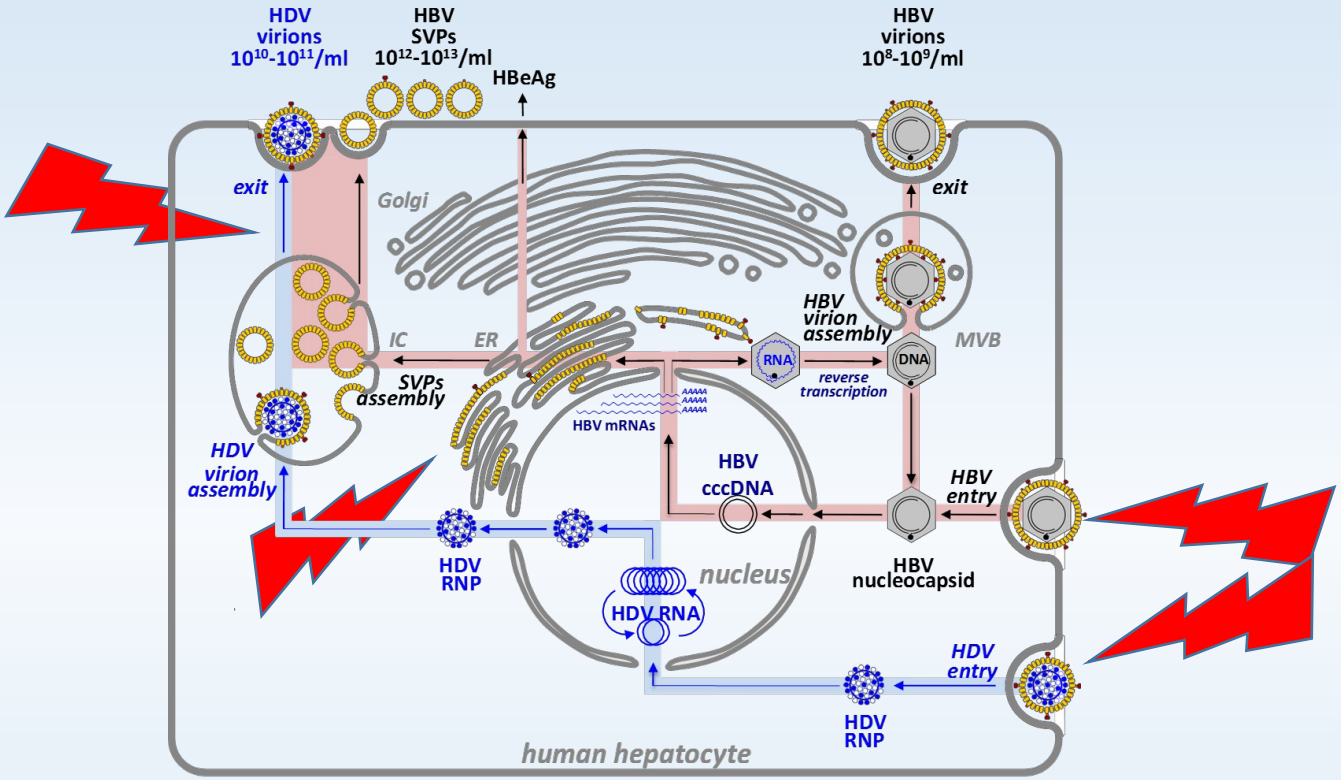
Long term FU preliminary results

- **Impressive:**
  - **Follow-up 24w after treatment (n= 25)**
    - **SVR → 72% (n= 18) HDV RNA (-)**
  - **24% (n= 6) resulted in HBsAg negative**
  - **20% (n=5) Anti-HBs seroconversion**

Different virus ?

Alike HBV
HDV will be
CURED

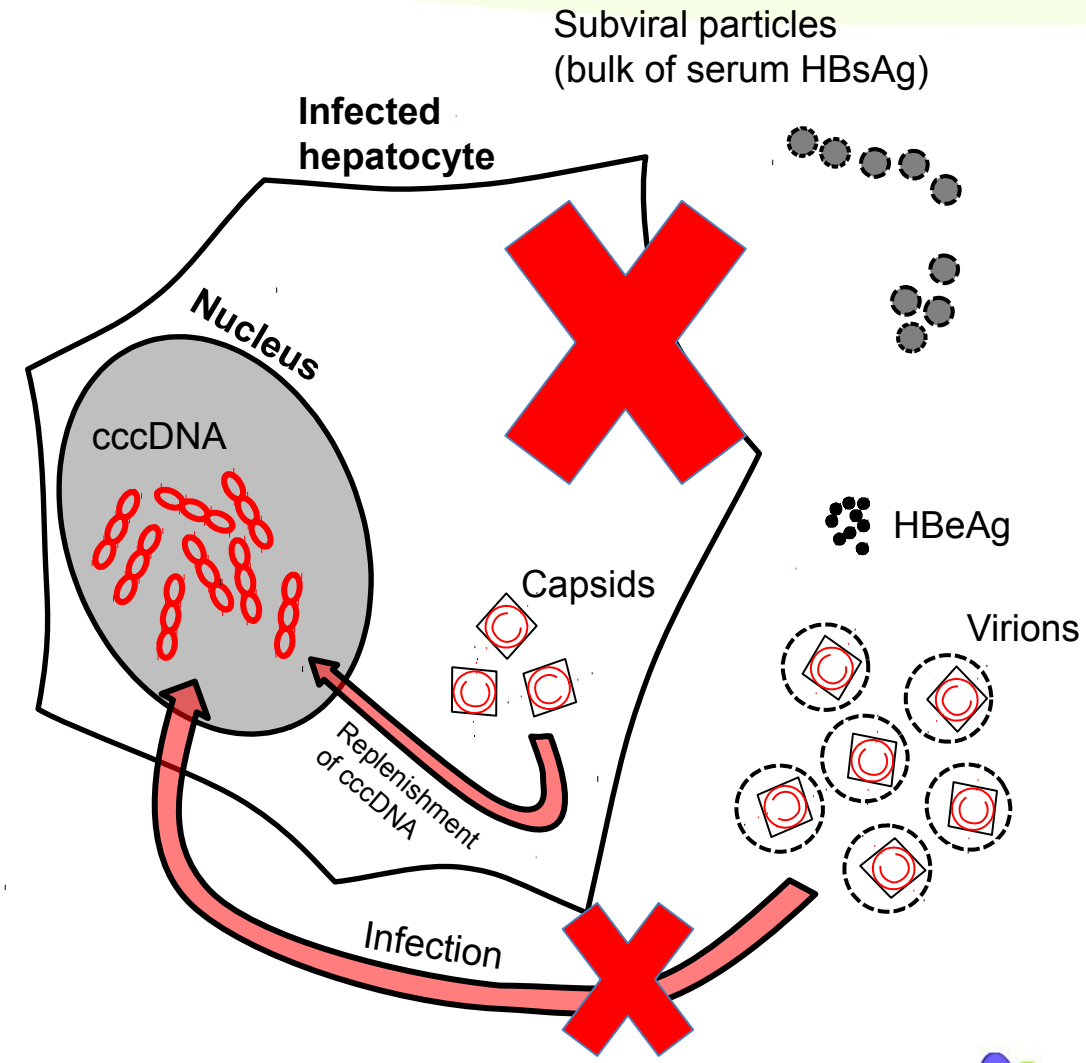
New therapies?



Camille Sureau

- Viral (HBV & HDV) entry – Myrcludex B
- Targeting of HDV RNP to ER – Lonafarnib
- HDV exit – Replicor (NAPs)

NAPs block the release of subviral particles (replicor)

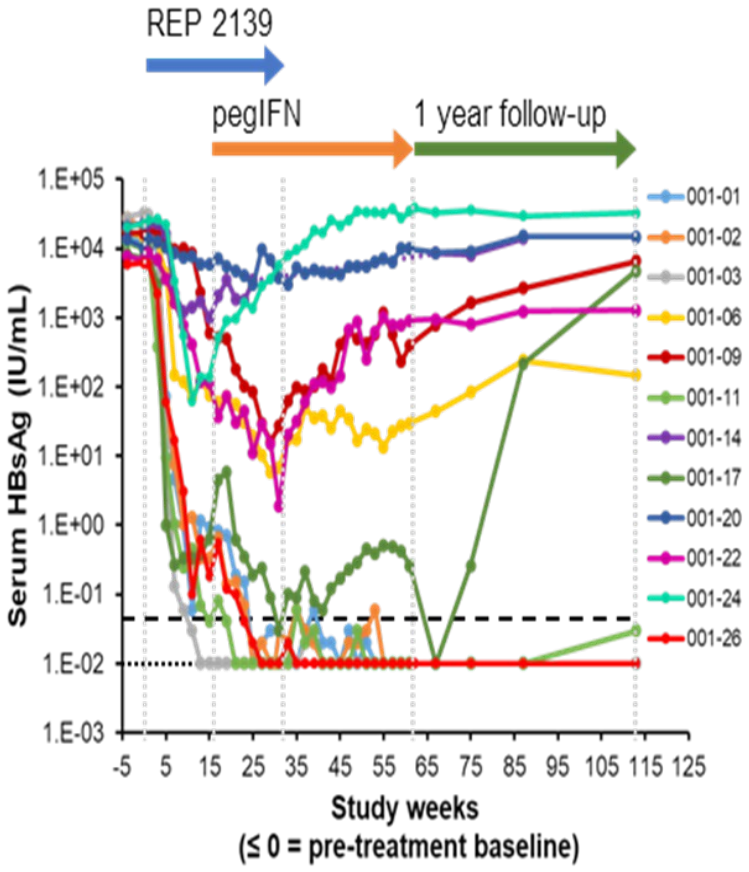


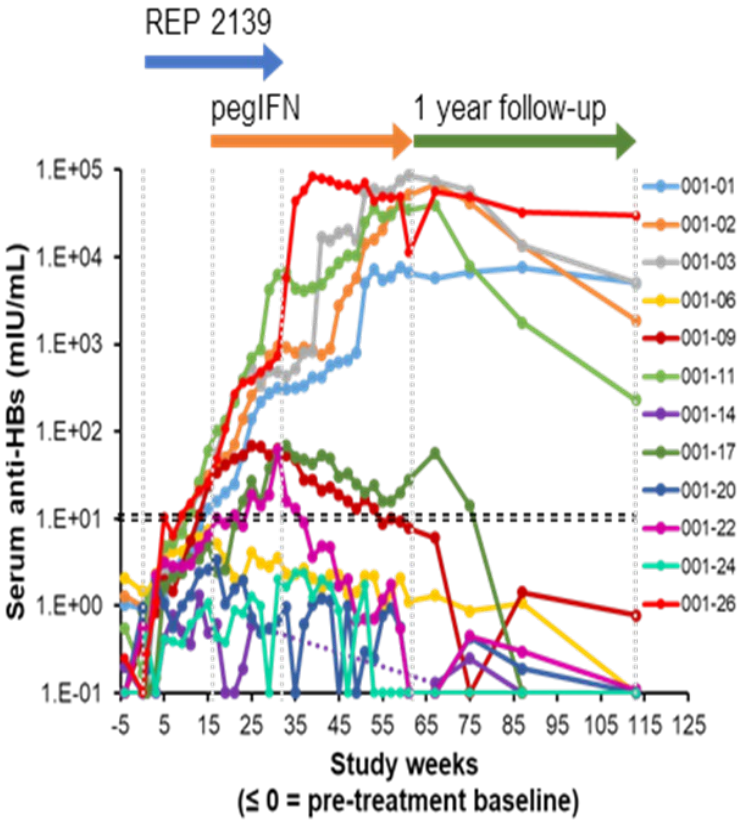
HBsAg is the key:

Sequesters anti-HBs
Suppresses innate immunity
Suppresses T-cell proliferation
Suppresses cytokine signaling
Suppresses immunotherapy

HBsAg removal will be required to achieve high SVR rates

HBsAg clearance in HBV / HDV co-infection





CURED !?

YES WE CAN !!!