The Precore Antigen evades Interferon response in Hepatitis B

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HBV Burden of Disease

240 million chronic HBV
Cirrhosis and hepatocellular carcinoma: 1 million deaths/ year
> 90% vertically transmitted HBV becomes chronic
Prevalence in Australia is rising

Locarnini et al. J of Hepatology 2015
Cowie et al. MJA 2015
Liver Disease Progression

Time 20-30 years

Normal

Cirrhosis

Cirrhosis

HCC

HBV

CHRONIC HEPATITIS B

CIRRHOSIS ESLD

HEPATOCELLULAR CARCINOMA

CHB is INCURABLE

Novel immunotherapy approaches require a deeper understanding of the host-viral interaction

PRIMARY PREVENTION

Vaccination

CURRENT TREATMENTS

Peg-IFN-α
Antivirals
TDF, ETV
HBV is an immune mediated disease

HBV has developed viral evasion strategies to establish chronicity and persistence
Interferon in HBV

• Interferon is a key player in the innate defense against viral infections
  – Hepatitis C, HIV
• HBV is highly susceptible to interferon *in vitro*
• Evades immune response *in vivo*
• Viral evasion strategy to establish chronicity

? How does HBV evade interferon
? Structural protein
The Precore antigen: HBeAg

Not required for infectivity or viral replication

?Immunomodulator ? Tolerogen

**Necessary for chronicity**

HBeAg negative HBV does not become chronic

Evades Toll-like receptor

Tolerises T cells

Response to interferon treatment is poor in HBeAg positive patients

*Visvanathan et al. Hepatology 2007*

*Milich et al. Hepatology 2003*
HBeAg in the natural history of HBV infection

HBeAg is only present in early phases of disease
Escape mutations are common
Basal core promoter and precore variants reduce/abolish HBeAg

PC Δ=G1896A
Translational stop codon
no HBeAg translated

BCP Δ = A1762T/G1764A
Relative reduction in transcription
Reduced HBeAg

BCP and PC mutations are independently associated with advanced fibrosis

Valaydon et al. EASL 2016
Hypothesis

HBeAg has a significant immunomodulatory effect in early HBV infection
Aims

To characterize and compare the viral kinetics of HBeAg-negative variants vs wild type HBV using a mouse model of HBV infection

Examine effect of HBeAg on immune mediators of HBV using a mouse model of HBV infection
Mutagenesis
Hydrodynamic injection (HDI) of HBV-DNA to induce HBV infection in mice

AAV cis vector

pAAV HBV1.2

8060bp

5'TR

HBV 1.2 overlength genome (3818bp)

3'TR

Genotype X, +/- mutation

Hydrodynamic injection

10μg DNA

Immuno competent C57/BL6 Mouse

Ebert, Pellegrini et al. PNAS 2015
BCP and PC vs WT

- Hydrodynamic injection of **immuno competent** C57BL/6

1.2 mer HBV A2 WILD TYPE VS 1.2 mer HBV A2 PC VS 1.2 mer HBV A2 BCP

- HBV DNA measured by qPCR
- Serology measured by ELISA
- Terminal bleeds for ALT/ AST levels
Rapid early viral suppression in PC and BCP mutants

* P<0.05
HBeAg was reduced in BCP mutants and absent in PC mutants.
No difference in HBsAg levels between mutants and WT
Levels of ALT and AST were significantly increased in BCP and PC mutants

ALT and AST levels (IU/ml) at week 3

- BCP (n=5)
- PC (n=6)
- WT (n=6)

p < 0.001

p < 0.002
The interferon response in HBV

- Monocytes
- HBV
- B cell
- CD4 T cell
- CD8 T cell
- Kupffer cell
- NK cell
- NK T cell
- IFN-α/β
- IFN-γ
- TNF

Dendritic cell
TNF was not a key mediator in viral suppression in mutants

Day 3
week 1
week 2
week 3
week 4
week 5
week 6
week 7
week 8
week 9
week 10

Time
DNA copies/ml
WT control (n=6) BCP control (n=6)
d0 3 7 10 14 17 21
HDI

p > 0.05
IFN gamma was not a key mediator in viral suppression in mutants
IFN alpha is a key mediator in viral suppression in mutants
IFN alpha is a key mediator in viral suppression in mutants

![Graph showing DNA copies/ml over time for WT BL6 (n=6) and WT IFNaR-KO (n=7). The graph displays a decline in DNA copies/ml over weeks, with a statistically significant difference (P > 0.05) between the two groups.]
IFN alpha is a key mediator in viral suppression in mutants

![Graph showing DNA copies/ml over time (Day 1 to week 7)](image-url)

- DNA copies/ml on the y-axis
- Time (Day 1 to week 7) on the x-axis
- BCP BL6 (n=6) line graph
IFN alpha is a key mediator in viral suppression in mutants

P < 0.05

P < 0.02
IFN alpha is a key mediator in viral suppression in mutants

![Graph showing DNA copies/ml over time for WT BL6, BCP BL6, WT IFNαR-KO, and BCP IFNαR-KO groups.](image)

- WT BL6 (n=6)
- BCP BL6 (n=6)
- WT IFNαR-KO (n=7)
- BCP IFNαR-KO (n=7)
Conclusions

• New small animal model to study the immuno pathogenesis of HBV
• Major differences in the viral kinetics of HBeAg negative mutants
• Mediated by Type 1 Interferon
• HBeAg may be an interferon resistance protein allowing immune evasion
• Significant therapeutic implications
  – Neutralisation of HBeAg may improve rate of viral clearance with interferon treatment
Future Directions

• In vivo testing of anti-Hbe neutralising antibodies to improve viral suppression and clearance
• Complementation studies
  – Mix of WT and mutant strains
• Liver transcriptome for further elucidation of the interferon pathway
• Proteomics to investigate associated proteins
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Does the preclinical mouse model recapitulate HBV replicative lifecycle in human hepatocytes?