Timeframe of HBeAg and HBsAg Levels of Developing Transgenic Mice and Correlation with Liver Hepatitis B DNA Levels

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Image from Hepatitis B by Steven A Gonzalez MD, MS
What is Hepatitis B?

• DNA virus spread through transmission of bodily fluids
• Causes a wide spectrum of liver disease
  • 5-10% of infected adults develop chronic infection
  • 20-30% of Chronic infections lead to cirrhosis or hepatocellular carcinoma
• ~300 million HBV carriers worldwide
  • 686,000 deaths annually

-Liang et al 2009
HBsAg and HBeAg

• Surface antigen:
  • Major surface protein
  • Chronic HBV infection: presence of hepatitis B surface antigen (HBsAg) for >6 months
  • key diagnostic marker

• E antigen
  • Indicated viral replication and infectivity

-Amini et al, 2017
-Nguyen et al, 2008
-Milich D and Liang T, 2003
Transgenic Mouse Model Used by the IAR

• Genome of C57BL genetically engineered to produce hepatitis B
• Liver and kidney tissue produce complete viral particle with associated DNA, HBsAg, HBeAg, HBcAg
• No associated cytopathology

-Guidotti et al, 1995
What We Know

• Weak observed correlation between HBeAg levels and expressed viral DNA
• No record of correlation between HBsAg levels and expressed viral DNA
• Data suggest that HBsAg levels decrease over time in males
  • No data regarding decrease in females
Purpose of experiment

• Develop a timeline showing HBsAg levels over time in both males and females
• Determine correlation between HBsAg levels and expressed viral DNA
• Confirm correlation between HBeAg levels and expressed viral DNA
Methods: Samples

• Serum collected from pups weekly from 4-10 weeks of age
• Mice were euthanized and livers were harvested at 10 weeks
Methods: Antigen Analysis

- HBsAg-specific ELISA with calibrator of known WHO (IU/mL) units
  - Absorbance was matched to standard curve to determine HBsAg levels from each collection date
- HBeAg-specific ELISA with calibrator of known PEI units
  - Absorbance was matched to standard curve to determine HBsAg levels from each collection date
Methods: DNA Analysis

- Serum DNA was extracted using QIAamp cador Pathogen Mini Kit
- Liver DNA was extracted by phenol-chloroform extraction
- Extracted DNA was analyzed by real-time qPCR
Results

HBV Serum Over Time

HBV Serum Week 4 vs. Week 10

No significant difference
Results

HBV Serum DNA vs Liver DNA at Necropsy

$R^2 = 0.7612$
**Results**

- **HBeAg 1:100**
  - Upper limit of confidence (3OD)
  - Normal Experimental Range

- **HBeAg Wk 4 vs Wk 10**
  - No significant difference
**Results**

**HBeAg vs Serum HBV DNA Linear Regression**

**Week 4 HBeAg vs HBV Serum DNA**

$R^2 = 0.2595$

**Week 5:** $R^2 = 0.2$

**Week 6:** $R^2 = 0.59$

**Week 7:** $R^2 = 0.72$

**Week 8:** $R^2 = 0.46$

**Week 9:** $R^2 = 0.28$

**Week 10 HBeAg vs HBV Serum DNA**

$R^2 = 0.6211$
Results

HBeAg vs Liver DNA at Necropsy

$R^2 = 0.7962$
Results

HBsAg wk 4 vs wk 10 ALL

p=0.0066 **

Individual HBsAg 1:200

Upper limit of confidence (3OD)
Results

HBsAg Wk 4 vs Wk 10 between genders

No Significant Difference Between Females Groups
p=0.058

p=0.016 **
HBeAg vs Viral DNA

- HBeAg vs. Serum HBV DNA: Weak/moderate correlation
- HBeAg vs. Liver HBV DNA: Strong correlation
- HBeAg useful as surrogate marker for liver and serum HBV DNA
Serum HBV DNA vs. Liver HBV DNA

- Strong positive correlation
- Serum HBV DNA useful as surrogate marker for liver HBV DNA
HBsAg Levels

• Significantly decrease over time

• Implications:
  • HBsAg commonly used as diagnostic marker
  • Potential erroneous results in mouse studies
  • Not a reliable indicator of liver HBV DNA levels
Conclusion

• Serum HBV DNA levels remain constant overtime, suggesting constant liver HBV DNA over time

• HBsAg levels significantly decrease over time. Why?
Hypothesis

• HBsAg-specific immunoglobulins begin to be produced by the mice
  • HBsAg is neutralized and degraded by phagocytosis

• Suggests possible autoimmune response
Further research

- Repeat experiment with larger sample size
  - Support evidence that HBsAg levels significantly decrease in male mice
  - Clarify non-significant decrease in female mice
- Perform IgG-specific ELISA alongside HBsAg-specific ELISA
  - Analyze suspected inverse relationship between IgG and HBsAg levels
Works Cited


Thank You!