Serum HBsAg has minimal impact on CD8+ T cell responses in mouse models of HBV pathogenesis – practical implication for public health –

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Immunobiology and pathogenesis of Hepatitis B Virus (HBV) infection

Hepatitis B Virus (HBV)

**Virions (42nm)**
- Envelope: L, M, S-HBsAg
- Core: HBcAg
- Partially dsDNA

**Sub viral particles (24nm)**
- Serum concentration: $10^6$ fold higher than Virions
- S-HBsAg

Outcome of infection is mainly determined by kinetics, breadth, vigour and effector functions of HBV-specific CD8$^+$ T cell responses.

Detectable HBsAg is the serological hallmark of persistent HBV infection
Serum HBsAg loss: marker of therapeutic success
Aim

Does circulating HBsAg induce tolerance?

Decoy for anti-HBsAg Antibodies

Induction of Immune Tolerance

Boni et al., Gastroenterology, 2012
Zhou et al., J immunol, 2016
Bazinet et al., Gastroenterology, 2020

HBsAg loss

Lack of a causative link
Mouse models to study immune-mediated HBV pathogenesis

Virus replication in hepatocytes at high level without cytopathology

T cells tolerance

$10^7/10^8$ serum viral particles/ml

HBV-specific TCR CD8$^+$ T effector cells  $\rightarrow$  Transient liver disease

Guidotti et al., Immunity, 1996

HBV-specific TCR CD8$^+$ T naïve cells  $\rightarrow$  Dysfunctional response

Benechet, De Simone et al., Nature, 2019

Guidotti et al., J.Virol., 1995

Methods
Models to study the role of circulating HBsAg in affecting CD8⁺ T cell response

HBV-replication competent Tg mice

HBcAg HBsAg (70-80%)

HBsAg

Spontaneous HBsAg loss

Anti-HBsAg Abs

Gilead Sciences

Endogenous CD8⁺

Transferred HBV-specific CD8⁺ T
Results

A fraction of HBV replication competent transgenic mice spontaneously clear serum HBsAg

Spontaneous HBsAg loss

- HBV Tg mice (C57BL/6) (n = 12-59)

3 Experiments; Log-rank (Mantel-Cox); two-way ANOVA
Results

HBsAg clearance does not cause overt reactivation of tolerant T cells present in HBV Tg mice

Spontaneous HBsAg loss

? (question mark)

Endogenous tolerant T cells

Graph showing serum ALT (U/L) levels over weeks of age:
- HBsAg + mice ($n = 4$)
- HBsAg - mice ($n = 4$)

3 Experiments
Results

Spontaneous HBsAg loss

Transferred HBV-specific CD8+ T cells

HBV-specific TCR CD8+ T EFFECTOR cells

Guidotti et al., Immunity, 1996

HBV-specific TCR CD8+ T NAÏVE cells

Benechet, De Simone et al., Nature, 2019

Transient liver disease

Dysfunctional response

3 Experiments
HBsAg clearance has minimal impact on HBV-specific CD8+ T cell responses

**Results**

HBV-specific TCR CD8+ T **EFFECTOR** cells

Transient liver disease

HBV Tg mice

Env28 T<sub>E</sub>

0 Analyses (days 0-5)

HBsAg + HBsAg - mice

HBV-specific TCR CD8+ T **NAÏVE** cells

Dysfunctional response

HBV Tg mice

Env28 T<sub>N</sub>

0 Analyses (day 5)

HBsAg + HBsAg - mice

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2 Experiments; Mann-Whitney U-test; two-way ANOVA
HBsAg clearance has minimal impact on HBV-specific CD8+ T cell responses

Results

HBsAg has no impact on the capacity of HBV-effector CD8+ T cell to induce liver immunopathology

HBsAg has minimal effect on HBV-CD8+ T cell expansion without affecting their differentiation in dysfunctional cells

Same results were obtained upon anti-HBsAb treatment to reduce HBsAg levels

2 Experiments; Mann-Whitney U-test; two-way ANOVA
Functional improvement of intrahepatically primed CD8+ T cells by IL-2 based immunotherapeutic strategies

Results

HBV-specific TCR CD8+ T NAÏVE cells

Dysfunctional response

Benechet, De Simone et al., Nature, 2019

IL-2 based therapy

2 Experiments; Mann-Whitney U-test; two-way ANOVA
Circulating HBsAg does not affect the functional improvement of intrahepatically primed CD8+ T cells by IL-2 based immunotherapeutic strategies

HBV-specific TCR CD8+ T NAÏVE cells
Dysfunctional response
Benechet, De Simone et al., Nature, 2019
IL-2 based therapy

HBsAg clearance do not improve the functional restoration of intrahepatically primed CD8+ T cells by IL-2-based immunotherapeutic strategies
Conclusion

HBV-specific CD8+ T cell response is not affected by the levels of circulating HBsAg

Initial hypothesis

HBsAg clearance

Tolerance

Immune active
HBV-specific CD8+ T cell response is not affected by the levels of circulating HBsAg. Although anti-HBV Ab therapy alone might reduce the infection of new hepatocytes, it is unlikely to increase virus-specific CD8+ T cell immunity.
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