Is it possible to restore innate and adaptive immunity in HBV and T cell Exhaustion?

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INTRAHEPATIC INHIBITORY MECHANISMS

Modified from U. Protzer et al. Nature Reviews in Immunology 2012
List of topics

- HBV-specific T cells in chronic infection
- NK cell response and its regulatory role on HBV-specific T-cell response
- Potential strategies to reconstitute the anti-viral T cell function
- Molecular basis of CD8 cell dysfunction in chronic HBV infection
The five phases of the natural history of chronic HBV infection

HBsAg

Immunotolerant phase

HBeAg(+)

HBV DNA

10^9-10^{12} IU/mL

Occult infection

HBV DNA

>2000 IU/mL

<2000 IU/mL

HBeAg(-)/anti-HBe(+)

Immuno-active phase

Low replication

<2000 IU/mL

>2000 IU/mL

Immunocompetent phase

HBeAg(-)/anti-HBe(+)

Inactive phase

<2000 IU/mL

>2000 IU/mL

Inactive phase

<2000 IU/mL

Moderate to severe CH

CH

Moderate to severe CH

CH

Remission

Inactive cirrhosis

Cirrhosis

Cirrhosis

Adapted from Fattovich G. Sem Liver Dis. 2003
HBV-specific T cells in chronic infection
Different levels of functional T cell efficiency in different conditions of HBV control

HBV-SPECIFIC CD8 CELLS ARE PREFERENTIALLY CONCENTRATED WITHIN THE LIVER IN PATIENTS WITH CHRONIC HBV INFECTION

(Fisicaro P. et al. Gastroenterology 2010)
INTRAHEPATIC HBV-SPECIFIC T CELLS ARE MORE DEEPLY EXHAUSTED THAN THEIR PERIPHERAL BLOOD COUNTERPARTS IN CHRONIC HBV INFECTION

(Fisicaro P. et al. Gastroenterology 2012)
Is it possible to restore innate and adaptive immunity for HBV?
PUTATIVE MECHANISMS OF T CELL EXHAUSTION IN HBV INFECTION

Tolerizing liver environment

High viral load with massive production of secretory proteins (HBsAg, HBeAg)
T CELL FUNCTIONAL IMPAIRMENT IN CHRONIC HBV INFECTION

Acute Self-limited Infection
Efficient T cell function/differentiation

Naïve CD8 cell +Ag Effector CD8 cell Rapid Proliferation / Differentiation Effector memory Central memory Antigen cleared Antigen persistence Inefficient T cell function/differentiation

Chronic HBV infection
CAN THE HBV-SPECIFIC T CELL FUNCTION BE RESTORED BY ANTIGEN DECLINE IN CHRONIC HBV PATIENTS?

Efficient T cell function/differentiation

Acute Self-limited Infection

Naïve CD8 cell Effector CD8 cell

+Ag

Rapid Proliferation / Differentiation

Effector memory Central memory

Antigen cleared Antigen persistence

Antigen decline

Restored T cell function/differentiation

?
Effect of long-term NUC therapy on T cell responses
Stable restoration of the T cell function after long-lasting suppression of HBV replication induced by NUC therapy and detected following expansion in vitro

A stable restoration of the T cell function is detectable upon in vitro expansion in NUC treated patients after long-lasting suppression of HBV replication.

Boni C. et al. Gastroenterology 2012
PD-1 expression by HBV-specific CD8 cells in NUC treated patients

Boni C. et al. Gastroenterology 2012
T cell restoration following NUC treatment is efficient in vitro

Boni C. et al. Gastroenterology 2012
T cell restoration following NUC treatment is partial ex vivo

Boni C. et al. Gastroenterology 2012

[Graph showing % of HBV dextramer+ CD8 T cells producing IFN-γ, IL-2, and CD107a under different conditions: Naive CHB, NUC treated HBsAg neg, NUC treated HBsAg pos, Acute hepatitis B (follow-up).]
Ex vivo frequencies of IFN-γ producing HBV-specific T cells after peptide stimulation

(ELISPOT analysis)

Boni C. et al. Gastroenterology 2012
NK cell function in NUC treated patients

Is functional HBV-specific T cell restoration associated with a parallel improvement of NK cell function in NUC treated patients?
Functional NK cell dichotomy in chronic HBV infection

*Impaired IFN-γ production with normal cytotoxicity*

**IFN-γ**

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**CD107a**

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*Peppa D. et al Plos Pathogens 2010*

IFN-γ production by NK cells is not significantly improved by NUC therapy

Capacity to degranulate of NK cells is not significantly affected by NUC treatment

Reciprocal behaviour of NK cell and HBV-specific T cell responses in NUC-treated patients

Reciprocal behaviour of NK cell and HBV-specific T cell responses in NUC-treated patients

Inverse correlation between global NK cell and global HBV-specific T cell functions in 16 NUC-treated patients

Inverse correlation between NK cell IFN-γ production and HBV-specific T cell cytokine production in NUC-treated patients
Improvement of HBV-specific T cell function after NK cell depletion by cell sorting in NUC-treated patients (1).

**Figure:**
- **Graph 1:** Improvement of CD4+ and CD8+ T cell function after NK cell depletion. The graph shows the percentage of CD4+ and CD8+ T cells producing cytokines (IFN-γ and TNF-α) in PBMC, NK-depleted cells, and +NK (day 0) conditions. The bars indicate a significant increase in cytokine production in the NK-depleted and +NK conditions compared to PBMC, with a statistical significance of 0.003 and 0.03, respectively.

- **Graph 2:** Global HBV-specific CD4+ and CD8+ T cell function. The graphs show the percentage of global HBV-specific CD4+ and CD8+ T cells producing cytokines (IFN-γ and TNF-α) in different conditions. The bar charts illustrate the increase in cytokine production in the NK-depleted and +NK conditions compared to PBMC, highlighting a significant improvement with statistical significance levels.

**Legend:**
- PBMC: Peripheral Blood Mononuclear Cells
- NK DEPLETED (Δ NK): NK-depleted cells
- +NK (DAY 0): +NK cells (day 0)

**HBV Core and Polymerase:**
- HBV-Core and Polymerase expression was measured in different conditions, showing an increase in cytokine production. The graph indicates a significant increase in CD4 and CD8 T cell function with NK cell depletion and +NK conditions.
NK cell depletion does not affect the function of T cells of different specificity in NUC-treated patients

Boni C. et al. submitted
- HBV-specific T cells in chronic infection
- NK cell response and its regulatory role on HBV-specific T-cell response
- **Potential strategies to reconstitute the anti-viral T cell function**
- Molecular basis of CD8 cell dysfunction in chronic HBV infection
Expression of various inhibitory receptors on circulating and intrahepatic virus-specific CD8 cells of patients with chronic HBV infection
Expression of PD-1 and CD127 on circulating and intrahepatic virus-specific CD8 cells of patients with chronic HBV infection and effect of PD-1/PD-L1 blockade on the T cell function

Strategies based on the correction of individual dysregulated pathways can only induce a partial restoration of the T cell function.
HBV-specific T cells

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Genome-wide expression profiling

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Mysregulated genes and pathways associated with T cell exhaustion

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Correction strategies to restore anti-viral T cell functions
TRANSCRIPTOME STUDY IN ACUTE, RESOLVED AND CHRONIC HBV INFECTION

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Isolation of HBV/FLU-specific CD8+ T cells by cell sorting

RNA extraction and amplification

Gene expression by microarray analysis (4x44K Agilent)

VALIDATION AND DISCOVERY OF NEW TARGETS
Visualization of overall sample distribution and distances between groups of patients by principal component analysis (PCA)
Results and conclusion of molecular analysis of HBV-specific CD8 T-cells

1. Downregulation of genes involved in mithocondrion function
2. Dna repair
3. Proteasome

Exhausted HBV-specific CD8 cells are deeply impaired at a metabolic and energetic level with a prevalent down-regulation of various core cellular processes centered on extensive mitochondrial alterations.

A significant improvement of mitochondrial and antiviral CD8 functions was elicited by mitochondrion-targeted antioxidants
Main message

A deep metabolic and energetic impairment is typical of exhausted T cells

Question

Is restoration of an efficient anti-viral T cell function an achievable objective?

Evidence

Multiple levels of correction will likely be needed to restore an efficient anti-viral T cell function

(unless correction affects specific core central processes able to indirectly affect other distal regulatory pathways)
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