Immunology of chronic viral hepatitis and HCC

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## Viral hepatitis

- Most common liver disease
- Liver inflammation due to virus infection

<table>
<thead>
<tr>
<th></th>
<th>Hepatitis A</th>
<th>Hepatitis B</th>
<th>Hepatitis C</th>
<th>Hepatitis Delta</th>
<th>Hepatitis E</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Virus family</strong></td>
<td>Picornavirus</td>
<td>Hepadnavirus</td>
<td>Flavivirus</td>
<td>Circular RNA similar to plant viroid</td>
<td>Similar to Calicivirus</td>
</tr>
<tr>
<td><strong>Nucleic acid</strong></td>
<td>RNA (+ sense)</td>
<td>DNA (partially double strand)</td>
<td>RNA (+ sense)</td>
<td>RNA (- sense)</td>
<td>RNA (+ sense)</td>
</tr>
<tr>
<td><strong>Disease caused</strong></td>
<td>Infectious hepatitis</td>
<td>Serum hepatitis</td>
<td>Non-A, non-B hepatitis</td>
<td></td>
<td>Enteric non-A, non-B hepatitis</td>
</tr>
<tr>
<td><strong>Size</strong></td>
<td>~28nm</td>
<td>~40nm</td>
<td>30 - 60nm</td>
<td>~40nm</td>
<td>30 - 35 nm</td>
</tr>
<tr>
<td><strong>Envelope</strong></td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>
Chronic viral hepatitis: a global burden

HBV: discovered in 1970
360 million people chronically infected
1,000,000 deaths/year

HCV: discovered in 1989
170 million people chronically infected
leading cause of liver transplantation
500,000 deaths/year
Chronic viral hepatitis: a global burden

**HBV:** route of transmission

- blood-blood contact
- blood transfusion, contaminated needles,
  mother to child (vertical transmission)

chronicity at young age: very high (90%)
chronicity at adult age: very low (10%)

**HCV:** route of transmission

- blood-blood contact
  - blood transfusion, contaminated needles,
  - sexual contact

chronicity at adult age: 70%
Viral hepatitis -- disease progression

30–50 years

Decompensation
Transplantation
Death
Clinical characteristics of infections with HBV and HCV

- **Hepatitis B (chronically evolving)**
  - HBcAg-specific antibodies
  - Serum HBeAg
  - HBeAg-specific antibodies
  - Serum HBsAg

- **Hepatitis C (chronically evolving)**
  - HCV-specific antibodies

**Outcome**
- **Chronic hepatitis**
- **Serum ALT activity**

**Time after infection (years)**
- 0
- 10
- 20
- 30
- 40
- 50

**Increase (% of maximum)**
- 0
- 50
- 100

- **Outcome**
  - Incubation phase
  - Acute phase
  - Viral persistence, chronic hepatitis

**Time after infection (months)**
- 0
- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8

**Increase (% of maximum)**
- 0
- 50
- 100
Develop a novel effective immune modulatory therapy to treat chronic hepatitis

Identify immunological parameters / biomarkers to predict/improve:
- Response to therapy
- Disease progression

Focus on the liver
Hepatitis C: Treatment

4Lindsay, Hepatology 2001; 5Manns, Lancet 2001; 6Hadziyannis, Ann Intern Med 2004
Scarcity of suitable *in vitro* and *in vivo* models

- Scientific progress in the field of human viral hepatitis severely hampered
- Restricted species and cell tropism of most human hepatitis viruses
- Robust replication of HBV and HCV has only been documented in hepatocytes
  - Extremely hard to culture
- Animal models are essential for pathogenesis studies as well as preclinical antiviral efficacy and toxicity studies

Boonstra, Hepatology 2009
Experimental models for HBV/HCV infection

In vitro

**HepG2.2.15**
- Stably transfected hepatoblastoma cell line
- Complete HBV genome
- All viral RNAs and proteins
- Produce viral genomes
- Secrete infectious particles
- Secrete virus-like particles

Note:
Hepatoma cell lines are difficult to infect
Primary hepatocytes are difficult to obtain/culture
Adaptive mutations

Huh7 Subgenomics replicons

electroporate

antibiotic selection

Adaptive mutations

Lohmann, Science 1999

Blight, Science 2000
Lohmann, JVI 2001

Improvement by using Huh7.5 (Blight, JVI 2002)
JFH-1- culture of infectious virus

- Electroporate
- Huh7
- HCV virions
- Naïve cells
- Chimpanzee
- Chimeric mice

Wakita, 2005
Lindenbach, 2005
Zhong, 2005
Sofosbuvir, Ledipasvir
Daclatasvir, Dasabuvir, Ombitasvir

Entry inhibitors

Cyclophilin inhibitors
NS5A/B inhibitors

Assembly inhibitors

Monoclonal antibodies

Legend:

1. Nucleus
2. ER

Bar chart:

Overall SVR
HARVONI / 12 weeks
n=210/213 99%

Without cirrhosis
HARVONI / 12 weeks
n=176/177 99%

With cirrhosis
HARVONI / 12 weeks
n=32/34 94%
Regression of hepatic fibrosis

• Paired liver biopsy in a single patient with HCV-induced cirrhosis who attained SVR
Histological appearance of HCV infection in the liver

Healthy

HCV
Most chronic HCV patients have a weak or absent HCV-specific CD4+ T cell response

n=48 patients
HCV protein mix: Core, NS3, NS4, NS5a
Day 5-6 overnight 3H-thymidine incorporation

T-cell responses in HBV infection are weak or absent

Boni et al, Gastroenterology 2012
Host mechanisms that promote persistence of HBV in the liver

Resides in the liver in an immunosuppressive environment

Host mechanisms that promote persistence of HBV in the liver

Regulatory T cells

IL-10 and/or TGF-β

Regulatory B cells

Active elimination of T cells

Impaired NK cells

Myeloid derived suppressor cells

Impaired dendritic cells

High viral load leading to T cell exhaustion

Mitochondrial dysfunction
Relative high numbers of HBV-specific T cells in liver inversely correlate with HBV DNA load.
# T cell exhaustion during chronic viral infections

<table>
<thead>
<tr>
<th>State</th>
<th>IFN-γ</th>
<th>TNF-α</th>
<th>IL-2</th>
<th>CTL</th>
<th>Proliferative potential</th>
<th>Apoptosis</th>
<th>Antigen load</th>
<th>CD4 help</th>
<th>PD-1 expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional T cell</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>–</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Partial exhaustion I</td>
<td>++</td>
<td>+/-</td>
<td>–</td>
<td>–</td>
<td>++</td>
<td>+/-</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Partial exhaustion II</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>++</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Full exhaustion</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+++</td>
<td>Low</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Deletion</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>High</td>
<td>Low</td>
<td>High</td>
</tr>
</tbody>
</table>

Freeman, J Exp Med 2006
High PD-1 expression on HBV-specific CD8+ T cells
PD-1 blockade enhances HBV-specific T cell function

Fisicaro, Gastro 2010
**CD4+CD25+FoxP3+ Treg are abundantly present in HCV infected livers, while absent from healthy livers**

Regulation of HCV-specific CD4+ and CD8+ T cell responses differs between chronic HCV patients.
The balance between protective immunity and pathology

Protective immunity + pathology

chronicity + mild pathology

Claassen, Boonstra. Curr Opin Virol 2013
Phases of chronic infection in HBV patients

- Immunotolerant Phase
  - Minimal inflammation
- Immuno-active Phase
  - Chronic active inflammation
- Inactive carrier Phase
  - Mild hepatitis
- HBeAg-negative Chronic Hepatitis
  - Active inflammation

- HBV DNA
- ALT

HBeAg
Anti-HBe
Phases of chronic infection in HBV patients

- **HBV DNA**
  - Log IU/ml
  - IT, IA, IC, ENEG

- **ALT**
  - IU/ml
  - IT, IA, IC, ENEG

- **HBeAg**
  - Log IU/ml
  - IT, IA, IC, ENEG

- **HBsAg**
  - Log IU/ml
  - IT, IA, IC, ENEG

**Legend**
- **IT** immunotolerant
- **IA** immuno active
- **IC** inactive carrier
- **ENEG** HBeAg-neg hepatitis
Quantification of T and B cells in livers from HBV patients at different clinical phases

Intrahepatic T cells
- Immunotolerant
- Immuno-active
- Inactive carrier
- HBsAg-neg hepatitis

Intrahepatic B cells
- Immunotolerant
- Immuno-active
- Inactive carrier
- HBsAg-neg hepatitis
Phases Of Infection

- Immunotolerant Phase
- Immuno-active Phase
- Immune control Phase
- HBeAg-negative Chronic Hepatitis

HBV DNA

ALT

HBeAg

Anti-HBe

Treatment indication

Treatment indication

Buster, Neth J Med 2006
Identify virus or host biomarkers in peripheral blood that distinguish HBV clinical phases
Nucleos(t)ide analogues cause chain termination and give viral suppression, but treatment is generally lifelong.

Options:
- Lamivudine
- Adefovir
- Telbivudine
- Entecavir
- Tenofovir

Peg-interferon –ISG induction
NUC therapy restores T-cell responses in HBV infection

However, NUC therapy generally does not lead to HBV cure or functional cure. Stopping of NUC therapy leads to reactivation in many patients
Most patients achieve HBV DNA undetectability during antiviral therapy

Clearance of HBsAg is rare in patients treated with nucleo(s)tidine analogues

Marcellin, AASLD 2011
**Hepatitis B Particle Types**

- **Hepatitis B virion (Dane particle)**
  - Infectious
  - Consists of HBsAg, HBcAg, polymerase and HBV DNA

- **Hepatitis B filament**
  - Non-infectious
  - Consists of HBsAg (small + large)

- **Hepatitis B sphere**
  - Non-infectious
  - Consists of HBsAg (small only)
Structural Components of HBV and HBsAg Particles

- **virus**
- **preS1**
- **preS2**
- **filaments**
- **LHBs**
- **SHBs**
- **MHBs**
- **spheres**
- **52 nm**
- **17-25 nm**
- **RT**
- **3.2kb DNA**
- **pr**

Erasmus MC
Closed coiled circular DNA (cccDNA)
Improvement of current therapy in order to:

- Get true immune control
- HBsAg seroconversion
- Eradication of cccDNA
Novel therapeutic strategies for HBV and HCV

Nucleotide analogues and DAA
Peg-IFN lambda
TLR 7 agonists
Interfering RNA’s
Entry blockers
Therapeutic vaccines (T cell-based)
Reversal of exhausted phenotype

HCV-spec T cell prolif

T cell, Treg, NK cell
Hepatocellular carcinoma, epidemiology and risk factors
Hepatocellular carcinoma (HCC) is the most common of the three hepatobiliary malignancies.
Globally, HCC is the second leading cause of cancer mortality
Epidemiology of HCC

Age of diagnosis: 62 years
Underlying cause:

- Hepatitis C: North America, Europe, Japan
- Hepatitis B: Rest of the world
Cirrhosis: 90%
Risk factors of HCC

- Hepatitis B
- Hepatitis C
- Alcohol

- but also NAFLD
- Hemochromatosis
- PBC
- Autoimmune hepatitis

Other host risk factors are:
- Male
- Older age
- Asian or African ancestry
- Family history of HCC
- Aflatoxin exposure
- Alcohol or tobacco use
- Cirrhosis

90%
Risk factors of HCC

**HBV**

Globally, 54% of HCC can be attributed to HBV infection

Factors that play a role are:

- HBV DNA viral load
- HBV genotype C
- High serum HBsAg

Chen CJ, JAMA 2006; 295:65-73
Chan HL, Gut 2004; 53:1494-1498
Tseng TC, Gastro 2012; 142:1140-1149

**Gender disparity**

Table 1 | Age-standardized incidence rates for HCC*

<table>
<thead>
<tr>
<th>Countries</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low resource</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mongolia</td>
<td>116.6</td>
<td>74.8</td>
</tr>
<tr>
<td>Middle Africa</td>
<td>18.9</td>
<td>9.6</td>
</tr>
<tr>
<td>Eastern Africa</td>
<td>7.2</td>
<td>3.6</td>
</tr>
<tr>
<td>South-Eastern Asia</td>
<td>21.4</td>
<td>9.0</td>
</tr>
<tr>
<td>Melanesia</td>
<td>12.9</td>
<td>5.0</td>
</tr>
<tr>
<td>Western Africa</td>
<td>16.6</td>
<td>8.0</td>
</tr>
<tr>
<td>Polynesia</td>
<td>10.8</td>
<td>3.4</td>
</tr>
<tr>
<td><strong>Intermediate resource</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>China</td>
<td>37.4</td>
<td>13.7</td>
</tr>
<tr>
<td>Caribbean</td>
<td>6.3</td>
<td>4.4</td>
</tr>
<tr>
<td>South Africa</td>
<td>13.9</td>
<td>5.1</td>
</tr>
<tr>
<td>Central America</td>
<td>7.3</td>
<td>7.0</td>
</tr>
<tr>
<td>Western Asia</td>
<td>4.4</td>
<td>2.3</td>
</tr>
<tr>
<td>Northern Africa</td>
<td>7.5</td>
<td>2.5</td>
</tr>
<tr>
<td>South America</td>
<td>5.3</td>
<td>3.9</td>
</tr>
<tr>
<td>South Central Asia</td>
<td>3.4</td>
<td>1.6</td>
</tr>
<tr>
<td><strong>High resource</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Korea</td>
<td>38.4</td>
<td>10.6</td>
</tr>
<tr>
<td>Southern Europe</td>
<td>9.8</td>
<td>3.2</td>
</tr>
<tr>
<td>Western Europe</td>
<td>7.2</td>
<td>2.1</td>
</tr>
<tr>
<td>Eastern Europe</td>
<td>4.6</td>
<td>1.9</td>
</tr>
<tr>
<td>Northern America</td>
<td>6.8</td>
<td>2.2</td>
</tr>
<tr>
<td>Australia/New Zealand</td>
<td>5.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Northern Europe</td>
<td>3.8</td>
<td>1.6</td>
</tr>
</tbody>
</table>

*All values expressed per 100,000 of the population in 2008 (data from GLOBOCAN 2008). Abbreviation: HCC, hepatocellular carcinoma.
Epidemiology of HCC in Africa

2000 patients with HCC

55% Hepatitis B

Map of Africa highlighting regions with the highest incidence of HCC due to Hepatitis B.
HCC in Africa occurs at a relatively young age (mean age 42 (range 34-55))

In 14 non-African countries the mean age of HCC diagnosis ranged from 52-69
Aflatoxins

A fungal toxin that contaminates maize, nuts etc. Metabolized in the liver, and forms adducts with DNA, it is a known carcinogen. Exposure leaves a p53 signature (249ser).

HCC risk:
4x higher in persons with high aflatoxin levels
7x higher in persons with chronic HBV
60x higher in persons with high aflatoxin+ chronic HBV

Likely, also synergy with HCV, but aflatoxin is more prevalent in areas with high HBV.

Coffee and aspirin

Reduction of HCC has been described for coffee (40%), the anti-diabetic drug metformin and aspirin (49%).
Global differences in HCC risk factors

In high rate countries:
HBV and aflatoxin are major risk factors

In low rate countries:
HCV, alcohol and diabetes/obesity are major factors
So, what’s going on?

Why are individuals with HBV more likely to get HCC?
The development of HCC is accelerated by HBV, since host genes are modified near the HBV integration site, which may cause host cell genome instability and carcinogenic proteins.

Integration of HBV DNA into the host hepatocyte genome is key to HCC.

Integration of HBV DNA is found in 80-90% of cancer cells, and in 30% of liver tissue adjacent to the tumor. Sung WK, Nature Genetics 2012; 44:765-769

Integration can affect the function of host genes as a cis-acting element, and activate proto-oncogenes and silence tumor-suppressor genes.

HBV DNA integration can also cause host genome instability, and induce mutations, deletions, and rearrangements.

HBV expresses diverse active proteins, such as HBx, which can alter apoptosis, intracellular signaling and epigenetics. HBx is highly expressed in the tumor.
HBV integration is not a random event

41% of all inserted genes are the TERT, MLL4 and CCNE1 genes
- TERT: Telomerase reverse transcriptase
- MLL4: mixed-lineage leukemia 4
- CCNE1: cyclin E1

40% of breakpoints are near the 1800th nt of the HBV genome (area that contains an enhancer, X gene and core promoters)

Sung, Nat Gen 2012; 44: 765-769
Murakami, Gut 2005; 54: 1162-1168
Paterlini, Oncogene 2003; 22: 3911-3916
Role of necroinflammation in development of HCC
The development of HCC is accelerated by prolonged HBV-induced hepatic inflammation. Also induction of IL-6/STAT3 and TGF-β has been reported.
Tolerance inducing mechanisms in the liver

Immune suppression  

→

Immune activation

Blood-borne pathogens and host antigens

Toxic waste

Gut antigens

Immune surveillance

IL-10

TGF-β
Immune response and HCC

Tumor-associated **macrophages** express exhaustion/inhibitory receptors

**Natural killer cells** are less frequent, show reduced cytokine production, and have a reduced lytic activity

**Dendritic cells** produce less IL-12 and more IL-10, and have a poor maturation

**T cells** have an exhausted phenotype and are functionally impaired

**Regulatory T cells** are present at higher frequencies and express high levels of inhibitory molecules
T cells do not efficiently eliminate tumor cells

**Tumor-cell intrinsic factors**
- Low antigenicity of HCC-associated Ags
- ↓ MHC molecules
- ↑ PD-L1

**Microenvironment-related suppressive factors**
- Immunosuppressive innate immunity: defective DC, ↑ MDSC and TAMs
- Abundance of suppressive cytokines (IL-10, TGF-β...)
- ↑ inhibitory checkpoints: PD-L1/PD-1 → T cell exhaustion

Tumorantigen-specific T cell responses and patient survival
Tumor antigens in HCC

- Mutated proteins (e.g. p53)
- Viral proteins (e.g. HBV proteins)
- Cell type-specific proteins (e.g. AFP, Glypican-3)
- Shared tumor-specific proteins (e.g. MAGE-A1, NY-ESO-1)

![Graphs showing response to overlapping peptides for different proteins: AFP, Glypican-3, MAGE-A1, NY-ESO-1.]

CD8+ T cells are shown interacting with the HCC cell.
Effector T cells are exhausted and functionally impaired

**PD-1**

**Tim3**

**LAG3**

TFL = tumor free liver
Enrichment of regulatory T cells in HCC

Ormandy, 2005
Globally, the estimated numbers of HCC-related deaths are still increasing, 2000-2015
HCC is a preventable cancer

Main risk factor worldwide is hepatitis B
  Vaccine preventable disease

Main risk factor in the “West” is hepatitis C
  Fully curable disease

Second risk factor is alcohol
  Fully preventable

Prevention of HCC development

HBV vaccination

Early surveillance of HCC – most HCC cases are diagnosed (too) late

Antiviral treatment
In 1984, none of the subjects were under universal vaccination coverage. In 1989, only children <5 years of age were covered.
Prediction or diagnostic markers for HCC

Diagnostic blood markers include PIVKA, CA19, AFP, AFP-L3 (not CEA)
BRIEF REPORT

Levels of Cytokines in Serum Associate With Development of Hepatocellular Carcinoma in Patients With HCV Infection Treated With Direct-Acting Antivirals

Jose D. Debes,1,2,* Marjolein van Tilborg,2,* Zwier M. A. Groothuismink,2 Bettina E. Hansen,2,3,4 Julian Schulze zur Wiesch,5 Johann von Felden,5 Robert J. de Knecht,2 and Andre Boonstra2

12 cytokine signature in patients with HCV treated with direct acting antivirals (sofosbuvir/ledipasvir)

Control = HCV patients treated with DAA, no HCC
De novo = HCV patients treated with DAA, who develop de novo HCC within 2 years
Recurrent = HCV patients treated with DAA, who develop recurrent HCC within 2 years
Immunotherapy for HCC

Clinical trials with antibodies against CTLA-4 and PD1/PD-L1

Good safety profile (pruritis, diarrhea, rash)

15-20% of patients had tumor responses, which lasted for a median of 17 months
45% of patients had stable disease, lasting more than 6 months in most cases
Hepatocellular carcinoma, epidemiology and risk factors

Healthy Liver  Fibrosis/ Cirrhosis  Hepatocellular carcinoma