

# Role of Immune system in HBV cure: Innate and adaptive immunity

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Toronto Centre for Liver Disease  
University Health Network (UHN)

Assistant Professor  
Department of Immunology  
University of Toronto



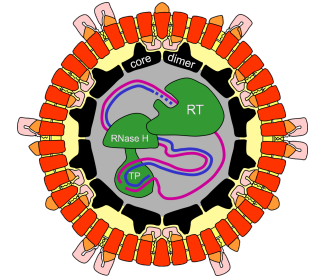
UNIVERSITY OF  
TORONTO



# Hepatitis B Virus

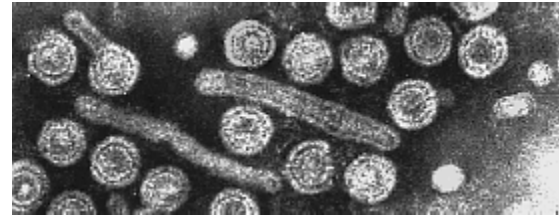
## ➤ Hepatotropic DNA virus

- Small (3.2kb) DNA virus that infects hepatocytes
  - 4 proteins
  - Can sustain up to  $10^{10}$  HBV virions/ml serum in chronic patients
  - viral antigen can reach mg/ml in serum



## ➤ Not directly cytopathic

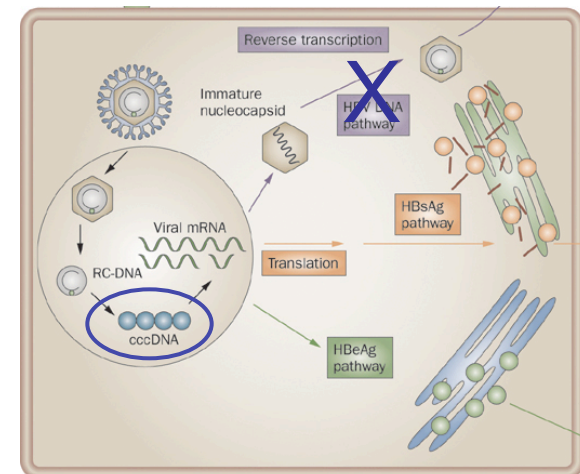
- Immunopathology



## ➤ Antiviral therapy rarely eliminates HBV

## ➤ No effect on cccDNA

- HBV minichromosome in the hepatocyte nucleus

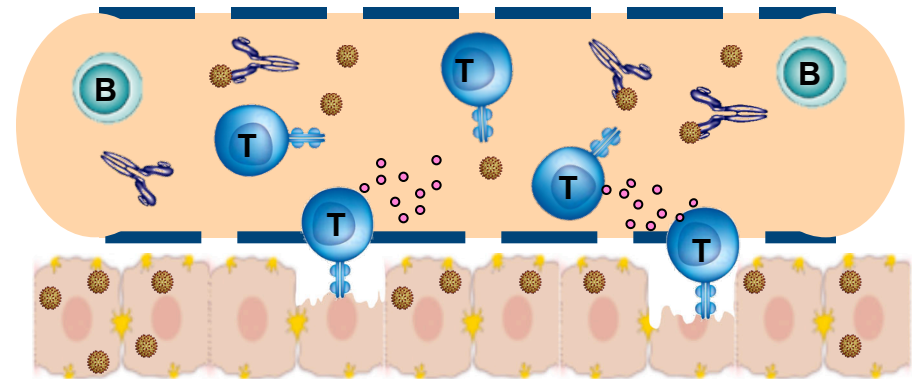


# Post-infection Clearance of HBV Requires T cells

## Acute

### **Multi-specific T cell response + anti-core/anti-surface Abs**

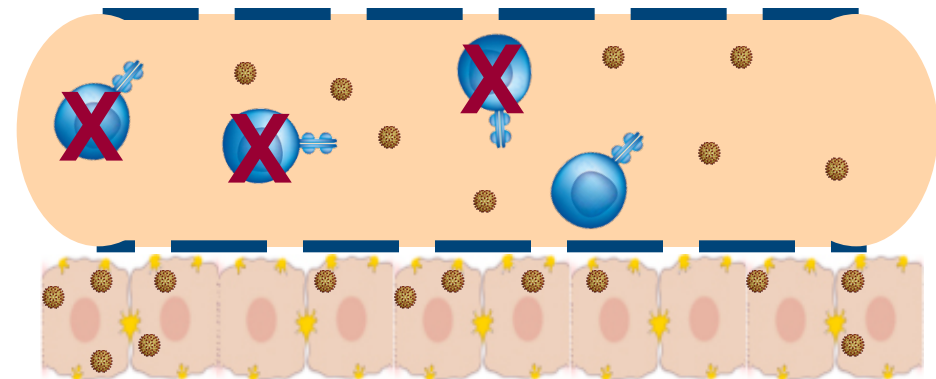
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2. CD8 T cells mediate clearance of infected cells
3. anti-HBs marker of resolution – B cells
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## Chronic

### **Weak T cell response + no effective antibodies**

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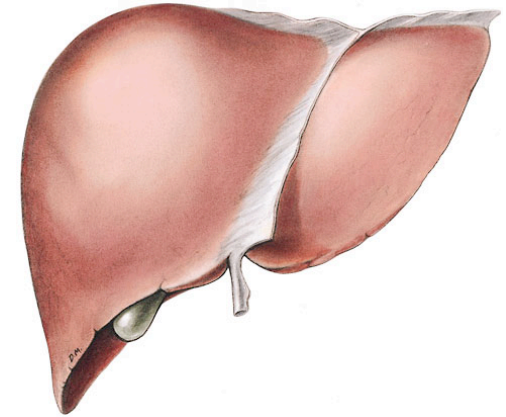


# Hepatitis B Virus Immune Evasion/Exhaustion

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## ➤ Hepatotropic virus

- ◆ Liver is generally a tolerizing organ
  - High IL-10, TGF- $\beta$ , PD-L1, enzymes degrading essential amino acids
  - Low MHC class-I and co-stimulatory molecule expression on hepatocyte
  - Suppressive Dendritic cells, macrophages



## ➤ High Antigen burden

- ◆ Small (3.2kb) DNA virus that infects hepatocytes
  - Can sustain up to  $10^{10}$  HBV DNA copies/ml serum in chronic patients
  - viral antigen can reach 1 mg/ml in serum

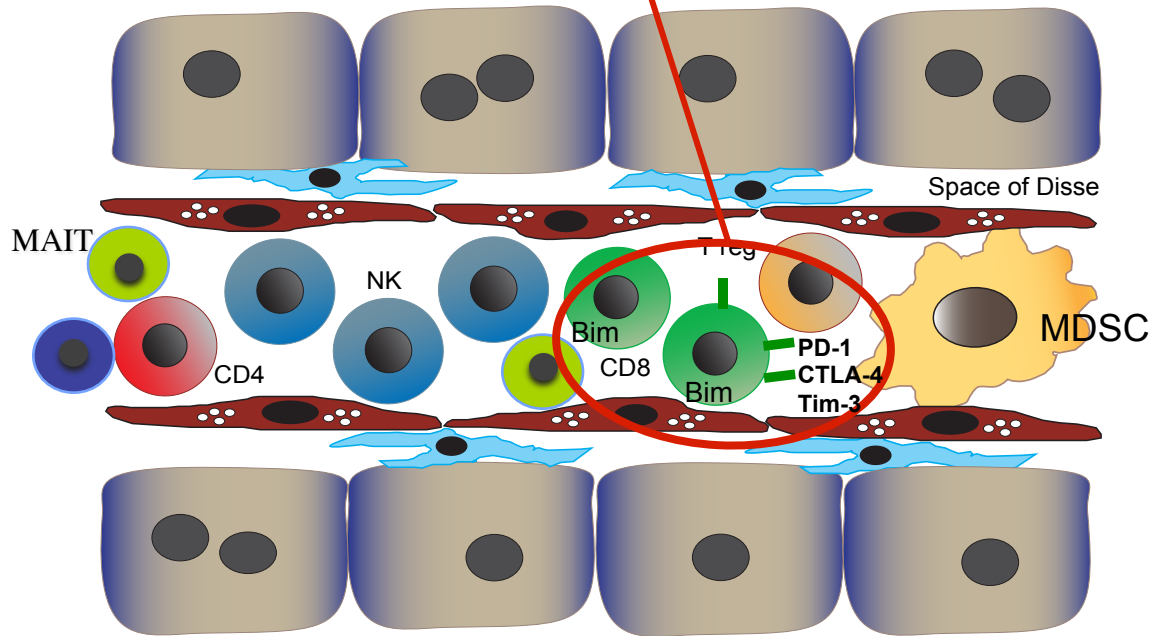


## ➤ Immune regulation at the innate and adaptive level

# Multiple Levels of Immune Defects in Chronic Infection

HBV-specific T cells are exhausted and pro-apoptotic

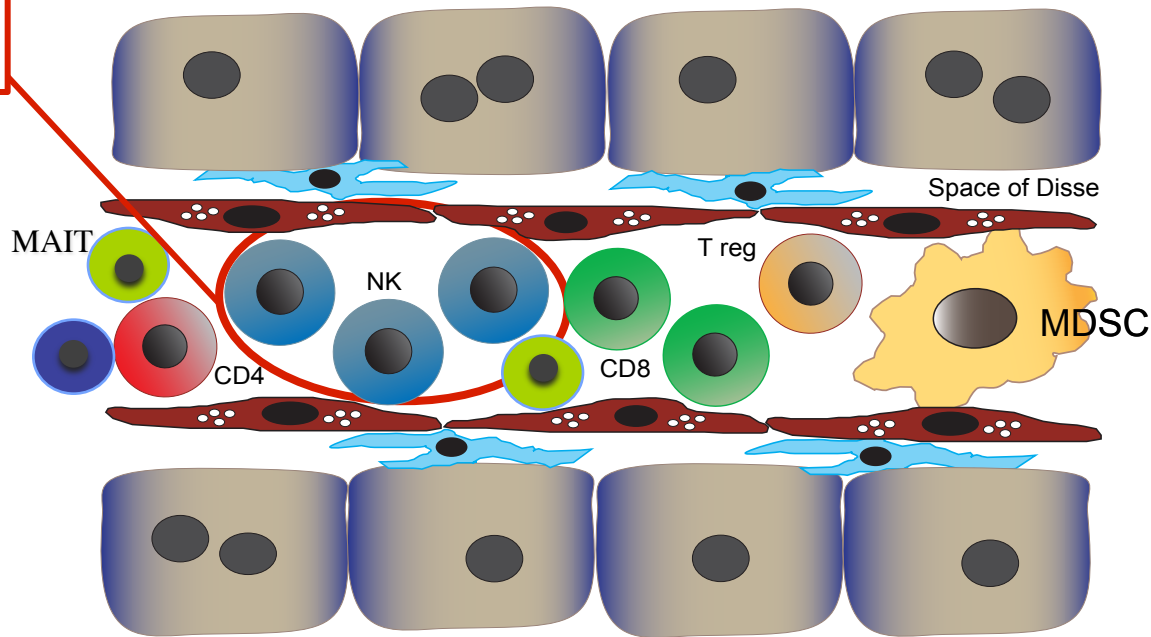
*Boni J Virol 2007*  
*Lopes JCI 2008*  
*Fisicaro et al Gastro 2010*  
*Schurich Hepat 2011*  
*Nebbia PLoS One 2012*



# Multiple Levels of Immune Defects in Chronic Infection

NK cells have defective IFN- $\gamma$  production

HBV-specific T cells are exhausted and pro-apoptotic

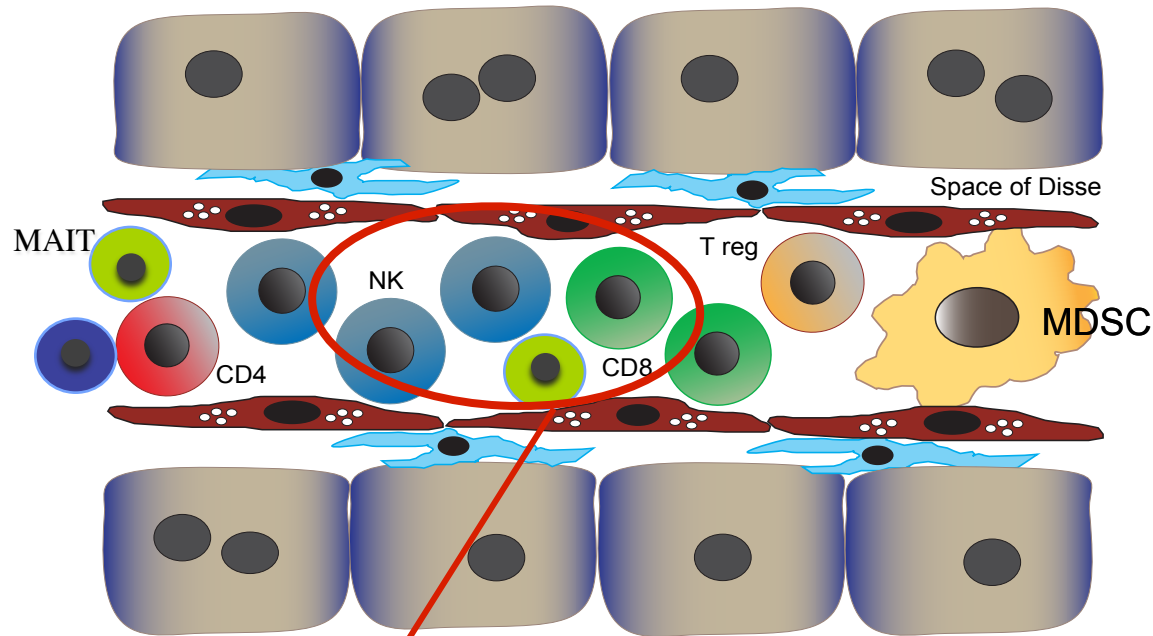


*Oliviero Gastro 2009*  
*Bonorino J Hep 2009*  
*Peppa PLoS Path 2010*  
*Tjwa J Hep 2011*

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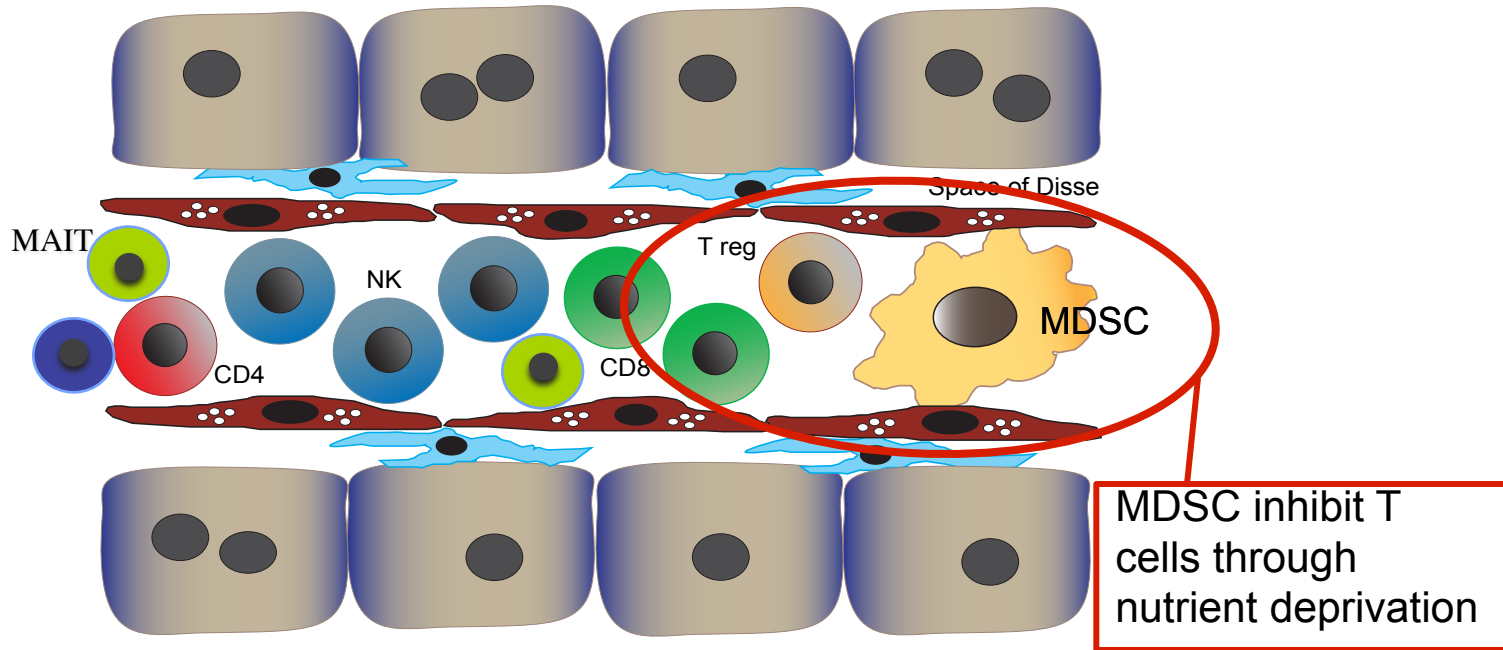


NK cells delete HBV-specific T cells

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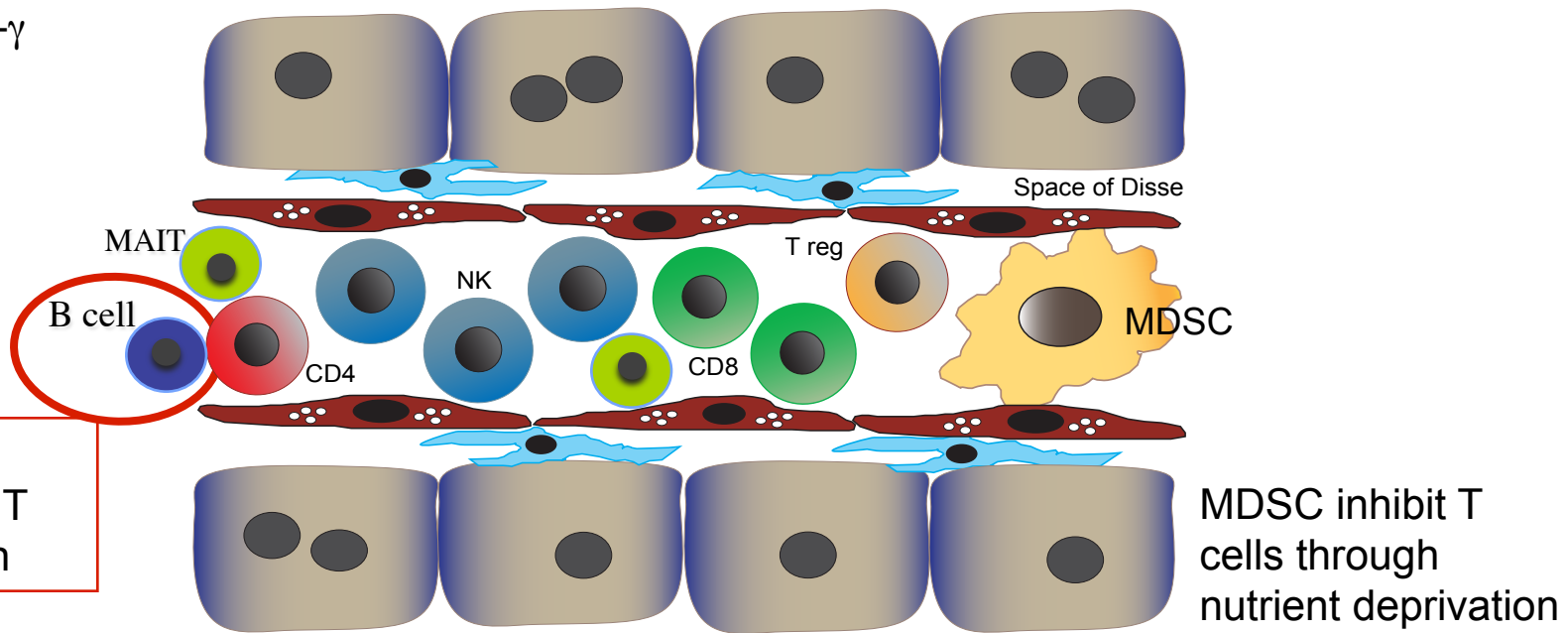
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# Multiple Levels of Immune Defects in Chronic Infection

NK cells have defective IFN- $\gamma$  production

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NK cells delete HBV-specific T cells

# Rationale for Immunotherapy in HBV

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- **Spontaneous sustained immune control in adults following acute infection**
- **Bone marrow transplant > Immune reconstitution > clearance of chronic HBV**
- **Patients that spontaneously clear chronic HBV display robust T cell responses**

# Potential Immunological Targets for HBV Therapy

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## Innate

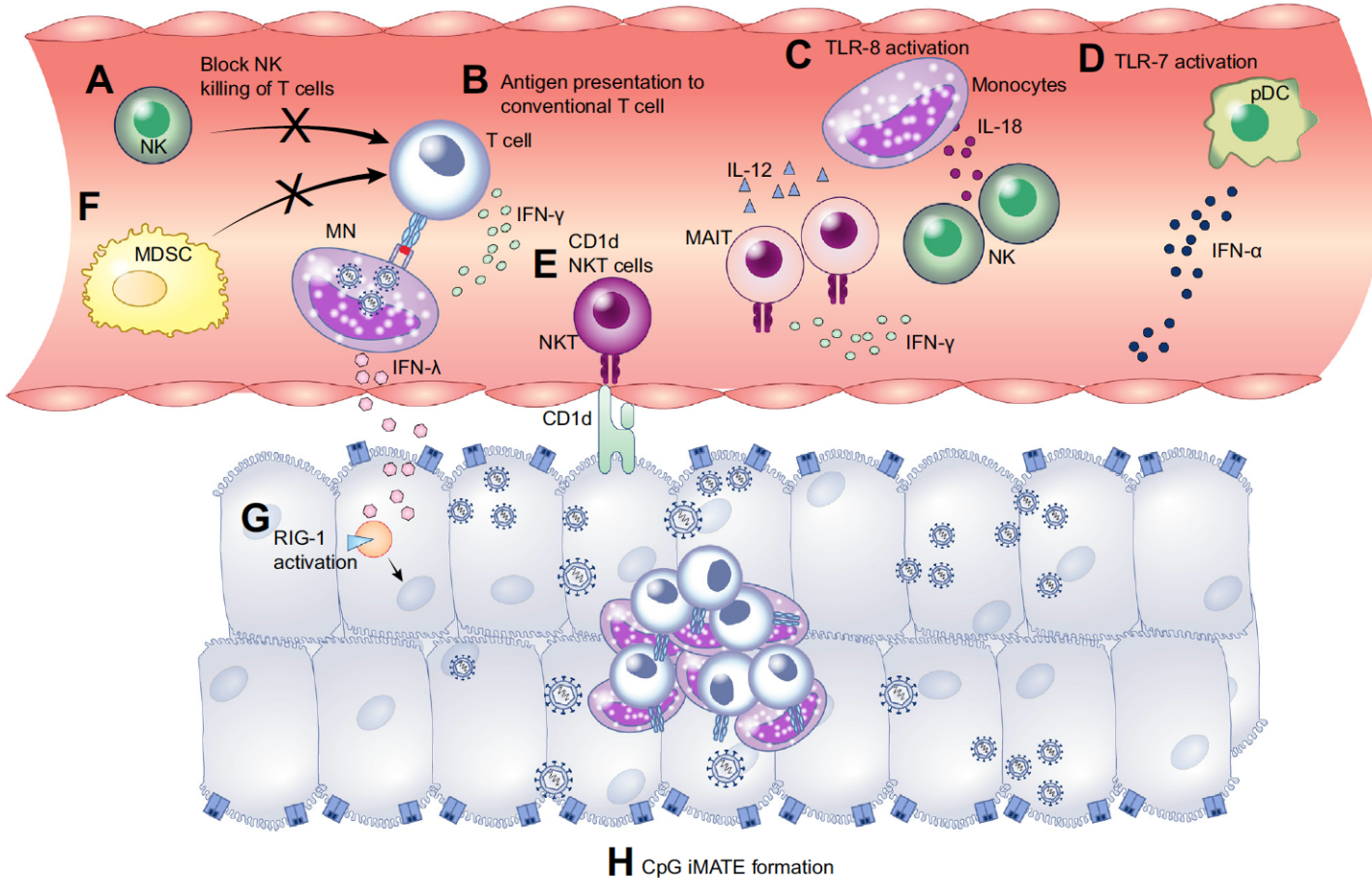
- Blocking negative regulation
- Stimulating antiviral cytokine production

## Adaptive

- Therapeutically boost HBV-specific T cell immunity
- Checkpoint blockade to release negative regulation

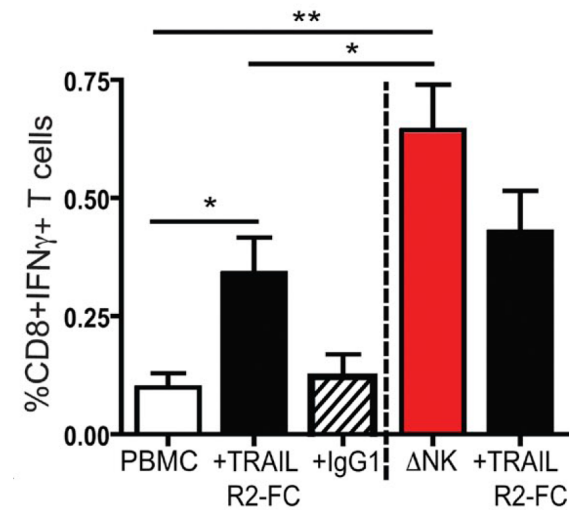
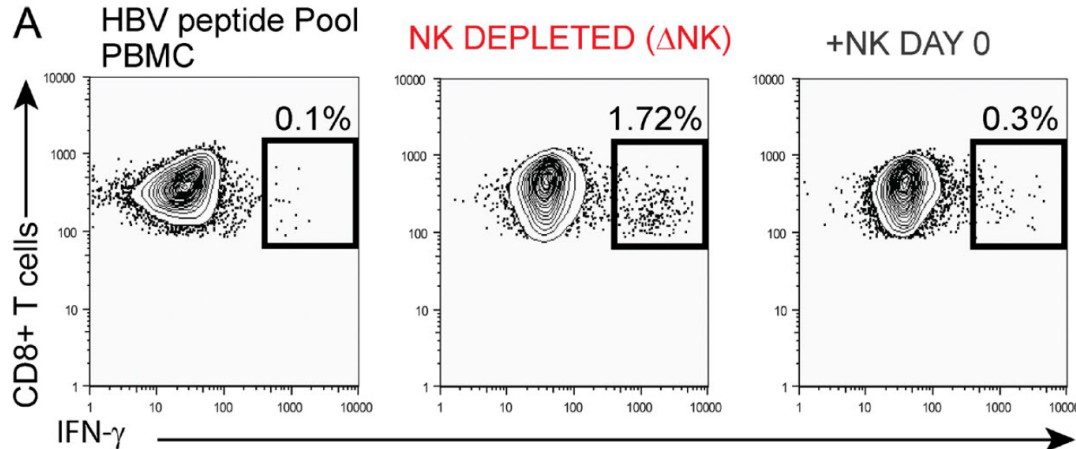
***Coordinated, integrated immune response important***

# Opportunities for Innate-targeted Immunotherapy - Blocking Negative regulation -

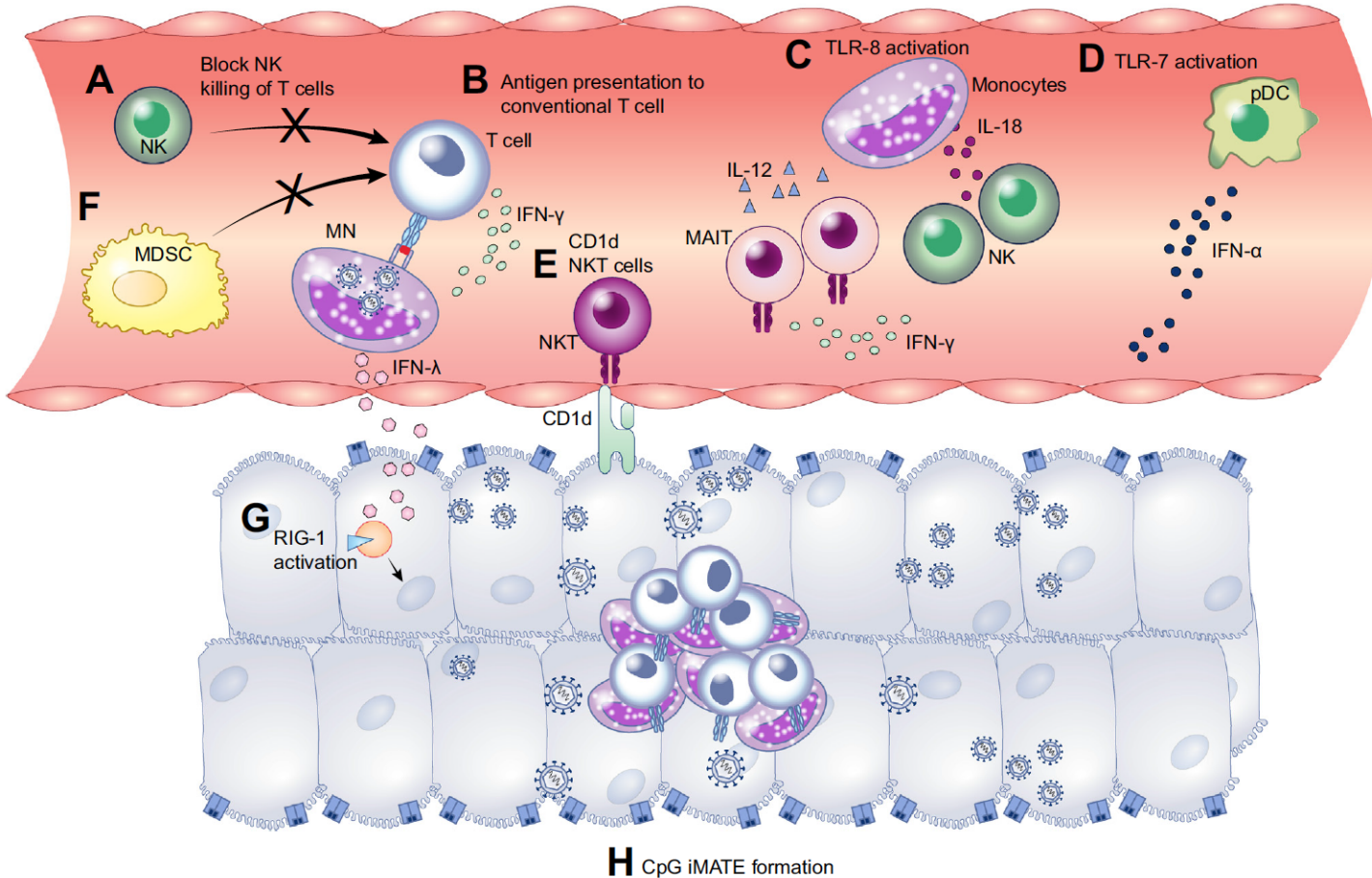


- A) Blocking NK TRAIL-mediated killing of HBV-specific T cells**
- F) Blocking MDSC-mediated suppression**

# Blocking NK TRAIL-mediated killing of HBV-specific T cells.

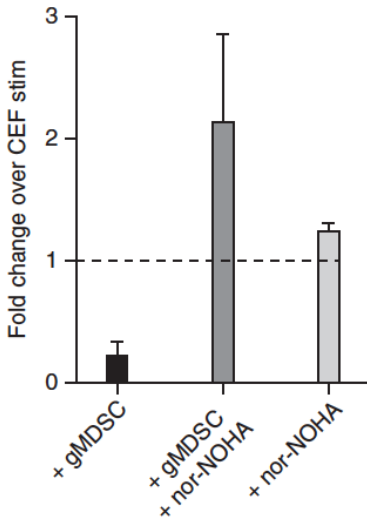
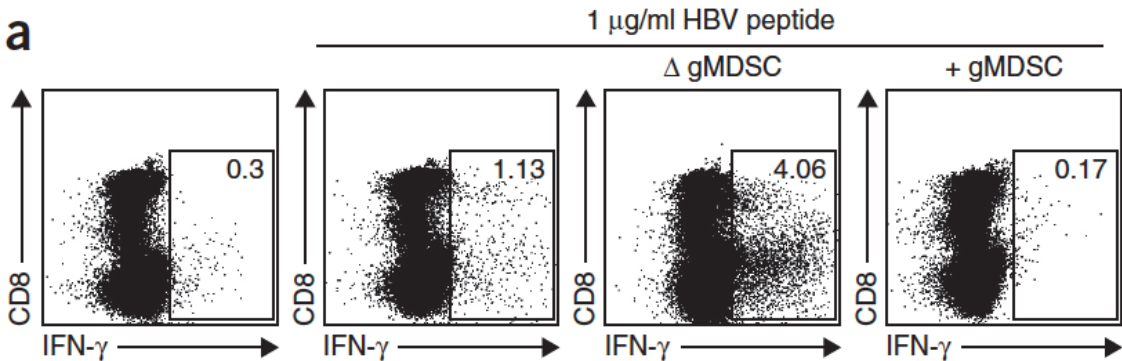
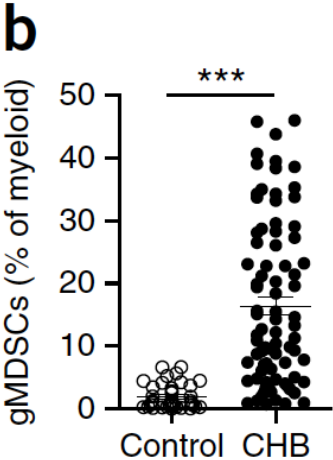
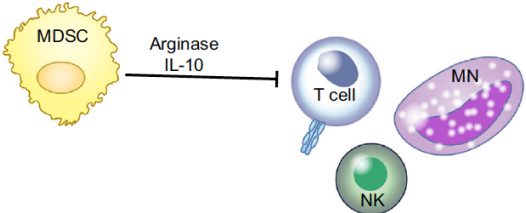


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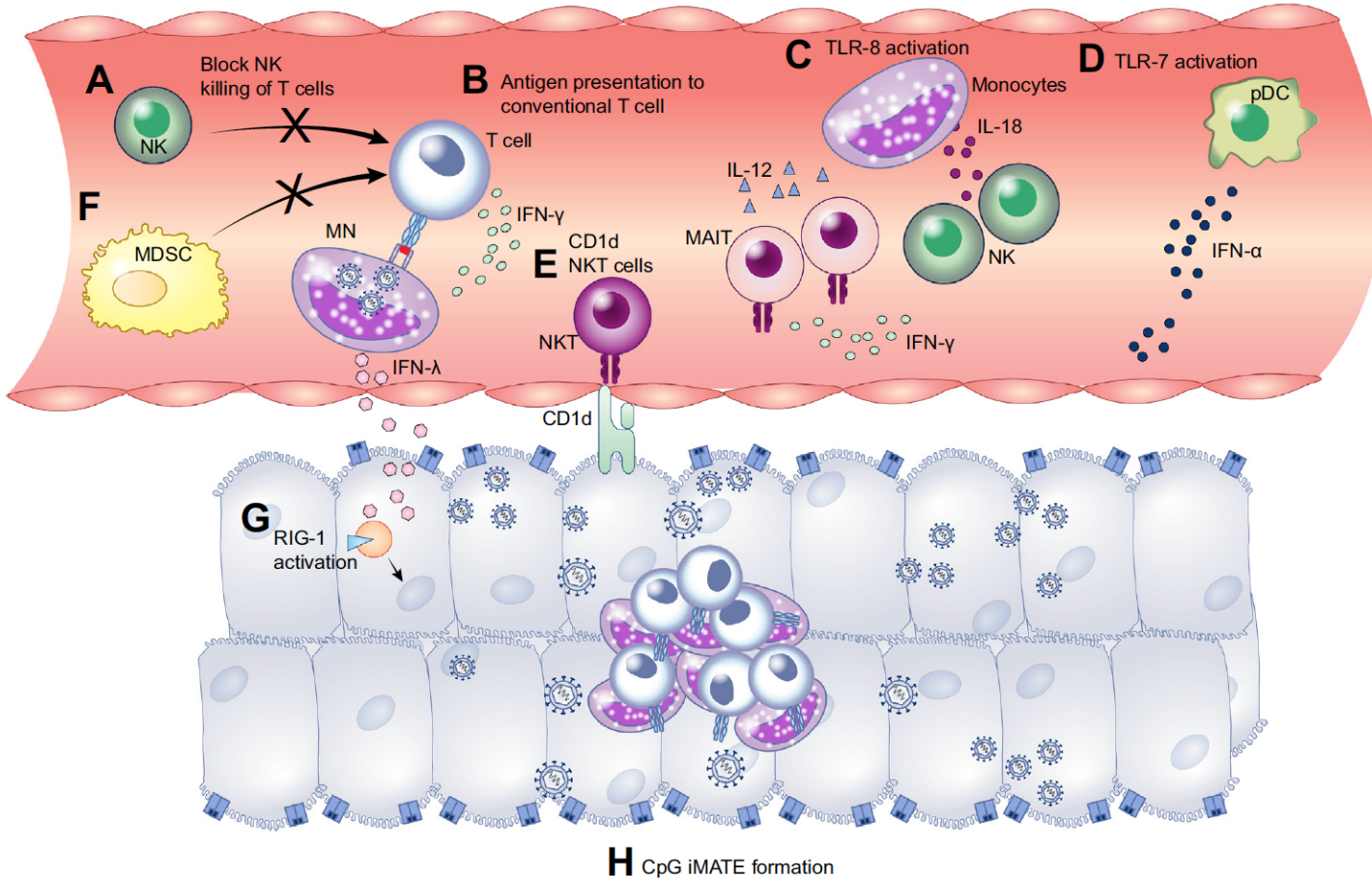
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# Blocking MDSC-mediated suppression



# Opportunities for Innate-targeted Immunotherapy

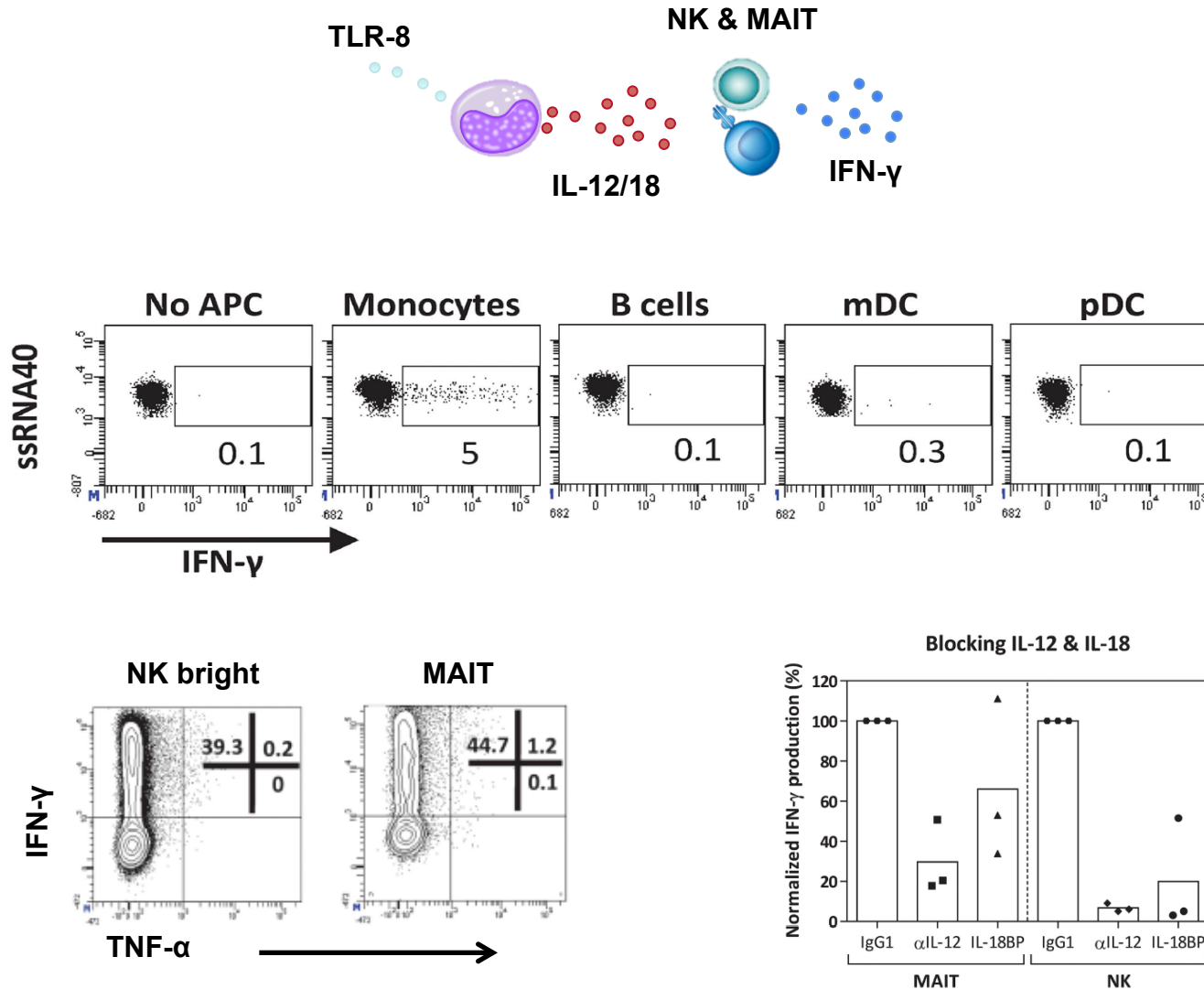
## - Stimulating antiviral cytokine production --



- C) TLR-8 activation of intrahepatic monocytes stimulating IL-12 and IL-18
- D) TLR-7 mediated IFN- $\alpha$  production from plasmacytoid DC.
- G) Direct triggering of RIG-1 in infected hepatocytes
- H) CpG induction of iMATEs

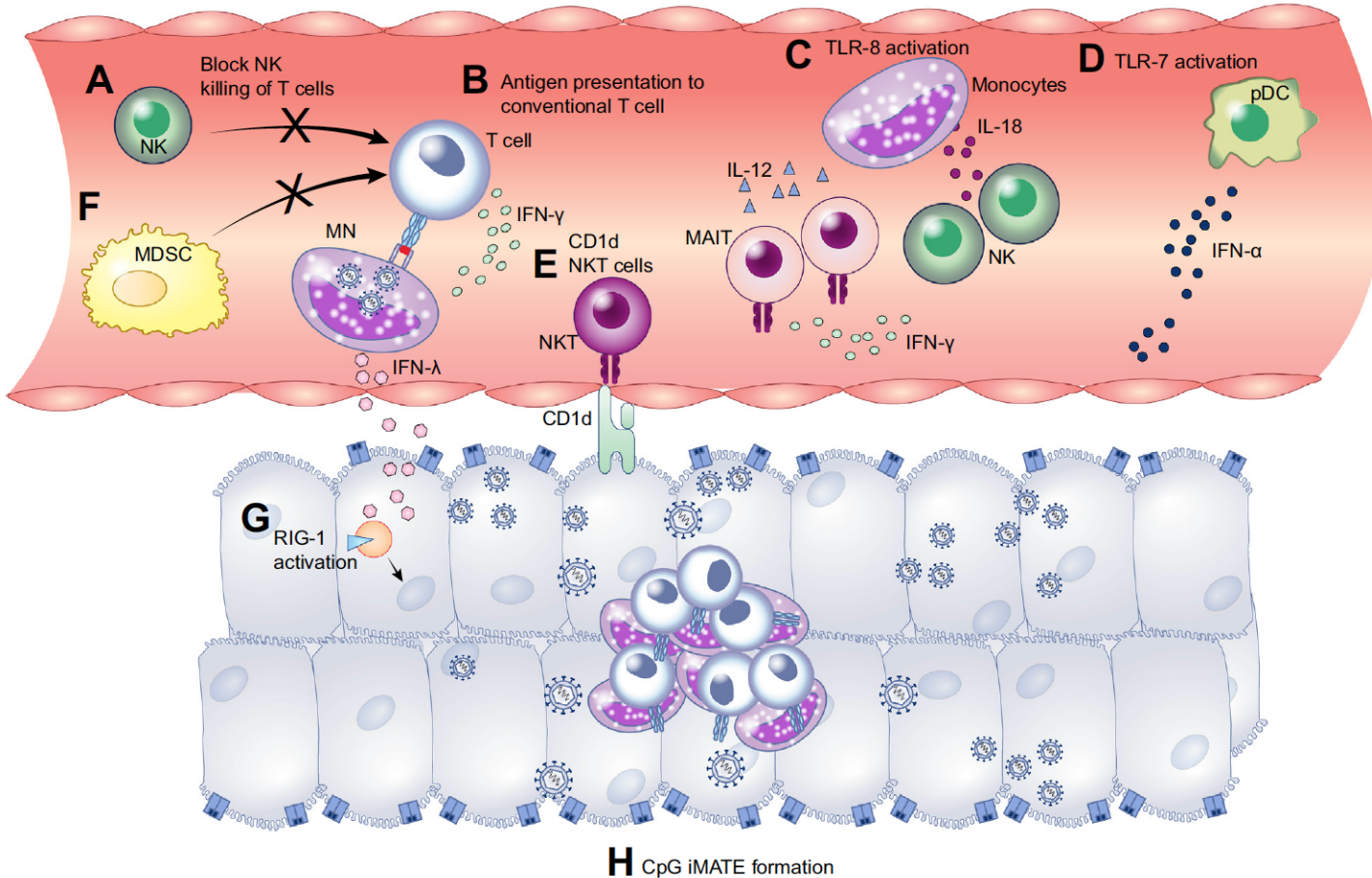


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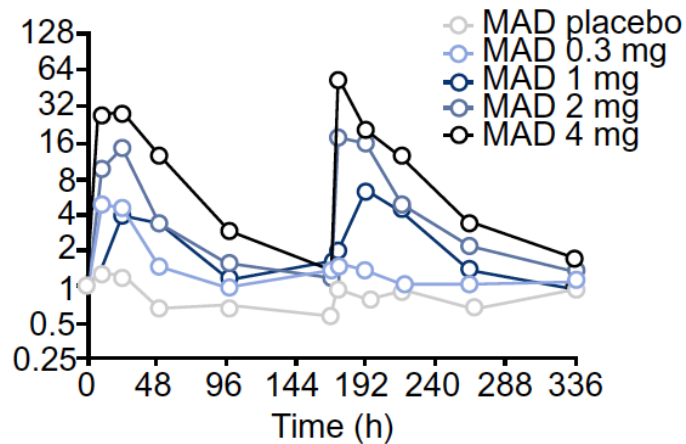
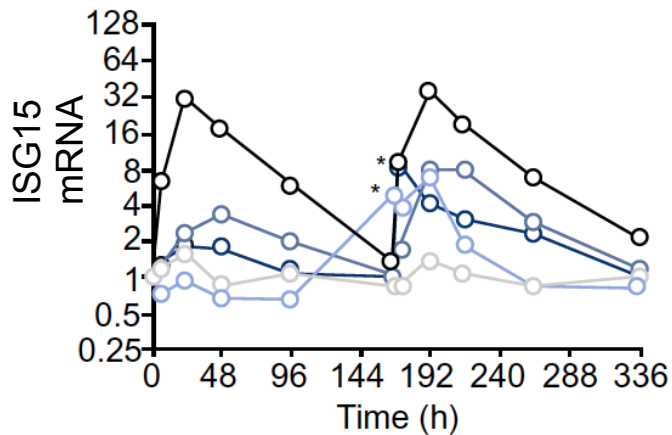
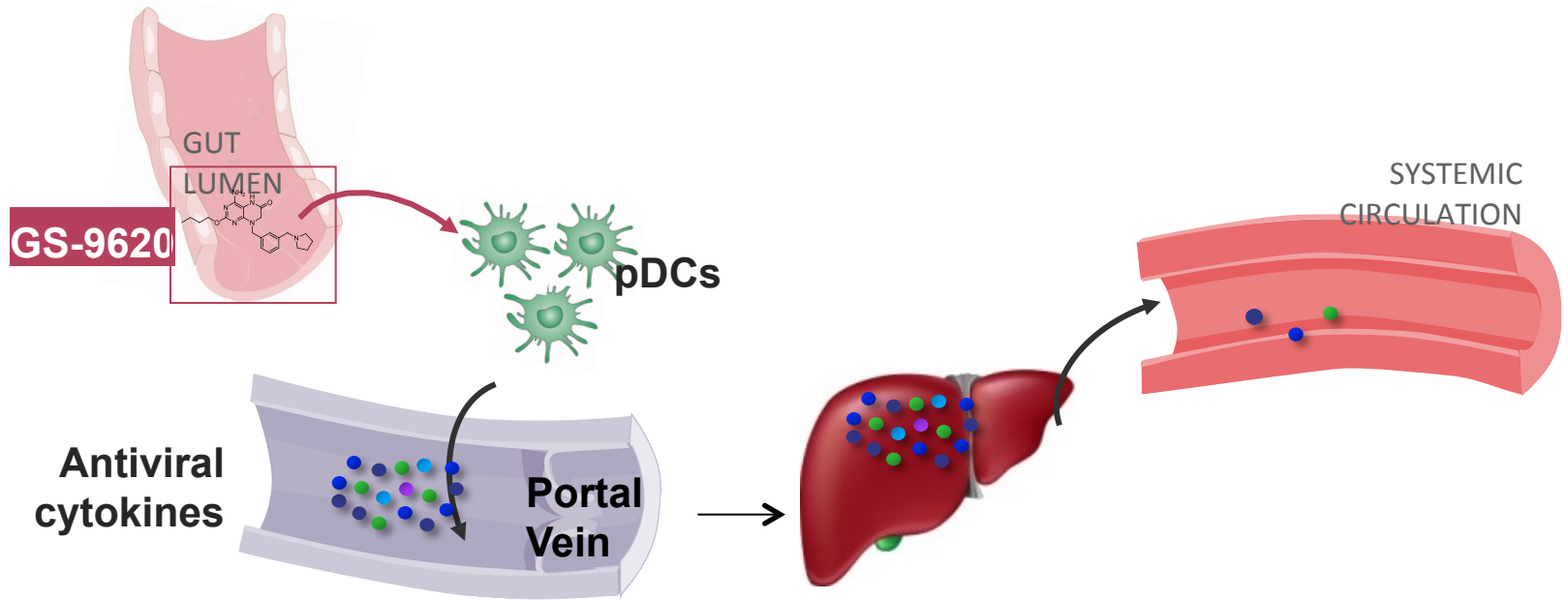
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## - Stimulating antiviral cytokine production --



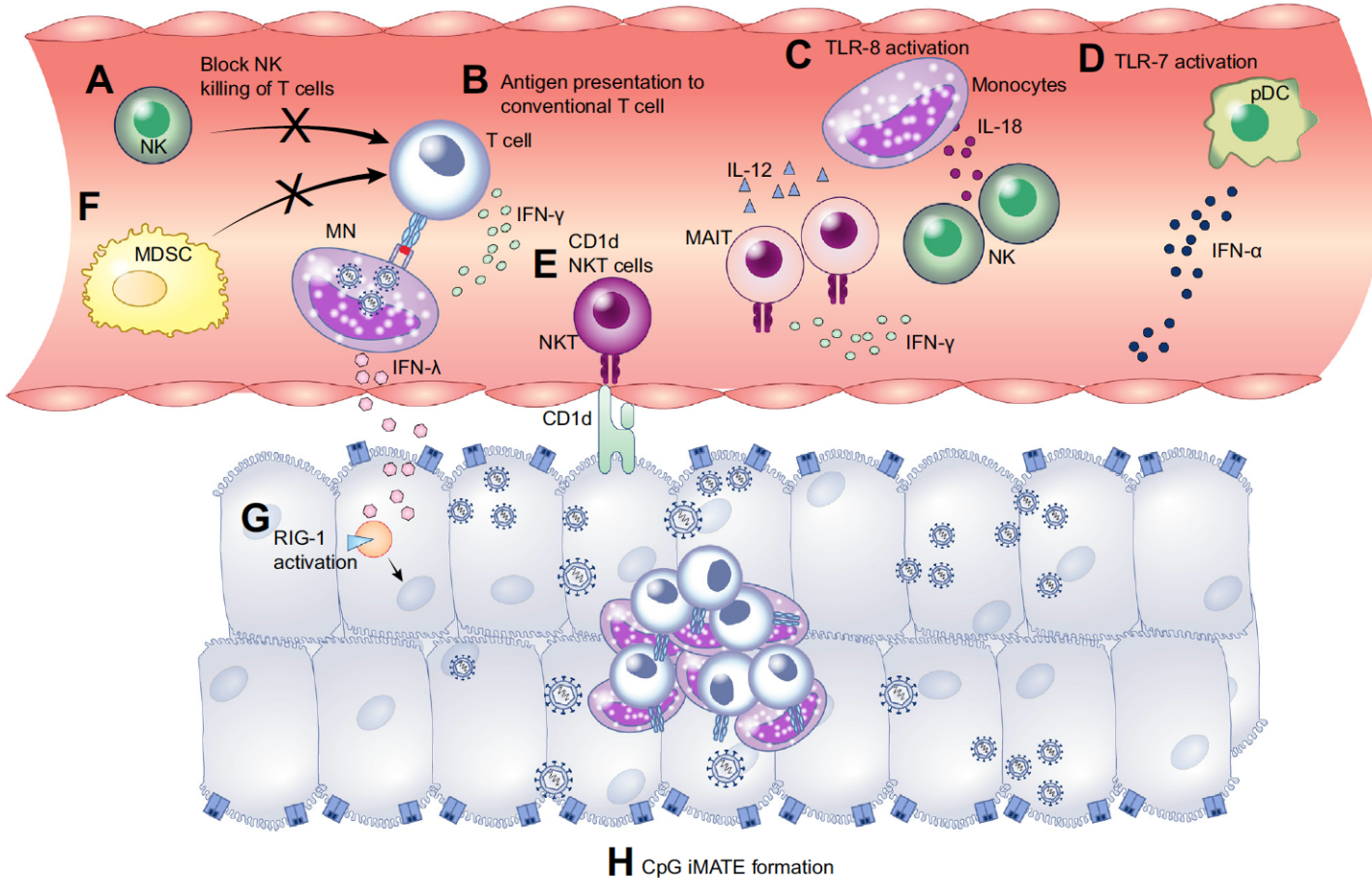
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# TLR-7 mediated IFN- $\alpha$ production from plasmacytoid DC.



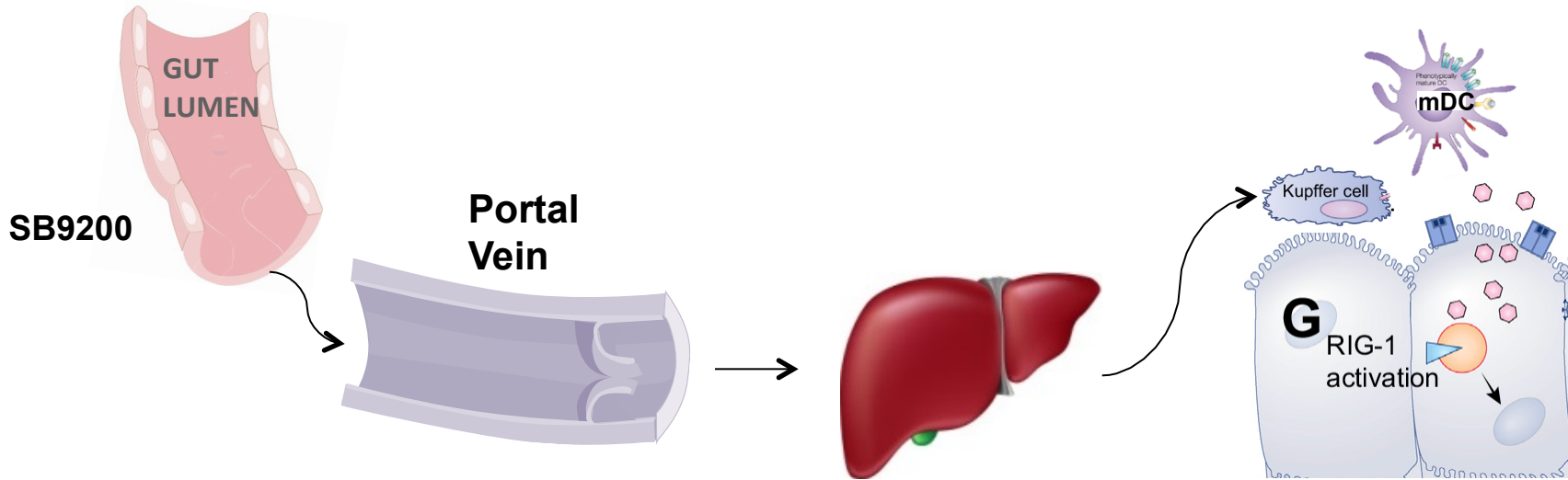
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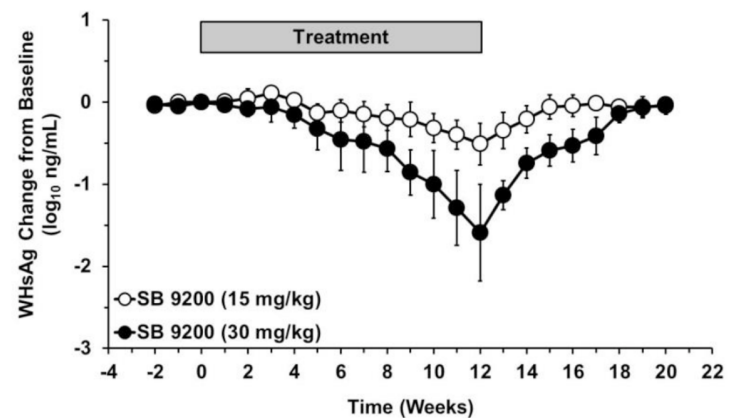
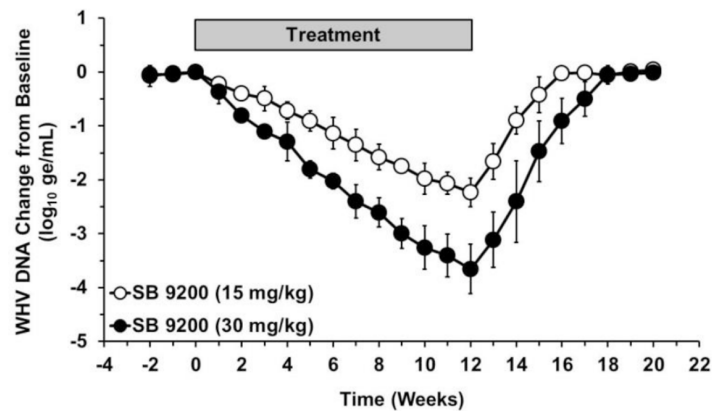


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# Direct triggering of RIG-I in infected hepatocytes

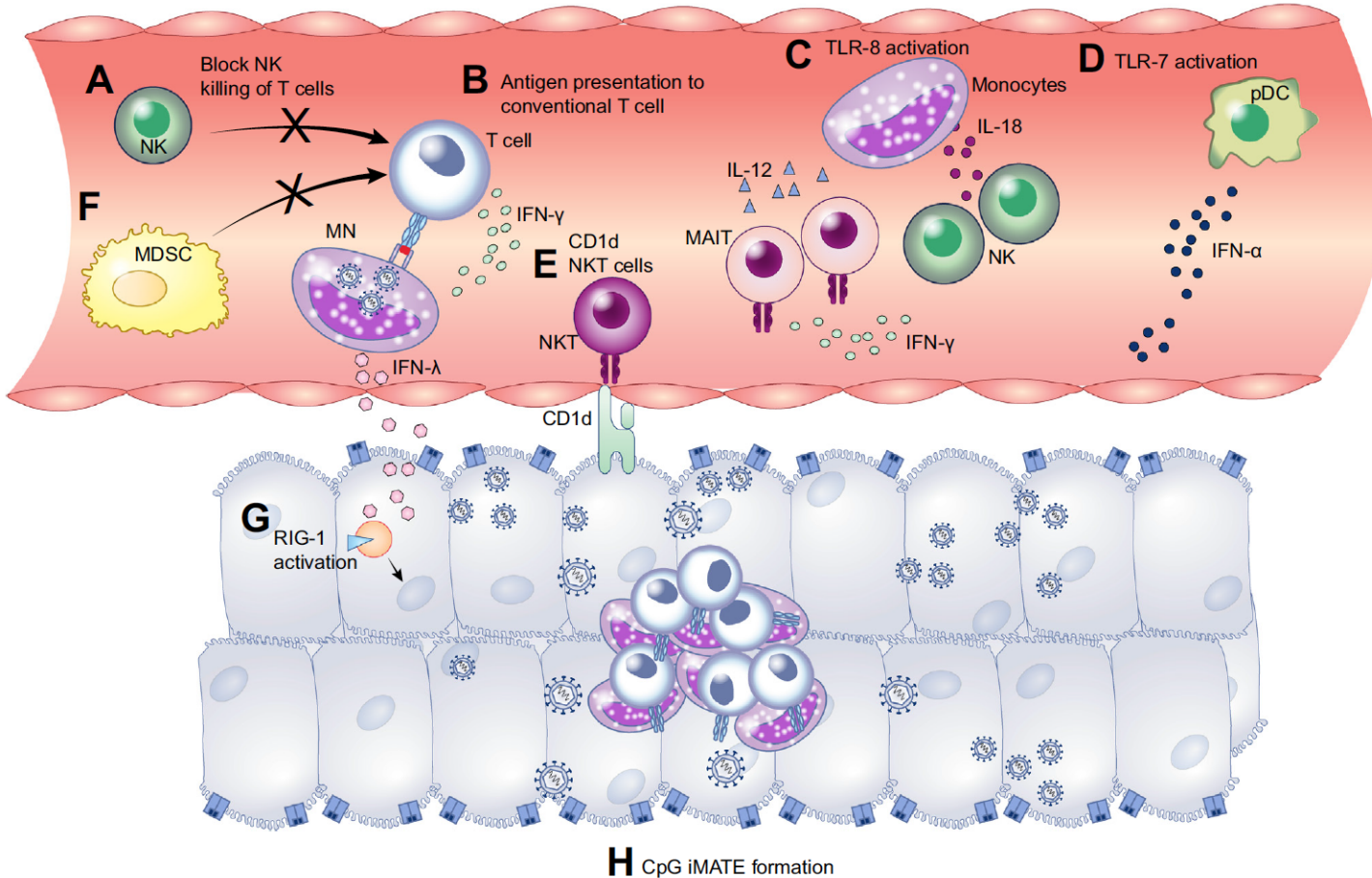


## Chronic Woodchuck Hepatitis Virus Infection model



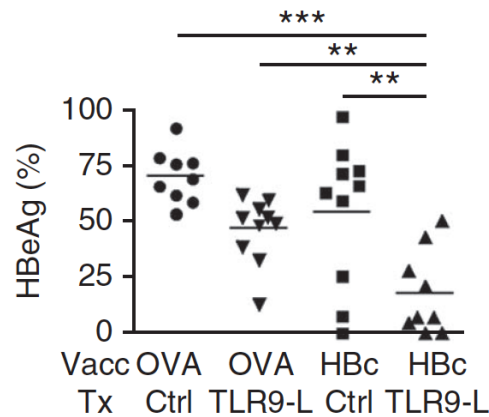
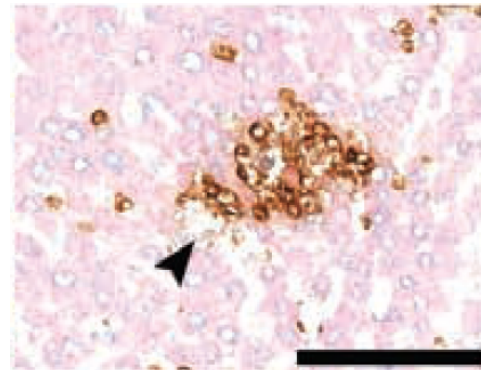
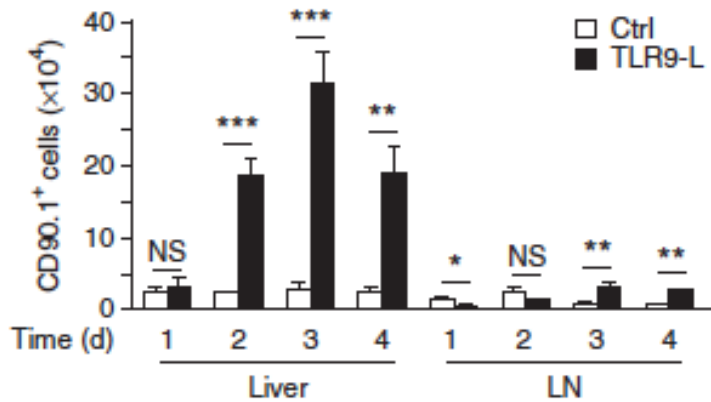
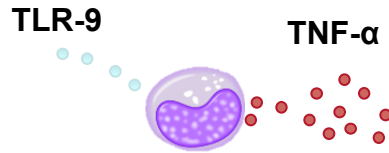
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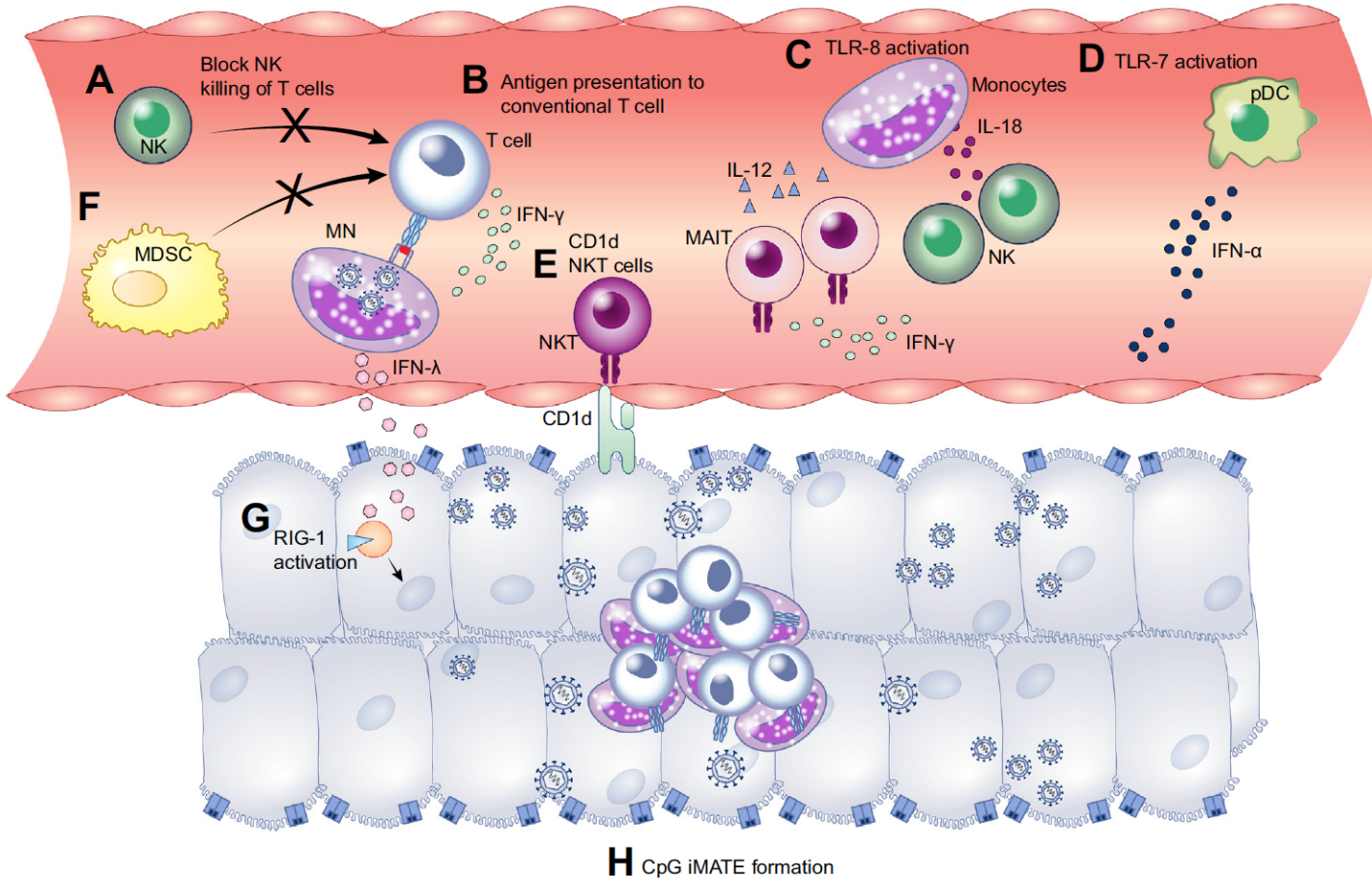


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# CpG induction of iMATEs



# Opportunities for Innate-targeted Immunotherapy



**Direct antiviral effect & potential environmental effect**

**Concern: Innate immunity is inherently non-specific**



# Potential Immunological Targets for HBV Therapy

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## Innate

- Blocking negative regulation
- Stimulating antiviral cytokine production

## Adaptive

- Therapeutically boost HBV-specific T cell immunity
- Checkpoint blockade to release negative regulation

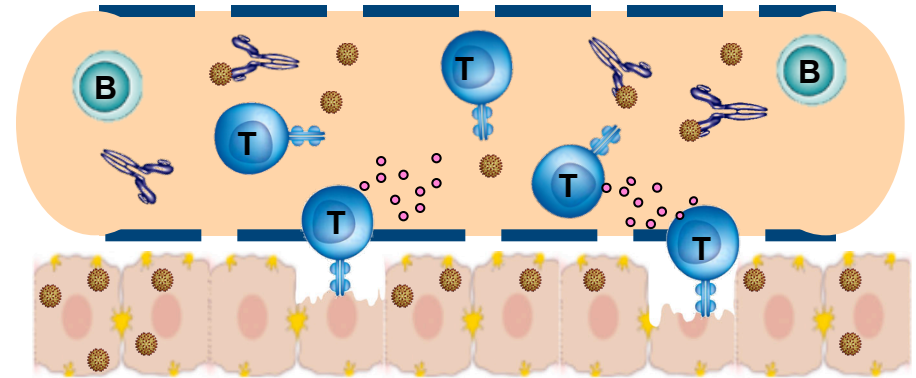
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# Post-infection Clearance of HBV Requires T cells

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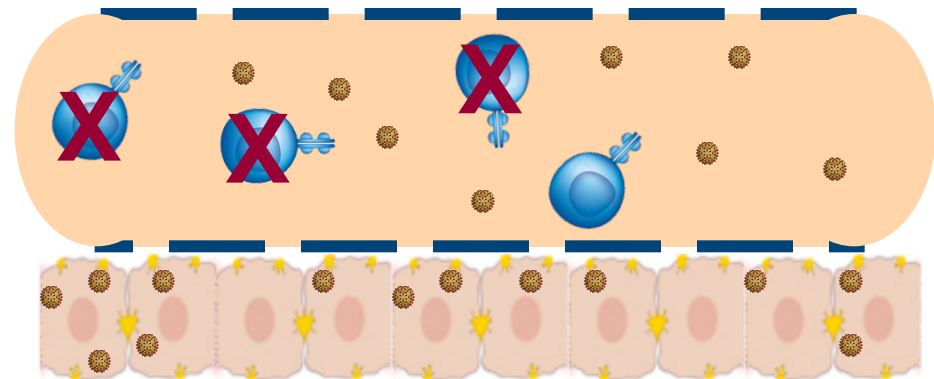
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  - c. Schurich Hepatology. 2011; 53: 1494–1503.



# Therapeutic Vaccination in Chronic HBV Infection

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## ➤ Re-tooled prophylactic vaccine

### ❖ Proliferative response – no viral clearance

- Vandepapelière, Vaccine. 2007 Dec;25(51):8585–97.
- Pol, J Hepatol. 2001 Jun;34(6):917–21.
- Couillin, J Infect Dis. 1999;180:15–26.
- Jung, Vaccine. 2002 Oct 4;20(29-30):3598–612.

## ➤ Lipopeptide vaccine with immunodominant epitope

### ❖ no T cell responses

- Heathcote, Hepatology. 1999 Aug;30(2):531–6.

## ➤ DNA vaccination

### ❖ Induction of transient T cell responses

### ❖ Transient drop in viral DNA

- Yang Journal of Viral Hepatitis 2012;19(8):581–93.
- Mancini-Bourgine, Vaccine. 2006 May 22;24(21):4482–9.
- Mancini-Bourgine, Hepatology. 2004;40(4):874–82.

## ➤ Immune Complexes + Alum

### ❖ Responses no better than alum alone

- Xu, J Hepatol. 2013 May.

**Transient responses and no significant induction of CD8 T cell immunity**

# Therapeutic Vaccination in Chronic HBV Infection

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- **ABX203 – AbiVax**
  - HBcAg & HBsAg virus-like particles (VLP)
  - Enrolling Phase IIb/III in chronic HBV patients on NUC treatment
  
- **GS-4774 – Globelimmune** (Gaggar et. al. Vaccine. 2014 Sep 3;32(39):4925-31)
  - Recombinant yeast expressing HBV X,S, and C antigen
  - Enrolling Phase II in chronic HBV patients +/- NUC treatment
  
- **TG1050 – Transgene** (Martin et. al. Gut. 2014 Nov 26 PMID: 2542905)
  - Adenovirus with modified/truncated HBV Core, Pol, Env fusion protein
  - Enrolling Phase I chronic HBV patients on NUC treatment
  
- **INO-1800 – Inovio**
  - DNA plasmids encoding HBsAg&HBcAg +/- IL-12 plasmid DNA delivered by electroporation
  - Enrolling Phase I NUC treated chronic HBV patients

# Potential Immunological Targets for HBV Therapy

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## Innate

- **Blocking negative regulation**
- **Stimulating antiviral cytokine production**

## Adaptive

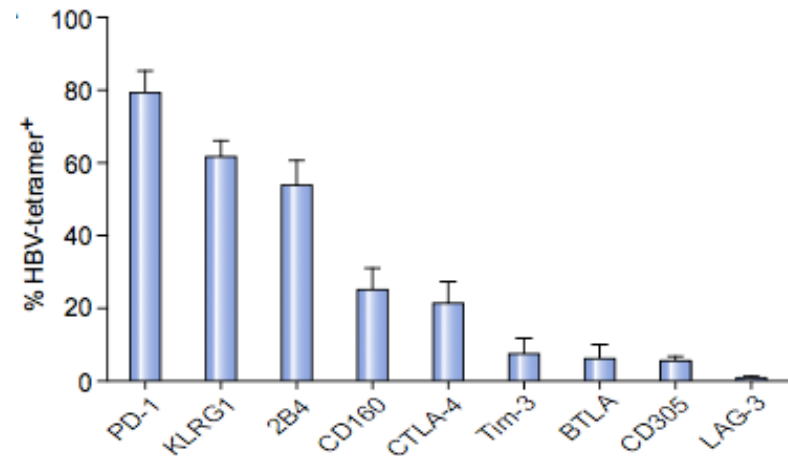
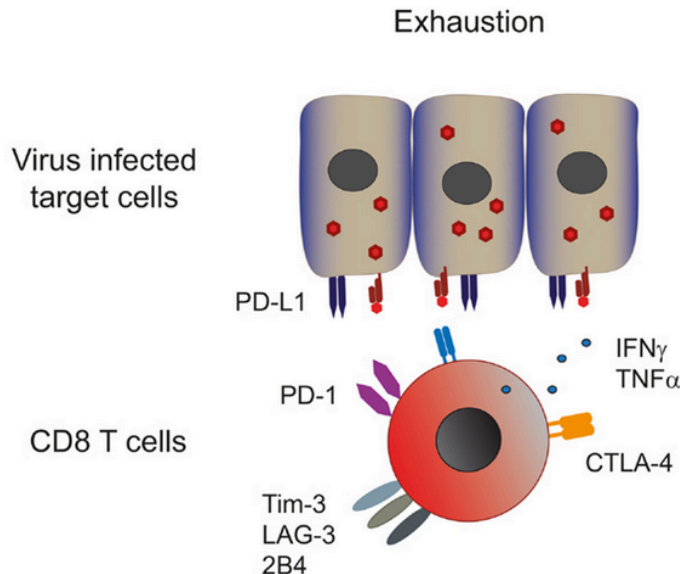
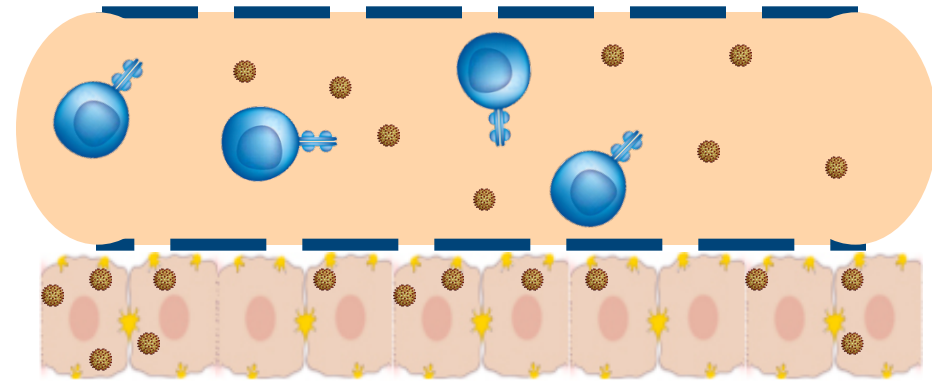
- **Therapeutically boost HBV-specific T cell immunity**
- **Checkpoint blockade to release negative regulation**

# Inhibition of HBV-specific T cell Function

## Chronic

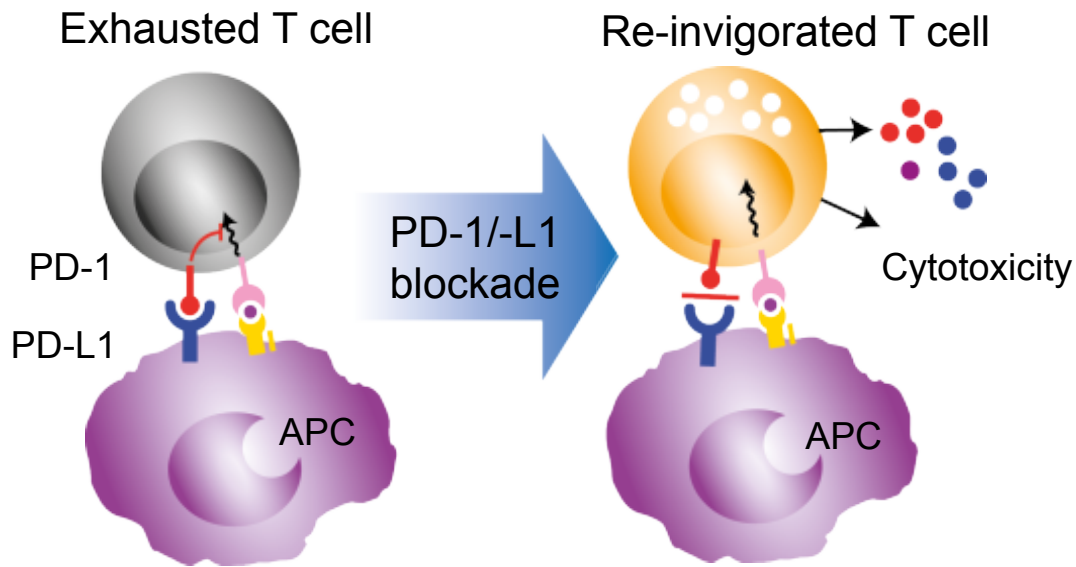
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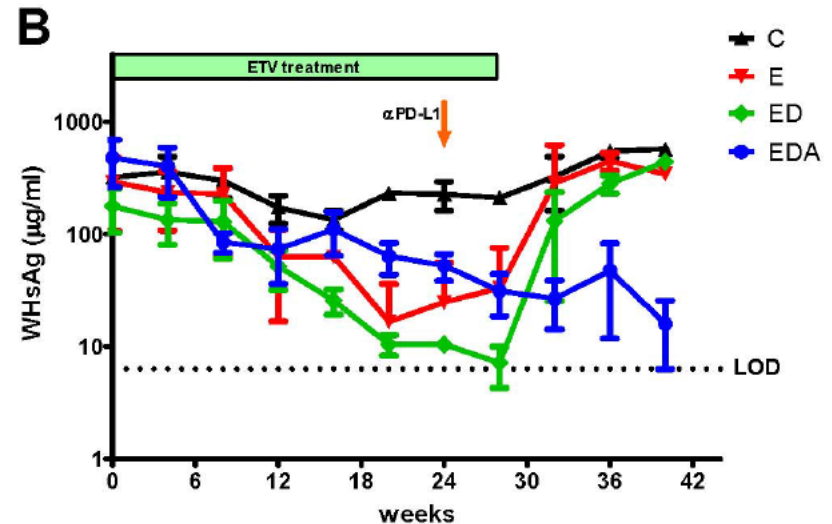
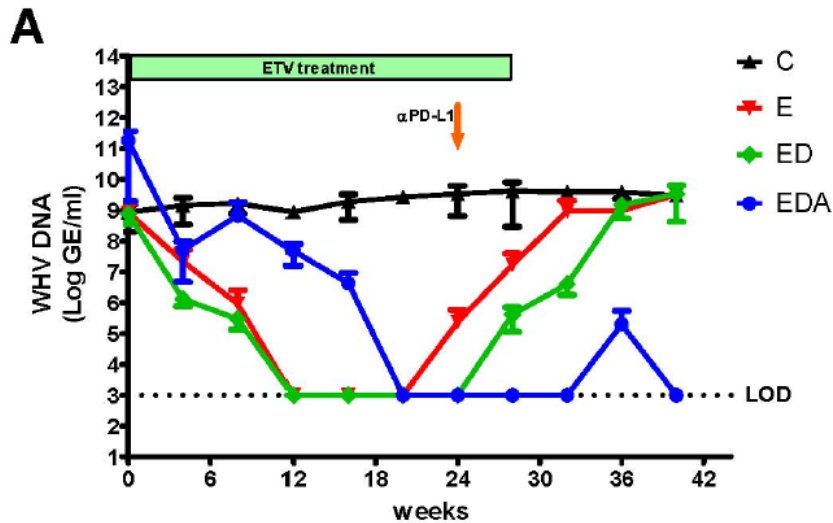
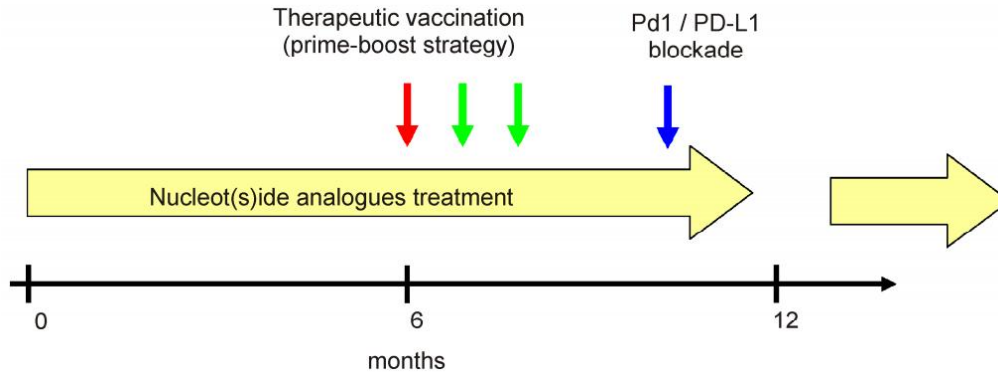


# Therapeutic vaccination with checkpoint modulation

*The NEW ENGLAND JOURNAL of MEDICINE*  
Safety and Activity of Anti-PD-L1 Antibody  
in Patients with Advanced Cancer  
N ENGL J MED 366;26 NEJM.ORG JUNE 28, 2012



# Therapeutic vaccination with checkpoint modulation





# Balance Checkpoint Inhibition with Co-stimulation

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## Co-inhibitory signals

~~PD-1~~  
~~CTLA-4~~  
~~Tim-3~~  
~~Lag-3~~

## Co-stimulatory signals

41BB  
OX-40  
ICOS  
IL-12

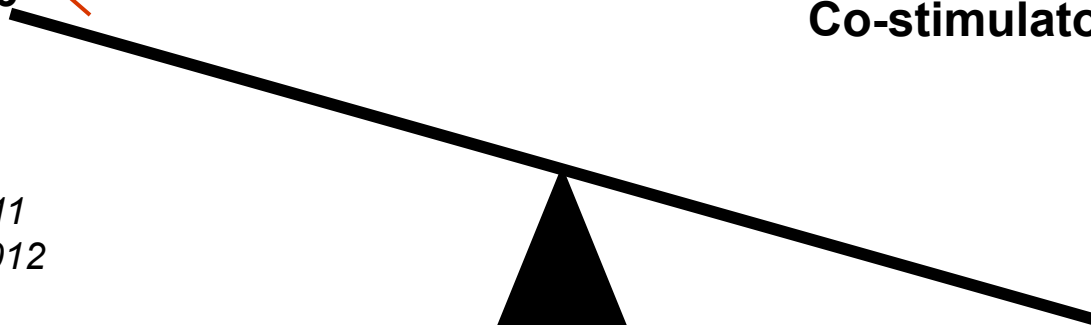
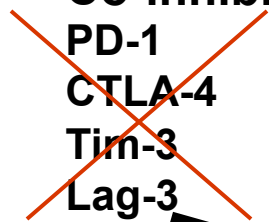
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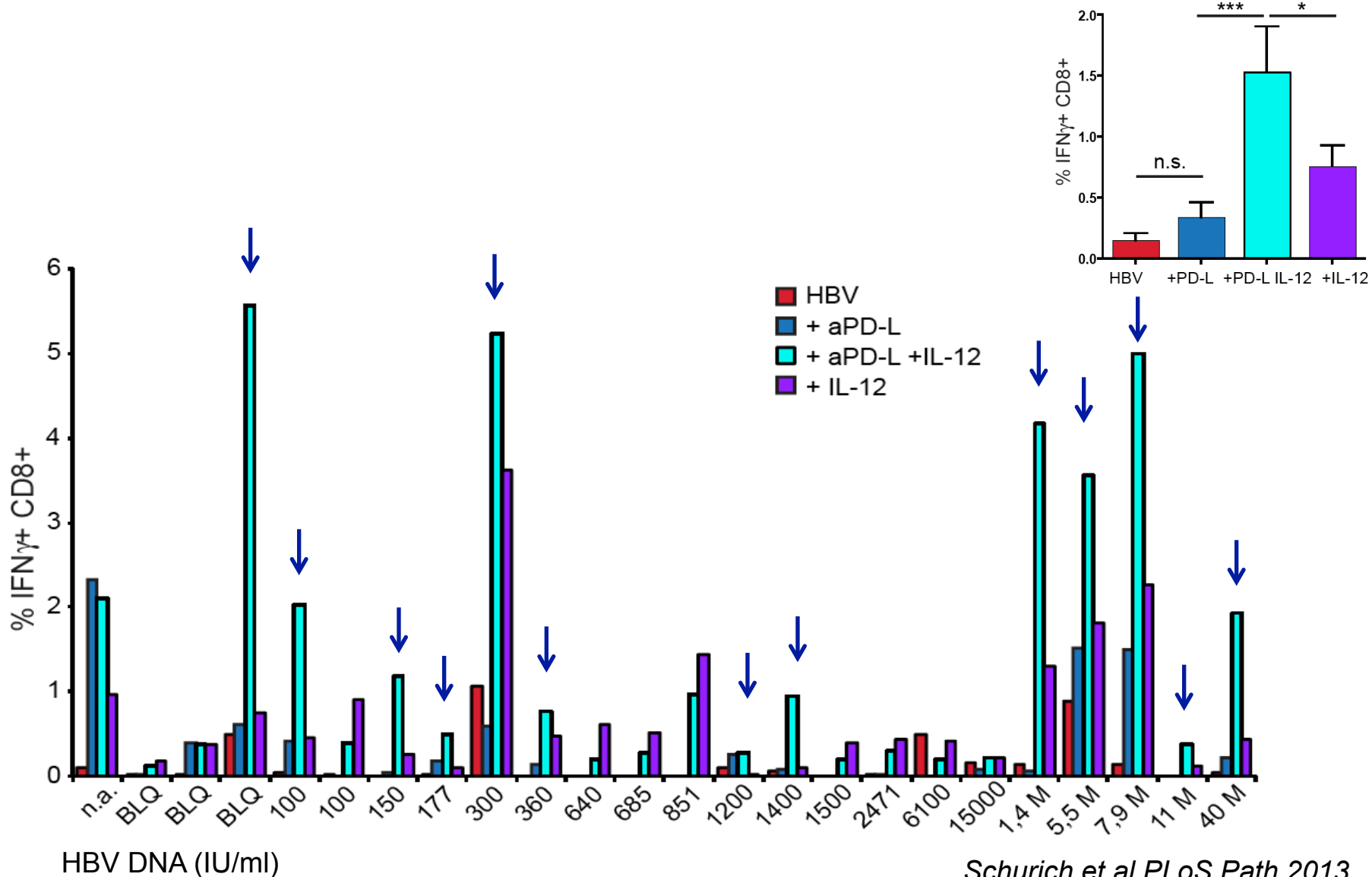
CD8 T cell



Revived



# PD-L blockade in combination with IL-12 Co-stimulation to Boost T cell recovery in vitro



# Environmental Modulation to Boost T cell immunity

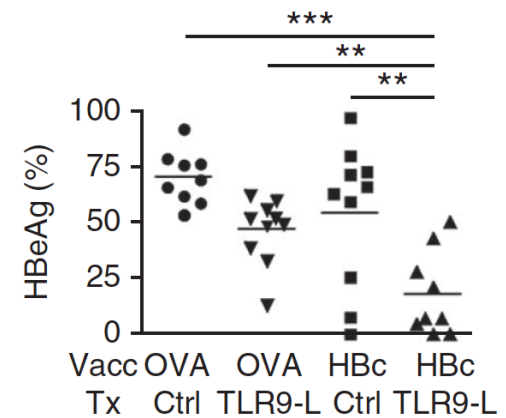
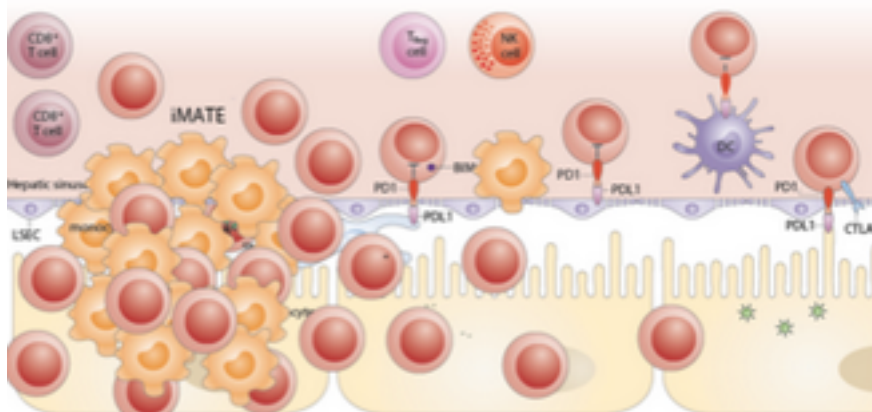
e.g. Combining therapeutic vaccination with TLR-9 agonist to induce i-mates

NATURE IMMUNOLOGY | ARTICLE



## Intrahepatic myeloid-cell aggregates enable local proliferation of CD8<sup>+</sup> T cells and successful immunotherapy against chronic viral liver infection

Li-Rung Huang, Dirk Wohlleber, Florian Reisinger, Craig N Jenne, Ru-Lin Cheng, Zeinab Abdullah, Frank A Schildberg, Margarete Odenthal, Hans-Peter Dienes, Nico van Rooijen, Edgar Schmitt, Natalio Garbi, Michael Croft, Christian Kurts, Paul Kubes, Ulrike Protzer, Mathias Heikenwalder & Percy A Knolle



# Potential Immunological Targets for HBV Therapy

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## Innate

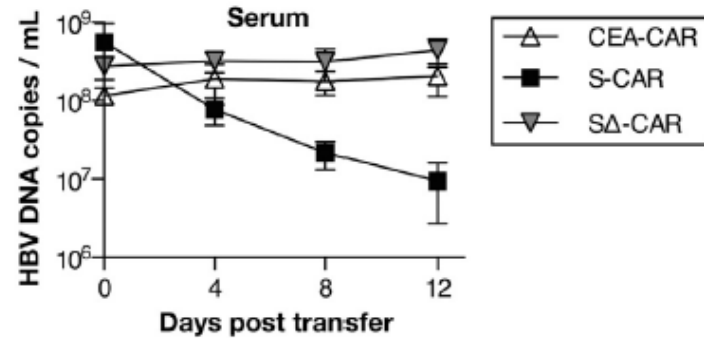
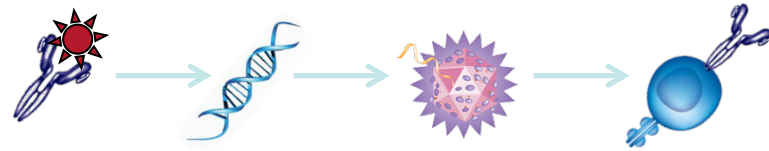
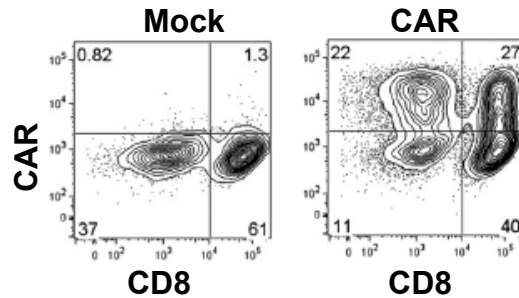
- **Blocking negative regulation**
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## Adaptive

- **Therapeutically boost HBV-specific T cell immunity**
- **Checkpoint blockade to release negative regulation**
- **What happens if adaptive immunity is beyond restoration?**

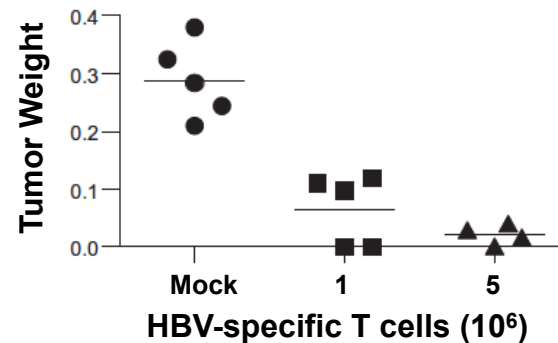
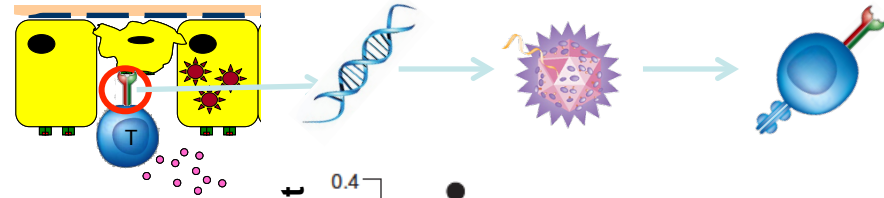
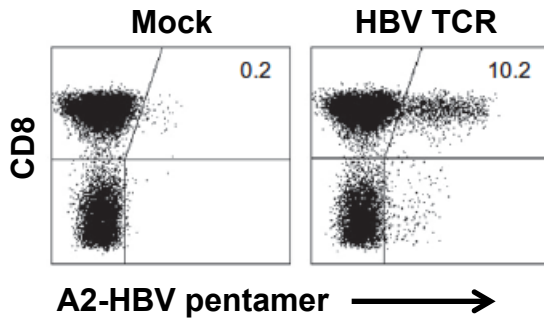
# Engineering anti-HBV Immunity

## Chimeric antigen receptor (CAR)



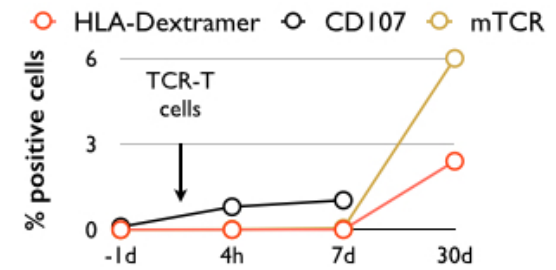
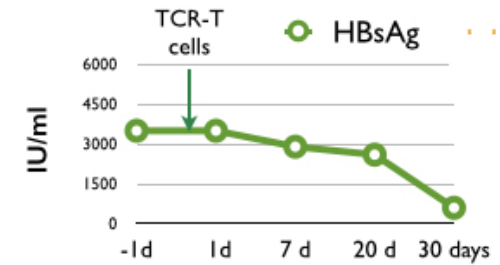
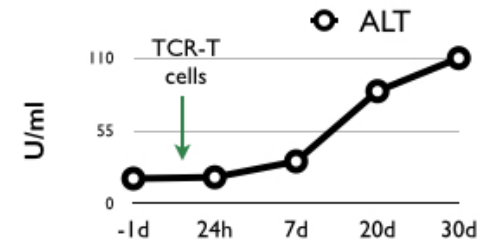
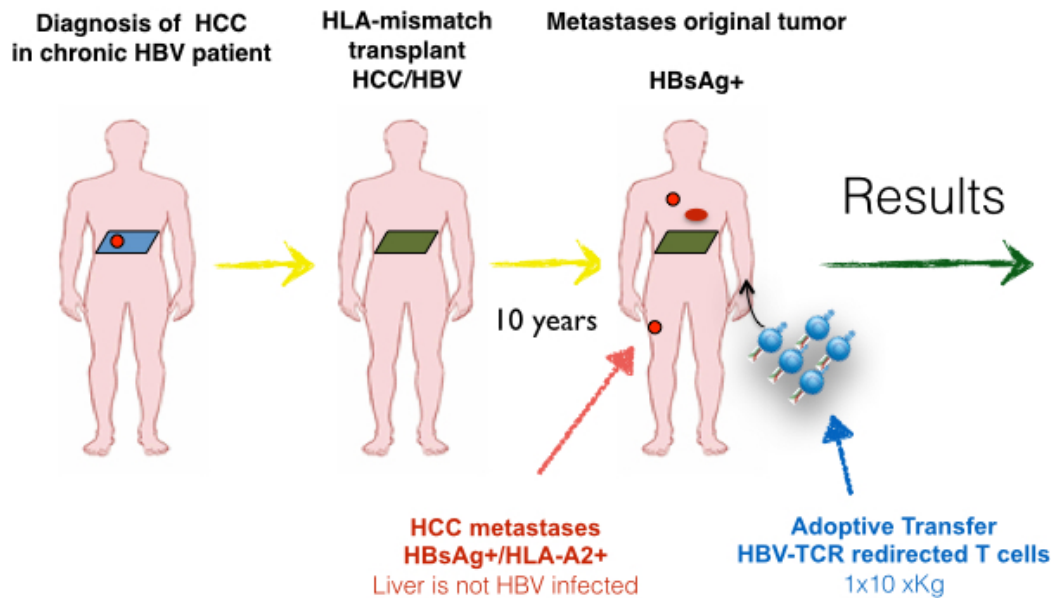
Krebs et. al. Gastroenterology. 2013 Aug;145(2):456-65.

## T cell receptor (TCR) gene therapy



Gehring et. al. J Hepatol. 2011 Jul 1;55(1):103-10.

# Engineering anti-HBV Immunity



# Potential Immunological Targets for HBV Therapy

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## Innate

- Blocking negative regulation of adaptive immunity
- Stimulating antiviral cytokine production through pattern recognition receptor targeted drugs
- Modulate the immunological environment to permit effective immunity

## Adaptive

- Therapeutically boost HBV-specific T cell immunity
- Checkpoint blockade +/- co-stimulation to release negative regulation
- Engineering HBV-specific immunity

***Coordinated, integrated immune response important***