The Liver as an Immune Organ

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Immunological Role of the Liver

Health

Disease

Tolerance
- dietary antigens
- environmental toxin
- bacterial products
- endotoxin
- allogeneic liver grafts without immunosuppression

Inflammation
- infection
- autoimmune disease

(Calne et al., Nature 1969)
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*(Calne et al., Nature 1969)*

**Inflammation**
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Cellular Anatomy of the Healthy Liver

Hepatocytes (70-80%)

Non-Hepatocytes (20-30%)

Endothelial Cells (~50%)

Kupffer Cells (~25%)

Lymphocytes (~25%)

Biliary Cells (~5%)

Stellate Cells (<1%)

NK Cells (~31%)

NKT Cells (~26%)

T Cells (~37%)

B Cells (~6%)

Mackay, Immunol Cell Biol 2002
How is Tolerance Maintained in the Liver?

Kupffer cells (liver - resident macrophages)

**Effect on Antigen-Presenting Cells:**
- ⇒ receptor-mediated antigen uptake ↓
- ⇒ MHC class II ↓
- ⇒ immunogenicity (DCs) ↓
- ⇒ CCR7 ↓ and migration to LN (DCs) ↓

**Effect on T cells:**
- ⇒ activation ↓
- ⇒ suppression

Knolle et al., J Hepatol 1995
Groux et al., J Exp Med 1996
How is Tolerance Maintained in the Liver?

Liver sinusoidal endothelial cells (LSEC)

IL-2, IFN-γ
IL-4, IL-10
cytotoxicity, IL-2, IFN-γ
apoptosis

CD4+ T cell
CD8+ T cell
Liver Sinusoid
Space of Dissé

IL-10

Effect on T cells:
⇒ IL-4/IL-10 secretion
⇒ IL-2/IFN-γ ↓
⇒ Loss of effector function

Knolle et al., Gastroenterology 1999
Limmer et al., Nat Med, 2000
Liver Tolerance

**Kupffer cells:**
- ↓ antigen uptake by antigen presenting cells
- ↓ migration of dendritic cells to lymph node
- ↓ immunogenicity of dendritic cells
- ↓ T cell activation via nitric oxide

**Liver sinusoidal endothelial cells (LSEC):**
- ↓ CD8 T cells: effector functions
- ↓ CD4 T cells: IL-2/IFN-γ → promote IL-4/IL-10 production

**Dendritic cells:**
- ↓ maturation due to cytokine milieu
- ↓ T cell proliferation and effector function (CTLA-4 and PD-1)
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Lymphocyte Populations - Liver versus Blood

Blood

- B cell (10%)
- NK cell (13%)
- NKT + γδT cell (5%)
- αβT cell (72%)

Liver

- B cell (6%)
- NK cell (31%)
- αβT cell (37%)
- NKT + γδT cell (26%)

=> Predominantly innate effector lymphocytes in the liver

Doherty, Immunol Rev 2000
Effect on NK cells:
- Activation ↑
- Cytotoxicity ↑
- IFN-γ release ↑

Effect on Neutrophils:
- Chemotaxis
- Phagocytosis of bacteria
- Recruitment of T cells
- T cell activation

Racanelli et al, Hepatology 2006
Inflammatory Response: Natural Killer Cells

- IFN-α: < 24h hours post infection
- Cytotoxicity ↑, TRAIL ↑
- Killing/apoptosis of infected cells
- MIP-α ↑, MIP-β ↑
- Infiltration of NK cells, T cells, iDCs, monocytes

- IFN-γ ↑, TNF-α ↑
- Antiviral effects
- Polarization of T cells (Th1)

- IL-12

- NK cell

< 24h hours post infection
Pro-inflammatory

- CD1d restricted pathogen recognition
- NKT cell
- IL-12
- IFN-γ↑, TNF-α↑, FasL↑
- apoptosis of infected cells
- antiviral effects

Anti-inflammatory

- IL-2
- IL-4, IL-10, IL-13
- downregulation of inflammation

Inflammatory Response: Natural Killer T Cells
Inflammatory Immune Response in the Liver

Infected hepatocyte

Kupffer cell

NK cell

NKT cell

CD4+ and CD8+ T cells

Pathogen

Migration to LN, Priming of T cells

Cytotoxicity, TNF-α

IL-12

MIP-1α

IFN-γ

FasL, TNF-α

IFN-α

IL-2

MIP-1α

IFN-γ

-> promotes TH1

IL-12

IFN-γ

Migration to the liver

Cytotoxicity, TNF-α

IL-12+IL-18

IFN-α

IFN-γ

???
Inflammatory Response - Stellate cells

Seki et al., Nature Med 2007
Liver Inflammation

1. The liver is enriched with NK and NKT cells.
   ⇒ Resident innate immune system

2. Infection starts an expanding cascade of events:
   ⇒ NK cell and NKT cell activation (within hours)
   ⇒ DC maturation and migration to lymph node
   ⇒ T cell priming and activation
   ⇒ Recruitment of T cells, NK cells and NKT cells

3. Cytokines/chemokines mediate and enhance this process.

4. Chronic activation of stellate cells results in induction of fibrosis.
What is the Role of the KIR/HLA System in Liver Disease?
Killer Cell Immunoglobulin-Like Receptor (KIR)

No regulation

NK cell

Target Cell

Tolerance

KIR

HLA

Inflammation

HLA↓
Killer Cell Immunoglobulin-Like Receptor (KIR) Diversity

Ligand: HLA-C

HLA-C Dimorphism

**HLA-C1**
- Ser\textsubscript{77}Asp\textsubscript{80}
- Cw1
- Cw3
- Cw7
- Cw8
- Cw12
- Cw13
- Cw14

**HLA-C2**
- Asp\textsubscript{77}Lys\textsubscript{80}
- Cw2
- Cw4
- Cw5
- Cw6
- Cw15
- Cw17

**KIR2DL3** → **NK cell inhibition** → **KIR2DL1**
Role of KIR/HLA-C in Liver Disease

**HLA-C1/C1 (KIR2DL3)**
- Spontaneous recovery
  - Partial protection
- Strong NK cell response

**Liver disease:**
- HCV infection
- Primary sclerosing cholangitis
- Hepatocellular carcinoma
- Liver transplant

**HLA-C2/C2 (KIR2DL1)**
- Chronic infection
  - Partial protection
  - Longer graft survival
- Weak NK cell response

Partial protection
Longer graft survival
Analysis of 352 spont. recovered and 685 chronic patients:

**HLA-C1** homozygous:

- Spontaneous recovery: 37.5%  \( P = 0.01, \ OR = 1.4 \)
- Chronic: 29.9%

**HLA-C2** homozygous:

- Spontaneous recovery: 14.5%  \( P = 0.02, \ OR = 0.67 \)
- Chronic: 20.2%

*Khakoo et al., Science 2004*
HCV AND KIR/HLA Genotypes (II)

Odds ratio for viral clearance

KIR2DL3/HLA-C1
- homozygous
- heterozygous
- null

X² for trend = 12.23
P = 0.0004

Khakoo SI et al., Science 2004
HLA-C Dimorphism

HLA-C1
- Ser_{77}Asp_{80}
- Cw1
- Cw3
- Cw7
- Cw8
- Cw12
- Cw13
- Cw14

KIR2DL3

NK cell inhibition

HLA-C2
- Asp_{77}Lys_{80}
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- Cw5
- Cw6
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- Cw17

KIR2DL1
Question

Does KIR2DL3/HLA-C1 homozygosity lead to stronger NK cell responses than KIR2DL1/HLA-C2 homozygosity?
Study Cohort

All donors have KIR haplotype A

(KIR2DL1, KIR2DL3, KIR2DL4, KIR3DL1, KIR3DL2, KIR2DS4)

HLA-C Group 1 homozygous (n = 13)

HLA-C Group 2 homozygous (n = 10)

Is There an Intrinsic Difference Between NK Cells of HLA-C1+ and HLA-C2+ Subjects?

PBMC

Depletion of all non-NK cells

Stimulation with K562 cells (HLA-negative targets)

1, 3, 6, 7, 9h

Cytotoxicity: $^{51}$Chromium release

Cytokine induction: IFN-γ release (EIA)

In the Absence of HLA-C on Target Cells, NK Cells from both Groups do not Differ.

HLA-C1 homozygous

HLA-C2 homozygous

=> Also no difference in IFN-γ release

Question

*Does the presence HLA-C result in a functionally different NK cell response?*
Is There a Difference Between NK Cells of HLA-C1+ and HLA-C2+ Subjects in the Presence of HLA-C?

PBMC

↓

CD3+ T cells

↓

Influenza A virus Infection

↓

3, 5, 7, 9, 12, 15, 17h

 Degranulation assay: CD107a expression

 Supernatant: IFN-γ release (EIA)

 Cells: % IFN-γ+ NK cells

KIR2DL3+ NK Cells Degranulate More in Response to Influenza than KIR2DL1+ NK Cells

KIR2DL3+ NK Cells Degranulate More in Response to Influenza than KIR2DL1+ NK Cells

NK Cells from HLA-C1+ Subjects Release IFN-γ Faster than HLA-C2+ Subjects in Influenza Infection

KIR2DL3+ NK Cells Respond Faster to Influenza than KIR2DL1+ NK Cells

Summary

Stimulation in absence of HLA-C:
• cytotoxicity against K562 \[C_1 = C_2\]
• IFN-\(\gamma\) production in response to K562 \[C_1 = C_2\]

Stimulation in presence of HLA-C:
• bulk NK cells:
  • kinetics of IFN-\(\gamma\) release \[C_1 > C_2\]
• HLA-C inhibited NK cell subset:
  • degranulation (CD107a assay) \[C_1 > C_2\]
  • kinetics of IFN-\(\gamma\) release \[C_1 > C_2\]
Conclusions

1. Liver-resident, non-parenchymal cells induce tolerance to antigens in the healthy liver.

2. During infection liver-resident NK and NKT cells respond within early hours to the pathogen and promote T cell priming and recruitment.

3. The level of NK cell inhibition is genetically influenced by KIR/HLA interactions. This may impact the course of liver diseases, such as viral hepatitis, tumor development and liver transplantation.
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