INFLAMMATION
AND IMMUNE-REGULATION

Nikoletta Argentou
Department of Immunology & Histocompatibility, University of Thessaly
Inflammation

Medzhitov R. *Nature* 2008; **454**: 428-435
Tissue damage causes release of vasoactive and chemotactic factors that trigger a local increase in blood flow and capillary permeability.

Permeable capillaries allow an influx of fluid (exudate) and cells.

Exudate (complement, antibody, C-reactive protein) leads to:

1. Phagocytes and antibacterial exudate destroy bacteria.
2. Phagocytes migrate to site of inflammation (chemotaxis).
3. Extravasation
4. Margination

Capillary
Inflammation

<table>
<thead>
<tr>
<th>Inducers</th>
<th>Sensors</th>
<th>Mediators</th>
<th>Effectors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipopolysaccharide</td>
<td>TLR4</td>
<td>TNF-α, IL-6 and PGE₂</td>
<td>Endothelial cells, hepatocytes, leukocytes, the hypothalamus, and others</td>
</tr>
<tr>
<td>Allergens</td>
<td>IgE</td>
<td>Vasoactive amines</td>
<td>Endothelial cells and smooth muscle cells</td>
</tr>
<tr>
<td>Monosodium urate crystals and calcium pyrophosphate dihydrate crystals</td>
<td>NALP3</td>
<td>IL-1β</td>
<td>Endothelial cells, hepatocytes, leukocytes, the hypothalamus, and others</td>
</tr>
<tr>
<td>Collagen</td>
<td>Hageman factor</td>
<td>Bradykinin</td>
<td>Endothelial cells and smooth muscle cells</td>
</tr>
</tbody>
</table>

- **Exogenous**
  - **Microbial**
    - PAMPs
    - Virulence factors
  - **Non-microbial**
    - Allergens
    - Irritants
    - Foreign bodies
    - Toxic compounds

- **Endogenous**
  - Cell derived
  - Tissue derived
  - Plasma derived
  - ECM derived

- Signals released from stressed, malfunctioning or dead cells and from damaged tissues
- Endogenous crystals
- Products of ECM breakdown

Medzhitov R. Nature 2008; 454: 428-435
Regulatory T cells

Normal

Immunologic Ignorance

Deletion

Inhibition

Suppression

Antigen-presenting cell

CD80

MHC

Peptide

Anatomical barrier

CD28

CD3

CD4

T-cell receptor

Activated T cell

T cell

Apoptosis

No activation

No activation

CD152

Fas ligand

Fas

Regulatory T cell
Regulatory T cells (Treg) are an essential component of the immune system, balancing necessary aggressiveness against foes with tolerance for self-constituents.


Dolganiuc A. J Leuc Biol 2008; 84: 614-622
Regulatory T cells- Foes?

Chronic Hepatic Infection

- Accumulation of Tregs in the liver of patients with chronic HBV infection
  
  Franzese et al, 2005

- Positive correlation between the HBV DNA level and the frequency of Tregs in the blood of chronically infected patients
  
  Stoop et al, 2007

- Presence of CD4⁺FOXP3⁺ T cells in the liver of chronically HCV infected persons
  
  Scott et al, 2007

Autoimmune Hepatic Diseases

- Reduced levels of circulating CD4⁺CD25<sup>high</sup> Tregs
  
  Longhi et al, 2004

- Reduced levels in correlation with higher disease activity or poorer prognosis
  
  Longhi et al, 2004; Boyer et al, 2004
Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice

Jeong M Kim¹, Jeffrey P Rasmussen¹ & Alexander Y Rudensky¹,²

- CD4⁺CD25⁺Foxp3 regulatory T Cells protect against T Cell-mediated fulminant hepatitis in a TGF-β-dependent manner in mice

Wei et al, 2008
Apoptosis: A major homeostatic mechanism

Physiological role
- Development
- Differentiation
- Immune regulation

Pathophysiologica role
- Tumorigenesis
- Autoimmune diseases
- Neurodegenerative diseases
Apoptosis and Liver Disease

Lethal effect of the anti-Fas antibody in mice

Apoptosis: Inhibitor or Instigator of Carcinogenesis?
Manning et al, *Cancer Invest* 1996

Apoptosis in human hepatocellular carcinoma and in liver cell dysplasia is correlated with p53 protein immunoreactivity.
Apoptosis and Liver

Canbay et al, Hepatology 2004
Apoptosis Pathway

Kilicarslan A et al, Turk J Gastroenterol 2009
Aim

Clarify the contribution of Tregs in pathogenesis of apoptosis-induced liver inflammation
Chronic liver diseases

1. chronic HBV infection at diagnosis; CHB/d
2. chronic HBV infection after treatment/relapse; CHB/nr
3. chronic HBV infection after treatment/remission; CHB/r
4. chronic HCV infection; CHC
5. Non Alcoholic Fatty Liver Disease; NAFLD
6. Autoimmune hepatic diseases; AD

Control group (with minimal disease)
<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>CHB/d</th>
<th>CHB/nr</th>
<th>CHB/r</th>
<th>CHC</th>
<th>NAFLD</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>8</td>
<td>34</td>
<td>5</td>
<td>23</td>
<td>19</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>5/3</td>
<td>16/18</td>
<td>3/2</td>
<td>18/5</td>
<td>14/5</td>
<td>7/5</td>
<td>2/6</td>
</tr>
<tr>
<td>Age (median, range)</td>
<td>58, 45-82</td>
<td>48, 21-64</td>
<td>57, 22-65</td>
<td>52, 23-67</td>
<td>43, 27-68</td>
<td>45, 21-71</td>
<td>57, 37-73</td>
</tr>
<tr>
<td>ALT (U/μL), (median, range)</td>
<td>32, 21-48</td>
<td>58, 15-1478</td>
<td>97, 32-332</td>
<td>27, 16-48</td>
<td>73, 32-213</td>
<td>54,15-141</td>
<td>41,31-212</td>
</tr>
<tr>
<td>Inflammation grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-0</td>
<td>8</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>I-1</td>
<td>—</td>
<td>8</td>
<td>—</td>
<td>18</td>
<td>2</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>I-2</td>
<td>—</td>
<td>16</td>
<td>4</td>
<td>4</td>
<td>10</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>I-3</td>
<td>—</td>
<td>8</td>
<td>1</td>
<td>—</td>
<td>7</td>
<td>—</td>
<td>5</td>
</tr>
<tr>
<td>I-4</td>
<td>—</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Fibrosis (median, range)</td>
<td>—</td>
<td>3, 0-6</td>
<td>4, 1-5</td>
<td>2, 0-4</td>
<td>3.0, 1-6</td>
<td>0.5, 0-2</td>
<td>6, 2-6</td>
</tr>
<tr>
<td>HAI-score (median, range)</td>
<td>—</td>
<td>6, 1-15</td>
<td>8, 5-11</td>
<td>2, 0-7</td>
<td>7, 2-12</td>
<td>2, 0-5</td>
<td></td>
</tr>
<tr>
<td>Viral load (median, range)</td>
<td>—</td>
<td>10^5 Meq/mL (0.007-521)</td>
<td>0.10 Meq/mL (0-44.5)</td>
<td>0 Meq/mL (0-0.008)</td>
<td>0.70 Meq/mL (0.10-6.25)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Biopsy Material**

**Department of Immunology & Histocompatibility – University of Thessaly**
<table>
<thead>
<tr>
<th>Immune Processes</th>
<th>Examined Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treg markers</td>
<td>FOXP3, IL10, TGFB</td>
</tr>
<tr>
<td>Immune-suppression</td>
<td>IL10, TGFB, PD1, PDL1, PDL2</td>
</tr>
<tr>
<td>Inflammation</td>
<td>IL1B, TNFA, IFNG,</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>FAS/FASL, TNFA, TRAIL</td>
</tr>
<tr>
<td>T cell markers</td>
<td>CD4, CD8</td>
</tr>
<tr>
<td>T cell effector restoration</td>
<td>IL2, IFNG</td>
</tr>
<tr>
<td>T cell exhaustion</td>
<td>PD1, PDL1, PDL2</td>
</tr>
<tr>
<td>Fibrotic Pathway</td>
<td>TGF-B (-B1,-B2,-B3), TGFBRs, SMADs (-2,-3,-4,-7), Activins (INHB-A,-B,-C,-E)</td>
</tr>
</tbody>
</table>
RNA extraction from **BIOPSY MATERIAL**

↓

**cDNA synthesis**

↓

**Real Time PCR**

Reference gene: **b2M**

Relative expression analysis: **ΔΔCT** method

Livak and Schmittgen, 2001
Liver Diseases vs control group

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chronic HCV hepatitis</th>
<th>Chronic HCV cirrhosis</th>
<th>Chronic HCV non-cirrhosis</th>
<th>Autoimmune diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60</td>
<td>55</td>
<td>15</td>
<td>(no. 8)</td>
</tr>
</tbody>
</table>

**Results-1**

**Foxp3, 48 KDa**

**GAPDH, 38 KDa**

**Densitometric Foxp3/GAPDH ratio**

| Caspase-3 | 5 | 0 | 0 | 0 | 3.19 ± 2.23 |
| TNF-α     | |  | -5 | 0 | 6.63 ± 9.11 |
| IFN-γ     | | | | | 8.43 ± 7.25 |
| IL-1β     | 1.37 ± 1.05 | | | | 1.15 ± 1.12 |

N = 19, 7, 14, 11, 8, 8, 8
Liver Diseases vs control group

- Apoptosis-induced inflammation, independently of the cause of tissue damage, may be responsible for the accumulation of Tregs in liver.
Research Article

Foxp3 Expression in Liver Correlates with the Degree but Not the Cause of Inflammation

Matthaios Speletas,¹ Nikoletta Argentou,¹ Georgios Germanidis,² Themistoclis Vasiliadis,³ Konstantinos Mantzoukis,² Kalliopi Patsiaoura,⁴ Pavlos Nikolaidis,² Vaios Karanikas,¹ Konstantinos Ritis,⁵ and Anastasios E. Gemenis¹

Acknowledgments

This study was supported by grants from the “Hellenic Society for the Study of the Liver” and the “Basic Research Scholarship Hrakleitos-II, National Strategic Reference Framework 2007–2013, Greece.”
Gene expressions with significant alteration of mRNA levels in the liver in CHB (Mann-Whitney U test)
Results-2

CHB at diagnosis vs CHB at remission

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Remission</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4</td>
<td></td>
</tr>
<tr>
<td>CD8</td>
<td></td>
</tr>
<tr>
<td>FOXP3</td>
<td></td>
</tr>
</tbody>
</table>
CHB at diagnosis vs CHB at remission

Diagnosis

Remission

PD1

PDL1
Gene expressions in the liver in CHB, according to the intensity of liver inflammation and fibrosis.
Correlations of FOXP3 and PD-1/PD-L1 with the other studied genes in the liver in CHB at diagnosis vs CHB at remission.
• The immunosuppressive liver environment is down-regulated in the maintained on-treatment long-term remission state and correlates with the intensity of liver inflammation, but not liver T-cell restoration.

“Liver FOXP3 and PD1/PDL1 expression is down-regulated in chronic HBV hepatitis on maintained remission, related to the degree of inflammation”.

*Frontiers in Immunology*
Overexpression of SMAD7 protects liver from TGFβ/Smad-mediated fibrogenesis

N. Argentou, * G. Germanidis, † E. Apostolou, † T. Vasiliadis, § P. Sideras, † A. E. Germenis* & M. Speletas*

DEPARTMENT OF IMMUNOLOGY & HISTOCOMPATIBILITY – UNIVERSITY OF THESSALY

Glasgow ECI 2012
Immunology 2012;137 (Suppl 1):506 (poster)
Manuscript in preparation

Error bar diagrams presenting the expression of genes, for which a significant alteration of their mRNA levels was observed. $p$ values in each diagram refer to Mann-Whitney $U$ test.
Boxplot diagrams presenting the expression of mediators of the TGFb/Activin signaling pathway according to the intensity of liver inflammation. *p* values in each diagram refer to Kruskal-Wallis *H* test.

- **SMAD7** mRNA expression, *p* = 0.029
- **ALK5** mRNA expression, *p* = 0.001
- **INHBC** mRNA expression, *p* = 0.035
- **ALK4** mRNA expression, *p* = 0.040

Glasgow ECI 2012
**Immunology 2012;137 (Suppl 1):506 (poster)**
Manuscript in preparation
Overexpression of SMAD7 protects liver from TGFβ/Smad-mediated fibrogenesis

N. Argentou,* G. Germanidis,† E. Apostolou,‡ T. Vasiliadis,§ P. Sideras,† A. E. Germenis* & M. Speletas*

- **SMAD7** overexpression might be a mechanism limiting the fibrogenic effect of TGFβ suggesting that its induction may provide a target for novel therapeutic approaches.

- The completion of Immunohistochemistry experiments for the analysis of TGF-b(-b1,-b2,-b3), and SMAD7 protein expression
Examination of the hypothesis

in another model of Chronic Inflammation

• **Osteoarthritis of hip and knee**
  
  27 patients (3M/24F); median age 74 years
  
  *Normal synovium*; 16 patients (3M/13F)
  
  *Hypertrophic synovium*; 6 patients (6F)
  
  *Atrophic synovium*; 5 patients (5F)

• **Control group**
  
  5 patients (3M/2F); median age 85 years
Results

DEPARTMENT OF IMMUNOLOGY & HISTOCOMPATIBILITY – UNIVERSITY OF THESALY

p<0.001

FASL mRNA expression

TRAIL mRNA expression

Controls  Normal  Hypertrophic  Atrophic

p=0.018

R² Linear=0.351

FAS mRNA expression

p=0.001

R² Linear=0.588

FASL mRNA expression

p<0.001

R² Linear=0.161

TRAIL mRNA expression
Collaborating Groups

• A’ Department of Internal Medicine, A.H.E.P.A Hospital, Aristotle University of Thessaloniki

• Gastroenterology and Hepatology Division, Hippokration Hospital, Aristotle University of Thessaloniki

• Department of Pathology, AHEPA Hospital, Aristotle University of Thessaloniki

• Center of Immunology and Transplantation, Biomedical Research Foundation, Academy of Athens

• A’ Department of Internal Medicine, Medical School, Democritus University of Thrace,

• Department of Orthopaedic Surgery and Musculoskeletal Trauma, University General Hospital of Larissa, University of Thessalia
Thank you
for your attention...