

5^ο Συνέδριο Παθολογίας Κεντρικής Ελλάδος

με διεθνή συμμετοχή

28-30 Μαρτίου 2013, Λάρισα

Οργάνωση:



ΙΝΣΤΙΤΟΥΤΟ ΕΣΩΤΕΡΙΚΗΣ
ΠΑΘΟΛΟΓΙΑΣ ΚΑΙ ΗΠΑΤΟΛΟΓΙΑΣ



ΠΑΘΟΛΟΓΙΚΗ ΚΛΙΝΙΚΗ ΚΑΙ ΟΜΟΝΥΜΟ ΕΡΕΥΝΗΤΙΚΟ ΕΡΓΑΣΤΗΡΙΟ
ΠΑΝΕΠΙΣΤΗΜΙΟΥ ΘΕΣΣΑΛΙΑΣ
Διευθυντής: Καθηγητής Γ. Ν. Νταλέκος

Ξενοδοχείο: Larissa Imperial

Υπό την αιγίδα:



Ιατρικής Σχολής Πανεπιστημίου Θεσσαλίας



Ελληνικής Εταιρείας Εσωτερικής Παθολογίας (Ε.Ε.Ε.Π.)



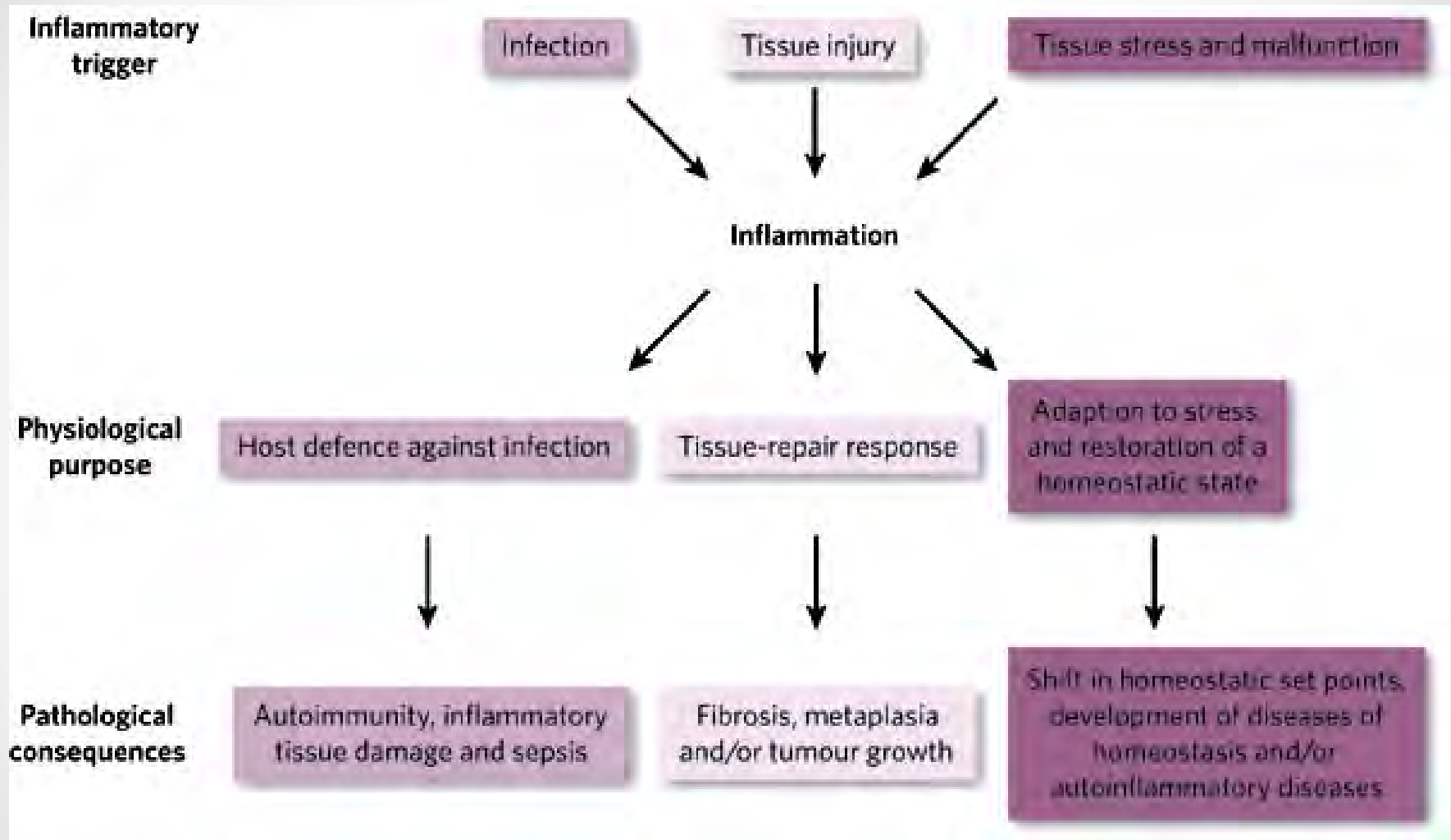
Εταιρείας Παθολογίας Βορείου Ελλάδος (Ε.Π.Β.Ε.)

<http://www.internalmedicine-uth.gr>

INFLAMMATION AND IMMUNE-REGULATION

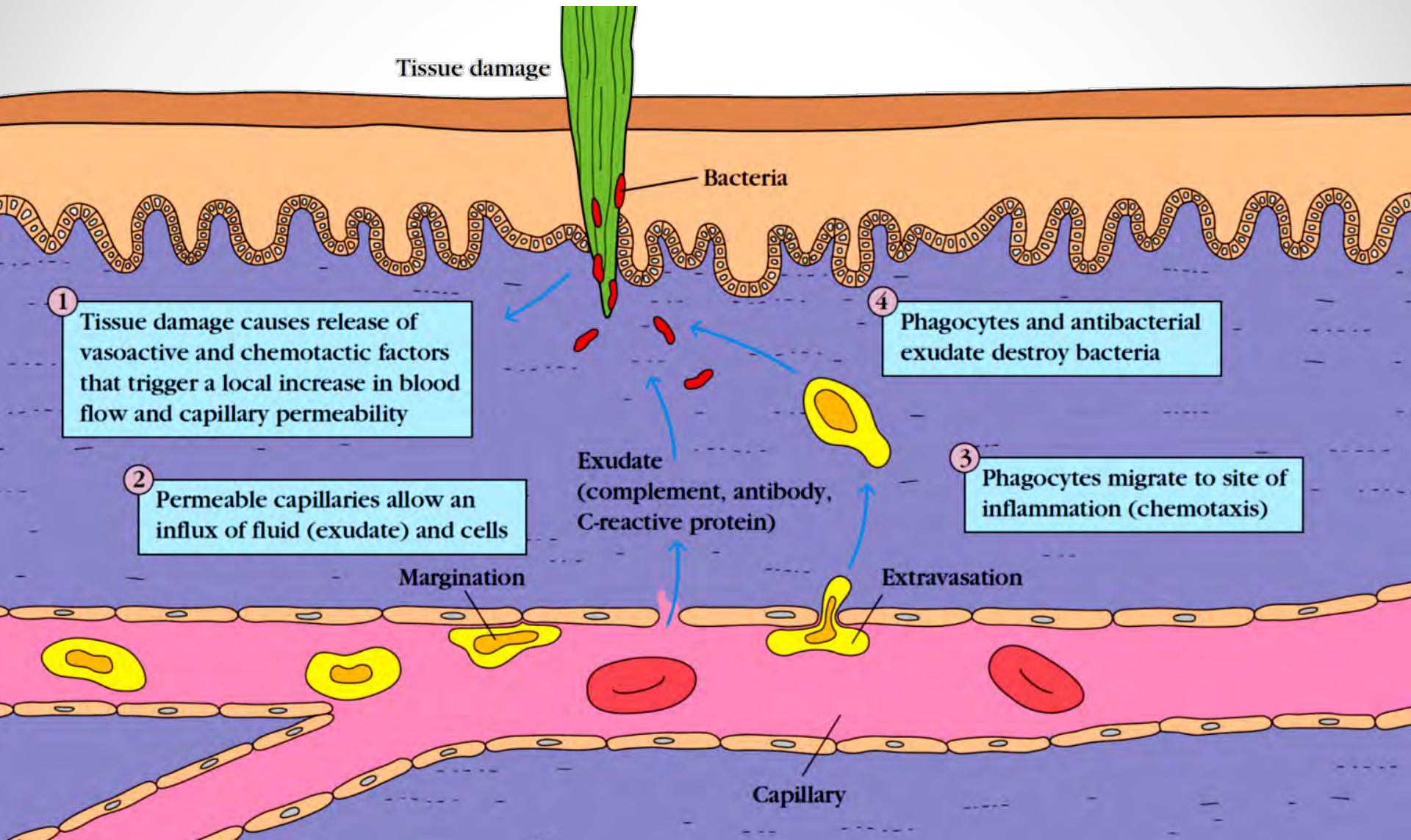
Nikoletta Argentou

**Department of Immunology &
Histocompatibility, University of Thessaly**



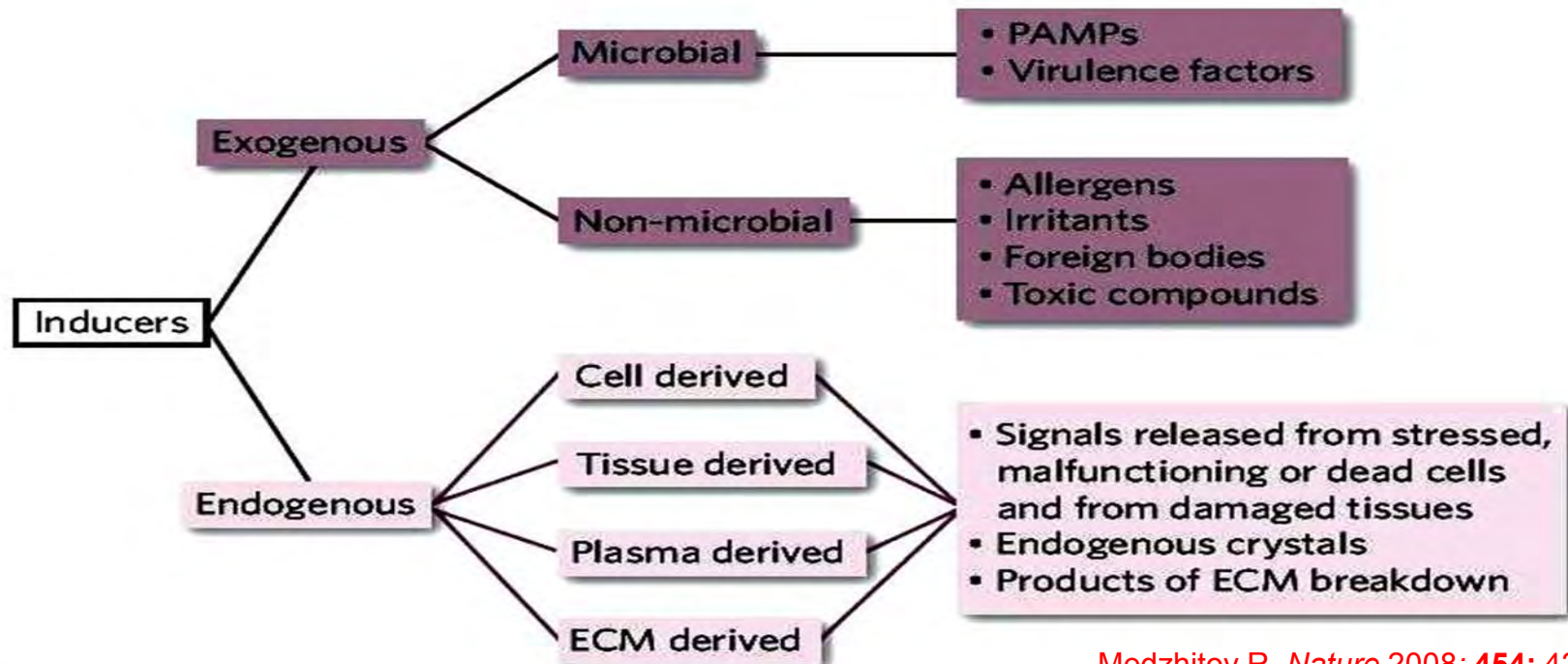
Medzhitov R. *Nature* 2008; **454**: 428-435





Inducers → Sensors → Mediators → Effectors

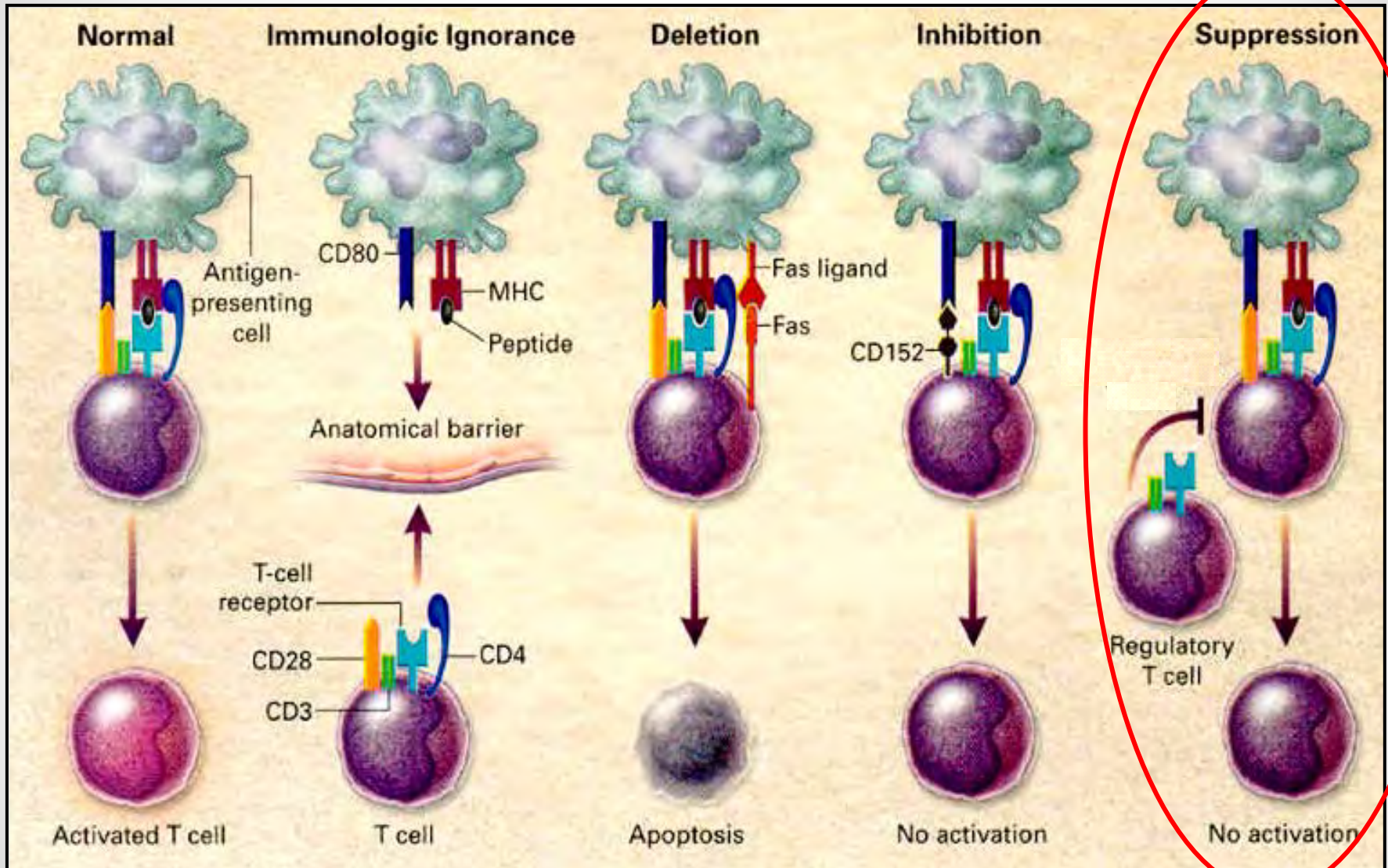
Lipopolysaccharide	TLR4	TNF- α , IL-6 and PGE ₂	Endothelial cells, hepatocytes, leukocytes, the hypothalamus, and others
Allergens	IgE	Vasoactive amines	Endothelial cells and smooth muscle cells
Monosodium urate crystals and calcium pyrophosphate dihydrate crystals	NALP3	IL-1 β	Endothelial cells, hepatocytes, leukocytes, the hypothalamus, and others
Collagen	Hageman factor	Bradykinin	Endothelial cells and smooth muscle cells



Medzhitov R. *Nature* 2008; **454**: 428-435



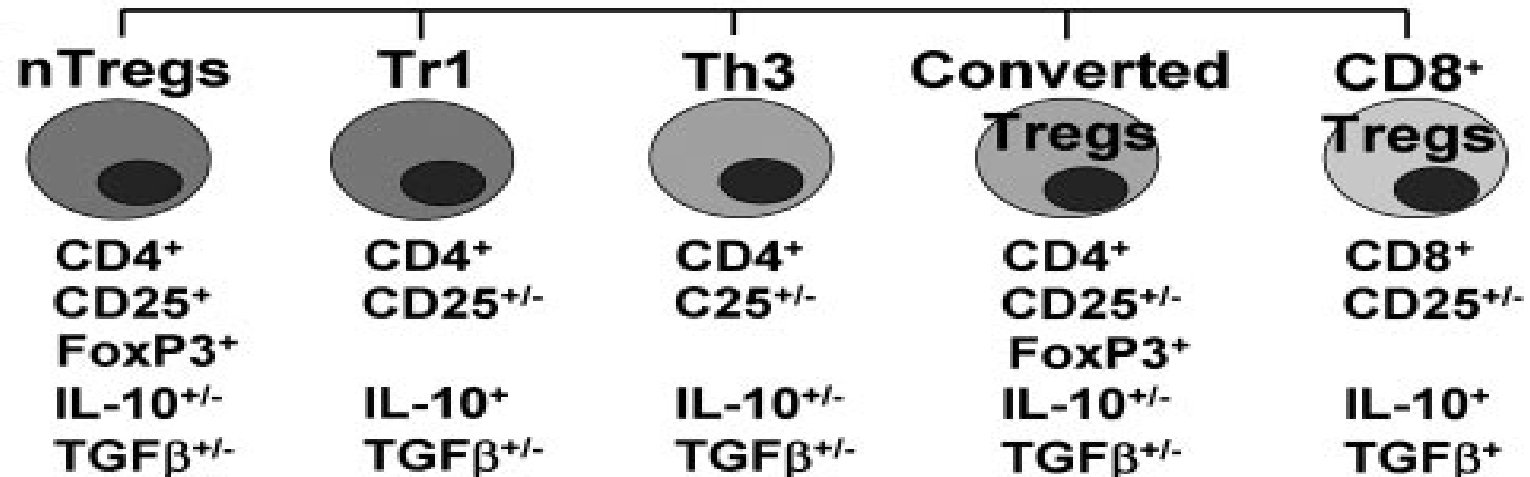
Regulatory T cells



Regulatory T cells (Treg)

are an essential component of the immune system,
balancing necessary aggressiveness against foes
with tolerance for self-constituents

Sakaguchi S. *Annu Rev Immunol* 2004; **22**: 531-562

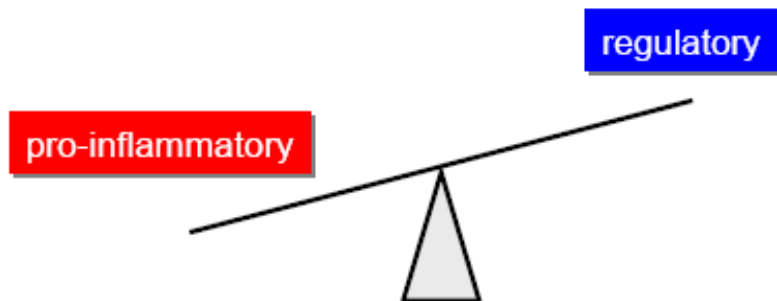


Dolganiuc A. *J Leuc Biol* 2008; **84**: 614-622

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^{high} 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

Kim et al, *Nature Immunol* 2007;8: 191-197

Jeong M Kim¹, Jeffrey P Rasmussen¹ & Alexander Y Rudensky^{1,2}



- 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

Pathophysiological
role

Tumorigenesis

Autoimmune diseases

Neurodegenerative diseases



Apoptosis and Liver Disease

Christian Rust, MD, Gregory J. Gores, MD

Am J Med. 2000;108:567–574.

Lethal effect of the anti-Fas antibody in mice

Ogasawara et al, *Nature* 1993

Apoptosis: Inhibitor or Instigator of Carcinogenesis?

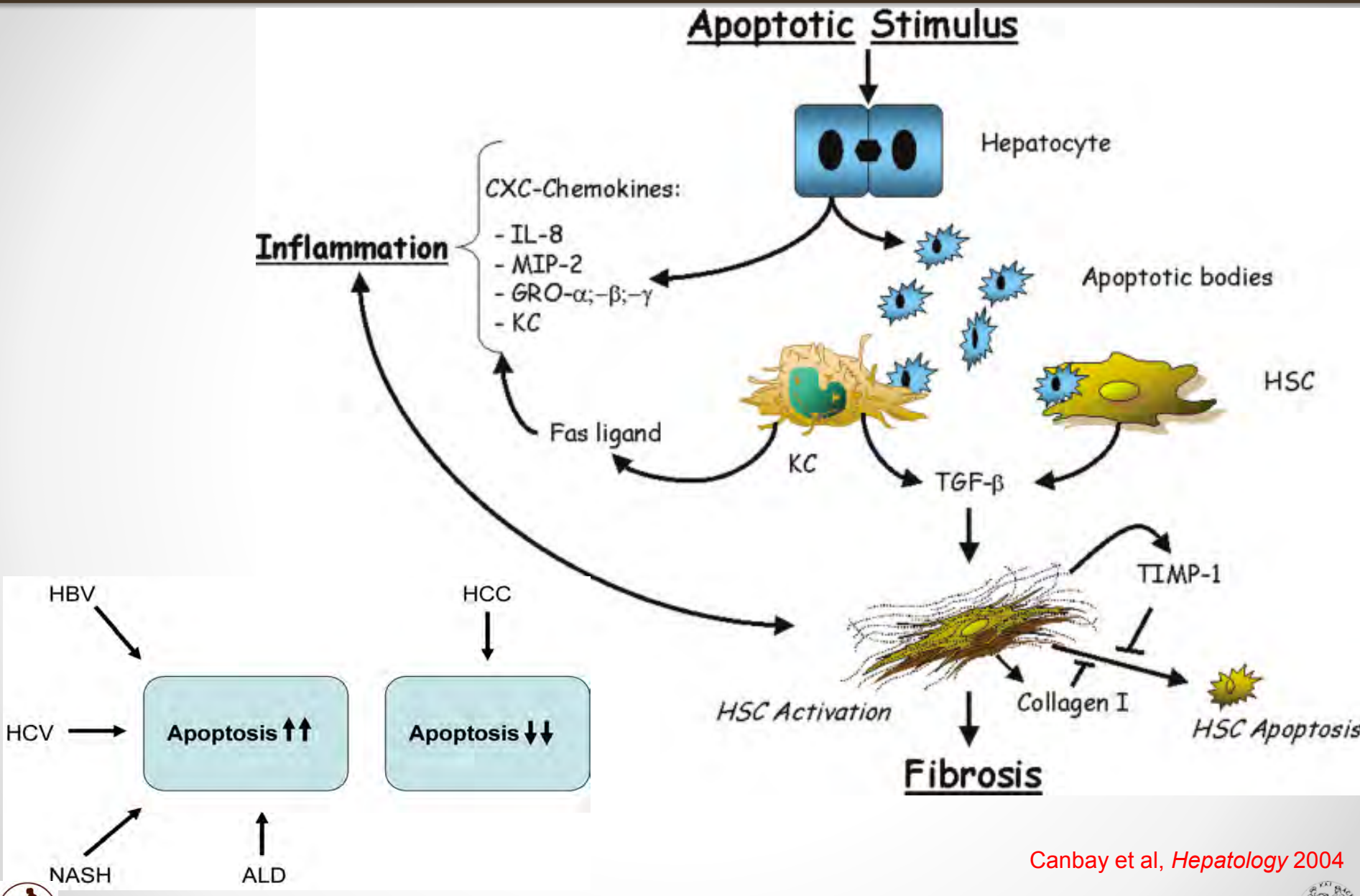
Manning et al, *Cancer Invest* 1996

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

Zhao et al, *J Clin Pathol* 1997



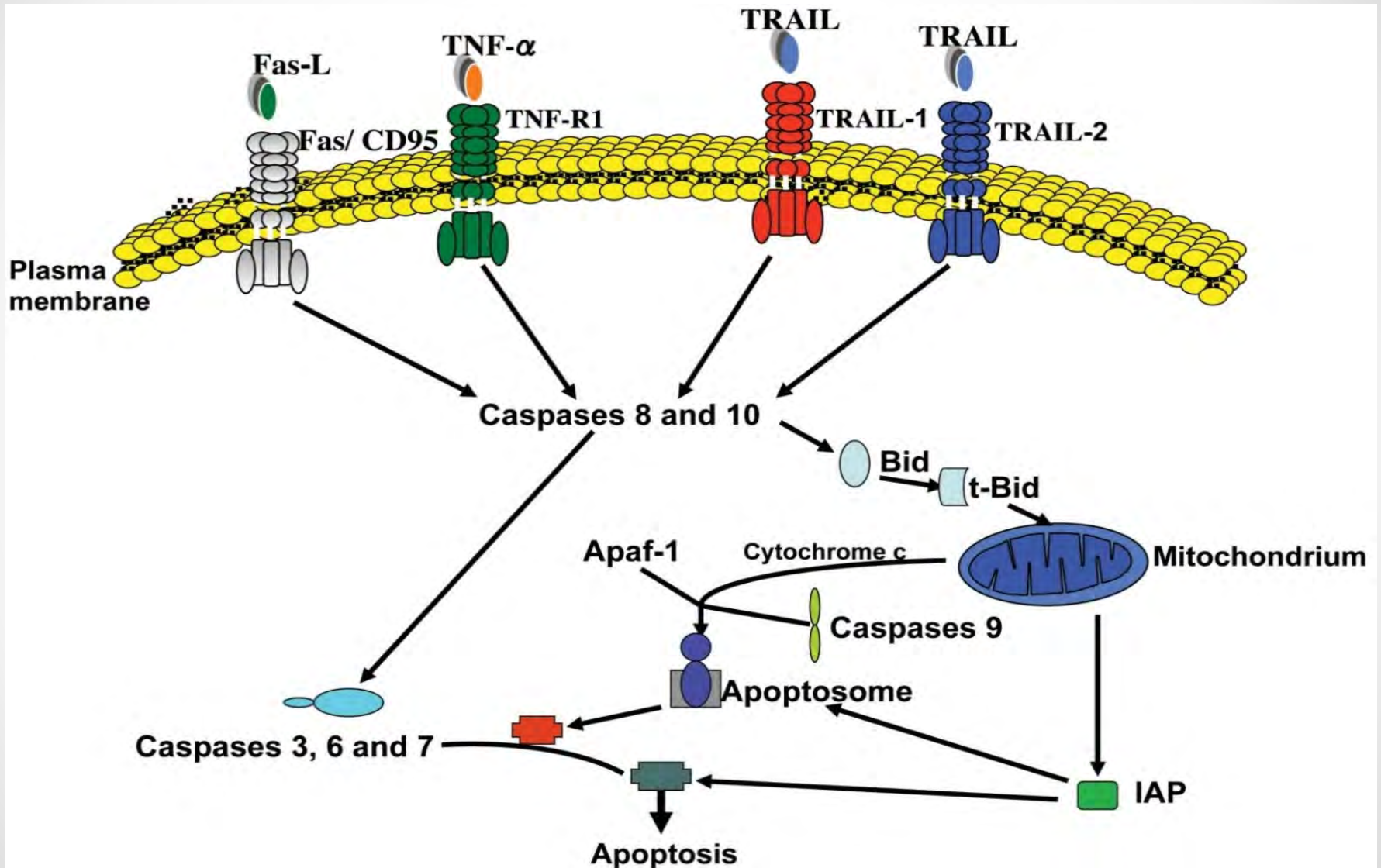
Apoptosis and Liver



Canbay et al, *Hepatology* 2004



Apoptosis Pathway



Kilicarslan A et al, *Turk J Gastroenterol* 2009



**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)



Biopsy Material

	Controls	CHB/d	CHB/nr	CHB/r	CHC	NAFLD	AD
No	8	34	5	23	19	12	8
Sex (M/F)	5/3	16/18	3/2	18/5	14/5	7/5	2/6
Age (median, range)	58, 45-82	48, 21-64	57, 22-65	52, 23-67	43, 27-68	45, 21-71	57, 37-73
AST (U/ μ L), (median, range)	42, 36-45	48, 17-1969	49, 32-277	24, 15-51	48, 24-237	32, 19-70	49,25-225
ALT (U/ μ L), (median, range)	32, 21-48	58, 15-1478	97, 32-332	27, 16-48	73, 32-213	54,15-141	41,31-212
Inflammation grade							
I-0	8	—	—	1	—	6	
I-1	—	8	—	18	2	5	2
I-2	—	16	4	4	10	1	—
I-3	—	8	1	—	7	—	5
I-4	—	2	—	—	—	—	1
Fibrosis (median, range)	—	3, 0-6	4, 1-5	2, 0-4	3.0, 1-6	0.5, 0-2	6, 2-6
HAI-score (median, range)	—	6, 1-15	8, 5-11	2, 0-7	7, 2-12	2, 0-5	
Viral load (median, range)	—	10 ⁵ Meq/mL (0.007-521)	0.10 Meq/mL (0-44.5)	0 Meq/mL (0-0.008)	0.70 Meq/mL (0.10-6.25)		



Immune Processes	Examined Molecules
Treg markers	FOXP3, IL10, TGFB
Immune-suppression	IL10, TGFB, PD1, PDL1, PDL2
Inflammation	IL1B, TNFA, IFNG,
Apoptosis	FAS/FASL, TNFA, TRAIL
T cell markers	CD4, CD8
T cell effector restoration	IL2, IFNG
T cell exhaustion	PD1, PDL1, PDL2
Fibrotic Pathway	TGF-B (-B1,-B2,-B3), TGFBRs, SMADs (-2,-3,-4,-7), Activins (INHB-A,-B,-C,-E)



RNA extraction from **BIOPSY MATERIAL**

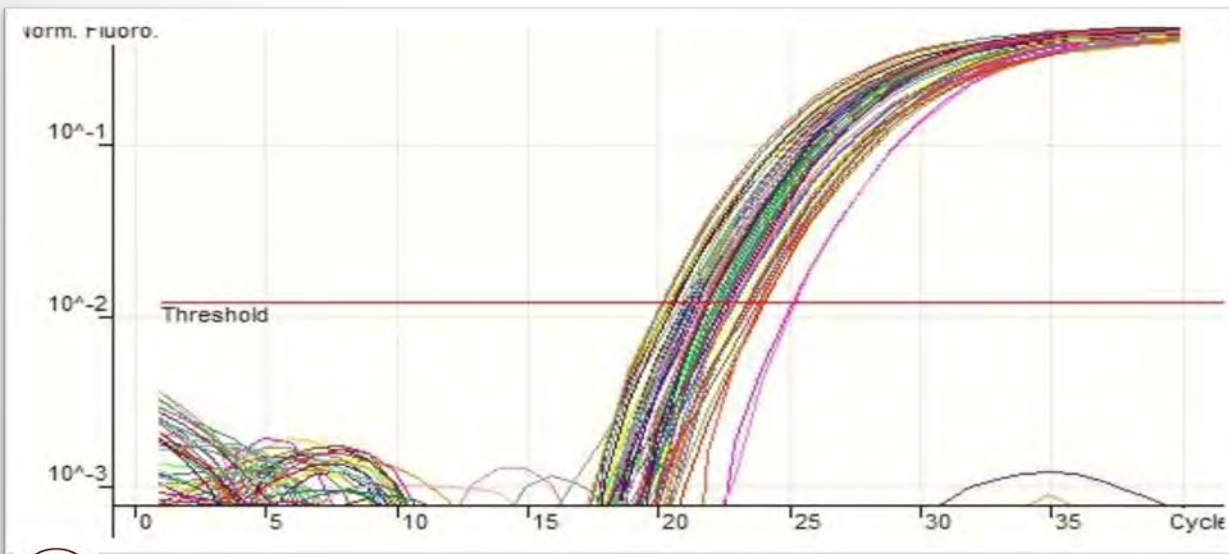


cDNA synthesis



Real Time PCR

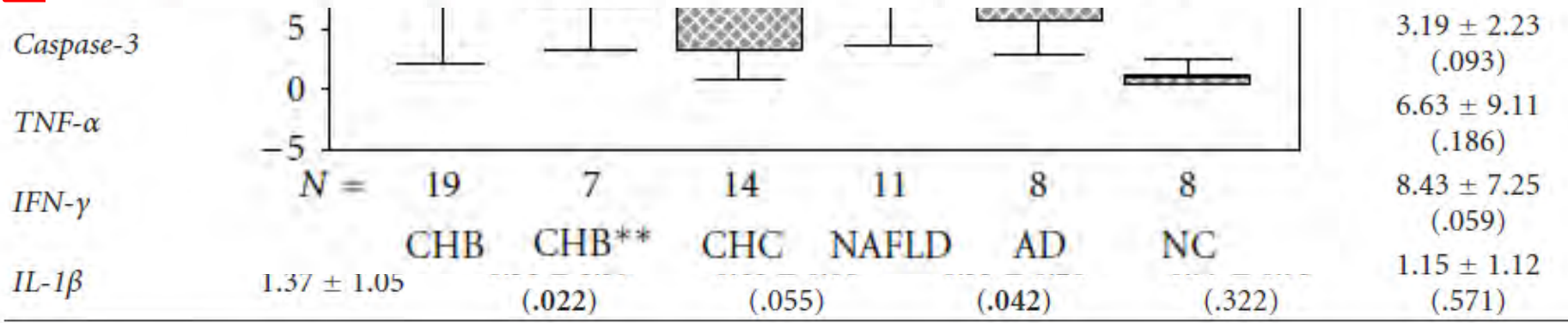
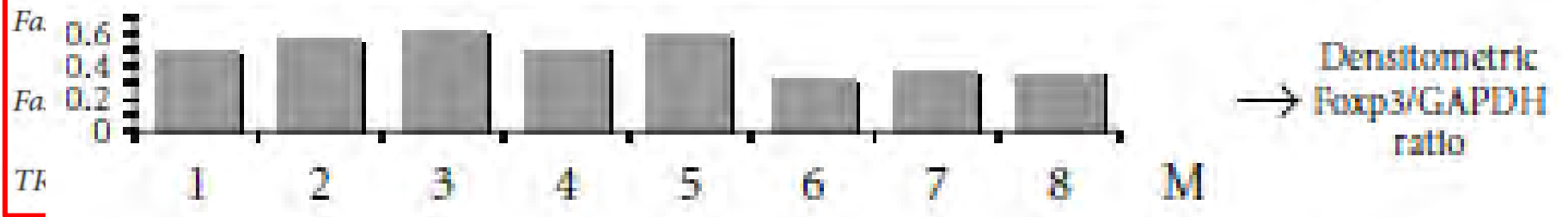
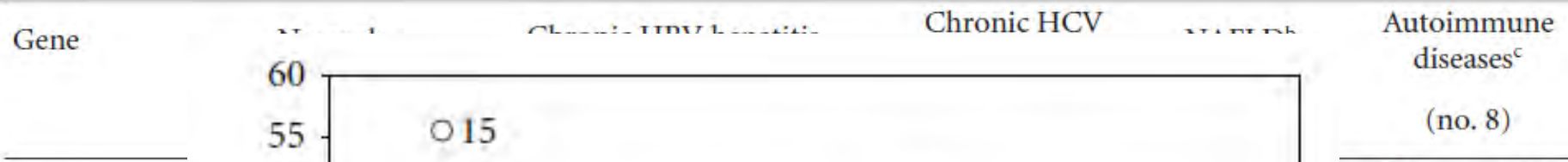
Reference gene: b2M



Relative expression
analysis: $\Delta\Delta CT$ method

Livak and Schmittgen, 2001





- **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

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2011, Article ID 827565, 9 pages
doi:10.1155/2011/827565

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. Germenis¹



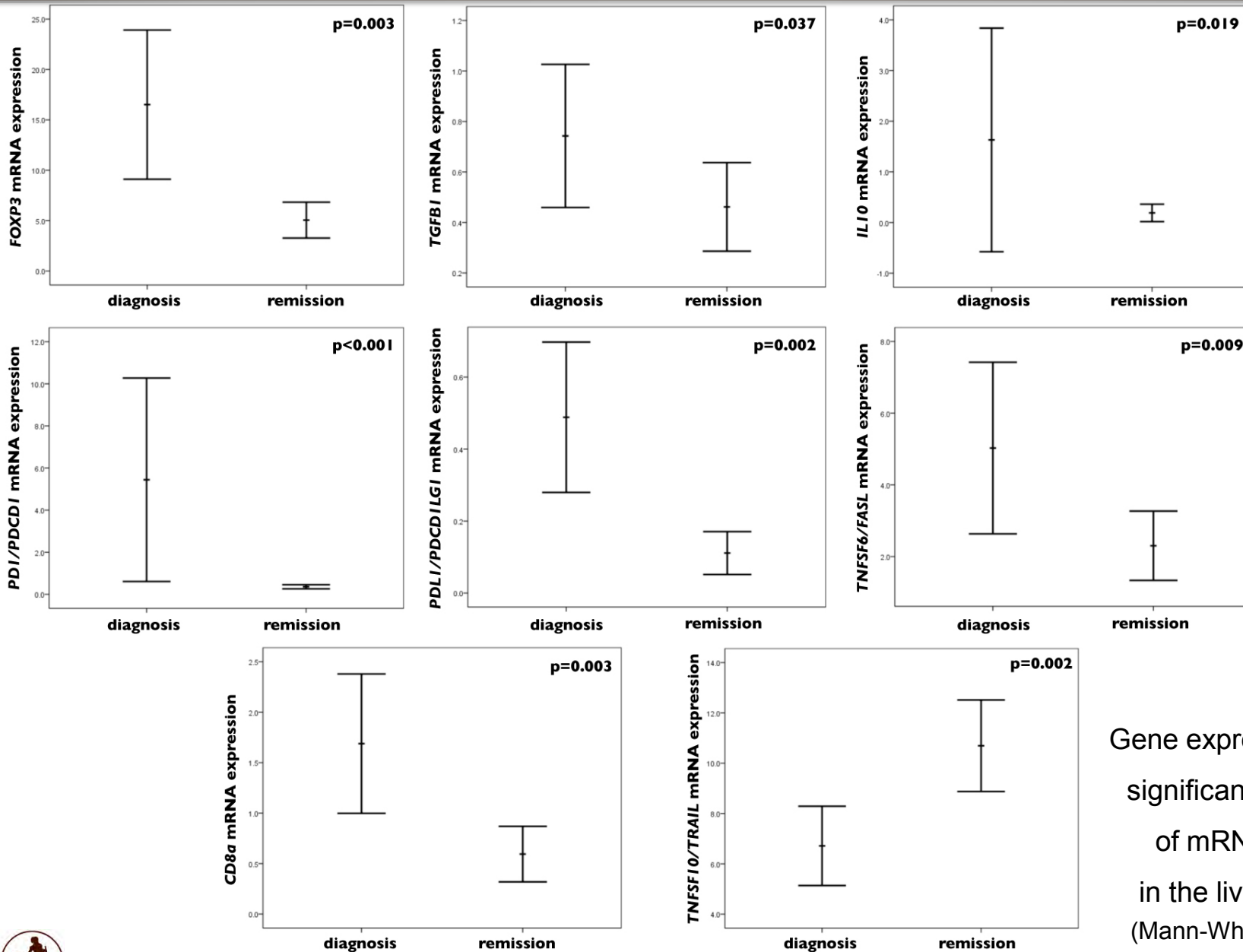
expansion **Acknowledgments**



Prevention of catastrophic

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.”



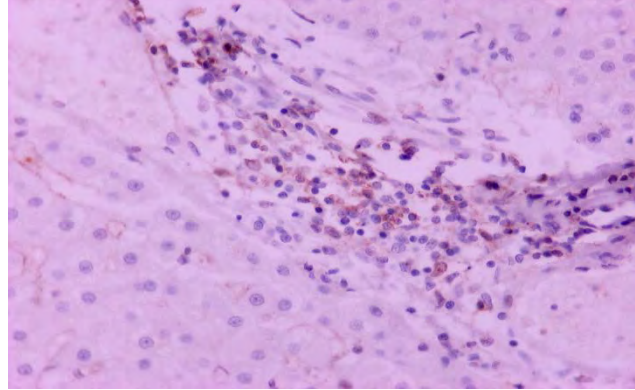
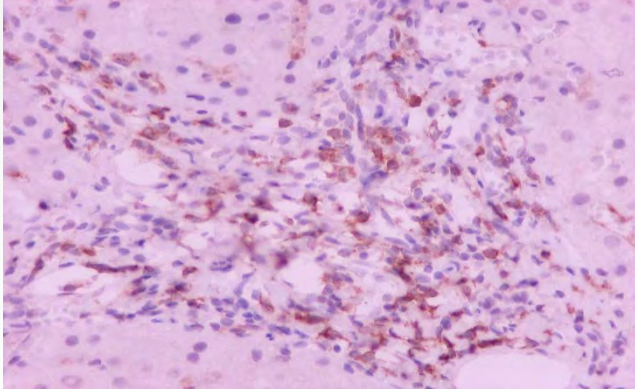


Gene expressions with significant alteration of mRNA levels in the liver in CHB (Mann-Whitney U test)

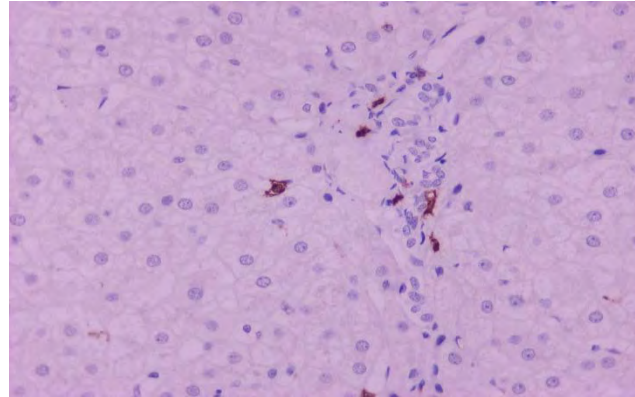
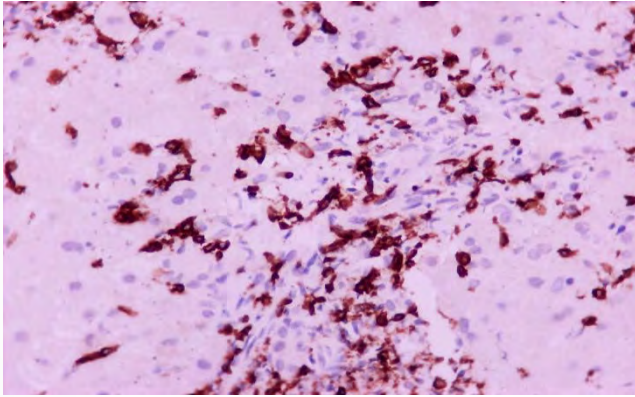


Diagnosis

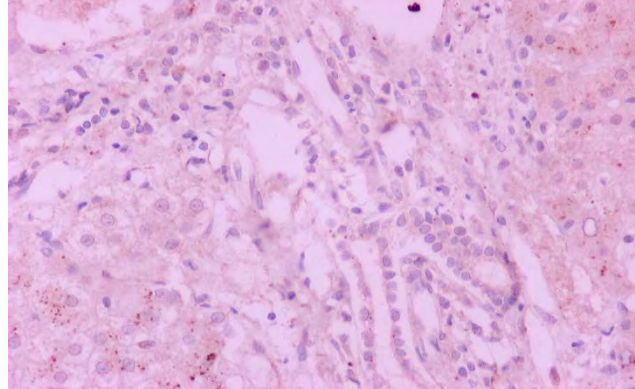
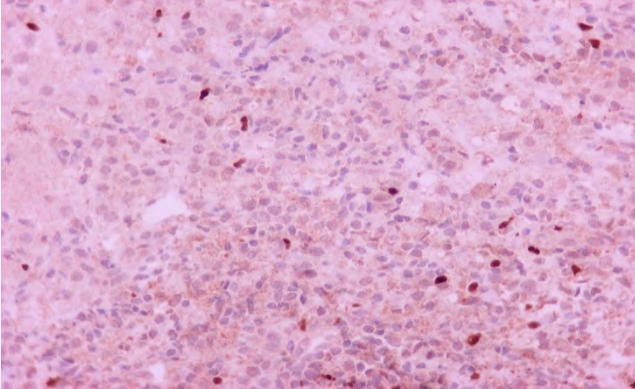
Remission



CD4



CD8



FOXP3

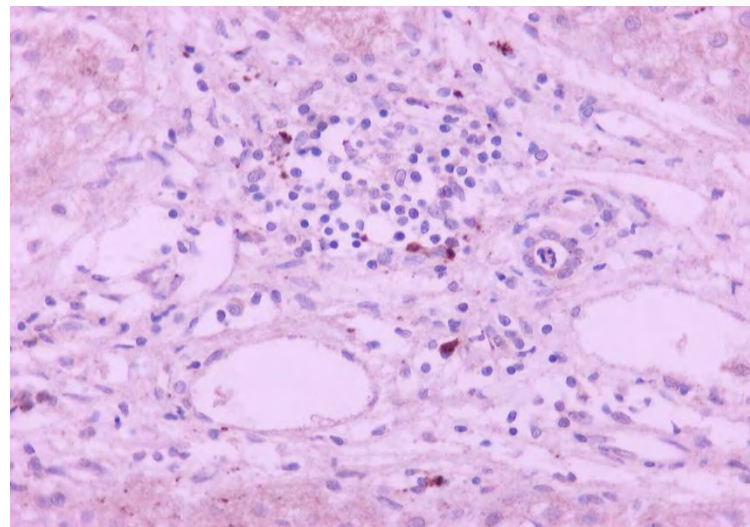
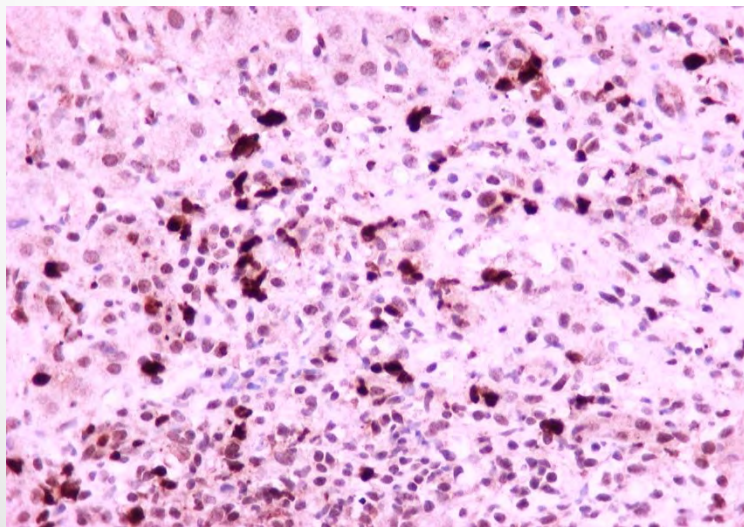
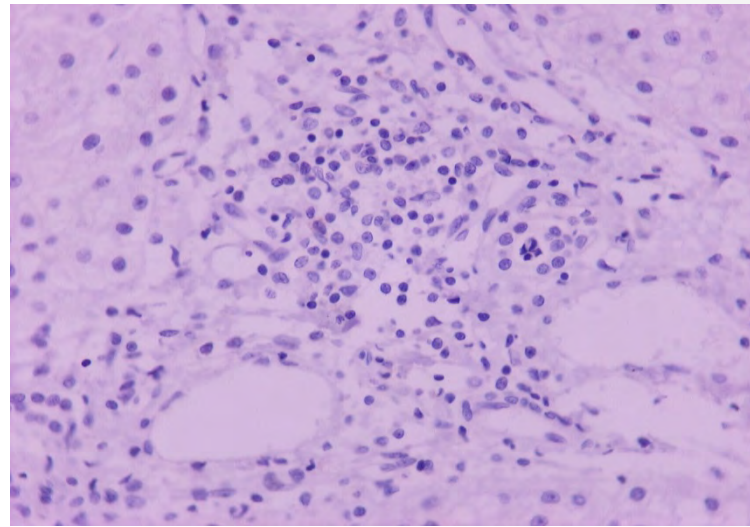
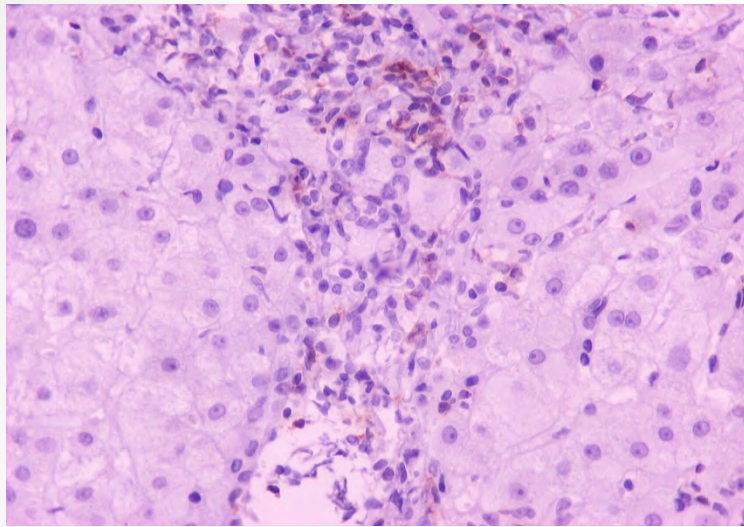


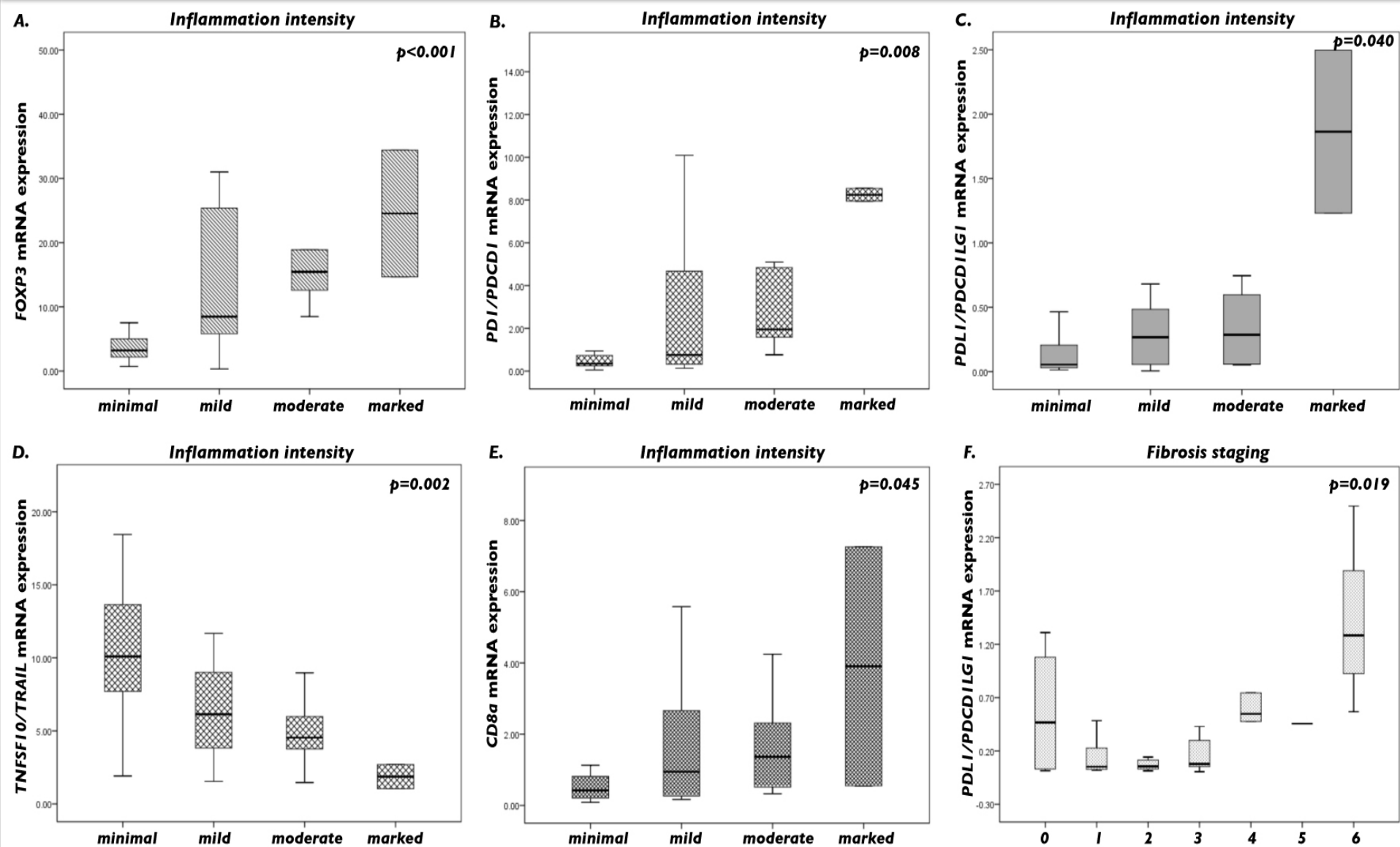
Diagnosis

Remission

PD1

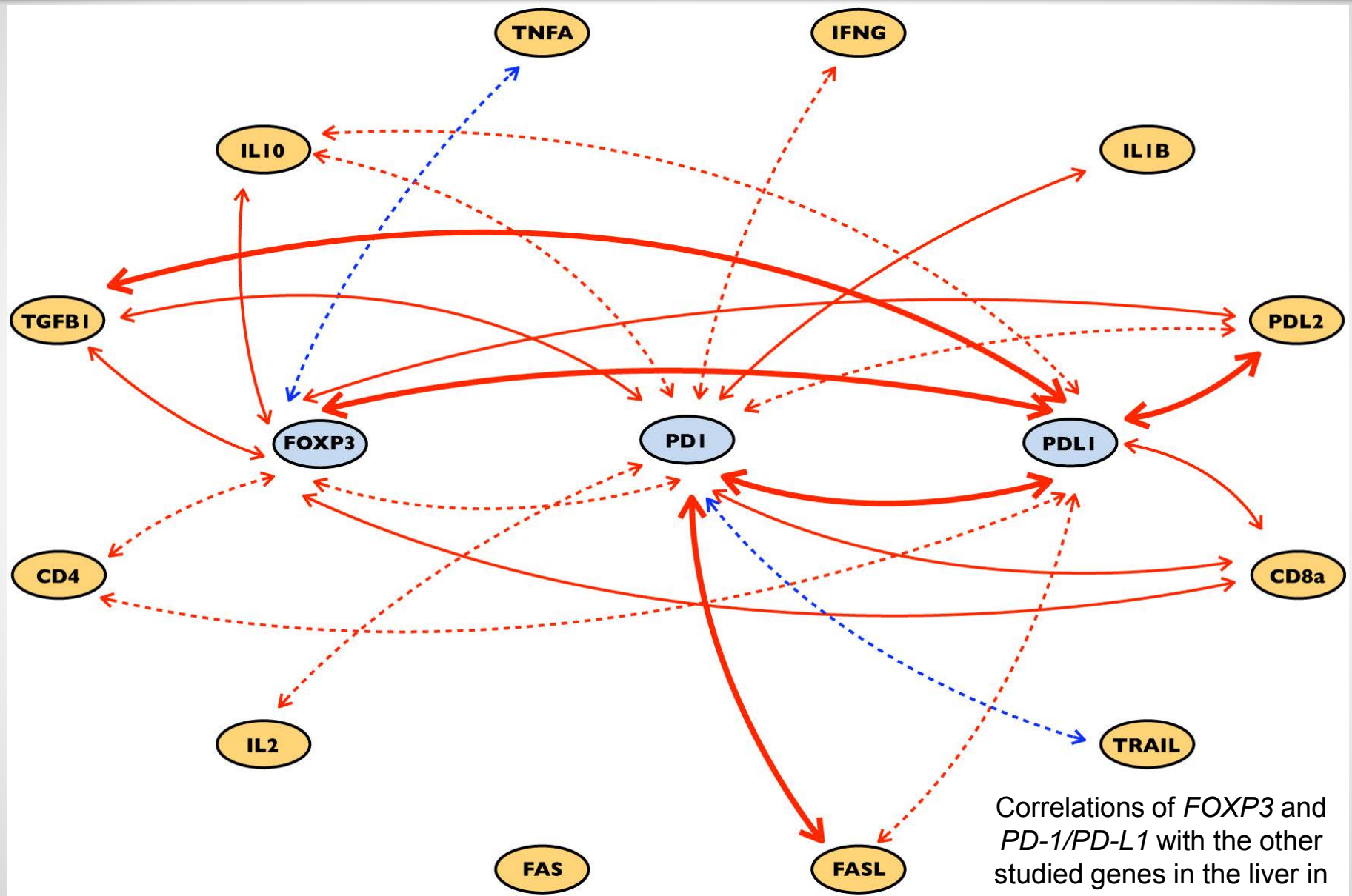
PDL1





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



- **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”.

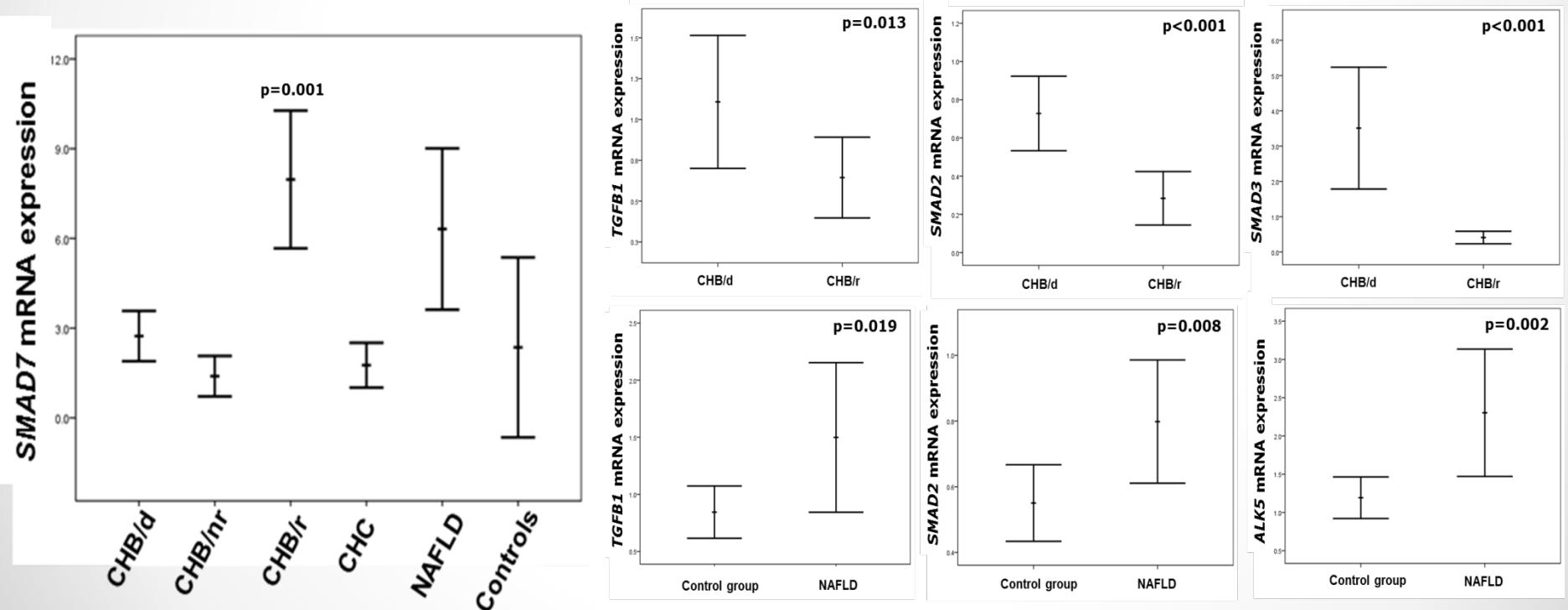
Frontiers in Immunology



Overexpression of SMAD7 protects liver from TGFb/Smad-mediated fibrogenesis

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

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.

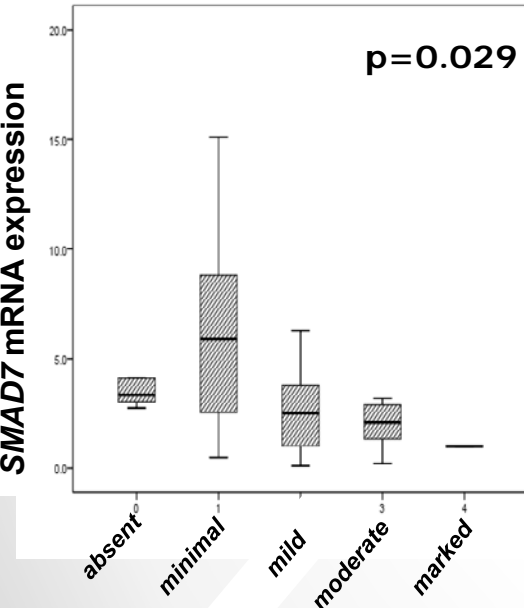


Overexpression of SMAD7 protects liver from TGFb/Smad-mediated fibrogenesis

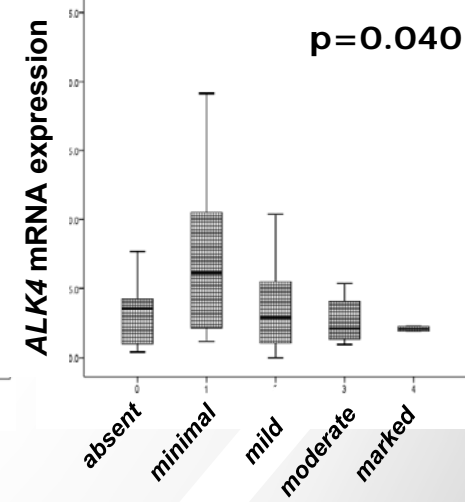
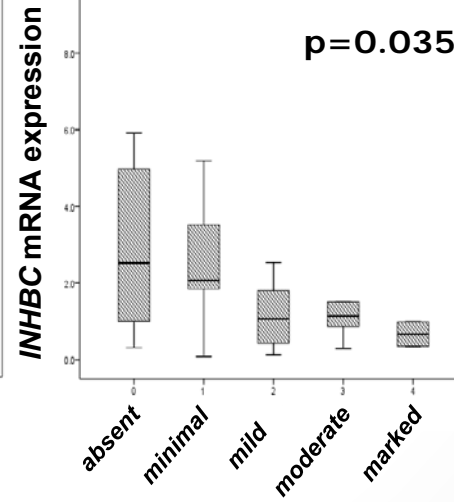
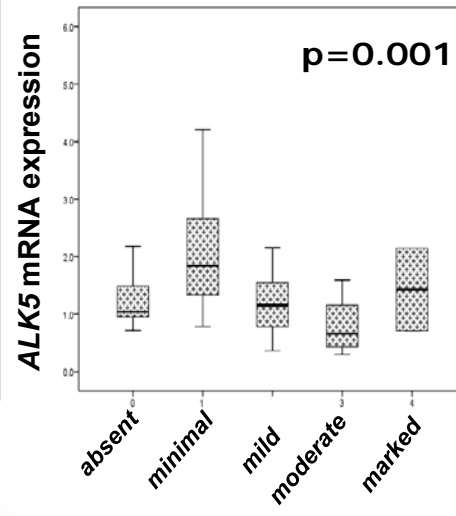
N. Argentou,^{*} G. Germanidis,[†] E. Apostolou,[‡] T. Vasiliadis,[§]
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Inflammation intensity



Inflammation intensity



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.



Overexpression of SMAD7 protects liver from TGFb/Smad-mediated fibrogenesis

N. Argentou,^{*} G. Germanidis,[†] E. Apostolou,[‡] T. Vasiliadis,[§]
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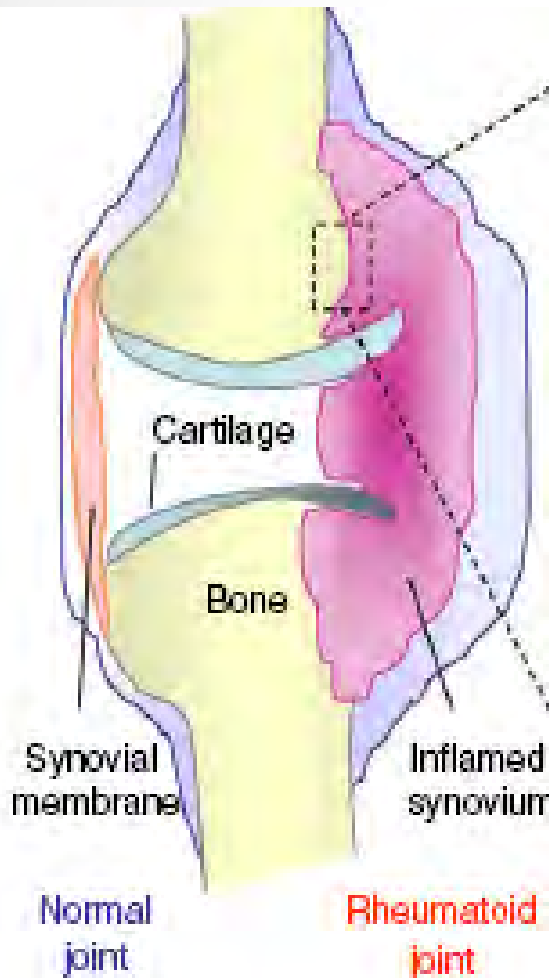
Immunology 2012;137 (Suppl 1):506 (poster)

Manuscript in preparation

- **SMAD7** overexpression might be a mechanism limiting the fibrogenic effect of TGFb 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

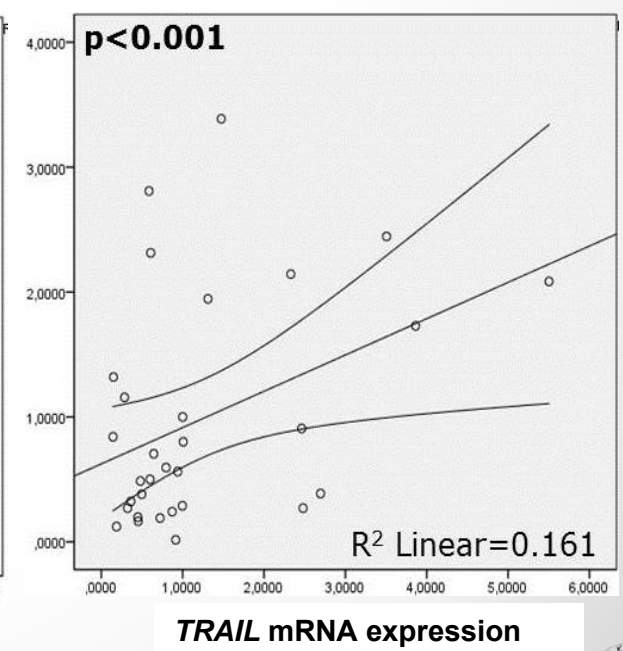
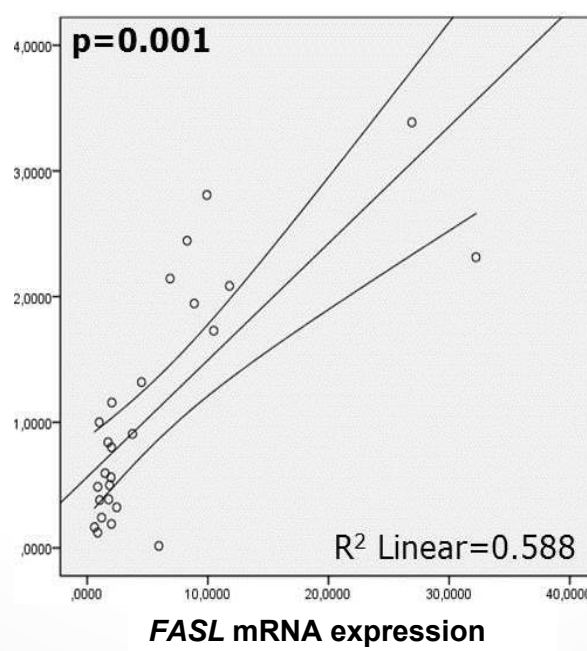
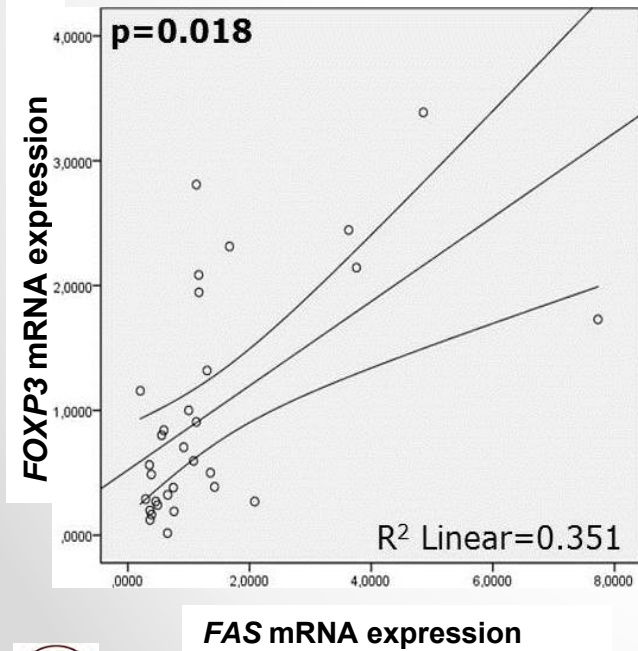
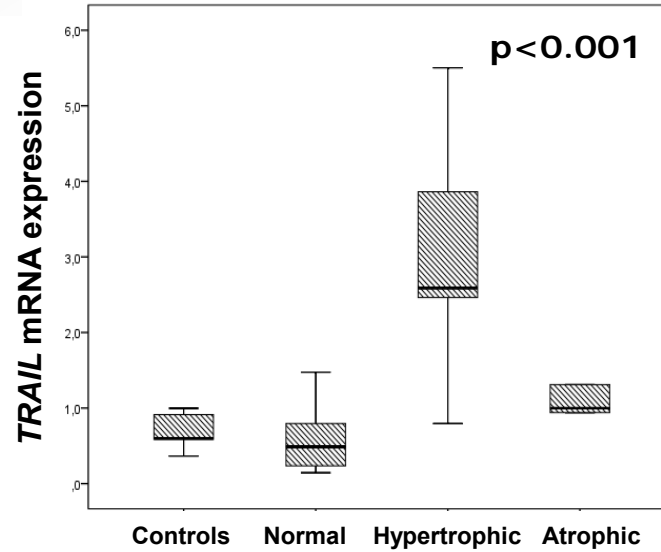
Normal synovium; 16 patients (3M/13F)

Hypertrophic synovium; 6 patients (6F)

Atrophic synovium; 5 patients (5F)

- **Control group**

5 patients (3M/2F); median age 85 years



- **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**





European Union
European Social Fund



Co- financed by Greece and the European Union



*Thank you
for your attention...*

