Foxp3 at the crossroad of immunoregulation, apoptosis and fibrosis in liver diseases

Speletas M,[1] Loules G,[1] Germanidis G,[2] Ritis K,[3] Germenis AE[1]

[1] Department of Immunology and Histocompatibility, School of Medicine, University of Thessaly, University Hospital of Larissa, GR-411 10 Larissa, Greece; [2] A' Department of Internal Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece; [3] A' Department of Internal Medicine, Democritus University of Thrace, Alexandroupolis, Greece

T-regulatory cells (Tregs) are important mediators of immune suppression since their presence prevents anti-self immune responses by inducing regulatory signals to antigen-presenting cells (APCs) and/or effector T cells.[1,2] They are, also, implicated in the inhibition of antineoplastic, antiparasitic and antiviral immune responses. In previous studies, the persistence of viral infection of the liver has been associated with the expansion of Tregs in the peripheral blood. [1,2] This study aimed to examine whether the possible presence of Tregs in hepatic tissue relates to the cause and/or the degree of liver damage.

Materials

Samples

Liver biopsies
- 48 patients
  - 12 chronic HBV hepatitis and/ or cirrhosis
  - 11 chronic HCV-hepatitis and/ or cirrhosis
  - 11 non-alcoholic steatohepatitis
  - 2 methotrexate-related toxicity
  - 2 autoimmune hepatitis/ cirrhosis
- 5 control individuals (patients with minimal liver disease)

Genes studied

TGFβ pathway	Apoptosis mediators		
TGF-β1	Fas	Foxp3	IL-10
ALK-5	FasL		
Smad2	TRAIL		
Smad3	Casp3		
Smad4			
Smad7			

All the primers used were supplied by SupperArray and B2M (beta2-microglobulin was used as reference gene.

Methods

Liver tissue from patients or controls

↓

RNA isolation via TRI

↓

cDNA synthesis from 1 μg RNA with M-MLV RT

↓

RT-PCR

↓

Relative expression with the use of REST

Results

An accumulation of natural Tregs was observed in all tissue samples obtained from patients and was independent of the origin of liver damage (viruses, drugs, non-alcoholic steatohepatitis, autoimmunity) [$p<0.01$] (Figure 1). The dramatic increase of Foxp3 expression was accompanied by a significant increase in mediators of apoptosis (Fas-FasL, TRAIL) compared with normal controls ($p<0.01$) (Figure 2). No difference in TGF-β1 and IL-10 expression between normal and patients was observed (Figure 3), implying the absence of inducible Tregs (Th3 and Tr1, respectively). Moreover, no difference in the mRNA expression of TGFβ receptors and mediators was observed, although an increase in the expression of Smad7 was observed in patients with cirrhosis compared with normal controls ($p=0.012$) (Figure 4).

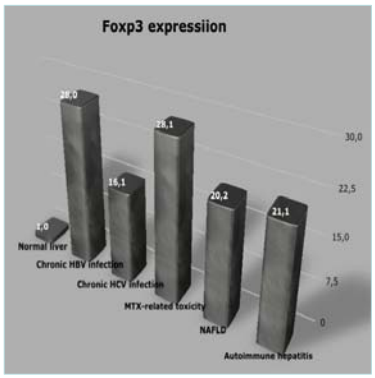


Figure 1. Foxp3 expression in liver biopsies of samples tested

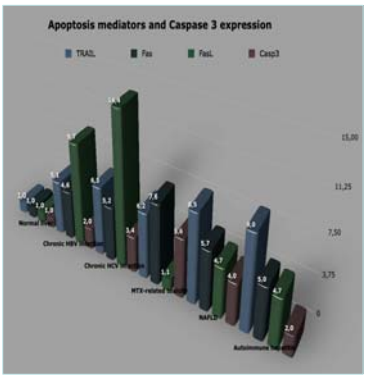


Figure 2. Expression of TRAIL, Fas, Fas-L, and caspase 3 in liver biopsies of samples tested

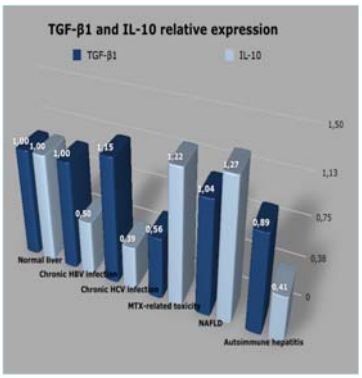


Figure 3. Expression of TGF-β1 and IL-10 in liver biopsies of samples tested

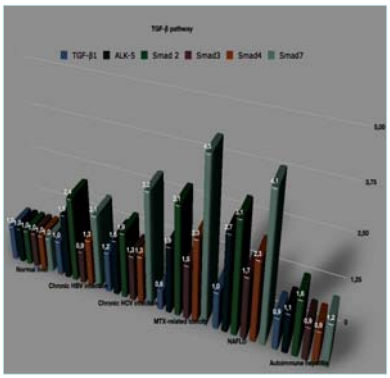
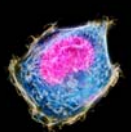


Figure 4. Expression of TGF-β receptors and mediators of samples tested.

Conclusion

Our results offer strong evidence that acute or chronic tissue damage, irrespective of its origin, results in increased apoptosis possibly leading to increased phagocytosis and APC presentation of self-antigens. Expansion of natural Tregs is induced to prevent catastrophic autoimmunity. Elucidation of the pathways mediating this model could increase our understanding of the role that Tregs play in disease propagation and, for the first time, could have wider implications for the manipulation of Tregs in a therapeutic setting.



References

1. Kim JM et al. Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. Nat Immunol 2007; 8: 191-7
2. Parker GA, Picut CA. Liver Immunobiology. Toxicologic Pathology 2005;33:52-62

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