

Regulatory T cells and role in liver disease

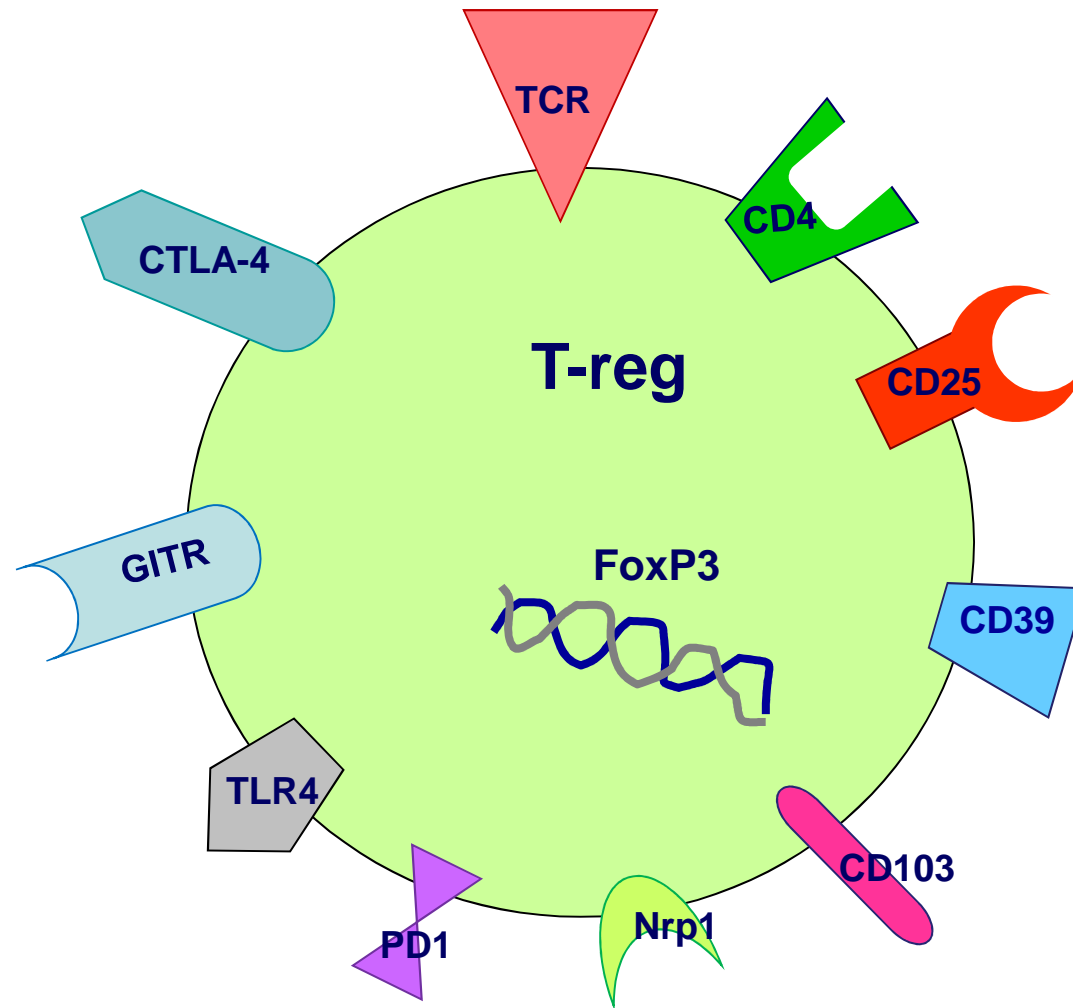
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CD4+CD25^{high} regulatory T cells

- Thymus-derived naturally occurring CD4+CD25+ T-regs and peripherally-induced T-regs
- Top 2% of the CD4 T cell subset
- Control adaptive and innate immune responses
- Mechanism of action: 1) direct control of target cell effector function; 2) control mediated through antigen presenting cells
- Express GITR, CD45RO, CD45RA, CD62L, CD40L^{low}, CD127^{low/-}, LAP, IL-1 receptor type I/II, galectins, CD39, CTLA-4, FOXP3

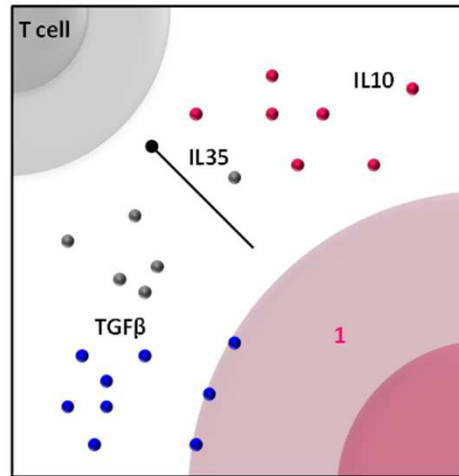
T-reg surface and intracellular markers



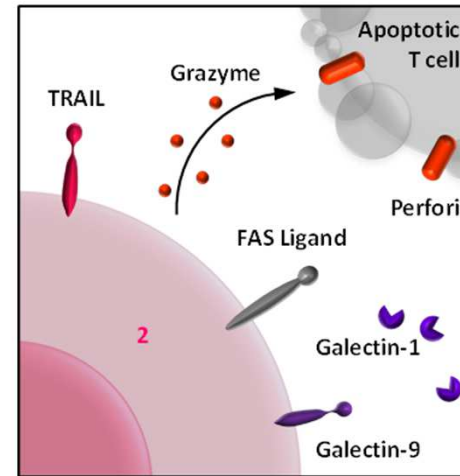
Adapted: Wing et al Scand J Immunol 2005

CD4+CD25^{high} regulatory T cells: mechanisms of suppression

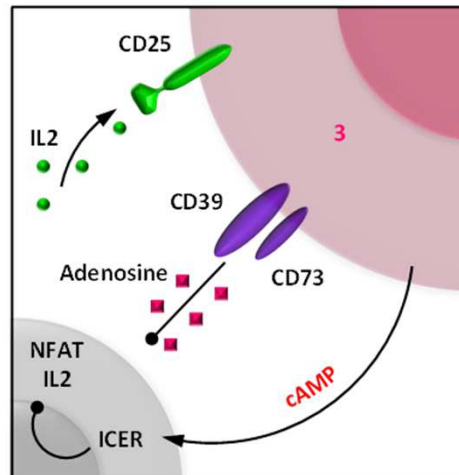
***Inhibitory
cytokines***



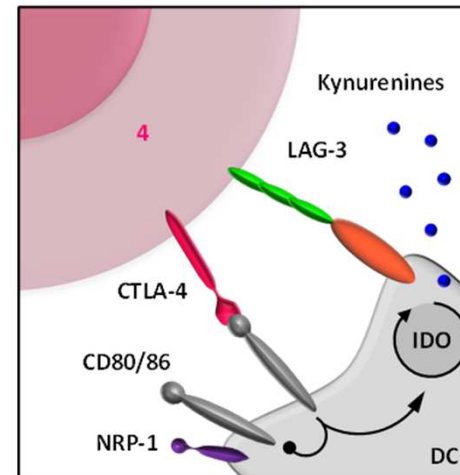
Cytolysis



***Metabolic
disruption***



***Targeting
dendritic cells***



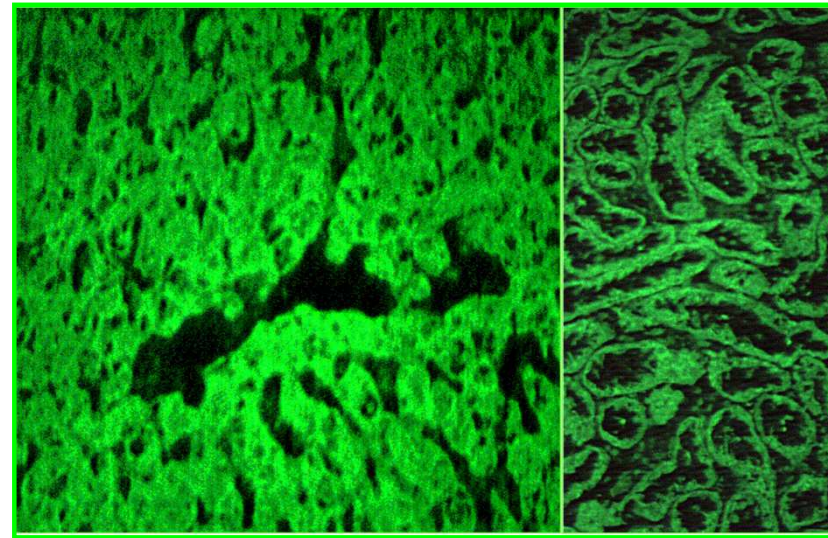
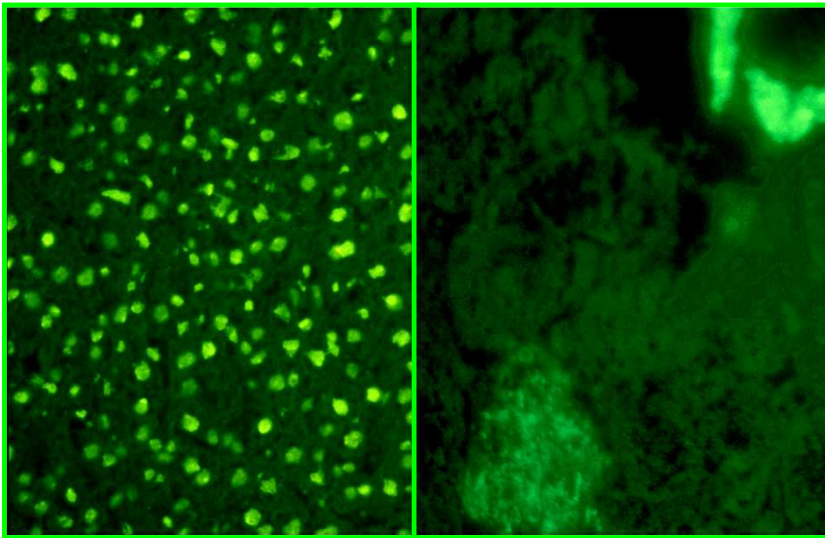
Autoimmune Hepatitis (AIH)

- progressive liver disorder
- female preponderance (4:1)
- hypergammaglobulinaemia
- seropositivity for circulating autoantibodies
- response to immunosuppressive treatment (corticosteroids ± azathioprine)

Autoantibodies

AIH-1

AIH-2



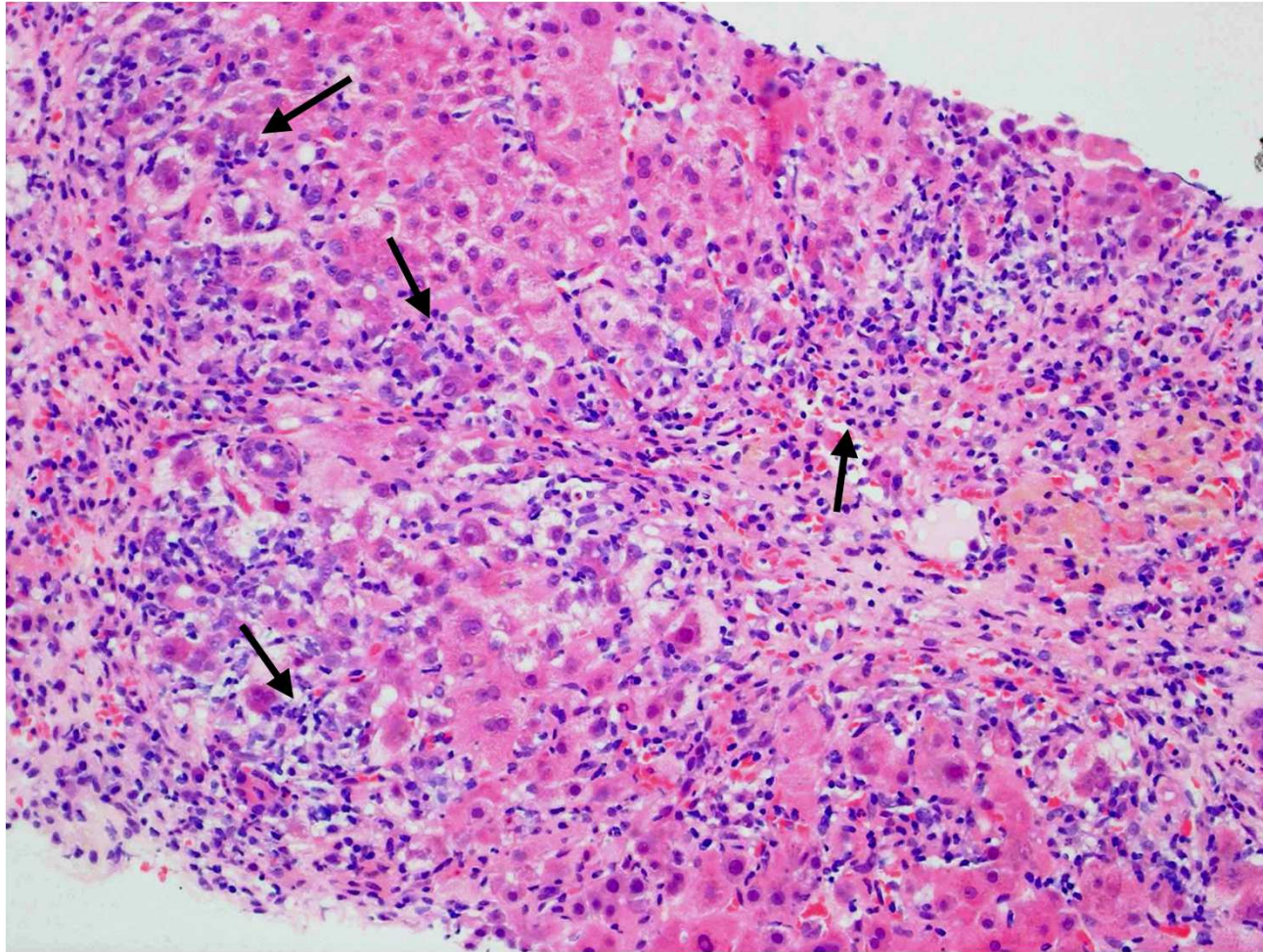
ANA

SMA

LKM-1

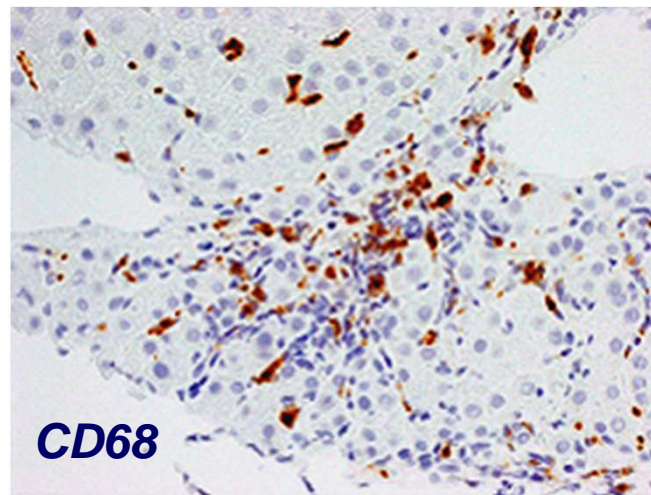
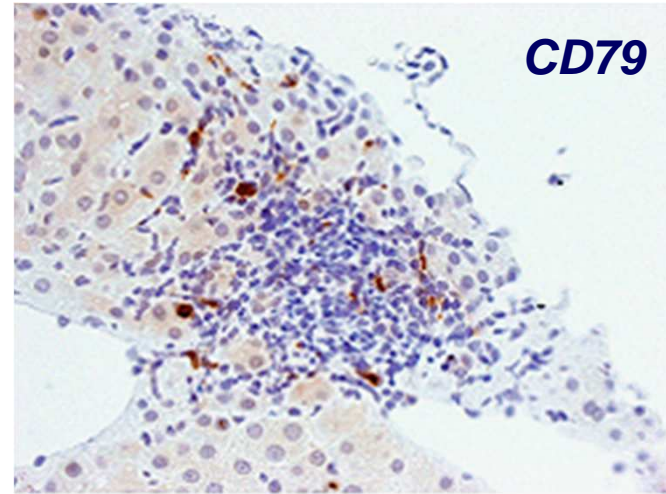
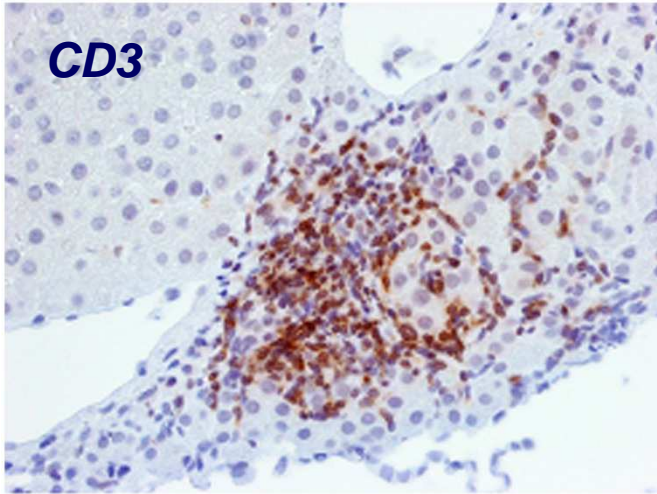
Additional antibodies to asialoglycoprotein receptor (**ASGPR**), soluble liver antigen (**SLA**), alcohol dehydrogenase (**ADH**), liver cytosol 1 (**LC1**)

Histology

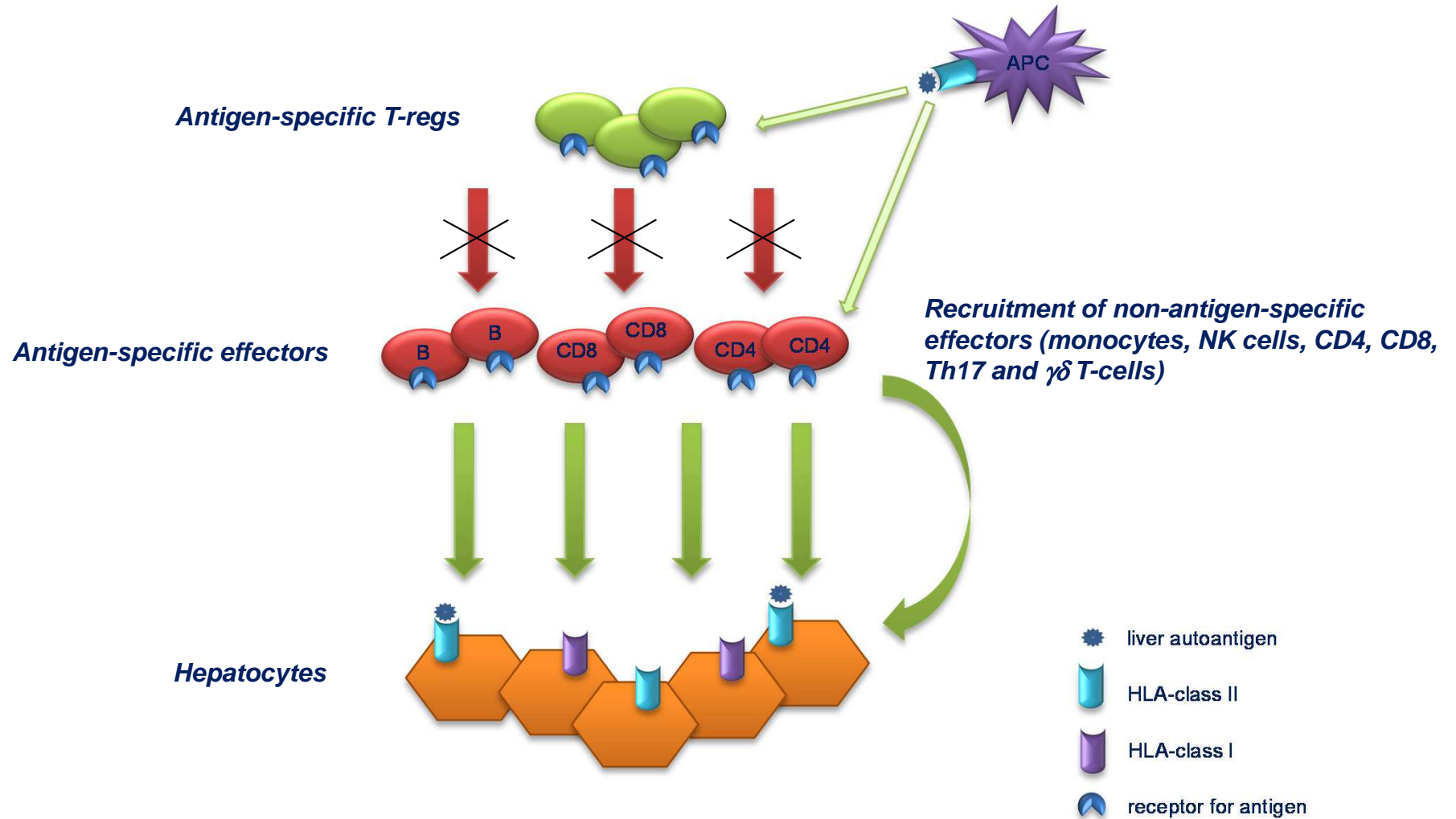


Interface hepatitis

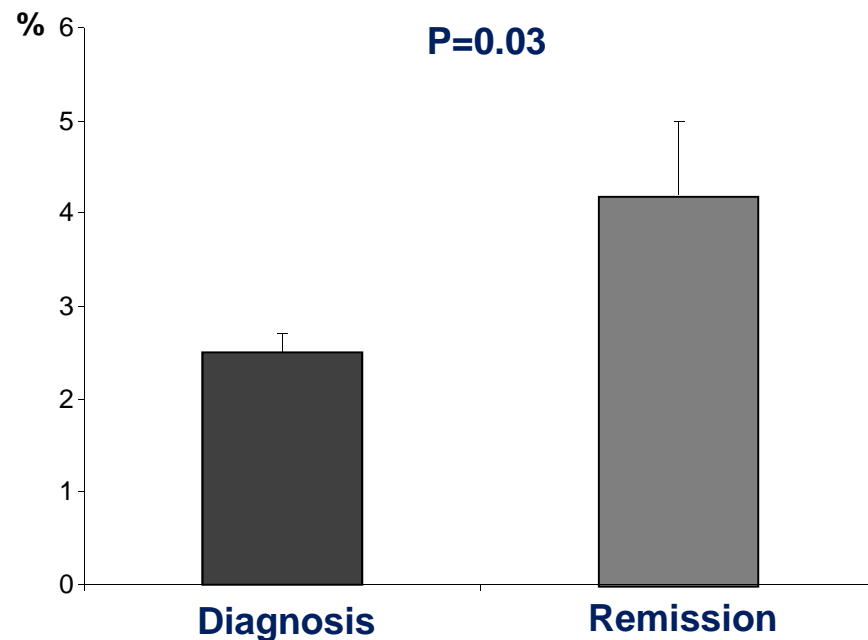
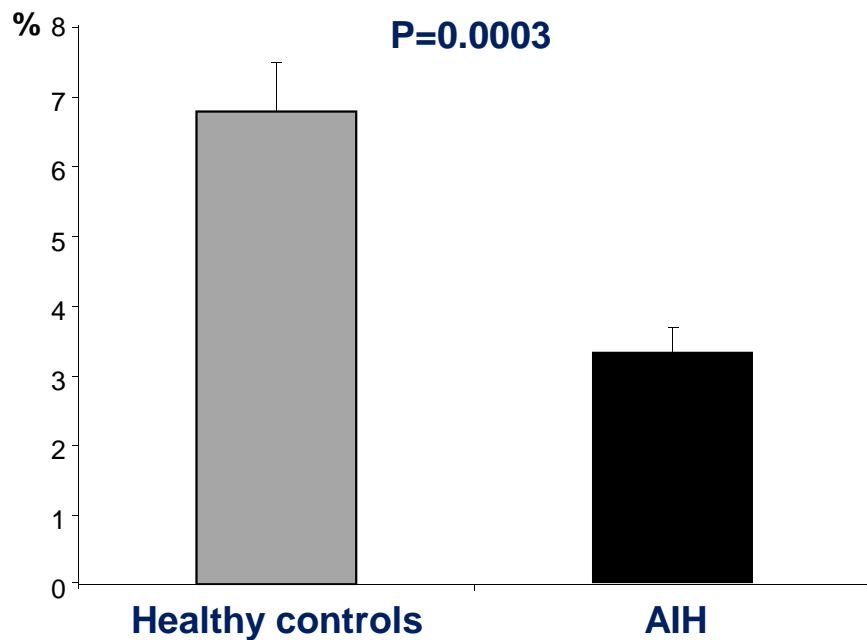
Hepatic infiltrate



Mechanisms of liver damage



Frequency of T-regs in AIH

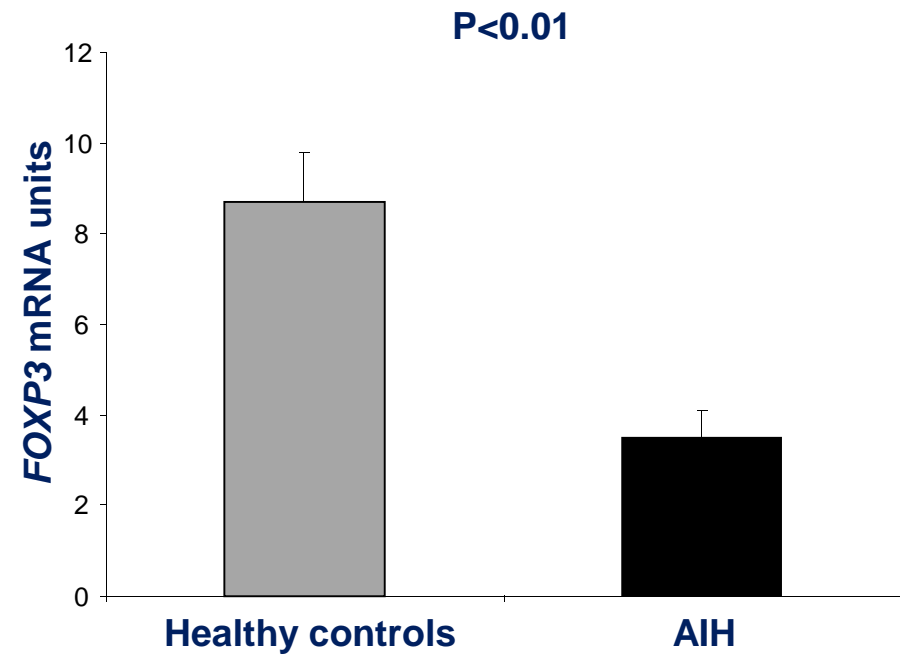


Longhi et al, J Hepatol 2004

Correlation with indices of disease activity

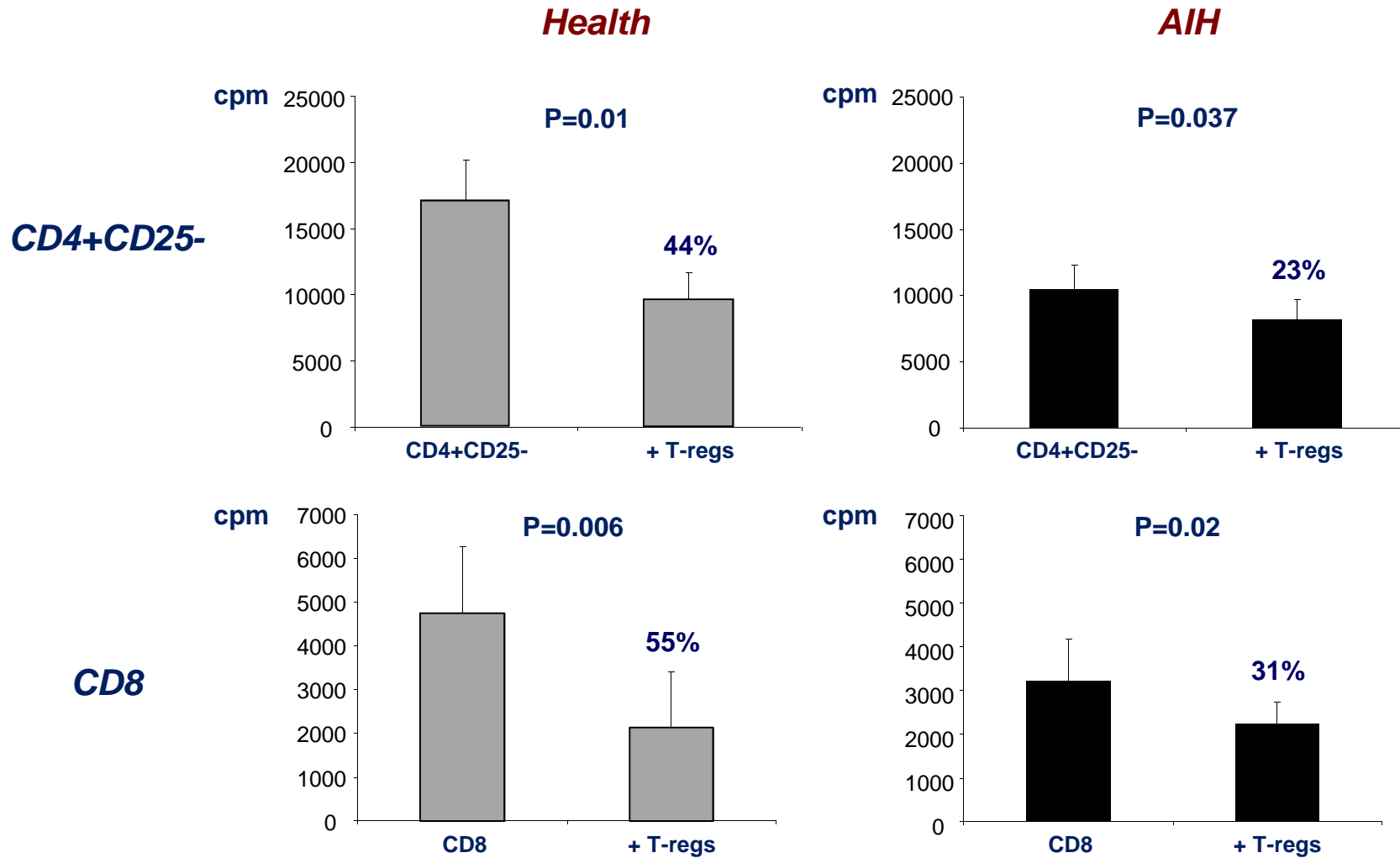
- Inverse correlation between titres of LKM-1 and T-reg frequency (R=-0.879; P<0.001)
- Inverse correlation between titres of anti-SLA and T-reg frequency (R=-0.6; P<0.05)
- No correlation between transaminases, IgG levels, ANA/SMA autoantibody titres and T-reg frequency

FOXP3 expression



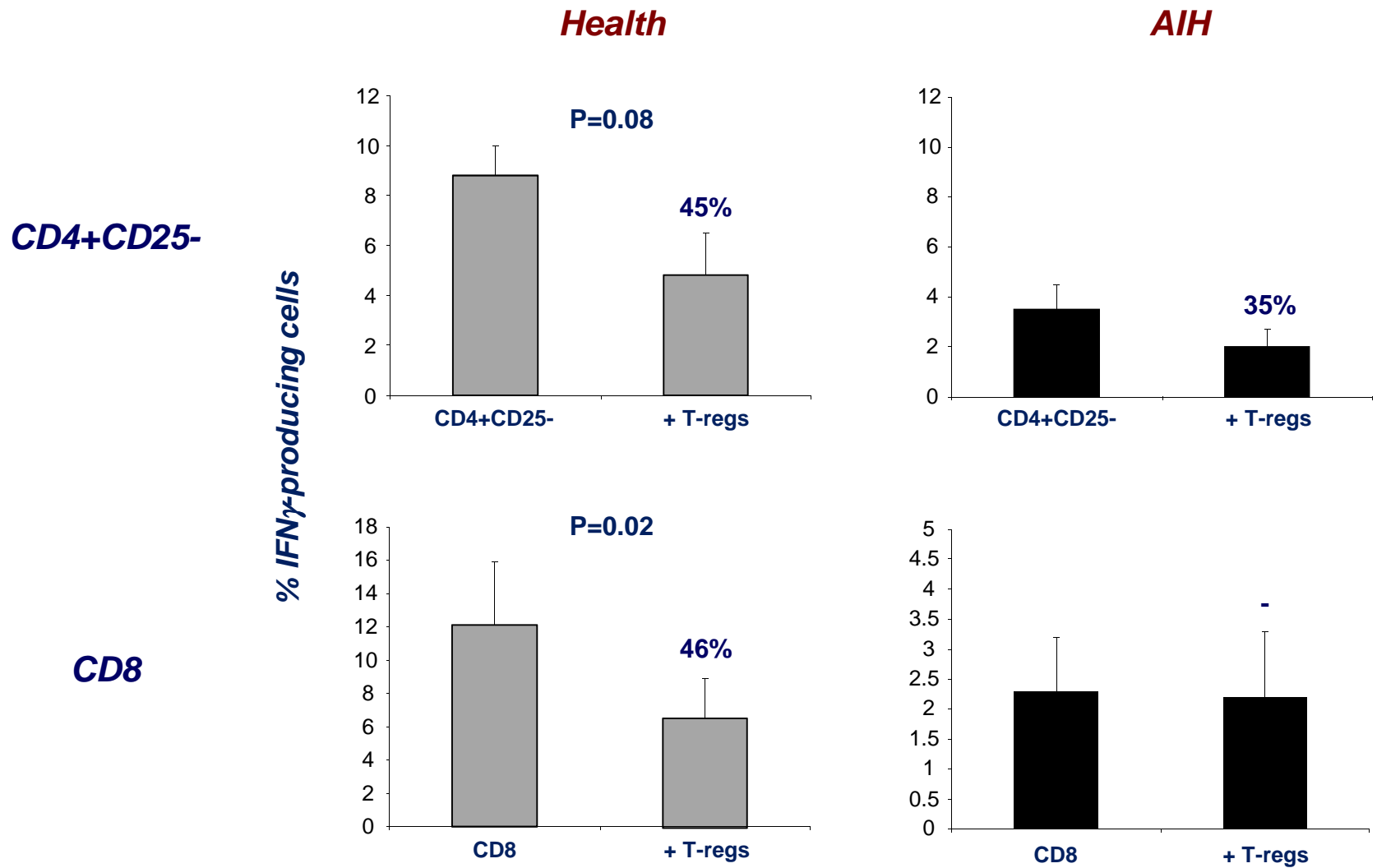
Longhi et al, J Immunol 2006

T-reg suppressive function



Longhi et al, J Hepatol 2004

T-reg suppressive function



Longhi et al, J Autoimmun 2005

Regulation of immuno-modulatory cytokines

	IL-4 (pg/ml)	IL-10 (pg/ml)	TGF- β (pg/ml)
Healthy controls			
<i>CD4+CD25+</i>	not detectable	not detectable	not detectable
<i>CD4+CD25-</i>	77.2 \pm 22.5	47 \pm 13	4 \pm 0.4
<i>CD4+CD25- and T-regs</i>	158.6 \pm 36.3**	769 \pm 234*	2,157 \pm 1,234**
AIH patients			
<i>CD4+CD25+</i>	not detectable	not detectable	not detectable
<i>CD4+CD25-</i>	71 \pm 30.8	267 \pm 139	53 \pm 20.9
<i>CD4+CD25- and T-regs</i>	155 \pm 40.6*	1,851 \pm 585§	285 \pm 93.7*

359-fold

2.9-fold

*: P<0.05

** : P<0.01

§: P=0.05

Longhi et al, J Immunol 2006

Summary

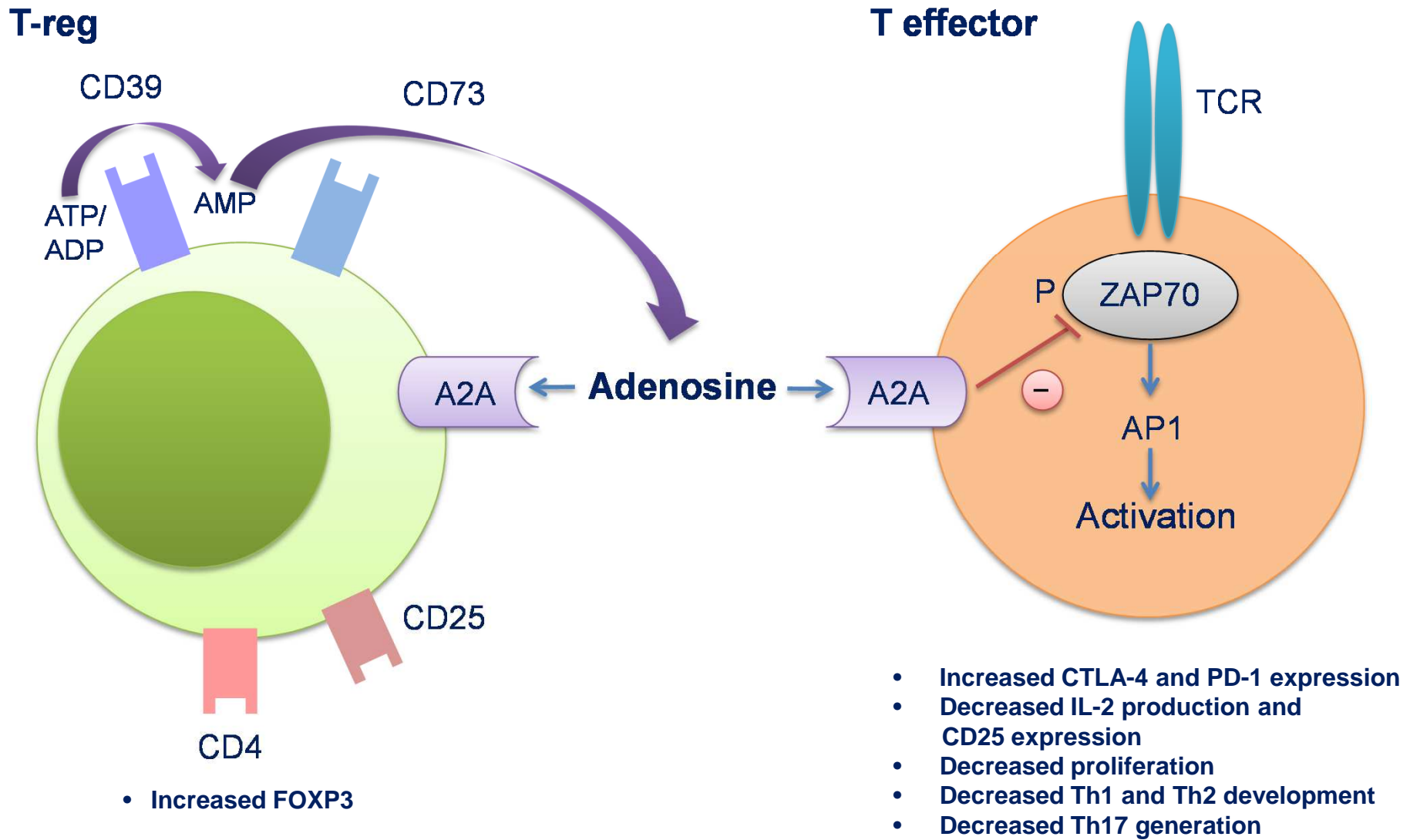
- T-regs from AIH patients:
 - are numerically defective
 - express lower levels of *FOXP3*
 - are impaired in their ability to control T cell proliferation and IFN γ production
 - are defective in promoting immuno-regulatory cytokine (e.g. TGF- β) secretion

- T-reg impairment is more marked during active disease

Causes of T-reg impairment: role of CD39

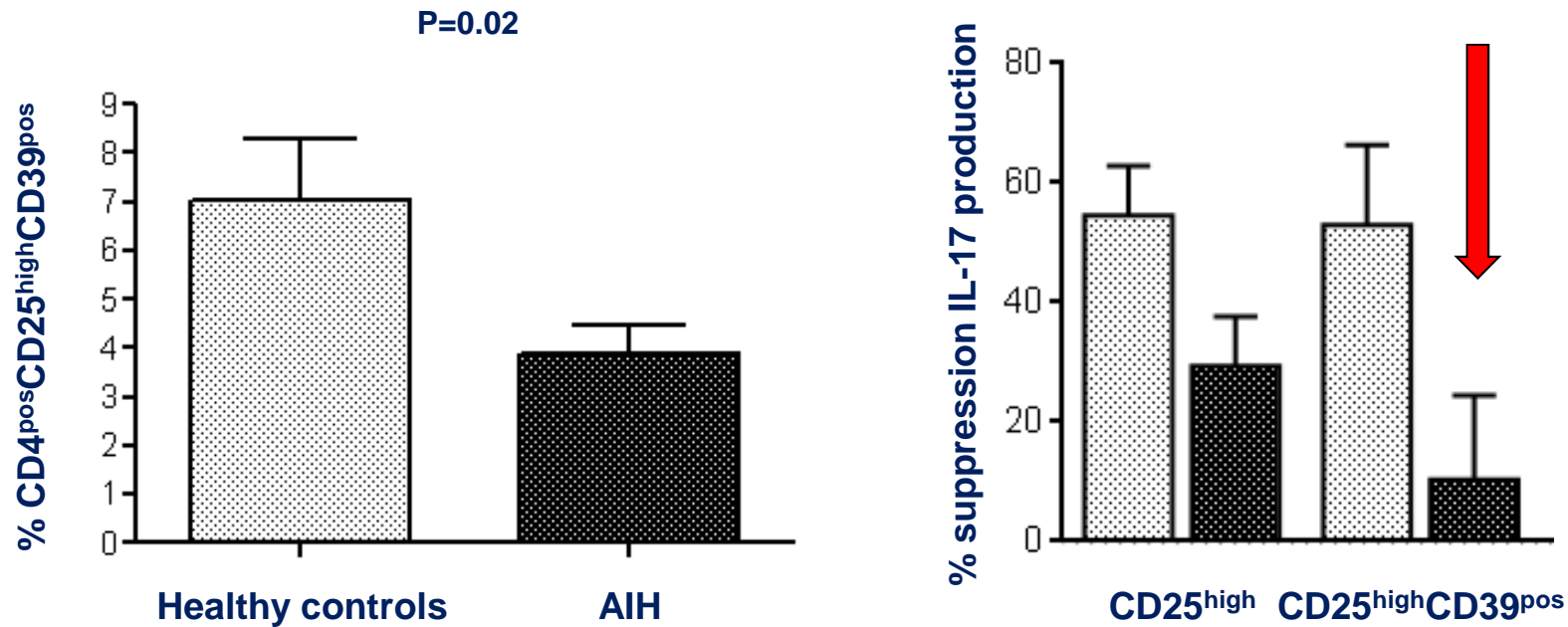
- CD39: key to T-reg suppressive function - initiation of an ATP hydrolysis cascade culminating with the generation of adenosine (immunosuppressive properties)
- CD39^{pos} T-regs are numerically and functionally impaired in patients with multiple sclerosis
- CD39 polymorphism (AA at rs10748643 next to the CD39 promoter region) associated with susceptibility to Crohn's disease

Adenosinergic effects on effector T cells



Adapted from Hasko, Linden, Cronstein et al. Nat Rev Drug Discov 2008

CD39^{pos}T-regs in AIH



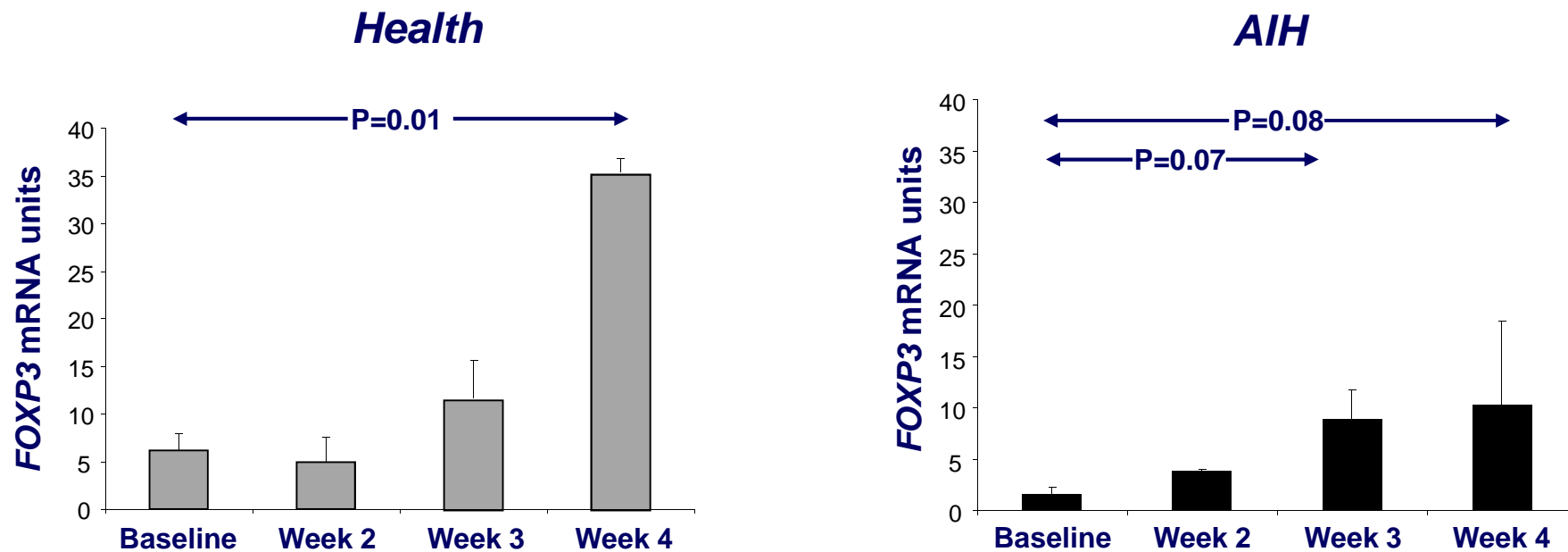
CD39^{pos}T-regs are decreased in number and defective in their ability to control IL-17 production in AIH

Polyclonal T-reg expansion

- At the end of week 4, the number of T-regs expanded from CD4+CD25+ T cells increased by a median of
 - 33.4-fold (range 1-450) in normal controls
 - 11.1-fold (range 1-96) in patients

Polyclonal T-reg expansion

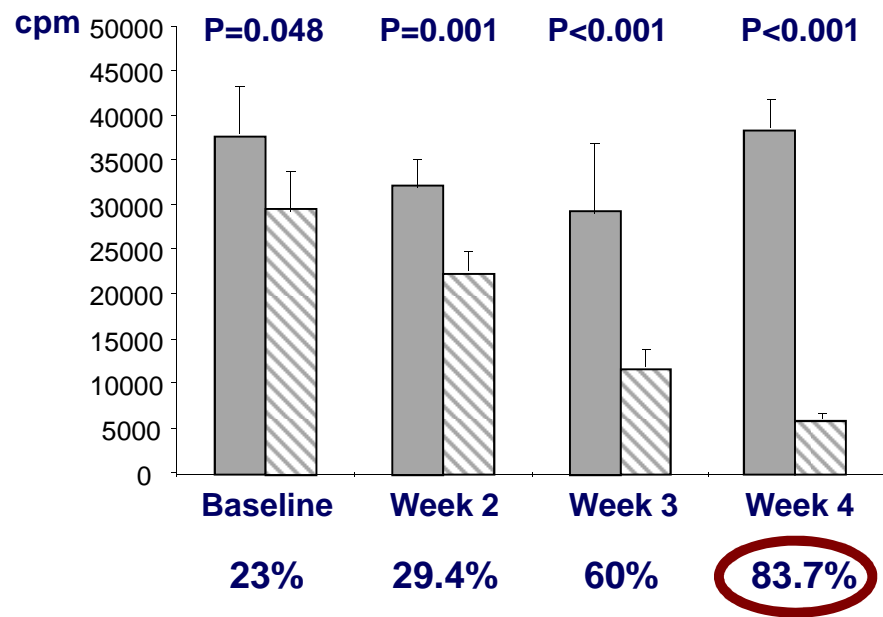
FOXP3 gene expression



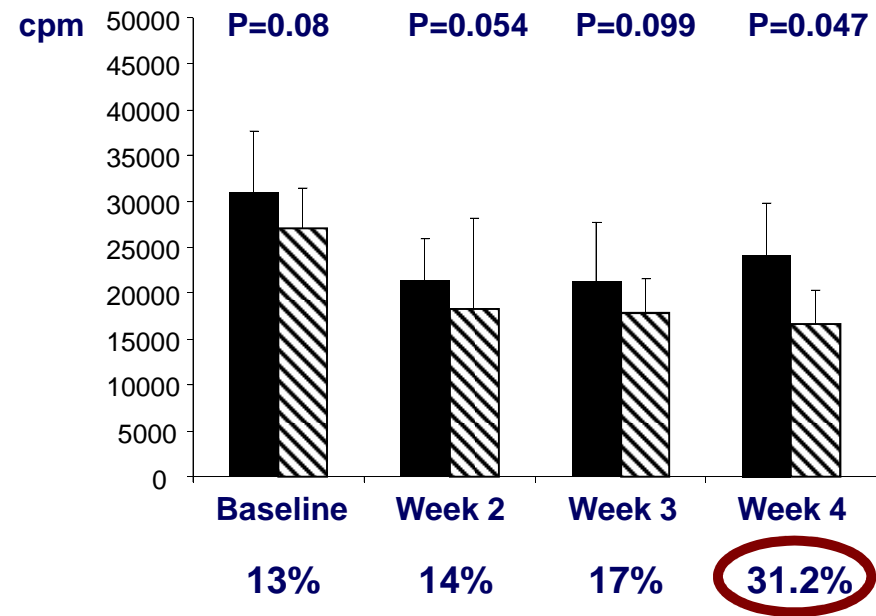
Longhi et al, Hepatology 2008

Polyclonal T-reg expansion

T-reg suppressive function



Health



AIH

Longhi et al, Hepatology 2008

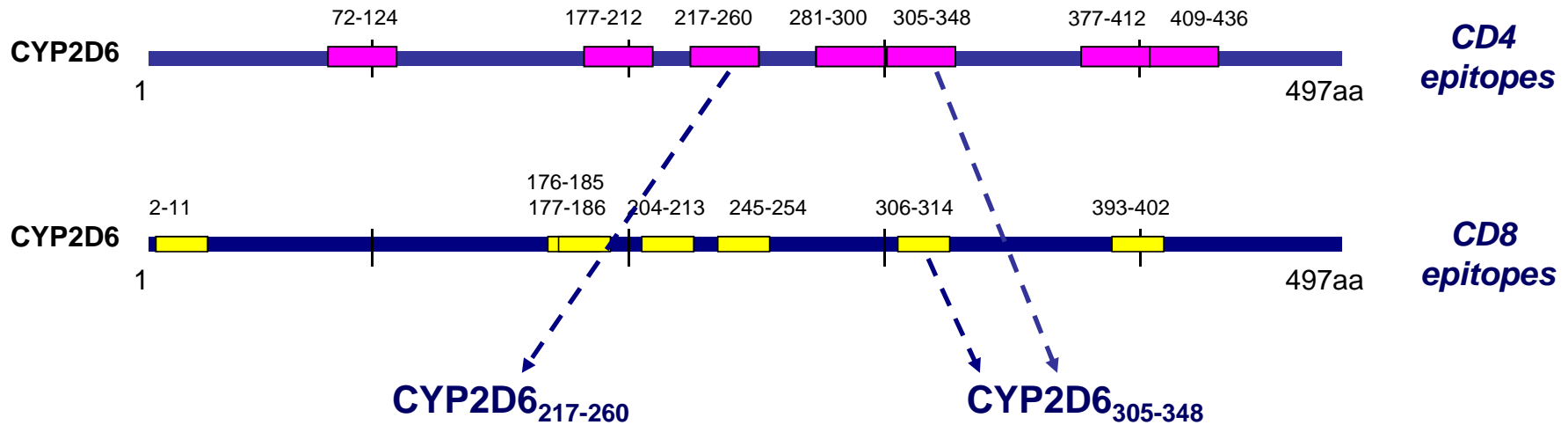
Summary

- T-regs can be polyclonally expanded from CD4+CD25+ T cells
 - higher FOXP3 gene and protein expression
 - stronger suppressor activity

Antigen-specific T-regs

- Evidence indicating that antigen-specific suppress more efficiently than non-antigen-specific T-regs
- Small numbers of antigen-specific T-regs can reverse diabetes after onset in NOD mice
- In transplantation, alloantigen-specific T-regs have stronger suppressor ability than non-alloantigen-specific T-regs

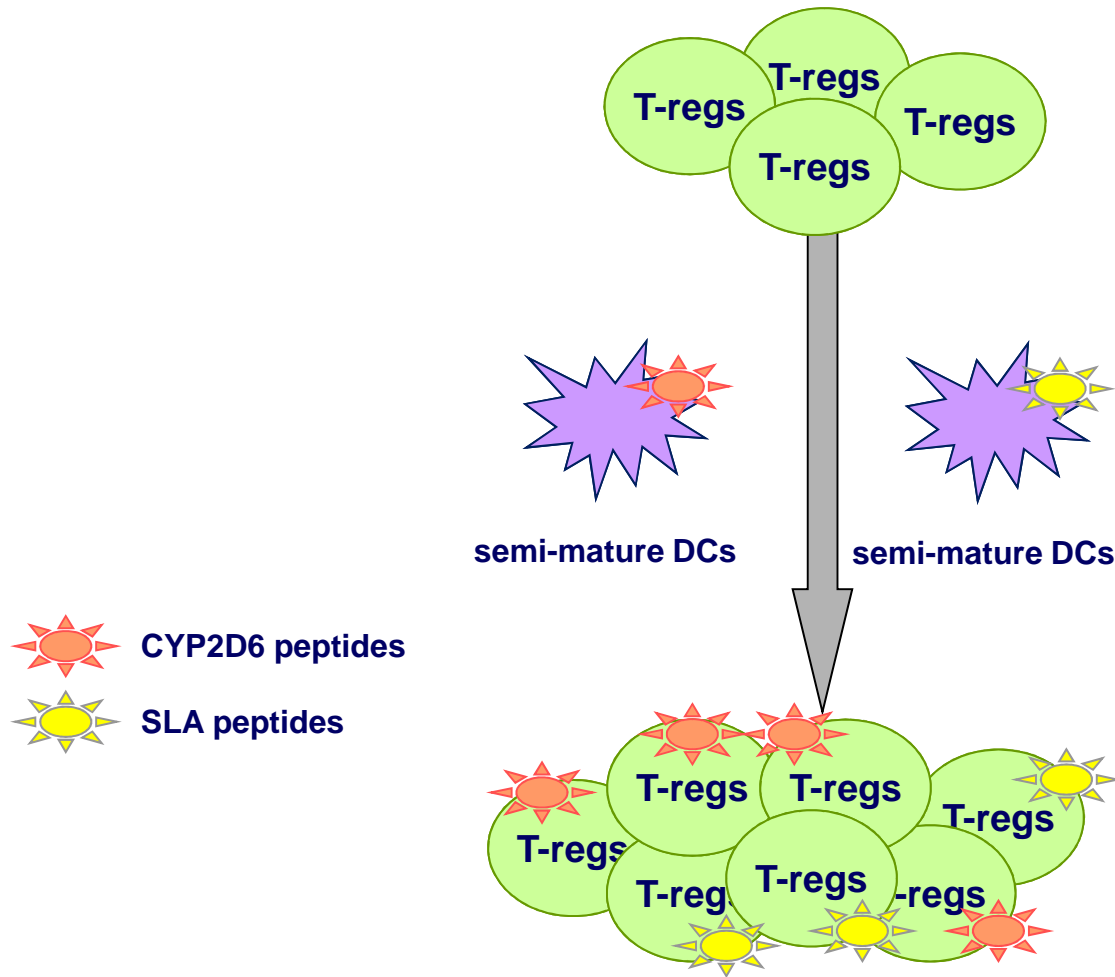
AIH-2 and CYP2D6 immunodominant regions



Ma et al, Gastroenterology 2006

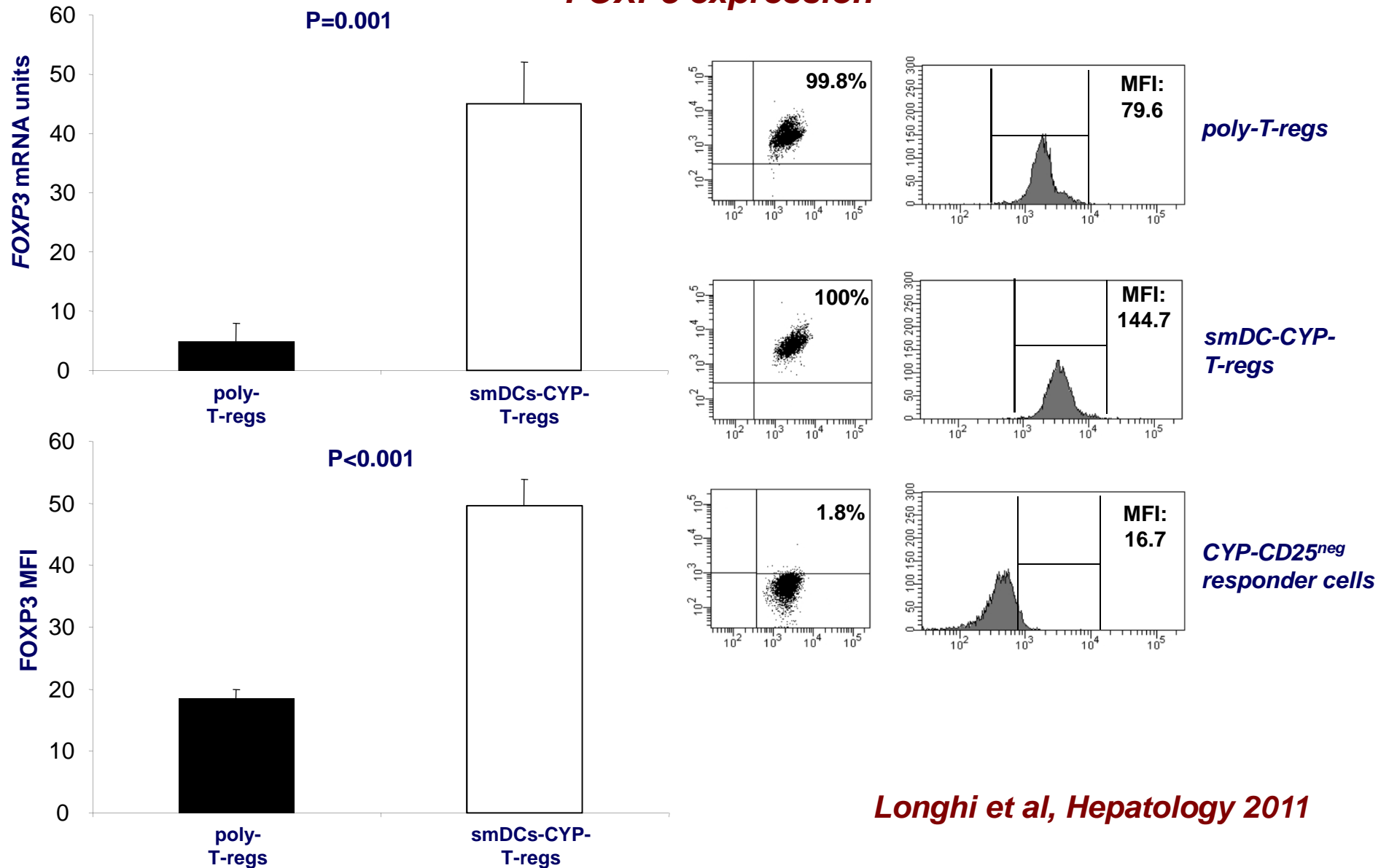
Longhi et al, Hepatology 2007

Generation of antigen-specific T-regs



Antigen-specific T-regs

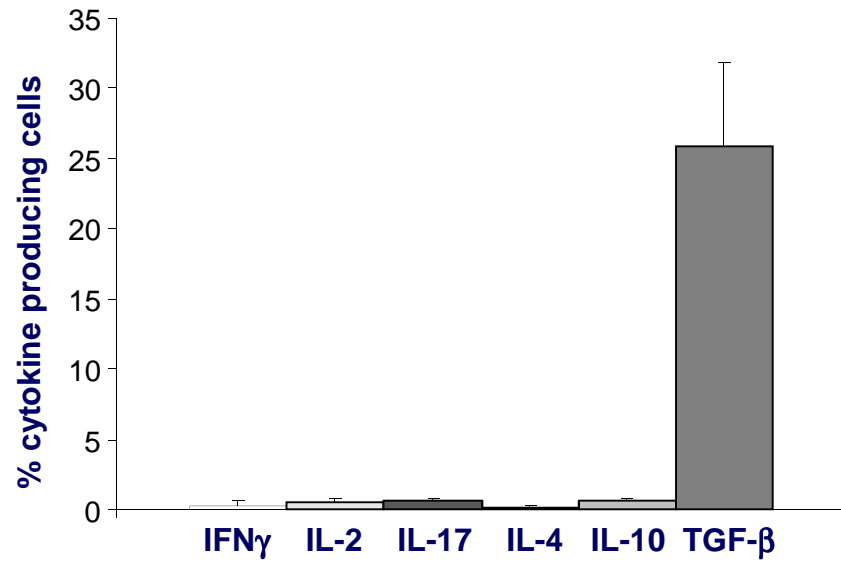
FOXP3 expression



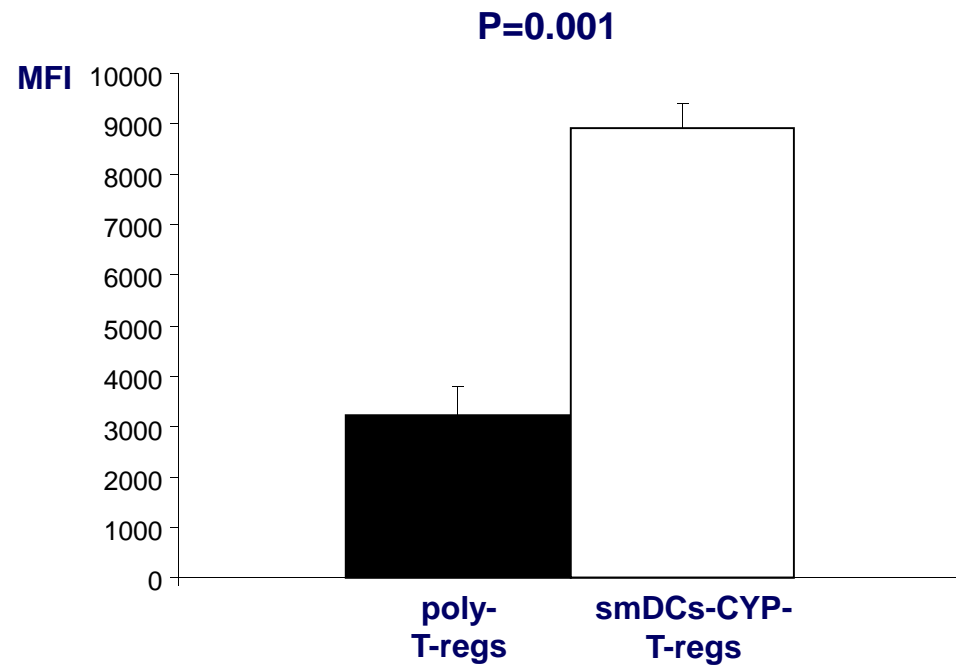
Longhi et al, Hepatology 2011

Antigen-specific T-regs

*smDCs-CYP-T-reg
cytokine profile*

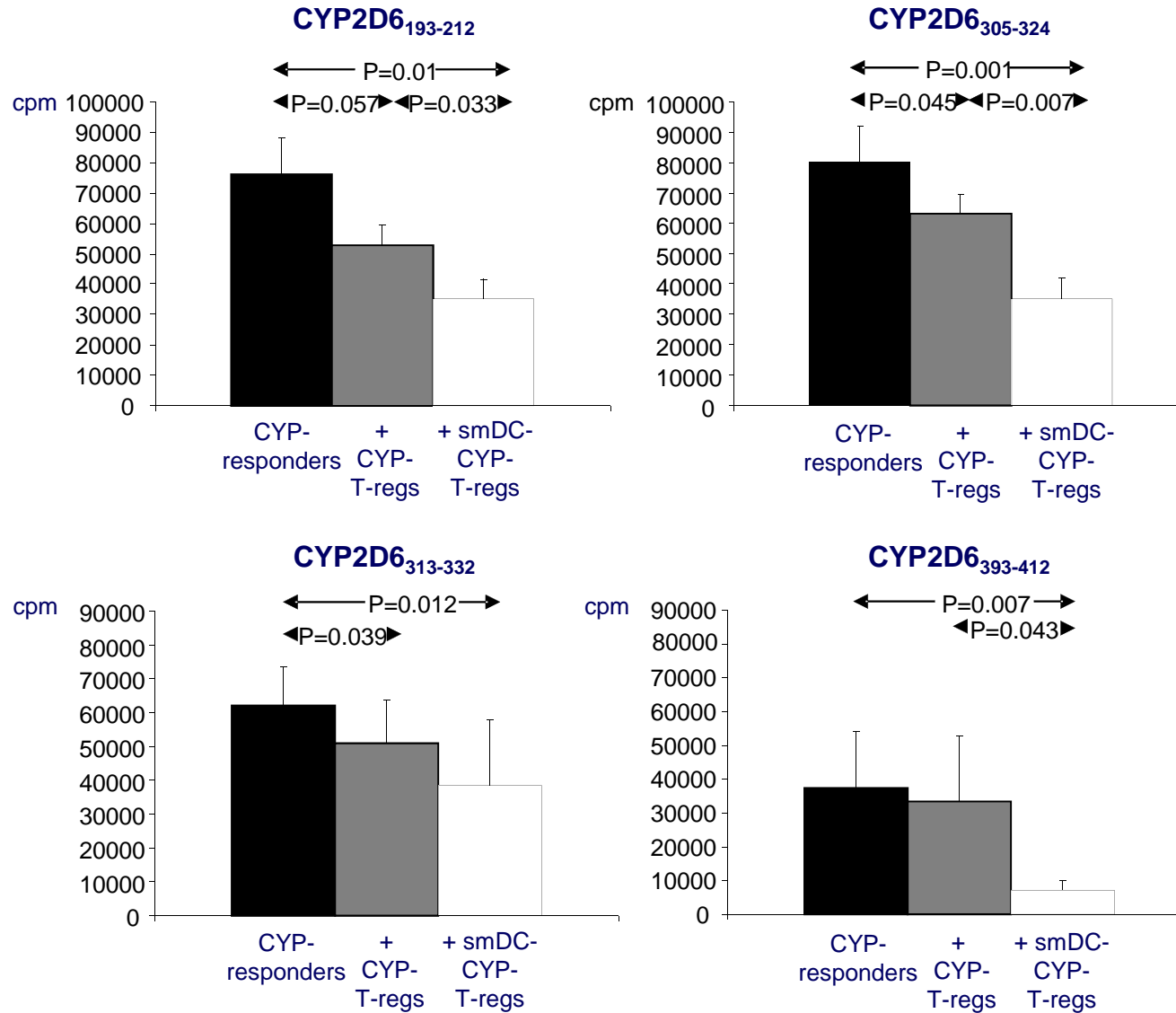


CXCR3 expression



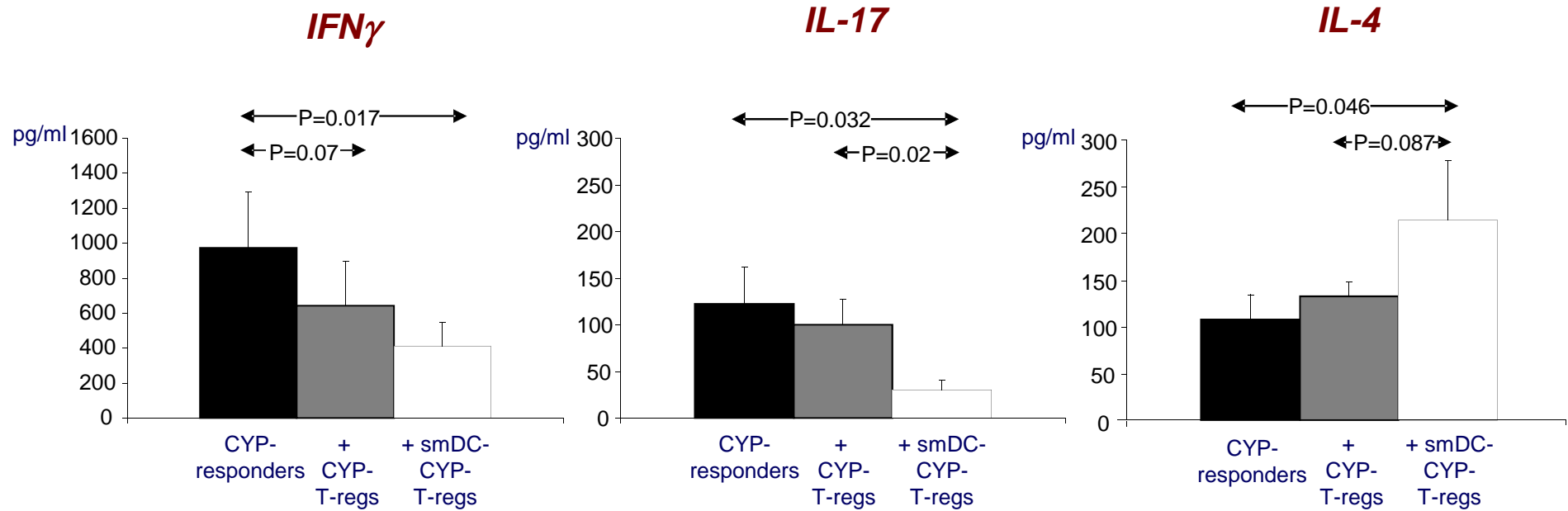
Antigen-specific T-regs

Suppressor function - Regulation of CD4 T-cell proliferation



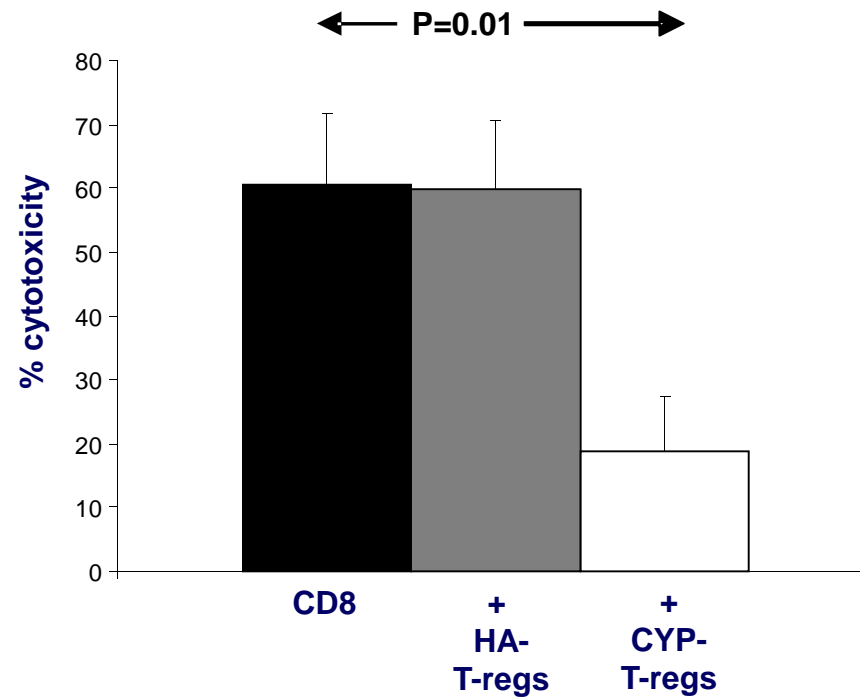
Antigen-specific T-regs

Suppressor function - Regulation of CD4 T-cell cytokine secretion

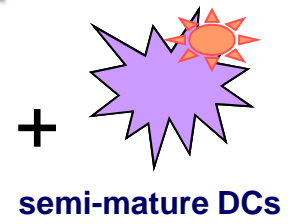
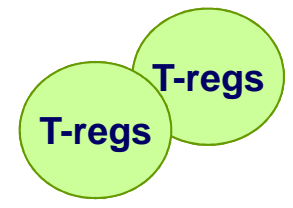
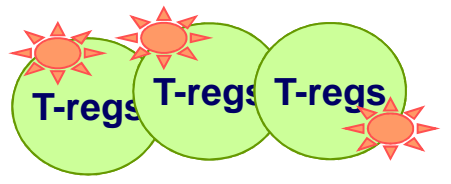
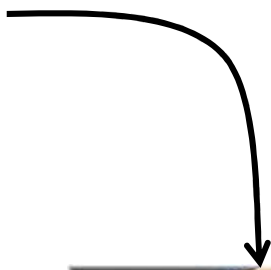
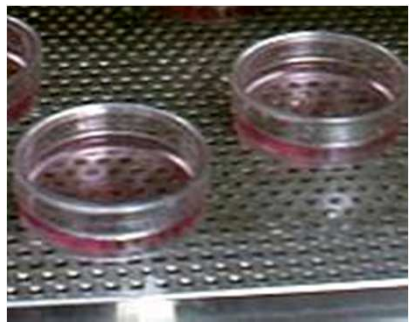
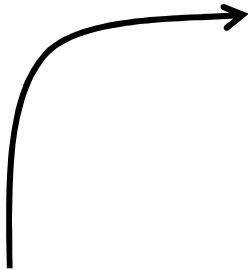


Antigen-specific T-regs

CYP2D6-T-regs: inhibition of CD8 cell cytotoxicity



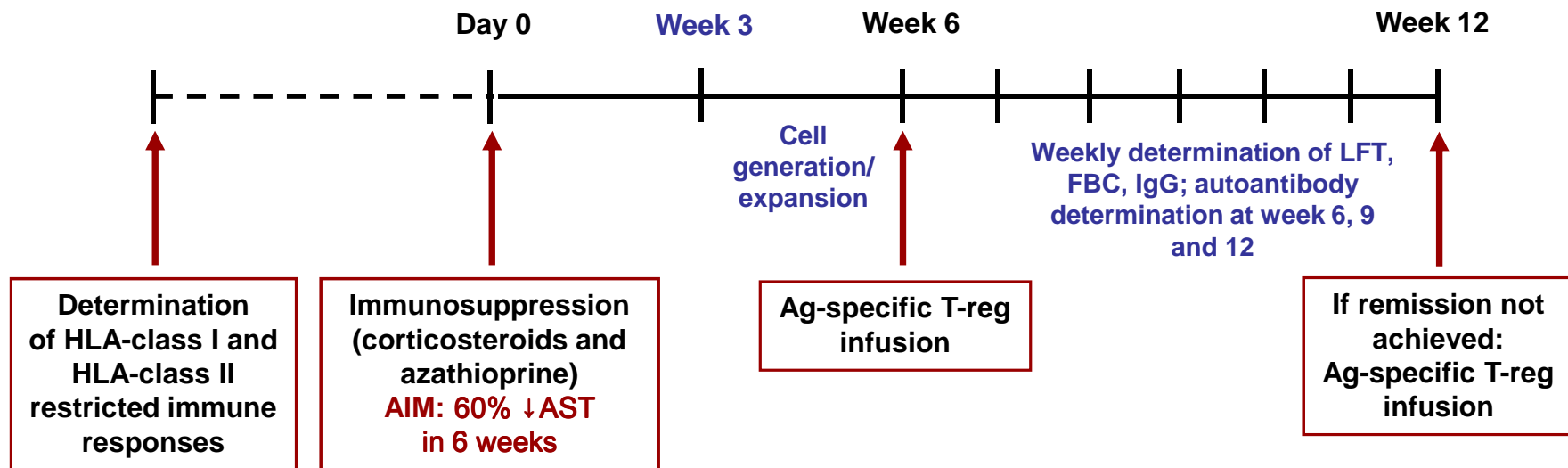
First-in-human clinical trial



First-in-human clinical trial

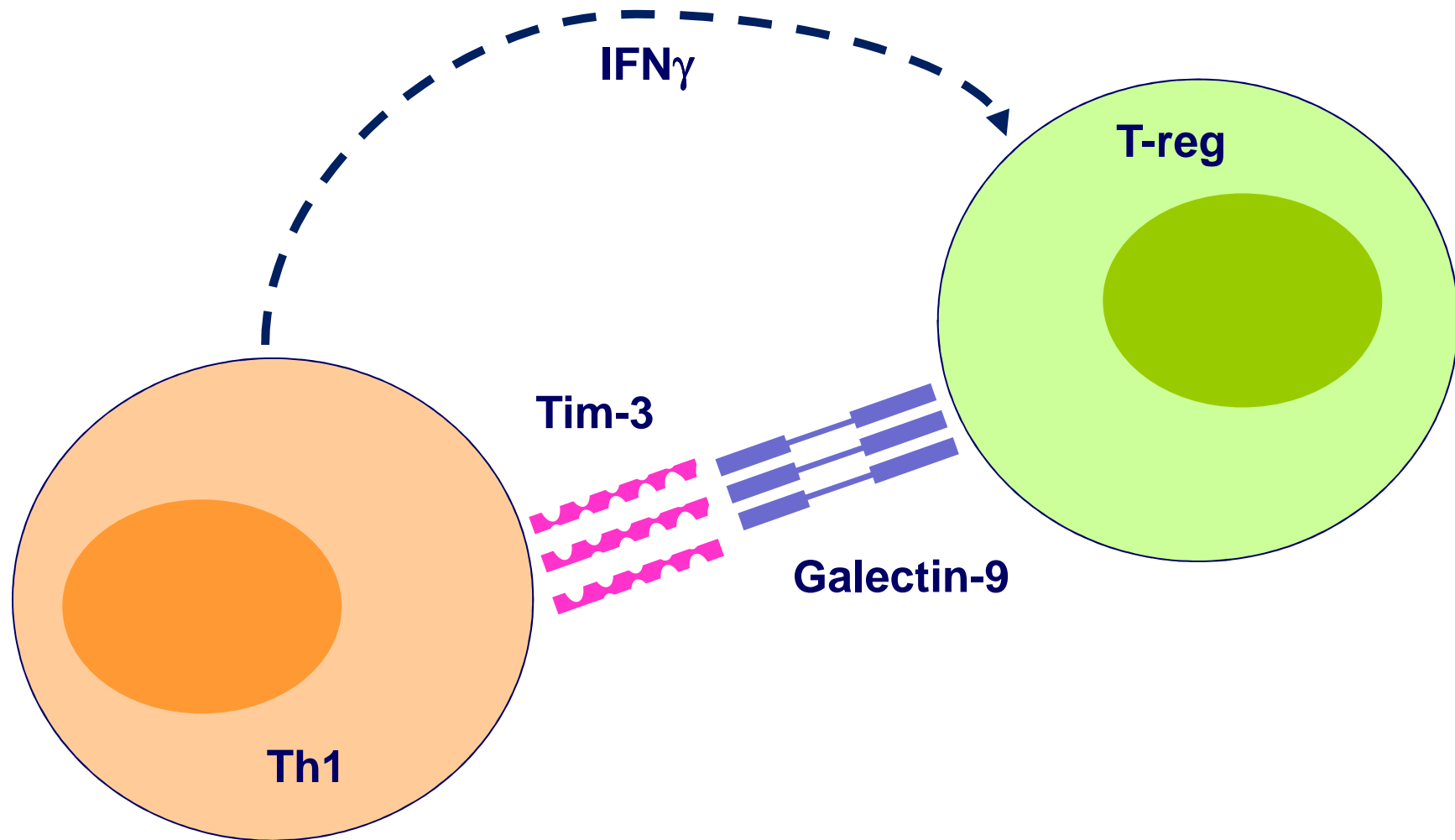
- **Number of cells to use:** 1×10^6 cells/kg?
- **Patients who could benefit**
 - Relapsers (20%), not responding to standard immuno-suppression (10%)
 - Patients at remission (to reduce the side-effects of life-long immunosuppressive treatment)
- **How T-regs will be administered**
 - First immuno-suppressive drugs, then T-regs

*Proposed protocol for T-reg immunotherapy in patients**



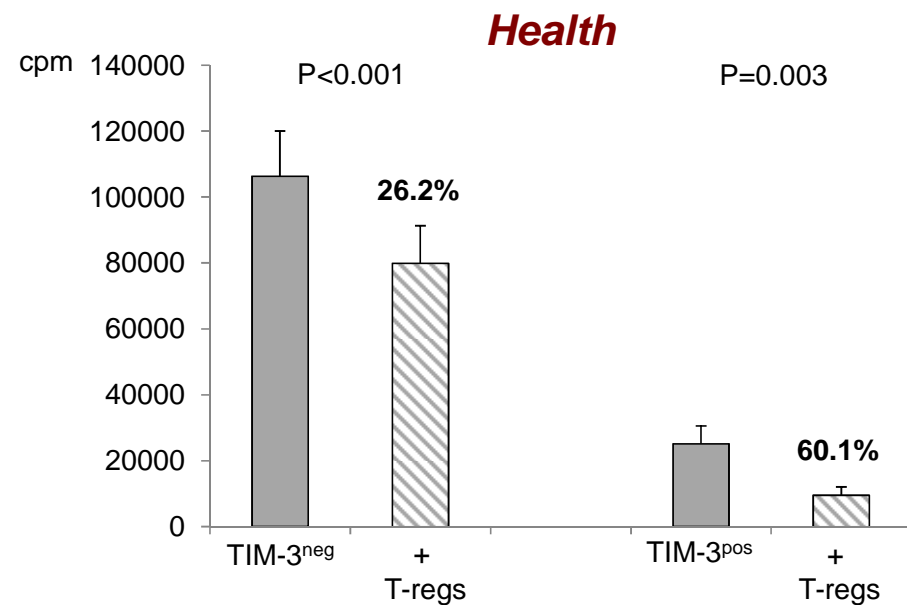
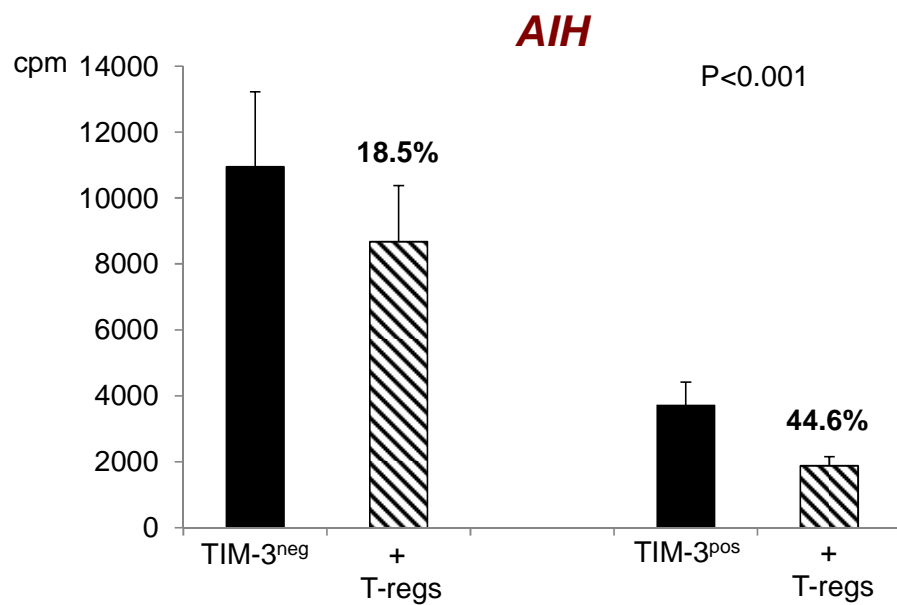
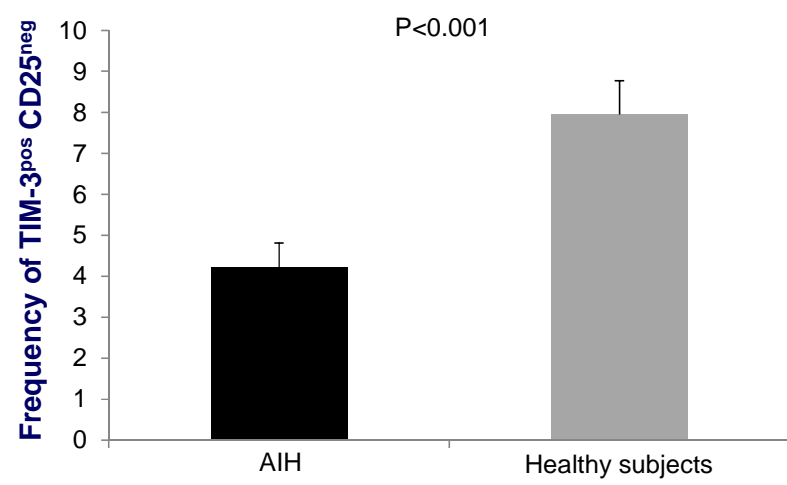
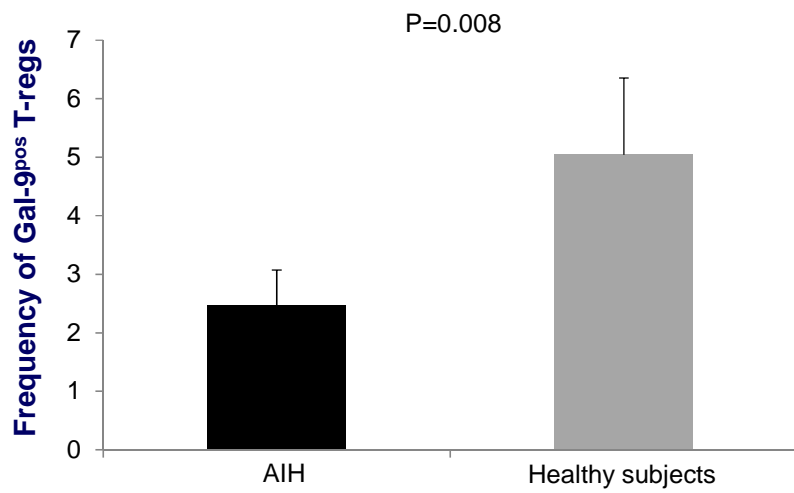
**Patients to start with: frequent relapsers*

Are effector T cells susceptible to T-reg control?



The Galectin-9/Tim-3 pathway

*Liberal et al,
Hepatology 2012*



T-regs in primary biliary cirrhosis

- T-reg numerical reduction in PBC patients (peripheral blood and liver) (*Lan et al, 2006*)
- T-reg numerical deficiency in daughters and sisters of PBC patients (*Lan et al, 2006*)
- No functional T-reg defect (*Lan et al, 2006*)
- IL-2R^(-/-) mice develop portal inflammation and biliary ductular changes similar to PBC patients (*Wakabayashi et al, 2006*)

T-regs in primary sclerosing cholangitis

- Presence of CCR10⁺ regulatory T-cells with anti-inflammatory properties in liver tissue from PSC patients (*Eksteen et al, 2006*)
- Interaction between CCR10 and CCL28 secreted by epithelial cells (*Eksteen et al, 2006*)
- Liver derived T-regs express CXCR3 and CCR4, with CXCR3 mediating T-reg recruitment via hepatic sinusoidal endothelium and CCR4 determining T-reg positioning at sites of inflammation (*Oo et al, 2010*)

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Boston)

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AIH patients

